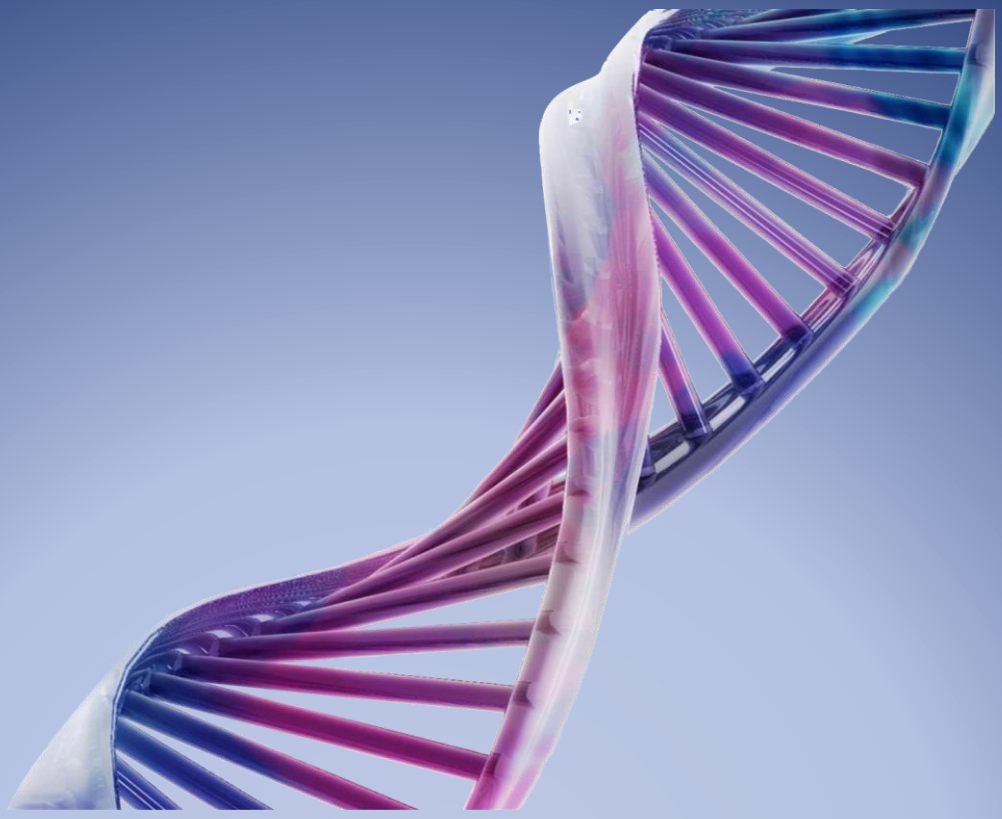


BIOCHEMISTRY II

BIO303

Compiled by: Sir Mughal
Contact: +923152113633

BIOENERGETICS

Bioenergetics is the quantitative study of the **energy transductions,** that is.. changes of one form of energy into another—that occur in living systems, and of the nature and function of the chemical processes underlying these transductions.

Bioenergetics' processes

1. Glycolysis is a biological process in which glucose is broken down into **pyruvate** thus generating two molecules of ATP (Adenosine triphosphate) per molecule of glucose
2. Gluconeogenesis is another process in which the cell synthesizes glucose from biomolecules such as proteins, amino acids and fats
3. Citric acid cycle generates molecules of ATP by oxidation of energy stored in food material.

Laws of Thermodynamics

• There are two fundamental laws of thermodynamics.

1. First law

The first law is the principle of the conservation of energy: it states that:
"for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed."

Entropy

• The term "entropy," was first used in 1851 by Rudolf Clausius, which literally means "a change within,".
• **Entropy** is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.
• Denoted by **S**
• ΔS has a +ve sign when entropy increase and –ve when entropy decreases
• units of entropy are joules/mole.Kelvin (J/mol.K) (joules/mole **multiply by** Kelvin)

2. Second law

The second law of thermodynamics states that "***the universe always tends towards increasing disorder: in all natural processes, the entropy of the universe increases.***

Enthalpy (*H*)

• Enthalpy is the heat content of the reacting system.
• It reflects the number and kinds of chemical bonds in the reactants and products
• When a chemical reaction releases heat, it is said to be **exothermic** and **value of H is negative**
• When a chemical reaction takes up heat from surroundings, it is called as endothermic and have **positive values of *H*.**

Gibbs free energy *(G)*

• **Gibbs free energy, *G*** expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.
• When a reaction proceeds with the release of free energy the free energy change, ***G*, has a negative value** and the reaction is said to be **exergonic.**
• In endergonic reactions, **the system gains free energy and *G* is positive.**
• The units of ***G* and *H*** are joules/mole or calories/mole (1 cal is equal to 4.184 Joules).
• Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation:

$\Delta G = \Delta H - T\Delta S$

ΔG: is the change in Gibbs free energy

ΔH: the change in enthalpy

T: the absolute temperature

ΔS: change in entropy

Cells require sources of Free Energy (G)

- amount of energy capable of doing work during a chemical reaction (G)
- Cells work at constant temperature and pressure (isothermal)
- Heat flow is NOT energy source
- Change in Gibbs Free energy (ΔG)

Significance of Free-Energy Changes (ΔG)

1. Actual free-Energy changes depend on **Reactant and Product Concentrations**

2. Allows prediction of the direction of chemical reactions, their exact equilibrium position and the amount of work done.

3. The free-energy change for a reaction is **independent of the pathway by which the reaction occurs.**

4. Free-energy changes are additive; the net chemical reaction that results from successive reactions sharing a common intermediate has an overall free-energy change that is the sum of the ΔG values for the individual reactions.

Standard free-energy $G°$

The free energy of reaction at under standard conditions:

- **When Temperature: *298K or 25°C*, when reactants are initially present at 1 M concentrations or, for gases, at partial pressures of 101.3 kilopascals (kPa), or 1 atm, the force driving the system toward equilibrium is defined as the standard free-energy change,**

$$\Delta G° = \Delta H° - T\Delta S°$$

Key conventions in biochemical reactions

- Most biochemical reactions occur near pH 7 (buffered)
- Standard biochemical state:
    a) (H_+) is 10^{-7} M
    b) concentration of water is 55.5 M.
- Both the pH and the concentration of water are essentially constant
- Under these conditions, ΔG *is written as* $\Delta G'°$***(with prime).***
- **Concentration of water, H+ and Mg+ are not included in equations for simplicity**

Molar concentration of water

- Density of pure water = 1000 g/L
- Molar mass of water = 18 g/mol (2H + 1 Oxygen=2+16=18)
- Molar concentration of water = d/m

= 1000 / 18.02

= 55.5 mol/L

Difference between ΔG and $\Delta G°$?

• The actual free-energy change, ΔG

• The standard free-energy change, $\Delta G'°$

• Each chemical reaction has a characteristic standard free-energy change, which may be positive, negative, or zero, depending on the **equilibrium constant** of the reaction.

• **Thus $\Delta G'°$ is a constant: it has a characteristic, unchanging value for a given reaction**

• But the *actual* free-energy change, ΔG, is a function of reactant and product ***concentrations and of the temperature prevailing during the reaction,*** none of which will necessarily match the standard conditions as defined above.

• The criteria for spontaneity of a reaction is the value of ΔG not ΔG'0

• If ΔG is negative (if the free energy of products is less than that of reactants).. A reaction is spontaneous.. the reaction tends to go in the forward direction.

• If ΔG is positive (if the free energy of products is more than that of reactants).. A reaction is NOT spontaneous… the reaction tends to go in the reverse direction.

• if ΔG is ZERO (if the free energy of products is equal to that of reactants).. A reaction is At equilibrium.

The Standard Free-Energy is directly related to the Equilibrium constant

What is Equilibrium constant (K)?

• The composition of a reacting system tends to continue changing until **equilibrium** is reached

• At the equilibrium **concentration of reactants and products**, the **rates of the forward and reverse reactions are exactly equal** and no further net change occurs in the system.

• The number that expresses the relationship between the amounts of products and reactants present at **equilibrium** in a reversible chemical reaction is known as the equilibrium constant, *K*eq.

• In the general reaction

$$aA + bB \; cC + dD$$

How to calculate Equilibrium constant?

• The equilibrium constant is given by following equation where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium, equilibrium constant can be written as:

$$Keq = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

Standard free-energy change

The standard free-energy change of a chemical reaction is an alternative mathematical way of expressing its equilibrium constant.

• ΔG is characteristic for a given reaction and can be calculated from the equilibrium constant for the reaction:

$\Delta G'° = -RT \ln K'eq$

Where,

• ***R is the gas constant (8.31 cal/Mol.K)***

• ***T is standard temperature (25 C or 298K)***

• ***K'eq is the equilibrium constant***

• If the *K*eq is equal to 1.0

The standard free-energy change ($\Delta G°$) of that reaction is 0.0 (the natural logarithm **(ln)** of 1.0 is zero)…**EQUILIBRIUM**

- **If *K*eq of a reaction is greater than 1.0**

$\Delta G°$ is negative, **forward direction is favored**

- **If *K*eq is less than 1.0**

$\Delta G°$ is positive, **reverse direction is favored**

WORKED EXAMPLE 13–1 **Calculation of $\Delta G'°$**

Calculate the standard free-energy change of the reaction catalyzed by the enzyme phosphoglucomutase

Glucose 1-phosphate ⇌ glucose 6-phosphate

given that, starting with 20 mM glucose 1-phosphate and no glucose 6-phosphate, the final equilibrium mixture at 25°C and pH 7.0 contains 1.0 mM glucose 1-phosphate and 19 mM glucose 6-phosphate. Does the reaction in the direction of glucose 6-phosphate formation proceed with a loss or a gain of free energy?

Solution: First we calculate the equilibrium constant:

$$K'_{eq} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose 1-phosphate}]} = \frac{19\ \text{mM}}{1.0\ \text{mM}} = 19$$

We can now calculate the standard free-energy change:

$$\begin{aligned}\Delta G'° &= -RT \ln K'_{eq} \\ &= -(8.315\ \text{J/mol·K})(298\ \text{K})(\ln 19) \\ &= -7.3\ \text{kJ/mol}\end{aligned}$$

Because the standard free-energy change is negative, the conversion of glucose 1-phosphate to glucose 6-phosphate proceeds with a loss (release) of free energy. (For the reverse reaction, $\Delta G'°$ has the same magnitude but the *opposite* sign.)

ΔG and $\Delta G'°$ are related

- The actual free-energy change, ΔG is a variable that depends on $\Delta G'°$ and on the concentrations of reactants and products:

$$aA + bB \longleftrightarrow cC + dD$$

$\Delta G = \Delta G'° - RT$ **ln ([products]/[reactants])**

$$\Delta G = \Delta G'° + RT \ln \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

- The concentration terms in this equation express the effects commonly called mass action, and the term $[C]_c[D]_d/[A]_a[B]_b$ is called the **mass-action ratio**, ***Q***.
- Thus the equation can be written a follows:

$\Delta G = \Delta G'° + RT \ln Q$

- Lets suppose that the reaction is under standard conditions..

$$aA + bB \longleftrightarrow cC + dD$$

- But the conc. Of reactants and products is NEITHER equal to each other… Nor the conc. Is 1M
- **non-standard concentrations**
- **Actual conc. Of reactants and products but standard values of $\Delta G'°$, *R and T***
- **Value of ΔG *at equilibrium* (for spontaneous reaction)??**

$$0 = \Delta G = \Delta G'^{\circ} + RT \ln \frac{[C]^{eq}\,[D]^{eq}}{[A]^{eq}\,[B]^{eq}}$$

$\Delta G'^{\circ} = -RT \ln K'_{eq}$

Standard Free-Energy Changes Are Additive

• Consider two sequential chemical reactions

$A \rightleftharpoons B \qquad \Delta G'^{0}_{1}$

$B \rightleftharpoons C \qquad \Delta G'^{0}_{2}$

• Since the two reactions are sequential, we can write the overall reaction as

$A \rightleftharpoons C \qquad \Delta G'^{0}_{total}$

• The ΔG'0 values of sequential reactions are additive.

$$\Delta G'^{0}_{total} = \Delta G'^{0}_{1} + \Delta G'^{0}_{2}$$

• **This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate.**

Example

• For example, the synthesis of glucose 6-phosphate is the first step in the utilization of glucose by many organisms:

Glucose + Pi ⟶ glucose6-phosphate + H2O

$\Delta G'^{\circ}$ = 13.8 kJ/mol

• The positive value of $\Delta G'^{\circ}$ predicts that under standard conditions the reaction will tend not to proceed spontaneously in the direction written.

• Hydrolysis of ATP to ADP and Pi, is very exergonic:

ATP + H2O = ADP + Pi

$\Delta G'^{\circ}$ = -30.5 kJ/mol

• These two reactions share the common intermediates Pi and H2O and may be expressed as sequential reactions:

(1) Glucose + Pi ⟶ glucose6-phosphate + H2O ____reaction (1)

(2) ATP + H2O ⟶ ADP + Pi ____reaction (2)

Sum: ATP + Glucose ⟶ ADP + glucose6-phosphate

• The overall standard free-energy change is obtained by adding the $\Delta G'^{\circ}$ values for individual reactions:

$\Delta G'^{\circ}$=13.8 kJ/mol + (-30.5 kJ/mol) = -16.7 kJ/mol

As $\Delta G'^{\circ}$ is a way of expressing the equilibrium constant for a reaction. For reaction (1) above,

$$K'_{eq_1} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose}]\,[P_i]} = 3.9 \times 10^{-3}\ \text{M}^{-1}$$

• The equilibrium constant for the hydrolysis of ATP is

$$K'_{eq_2} = \frac{[ADP]\,[P_i]}{[ATP]} = 2.0 \times 10^{5}\ \text{M}$$

• The equilibrium constant for the two coupled reactions is

$$K'_{eq_3} = \frac{[\text{glucose 6-phosphate}]\,[ADP]\,[P_i]}{[\text{glucose}]\,[P_i]\,[ATP]}$$

$$= (K'_{eq_1})(K'_{eq_2}) = (3.9 \times 10^{-3}\ \text{M}^{-1})(2.0 \times 10^{5}\ \text{M})$$

$$= 7.8 \times 10^{2}$$

• This calculation illustrates that equilibrium constants: although the $\Delta G'^{\circ}$ values for two reactions that sum to a third, overall reaction are *additive*, the *K*.eq for the overall reaction is the *product* of the individual *K*.eq values for the two reactions.

• **Equilibrium constants are *multiplicative.***

• Energy stored in ATP is used to drive to synthesis of glucose 6-phosphate, even though its formation from glucose and Pi is endergonic.

• **This strategy works only if compounds such as ATP are continuously available.**

• **Homogeneous chemical reaction** **chemical reactions** in which the reactants and products are in the same phase.

• **Heterogeneous chemical reaction** have reactants in two or more phases.

• **Le Châtelier Principle** ("The Equilibrium Law") states that when a system experiences a disturbance (such as concentration, temperature, or pressure changes), it will respond to restore a new equilibrium state.

TYPES OF CHEMICAL REACTIONS IN CELLS

5 types of chemical reactions in cells

The reactions going on in biological systems can be categorized into 5 types:

1. Reactions that make or break carbon-carbon bonds
2. Internal rearrangements, isomerization, and eliminations
3. Free-radical reactions
4. Group transfer
5. Oxidation-reduction reactions

2 basic chemical principles

(1) A covalent bond can be broken in two general ways

• Homolytic cleavage

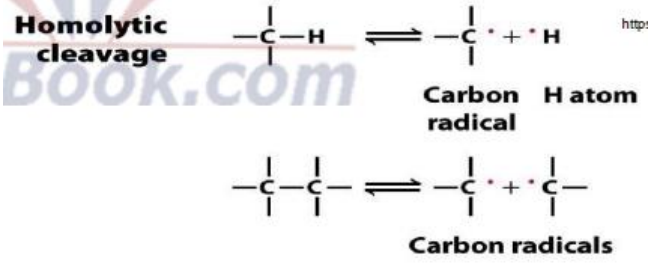
• Heterolytic cleavage

• A **Covalent bond**, also called a molecular *bond*, involves the sharing of electron pairs between atoms.

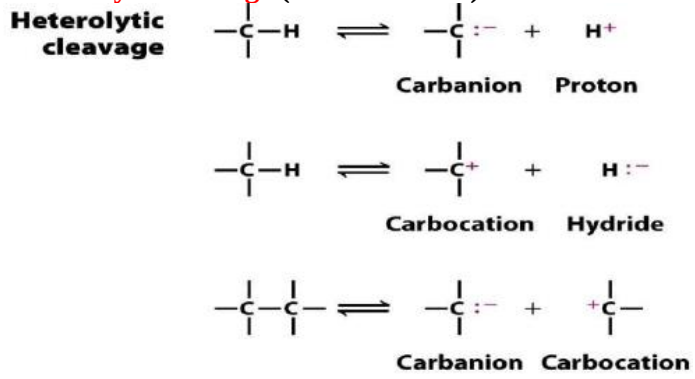
• These electron pairs are known as shared pairs or *bonding* pairs, and the stable balance of attractive and repulsive forces between atoms, when they share electrons, is known as *covalent bonding*.

2 mechanisms for breaking a C-C or C-H bond

- **Homolytic cleavage: each atom leaves the bond as a radical, carrying one unpaired electron**



- **Heterolytic cleavage (more common): one atom retains both bonding electrons**



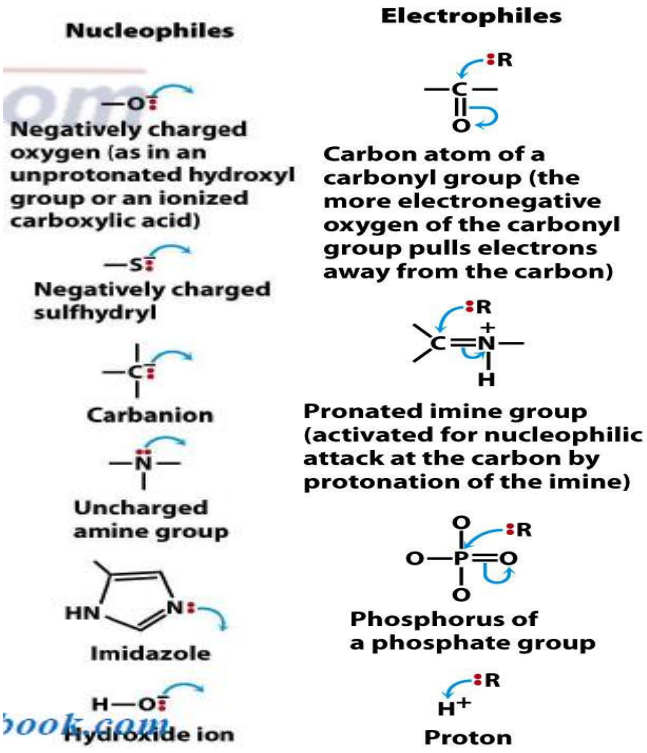
Second principle

2. Many biochemical reactions involve interactions between nucleophiles and electrophiles.

- Nucleophiles: functional groups rich in and capable of donating electrons. Nucleophiles are Lewis bases.
- Electrophiles: electron-deficient functional groups that seek electrons

Common nucleophiles and electrophiles in biochemical reactions

FIGURE 13–2 Common nucleophiles and electrophiles in biochemical reactions. Chemical reaction mechanisms, which trace the formation and breakage of covalent bonds, are communicated with dots and curved arrows, a convention known informally as "electron pushing." A covalent bond consists of a shared pair of electrons. Nonbonded electrons important to the reaction mechanism are designated by dots (:). Curved arrows (↷) represent the movement of electron pairs. For movement of a single electron (as in a free radical reaction), a single-headed (fishhook-type) arrow is used (↷). Most reaction steps involve an unshared electron pair.



Electron pushing: The movement of a pair of electrons (a lone pair of electrons or a bond) from an electron rich site to an electron poor site.

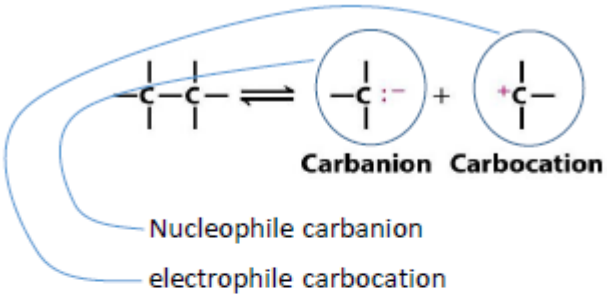
Significance of Carbonyl group

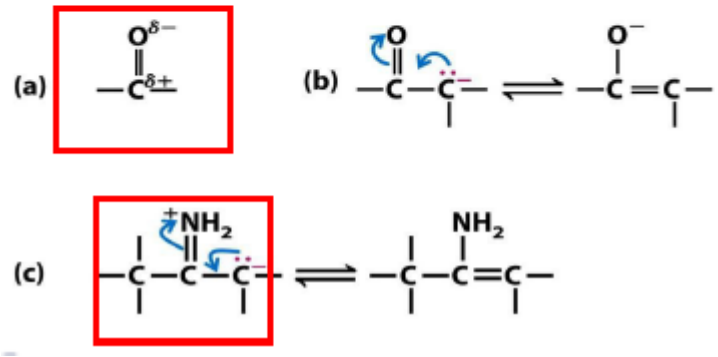
• Carbonyl groups are particularly important in the chemical transformations of metabolic pathways…

(1)The carbon of a **carbonyl group has a partial positive** charge due to the electron-withdrawing property of the **carbonyl oxygen** ⟶ **electrophilic carbon**

(2)Carbonyl group facilitate the formation of a carbanion's on an adjoining carbon by delocalizing the carbanion's negative charge

(3)An imine group can serve a similar function





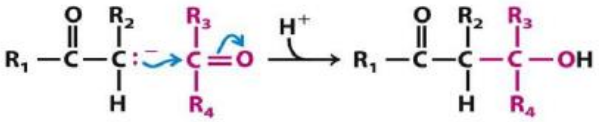
1. Types of reactions that make or break C-C bonds

• Three major class of reaction in which **carbon-carbon** bonds are formed or broken

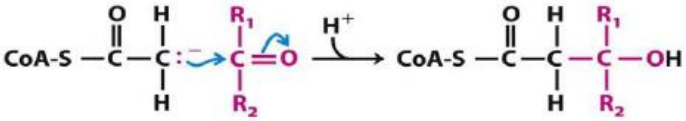
(1)Aldol condensation

(2)Claisen condensation

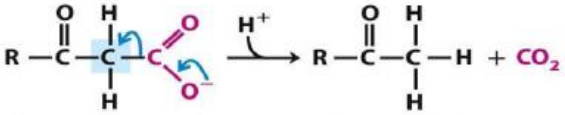
(3)Decarboxylation



Aldol condensation



Claisen ester condensation

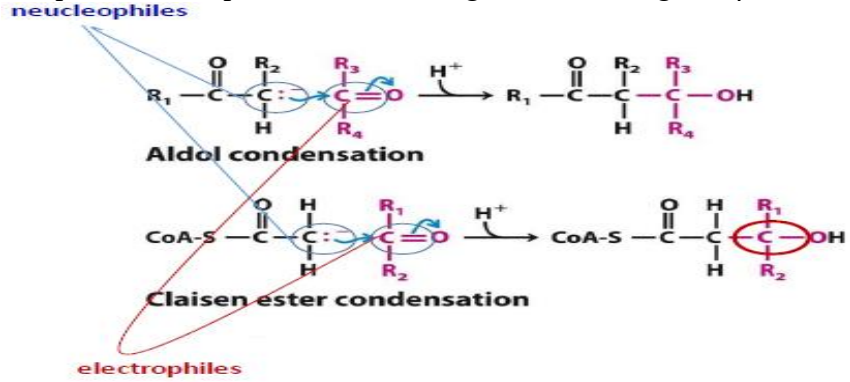


Decarboxylation of a β-keto acid

1. Aldol condensation: When the enolate of an aldehyde or a ketone reacts at the α-carbon with the carbonyl of another molecule to obtain β-hydroxy aldehyde or β-hydroxy ketone

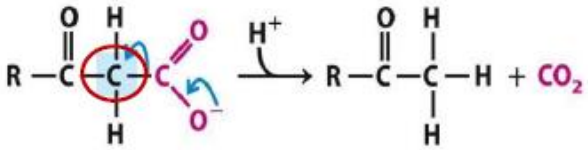
2. Claisen condensation:

A carbon–carbon bond forming **reaction** that occurs between two esters or one ester and another carbonyl compound in the presence of a strong base, resulting in a β-keto ester.



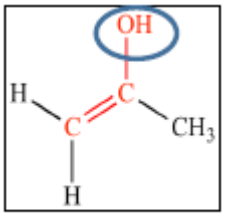
In **Aldol condensation and Claisen condensation** a carbanion serves as nucleophile and the carbon of a carbonyl group serves as electrophile, but **,** in **Claisen condensation,** the carbanion is stabilized by the carbonyl of an adjacent thioester

3. Decarboxylation: involves the formation of a carbanion stabilized by a carbonyl group. eg, during formation of
ketone bodies during fatty acid metabolism



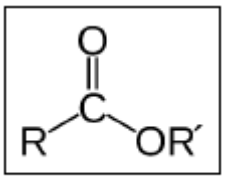
Decarboxylation of a β-keto acid

• **ENOL:** also known as, alkenol, is a type of reactive structure or intermediate in organic chemistry that is represented as an alkene with a **hydroxyl group attached to one end of the alkene double bond.**



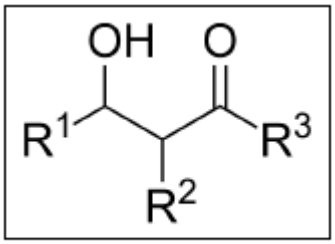
ENOL

• **ESTER:** is a chemical compound derived from an acid (organic or inorganic) in which at least one –OH (hydroxyl) group is replaced by an –O–alkyl (alkoxy) group. Usually, **esters** are derived by the reaction between a carboxylic acid and an alcohol.



Ester

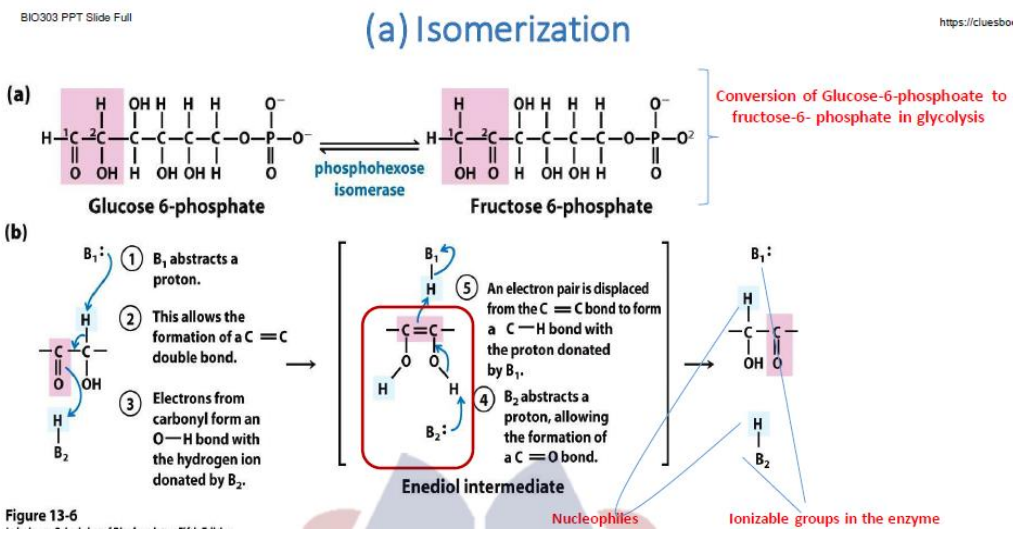
• **ALDOL:** An **aldol** (from "Aldehyde alcohol") is a hydroxyl ketone or aldehyde, and is the product of **aldol** addition (as opposed to **aldol** condensation.



Aldol

2. Internal rearrangements, isomerization, and eliminations

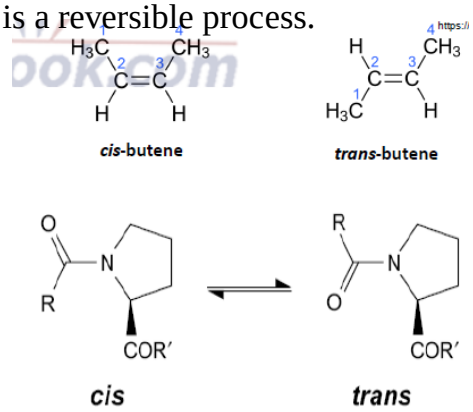
• **Intramolecular rearrangement**: redistribution of electrons results in alterations of many different types without a change in the overall oxidation state of the molecule.
• different group in the molecule may undergo oxidation-reduction, with no net change in oxidation state of the molecule.
• groups at a double bond may undergo a cis-trans rearrangement
• the positions of double bonds may be transposed



(b) Cis-trans rearrangement

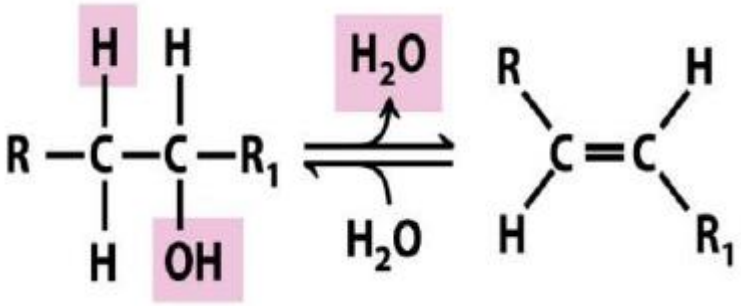
• In general, Peptide bonds formed between amino acid residues with alpha carbons in trans-configuration… (more than 99.95% have **trans configuration**.)
• For peptide bonds involving the **imino nitrogen of proline**, **peptide bonds** are able to exist both in **cis and trans configuration.. (6% are in the cis configuration)** through peptidylprolyl (proline) isomerase enzyme

The cis and trans isomers of proline. The process of formation of cis and trans conformation is via isomerism, ant the reaction is a reversible process.



(c) Elimination

• **The loss of water from an alcohol, resulting in the introduction of a C=C** bond b/w 2 adjacent carbon atoms
• **An elimination reaction with no changes in oxidation state**



3. Free-Radical reactions

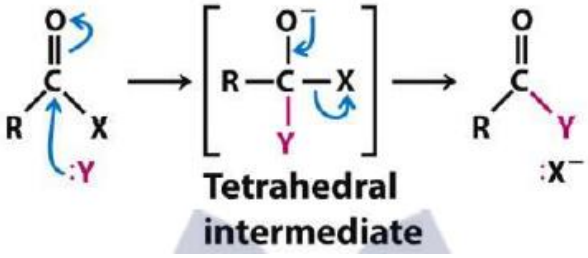
• Homolytic cleavage of covalent bond ⟶ produce free radical
1) Isomerizations that make use of adenosylcobalamin (vitamin B12) for methylmalonyl-CoA mutase (MCM) reactions
2) Radical-initiated **decarboxylation** reaction
3) Some **reduction** reaction, such as that catalyzed by **ribonucleotide reductase (converts ribonucleotides to deoxyribonucleotides)**
4) Some **rearrangement reactions**, such as that catalyzed by **DNA photolyase**

Oxygen-independent **coproporphyrinogen III oxidase** (HemN) catalyzes decarboxylation

4. Group Transfer reactions

• The transfer of **acyl, glycosyl, and phosphoryl** is common in living cells

1) Acyl transfer: acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an **acyl group** to form a tetrahedral intermediate, eg. Chymotrypsin reaction



2) Phosphoryl group activates the metabolic intermediate for subsequent reaction

• Nucleophilic substitutions reactions and attachment of a phosphoryl group to functional groups like (-OH) occur in hundreds of metabolic reactions

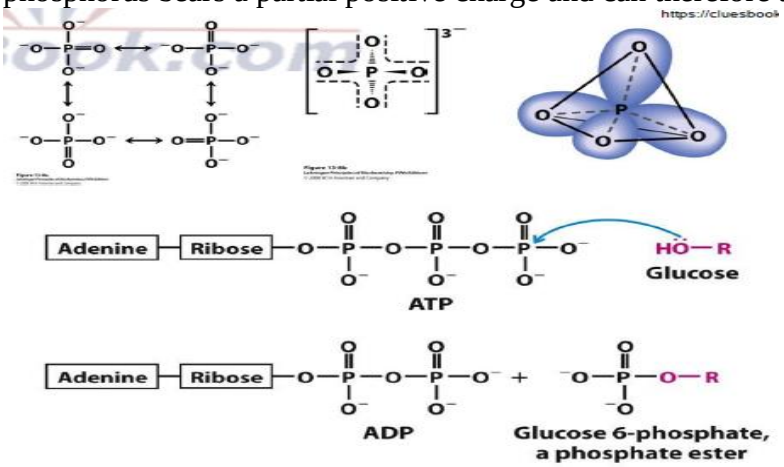
• A nucleophile (OH) on c-6 of glucose attacks ATP, it displaces ADP..and a pentacovalent intermediate can be formed.

• A phosphoryl group (-PO32-) from ATP is transferred to an alcohol (→ forming a phosphate ester)

• When nucleophile attacks the electrophilic phosphorus atom in ATP, a relatively stable pentacovalent structure forms as a reaction intermediate.

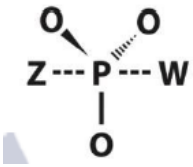
Phosphorous can form five covalent bonds

• Because oxygen is more electronegative than phosphorus, the sharing of electrons is unequal: the central phosphorus bears a partial positive charge and can therefore act asan electrophile.



• Kinases: enzymes that catalyze phosphoryl group transfer) using ATP as donor)

• Thioalcohols (thiols): the oxygen atom of an alcohol is replaced with a sulfur atom



Z = R — OH

W = ADP

5. Oxidation-Reduction reactions

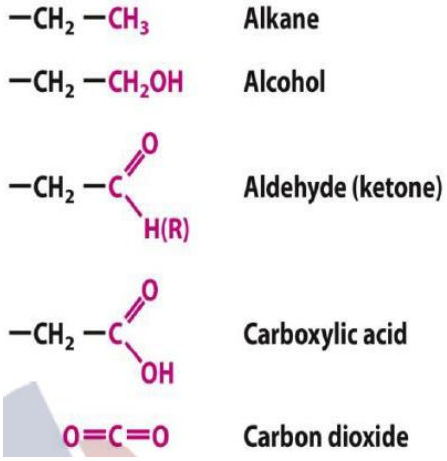
Oxidation

• Addition of oxygen
• **loss** of electrons
• **Loss** of hydrogen

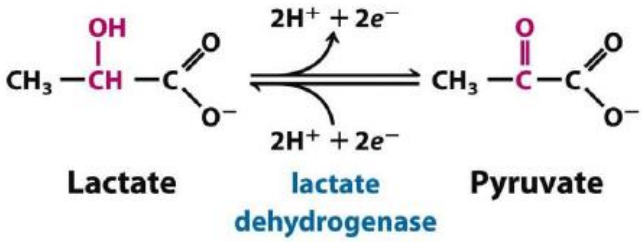
Reduction

• Removal of oxygen
• **Gain** of electrons
• **Gain** of hydrogen

• Carbon **atoms can exist in five oxidation states**
• The transition of these status are of crucial importance in metabolism
• Biological reaction (commonly), a compound loses two electrons and two hydrogen ions: **dehydrogenations (catalyzed by dehydrogenases)**
• In some biological oxidation, a carbon atom becomes covalently bonded to an oxygen atom (a enzyme catalyze these reaction calls **oxidases**).

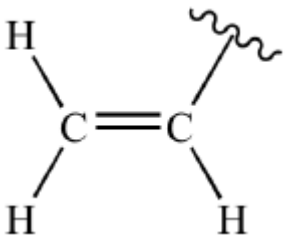


Oxygenases: if oxygen is derived directly from molecular oxygen (O_2).



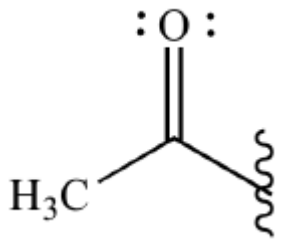
Vinyl group

In chemistry, vinyl or ethenyl is the functional group with the formula −CH=CH2. It is the ethylene (IUPAC ethene) molecule ($H_2C=CH_2$) less one hydrogen atom.



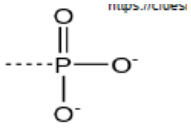
Acyl group

It contains a double bonded oxygen atom and an **alkyl group**. In organic chemistry, the **acyl group** (IUPAC name: alkanoyl) is usually derived from a carboxylic acid. **Acyl group is electron withdrawing due to the electronegativity of the oxygen atom.**

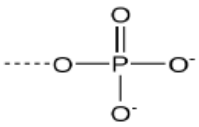


Phosphoryl group

A *phosphoryl group* is the chemical entity $PO3^{2-}$. The term is usually used for the compounds in which the *phosphoryl group* is attached to other atoms, e.g. *phosphoryl* chloride, or in the description of catalytic mechanism.



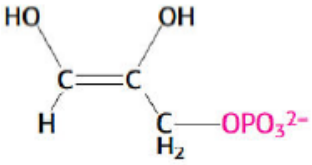
phosphoryl group



phosphate group

Enediol

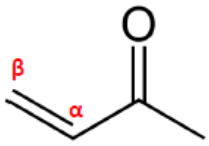
An **alkene enol** with a **hydroxyl group attached to both carbon atoms of the carbon double bond**. A reducing sugar can form an enediol.



Enediol intermediate

Enone:

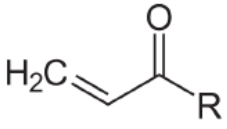
An enone, also called an α, β-unsaturated carbonyl, is a type of organic compound consisting of an alkene conjugated to a ketone. The simplest enone is methyl vinyl ketone or $CH_2{=}CHCOCH_3$.



Enone

Acryloyl group

In organic chemistry, the **acryloyl group is form of enone** with structure $H_2C{=}CH–C(=O)–$**.** It is the **acyl group derived from acrylic acid**. The preferred IUPAC name for the group is prop-2-enoyl, and it is also known as **acrylyl or simply acryl.**



Acryloyl group

Phosphoryl Group Transfers and ATP

• **Energy currency** of living cells
• ATP is used for **Phosphoryl transfer reactions**
• Heterotrophic organisms obtain energy from ATP by breakage of covalent bond, and can undergo one of the two following reactions.

A) ATP $\longrightarrow$ ADP+ P_i OR

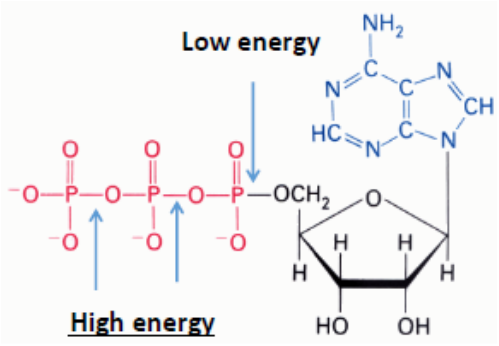
B) ATP $\longrightarrow$ AMP+ 2 P_i

ATP donates some of its chemical energy to
1. Endergonic processes such as the synthesis of **metabolic intermediates** and macromolecules from smaller precursors
2. The **transport of substances across membranes** against concentration gradients
3. Mechanical motion (**muscle contraction**), etc.

Energy donation involves group transfer and hydrolysis of ATP/energy rich phosphate compounds.

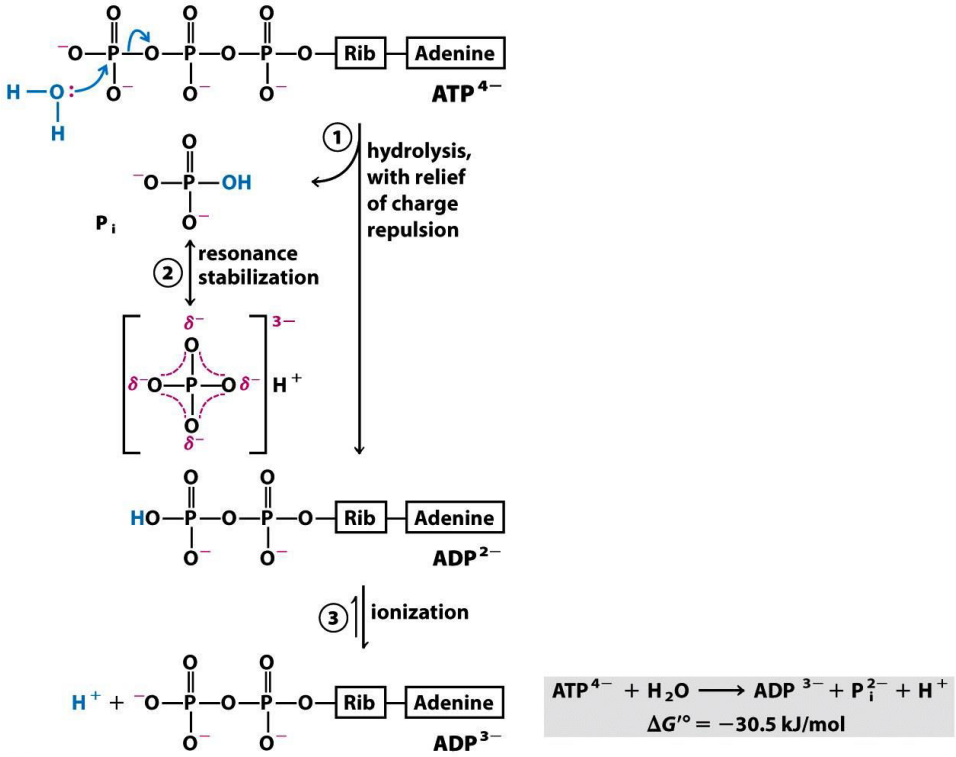
The Free-Energy Change for ATP Hydrolysis Is Large and Negative

1. The hydrolytic cleavage of the terminal **phosphoanhydride bond** in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP
2. The terminal two **phosphoanhydride bonds**, are known as high energy **bonds** because their cleavage releases tremendous amount of free energy
3. Released Pi and ADP are highly soluble.



Adenosine Triphosphate

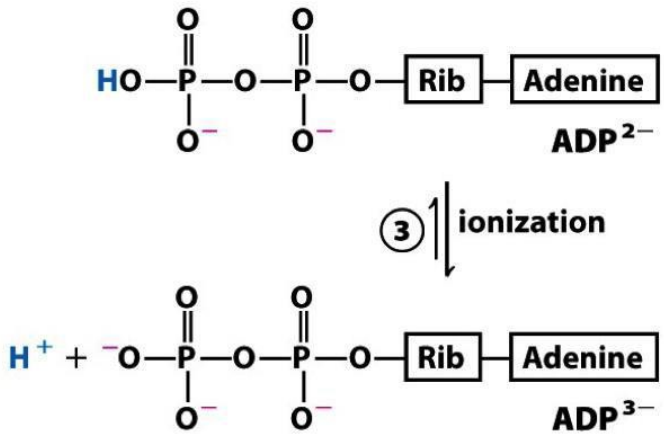
FIGURE 13–11 Chemical basis for the large free-energy change associated with ATP hydrolysis. ❶ The charge separation that results from hydrolysis relieves electrostatic repulsion among the four negative charges on ATP. ❷ The product inorganic phosphate (P_i) is stabilized by formation of a resonance hybrid, in which each of the four phosphorus-oxygen bonds has the same degree of double-bond character and the hydrogen ion is not permanently associated with any one of the oxygens. (Some degree of resonance stabilization also occurs in phosphates involved in ester or anhydride linkages, but fewer resonance forms are possible than for P_i.) A third factor (not shown) that favors ATP hydrolysis is the greater degree of solvation (hydration) of the products P_i and ADP relative to ATP, which further stabilizes the products relative to the reactants.



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -30.5\ kJ/mol$$

Figure 13-11
Lehninger Principles of Biochemistry, Fifth Edition

• ADP2- is the other product of hydrolysis and it immediately ionizes, releasing H+ into a medium of very low [H+] (~10-7 M)
• the hydrolysis of ATP is highly **exergonic (ΔG'0 = -30.5 kj/mol)**, the ATP is stable at pH 7
• Rapid hydrolysis of ATP occurs only when catalyzed by an enzyme.



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -30.5\ kJ/mol$$

• Actual free energy of hydrolysis (ΔG) of ATP in living cells is very different because
(1)the cellular concentration of ATP, ADP, and Pi are not identical
(2)ATP, ADP, and Pi concentration are much lower than the standard condition of 1M
(3)Mg^{2+} in the cytosol binds to ATP and ADP

TABLE 13–5 Adenine Nucleotide, Inorganic Phosphate, and Phosphocreatine Concentrations in Some Cells

	Concentration (mM)*				
	ATP	ADP†	AMP	P_i	PCr
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Rat neuron	2.59	0.73	0.06	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	1.65	0
E. coli cell	7.90	1.04	0.82	7.9	0

*For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 510.

†This value reflects total concentration; the true value for free ADP may be much lower (p. 503).

Table 13-5
Lehninger Principles of Biochemistry, Fifth Edition

• **For ATP as phosphoryl group donor, the true substrate is $MgATP^{-2}$.**

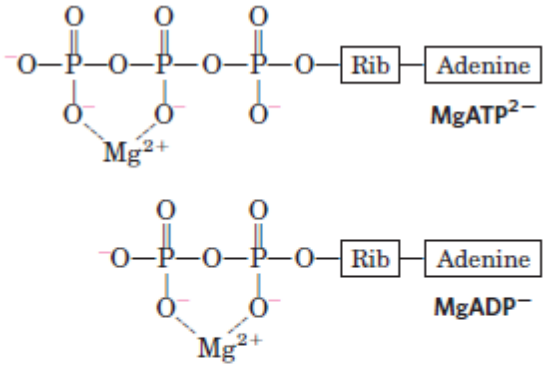


FIGURE 13–12 Mg^{2+} and ATP. Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

Phosphorylation potential: The actual free energy of hydrolysis of ATP under intracellular conditions is often called its **phosphorylation potential**, ΔG.

WORKED EXAMPLE 13–2 Calculation of ΔG_p

Calculate the actual free energy of hydrolysis of ATP, ΔG_p, in human erythrocytes. The standard free energy of hydrolysis of ATP is −30.5 kJ/mol, and the concentrations of ATP, ADP, and P_i in erythrocytes are as shown in Table 13–5. Assume that the pH is 7.0 and the temperature is 37 °C (body temperature). What does this reveal about the amount of energy required to *synthesize* ATP under the same cellular conditions?

Solution: The concentrations of ATP, ADP, and P_i in human erythrocytes are 2.25, 0.25, and 1.65 mM, respectively. The actual free energy of hydrolysis of ATP under these conditions is given by the relationship (see Eqn 13–4)

$$\Delta G_p = \Delta G'^{\circ} + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

Substituting the appropriate values we get

$$\begin{aligned}\Delta G_p &= -30.5\ \text{kJ/mol} + \left[(8.315\ \text{J/mol}\cdot\text{K})(310\ \text{K}) \ln \frac{(0.25\times10^{-3})(1.65\times10^{-3})}{(2.25\times10^{-3})}\right] \\ &= -30.5\ \text{kJ/mol} + (2.58\ \text{kJ/mol}) \ln 1.8\times10^{-4} \\ &= -30.5\ \text{kJ/mol} + (2.58\ \text{kJ/mol})(-8.6) \\ &= -30.5\ \text{kJ/mol} - 22\ \text{kJ/mol} \\ &= -52\ \text{kJ/mol}\end{aligned}$$

(Note that the final answer has been rounded to the correct number of significant figures (52.5 rounded to 52), following rules for rounding a number that ends in a 5 to the nearest even number.) Thus ΔG_p, the actual free-energy change for ATP hydrolysis in the intact erythrocyte (−52 kJ/mol), is much larger than the standard free-energy change (−30.5 kJ/mol). By the same token, the free energy required to *synthesize* ATP from ADP and P_i under the conditions prevailing in the erythrocyte would be 52 kJ/mol.

Phosphorylated compounds

• Phosphoenolpyruvate
• 1,3-bisphosphoglycerate
• Phosphocreatine
• ADP
• ATP
• AMP
• PPi
• Glucose 1-phosphate
• Fructose 6-phosphate
• Glucose 6-phosphate

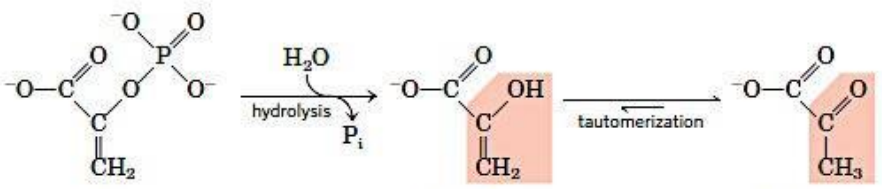
Large Free Energies of Hydrolysis Phosphorylated Compounds and Thioesters

Other compounds that have large free energy of Hydrolysis
1. Phosphorylated compounds
2. Thioesters (Acetyl-CoA)

Phosphoenolpyruvate (PEP)

• Phosphoenolpyruvate contains a **phosphate ester bond** that undergoes hydrolysis to yield to **enol form of pyruvate**
• The **enol** form of pyruvate can immediately tautomerize to the more stable **keto** form of pyruvate
• Because phosphoenolpyruvate has only one form (enol) and the product, pyruvate, has two possible forms, the product is more stabilized relative to the reactant.
• This is the greatest contributing factor to the high standard free energy change of hydrolysis of phosphoenolpyruvate (ΔG'0 = -61.9 kj/mol).

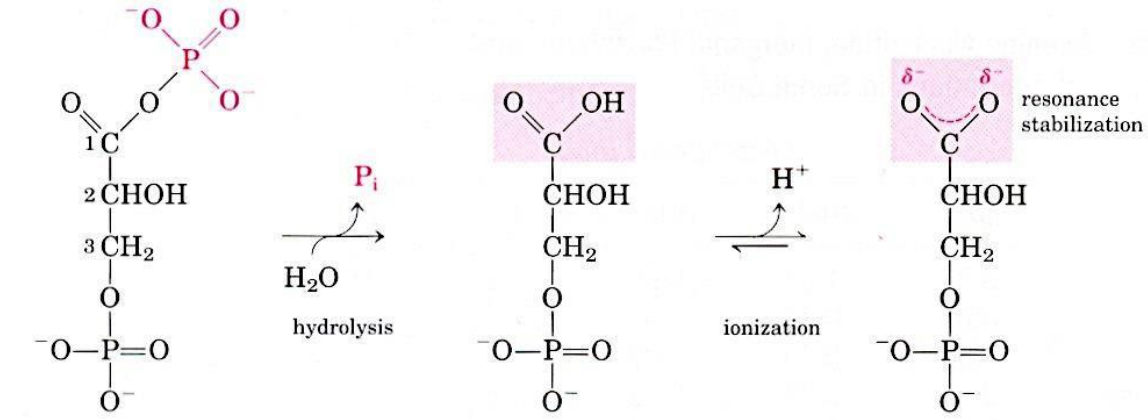
FIGURE 13–13 Hydrolysis of phosphoenolpyruvate (PEP). Catalyzed by pyruvate kinase, this reaction is followed by spontaneous tautomerization of the product, pyruvate. Tautomerization is not possible in PEP, and thus the products of hydrolysis are stabilized relative to the reactants. Resonance stabilization of P_i also occurs, as shown in Figure 13–11.



$$PEP^{3-} + H_2O \longrightarrow pyruvate^- + HPO_4^{2-}$$
$$\Delta G'^{\circ} = -61.9\ kJ/mol$$

1,3-bisphosphoglycerate

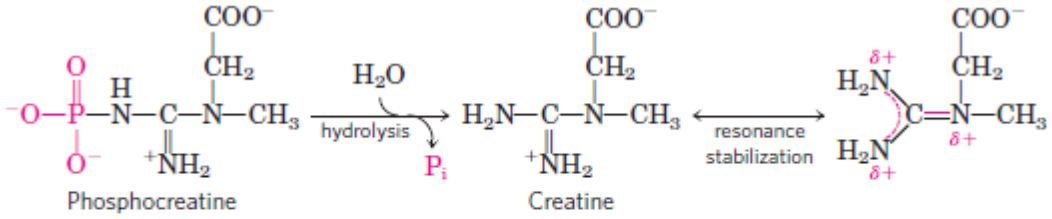
• 1,3-bisphosphoglycerate contains an **anhydride bond** between the carboxyl group at C-1 and phosphoric acid.
• Hydrolysis of this **acyl phosphate** is accompanied by a large, negative, standard free energy change (ΔG'0 = -49,3 kj/mol).
• This large, negative ΔG'0 can, again, be explained in terms of the structure of reactants and products.
• Addition of water to anhyhride bond of 1,3-**bisphosphoglycerate, one of the direct products, 3-phosphoglyceric acid,** immediately leads to the lose a proton to give the **carboxylate ion,** 3-phosphoglycerate, which has two equally probable resonance forms.
• Removal of a direct product, **3-phospho-glyceric acid**, and formation of resonance-stabilized ion favor the forward reaction.



$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^\circ = -49.3\ \text{kJ/mol}$$

Phosphocreatine (Pcr)

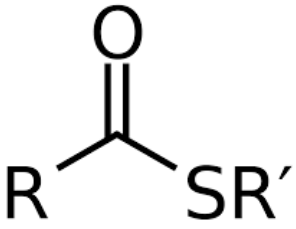
- In the **phosphocreatine**, the P-N bond can be hydrolyzed to generate free creatine and Pi.
- The release of Pi and the resonance stabilization of creatine favor the forward reaction.
- Pi is also resonance stabilized.
- The standard free energy change of phosphocreatine is large and negative (ΔG'0 = -43 kj/mol).



$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + HPO_4^{2-}$$
$$\Delta G'^\circ = -43.0\ \text{kJ/mol}$$

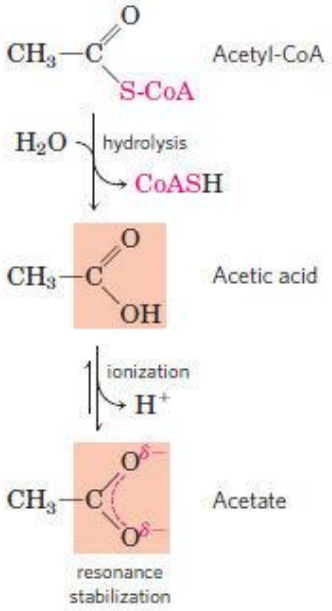
Thioesters

- Thioesters **contain a sulfur** atom in the position occupied by an oxygen
- Thioesters have large, negative standard free energy change of hydrolysis.
- **Hydrolysis of acetyl-coenzyme A..** important in metabolism and has a large, negative, standard free energy of hydrolysis.
- **The acyl group in these compounds is activated for trans-acylation**, condensation or oxidation-reduction reactions.
- Hydrolysis of the **ester bond generates a carboxylic acid**
- ΔG'0 = -31,4 kj/mol for acetyl-CoA hydrolysis



Hydrolysis of acetyl-coenzyme A.

Acetyl-CoA is a thioester with a large, negative, standard free energy of hydrolysis.



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4 \text{ kJ/mol}$$

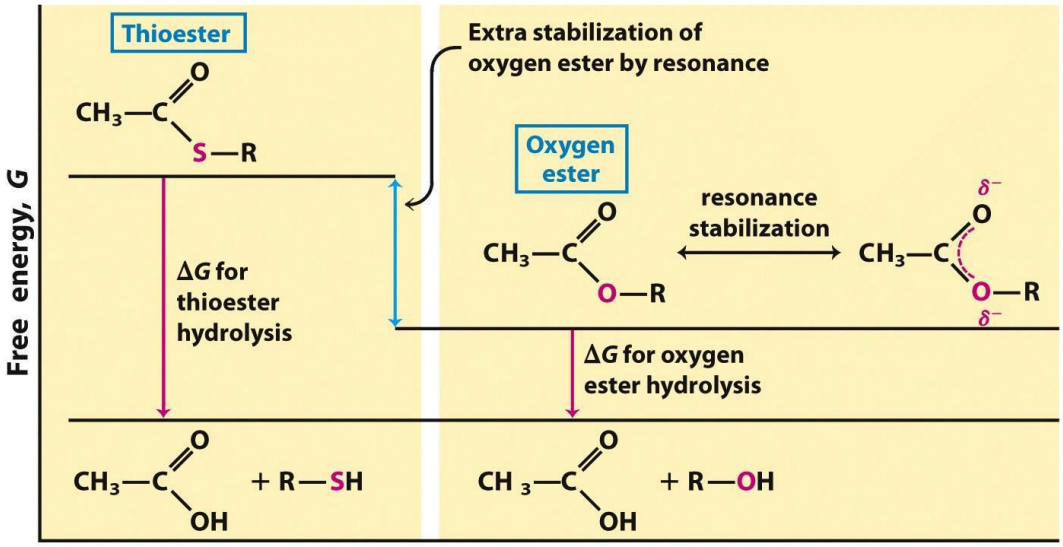


Figure 13-17
Lehninger Principles of Biochemistry, Fifth Edition

Free energy released by hydrolysis of THIOESTERS is more than OXYGEN esters

Summary for hydrolysis reactions

For hydrolysis reactions with large, negative standard free energy changes, the products are more stable than the reactants for one or more of the following reasons:

1. The bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as for ATP.

2. The products are stabilized by **ionization**, as for ATP, acyl phosphates, thioesters.

3. The products are stabilized by isomerization (tautomerization) as for phosphoenolpyruvate

4. The products are stabilized by **resonance** as for creatine released from phosphocreatine, **carboxylate ion released from acyl phosphates** and thioesters and phosphate released from **anhydride or ester linkages.**

• The phosphate compounds found in living organisms can be arbitrarily divided into two groups based on their standard free energy changes of hydrolysis.

1. 'High-energy' compounds have a $\Delta G'_0$ of hydrolysis more negative than -25 kj/mol
2. 'low-energy' compounds have a less negative $\Delta G'_0$

• ATP with a $\Delta G'_0$ of hydrolysis of -30 kj/mol is a high-energy compound

• glucose 6-phosphate is a low-energy compound ($\Delta G'^0$ = -13,8 kj/mol)

TABLE 13–6 Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP (→ AMP + P_i)	−32.8	−7.8
ATP (→ ADP + P_i)	−30.5	−7.3
ATP (→ AMP + PP_i)	−45.6	−10.9
AMP (→ adenosine + P_i)	−14.2	−3.4
PP_i (→ 2P_i)	−19.2	−4.0
Glucose 3-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

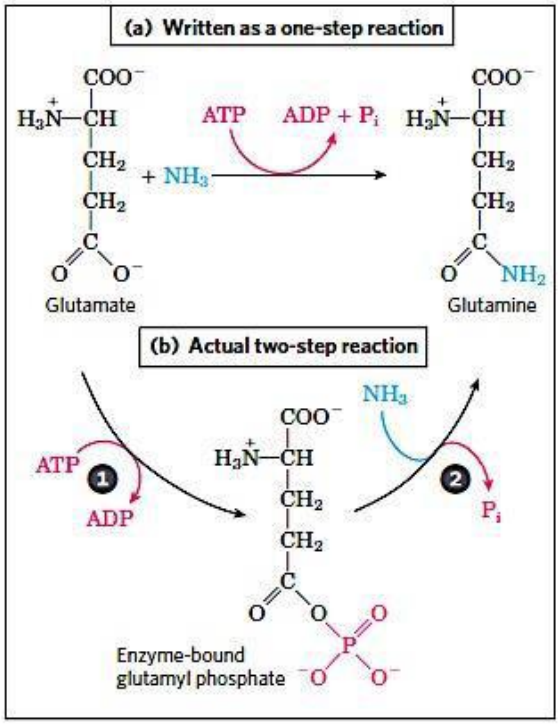
Source: Data mostly from Jencks, W.P. (1976) in *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data*, Vol. 1, pp. 296–304, CRC Press, Boca Raton, FL. The value for the free energy of hydrolysis of PP_i is from Frey, P.A. & Arabshahi, A. (1995) Standard free-energy change for the hydrolysis of the α-β-phosphoanhydride bridge in ATP. *Biochemistry* **34**, 11,307–11,310.

Table 13-6
Lehninger Principles of Biochemistry, Fifth Edition

ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

ATP hydrolysis in two steps.

• The contribution of ATP to a reaction is often shown as a single step, but is almost always a two-step process.
• Shown here is the reaction catalyzed by ATP-dependent **glutamine synthetase.**
• A phosphoryl group is transferred from ATP to glutamate
• the phosphoryl group is displaced by NH_3 and released as Pi.



Hydrolysis accompanied biological processes

1. Noncovalent binding of ATP (or GTP), followed by its hydrolysis to ADP (or GDP) and Pi, (to cycle some proteins between two conformations), in muscle contraction.
2. In the movement of enzymes along DNA
3. In the movement of ribosomes along messenger RNA
4. The energy-dependent reactions catalyzed by helicases and some topoisomerases GTP-binding proteins that act in signaling pathways to drive conformational changes.

• The phosphate compounds found in living organisms can be divided into two groups based on their standard free energy changes of hydrolysis.

1. 'High-energy' compounds have a ΔG'0 of hydrolysis more negative than -25 kj/mol
2. 'low-energy' compounds have a less negative ΔG'0

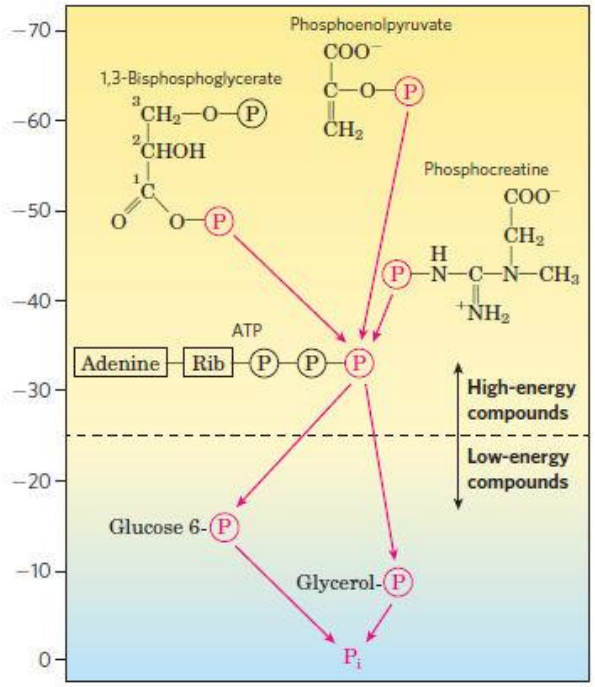
• ATP with a ΔG'0 of hydrolysis of -30 kj/mol is a high-energy compound
• glucose 6-phosphate is a low-energy compound (ΔG'0 = -13,8 kj/mol)
• The term "high-energy phosphate bond," used to describe the P-O bond broken in hydrolysis reactions, is incorrect and misleading as it wrongly suggests that the bond itself contains the energy.
• the breaking of all chemical bonds requires an *input* of energy
• The additivity of free energy changes of sequential reactions
• any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative standard free energy change of hydrolysis.

• **Example:**		$\Delta G'^0$
• **PEP + H_2O**	**Pyruvate + P_i**	**-61,9**
• **ADP+ P_i**	**ATP+ H_2O**	**+30,5**
• **PEP + ADP**	**Pyruvate + ATP**	**-31,4**

• **Clevage of Pi from PEP releases more energy than is needed to drive to condensation of Pi with ADP, the direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible.**
• Notice that while the energy of overall reaction is represented as the algebraic sum of first two reactions, the overall reaction (third) does not involve Pi ; PEP donates a phosphoryl group directly to ADP.
• On the basis of their standard free energy changes of hydrolysis, phosphorylated compounds as having a high or low **phosphoryl group transfer potential**.
• The transfer of a phosphoryl group to a compound effectively puts free energy into that compound that can be used in subsequent metabolic transformations.

Ranking of biological phosphate compounds by standard free energies of hydrolysis

• flow of phosphoryl groups, **represented by P**, from **high-energy phosphoryl group donors via ATP to acceptor molecules** (such as glucose and glycerol) to form their low-energy phosphate derivatives.
• This flow of phosphoryl groups is catalyzed by **kinases**, and proceeds with an overall loss of free energy under intracellular conditions.

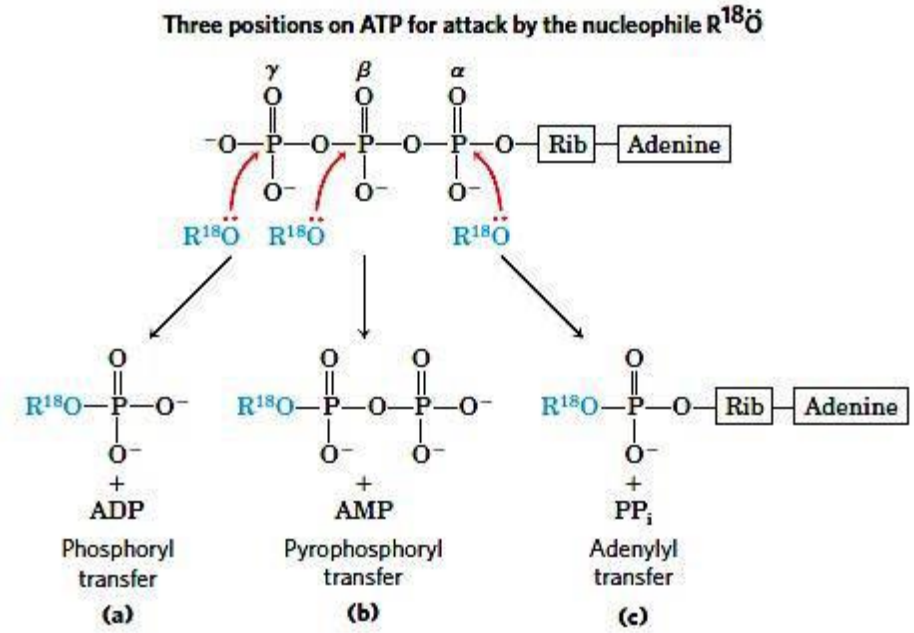


(The location of each compound's donor phosphoryl group along the scale approximately indicates the $\Delta G'^{\circ}$ of hydrolysis.)

• in aqueous solution ATP is thermodynamically unstable and is therefore a good phosphoryl group donor, it is kinetically stable.
• Because of high activation energies required for uncatalyzed reaction ATP does not spontaneously donate phosphoryl groups to water or to the other potential acceptors in the cell.
• ATP hydrolysis occurs only when specific enzymes which lower the energy of activation are present
• **thermodynamically unstable**: when energy level of products is lower than that of reactants
• kinetically stable is when reaction has high activation energy due to some strong bonds.

ATP Donates Phosphoryl, Pyrophosphoryl, and Adenylyl Groups

Nucleophilic displacement reactions of ATP.
• Any of the three P atoms (α, β or γ) may serve as the electrophilic target for nucleophilic attack.
• By the labeled nucleophile $R^{-18}O$: The nucleophile may be an alcohol (ROH), a carboxyl group (RCOO-), or a phosphoanhydride (a nucleoside mono- or diphosphate, for example).
• When the oxygen of the nucleophile attacks the position, the bridge oxygen of the product is labeled, indicating that the group transferred from ATP is a phosphoryl ($2PO3^{-2}$), not a phosphate ($OPO3^{-2}$).



• Attack on the position displaces AMP and leads to the transfer of a pyrophosphoryl (not pyrophosphate) group to the nucleophile.
• Attack on the a position displaces PPi and transfers the adenylyl group to the nucleophile.
• 5-**phosphoribosyl-1-pyrophosphate** is a key intermediate in nucleotide synthesis
1. Hydrolysis of α-β phosphoanhydride bond: ~46 kJ/mol
2. Hydrolysis of β-γ phosphoanhydride bond: ~31 kJ/mol
3. PPi ⟶ 2 Pi: -19.2 kJ/mol: total = -64.8 kJ/mol (**Adenylation: produces Ppi hydrolysed to 2Pi by inorganic pyrophosphatase enzyme).**

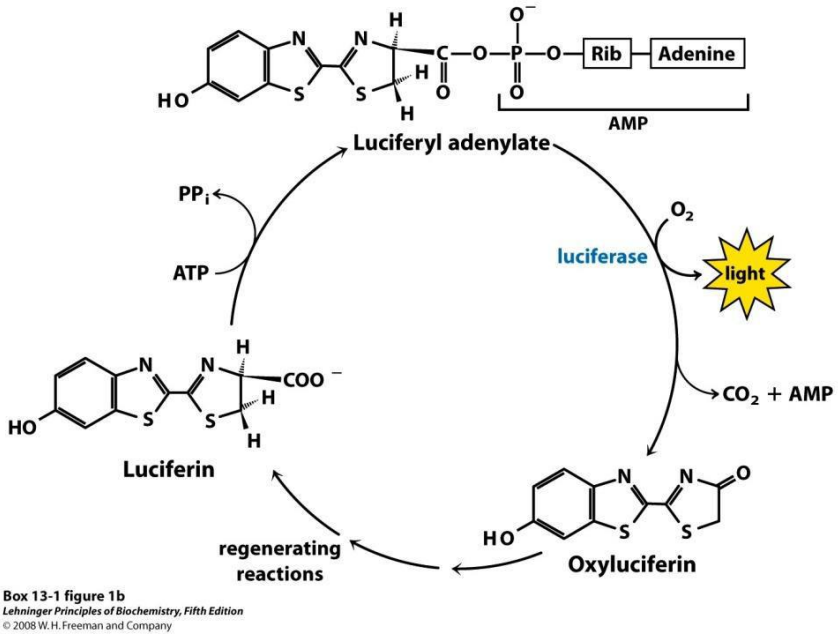
Activation of fatty acids

a) Energy yielding oxidation
b) Synthesis of more complex lipids (thiol esters)

1. First, adenylate (AMP) is transferred from ATP to the carboxyl group of the fatty acid, forming a mixed anhydride (fatty acyl adenylate) and liberating PPi.
2. The thiol group of coenzyme A then displaces the adenylyl group and forms a thioester with the fatty acid.
3. sum of these two reactions is energetically equivalent to the exergonic hydrolysis of ATP to AMP and Ppi
4. The formation of fatty acyl–CoA is endergonic but made energetically favorable (exergonic) by hydrolysis of the PPi by inorganic pyrophosphates.
5. Thus, in the activation of a fatty acid, both phosphoanhydride bonds of ATP are broken.

Luciferin

• William McElroy and his colleagues at the Johns Hopkins University isolated the principal biochemical components: luciferin, a complex carboxylic acid, and luciferase, an enzyme.
• The generation of a light flash requires activation of luciferin by an enzymatic reaction involving pyrophosphate cleavage of ATP to form luciferyl adenylate.
• In **the presence of molecular oxygen and luciferase**, the luciferin undergoes a multistep **oxidative decarboxylation to oxyluciferin**.
• This process is accompanied by emission of light.
• **Luciferin is regenerated from oxyluciferin** in a subsequent series of reactions.
• **In the laboratory, pure firefly luciferin and luciferase are used to measure minute quantities of ATP by the intensity of the light flash produced. As little as a few picomoles (10-12 pico mol) of ATP can be measured in this way.**
• **Next-gen pyrosequencing of DNA relies on flashes of light from the luciferin-luciferase reaction to detect the presence of ATP after addition of nucleotides to a growing strand of DNA.**



Box 13-1 figure 1b
Lehninger Principles of Biochemistry, Fifth Edition

Assembly of Informational Macromolecules Requires Energy

Pi and Ppi groups from ATP

• The precursors for DNA and RNA synthesis are nucleoside triphosphates.
• Polymerization of DNA and RNA is accompanied by cleavage of the **phosphoanhydride linkage** between α **and β phosphates, with the release of PPi.**
• The moieties transferred to the growing polymer in these reactions are **adenylate (AMP), guanylate (GMP), cytidylate (CMP), or uridylate (UMP)** for RNA synthesis, and their deoxy analogs (with TMP in place of UMP) for DNA synthesis.

Adenylyl groups from ATP

• required for the **activation of amino acids for protein synthesis,** several steps on the ribosome are also accompanied by **GTP hydrolysis**.

ATP energizes active transport and muscle Contraction

• Transport processes are major consumers of energy
• In brain and kidney, two third of total is consumed to pump sodium and potassium ions across plasma membrane (Na + K+ ATPase).
• Na+ dependent phosphorylation of Na+ K+ ATPase changes protein conformation, K+ dependent dephosphorylation brings back original conformation.
• Phosphoryl group transfer: to enzyme, not to substrate.
• Direct hydrolysis of ATP (ATP ⟶ ADP+Pi) is the source of energy in the conformational changes that produce muscle contraction

• ATP bind with head of myosin (not covalent) dissociate from actin → ATP hydrolysis induces conformational change (still ADP and Pi bound tightly) → Pi release → conformational change of the head of myosin → actin move.

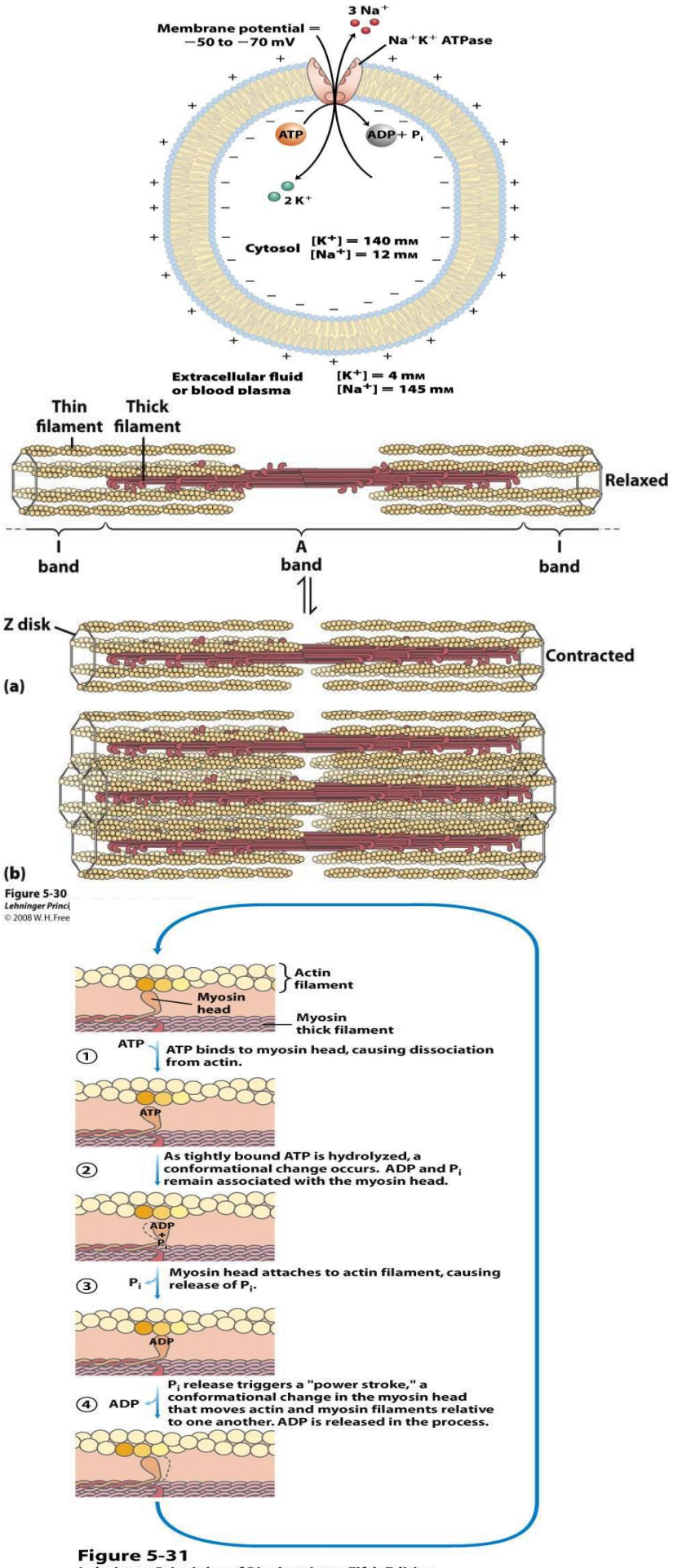
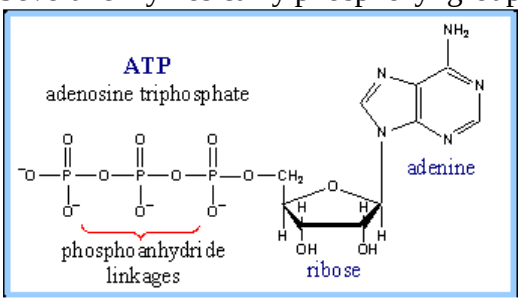


Figure 5-31
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Transphosphorylations between nucleotides occur in all cell types

• ATP is considered as the cell's energy currency and donor of phosphoryl groups, all other nucleoside triphosphates (GTP, UTP, CTP) and all the deoxynucleoside triphosphates (dATP, dGTP, dTTP and dCTP) are energetically equivalent to ATP.
• The free energy changes associated with hydrolysis of their **phosphoanhydride linkages** are very nearly identical with those for ATP.
• In preparation for their various biological roles, other nucleotides are generated as the nucleoside triphosphate (NTP) forms by phosphoryl group transfer to the corresponding nucleoside diphosphates (NDPs) and monophosphates (NMPs)
• ATP is the primary high-energy phosphate compound produced by catabolism in the processes of glycolysis, oxidative phosphorylation. Several enzymes carry phosphoryl groups from ATP to the other nucleotides.



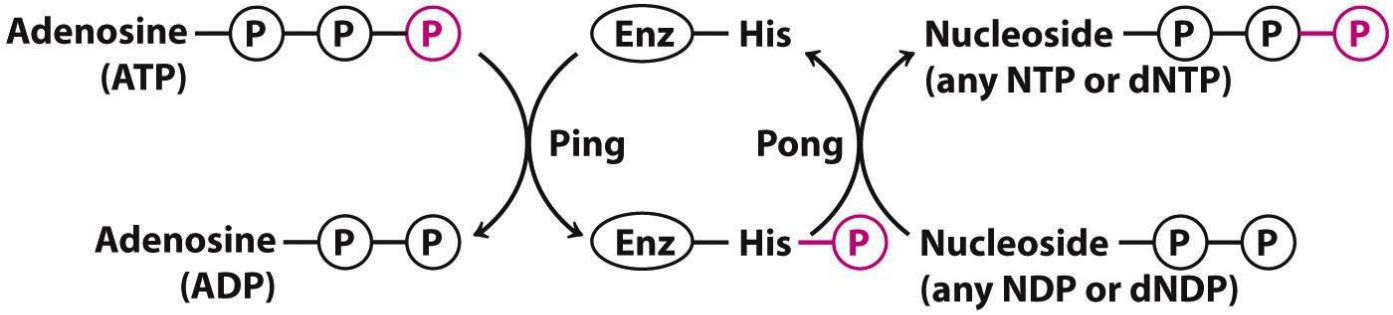
• Nucleoside diphosphate kinases, found in all cells, catalyzes the reaction.

$$\text{ATP+NDP(or dNDP)} \underset{}{\overset{Mg^{2+}}{\rightleftharpoons}} \text{ADP+NTP(or dNDP)}$$

• Although this reaction is fully reversible the relatively high ATP/ADP ratio in cells normally drives the reaction to the right, with the net formation of NTPs and dNTPs.
• Phosphoryl group transfers from ATP results in an accumulation of ADP. eg, when muscle is concracting vigorously ADP accumulates and interferes with ATP dependent contraction.
• During intense demand for ATP, the cell lowers the ADP concentration, and at the same time acquires ATP, by the action of adenylate kinase:

$$2\text{ADP} \underset{}{\overset{Mg^{2+}}{\rightleftharpoons}} \text{ATP + AMP}$$

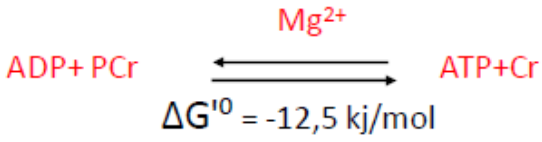
Ping-pong mechanism of nucleoside diphosphate Kinase



Ping-pong mechanism of nucleoside diphosphate kinase: The enzyme binds its first substrate (ATP in our example), and a phosphoryl group is transferred to the side chain of a **His** residue. ADP departs, and another nucleoside (or deoxynucleoside) diphosphate replaces it, and this is **converted to the corresponding triphosphate by transfer of the phosphoryl group** from the phosphohistidine residue.

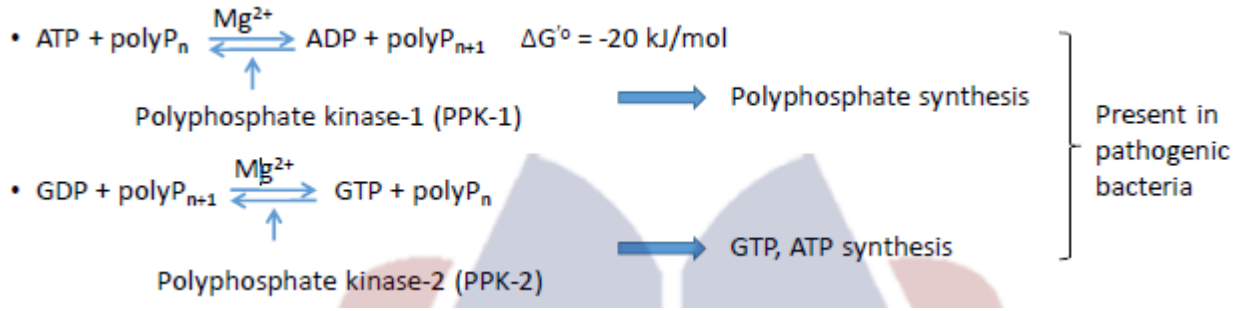
• This reaction is fully reversible, so after the intense demand for ATP ends, the enzyme can recycle AMP by converting it to ADP which can then be phosphorylated to ATP in mitochondria.
• A similar enzyme **guanylate kinase, converts GMP to GDP** at the expense of ATP.
• **By these pathways energy conserved in the catabolic production of ATP is used to supply the cell with all required NTPs and dNTPs**

• **Phosphocreatine (PCr)** serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The phosphocreatine concentration in skeletal muscle is considerably higher than those in the other tissues. The enzyme **creatine kinase** catalyzes the reversible reaction.



Inorganic polyphosphate is a donates phosphoryl group

• Inorganic polyphosphate ((polyP)n): linear polymer of Pi, Present in simple organisms of other phyla like yeast.
• In yeast, polyP accumulated in vacuoles (= 200 mM)
• Serve as a **phosphagen (Reservoir of phosphoryl group).. Like PCr**
• The shortest polyP is PPi (n=2): can serve as the energy source for active transport of H+ in plant cells (across vacuolar membrane)
• In bacteria, the enzyme polyphosphate kinase-1 (PPK-1) catalyzes the reversible reaction
• **polyphosphate kinase-2** (PPK-2), catalyzes the reversible synthesis of GTP (or ATP) from polyphosphate and GDP (or ADP):



Biological oxidation-reduction reactions

• Central feature of metabolism:
1) Phosphoryl group transfer
2) Electron transfer in oxidation-reduction
• The flow of electrons in oxidation-reduction reactions is responsible for all work done by living organisms.
• In nonphotosynthetic organism, source of electron: food
• In photosythetic organism, source of electron: chemical compound excited by the absorption of light
• Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalyzed reaction & energy releases.

1. The flow of electrons can do biological work

• **Electromotive force (emf):** (measured in volts), is the voltage developed by any source of electrical energy such as a battery… In which two chemical species differ in their affinity for electrons, so the electrons flow spontaneously through the circuit, due to the difference in electron affinity.
• **Living cells have an analogous biological "circuit",** with relatively reduced compound such as glucose (the source of electrons)
• Glucose ⟶ enzymatically oxidized ⟶ release electron ⟶ spontaneously flow through electron-carrier intermediates to another chemical species, such as O2 this electron flow is **exergonic reaction because O2 has a higher affinity for electrons than do electron-carrier intermediates**
• The resulting emf provides energy to a variety of **molecular energy transducers** (**enzymes and other proteins**) that do biological work.
• In the mitochondrion, membrane-bound enzymes couple electron flow ⟶ a production of **transmembrane pH difference** and a **transmembrane electrical potential** ⟶ which **helps to accomplish osmotic and electrical work.**
• The **proton gradient** thus formed has potential energy, sometimes called the **proton-motive force** by analogy with electromotive force.
• Another enzyme, ATP synthase in the inner mitochondrial membrane, uses the proton-motive force to do chemical work: synthesis of ATP from ADP and Pi as protons flow spontaneously across the membrane.

- Bacterial flagellar motion: use proton-motive force
- Thus, the proton potential has potential energy: electron-motive force

Oxidation-Reductions Can Be Described as Half-Reactions

Half-Reactions:

• A **half reaction** is either the oxidation or reduction reaction component of a redox reaction.

• A half reaction is obtained by considering the change in oxidation states of individual substances involved in the redox reaction

Although oxidation and reduction occur together, we consider the two halves of an oxidation-reduction reaction separately.

• For example, the oxidation of ferrous ion by cupric ion is written as follows,

$$Fe^{+2} + Cu^{+2} \leftrightarrow Fe^{+3} + Cu^{+}$$

• Same can be described as 2 half reactions in the following form:

1. $Fe^{+2} \leftrightarrow Fe^{+3} + e^{-}$

2. $Cu^{+2} + e^{-} \leftrightarrow Cu^{+}$

Conjugate redox pair

• The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant

• the electron-accepting molecule is the oxidizing agent or oxidant.

• A given agent, such as an iron cation existing in the ferrous (Fe^{+2}) or ferric (Fe^{+3}) state functions as a conjugate reductant-oxidant pair (redox pair), just as an acid and corresponding base function as a **conjugate acid-base pair.**

• Acid-base reactions can be written as a general equation:

• proton donor ↔ H+ + proton acceptor

• In redox reactions we can write a similar general equation:

• electron donor ↔ *e* - + electron acceptor

• In the reversible half-reaction (1) above, Fe+2 is the electron donor and Fe^{+3} is the electron acceptor; together, Fe+2 and Fe+3 constitute a **conjugate redox pair**.

• The oxidation of reducing sugar by cupric acid

• Aldehydes and ketones with anomeric carbon can act **reducing sugars.. Having capability to reduce copper ions in medium..** The reducing sugar reduces the copper(II) ions in these test solutions to copper(I), which forms a brick red copper(I) oxide precipitate

• Basis of qualitative carbohydrate test (Benedict's test)

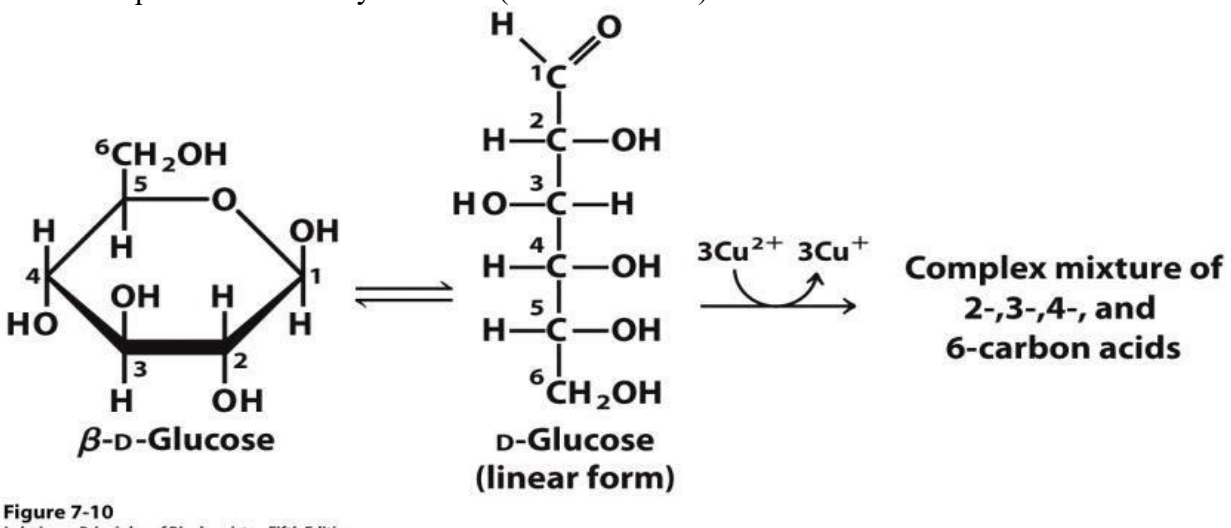
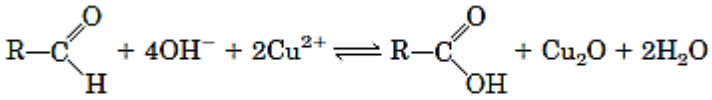
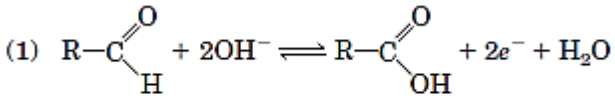


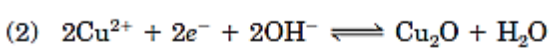
Figure 7-10
Lehninger Principles of Biochemistry, Fifth Edition

• **consider the oxidation of a reducing sugar by cupric ion:**



• **This overall reaction can be expressed as two half reactions:**





• **Because two electrons are removed from the aldehyde carbon, the second half reaction (the one-electron reduction of cupric to cuprous ion) must be doubled to balance the overall equation.**

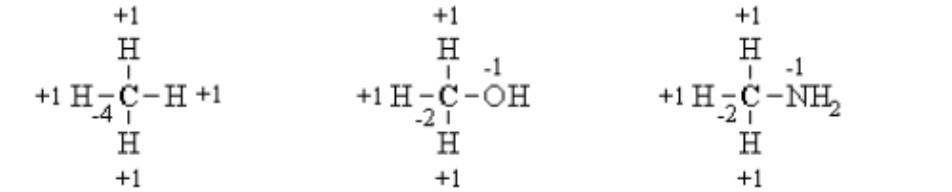
Biological Oxidations Often Involve Dehydrogenation

• In biological Systems oxidation (loss of electrons) is often synonymous with **dehydrogenation** and many enzymes that catalyze oxidation reactions are **dehydrogenases**.
• The carbon in living cells exists in a range of oxidation status
• Electronegativity**: H<C<S<N<O**

OXIDATION STATE

It is calculated by counting all the electrons bonded to carbon and add them in following way:

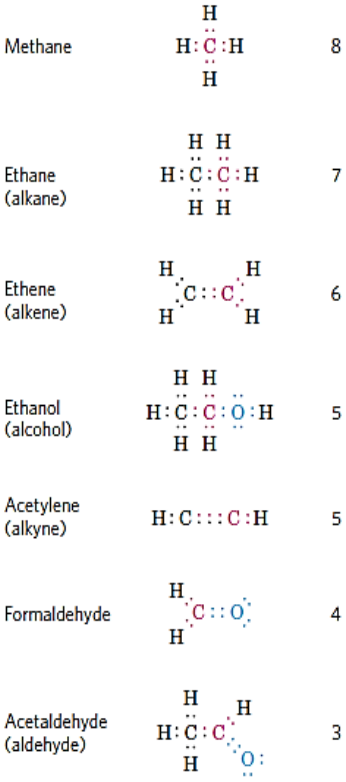
- for attached C atoms, *i.e.* C-C bonds electrons shared, count 0
- for attached X atoms, *i.e.* C-X bonds (X more electronegative), count -1 (per bond)
- for attached H atoms, *i.e.* C-H bonds (H is less electronegative than C), count +1
- Add the total for atoms attached to the C in question, then switch the sign.

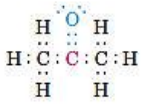
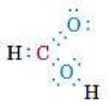
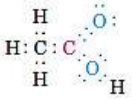


Oxidation Levels of carbon compounds

For the figure given below:

Different levels of oxidation of carbon compounds in the biosphere.
• In order to estimate the level of oxidation of these compounds, focus on the red carbon atom and its bonding electrons.
• **When this carbon is bonded to the less electronegative H atom, both bonding electrons (red) are assigned to the carbon.**
• When carbon is bonded to another carbon, bonding electrons are shared equally, one of the two electrons is assigned to the red carbon. When the red carbon is bonded to the **more electronegative O atom**, the bonding electrons are assigned to the oxygen.
• The number to the right of each compound is the number of electrons "owned" by the red carbon, a rough expression of the degree of oxidation of that compound. As the red carbon undergoes oxidation (loses electrons), the number gets smaller.



Acetone (ketone)		2
Formic acid (carboxylic acid)		2
Carbon monoxide		2
Acetic acid (carboxylic acid)		1
Carbon dioxide		0

Oxidation states of C reducing sugar (an aldehyde or ketone) by carbon in various compounds; from fully reduced (methane to fully oxidized (carbon dioxide).

Electrons are transferred from one molecule to another in 4 ways

1. Directly as electrons: Fe2+/Fe3+ redox pair can transfer an electron to the Cu/Cu2+ redox Pair

$$Fe^{2+} + Cu^{2+} \leftrightarrow Fe^{3+} + Cu^{+}$$

2. As hydrogen atoms: hydrogen atom consists of a proton (H+) and a single electron (*e*-)

$$AH_2 \leftrightarrow A + 2\,e^- + 2H^-$$

AH2/A: conjugate redox pair can reduce another compound B

$$AH_2 + B \leftrightarrow A^+ + BH_2$$

3. As a hydride ion (:H-) .. has two electrons. This reaction occurs in NAD-linked dehydrogenase

4. Through direct combination with oxygen, where Oxygen covalently incorporated into Product

$$R\text{-}CH_3 + 1/2O_2 \leftrightarrow R\text{-}CH_2\text{-}OH$$

Reduction potential measures affinity for Electrons

• Reduction potential:

Reduction potential (redox potential, oxidation/reduction potential), is a measure of the tendency of a chemical species to acquire electrons and thereby get **reduced**. Units: Volts (V), or millivolts (mV).. denoted by ***E.***

• Standard reduction potential:

The tendency for a chemical species to be reduced, **at standard conditions. The more positive the potential is the more likely it will be reduced…** denoted by ***EO.***

A Half-cell

• Reduction potential of a half-cell depends on the activity of reduced and oxidized species which is approximated by their concentrations

• On the anode, oxidation takes place.

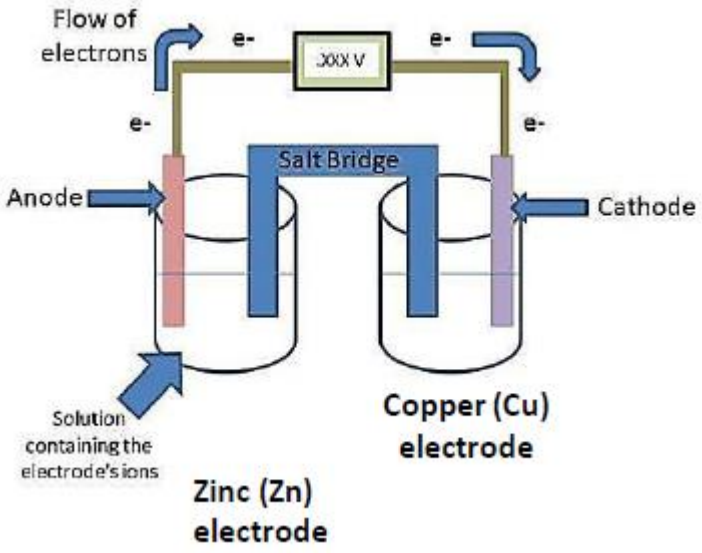
Zn Zn2+ + 2 e-

Zn is the reducing agent, and Zn2+ the oxidizing agent.

• On the cathode, reduction takes place.

Cu2+ + 2 e Cu

Cu2+ is the oxidizing agent and Cu the reducing agent.



A standard Half-cell

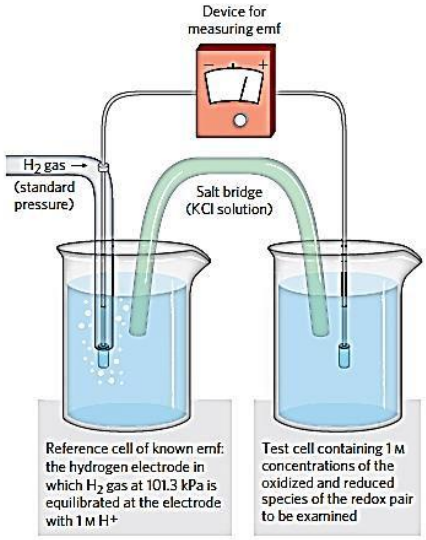
• Standard Hydrogen electrode..(SHE) is the primary reference electrode with platinum electrode dipped in solution with H ions under standard conditions

• The electromotive force (emf) of this electrode is designated 0.00 V. (At pH 7, 25 **O**C)

• Using observed emf (of test solution) and the known emf of the reference cell, the emf of the test cell with the redox pair can be calculated.

• **The cell that gains electrons has, by convention, the more positive reduction potential.**

• **The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned an *E*O of 0.00 V.**



Standard reduction potentials can be used to calculate free-energy change

• Electrons tend to flow to the half-cell with the more positive *E*, and the strength of that tendency is proportional to **Δ** *E*, the difference in reduction potential.

• We can then predict the direction in which electrons will tend to flow when the two half-cells are connected through an external circuit.

• Electrons tend to flow to the half-cell with the more positive *E* ⟶ the strength of flow depends on Δ*E* (difference in reduction potential)

• The energy made available by this spontaneous electron flow (the free-energy change, **Δ G**, for the oxidation-reduction reaction) is proportional to **Δ** *E*:

$$\Delta G = -n\mathcal{F}\Delta E \quad \text{or} \quad \Delta G^{\circ} = -n\mathcal{F}\Delta E^{\circ}$$

• where *n* is the number of electrons transferred in the reaction

• **F is Faraday** (**unit** of electricity), used in the study of electrochemical reactions and equal to the amount of electric charge that liberates one gram equivalent of any ion from an electrolytic solution

• With this equation we can calculate the actual free-energy change for any oxidation-reduction reaction from the values of ***E* 'o** (reduction potentials) and the concentrations of reacting species
• When acetaldehyde is reduced by the biological electron carrier NADH, ΔG = -35.3 kJ/mol

TABLE 13–7 Standard Reduction Potentials of Some Biologically Important Half-Reactions

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + $e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol + H_2	0.045
$Fumarate^{2-} + 2H^+ + 2e^- \longrightarrow succinate^{2-}$	0.031

Half-reaction	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
$Oxaloacetate^{2-} + 2H^+ + 2e^- \longrightarrow malate^{2-}$	−0.166
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−0.185
Acetaldehyde + $2H^+ + 2e^- \longrightarrow$ ethanol	−0.197
FAD + $2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione + $2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^- \longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

• **Conjugate redox pair**: An electron donor and its corresponding electron acceptor form; for example, Cu+ (donor) and Cu2+ (acceptor), or NADH (donor) and NAD+ (acceptor) form **Conjugate redox pair.**
• **Half cell:** A **half-cell** is one of the two electrodes in a galvanic **cell** or simple battery. For example, in the Zn-Cu battery, the two **half cells** make an oxidizing reducing couple.

Universal Electron Carriers

Cellular oxidation of glucose to carbon dioxide requires specialized electron carriers

• The principles of oxidation-reduction energetics □□can apply to the many metabolic reactions that involve electron transfers.

Glucose oxidation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O,$$

$\Delta G'^{\circ}$ = -2,840 kJ/mol > ATP synthesis in cells: 50-60 kJ/mol

• Cells does not convert to CO2 in a single, high energy releasing reaction but rather in a series of controlled reactions, some of which are oxidation for the ATP synthesis from ADP through NAD+ and FAD.

A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

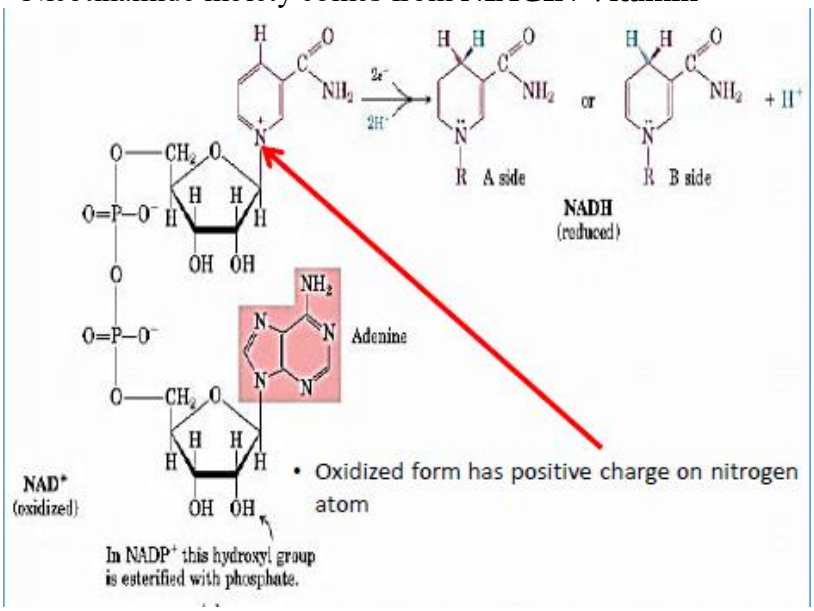
1. The nucleotides NAD and NADP move readily from one enzyme to another.
2. The **flavin nucleotides FMN and FAD** are usually very tightly bound to the enzymes, called **flavoproteins**, for which they serve as **prosthetic groups**
3. Lipid-soluble quinones: ubiquinone and plastoquinone act as **electron carriers and proton donors in the non-aqueous environment** of membranes.
4. Iron-sulfur proteins and cytochromes, which have tightly bound prosthetic groups that undergo reversible oxidation and reduction, serve as electron carriers in many oxidation-reduction reactions.
• **NAD, NADP, FMN, and FAD** are water-soluble coenzymes and undergo reversible oxidation and reduction during many of the electron-transfer reactions of metabolism.

NAD and NADP are soluble electron carriers that act with dehydrogenase enzymes

• Activated electron carriers, involved in oxidation-reduction and coupling reactions
• NAD+ (nicotinamide adenine dinucleotide)
• NADP+ (nicotinamide adenine dinucleotide phosphate)

For NAD: → NAD^+: oxidized form
For NADP:→ $NADP^+$: oxidized form

• Joined by phosphoanhydride bond
• Pyridine nucleotides
• Nicotinamide moiety comes from **NIACIN Vitamin**



• Both coenzymes undergo reversible reduction of the nicotinamide ring
• As a substrate molecule undergoes oxidation (dehydrogenation), giving up two hydrogen atoms, the oxidized form of the nucleotide (NAD+ or NADP+) accepts a hydride ion (:H2, the equivalent of a proton and two electrons) and is reduced (to NADH or NADPH).
• The second proton removed from the substrate is released to the aqueous solvent.

The half-reactions for these nucleotide cofactors are:

$$NAD^+ + 2e^- + 2H^+ + \ NADH \rightarrow NADH + H^+$$

$$NADP^+ + 2e^- + 2H^+ + NADH \rightarrow NADPH + H^+$$

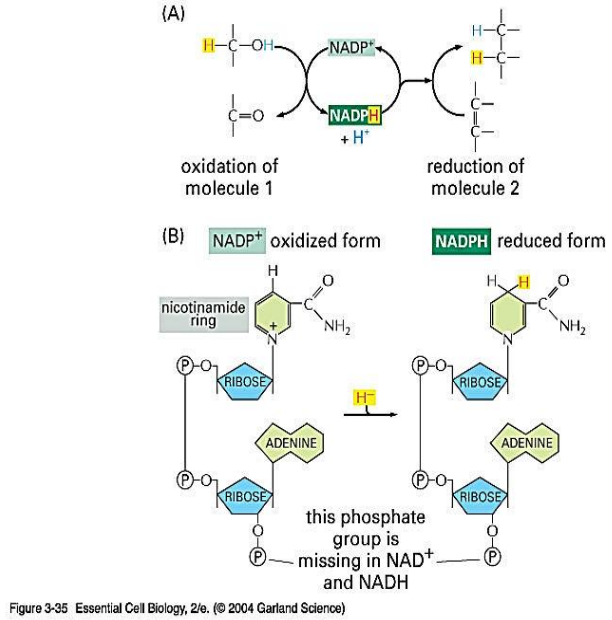


Figure 3-35 Essential Cell Biology, 2/e. (© 2004 Garland Science)

• Reduction of NAD or NADP converts the benzenoid ring of the nicotinamide moiety (with a fixed positive charge on the ring nitrogen) to the **quinonoid** form (with no charge on the nitrogen).
• The reduced nucleotides absorb light at **340 nm**; the oxidized forms do not (used for detection).
• Plus sign with NAD and NADP does *not* indicate the net charge (both are negatively charged) rather, it indicates that the nicotinamide ring is in its oxidized form, with a positive charge on the **Nitrogen atom**
• **"H" in NADH and NADPH, the denotes the added hydride ion**
• Total concentration of NAD+ + NADH in most tissues: 10-5 M
• Total concentration of NADP+ + NADPH in most tissues: 10-6 M
• Concentration ratio: NAD+ > NADH ⟶ favoring hydride transfer from a substrate to NAD+ to form NADH function in oxidation, occur in mitochondrial matrix
• Concentration ratio: NADP+ < NADPH ⟶favoring hydride transfer from NADPH to a substrate ⟶ function in reduction ⟶ occur in cytosol
• More than 200 enzymes are known to catalyze reactions in which NAD+ (or NADP+) accepts a hydride ion from a reduced substrate or NADPH (or NADH) donates a hydride ion to an oxidized substrate.

General reaction

$AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$ (NADH is Oxidized substrate) (AH_2 is reduced)

$A + NADPH + H^+ \rightleftharpoons AH_2 + NADP^+$ (NADP+ is reduced substrate, A is oxidized)

oxidoreductase

$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$

Ethanol → acetaldehyde

Alcohol dehydrogenase enzyme

NAD and NADP:

• NAD and its phosphorylated analog NADP undergo reduction to NADH and NADPH, accepting a hydride ion from an oxidizable substrate.
• The hydride ion is added to either the front (the A side) or the back (the B side) of the planar nicotinamide ring

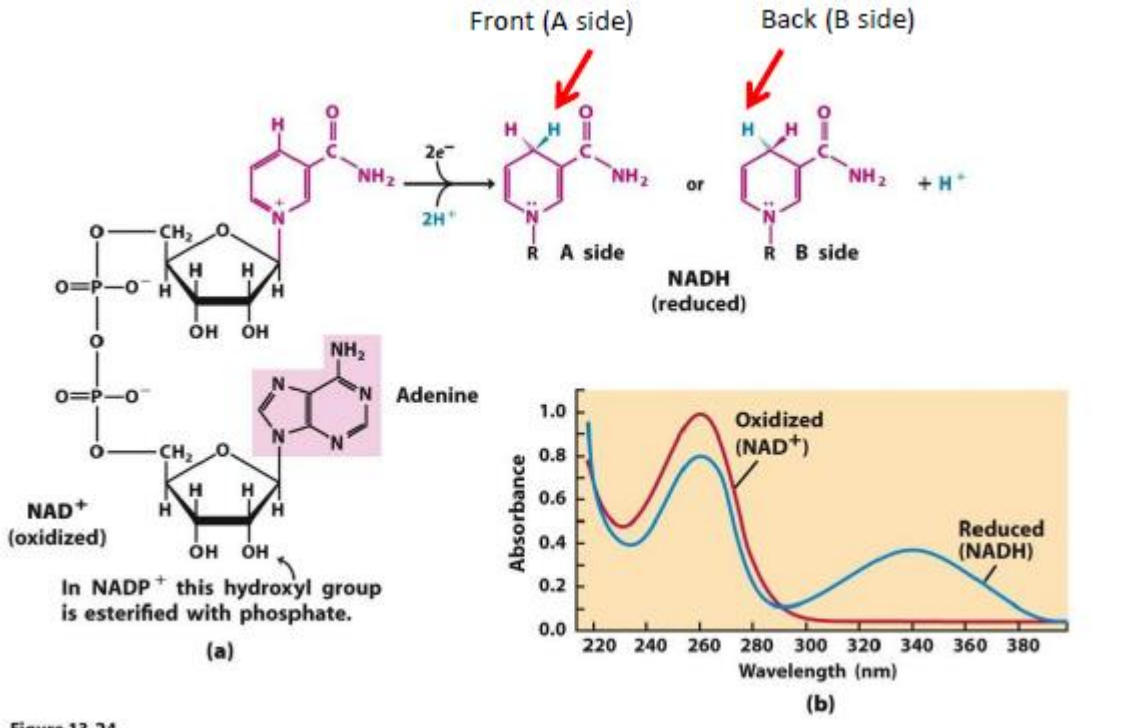


Figure 13-24
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company

The UV absorption spectra of NAD and NADH

TABLE 13–8 Stereospecificity of Dehydrogenases That Employ NAD^+ or $NADP^+$ as Coenzymes

Enzyme	Coenzyme	Stereochemical specificity for nicotinamide ring (A or B)	Text page
Isocitrate dehydrogenase	NAD^+	A	643
α-Ketoglutarate dehydrogenase	NAD^+	B	644
Glucose 6-phosphate dehydrogenase	$NADP^+$	B	577
Malate dehydrogenase	NAD^+	A	647
Glutamate dehydrogenase	NAD^+ or $NADP^+$	B	702
Glyceraldehyde 3-phosphate dehydrogenase	NAD^+	B	553
Lactate dehydrogenase	NAD^+	A	563
Alcohol dehydrogenase	NAD^+	A	565

TABLE 11–7 Some Diseases Resulting from Ion Channel Defects

Ion channel	Affected gene	Disease
Na^+ (voltage-gated, skeletal muscle)	*SCN4A*	Hyperkalemic periodic paralysis (or paramyotonia congenita)
Na^+ (voltage-gated, neuronal)	*SCN1A*	Generalized epilepsy with febrile seizures
Na^+ (voltage-gated, cardiac muscle)	*SCN5A*	Long QT syndrome 3
Ca^{2+} (neuronal)	*CACNA1A*	Familial hemiplegic migraine
Ca^{2+} (voltage-gated, retina)	*CACNA1F*	Congenital stationary night blindness
Ca^{2+} (polycystin-1)	*PKD1*	Polycystic kidney disease
K^+ (neuronal)	*KCNQ4*	Dominant deafness
K^+ (voltage-gated, neuronal)	*KCNQ2*	Benign familial neonatal convulsions
Nonspecific cation (cGMP-gated, retinal)	*CNCG1*	Retinitis pigmentosa
Acetylcholine receptor (skeletal muscle)	*CHRNA1*	Congenital myasthenic syndrome
Cl^-	*CFTR*	Cystic fibrosis

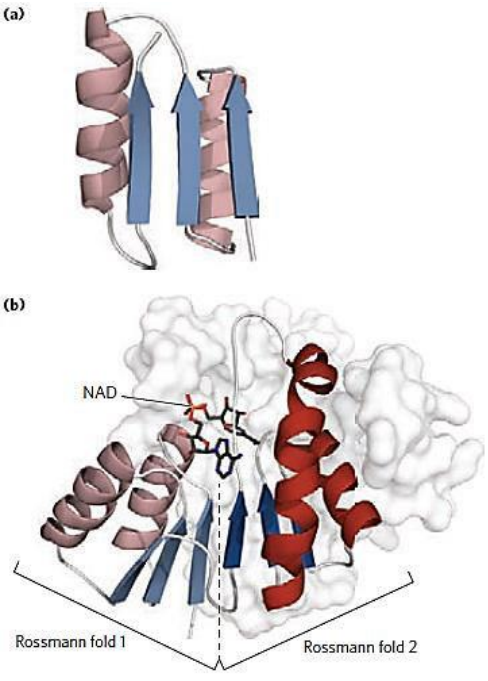
Rossmann fold

•Most dehydrogenases that use NAD or NADH contain a conserved protein binding domain called the Rossmann fold

• Rossmann fold: consists of a six-stranded parallel beta sheet and four associated alpha helices

• Nucleotide binding domain of lactate dehydrogenase with NAD is shown

• NAD is bound to paired beta-alpha motifs of Rossmann fold.



• The association between dehydrogenases and cofactor is loose ⟶ the coenzyme readily diffuse from one enzyme to another ⟶ act as a water-soluble carrier of electron form one metabolite to another
• In overall reaction, there is not net production or consumption of NAD+ or NADH ⟶ no concentration change of NAD+ or NADH .. and NAD+ or NADH get recycled repeatedly.

(1) Glyceraldehyde 3-phosphate + NAD^+ → 3-phosphoglycerate + NADH + H^+
(2) Acetaldehyde + NADH + H^+ → ethanol + NAD^+

Glyceraldehyde 3-phosphate + Acetaldehyde → 3-phosphoglycerate + ethanol

Dietary deficiency of Niacin causes Pellagra

• Most coenzyme: derived from vitamins
• NAD and NADP: derived from vitamin niacin (=nicotinic acid)
• **Human cannot synthesize sufficient quantities of niacin**
• **Niacin** derived from tryptophan
• In the laboratory, nicotinic acid was first produced by oxidation of the natural product.. nicotine—thus the name.
• Niacin deficiency: affects all the NAD(P)- dependent dehydrogenase and causes the serious human disease pellagra (=rough skin): characterized by the **Three Ds (dermatitis, diarrhea and dementia)and death**
• similar disease **black tongue in dogs**
• **Niacin is curative agent (scientists)**

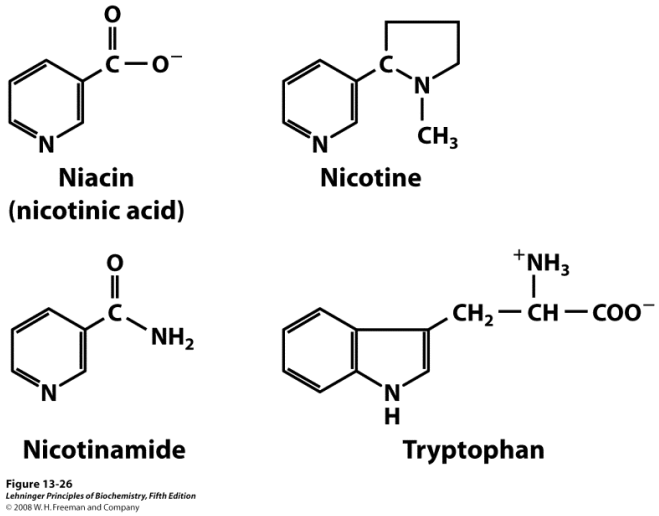


Figure 13-26
Lehninger Principles of Biochemistry, Fifth Edition

• Too much alcohol drinking □□reduce niacin absorption from the intestine
• Both nicotinic acid and nicotinamide cure pellagra, but nicotine (from cigarettes or elsewhere) has no curative activity.

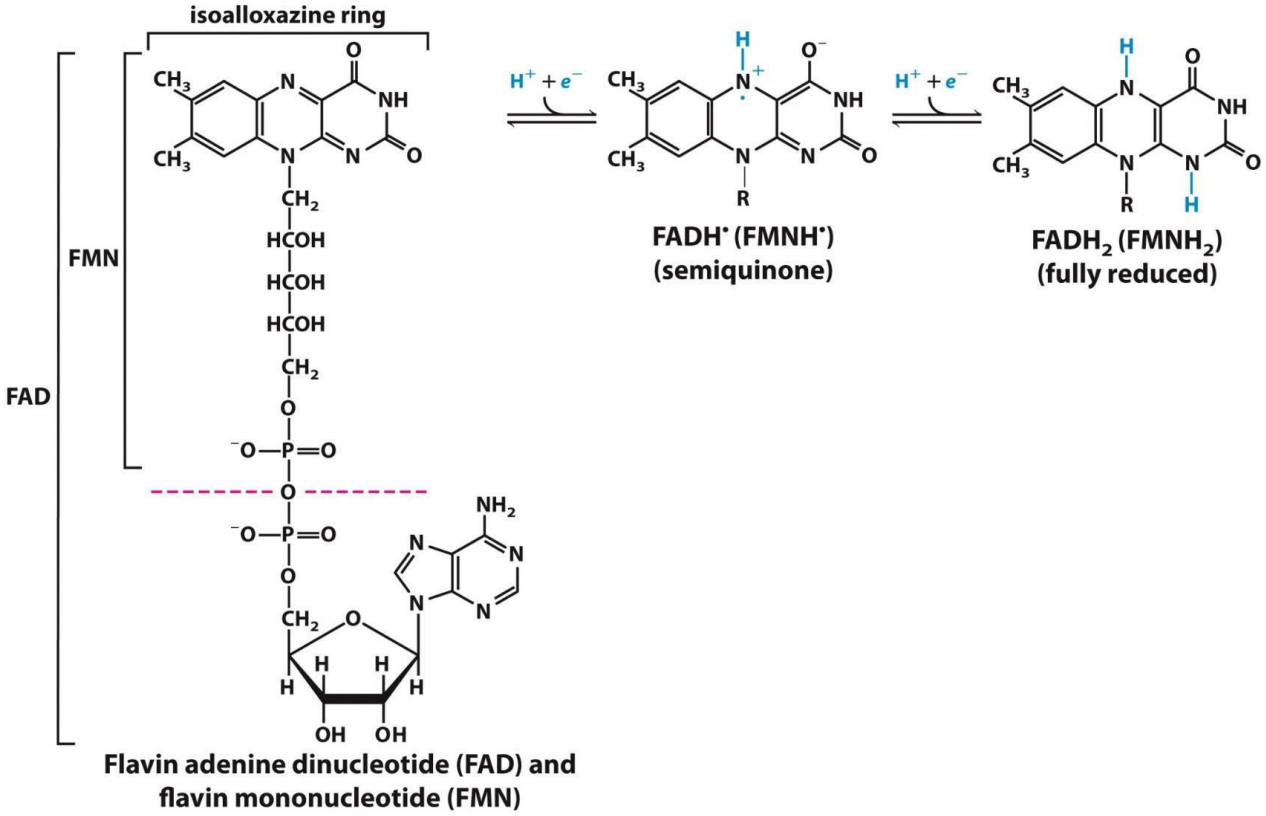
Flavoproteins

• Flavoproteins: enzymes that catalyze oxidation-reduction reactions using coenzyme
1. Flavin mononucleotide (FMN)
2. Flavin adenine dinucleotide (FAD)
3. Flavin nucleotides are derived from the vitamin riboflavin
4. Flavoproteins act in a quite different role, as light receptors
• The fused ring structure of the flavin nucleotide (isoalloxazine ring)
• **Isoalloxazine ring** undergoes reversible reduction, accepting either one or two electrons in the form of **one or two hydrogen atoms** (each atom an electron plus a proton) from a reduced substrate
• When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), **the semiquinone form of the isoalloxazine ring** is produced.
• The fully reduced forms are abbreviated FADH2 and FMNH2.

Oxidized and reduced forms of FAD and FMN

Some enzymes use flavin nucleotides for oxidationreduction

• **Flavin nucleotides are tightly bound in flavoproteins**



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

• **Fully reduced: 360 nm**

• **Partially reduced: 450 nm**

• **Fully oxidized: 370 and 440 nm**

FMN consists of the structure above the dashed line on the FAD (oxidized form). The flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system. When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable free radical, forms.

TABLE 13–9 Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin nucleotide	Text page(s)
Acyl-CoA dehydrogenase	FAD	673
Dihydrolipoyl dehydrogenase	FAD	637
Succinate dehydrogenase	FAD	646
Glycerol 3-phosphate dehydrogenase	FAD	759
Thioredoxin reductase	FAD	917
NADH dehydrogenase (Complex I)	FMN	738–739
Glycolate oxidase	FMN	813

Table 13-9
Lehninger Principles of Biochemistry, Sixth Edition

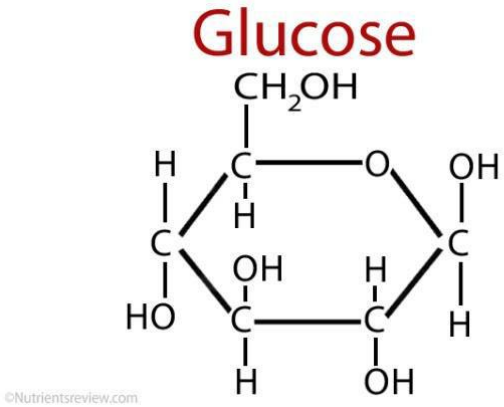
Enzymes that use FAD or FMN as coenzymes

Cryptochromes

• Family of flavoproteins,
• Found in both bacteria and eukaryotes
• mediate the effects of blue light on plant development and the effects of light on mammalian circadian rhythms (oscillations in physiology and biochemistry, with a 24-hour period)
• **Homologs of Photolyases**
• **Photolyases: Use energy of light to repair defects in DNA**

GLUCOSE METABOLISM

In animals and vascular plants, glucose has three major fates:
1. It may be stored (as a polysaccharide , i-e., glycogen or starch)
2. Oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates.
3. Oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.



Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$, which means that it is a molecule that is made of six carbon atoms, twelve hydrogen atoms, and six oxygen atoms.
Glucose circulates in the blood of animals as blood sugar.

• **Organisms that do not have access to glucose from other sources must make it.**
• **Photosynthetic organisms make glucose by first reducing atmospheric CO2 tortoises, then converting the trioses to glucose.**
• **Non-photosynthetic cells** make glucose from **simpler three and four-carbon precursors by the process of gluconeogenesis**, effectively reversing glycolysis in a pathway that uses many of the glycolytic enzymes.

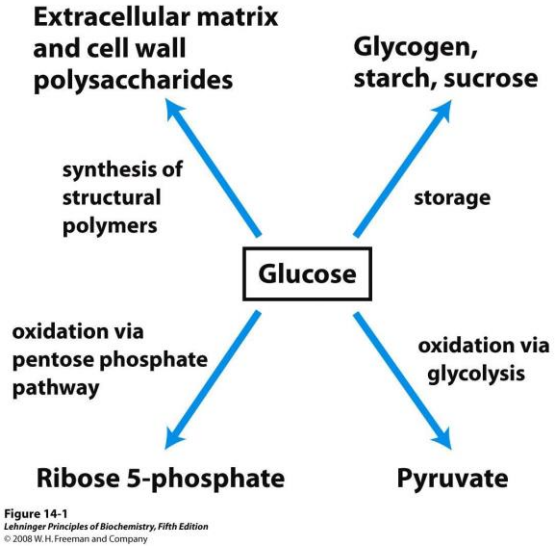


Figure 14-1
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company

GLYCOLYSIS

• In glycolysis (from the Greek *glykys*, "sweet" or "sugar," and *lysis*, "splitting"), a molecule of glucose $C_6H_{12}O_6$is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three carbon. compound pyruvate $CH_3COCOO^- + H^+$.

• During glycolysis, the free energy released is used to form the high energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

• The pathway of glycolysis was explained by Otto Warburg and Hans von Euler-Chelpin **in yeast** and in **muscle** by Gustav Embden and Otto Meyerhof in1930s.

• The most common type of glycolysis is the *Embden–Meyerhof–Parnas (EMP pathway)*, which was discovered by Embden and Meyerhof and J.K. Parnas.

• Glycolysis occurs in most organisms in the **cytosol** of the cell.

• **It is an oxygen independent metabolic pathway**, meaning that it does not use molecular oxygen (i.e. atmospheric oxygen) for any of its reactions.

• **The products of glycolysis, that is, pyruvate and NADH + H+)** are sometimes disposed of using atmospheric oxygen.

• Aerobic Glycolysis: When molecular oxygen is used in the disposal of the products of glycolysis the process is usually referred to as Aerobic Glycolysis.

• Anaerobic Glycolysis: When no oxygen is used, the process is said to be Anaerobic.

2 phases of GLYCOLYSIS

1. The Preparatory Phase – in which ATP is consumed, hence also known as the investment phase.

2. The Pay Off Phase – in which ATP is produced

• Occurs in 10 steps, the first 5 of which constitute the *preparatory phase.*

The Preparatory Phase

• **step 1:** The first step in glycolysis is phosphorylation of glucose at the hydroxyl group on C-6 by a family of enzymes called hexokinases to form glucose-6- phosphate (G6P).

• **step 2:** The D-glucose 6-phosphate thus formed is converted to D-fructose 6- phosphate,

• step 3: which is again phosphorylated, this time at C-1, to yield D-fructose 1,6- bisphosphate

• For both phosphorylations, ATP is the phosphoryl group donor.

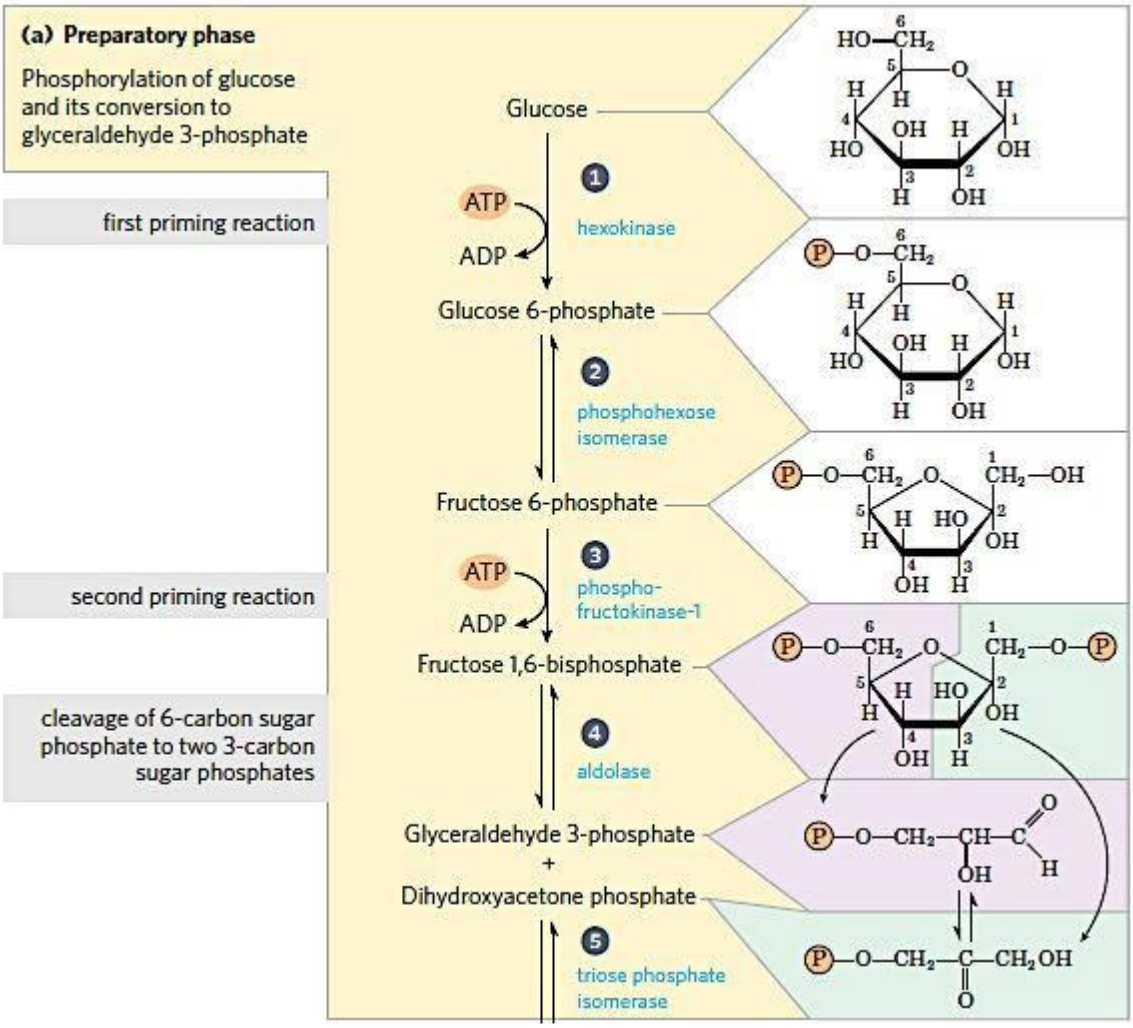
• **step 4:** Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step 4); this is the "lysis" step that gives the pathway its name.

• **step 5:** The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis.

To summarize:

In the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted to a common product, glyceraldehyde 3-phosphate.

Note that during preparatory phase, 2 molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces.



Preparatory phase of Glycolysis

For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde 3-phosphate are formed; both pass through the payoff phase

The Pay-off Phase

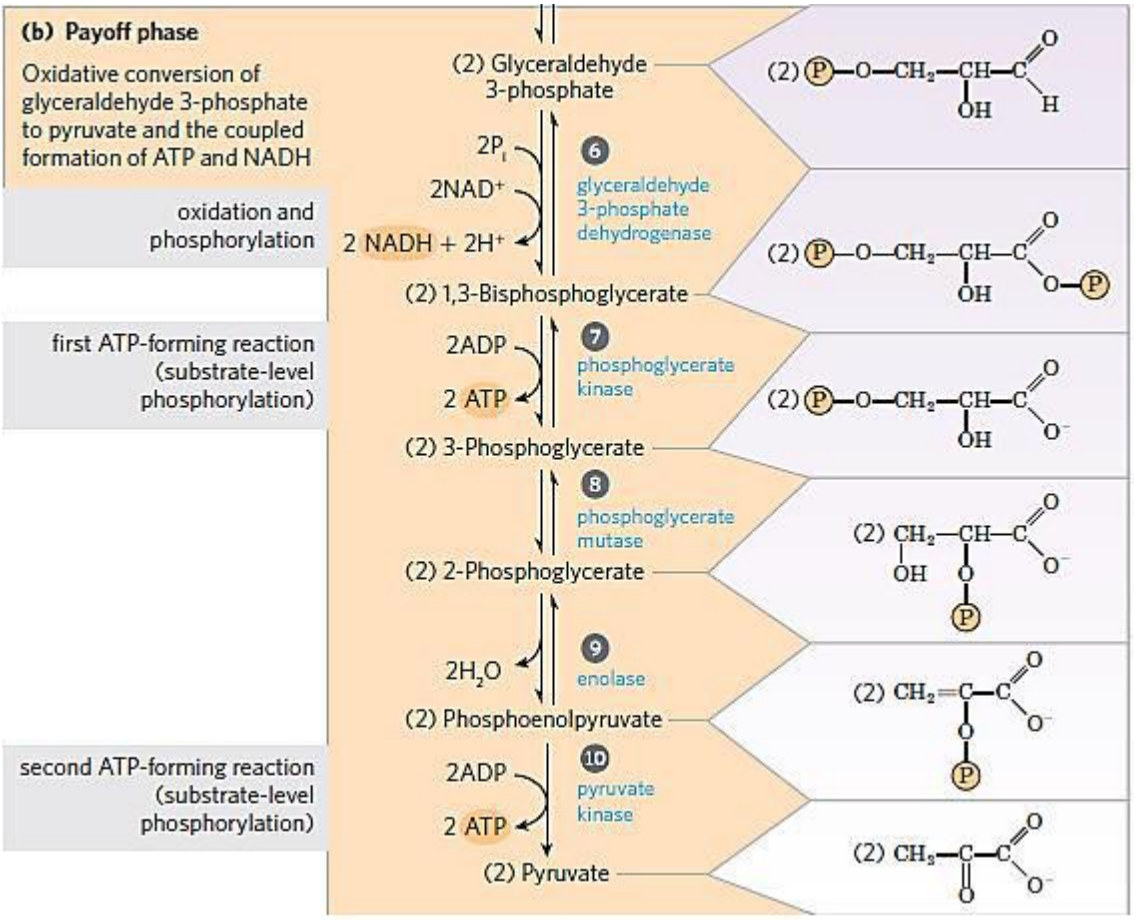
• The energy gain comes in the *payoff phase* of glycolysis

• step 6: Each molecule of glyceraldehyde 3-phosphate is oxidized and phosphorylated by inorganic phosphate (*not* by ATP) to form 1,3- bisphosphoglycerate

• Energy is also conserved in the formation of **two molecules of the electron carrier NADH per molecule of glucose**

• steps 7 through 10: Much of this energy is conserved by the coupled **phosphorylation of four molecules of ADP to ATP.**

• The net yield is two molecules of ATP per molecule of glucose, because two molecules of ATP were invested in the preparatory phase.



• Pyruvate is the end product of the **second phase of glycolysis.**
• For each glucose molecule, **two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase**,
• This gives a net yield of two ATP per molecule of glucose converted to pyruvate.
• each phosphoryl group, represented here as P, has two negative charges

Energy yield during Glycolysis

• Two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a net yield of two ATP per molecule of glucose converted to pyruvate. Note that each phosphoryl group, represented as P, has two negative charges ($2PO3^{-2}$)
• Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP.
• Energy is also conserved in the payoff phase in the formation of two molecules of the electron carrier NADH per molecule of glucose.
• In the sequential reactions of glycolysis, three types of chemical transformations are noteworthy:

(1) degradation of the carbon skeleton of glucose to yield pyruvate
(2) phosphorylation of ADP to ATP by compounds with high phosphoryl group transfer potential, formed during glycolysis
(3) transfer of a hydride ion to NAD+ forming NADH.

Preparatory phase of Glycolysis Requires ATP

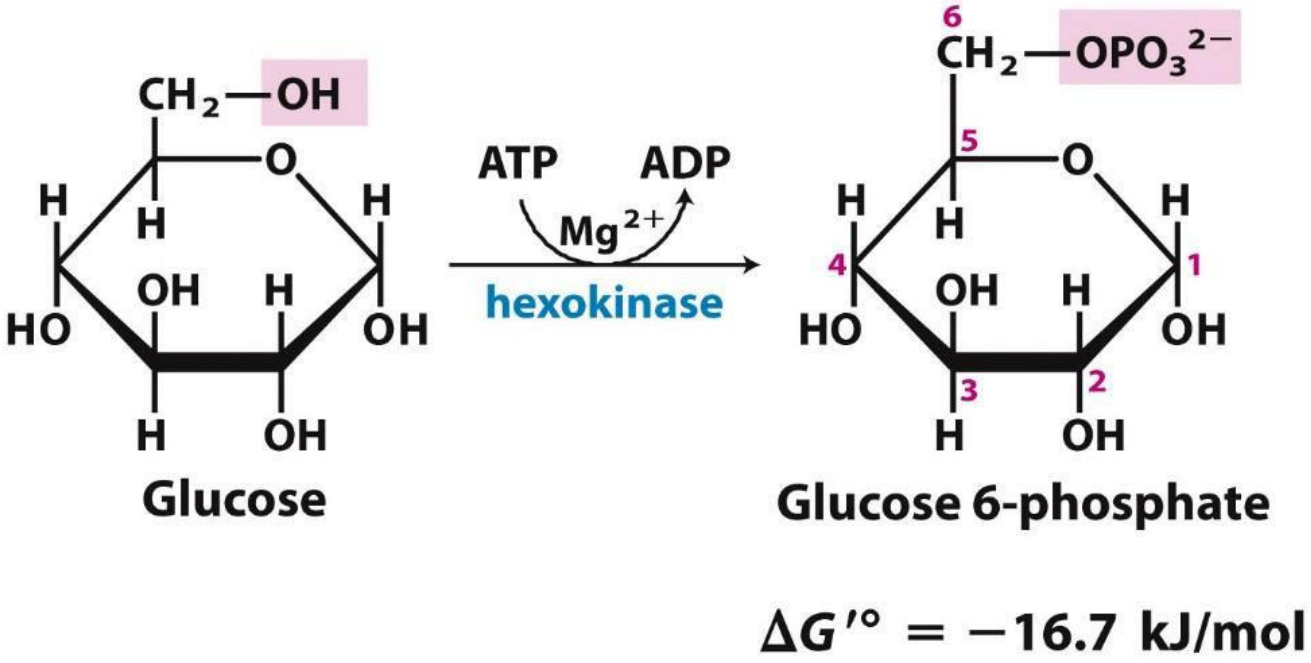
Experiment by A. Harden and W. Young

• Preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into **two triose phosphates**
• In 1906, Arthur Harden and William Young tested their hypothesis that **inhibitors of proteolytic enzymes would stabilize the glucose fermenting enzymes in yeast extract.**
• They added blood serum **(known to contain inhibitors of proteolytic enzymes)** to yeast extracts and observed the predicted stimulation of glucose metabolism.
• However, in a control experiment with the boiling serum, they discovered that boiled serum was just as effective at stimulating.

• **Harden and Young** soon discovered that glucose added to their yeast extract was converted to a hexose bisphosphate (the "Harden- Young ester," eventually identified as **fructose 1,6-bisphosphate**.
• Careful examination revealed that inorganic phosphate was responsible for the stimulation.
• This was the beginning of investigations on the role of organic esters and anhydrides of phosphate in biochemistry, which has led to our current understanding of the central role of **phosphoryl group transfer in biology.**

1. *Phosphorylation of Glucose*

• In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose 6-phosphate**, with ATP as the phosphoryl donor:
• This reaction is irreversible under intracellular conditions, and catalyzed by **hexokinase**.
• The acceptor in the case of **hexokinase is a hexose, normally D-glucose**, hexokinase also catalyzes the phosphorylation of other common hexoses, such as Dfructose and D-mannose, in some tissues.



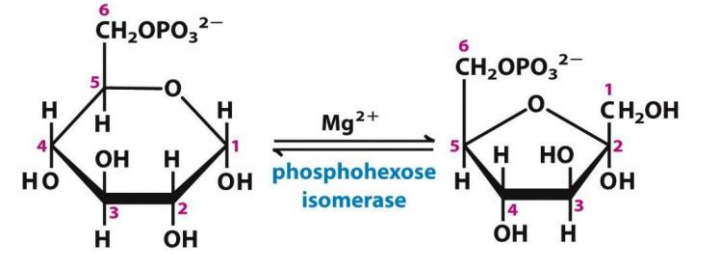
Unnumbered 14 p532a
Lehninger Principles of Biochemistry, Fifth Edition

• Hexokinase requires Mg+2 for its activity, because the true substrate of the enzyme is not ATP-4 but the $MgATP^{-2}$ complex.
• Hexokinase and other nine enzymes of are soluble cytosolic proteins.

Note:
• **Isozymes**
Two or more enzymes that catalyze the same reaction but are encoded by different genes are called **isozymes.**
• **Kinase:**
• Kinases are a subclass of transferases...catalyse transfer of phosphate group from phosphate donating molecule to specific substrates.

2. *Conversion of Glucose 6-Phosphate to Fructose 6- Phosphate*

• The enzyme **phosphohexose isomerase** (**phosphoglucose isomerase**) catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to **fructose 6-phosphate**, a ketose.
• The mechanism for this reaction involves an enediol intermediate
• **Enediol**: An **enediol** is an alkene enol with a hydroxyl group attached to both carbon atoms of the carbon double bond.



$\Delta G'^{\circ} = 1.7$ kJ/mol

3 steps for ***Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate*** **(phosphohexose isomerase reaction).**

1. opening of ring

2. Isomerization of glucose to fructose through formation of enediol intermediate

3. Closing of fructose ring

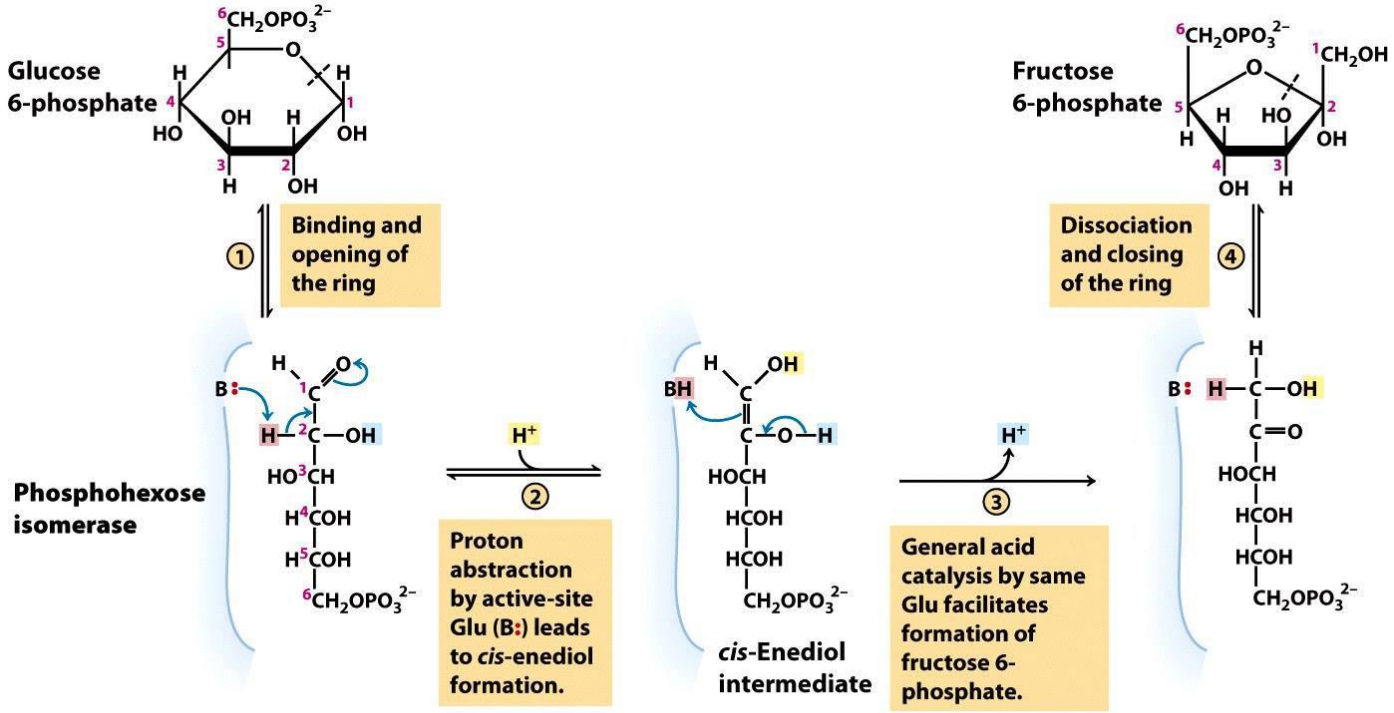


Fig 14-5: The phosphohexose isomerase reaction.

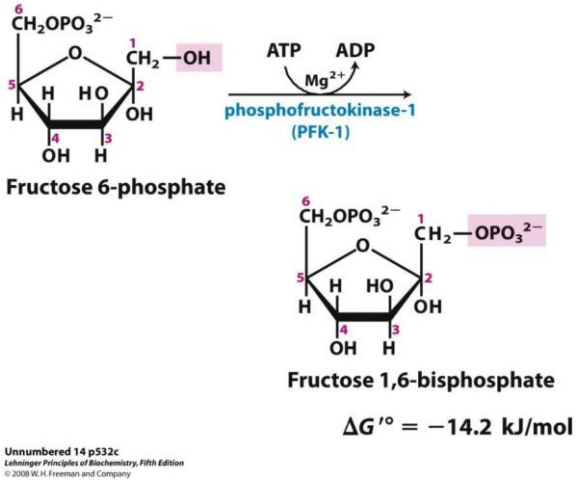
The ring opening and closing reactions (steps 1 and 4) are catalyzed by an active site/ **His residue**, by mechanisms omitted here for simplicity. The proton (light red) initially at C-2 is made more easily abstractable by electron withdrawal by the adjacent carbonyl and nearby hydroxyl groups. After its transfer from C-2 to the active site **Glu residue (a weak acid),** the proton is freely exchanged with the surrounding solution; that is, the proton abstracted from C-2 in step 2 is not necessarily the same one that is added to C-1 in step 3.

3. Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

Phosphofructokinase-1 (PFK-1) catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield **fructose 1,6-bisphosphate**.

KEY CONVENTION:

Compounds that contain two phosphate or phosphoryl groups attached at different positions in the molecule are named ***bisphosphates*** **(or *bisphospho* compounds);** for example, fructose 1,6-bisphosphate and 1,3-bisphosphoglycerate. **Compounds with two phosphates linked together as a pyrophosphoryl group are named *diphosphates*;** for example, adenosine diphosphate (ADP). **Similar rules apply for the naming of *trisphosphates*** (such as inositol 1,4,5-trisphosphate;) and *triphosphates* (such as adenosine triphosphate, ATP).



• The **PFK-1 reaction** is essentially irreversible under cellular conditions, and it is the first "committed" step in the glycolytic pathway

• Glucose 6-phosphate and fructose 6-phosphate have other possible fates, but **fructose 1,6-bisphosphate is targeted for glycolysis.**

• Some **bacteria and protists and perhaps all plants have a phosphofructokinase that uses pyrophosphate (PPi), not ATP**, as the phosphoryl group donor in the synthesis of fructose 1,6- bisphosphate.

• In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control

• **Phosphofructokinase-1** is subject to complex **allosteric regulation**

• The PFK1 has got binding sites for both, ATP-Mg2+ and F6P binding sites

• **The activity of PFK-1 enzyme and glycolysis is inhibited whenever the cell has high levels of ATP**

• In this way, the cell can increase or decrease the rate of glycolysis in response to the cell's energy requirements

• In Eukaryotes, **fructose 2,6-bisphosphate** (not to be confused with the **PFK-1 reaction product, fructose 1,6-bisphosphate**) is a potent allosteric activator of PFK-1.

• Ribulose 5-phosphate, an intermediate in the pentose phosphate pathway also activates phosphofructokinase indirectly.

• In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control

• **Phosphofructokinase-1** is subject to complex **allosteric regulation**

• The PFK1 has got binding sites for both, ATP-Mg2+ and F6P binding sites

• **The activity of PFK-1 enzyme and glycolysis is inhibited whenever the cell has high levels of ATP**

• In this way, the cell can increase or decrease the rate of glycolysis in response to the cell's energy requirements

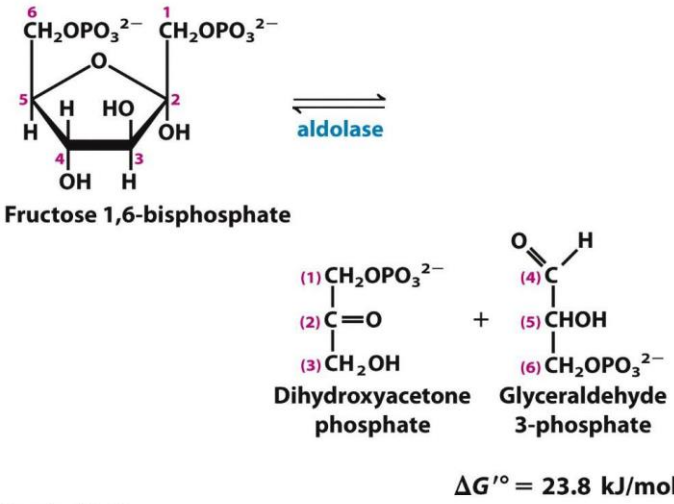
• In Eukaryotes, **fructose 2,6-bisphosphate** (not to be confused with the **PFK-1 reaction product, fructose 1,6-bisphosphate**) is a potent allosteric activator of PFK-1

• Ribulose 5-phosphate, an intermediate in the pentose phosphate pathway also activates phosphofructokinase indirectly.

4. Cleavage of Fructose 1,6-Bisphosphate

• The enzyme **fructose 1,6-bisphosphate aldolase**, often called simply **aldolase**, catalyzes a reversible aldol condensation.

• Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehyde 3-phosphate**, an aldose, and **dihydroxyacetone phosphate**, a ketose.



Two classes of aldolases

There are two classes of aldolases.

- **Class I,** found in animals and plants, form the Schiff base intermediate
- **Class II**, in fungi and bacteria, do not form the Schiff base Intermediate

- **Imine:** An **imine** is a functional group or chemical compound containing a carbon–nitrogen double bond. The nitrogen atom can be attached to a hydrogen or an organic group.
- **Schiff base intermediate:**

They can be considered a sub-class of imines, being either secondary ketimines or secondary aldimines depending on their structure. (R-CH=NR' where R' ≠ H)

- **Secondary amine:**

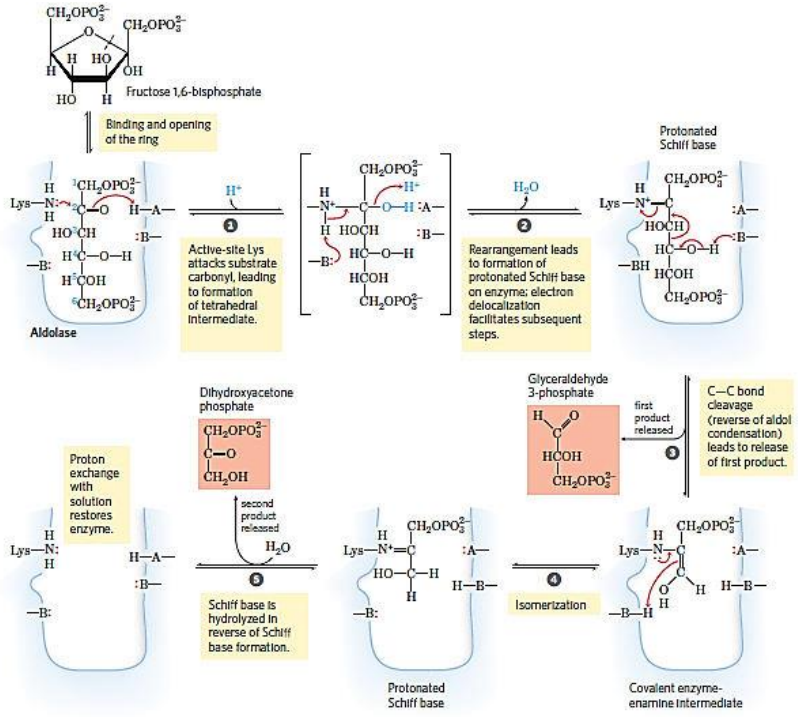
Secondary amines have two organic substituents (alkyl, aryl or both) bound to the nitrogen together with one hydrogen.

- **Enamine:**

An enamine is an unsaturated compound derived by the condensation of an aldehyde or ketone with a secondary amine.

MECHANISM

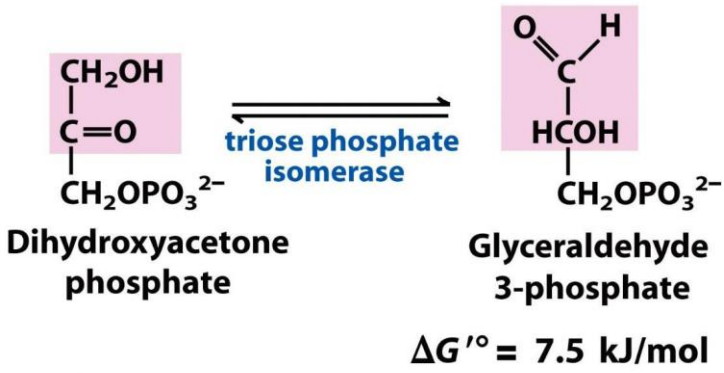
- FBPA, class I aldolase reaction. The reaction shownhere is the reverse of **an aldol condensation.**
- Cleavage between C-3 and C-4 depends on the presence of the **carbonyl group at C-2**, which is converted to an imine on the enzyme.
- A and B represent amino acid residues that serve as general acid (A) or base (B).



5. Interconversion of the Triose Phosphates

• Only one of the two triose phosphates formed by aldolase, glyceraldehyde 3- phosphate, can be directly degraded in the subsequent steps of glycolysis.

• The other product, **dihydroxyacetone phosphate**, is rapidly and reversibly converted to glyceraldehyde 3-phosphate by the fifth enzyme of the glycolytic sequence, **triose phosphate isomerase**:



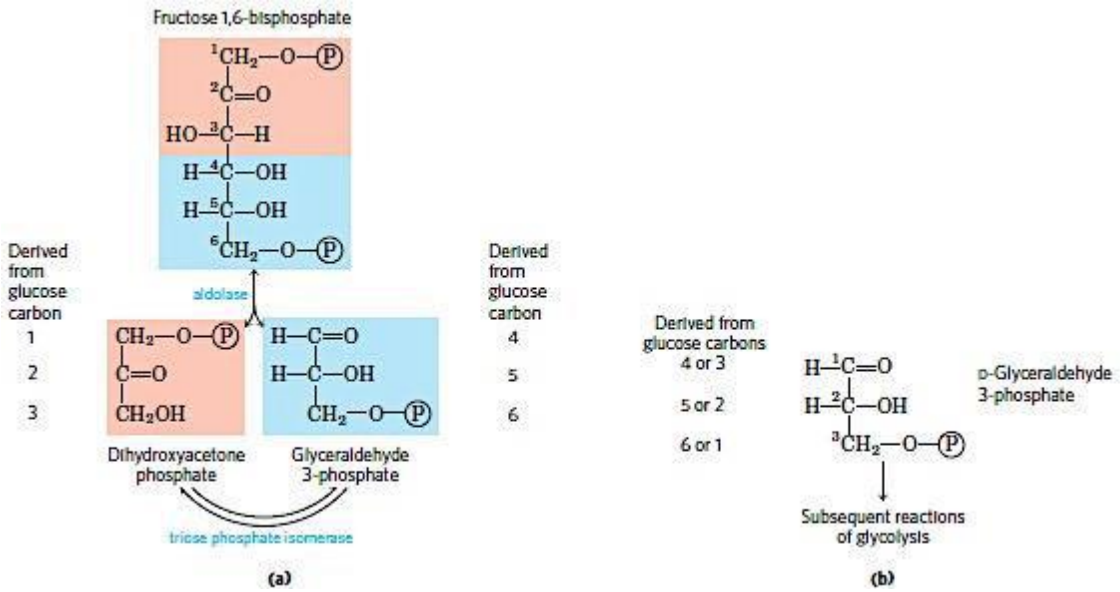
• The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis

• After the triose phosphate isomerase reaction, the carbon atoms derived from C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable

• from C-6, C-5, and C-4, respectively, the two “halves” of glucose have both yielded glyceraldehyde 3-phosphate.

• This reaction completes the preparatory phase of glycolysis.

• The hexose molecule has been phosphorylated at C-1 and C-6 and then cleaved to form two molecules of glyceraldehyde 3-phosphate.



Fate of the glucose carbons in the formation of glyceraldehyde 3-phosphate.

(a) The origin of the carbons in the two 3-carbon products of the aldolase and triose phosphate isomerase reactions. The end product of the two reactions is glyceraldehyde 3-phosphate (two molecules).

(b) Each carbon of glyceraldehyde 3-phosphate is derived from either of two specific carbons of glucose. Note that the numbering of the carbon atoms of glyceraldehyde 3-phosphate differs from that of the glucose from which it is derived. In glyceraldehyde 3-phosphate, the most complex functional group (the carbonyl) is specified as C-1. This numbering change is important for interpreting experiments with glucose in which a single carbon is labeled with a radioisotope.

Radioisotopes:

Atomic number (Z): no. of protons

Mass number (A): the total **number** of protons and neutrons in an atomic nucleus (together known as nucleons).

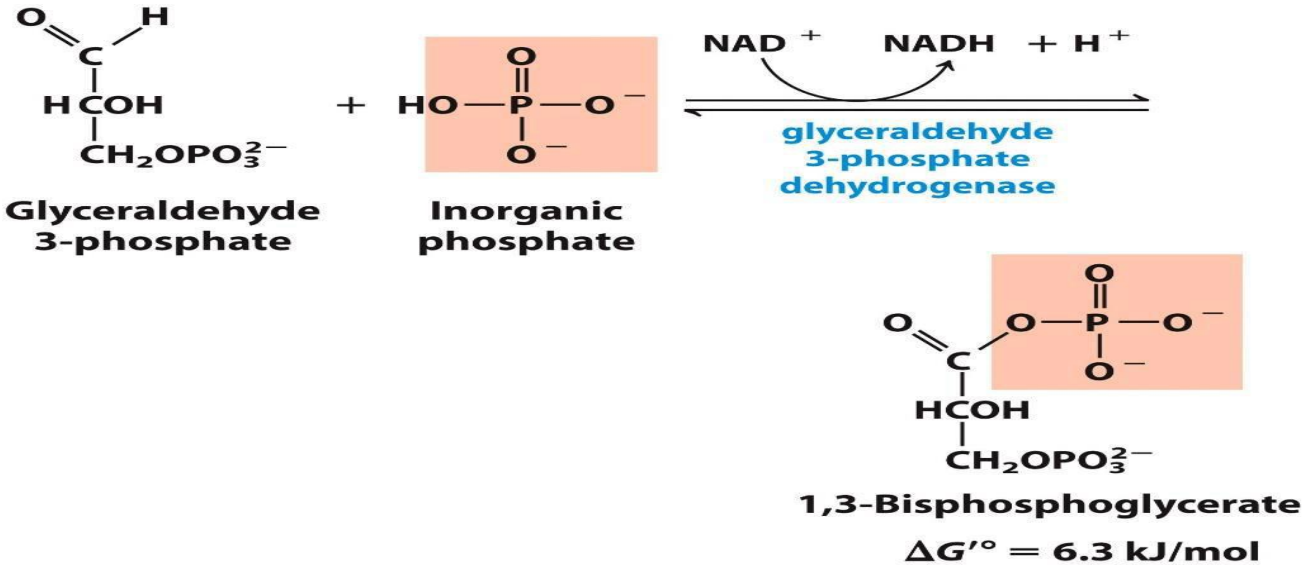
• Isotopes are the atoms in an element that have the same atomic number but a different atomic mass; that is, the same number of protons and thus identical chemical properties, but different numbers of neutrons and consequently different physical properties.
• Isotopes can be stable or unstable or radioisotopes.
• In the latter, their nuclei have a special property: they emit energy in the form of ionizing radiation while searching for a more stable configuration.

The Payoff Phase of Glycolysis Yields ATP and NADH

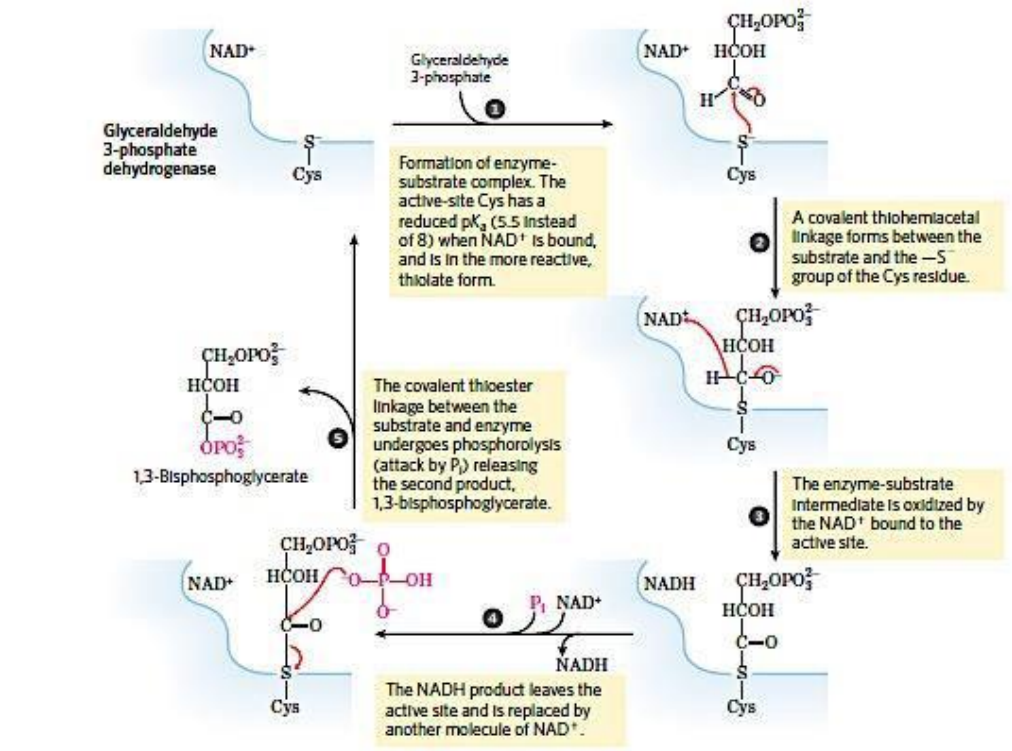
• The payoff phase of glycolysis includes the energy-conserving phosphorylation steps
• The chemical energy of the glucose molecule is conserved in the form of **ATP and NADH**
• The conversion of two molecules of glyceraldehyde 3-phosphate
to two molecules of pyruvate is accompanied **by the formation of four molecules of ATP from ADP.**
• the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

6. Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

• The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**, catalyzed by **glyceraldehyde 3-phosphate dehydrogenase.**
• This is the **first of the two energy-conserving reactions** of glycolysis that eventually lead to the formation of ATP.
• The aldehyde group of glyceraldehyde 3-phosphate (G3P) is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid.
• This type of anhydride, called an **acyl phosphate**, has a very high standard free energy of hydrolysis (-49.3 kJ/mol).



• Much of the free energy of oxidation of the aldehyde group of glyceraldehyde 3-phosphate is conserved by formation of the acyl phosphate group at **C-1 of 1,3-bisphosphoglycerate.**
• NAD+ is the e- acceptor (bound to Rossmann fold of dehydrogenase)
• G3P is covalently bound to the dehydrogenase
• The aldehyde group of glyceraldehyde 3-phosphate reacts with the —SH group of an essential **Cys residue** in the active site, in a reaction analogous to the formation of a **hemiacetal** producing a *thio*hemiacetal.
• Reaction of the essential Cys residue with a heavy metal such as Hg+2 irreversibly inhibits the enzyme.
• The amount of NAD+ in a cell is far smaller than the amount of glucose metabolized in a few minutes.
• Glycolysis would soon come to a halt if the NADH formed in this step of glycolysis were not continuously reoxidized and recycled.



MECHANISM FIGURE 14–8 The glyceraldehyde 3-phosphate dehydrogenase reaction.

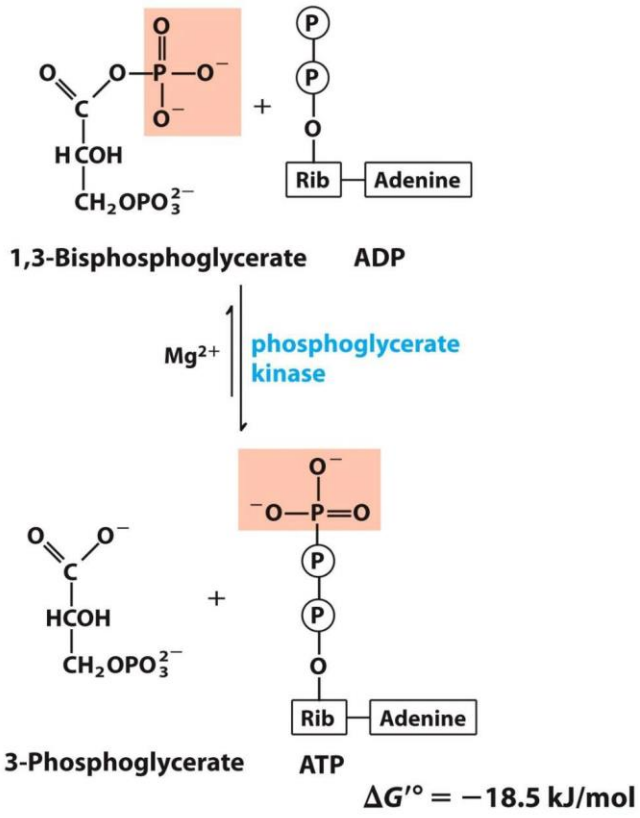
7. Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

• The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**.

• phosphoglycerate kinase is named for the reverse reaction, in which it transfers a phosphoryl enzymes, it catalyzes the reaction in both directions.

• In glycolysis, the reaction it catalyzes proceeds in the direction of ATP synthesis.

• Steps 6 and 7 of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). Overall reaction is exergonic.. The sum of these two reactions is



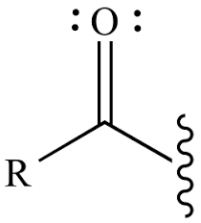
• The outcome of these reactions, both reversible under cellular conditions, is that the energy released on **oxidation of an aldehyde to a carboxylate** group is conserved by the coupled formation of ATP from ADP and Pi.
• The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**, to distinguish this mechanism from **respiration-linked phosphorylation**.

Substrate-level phosphorylation

• **Substrate-level phosphorylation** is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl (PO3) group to ADP or GDP from another **phosphorylated** compound.
• Substrate-level phosphorylations involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case).
• Respiration-linked phosphorylations, involve membrane-bound enzymes and transmembrane gradients of protons.

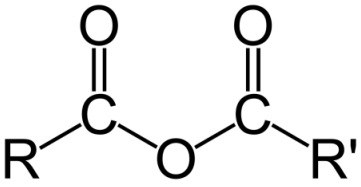
Acyl group

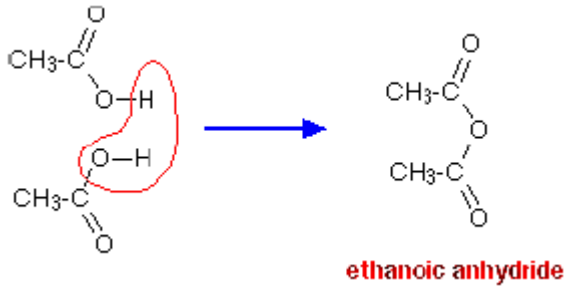
It contains a double bonded oxygen atom and an **alkyl group**. (R-C=O **group** is called **Acyl Group**). In organic chemistry, the **acyl group** (IUPAC name: alkanoyl) is usually derived from a carboxylic acid.



Acid anhydride

An acid anhydride is a compound that has two acyl groups bonded to the same oxygen atom. Thus, (CH3CO)2O is called **acetic anhydride**. Mixed (or unsymmetrical) **acid anhydrides**, such as **acetic** formic **anhydride.**

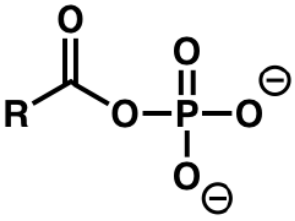




ethanoic anhydride

Acyl phosphate

A phosphate group is connected to the acyl group by a single bond and that the bond occurs between one of the oxygen atoms of the phosphate and the carbonyl carbon of the acyl group.

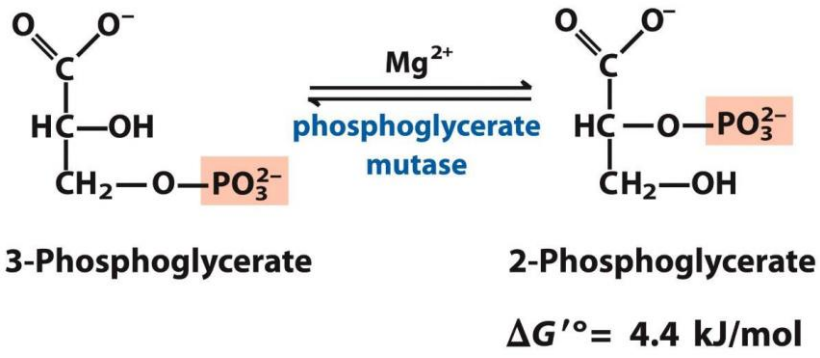


Step 8-10

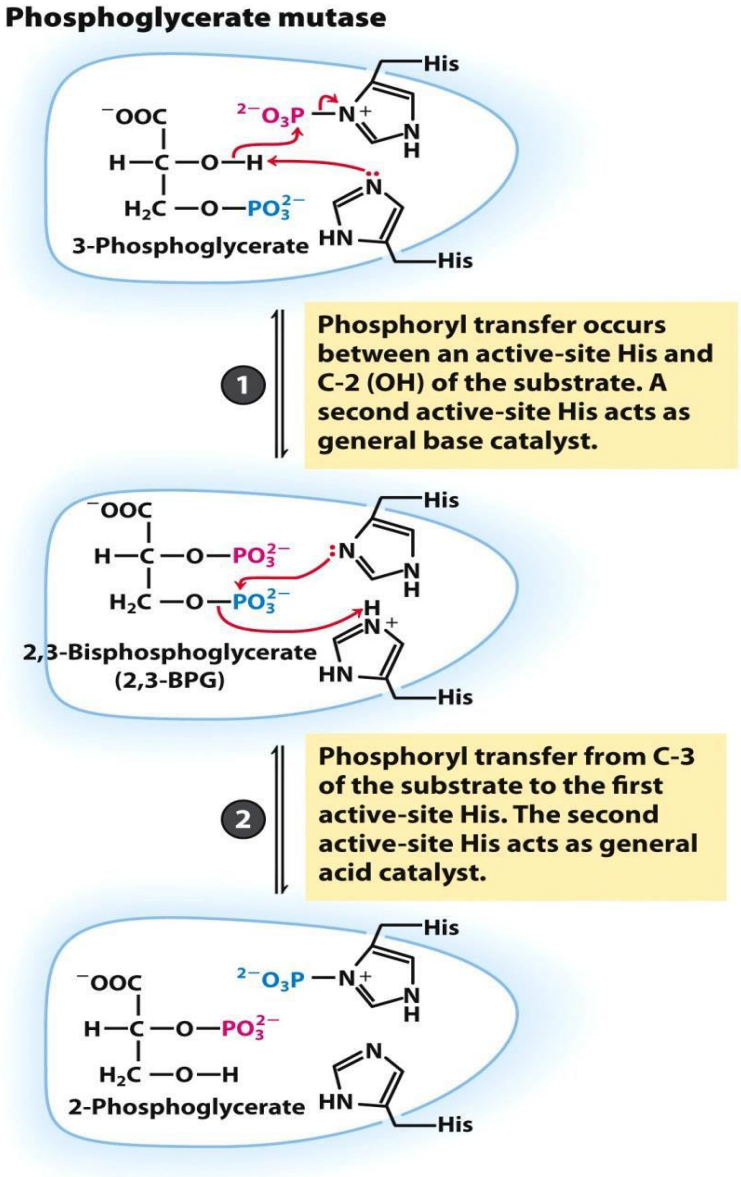
8. Conversion of 3-Phosphoglycerate to 2- Phosphoglycerate

• The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate
• Mg+2 is essential for this reaction. The reaction occurs in two steps **A phosphoryl group** initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG).
• The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated
enzyme.

• Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle

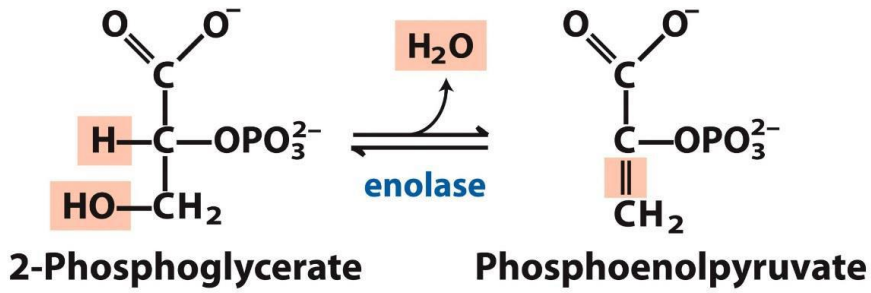


The phosphoglycerate mutase reaction.



9. Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

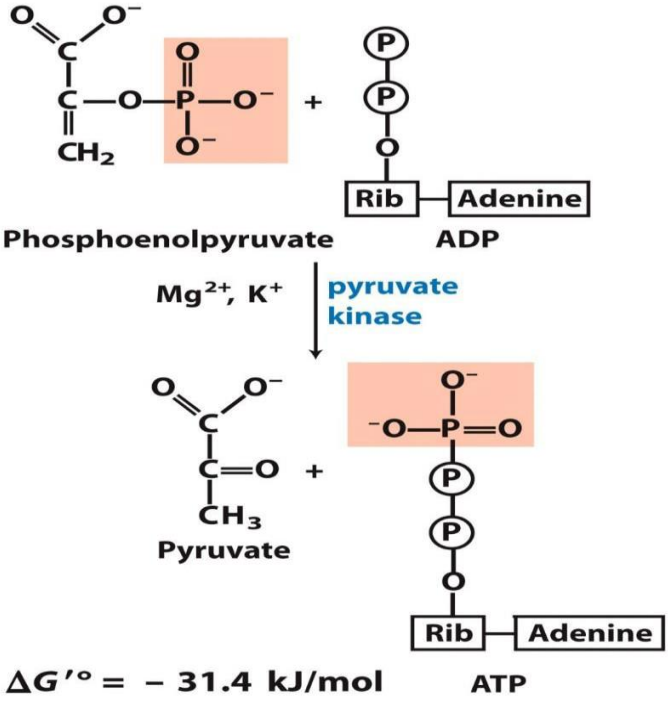
• In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential
• (the first was step 6), **enolase** promotes reversible removal of a molecule of water from 2- phosphoglycerate
• The mechanism of the enolase reaction involves an enolic intermediate stabilized by Mg+2
• The reaction converts a compound with a relatively low phosphoryl group transfer potential ($\Delta G'$ °for hydrolysis of 2-phosphoglycerate is -17.6 kJ/mol) to one with high phosphoryl group transfer potential ($\Delta G'$°for PEP hydrolysis is -61.9 kJ/mol).



$\Delta G'^{\circ} = 7.5$ kJ/mol

10. Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP

• The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase**, which requires K+ (potassium ions) and either Mg+2 or Mn^{+2}.
• In this substrate-level phosphorylation, the product **pyruvate** first appears in its enol form, then tautomerizes rapidly and nonenzymatically to its keto form, which predominates at pH 7.
• The overall reaction change, spontaneous conversion of the enol form of pyruvate to the keto form has a large, negative standard free energy
• About half of the energy released by PEP hydrolysis (- 61.9 kJ/mol) is conserved in the formation of the phosphoanhydride bond of ATP (-30.5 kJ/mol), and the rest (-31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis.



GLYCOLYSIS

ATP Formation Coupled to Glycolysis

• ***ATP Formation Coupled to Glycolysis*** **During glycolysis some of the energy of the glucose molecule is conserved in the form of ATP, while much remains in the product, pyruvate.**
• **The overall equation for glycolysis is**

$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2\ \text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O \quad \textbf{(Eqn I)}$$

• **For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and Pi.**

• **We can now resolve the equation of glycolysis into two processes:**
(1) the conversion of glucose into pyruvate is exergonic:

$$\text{Glucose} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+, \Delta G^{\circ\prime}_1 = -146\ \text{kJ/mol} \quad \textbf{(Eqn II)}$$

(2) and the formation of ATP from ADP and Pi is endergonic:

$$2\text{ADP} + 2\text{Pi} \longrightarrow 2\text{ATP} + 2\text{H}_2\text{O}, \Delta G^{\circ\prime}_2 = 2(30.5\ \text{kJ/mol}) = 61\ \text{kJ/mol} \quad \textbf{(Eqn III)}$$

• **If we now write the sum of Equations (II) and (III), we can also determine the overall standard free-energy change of glycolysis (Eqn I), including ATP formation, as the algebic sum, ΔG°'S, of ΔG°'1 and ΔG°'2**

$$\Delta G^{\circ\prime}_s = \Delta G^{\circ\prime}_1{}' + \Delta G^{\circ\prime}_2 = -146\ \text{kJ/mol} + 61\ \text{kJ/mol} = -85\ \text{kJ/mol}$$

Under standard and intracellular conditions, glycolysis is an essentially irreversible process, driven to completion by this large net decrease in free energy.

Energy Remaining in the Pyruvate Produced by Glycolysis

• The two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose.
• **This energy that can be extracted by oxidative reactions in the citric acid cycle and oxidative phosphorylation.**
• 4 electrons..in the form of 2 hydride ions are transferred from 2 G3P to 2NAD+
• 2 molecules of NADH are reoxidized to NAD+ by transfer of electrons to electron transport chain (ETC) within mitochondria.
• ETC passes these electrons to oxygen, that provides energy for ATP synthesis during respiration.

"Pasteur effect"

• The ATP yield from glycolysis under anaerobic conditions (2 ATP per molecule of glucose) is much smaller than that from the complete oxidation of glucose to CO2 under aerobic conditions (30 or 32 ATP per glucose)
• About 15 times more glucose would be consumed in anaerobic conditions to produce same amount of energy (ATP)
• This was discovered by Louis Pasteur while studying process of fermentation in yeast, and is known as the "Pasteur effect"

"Warburg effect"

• The German biochemist Otto Warburg first observed in 1928 that tumors of nearly all types carry out glycolysis at a much higher rate than normal tissue, *even when oxygen is available*.
• This is known as "Warburg effect" and is the basis for several methods of detecting and treating cancer
• Isolated 7 enzymes of glycolysis from tumor tissues
• Warburg manometer: experimental tool to measure amount oxygen consumed ..so oxidative activity
• Nobel prize of Physiology in 1931

Why glycolysis in tumor tissue?

• Most tumor cells grow under hypoxic conditions (i.e., with limited oxygen supply) because, at least initially, they lack the capillary network to supply sufficient oxygen.
• Cancer cells located more than 100 to 200 *u*m from the nearest capillaries must depend on glycolysis alone (without further oxidation of pyruvate) for much of their ATP production. The energy yield (2 ATP per glucose) is far lower than can be obtained by the complete oxidation of pyruvate to CO2 in mitochondria (about 30 ATP per glucose)
• To make the same amount of ATP, tumor cells must take up much more glucose than do normal cells, converting it to pyruvate and then to lactate as they recycle NADH.
• In general, the more aggressive the tumor, the greater is its rate of glycolysis. This increase in glycolysis is achieved at least in part by increased synthesis of the glycolytic enzymes and of the plasma membrane transporters GLUT1 and GLUT3 that carry glucose into cells.

• The **hypoxia-inducible transcription factor (HIF-1)** is a protein stimulates the production of at least eight glycolytic enzymes and the glucose transporters when oxygen supply is limited
• Another protein induced by HIF-1 is the peptide hormone VEGF (vascular endothelial growth factor), which stimulates the growth of blood vessels (angiogenesis) toward the tumor
• This heavier reliance of tumors than of normal tissue on glycolysis suggests a possibility for anticancer therapy: inhibitors of glycolysis might target and kill tumors by depleting their supply of ATP. Three inhibitors of hexokinase have shown promise as chemotherapeutic agents: 2-deoxyglucose, lonidamine, and 3-bromopyruvate.

Glucose Uptake Is Deficient in Type 1 Diabetes Mellitus

• The metabolism of glucose in mammals is limited by the rate of glucose uptake into cells and its phosphorylation by **hexokinase.**
• Glucose uptake from the blood is mediated by the **GLUT family** of glucose transporters
• The transporters of hepatocytes (GLUT1, GLUT2) and of brain neurons (GLUT3) are always present in plasma membranes.
• In contrast, the main glucose transporter in the cells of skeletal muscle, cardiac muscle, and adipose tissue (GLUT4) is sequestered in small intracellular vesicles and moves into the plasma membrane only in response to an insulin signal
• Thus in skeletal muscle, heart, and adipose tissue, glucose uptake and metabolism
• depend on the normal release of insulin by **pancreatic beta cells** in response to elevated blood glucose

Individuals with type 1 diabetes mellitus have too few cells and cannot release sufficient insulin to trigger glucose uptake by the cells of skeletal muscle, heart, or adipose tissue. Thus, after a meal containing carbohydrates, glucose accumulates to abnormally high levels in the blood, a condition known as hyperglycemia. Unable to take up glucose, muscle and fat tissue use the fatty acids of stored triacylglycerols as their principal fuel. In the liver, acetyl-CoA derived from this fatty acid breakdown is converted to "ketone bodies"—acetoacetate and -hydroxybutyrate—which are exported and carried to other tissues to be used as fuel These compounds are especially critical to the brain, which uses ketone bodies as alternative fuel when glucose is unavailable. (Fatty acids cannot pass through the blood-brain barrier and thus are not a fuel for brain neurons.)

In untreated type 1 diabetes, overproduction of acetoacetate and hydroxybutyrate leads to their accumulation in the blood, and the consequent lowering of blood pH produces **ketoacidosis**, a life-threatening condition.
Insulin injection reverses this sequence of events:
GLUT4 moves into the plasma membranes of hepatocytes and adipocytes, glucose is taken up into the cells and phosphorylated, and the blood glucose level falls, greatly reducing the production of ketone bodies.

Importance of Phosphorylated Intermediates

Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated. The phosphate groups appear to have three functions.
1. The phosphate groups are ionized at pH 7, thus giving each of the intermediates of glycolysis a net negative charge.
2. Because the plasma membrane is impermeable to molecules that are charged, the phosphorylated intermediates cannot diffuse out of the cell.
3. After the initial phosphorylation, the cell does not have to spend further energy in retaining phosphorylated intermediates despite the large difference between the intracellular and extracellular concentrations of these compounds.
• Phosphate groups are essential components in the enzymatic conservation of metabolic energy.
• Energy released in the breakage of phosphoric acid anhydride bonds (such as those in ATP) is partially conserved in the formation of phosphate esters such as glucose-6 phosphate.
• High-energy phosphate compounds formed in glycolysis (1,3- bisphosphoglycerate and phosphoenol pyruvate) donate phosphate groups to ADP to form ATP.
• Binding of phosphate groups to the active sites of enzymes provides binding energy that contributes to lowering the activation energy and increasing the specificity of enzyme-catalyzed reactions.

• The phosphate groups of ADP, ATP, and the glycolytic intermediates form complexes with Mg2+, and the substrate binding sites of many of the glycolytic enzymes are specific for these Mg2+ complexes.
• Nearly all the glycolytic enzymes require Mg2+ for activity.

Fates of Pyruvate

• The pyruvate formed by glycolysis is further metabolized via one of three catabolic routes.
• In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose.

1. Oxidation of Pyruvate:

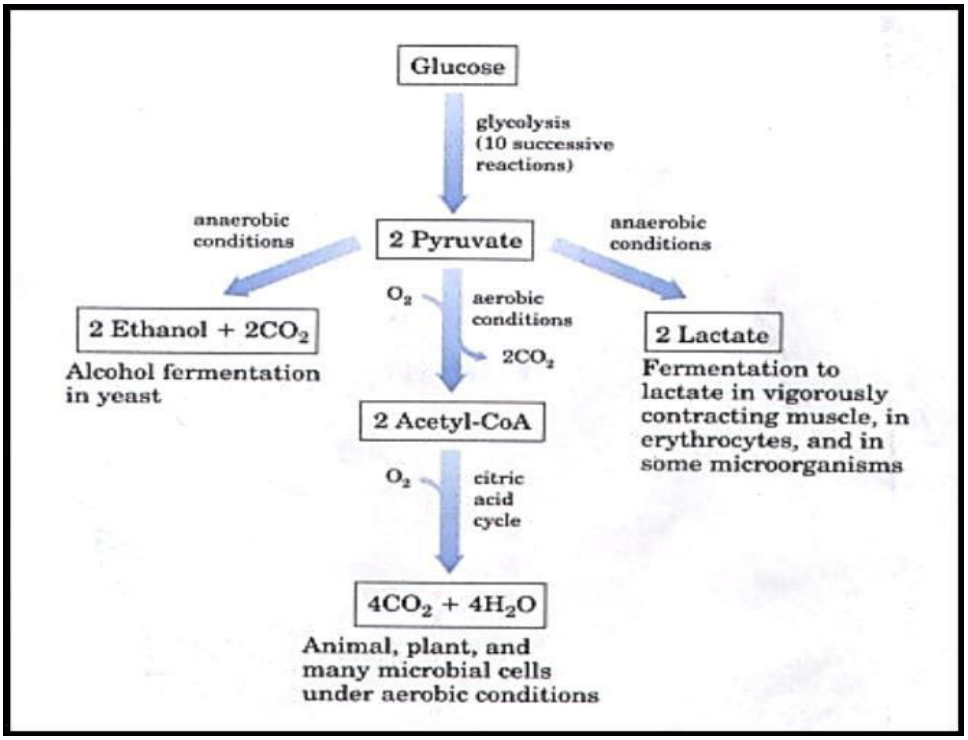
Pyruvate is oxidized, with loss of its carboxyl group as CO2, to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO2 by the citric acid cycle. The electrons from these oxidations are passed to O2 through a chain of carriers in mitochondria, to form H2O. The energy from the electron-transfer reactions drives the synthesis of ATP in mitochondria

2. Reduction to lactate

• The second route for pyruvate is its reduction to lactate via **lactic acid fermentation**.
• When vigorously contracting skeletal muscle must function under low oxygen conditions (**hypoxia**), NADH cannot be reoxidized to NAD+, but NAD+ is required as an electron acceptor for the further oxidation of pyruvate.
• Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD+ necessary for glycolysis to continue. Certain tissues and cell types (retina and erythrocytes, for example) convert glucose to lactate even under aerobic conditions.
• Lactate is also the product of glycolysis under anaerobic conditions in some microorganisms.

3. Ethanol (alcohol) fermentation

• The third major route of pyruvate catabolism leads to formation of ethanol.
• In some plant tissues and in certain invertebrates, protists, and microorganisms such as yeast, pyruvate is converted under hypoxic or anaerobic conditions to ethanol and CO2, a process called **ethanol (alcohol) fermentation**
• The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, provide the carbon skeleton for the synthesis of the amino acid alanine or for the synthesis of fatty acids.



Three possible catabolic fates of the pyruvate formed in the payoff phase of glycolysis

Fates of Pyruvate under Aerobic Conditions

• **Under Aerobic conditions**, the pyruvate formed in the final step of glycolysis is oxidized to **acetate (acetyl-CoA), which enters the citric acid cycle** and is oxidized to CO2 and H2O.
• The NADH formed by **dehydrogenation of glyceraldehyde 3-phosphate** is ultimately **reoxidized** to **NAD+** by passage of its electrons to O2 in mitochondrial respiration.

Fates of Pyruvate under Anaerobic Conditions

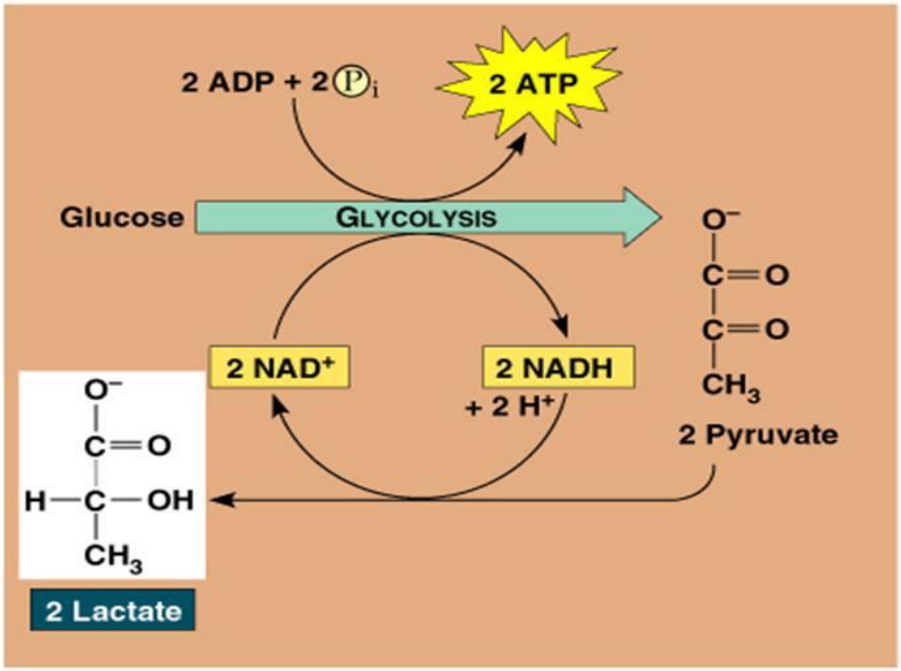
• **Under hypoxic (low-oxygen) conditions**, as in very active skeletal muscle, in submerged plant tissues, in solid tumors, or in lactic acid bacteria—**NADH generated by glycolysis cannot be reoxidized by O2.**
• Failure to regenerate NAD+ can leave the cell with **no electron acceptor** for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop.
• **There must be another way to regenerated NAD+**
• modern organisms continually regenerate NAD+ during anaerobic glycolysis **by transferring electrons from NADH to form a reduced end product such as lactate or ethanol (by fermentation).**

Fermentation

• **Fermentation actually comes from Latin word "fervere" which means "to boil"**
• **So the word "Fermentation" is referred to physical state of boiling/bubbling**
• **The bubbling appearance in fermentation is due to the production of CO2 bubbles caused by anaerobic catabolism of sugars**
• **Fermentation** is a metabolic process that converts sugars to **acids, gases, or alcohol**
• The science of fermentation is known as **ZYMOLOGY**
• French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes.
• It occurs in yeast and bacteria, and also in oxygen-starved muscle cells
• **In terms of BIOCHEMISTRY Fermentation relates to the generation of energy by catabolism of organic compounds**
• Industrial Fermentation is used to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product

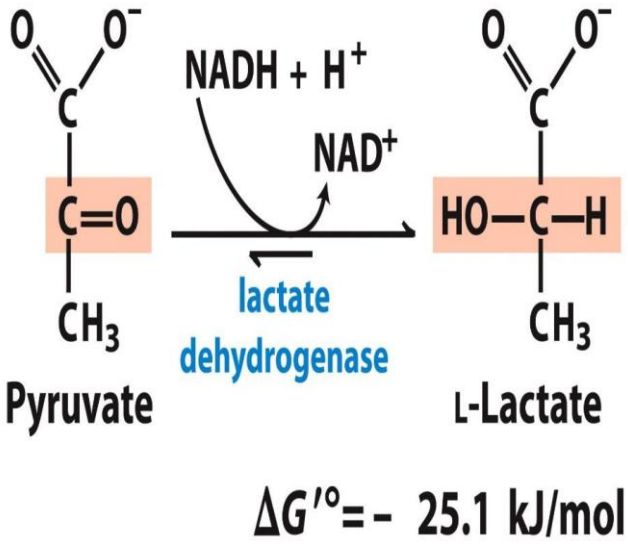
Lactic Acid Fermentation

• Lactic acid fermentation is done by some fungi, some bacteria like in yogurt, and sometimes by our muscles.
• Glucose carbon dioxide + lactic acid
• In the process of lactic acid fermentation, the 3-carbon pyruvic acid molecules are turned into lactic acid
•Allows glycolysis to continue
•Normally our muscles do cellular respiration like the rest of our bodies, using O2 supplied by our lungs and blood. However, under greater exertion when the oxygen supplied by the lungs and blood system can't get there fast enough to keep up with the muscles' needs, our muscles can switch over and do **lactic acid fermentation**.

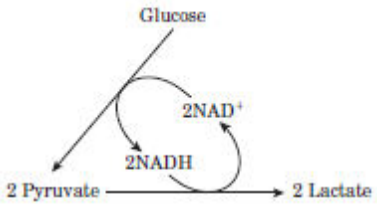


Pyruvate Is the Terminal Electron Acceptor in Lactic Acid Fermentation

• NAD+ is regenerated from NADH by the reduction of pyruvate to **lactate**.
• some tissues and cell types (such as erythrocytes, which have no mitochondria and cannot oxidize pyruvate to CO2) produce lactate from glucose even under aerobic conditions.
• The reduction of pyruvate in this pathway is catalyzed by **lactate dehydrogenase**, which forms lactate at pH 7.



• The overall equilibrium of the reaction strongly favors lactate formation, as shown by the large negative standard free-energy change.
• In glycolysis, dehydrogenation of the two molecules of glyceraldehyde 3- phosphate derived from each molecule of **glucose converts two**
molecules of NAD+ to two of NADH.
• Because the **reduction of two molecules of pyruvate** to two of lactate regenerates **two molecules of NAD+,** there is no net change in NAD+ or NADH.
• The lactate formed by active skeletal muscles (or by erythrocytes) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity.
• When lactate is produced in large quantities during vigorous muscle contraction (during a sprint, for example), the acidification that results
from ionization of lactic acid in muscle and blood limits the period of vigorous activity.
• **Although conversion of glucose to lactate includes two oxidation-reduction steps**, there is no net change in theoxidation state of carbon; in glucose (C6H12O6) and lactic acid (C3H6O3), the H:C ratio is the same.



- **Homolactic Fermentation**
- HOMOLACTIC In homolactic fermentation, one glucose molecule is converted into two molecules of lactic acid
- $C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$

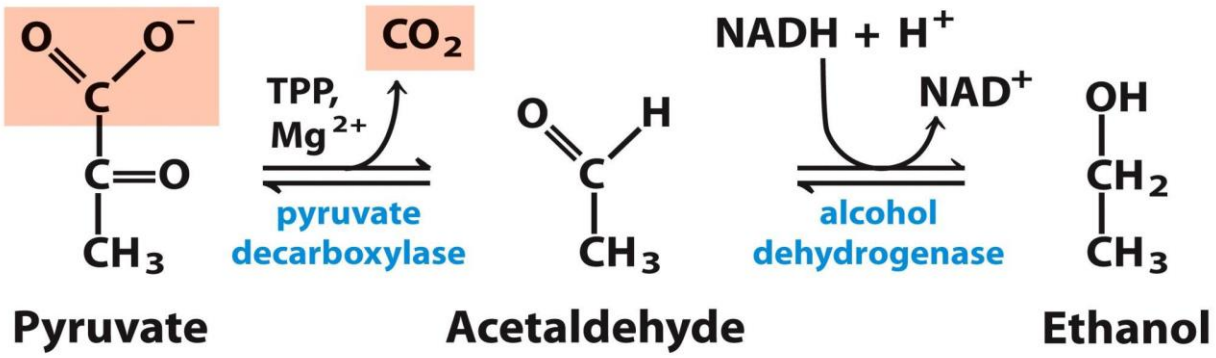
- **Heterolactic Fermentation**
- In Heterolactic Fermentation, one glucose molecule is converted into one molecule of lactic acid, one molecule of ethanol and one molecule of carbondioxide
- $C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

Ethanol Fermentation

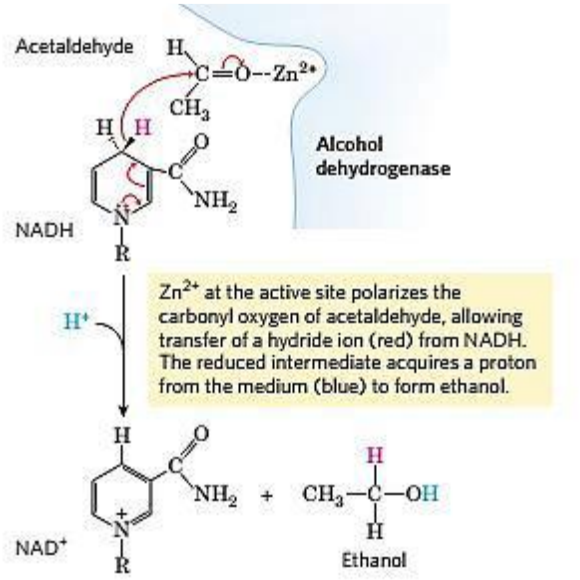
• Alcohol fermentation is done by yeast and some kinds of bacteria.
• Products: glucose undergoes fermentation to produce **ethanol and CO2**, instead of lactate.
• Substrate: glucose

$$6O_2 + \text{Glucose} \longrightarrow \textit{Pyruvate} \longrightarrow CO_2 + \textit{Ethanol}$$

two-step process:
• Yeast contain the enzyme pyruvate decarboxylase that decarboxylates pyruvate to form acetaldehyde.
• Acetaldehyde is reduced to ethanol under anaerobic conditions.



Ethanol Is the Reduced Product in Ethanol Fermentation



The alcohol dehydrogenase reaction.

• Final equation:
• As in lactic acid fermentation, there is no net change in the ratio of hydrogen to carbon atoms
• when glucose (H:C ratio = 12/6 = 2) is fermented to two ethanol and two CO2 (combined H:C ratio = 12/6 = 2).
• In all fermentations, the H:C ratio of the reactants and products remains the same.
• Alcohol dehydrogenase is present in many organisms that metabolize ethanol, including humans.
• In the liver it catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, **with the reduction of NAD+ to NADH.**
• **Pyruvate decarboxylase** is present in brewer's and baker's yeast (*Saccharomyces cerevisiae*) and in all other organisms that ferment glucose to ethanol, including some plants.
• The CO2 produced by pyruvate decarboxylation in brewer's yeast is responsible for the characteristic carbonation of champagne (ethanol fermentation).
• In **baking,** CO2 released by **pyruvate decarboxylase** when yeast is mixed with a fermentable sugar causes dough to rise.
• The enzyme is absent in organisms that carry out lactic acid fermentation.

Fermentation

Use of Fermentation In Industry

Industrial Fermentation

• Oldest form of microbiology and biotechnology which was used to make wine, beer, bread with use of bacteria and yeasts without knowing scientific basis
• Fermentation has different meaning to biochemistry and industrial microbiologists.
• Biochemical meaning relates to the generation of energy by the catabolism of organic compounds
• Microbiologists have extended the term - fermentation - to describe: "A process that generates a product by the mass culture of microorganism."
• **In Industrial Biotechnology,** fermentation means a process in which microorganisms that are cultured on a large-scale under aerobic or anaerobic conditions, convert a substrate into a product which is useful to man.
• The industrial microorganisms are grown under controlled conditions with an aim of optimizing the growth of the organism for production of a target microbial product.
• In 1910 Chaim Weizmann (later to become the first president of Israel) discovered that the bacterium *Clostridium acetobutyricum* ferments starch to butanol and acetone.
• This discovery opened the field of industrial fermentations, in which some readily available material rich in carbohydrate (corn starch or molasses, for example) is supplied to a pure culture of a specific microorganism, which ferments it into a product of greater commercial value.

Some important fermentation products

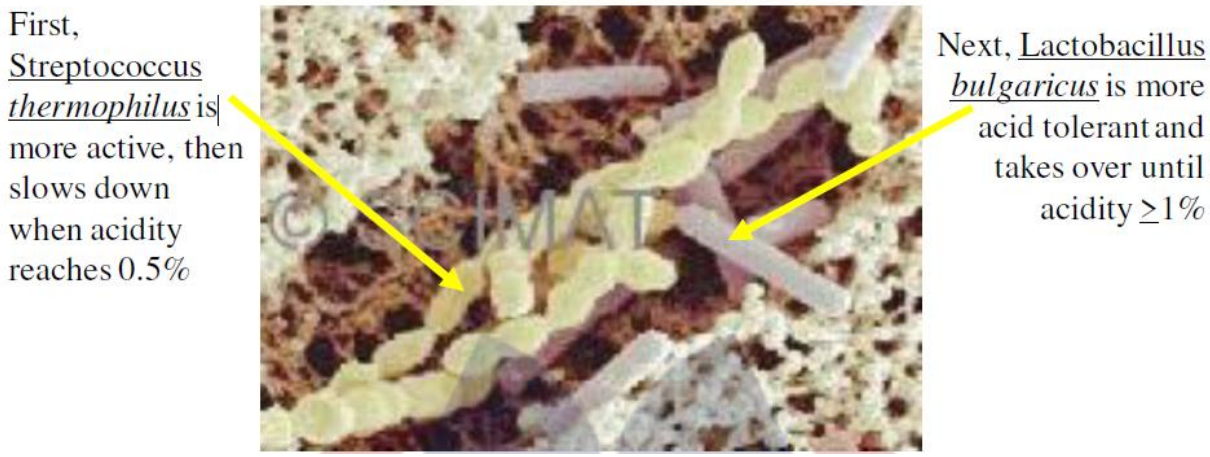
• Fermentations Are Used to Produce Some Common Foods and Industrial Chemicals

Product	Organism	Use
Ethanol	*Saccharomyces cerevisiae*	Industrial solvents, beverages
Glycerol	*Saccharomyces cerevisiae*	Production of explosives
Lactic acid	*Lactobacillus bulgaricus*	Food and pharmaceutical
Acetone and butanol	*Clostridium acetobutylicum*	Solvents
α-amylase	*Bacillus subtilis*	Starch hydrolysis

• Yogurt, is produced when the bacterium *Lactobacillus bulgaricus* ferments the carbohydrate in milk, producing lactic acid; the resulting **drop in pH causes the milk proteins to precipitate**, producing the thick texture and sour taste of unsweetened yogurt.
• **Cheese** is produced by using *Propionibacterium freudenreichii*, which ferments milk to produce ***propionic acid and CO2***; the propionic acid
• precipitates milk proteins, and bubbles of CO2 cause the holes characteristic of **Swiss Cheese**.

Commercial Yogurt

Contains 2 species of bacteria specialized to grow well in milk (but can't survive inside the human body):
These bacteria work in *symbiosis*. Each bacterium stimulates the growth of the other => acidifies the milk more rapidly than either partner on its own.



Use of Fermentation In agriculture

• In agriculture, plant byproducts such as corn stalks are preserved for use as animal feed by packing them into a large container (a silo) with limited access to air; microbial fermentation produces acids that lower the pH.
• **The silage** that results from this fermentation process can be kept as animal feed for long time without spoilage.

Fermentation Products and steps of industrial Fermentation

Fermentation Products

There are five major groups of commercially important fermentations:

1. Those that produce microbial cells (or biomass) as the product.
2. Those that produce microbial metabolites.
3. Those that produce microbial enzymes.
4. Those that produce recombinant products.
5. Those that modify a compound which is added to the fermentation – the transformation process.

Biomass Production

Baker's Yeast
SCP (Single Cell Protein)
Probiotics
Bio fertilizers
Vaccines
Bioweapons

1. Upstream processing

• **Upstream processing** includes ***formulation of the fermentation medium***, ***sterilization*** of air, fermentation medium and the fermenter, ***inoculum preparation*** and ***inoculation*** of the medium. The ***fermentation medium*** should contain an **energy source**, a **carbon source**, a **nitrogen source** and **micronutrients** required for the growth of the microorganism along with **water** and **oxygen**, if necessary.
A medium which is used for a large scale fermentation, in order to ensure the sustainability of the operation, should have the following characteristics;

1. It should be cheap and easily available
2. It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product
3. It should minimize the formation of undesired products
• Usually, waste products from other industrial processes, such as molasses, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentation.
• ***Sterilization*** is essential for preventing the contamination with any undesired microorganisms. **Air** is sterilised by memb**rane filtration** while the **medium** is usually **heat sterilized**. Any nutrient component which is **heat labile** is **filter-sterilised** and later added to the sterilised medium. The fermenter may be sterilised together with the medium or separately.
• ***Inoculum build up*** is the preparation of the seed culture in amounts sufficient to be used in the large fermenter vessel. This involves growing the microorganisms **obtained from the pure stock culture** in **several consecutive fermenters**. This process cuts down the time required for the growth of microorganisms in the fermenter, thereby increasing the rate of productivity. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

2. The fermentation process

• **The fermentation process** involves the ***propagation of the microorganism*** and ***production of the desired product***. The fermentation process can be categorized depending on various parameters.
It can be either **aerobic fermentation**, carried out in the presence of oxygen or **anaerobic fermentation**, carried out in the absence of oxygen. Many industrial fermentation are carried out under aerobic conditions where a few processes such as ethanol production by yeast require strictly anaerobic environments.

3. Downstream Processing

• **Downstream Processing** includes the **recovery of the products** in a pure state and the **effluent treatment**. **Product recovery** is carried out through a series of operations including ***cell separation*** by settling, centrifugation or filtration; ***product recovery*** by disruption of cells (if the product is produced intracellularly); ***extraction*** and ***purification*** of the product. Finally, the ***effluents are treated*** by chemical, physical or biological methods.

General Aspects of Fermentation Processes

What is fermentation technique?

Techniques for **large-scale production** of microbial products. It must both provide an **optimum environment** for the microbial synthesis of the desired product and be **economically feasible** on a large scale.

• Fermentation technique can be divided into following on the basis of moisture requirement
1. surface (emersion) fermentation
2. submersion fermentation technique
• The latter may be run in **batch, fed batch, continuous reactors**

1. Surface techniques:
• In the **surface techniques**, the microorganisms are cultivated on the surface of a liquid or solid substrate.
• These techniques are very complicated and used in industry for producing bread, cocoa

2. Submersion processes
• In the **submersion processes**, the microorganisms grow in a **liquid medium** provided with the substrate
• Except in traditional beer and wine fermentation, the medium is held in fermenters and stirred to obtain a homogeneous distribution of cells and medium.
• Most processes are **aerobic**, and for these the medium must be **vigorously aerated**.
• **Used** for pickling vegetables and producing soy sauce.
• All important industrial processes (production of biomass and protein, antibiotics, enzymes are carried out by submersion processes.

Fermenter/Bioreactors

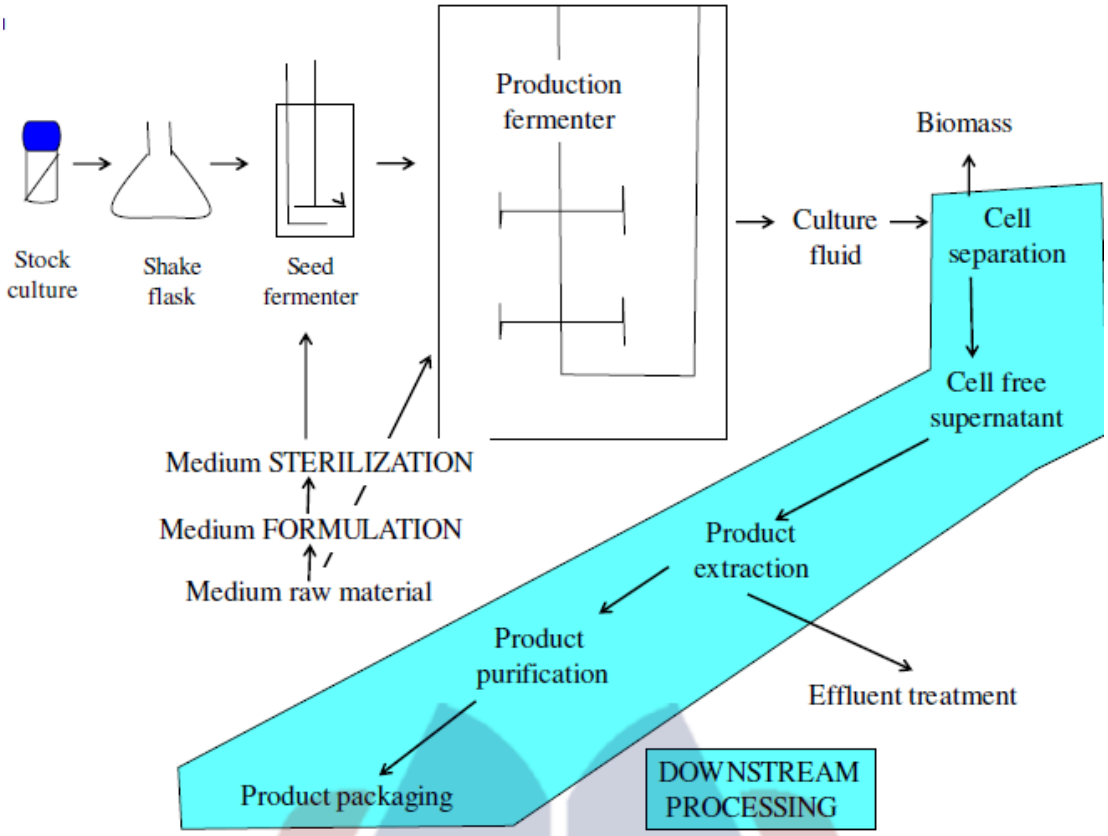
• The heart of the fermentation process is the fermenter.
• **A biorector is a device in which the organisms are cultivated and motivated to form a desired product**
• Closed vessel or containment designed to give a right environment for optimal growth and metabolic activity of the organism
• Fermenter: for microbes/ Bioreactor: for eukaryotic cells
• Size variable ranging from 20-250 million litres or Large scale production (..to1000-million L capacity)

Design of a Fermenter

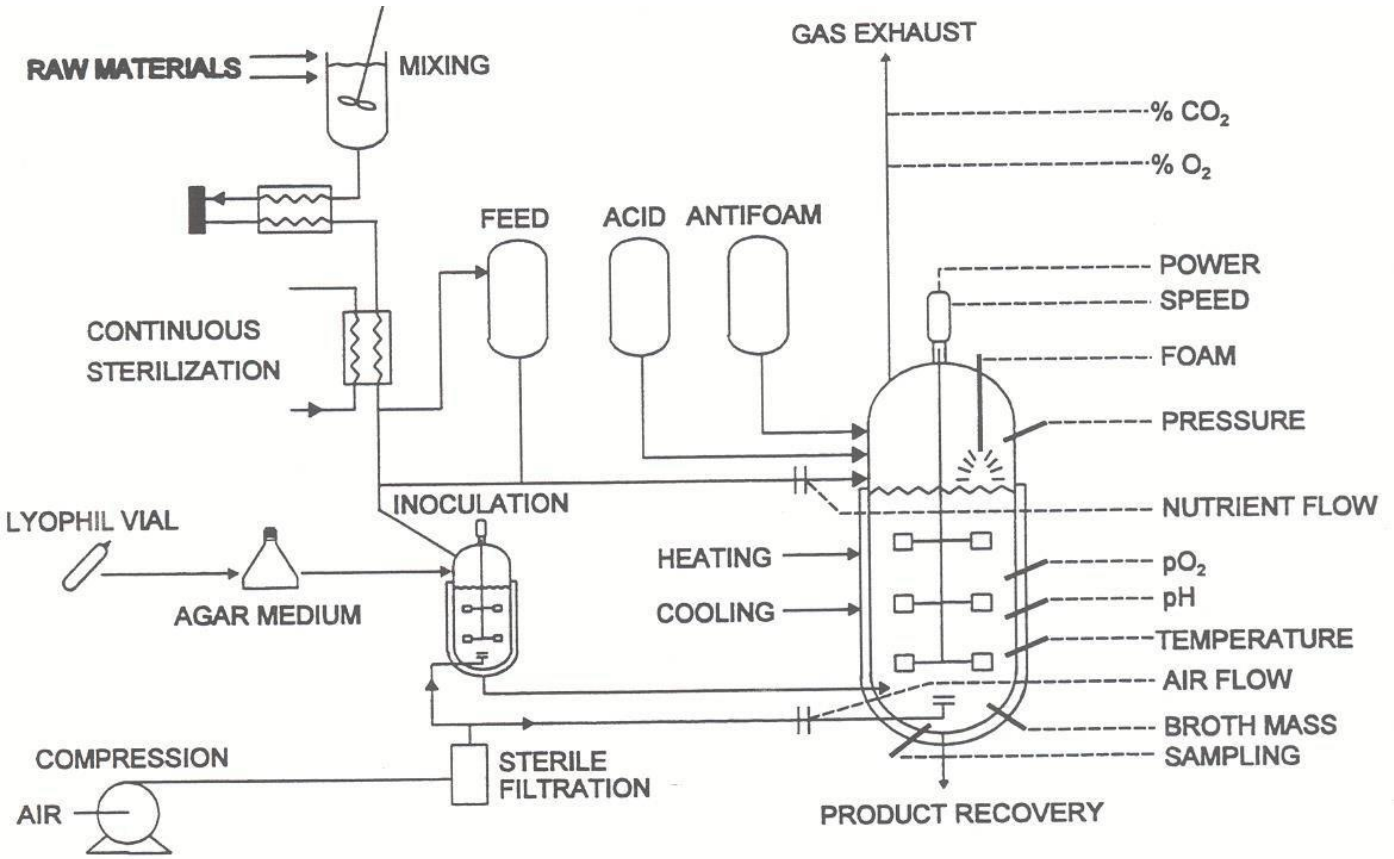
The success of a fermentation process is highly dependent on environmental factors. Factors to consider when designing a fermenter
• Aseptic and long-term reliability
• Adequate for aeration and agitation
• Low power consumption
• Temperature and pH controls
• Sampling facilities
• drain or overflow
• control systems
• sensors
• cooling to achieve maximum microbial yield

Types of Bioreactors

• Simple fermenters (batch and continuous)
• Fed batch fermenter
• Air-lift or bubble fermenter
• Cyclone column fermenter
• Tower fermenter
• Packed bed bioreactor
• photobioreactor



Flow sheet of a multipurpose fermenter and its auxiliary equipment



Basic modes for operating a fermenter

Types of fermentation on the basis of Process

Batch fermentation

• Closed system
• Batch fermentation refers to "A technique used to grow microorganisms or cells.
• A limited supply of Sterile nutrients (substrate) for growth is inoculated **(starter culture)** when these are used up, or some other factor becomes limiting, the **culture** declines.
• The time for which fermentation occurs is called 'fermentation time' or 'batch time'
• The only material added and removed during the course of a batch fermentation is oxygen (in the form of air, if the process is aerobic) and pH control solutions.
• The culture is allowed to grow until no more of the product is being made
• Cells, or products that the organisms have made, are "harvested" and fermenter is cleaned out for another run.
• The **principal disadvantage** of batch processing is the high proportion of unproductive time (down-time) between batches, comprising the charge and discharge of the fermenter vessel, the cleaning, sterilization and re-start process
• We take the example of *Aspergillus niger* and *Lactobacillus* are the microbes used to commercially produce citric acid and lactic acid, respectively.
• The production takes place in a batch fermenter.
• Rules of fermentation are applied on batch fermentation
– *Microbial Growth Kinetics*
– *Media for Industrial Fermentations*
– *Sterilization*
– *The Development of Inocula for Industrial Fermentations*
– *Design of a Fermenter*
– *Instrumentation and Control*
– *Aeration and Agitation*

Phases of Batch fermentation

lag phase (adapt to their surroundings)
exponential growth (grow in numbers)
stationary phase (stop growing)
death phase

Microbial Growth Kinetics

- Lag Phase

– This is the first phase in the fermentation process
– The cells have just been injected into a new environment and they need time to adjust accordingly
– Cell growth is minimal in this phase.

- Exponential Phase

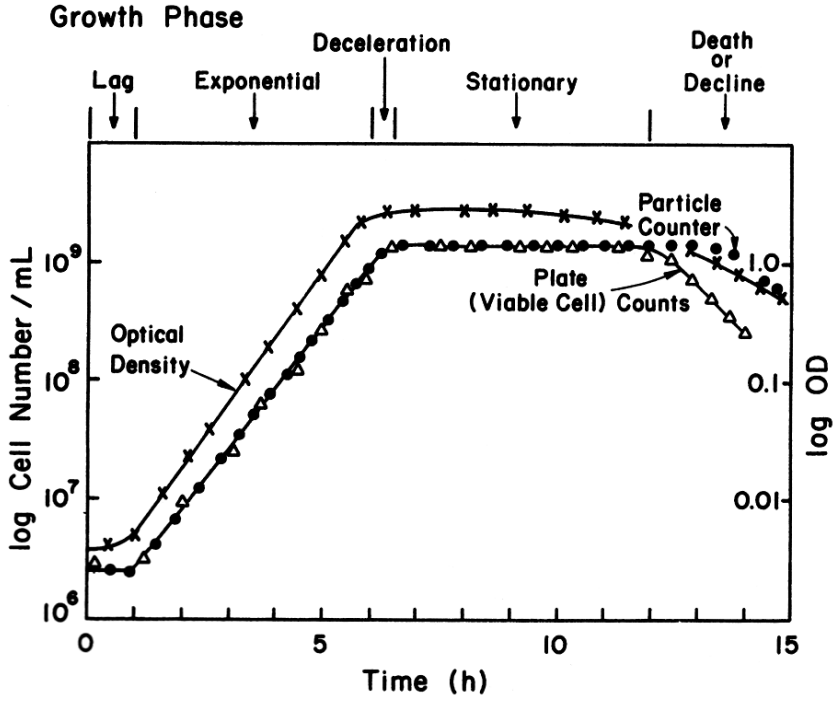
– The second phase in the fermentation process
– The cells have adjusted to their environment and rapid growth takes place
– Cell growth rate is highest in this phase
– At some point the cell growth rate will level off and become constant
– The most likely cause of this leveling off is substrate limited inhibition
• Substrate limited inhibition means that the microbes do not have enough nutrients in the medium to continue multiplying.

- Stationary phase

– This is the third phase in the fermentation process
– The cell growth rate has leveled off and become constant
– The number of cells multiplying equals the number of cells dying.

- Death phase

– The fourth phase in the fermentation process
– The number of cells dying is greater than the number of cells multiplying
• The cause of the death phase is usually that the cells have consumed most of the nutrients in the medium and there is not enough left for sustainability.



2. Continuous Fermentation

• A **Continuous fermentation** is a process in which fresh medium (substrate) is **continuously** added in the bioreactor ,
and biomass or products containing left over nutrients and microorganisms are **continuously** removed at the same rate to
keep the **culture** volume constant.
• Under these conditions the cells remain in the logarithmic phase of growth

3. Fed-batch Fermentation

• The fed-batch technique was originally devised by yeast producers in the early 1900s to regulate the growth in batch culture of *Saccharomyces* Yeast producers observed that in the presence of high concentrations of malt, a by-product - ethanol - was produced, while in low concentrations of malt, the yeast growth was restricted. The problem was then solved by a controlled feeding regime, so that yeast growth remained substrate limited.
• The concept was then extended to the production of other products, such as some enzymes, antibiotics, growth hormones, microbial cells, vitamins, amino acids and other organic acids.
• ***Fed-batch culture*** is, in the broad sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the **product(s)** remain in the bioreactor until the end of the run.
• An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion".
• It is also a type of **semi-batch culture**. In some cases, all the nutrients are fed into the bioreactor.
• ***Advantage of the fed-batch culture*** is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels).
• Generally speaking, fed-batch culture is superior to **conventional batch culture** when controlling concentrations of a nutrient (or nutrients) affect the yield or productivity of the desired metabolite.

Basically, cells are grown under a batch regime for some time, usually until close to the end of the exponential growth phase. At this point, the reactor is fed with a solution of substrates, without the removal of culture fluid. This feed should be balanced enough to keep the growth of the microorganisms at a desired specific growth rate and reducing simultaneously the production of by-products (that can be growth or product production inhibitory and make the system not as effective). By products may lead to cell death.

GLUCONEOGENESIS

The process of synthesis of glucose from non-carbohydrate sources is known as Gluconeogenesis (abbreviated GNG) ("new formation of sugar"), OR **Gluconeogenesis** is the pathway, which converts pyruvate and related noncarbohydrate carbon compounds to glucose.

Why the body needs Gluconeogenesis?

• For the human brain and nervous system, as well as the erythrocytes, testes, renal medulla, and embryonic tissues, glucose from the blood is the sole or major fuel source.
• The brain alone requires about 120 g of glucose each day
• more than half of all the glucose stored as glycogen in muscle and liver
• between meals and longer fasts, after vigorous exercise, glycogen is depleted
• For these times, organisms need a method for synthesizing glucose from noncarbohydrate precursors.

Site of Gluconeogenesis?

Organisms doing Gluconeogenesis: all animals, plants, fungi, and microorganisms. **In many microorganisms**, gluconeogenesis starts from simple organic compounds of two or three carbons, such as acetate, lactate, and propionate, in their growth medium. **Precursors of glucose in Gluconeogenesis:** The important precursors of glucose in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids.

Organs doing Gluconeogenesis:
• mainly in the liver
• To a lesser extent in renal cortex
• in the epithelial cells that line the inside of the small intestine
• In cytosol and mitochondria depending upon the substrate

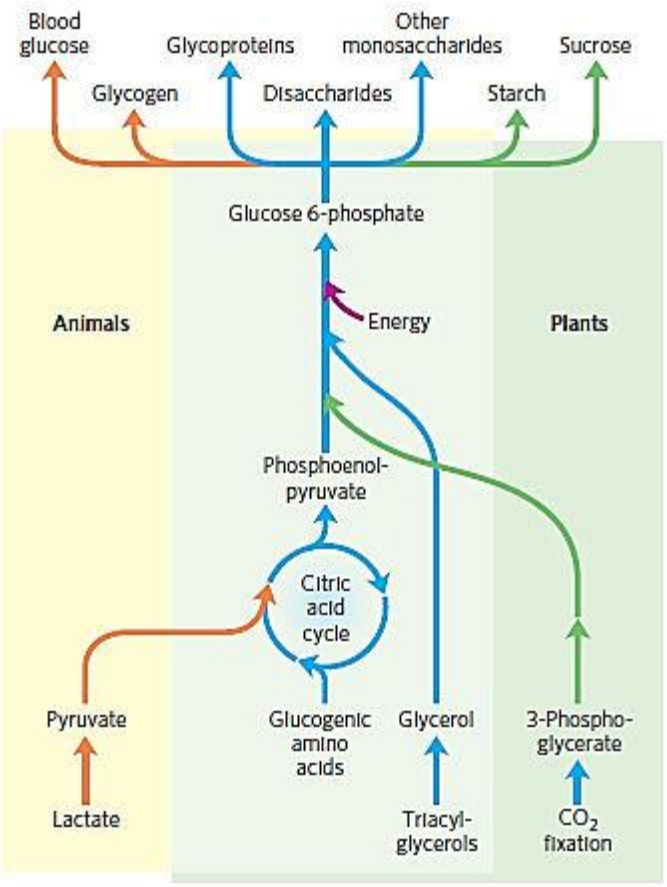
Where does this glucose go?
The glucose produced passes into the blood to supply other tissues.
Nature: Anabolic: 4 ATP are required for synthesis of 1 glucose from 1 pyruvate or lactate.

Pathways involved in Gluconeogenesis

• Reverse glycolysis (PEP to G6P)
• Tri carbocyclic acid cycle (TCA) or Kreb's cycle
• Cori cycle
• Glucose-alanine cycle

Precursors of glucose

• Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms.
• The reactions are essentially the same in all tissues and all species.



Biomedical importance of Gluconeogenesis

- To maintain the blood glucose concentration during prolonged fasting or starvation when sufficient carbohydrate is not available from the diet or glycogen reserves.
- It maintains the level of intermediates of the TCA cycle even when fatty acids are the main source of acetyl coA in the tissues.
- It clears the lactate produced by muscle and erythrocytes and glycerol produced by adipose tissue.

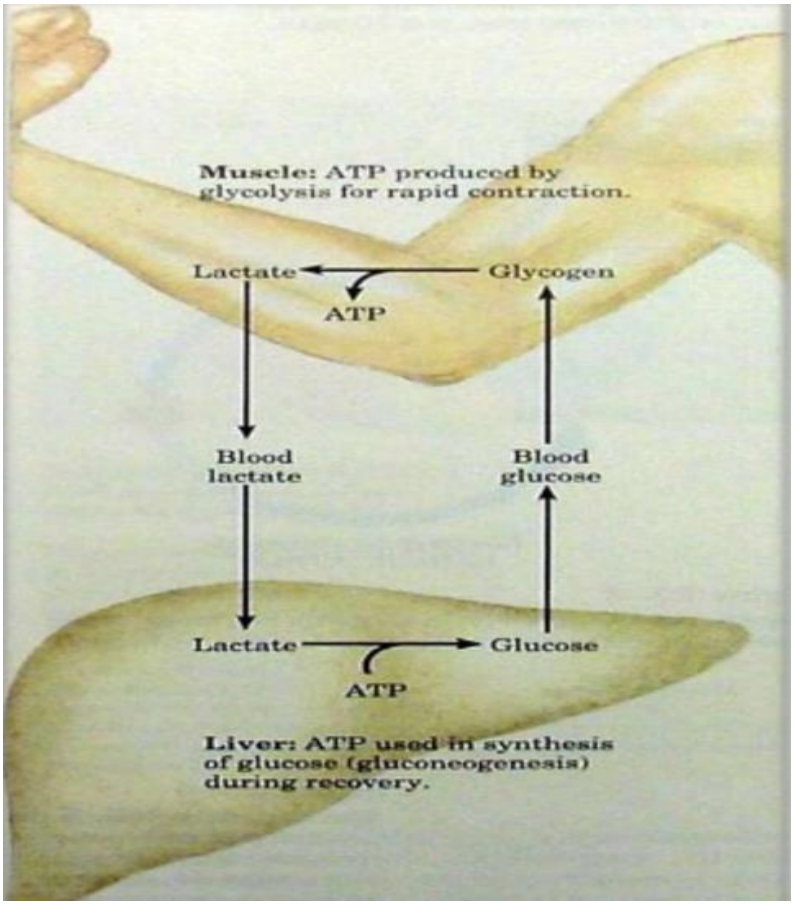
Cori cycle (the Lactic acid cycle)

• The pathway through which lactate produced by anaerobic glycolysis in skeletal muscle returns to the liver and is converted to glucose
• This moves back to muscle and is converted to glycogen is called the Cori cycle

Site: Liver

Substrate: Lactate

• **"Occurs due to absence of glucose-6- phosphate in liver".**



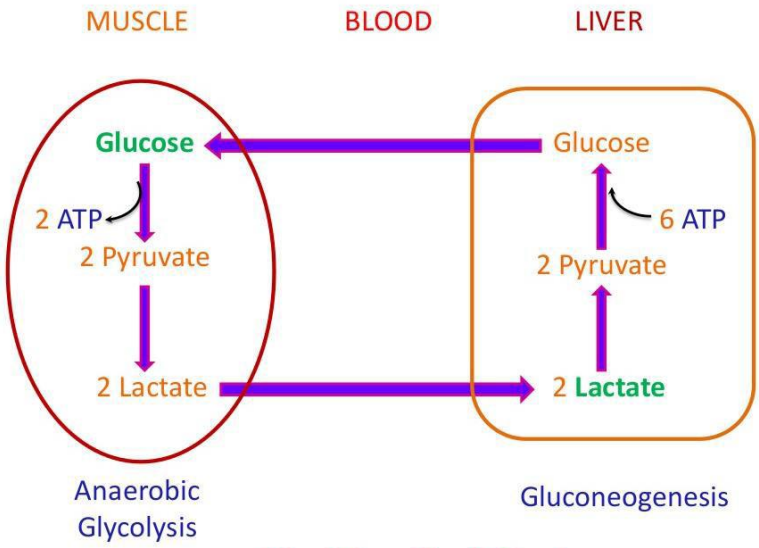


Fig: The Cori Cycle

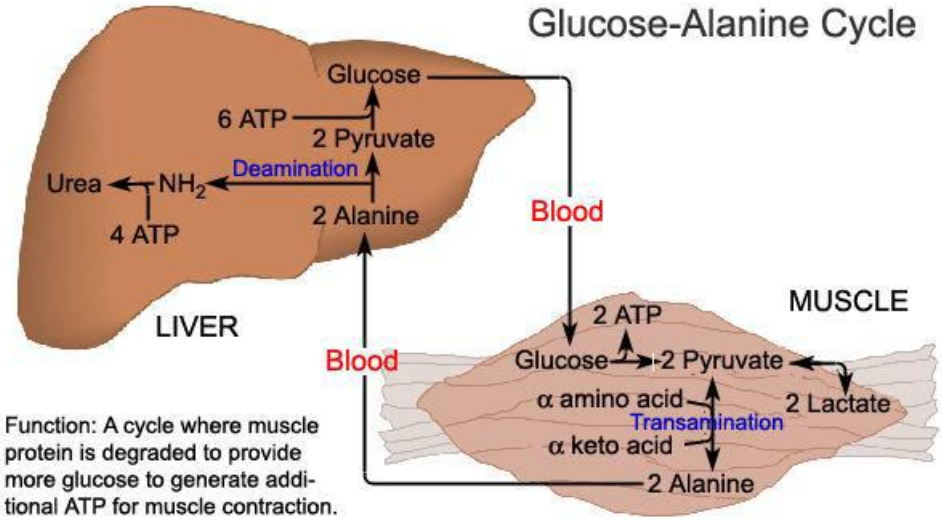
Significance of Cori cycle

1. Prevents lactic acidosis in the muscle under anaerobic conditions
2. The cycle is also important in producing ATP, an energy source, during muscle activity
3. The Cori cycle is a much more important source of substrate for gluconeogenesis than food.
4. The drug **METFORMIN** can cause lactic acidosis in patients because metformin inhibits the hepatic gluconeogenesis of the Cori cycle.

Glucose-Alanine cycle

• When in extrahepatic tissues amino acids are used for energy, pyruvate, derived from GLYCOLYSIS, is used as amino group acceptor, forming **alanine** • Alanine diffuses into the bloodstream and reaches the liver.
• In the liver, the amino group of alanine is transferred to **α- ketoglutarate** to form pyruvate
• The amino group enters the urea cycle, and in part acts as a nitrogen donor in many biosynthetic pathways.

• Pyruvate enters the GLUCONEOGENESIS and is used for glucose synthesis
• The newly formed glucose diffuses into the bloodstream and reaches the peripheral tissues where, due to GLYCOLYSIS, is converted into pyruvate that can accept amino groups from the free amino acids, thus closing the cycle.



Significance of Glucose-Alanine cycle

1. The Alanine cycle is less productive than the Cori cycle, which uses lactate, since a byproduct of energy production from alanine is production of Urea.
2. Removal of the urea is energy-dependent, requiring four "high-energy" phosphate bonds.
3. This pathway requires the presence of Alanine aminotransferase, restricted to muscle, liver and intestine.
4. Therefore, this pathway is used instead of the Cori cycle only when an aminotransferase is present, or a need to transfer ammonia to the liver.
5. The alanine cycle also Recycles carbon skeletons between muscle and liver
6. Transports ammonium to the liver and is converted into urea.

Gluconeogenesis and glycolysis

Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps.

7 of the 10 enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions

• three reactions of glycolysis are essentially irreversible in vivo and cannot be used in gluconeogenesis:
1. the conversion of glucose to glucose 6-phosphate by hexokinase,
2. the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate by phosphofructokinase-1
3. the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase

Glycolysis VS Gluconeogenesis

The reactions of glycolysis are on the left side, in red; the opposing pathway of gluconeogenesis is on the right, in blue.

• Both are energy generating pathways-**NOT identical**
• At one time only one of 2 can take place within a cell
• Glycolysis takes place essentially in the cytoplasm
• Gluconeogenesis occurs mutually in the cytosol and mitochondria
• Requirement of gluconeogenesis-**Glycogen**
• **Gluconeogenesis** from pyruvate share 7 reversible steps of glycolysis
• **Seven out of the 10 enzymes** used in the glycolytic pathway **are** used.
• The 3 irreversible steps of glycolysis that proceed with a large negative free energy change are bypassed during **gluconeogenesis** by using different enzymes.

• These three bypass reactions (during glycolysis) are catalyzed by:

1. Pyruvate kinase
2. Phosphofructokinase-1 (PFK-1)
3. Hexokinase/glucokinase

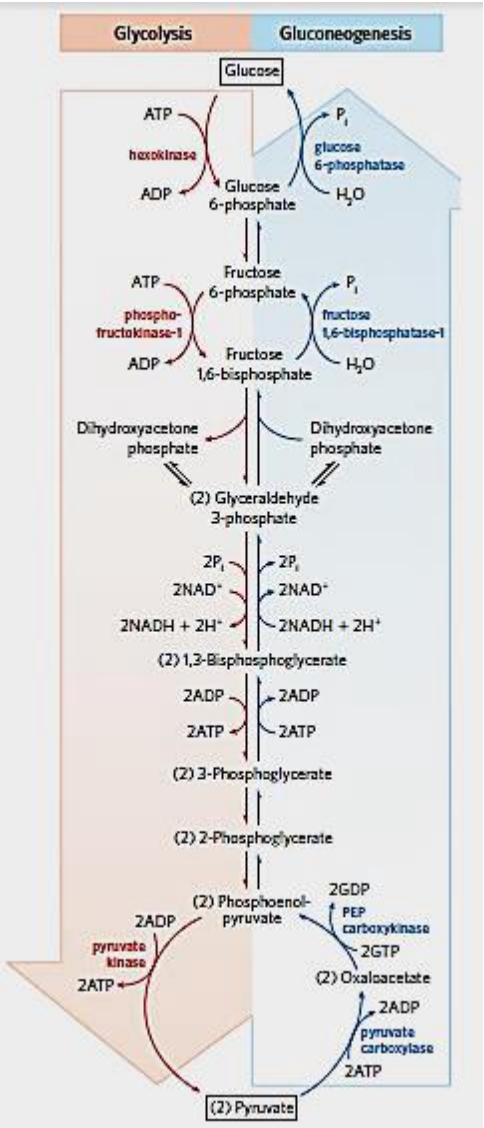


TABLE 14–2 Free-Energy Changes of Glycolytic Reactions in Erythrocytes

Glycolytic reaction step	$\Delta G'°$ (kJ/mol)	ΔG (kJ/mol)
1 Glucose + ATP ⟶ glucose 6-phosphate + ADP	−16.7	−33.4
2 Glucose 6-phosphate ⇌ fructose 6-phosphate	1.7	0 to 25
3 Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP	−14.2	−22.2
4 Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	23.8	−6 to 0
5 Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	7.5	0 to 4
6 Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	6.3	−2 to 2
7 1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	−18.8	0 to 2
8 3-Phosphoglycerate ⇌ 2-phosphoglycerate	4.4	0 to 0.8
9 2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	7.5	0 to 3.3
10 Phosphoenolpyruvate + ADP ⟶ pyruvate + ATP	−31.4	−16.7

Note: $\Delta G'°$ is the standard free-energy change, as defined in Chapter 13 (pp. 507–508). ΔG is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes, at pH 7. The glycolytic reactions bypassed in gluconeogenesis are shown in red. Biochemical equations are not necessarily balanced for H or charge (p. 517).

Table 14-2
Lehninger Principles of Biochemistry, Sixth Edition

1. Conversion of Pyruvate to Phosphoenolpyruvate

• The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate (PEP).
• This reaction cannot occur by simple reversal of the pyruvate kinase reaction
• Instead, the phosphorylation of pyruvate is achieved by a sequence of reactions that in eukaryotes requires 2 enzymes in both the **cytosol and mitochondria**.
• Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, in which the **alpha -amino group is transferred from alanine (leaving pyruvate) to an alpha-keto carboxylic acid**
• In mitochondria, pyruvate is converted to oxaloacetate in a biotin (coenzyme)-requiring reaction catalyzed by **pyruvate carboxylase**, a mitochondrial enzyme.

$$\text{Pyruvate} + HCO_3^- + ATP \longrightarrow \text{oxaloacetate} + ADP + P_i$$

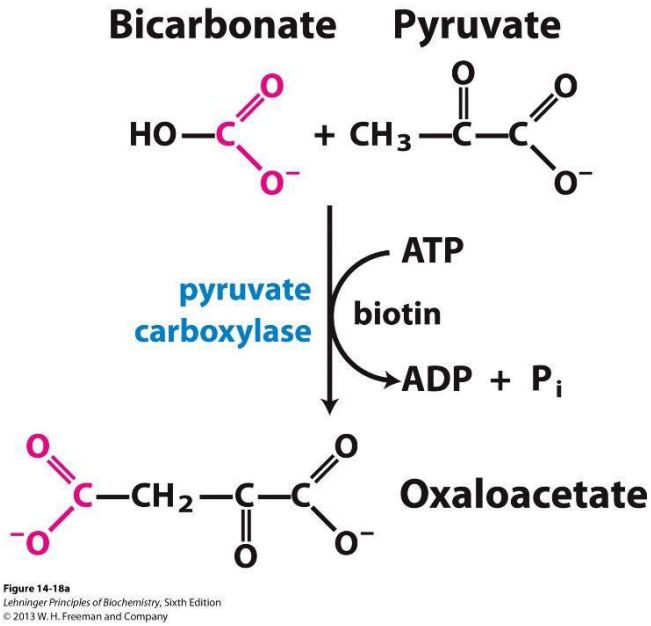


Figure 14-18a
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

• **Pyruvate carboxylase** is the first regulatory enzyme in the gluconeogenic pathway
• **Pyruvate carboxylase requires acetyl-CoA as a positive effector. (Acetyl-CoA is produced by fatty acid oxidation)**
• the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial **malate dehydrogenase**, at the expense of NADH.

Alternative paths from pyruvate to phosphoenolpyruvate

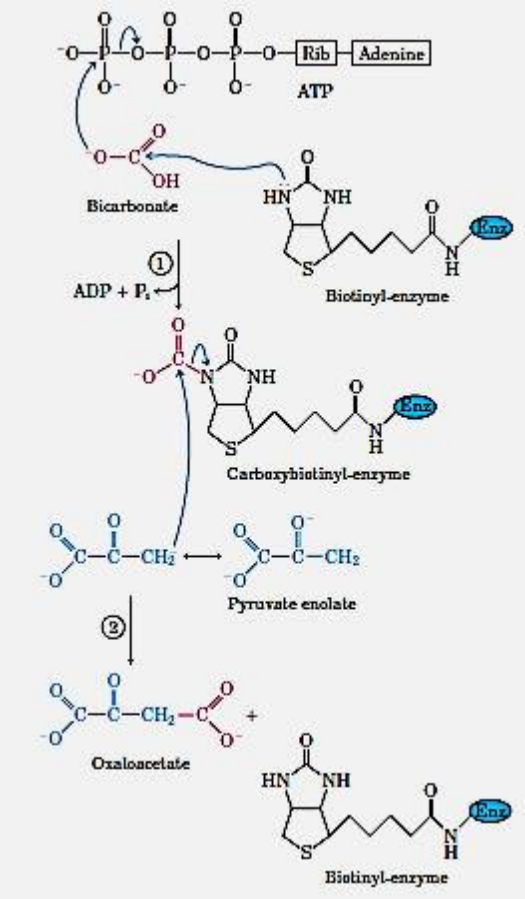
• Oxalo acetic acid (OAA) produced in mitochondria is impermeable through mitochondrial membrane.

$$\text{Oxaloacetate} + NADH + H^+ \longleftrightarrow \text{L-malate} + NAD^+$$

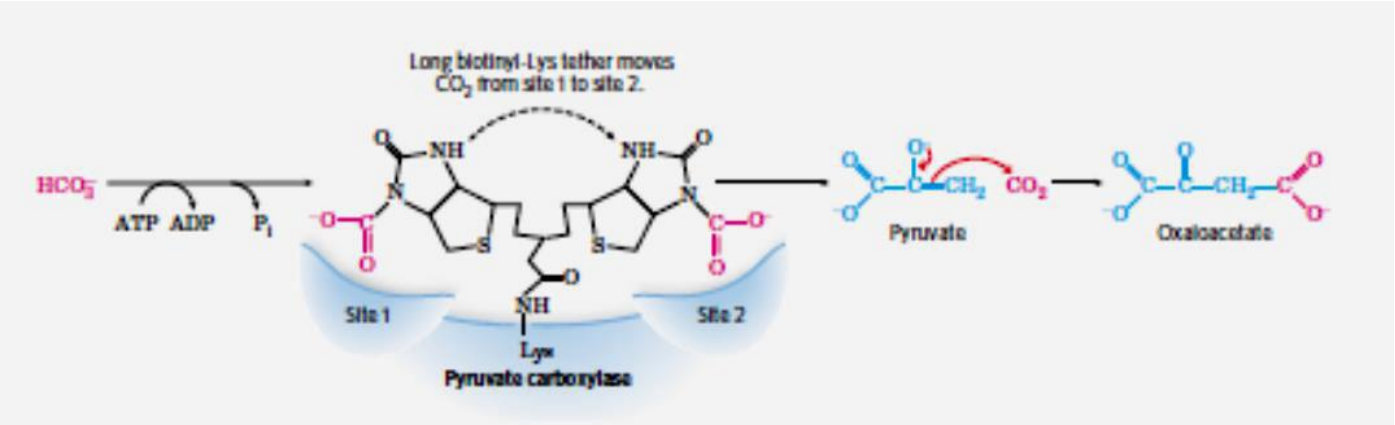
• Therefore, Oxalo acetic acid is converted into malate, exported from the mitochondrion into the cytoplasm, **converted back to oxaloacetate** inorder to allow gluconeogenesis
• In both mitochondria and cytoplasm, the transporter of malate is malate dehydrogenase

(a) In the cytosol, oxaloacetate is converted to phosphoenolpyruvate by **PEP carboxykinase**.
(b) The CO2 incorporated in the pyruvate carboxylase reaction is lost here as CO2. The decarboxylation leads to a rearrangement of electrons that facilitates attack of the carbonyl oxygen of the pyruvate moiety on the phosphate of GTP

• (b) In the cytosol, oxaloacetate is converted to phosphoenolpyruvate by PEP carboxykinase.
• The CO2 incorporated in the pyruvate carboxylase reaction is lost here as CO2.
• **The decarboxylation leads to a rearrangement of electrons that facilitates attack of the carbonyl oxygen of the pyruvate moiety on the phosphate of GTP**.



Role of biotin in the pyruvate carboxylase reaction.
• **The cofactor biotin is covalently attached to the enzyme through an amide linkage to the amino group of a Lys residue, forming a biotinylenzyme.**
• **The reaction occurs in two phases, which occur at two different sites in the enzyme.**
• **At catalytic site 1, bicarbonate ion is converted to CO2 at the expense of ATP. Then CO2 reacts with biotin, forming carboxybiotinyl- enzyme.**
• **The long arm composed of biotin and the Lys side chain to which it is attached then carry the CO2 of carboxybiotinyl enzyme to catalytic site 2 on the enzyme surface, where CO2 is released and reacts with the pyruvate, forming oxaloacetate and regenerating the biotinyl-enzyme.**



• There is a logic to the route of these reactions through the mitochondrion.
• The [NADH]/[NAD+] ratio in the cytosol is 8 X 10-4, about 10 times lower than in mitochondria.
• Because cytosolic NADH is consumed in gluconeogenesis (in the conversion of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate
• Glucose biosynthesis cannot proceed unless NADH is available.

• The transport of malate from the mitochondrion to the cytosol and its reconversion there to oxaloacetate effectively moves NAD to the cytosol, and converts it into NADH.
• This path from pyruvate to PEP therefore provides an important balance between NADH produced and consumed in the cytosol during gluconeogenesis.

Alternative paths from pyruvate to phosphoenolpyruvate
• The relative importance of the two pathways depends on the availability of lactate or pyruvate and the cytosolic requirements for NADH for gluconeogenesis.
• The path on the right predominates when lactate is the precursor, because cytosolic NADH is generated in the lactate dehydrogenase reaction and does not have to be shuttled out of the mitochondrion
• The requirements of ATP for pyruvate carboxylase and GTP for PEP carboxykinase are omitted for simplicity.

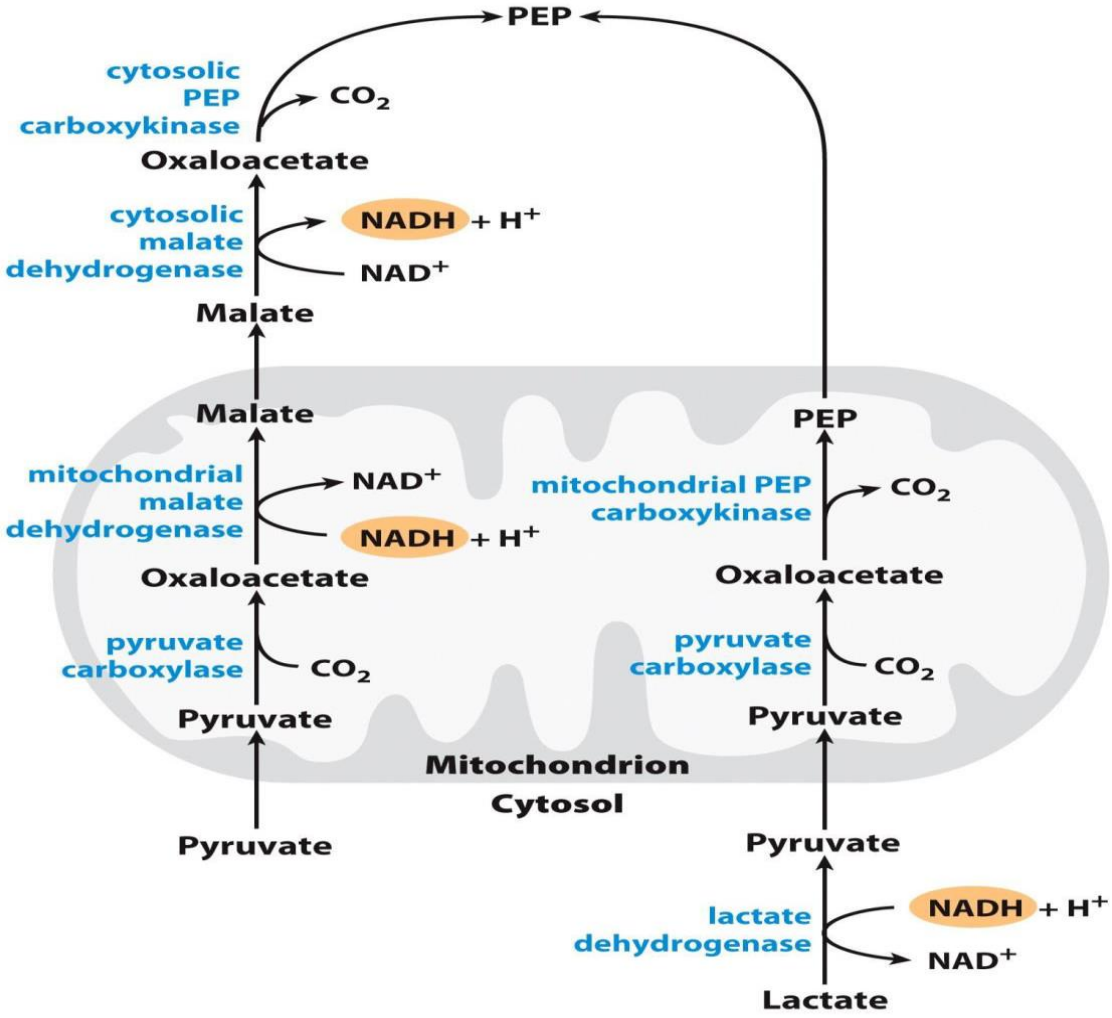


Figure 14-20
Lehninger Principles of Biochemistry, Sixth Edition

Bypass reactions in Gluconeogenesis-2

Conversion of Fructose 1,6-Bisphosphate to Fructose 6- Phosphate Is the Second Bypass

• The second glycolytic reaction that cannot participate in gluconeogenesis is the phosphorylation of fructose 6-phosphate by PFK-1
• Because this reaction is highly exergonic and therefore irreversible, the generation of fructose 6-phosphate from fructose 1,6- bisphosphate is catalyzed by a different enzyme, **fructose 1,6- bisphosphatase (FBPase-1)**
• **fructose 1,6-bisphosphatase (FBPase-1) is** Mg+2-dependent
• **FBPase-1** promotes the essentially irreversible *hydrolysis* of the C-1 phosphate (*not* phosphoryl group transfer to ADP

fructose 1,6-bisphosphate + H20 ⟶ fructose 6-phosphate + Pi

Conversion of Glucose 6-Phosphate to Glucose Is the Third Bypass

• The third bypass is the final reaction of gluconeogenesis, the dephosphorylation of glucose 6-phosphate to yield glucose.
• Reversal of the hexokinase reaction would require phosphoryl group transfer from glucose 6-phosphate to ADP, forming ATP… An energetically unfavorable reaction
• The reaction catalyzed by **glucose 6-phosphatase** does not require synthesis of ATP; it is a simple hydrolysis of a phosphate ester

Glucose 6-phosphatase + H20 ⟶ Glucose + Pi

• This Mg+2-activated enzyme (**glucose 6-phosphatase)** is found on the luminal side of the endoplasmic reticulum of hepatocytes, renal cells, and epithelial cells of the small intestine but not in other tissues, therefore other organs are unable to supply glucose to the blood.
• If other tissues had glucose 6-phosphatase, this enzyme's activity would hydrolyze **the glucose 6-phosphate needed within those tissues for glycolysis**.
• Glucose produced by gluconeogenesis in the liver or kidney or ingested in the diet is delivered to these other tissues, including brain and muscle, through the bloodstream.

Gluconeogenesis Is Energetically Expensive, but Essential

• The sum of the biosynthetic reactions leading from pyruvate to free blood glucose is as follows

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 4\text{H}_2\text{O} \longrightarrow \text{glucose} + 4\text{ADP} + 2\text{GDP} + 6\text{P}_i + 2\text{NAD}^+ \quad (14\text{–}9)$$

• For each molecule of glucose formed from pyruvate, six high-energy phosphate groups are required, four from ATP and two from GTP.
• In addition, two molecules of NADH are required for the reduction of two molecules of 1,3-bisphosphoglycerate.
• Clearly, Glycolysis is not simply the reverse of the equation for conversion of glucose to pyruvate by glycolysis, which would require only two molecules of ATP

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i + \text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

• The synthesis of glucose from pyruvate is a relatively expensive process.
• this high energy cost is necessary to ensure the irreversibility of gluconeogenesis
• Under intracellular conditions, the overall free-energy change of glycolysis is at least -63 kJ/mol.
• Under the same conditions the overall Delta*G* of gluconeogenesis is -16 kJ/mol.
• Thus both glycolysis and gluconeogenesis are essentially irreversible processes in cells.
• A second advantage to investing energy to convert pyruvate to glucose is **that if pyruvate were instead excreted, its considerable potential for ATP production by complete, aerobic oxidation would be lost (more than 10 ATP are produced per pyruvate)**

TABLE 14–3 Sequential Reactions in Gluconeogenesis Starting from Pyruvate

Pyruvate + HCO_3^- + ATP ⟶ oxaloacetate + ADP + P_i	×2
Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO_2 + GDP	×2
Phosphoenolpyruvate + H_2O ⇌ 2-phosphoglycerate	×2
2-Phosphoglycerate ⇌ 3-phosphoglycerate	×2
3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP	×2
1,3-Bisphosphoglycerate + NADH + H^+ ⇌ glyceraldehyde 3-phosphate + NAD^+ + P_i	×2
Glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate	
Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ fructose 1,6-bisphosphate	
Fructose 1,6-bisphosphate ⟶ fructose 6-phosphate + P_i	
Fructose 6-phosphate ⇌ glucose 6-phosphate	
Glucose 6-phosphate + H_2O ⟶ glucose + P_i	
Sum: 2 Pyruvate + 4ATP + 2GTP + 2NADH + $2H^+$ + $4H_2O$ ⟶ glucose + 4ADP + 2GDP + $6P_i$ + $2NAD^+$	

The bypass reactions are in red; all other reactions are reversible steps of glycolysis. The figures at the right indicate that the reaction is to be counted twice, because two three-carbon precursors are required to make a molecule of glucose. The reactions required to replace the cytosolic NADH consumed in the glyceraldehyde 3-phosphate dehydrogenase reaction (the conversion of lactate to pyruvate in the cytosol or the transport of reducing equivalents from mitochondria to the cytosol in the form of malate) are not considered in this summary. Biochemical equations are not necessarily balanced for H and charge.

Gluconeogenesis and Glycolysis are Reciprocally regulated

Reciprocal Regulation of Gluconeogenesis and Glycolysis in the Liver

• Gluconeogenesis and glycolysis are coordinated so that within a cell one pathway is relatively inactive while the other is highly active.
• The rate of glycolysis is determined by the concentration of glucose
• The rate of gluconeogenesis by the concentrations of **lactate and other precursors of glucose**
• **Pyruvate kinase** is inhibited by phosphorylation during starvation.
• The level of fructose 2,6-bisphosphate is high in the fed state and low in starvation.
• The interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate is stringently controlled
• **AMP** stimulates phosphofructokinase, whereas **ATP and citrate** inhibit it.
• Fructose 1,6-bisphosphatase, is inhibited by AMP and activated by citrate.
• **A high level of AMP indicates that the energy charge is low and signals the need for ATP generation.**
• Conversely, high levels of ATP and citrate indicate that the energy charge is high and that biosynthetic intermediates are abundant.
• Under these conditions, glycolysis is nearly switched off and gluconeogenesis is promoted.
• Phosphofructokinase and fructose 1,6-bisphosphatase are also reciprocally controlled by *fructose 2,6-bisphosphate in the liver*
• **The level of F-2,6-BP is low during starvation** and high in the fed state, because of the antagonistic effects of glucagon and insulin on the production and degradation of this signal molecule.
• *Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase.*
• Hence, glycolysis is accelerated and gluconeogenesis is diminished in the fed state.
• **During starvation**, gluconeogenesis predominates because the level of F- 2,6-BP is very low. Glucose formed by the liver under these conditions is essential for the viability of brain and muscle.
• The interconversion of phosphoenolpyruvate and pyruvate also is precisely regulated.
• High levels of ATP and alanine, which signal that the energy charge is high and that building blocks are abundant, inhibit the enzyme in liver.
• Conversely, pyruvate carboxylase, which catalyzes the first step in gluconeogenesis from pyruvate, is activated by **Acetyl CoA** and inhibited by **ADP**.
• Likewise, ADP inhibits phosphoenolpyruvate carboxykinase.
• Hence, gluconeogenesis is favored when the cell is rich in biosynthetic precursors and ATP.

Regulatory effect of hormones

• The regulators in this case are hormones.
• Hormones affect gene expression primarily by changing the rate of transcription, as well as by regulating the **degradation of mRNA**
• Insulin, which rises subsequent to eating, stimulates the expression of phosphofructokinase, pyruvate kinase, and the bifunctional enzyme that makes and degrades F-2,6-BP.
• Glucagon, which rises during starvation, inhibits the expression of these enzymes and stimulates instead the production of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase.

Pentose Phosphate Pathway (PPP) of Glucose Oxidation

• The *oxidative* pentose phosphate pathway
• **Phosphogluconate pathway**
• **Hexose monophosphate pathway**

• The major catabolic fate of **glucose 6-phosphate** is glycolytic breakdown to **pyruvate,** much of which is then oxidized via the **citric acid cycle**, leading to ATP formation .
• Glucose 6-phosphate does have other catabolic fates, which lead to specialized products needed by the cell.
• E.g. the **oxidation of glucose 6-phosphate to pentose phosphates** by the **pentose phosphate pathway.**
• Since it results in the synthesis of pentoses and NADPH, it is considered as an **anaerobic and anabolic pathway.**
• Occurs in cytosol

Outputs of PPP

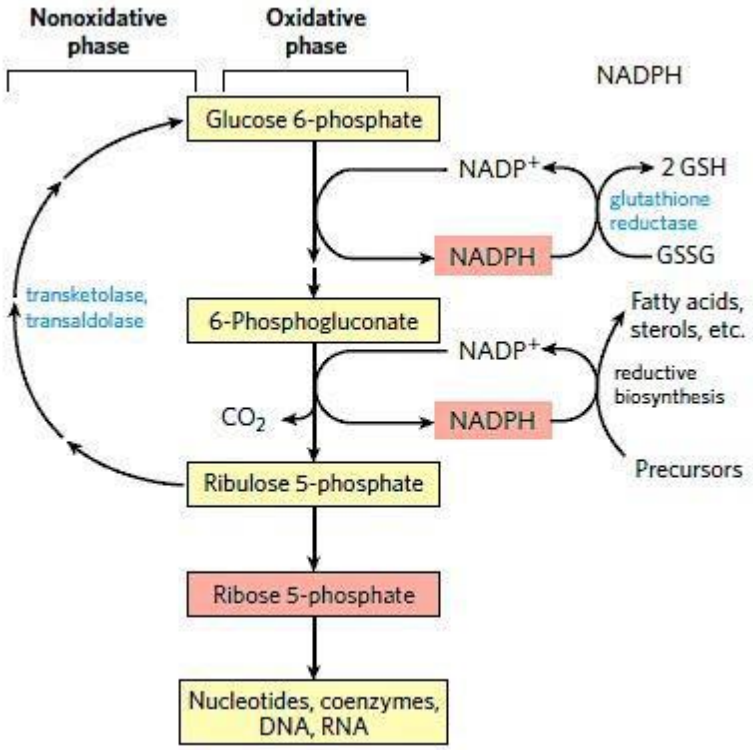
• **Reducing NADP+ to NADPH and producing pentose phosphates**. .
• brings about **oxidation and decarboxylation** of glucose 6-phosphate (G6P) at C-1, to form pentoses.. (bone marrow, skin, intestinal mucosa) to make RNA, DNA, ATP, NADH, FADH2 and coenzyme A.
• NADPH: to counter damaging effects of oxygen radicals (liver, adipose, erythrocytes, cornea)
• Erythrose 4 phosphate (E4P) used in the synthesis of aromatic amino acids

Two phases of PPP

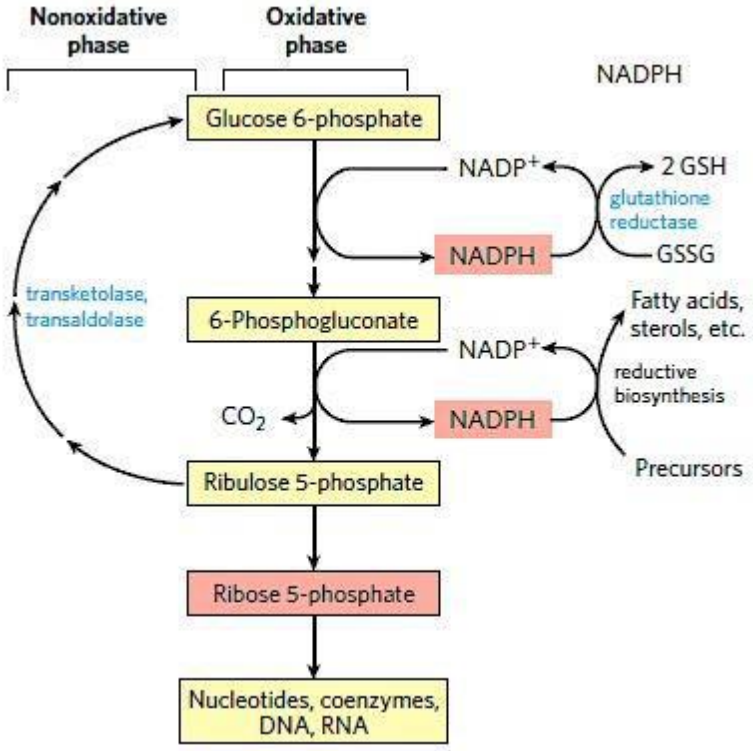
• There are **two phases** of the pentose phosphate pathway:
1. The oxidative phase
2. The non-oxidative phase

1. The Oxidative Phase

1. The Oxidative Phase Produces Pentose Phosphates and NADPH
2. The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase (G6PD)** to form 6- phosphoglucono-delta-lactone, an intramolecular ester.
3. NADP+ is the electron acceptor, and overall equilibrium is in the direction of NADPH formation.
4. The **lactone** is hydrolyzed to the free acid 6- phosphogluconate by a specific enzyme **lactonase**, then 6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase**



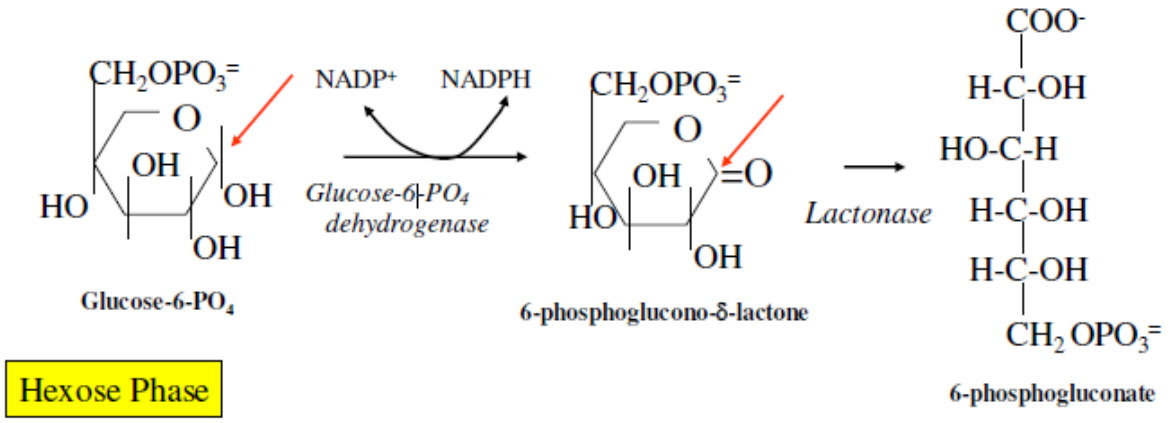
Pentose Phosphate Pathway NADPH formed in the oxidative phase is used to reduce glutathione to GSSG (Glutathione disulfide) and to support reductive biosynthesis. The other product of the oxidative phase is **ribose 5-phosphate**, which serves as a precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, the **non-oxidative phase recycles six molecules of the pentose into five molecules of the hexose glucose 6- phosphate,** allowing continued production of NADPH and converting glucose 6- phosphate to CO2.



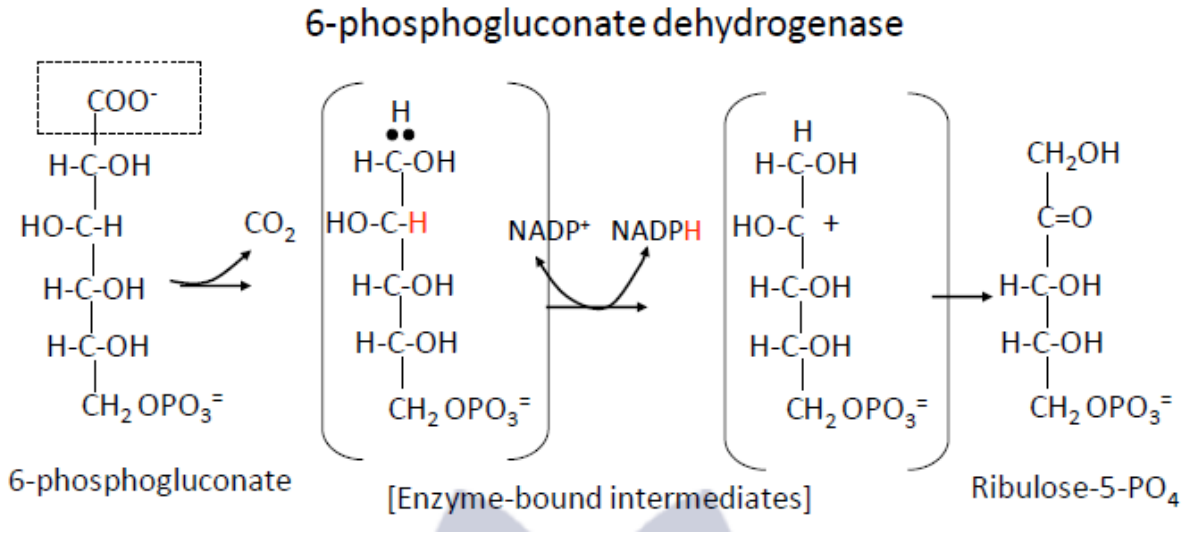
• The reaction generates a second molecule of NADPH.
• **Phosphopentose isomerase** converts ribulose 5- phosphate to its aldose isomer, ribose 5-phosphate.
• In some tissues, the pentose phosphate pathway ends at this point, and its overall equation for reactions is

$$G6\text{-}P + 2NADP^+ + H_2O \longrightarrow \text{ribulose 5-phosphate} + CO2 + 2NADPH + 2H^+$$

• The net result is the production of NADPH, a reducing agent for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.



Hexose Phase



Summary of The Oxidative Phase

Reactants	Products	Enzyme	Description
Glucose 6-phosphate + NADP+	→ 6-phosphoglucono-δ-lactone +NADPH	glucose 6-phosphate dehydrogenase	Dehydrogenation. The hydroxyl on carbon 1 of glucose 6-phosphate turns into a carbonyl, generating a lactone, and, in the process, NADPH is generated.
6-phosphoglucono-δ-lactone + H_2O	→ 6-phosphogluconate+ H^+	6-phosphogluconolactonase	Hydrolysis
6-phosphogluconate+ NADP+	→ ribulose 5-phosphate +NADPH + CO_2	6-phosphogluconate dehydrogenase	Oxidative decarboxylation. NADP+ is the electron acceptor, generating another molecule of NADPH, a CO_2, and ribulose 5-phosphate.

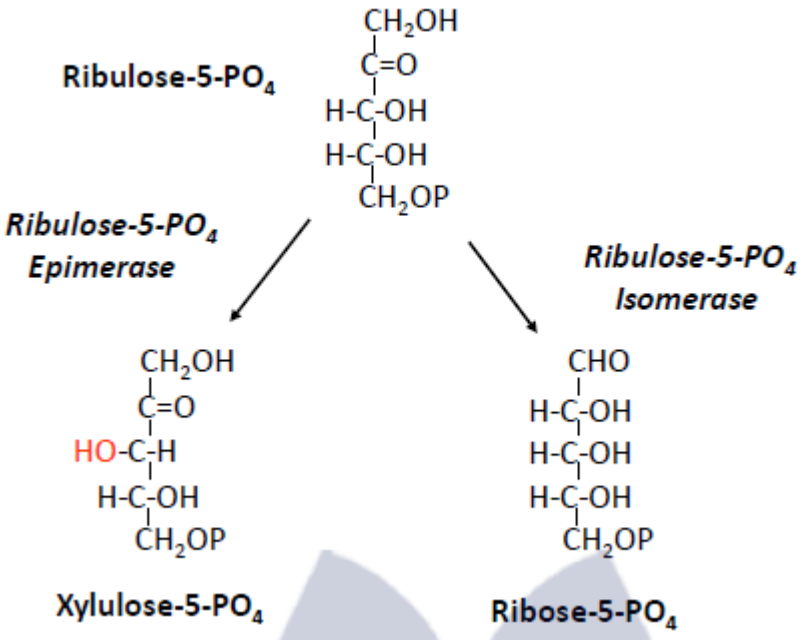
2. The Non-oxidative Phase

• The Non-oxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate

• In tissues that require **primarily NADPH**, the **pentose phosphates** produced in the oxidative phase of the pathway are **recycled into glucose 6-phosphate.**

• A series of rearrangements of the carbon skeletons takes place during which 6 **five-carbon sugar phosphates** are converted **to 5 six-carbon sugar phosphates**, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH

• In nonoxidative phase, ribulose 5-phosphate is first **epimerized** to xylulose 5- Phosphate



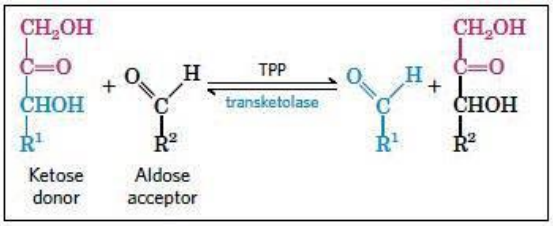
• Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO2.

• **Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars:** transketolase and transaldolase.

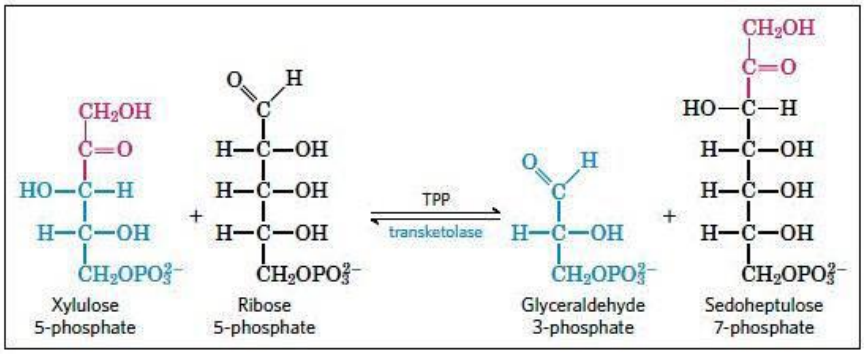
a. Transketolase

• Transketolase catalyzes the transfer of a two carbon fragment from **a ketose donor to an aldose acceptor** In its first appearance in the pentose phosphate pathway, transketolase transfers C-1 and C-2 of xylulose 5-phosphate to ribose 5- phosphate, forming the seven-carbon product sedoheptulose 7-phosphate.

• The remaining three carbon fragment from xylulose forms glyceraldehyde 3- phosphate.



(a)



The first reaction catalyzed by transketolase.
(a) The general reaction catalyzed by transketolase is the transfer of a two carbon group, carried temporarily on enzyme-bound TPP, (cofactor thymine pyrophosphate) from a ketose donor to an aldose acceptor.
(b) Conversion of two pentose phosphates to a triose phosphate and a seven-carbon sugar phosphate, sedoheptulose 7-phosphate.

b. Transaldolase

• Transaldolase catalyzes a reaction similar to the aldolase reaction of glycolysis, that is, a **three-carbon fragment is removed from sedoheptulose 7-phosphate** and condensed with glyceraldehyde 3-phosphate, forming fructose 6-phosphate and the tetrose erythrose 4-phosphate**.**
• Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3- phosphate from erythrose 4-phosphate and xylulose 5-phosphate. Two molecules of glyceraldehyde 3-phosphate formed by two repetition of the process can be converted to a molecule of fructose 1,6-bisphosphate as in gluconeogenesis
• finally FBPase-1 and phosphohexose isomerase convert fructose 1,6-bisphosphate to glucose 6-phosphate.
• Overall, six pentose phosphates have been converted to five hexose phosphates—the cycle is now complete.
• Transketolase requires the cofactor thiamine pyrophosphate (TPP), which stabilizes a two-carbon carbanion in this reaction.

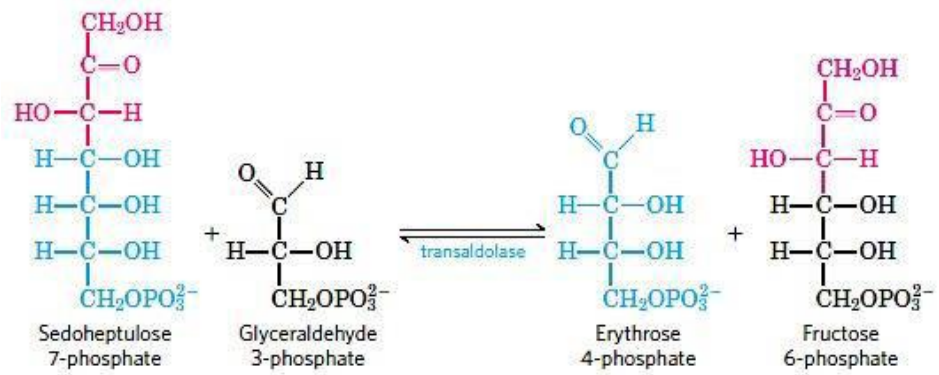


FIGURE 14–25 The reaction catalyzed by transaldolase.

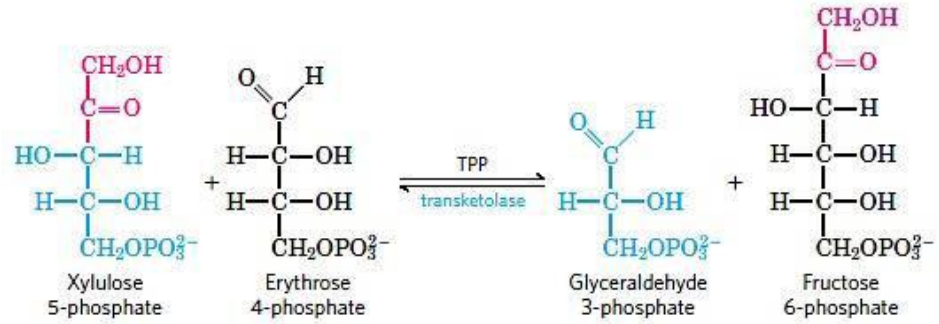
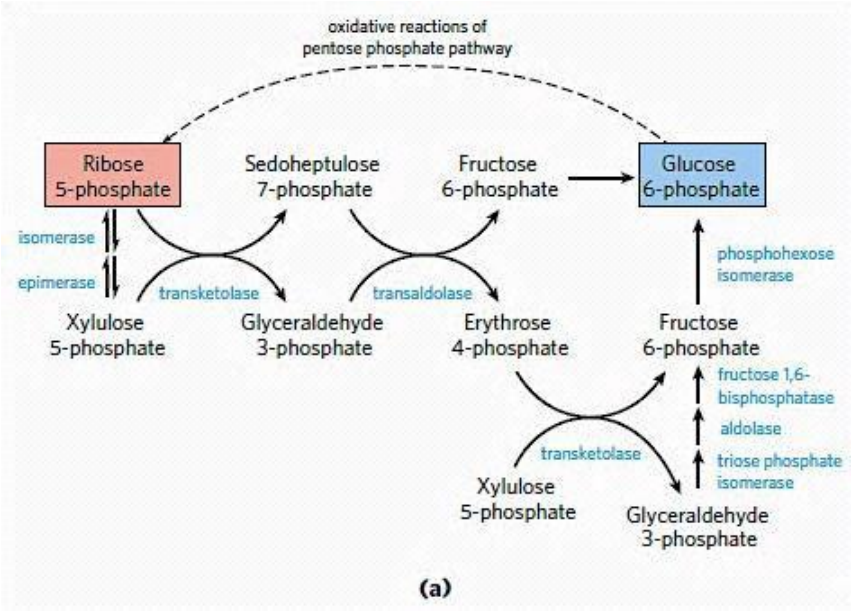
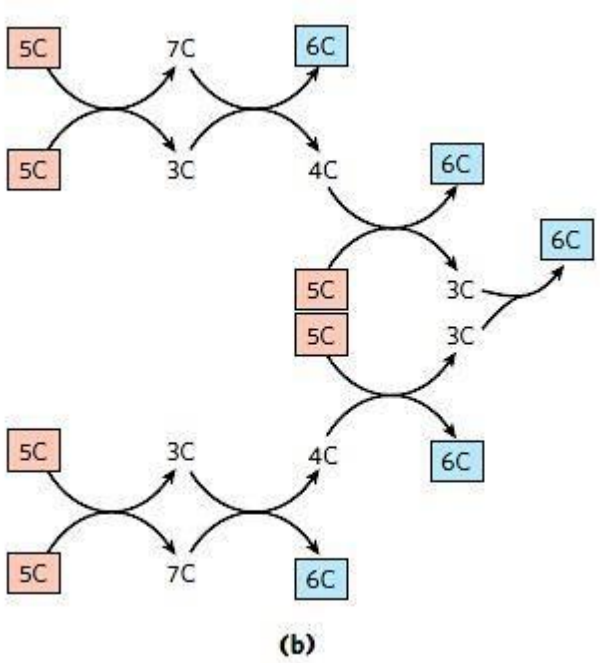


FIGURE 14–26 The second reaction catalyzed by transketolase.





(b)

Nonoxidative reactions of the pentose phosphate pathway.
(a) These reactions convert pentose phosphates to hexose phosphates, allowing the oxidative reactions. Transketolase and transaldolase are specific to this pathway; the other enzymes also serve in the glycolytic or gluconeogenic pathways.
(b) A schematic diagram showing the pathway from six pentoses (5C) to five hexoses (6C). Note that this involves two sets of the interconversions shown in (a). Every reaction shown here is reversible; unidirectional arrows are used only to make clear the direction of the reactions during continuous oxidation of glucose 6-phosphate. In the light-independent reactions of photosynthesis, the direction of these reactions is reversed

Summary of The Non-Oxidative Phas

Reactants	Products	Enzymes
ribulose 5-phosphate	→ ribose 5-phosphate	Ribulose 5-Phosphate Isomerase
ribulose 5-phosphate	→ xylulose 5-phosphate	Ribulose 5-Phosphate 3-Epimerase
xylulose 5-phosphate + ribose 5-phosphate	→ glyceraldehyde 3-phosphate +sedoheptulose 7-phosphate	transketolase
sedoheptulose 7-phosphate +glyceraldehyde 3-phosphate	→ erythrose 4-phosphate + fructose 6-phosphate	transaldolase
xylulose 5-phosphate + erythrose 4-phosphate	→ glyceraldehyde 3-phosphate + fructose 6-phosphate	transketolase

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway

• Whether glucose 6-phosphate enters glycolysis or the pentose phosphate pathway depends on the current needs of the cell and on the concentration of NADP + in the cytosol. Without this electron acceptor, the first reaction of the pentose phosphate pathway (catalyzed by G6PD) cannot proceed.
• When a cell is rapidly converting NADPH to NADP + in biosynthetic reductions, the level of NADP + rises, allosterically stimulating G6PD and increasing the flux of glucose 6-phosphate through the pentose phosphate pathway.
• When the demand for NADPH slows, the level of NADP + drops, the pentose phosphate pathway slows, and glucose 6-phosphate is instead used to fuel glycolysis.
• The ratio of NADPH to NADP + is 100:1 in liver cells (cytosol is a highly reducing environment)

Wernicke-Korsakoff Syndrome

• **Wernicke–Korsakoff** syndrome results from thiamine (Vitamin B12), deficiency. A component of TPP, by a Defect in Transketolase
• The syndrome is more common among **chronic** alcoholics.
• can be caused by a mutation in the gene for transketolase that results in an enzyme with a lowered affinity for TPP—an affinity one-tenth that of the normal enzyme.
• This defect makes individuals much more sensitive to a thiamine deficiency: even a moderate thiamine deficiency (tolerable in individuals with an unmutated transketolase) can drop the level of TPP below that needed to saturate the enzyme.
• The result is a slowing down of the whole pentose phosphate pathway.
• Symptoms: **memory loss, mental confusion, ataxia and partial paralysis.**

Feeder pathways of GLYCOLYSIS

Feeder pathways of glycolysis

• **Polysaccharides :** Glycogen and starch (Complex carbs)
• **Disaccharides:** Maltose, lactose, trehalose, sucrose
• **Monosaccharides:** Fructose, mannose, galactose

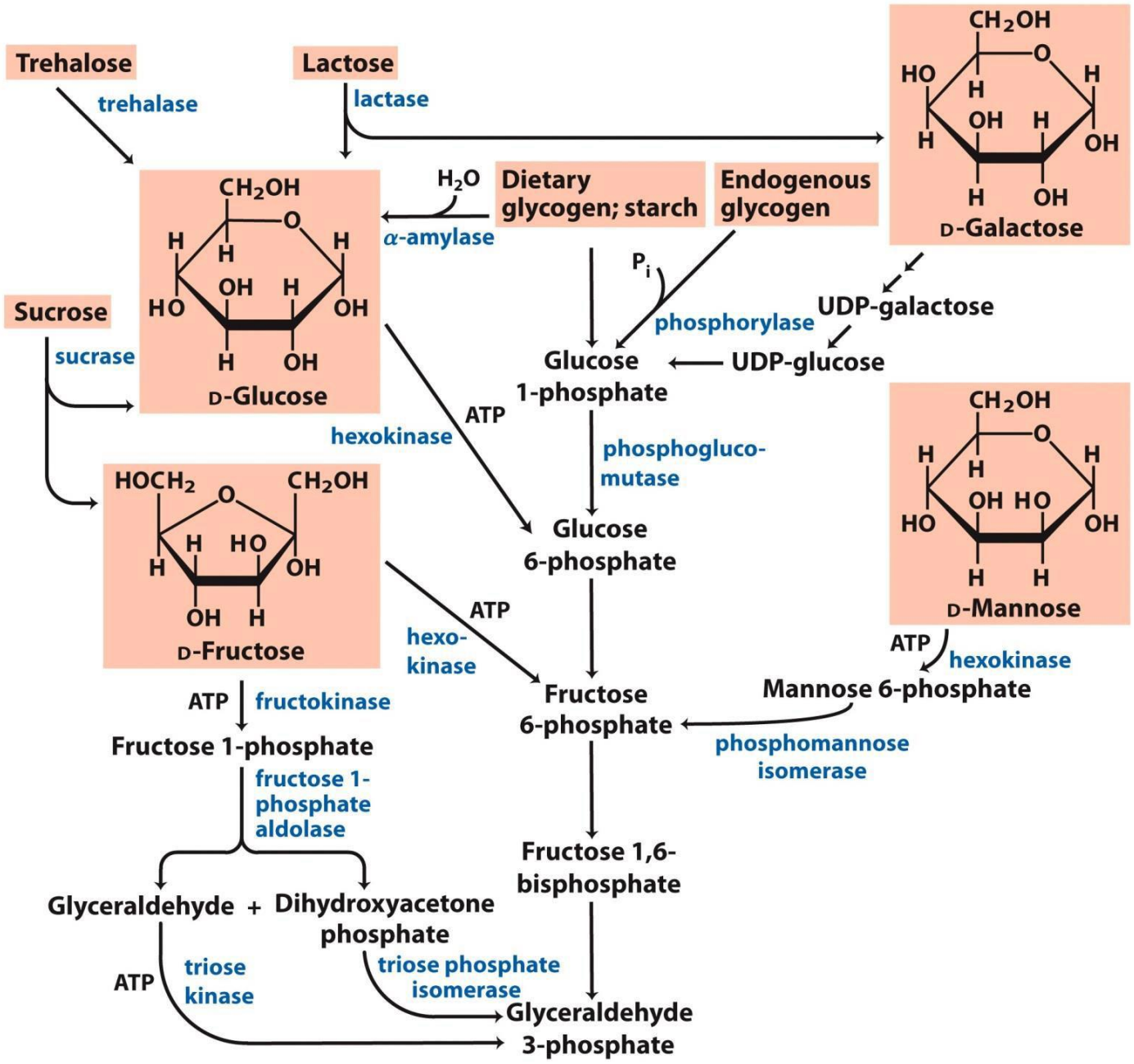
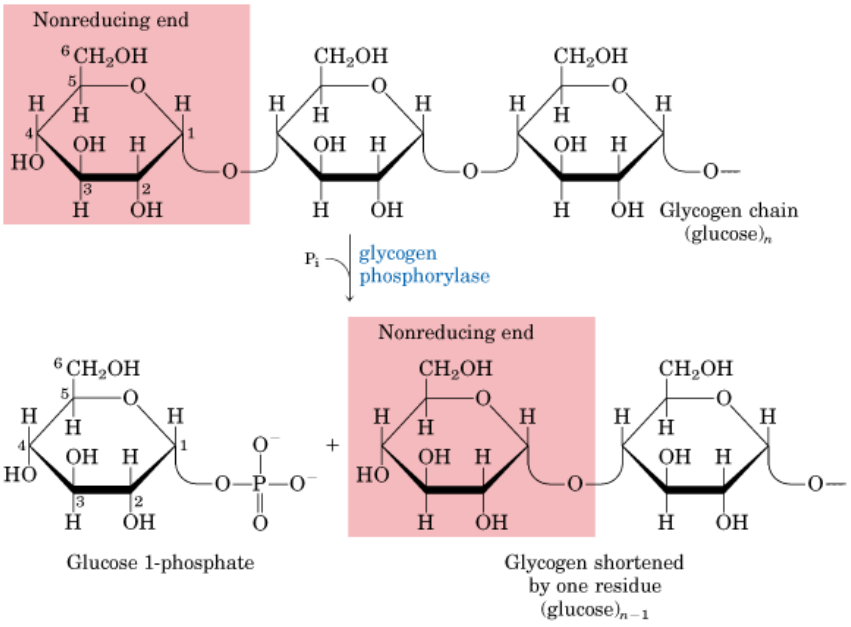


Figure 14-11
Lehninger Principles of Biochemistry, Sixth Edition

1. Catabolism of Polysachharides

• Alpha amylase in saliva and intestine breaks starch to oligosaccharides, maltose, dextrins, and finally glucose
• In ruminants, in the rumen, symbiotic microorganisms that produce cellulose break down cellulose into glucose molecules.
• These microorganisms use the resulting glucose in an anaerobic fermentation that produces propionate, used in gluconeogenesis to produce lactose of milk
• Glycogen phosphorylase (starch phosphorylase in plants) catalyzes an attack by Pi on the (a1→4) glycosidic linkage that joins the last two glucose
residues at a nonreducing end, generating glucose 1-phosphate and a polymer shortened by one residue



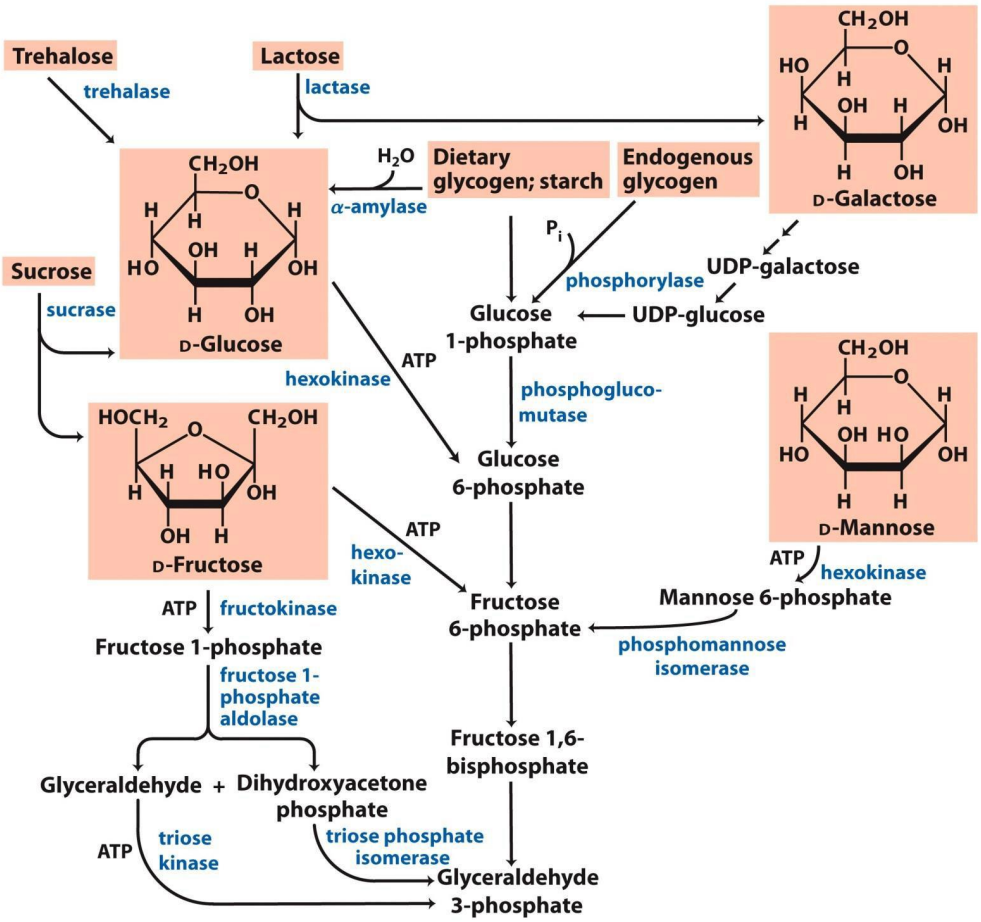


Figure 14-11
Lehninger Principles of Biochemistry, Sixth Edition

• Glucose 1-phosphate produced by glycogen phosphorylase is converted to glucose 6-phosphate by **phosphoglucomutase,** which catalyzes the reversible reaction

Glucose 1-phosphate ⇌ glucose 6-phosphate

1. Glycolysis
2. Pentose phosphate pathway

2. Metabolism of dietary polysaccharides and disaccharides

Dietary polysaccharides and disaccharides are hydrolyzed to monosaccharides by various enzymes

$$\text{Dextrin} + n\text{H}_2\text{O} \xrightarrow[\text{dextrinase}]{} n\ \text{D-glucose}$$

$$\text{Maltose} + \text{H}_2\text{O} \xrightarrow[\text{maltase}]{} 2\ \text{D-glucose}$$

$$\text{Lactose} + \text{H}_2\text{O} \xrightarrow[\text{lactase}]{} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + \text{H}_2\text{O} \xrightarrow[\text{sucrase}]{} \text{D-fructose} + \text{D-glucose}$$

$$\text{Trehalose} + \text{H}_2\text{O} \xrightarrow[\text{trehalase}]{} 2\ \text{D-glucose}$$

Feeder pathways of GLYCOLYSIS-2

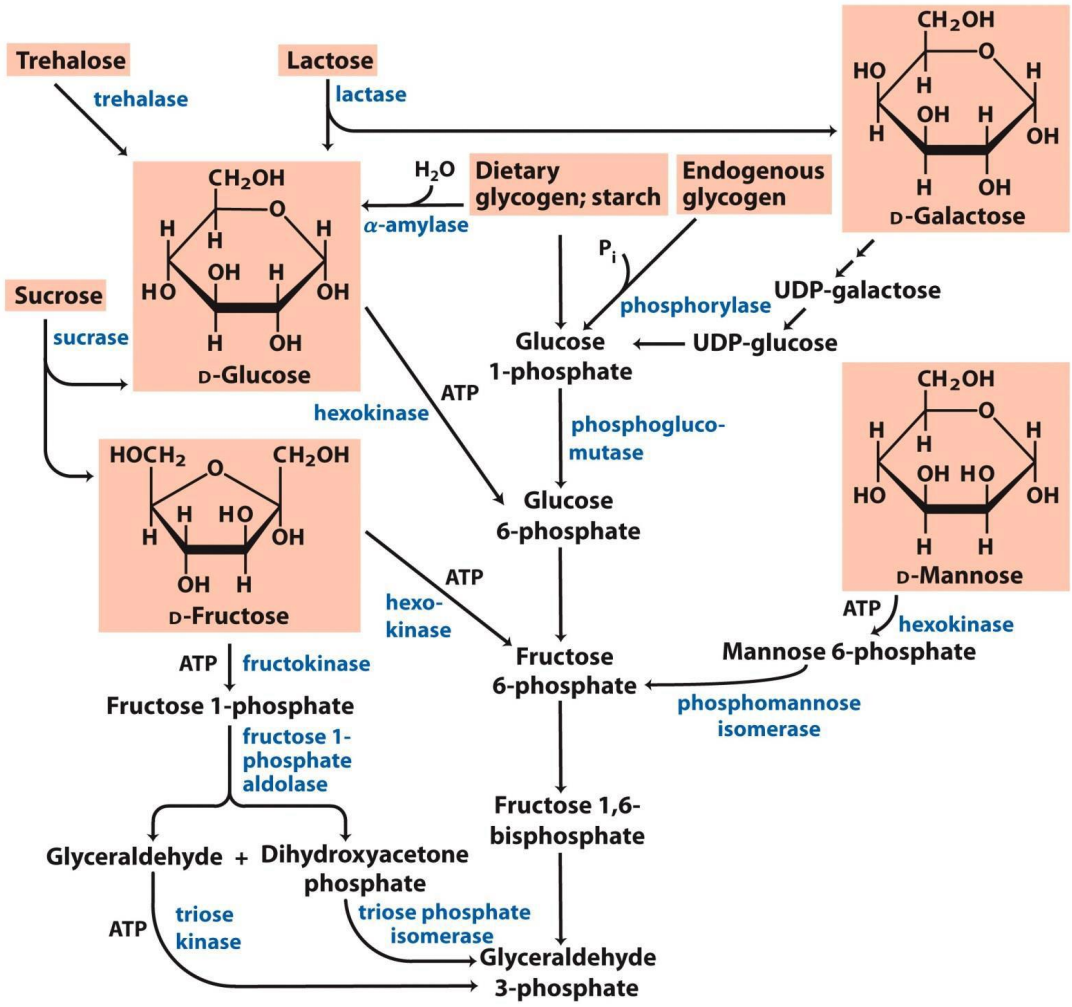
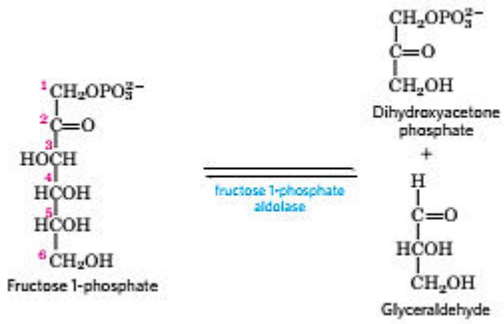


Figure 14-11
Lehninger Principles of Biochemistry, Sixth Edition

• In most organisms, hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated derivative.

FRUCTOSE:

• D-Fructose, present in free form in many fruits and formed by hydrolysis of sucrose in the small intestine of vertebrates, is phosphorylated by hexokinase
• Major pathway of fructose entry into glycolysis in the muscles and kidney.
• The liver enzyme **fructokinase** catalyzes the phosphorylation of fructose at C-1 rather than C-6
• The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by **fructose 1-phosphate aldolase.**

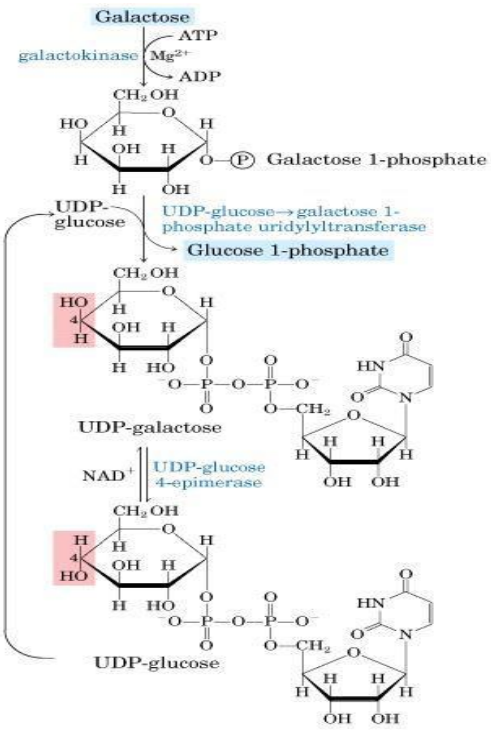


MANNOSE:

• D-Mannose, released in the digestion of various polysaccharides and glycoproteins of foods, can be phosphorylated at C-6 by hexokinase.
• Mannose 6-phosphate is isomerized by **phosphomannose isomerase** to yield fructose 6-phosphate, an intermediate of
glycolysis.

GALACTOSE:

• D-Galactose, a product of the hydrolysis of lactose (milk sugar), passes in the blood from the intestine to the liver,
where it is first phosphorylated at C-1, at the expense of ATP, by the enzyme **galactokinase**



Conversion of galactose to glucose 1- phosphate

Galactosemia

• A defect in any of the three enzymes in this pathway causes **galactosemia**

1. Galactokinase deficiency galactosemia

• high conc. are found in blood and urine.

• Infant develop cataracts, caused by deposition metabolite **galactitol in the lens**

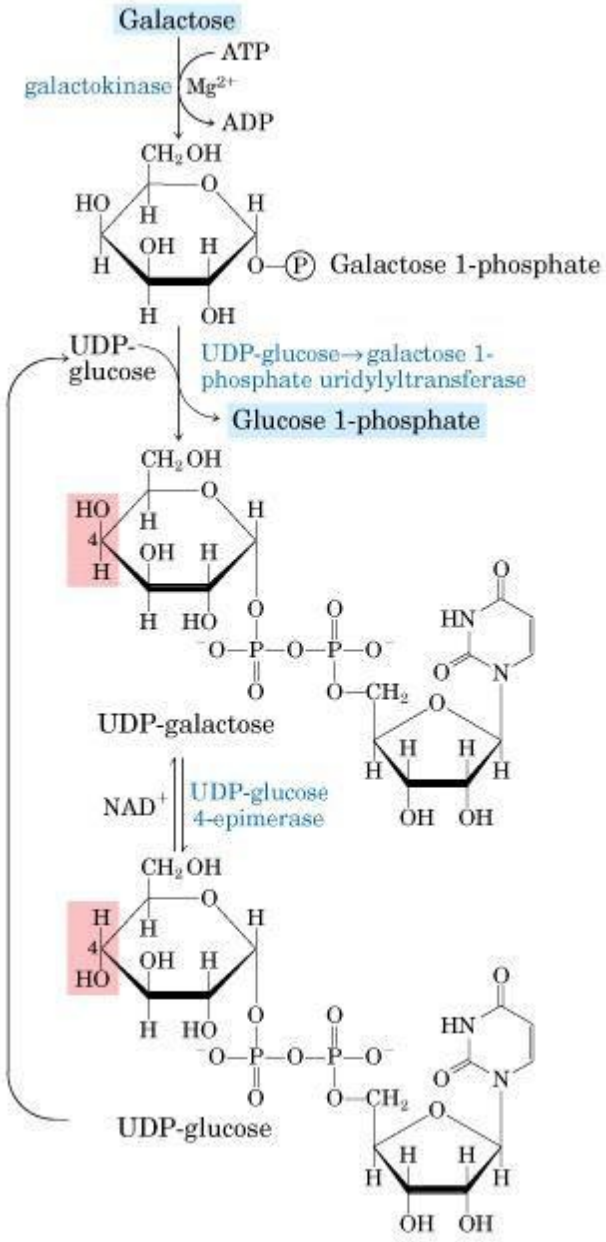
2. Transferase deficiency galactosemia

• more serious

• characterized by poor growth in children, mental deficiency and liver damage that may be fatal

3. Epimerase deficiency galactosemia

• similar symptoms, less severe when dietary galactose is carefully controlled



Lactose intolerance

• due to the lack of lactase synthesis

• common among adults

• Without intestinal lactase, lactose cannot be completely digested and absorbed in the small intestine, and it passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea.

• The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring retention of water in the intestine.

• In most parts of the world where lactose intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries.

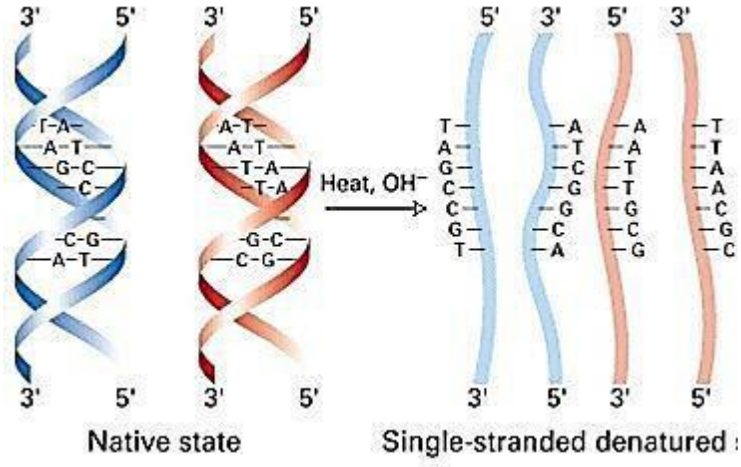
• The digestive disturbances can sometimes be minimized by a controlled diet

Mutase:
A **mutase** is an enzyme of the isomerase class that catalyzes the transfer of a functional group from one position to another within the same molecule.

Nucleotides and Nucleic Acids

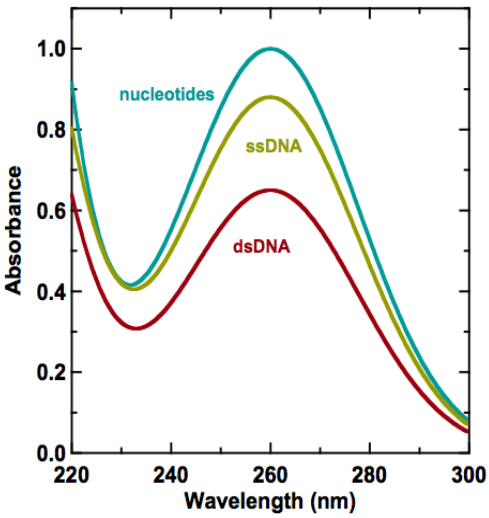
DNA Denaturation

• Separation of the two strands of the double helix when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if
• the pH or the salt concentration of the DNA solution is altered
• if the solution is heated above 80°C
• Disruption of the hydrogen bonds between paired bases and of base stacking causes unwinding of the double helix to form two single strands complete separation of DNA stands along the entire length.
• No covalent bonds in the DNA are broken



• RNA duplexes are more stable than DNA duplexes.
• At neutral pH denaturation of a double helical RNA often requires temperatures 20o C or more higher than those required for denaturation of a DNA molecule with a comparable sequence.

• **Tm:** When DNA is heated, the temperature at which one half of the helical structure is lost is defined as the melting temperature.
• A specific sequence of DNA has a characteristic denaturation temperature, or melting point (Tm)
• Careful determination of the melting temperature of a DNA specimen, under fixed conditions of pH and ionic strength, can yield an estimate of a DNA base composition.
• Concomitant with this denaturation of the DNA molecule is an increase in the optical absorbance of the purine and pyrimidine bases—a phenomenon referred to as hyperchromicity of denaturation.

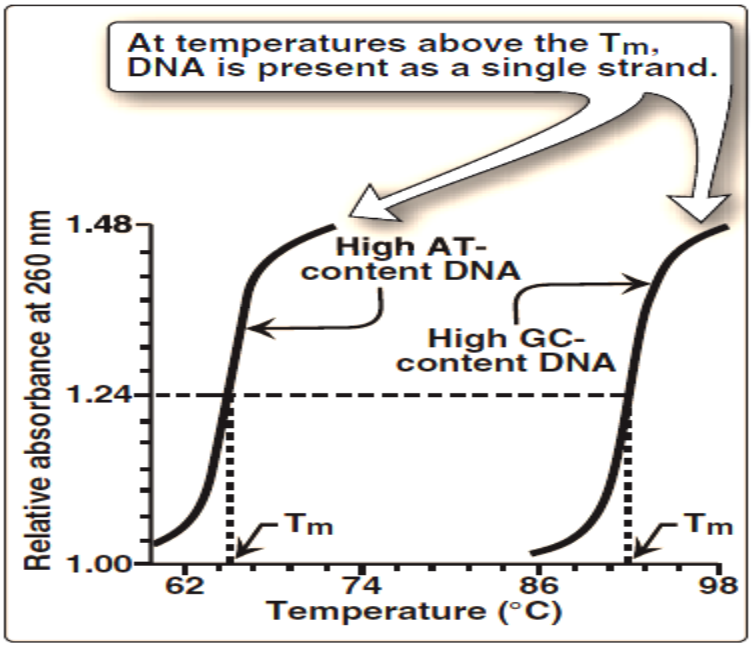


The absorbance of double-stranded DNA (dsDNA) at 260 nm is less than that of either single-stranded DNA (ssDNA) or the free bases. This is called "hyperchromism."

• So single-stranded DNA has a higher relative absorbance at this wavelength than does double-stranded DNA.
• Denaturation can be monitored by measuring its absorbance at 260 nm.

Factors affecting Tm

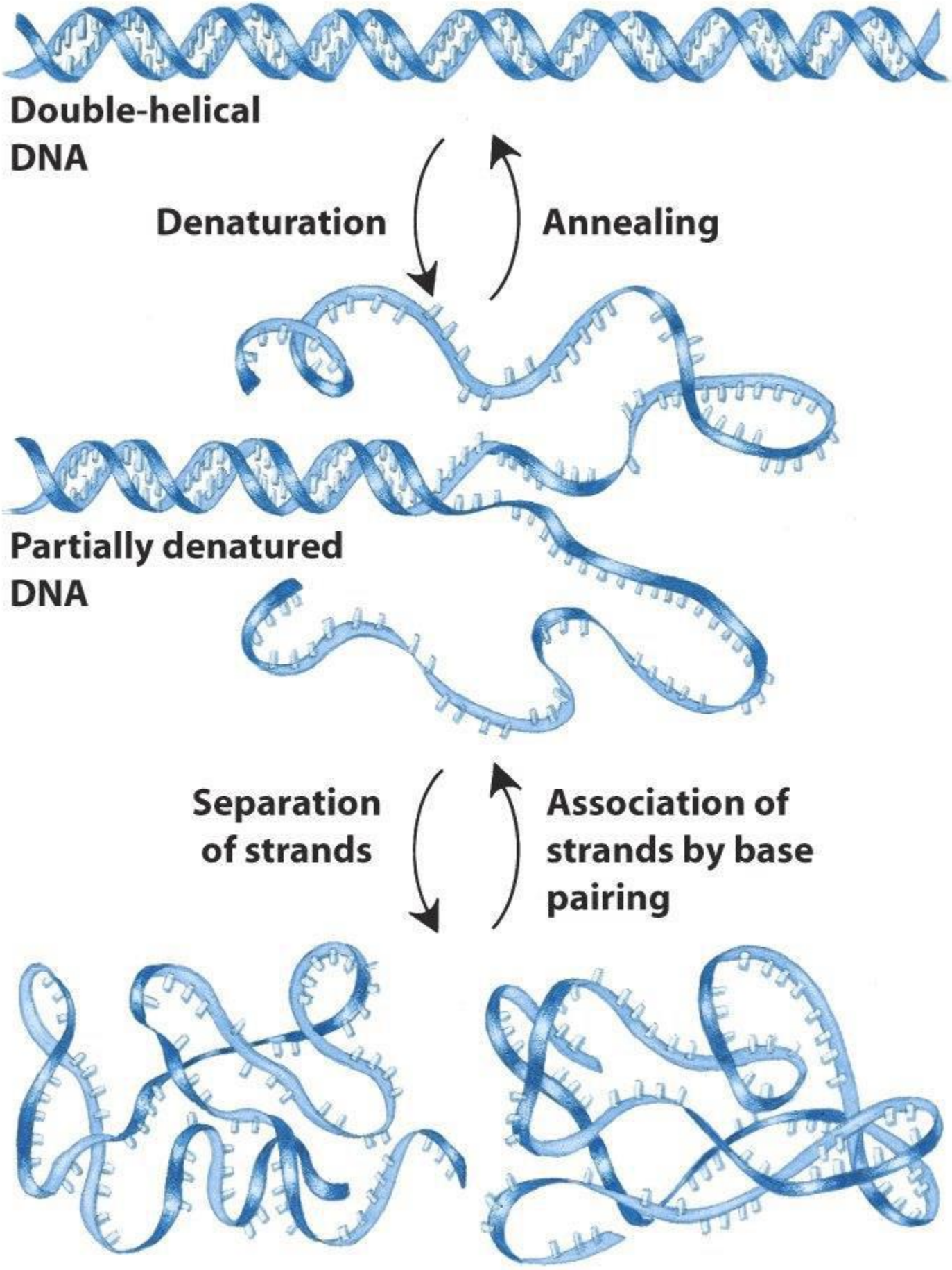
The Tm is influenced by
• the base composition of the DNA
• the salt concentration of the solution
• The higher the content of GC base pairs, the higher the melting point of the DNA.
• This is because GC base pairs, with three hydrogen bonds, require more heat energy to dissociate than AT base pairs.



• An *increase* in salt concentration *increases* and a
• decrease in salt concentration decreases the Tm

DNA Renaturation

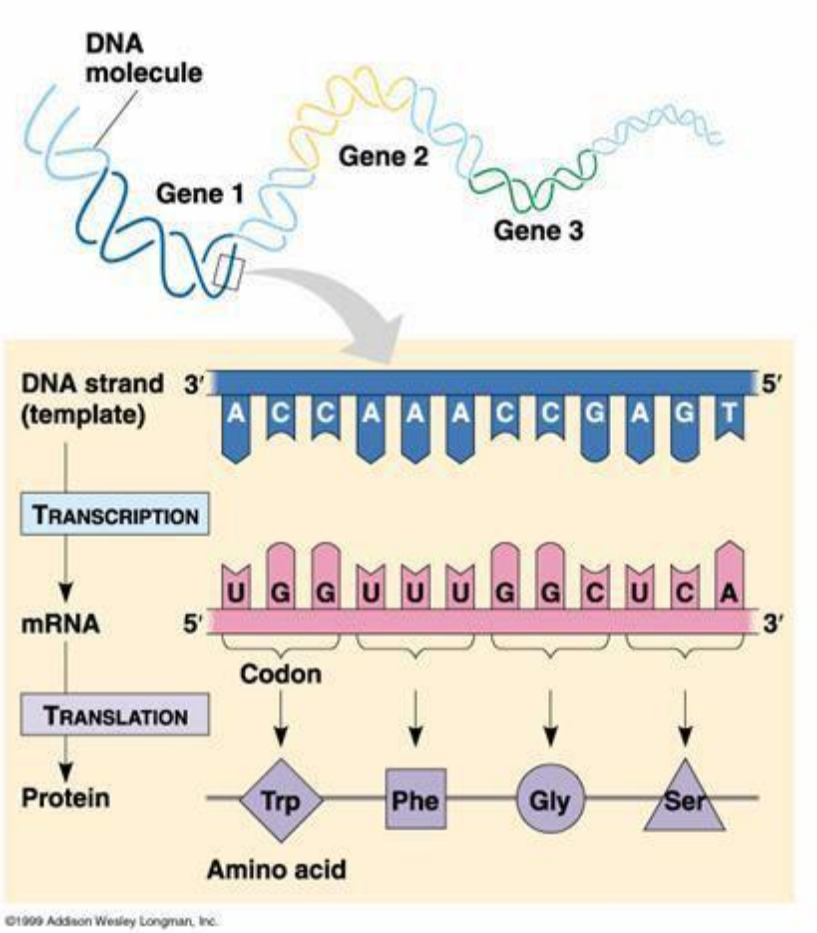
• Under appropriate conditions *(temp. & salt concentration)*, separated strands of DNA will renature or reassociate and form the double helix by the process called renaturation (or reannealing).
• This reannealing process is also referred to as hybridization.
• When the temperature or pH is returned to the range in which most organisms live, the unwound segments of the two strands spontaneously rewind, or anneal**,** to yield the intact duplex.


Double-helical DNA
Denaturation
Annealing
Partially denatured DNA
Separation of strands
Association of strands by base pairing
Separated strands of DNA in random coils

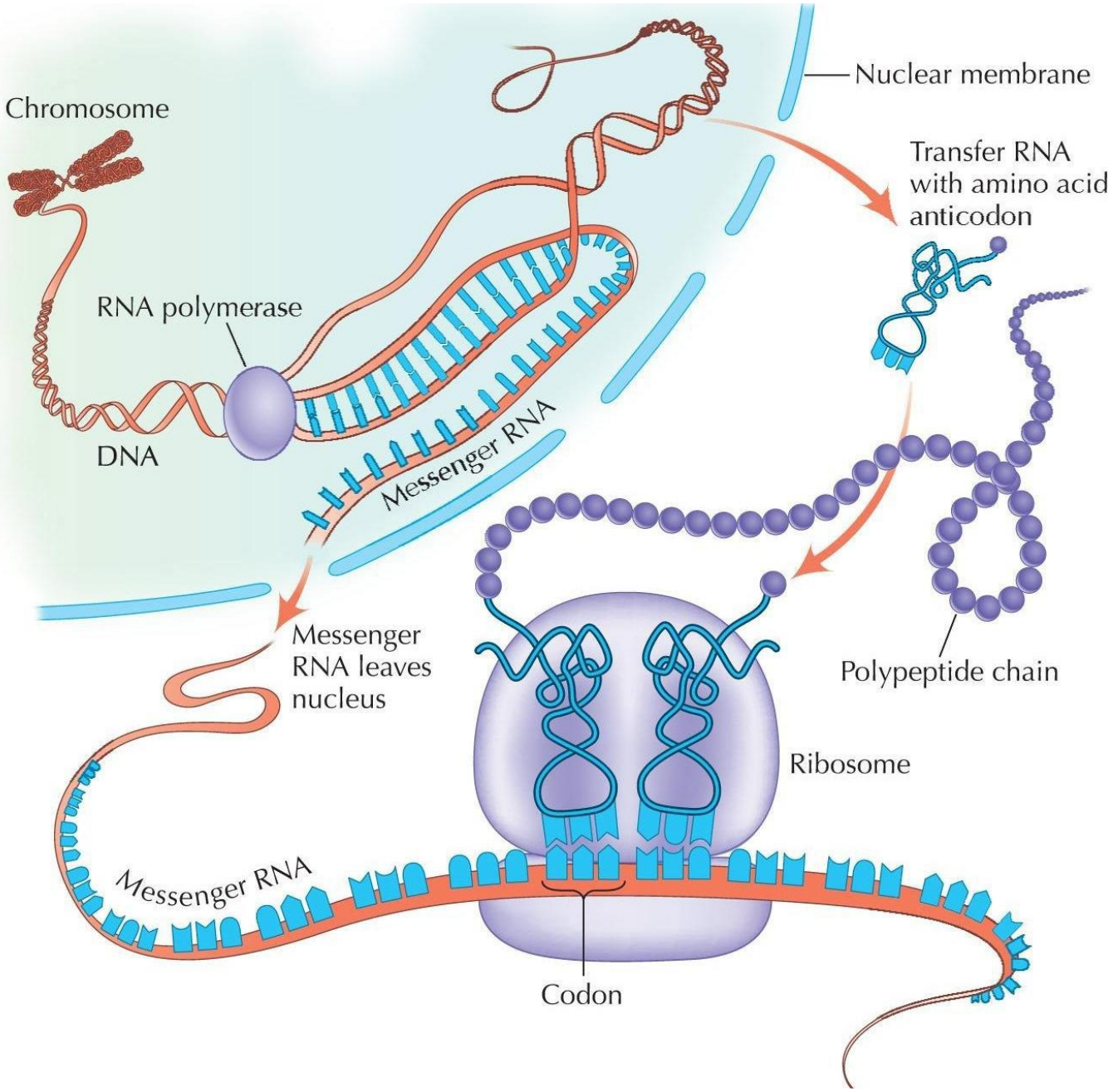
• Renaturation of a DNA molecule is a rapid one step process.
• The rate of re-association depends upon the concentration of the complementary strands.
• At a given temperature and salt concentration, a particular nucleic acid strand will associate tightly only with a complementary strand.
• This property is utilized in analyzing nucleotide sequencing of a given nucleic acid.
• Under appropriate conditions DNA will form a hybrid with a complementary DNA or with a complementary RNA.
• Hybridization is combined with gel electrophoresis techniques that separate nucleic acids by size,
• coupled with radioactive or fluorescent probe labeling to provide a detection of a nucleotide sequence.

RNA

• The genetic master plan is contained in the nucleotide sequence of DNA.
• It is through the ribonucleic acid (RNA)—the "working copies" of the (DNA) — that the master plan is expressed.



• RNA is a polymer of ribonucleotides of Adenine, Uracil, Guanine and Cytosine, joined together by 3´-5´phosphodiester bonds.
• RNA does not contain thymine except in rare cases.
• The pentose sugar of RNA is D-ribose.
• Location**:** RNA is found in the nucleolus, ribosomes, mitochondria, and cytoplasm.
• The genetic material for some animal and plant viruses is RNA rather than DNA.
• There is a wide variety of RNAs,
• messenger RNAs (mRNAs)- transfer genetic information from DNA to the protein-synthesizing machinery.
• ribosomal RNAs (rRNAs)- contribute to the formation and function of ribosomes
• transfer RNAs (tRNAs)- adapter molecules that carry specific amino acids for protein synthesis

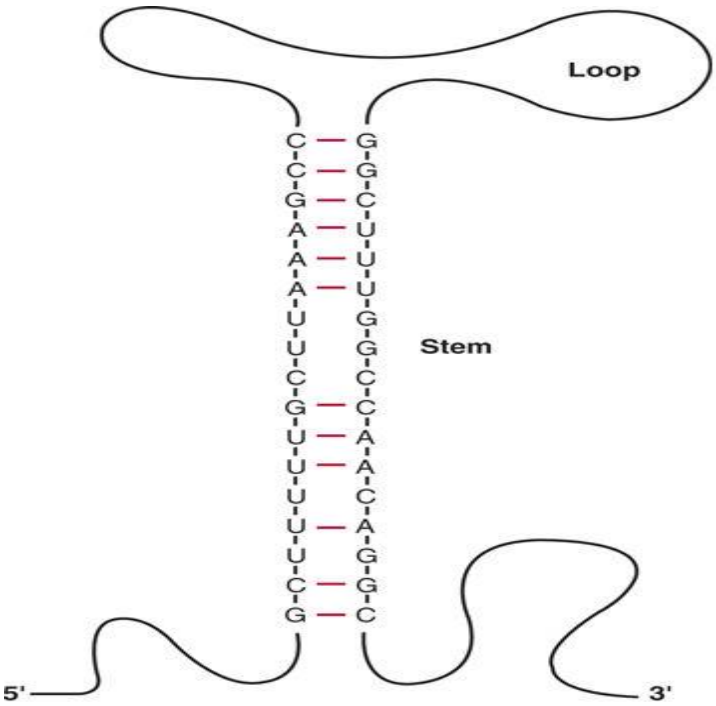


• small nuclear RNA (snRNA)- play pivotal roles in RNA processing, particularly mRNA processing
• ribozymes — some RNA molecules have intrinsic catalytic activity these RNA enzymes, are called Ribozymes.

Differences between DNA and RNA

• Although sharing many features with DNA, RNA possesses several specific differences.
• Size: They are considerably smaller than DNA
• Sugar**:** In RNA, the sugar moiety to which the phosphates and purine and pyrimidine bases are attached is ribose rather than the 2'-deoxyribose of DNA.
• Pyrimidine: The pyrimidine components of RNA differ from those of DNA.
• Instead of thymine, RNA contains the ribonucleotide of uracil.
• Thymine is present in the rare case of tRNA
• Single Strand**:**
• RNA typically exists as a single strand whereas DNA exists as a double-stranded helical molecule.

However, given the proper complementary base sequence with opposite polarity, the single strand of RNA is capable of self- folding like a hairpin and thus acquiring double stranded characteristics: G pairing with C, and A with U.

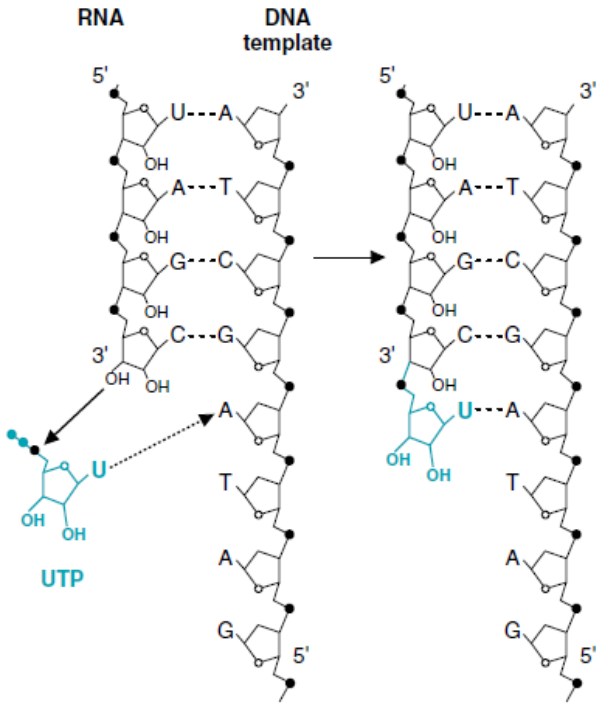


Chargaff's Rules Do Not Apply:

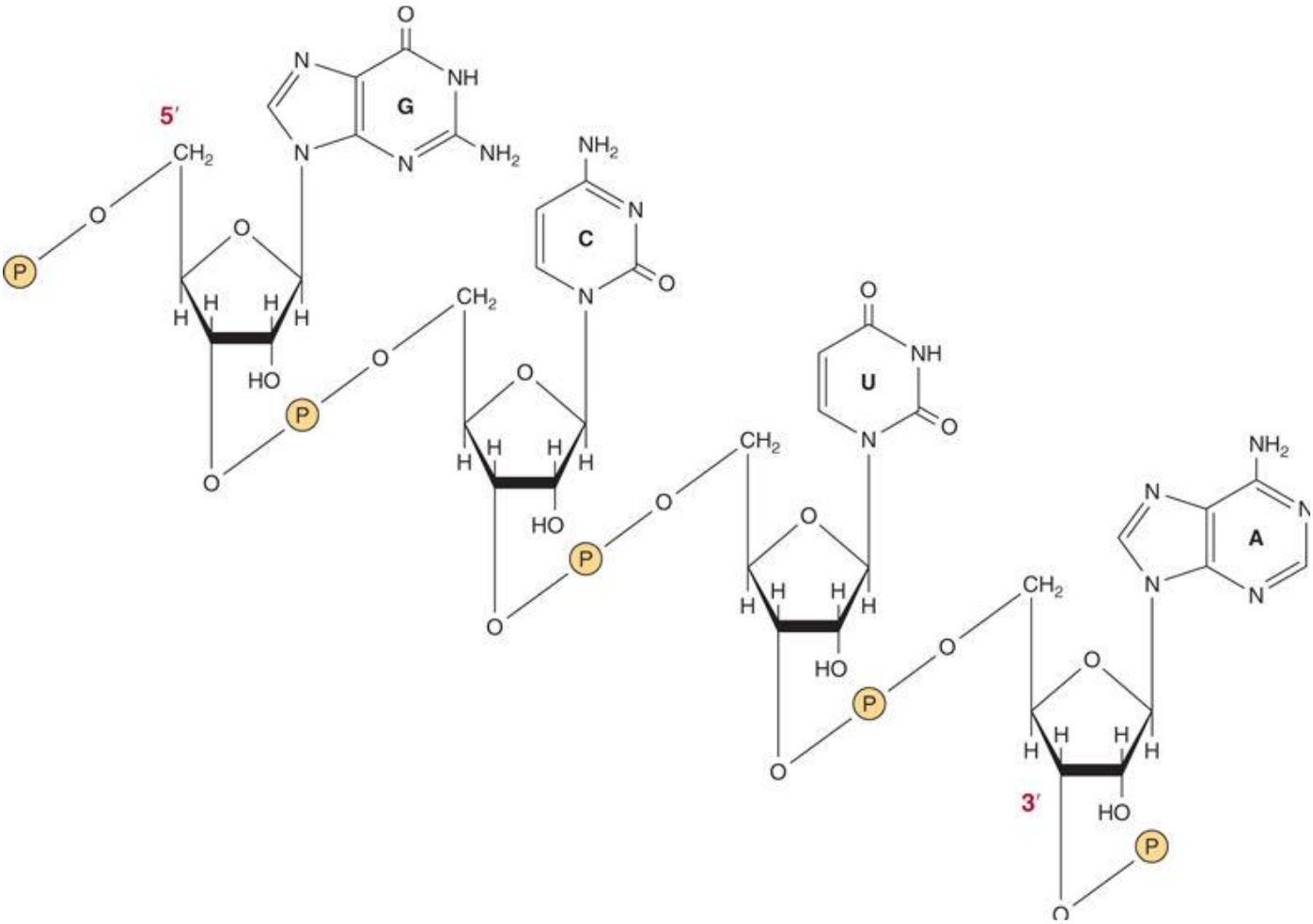
- Since the RNA molecule is a single strand complementary to only one of the two strands of a gene,
- G≠C
- A≠U
- Purines ≠ Pyrimidines
- Hydrolysis**:** RNA can be hydrolyzed by alkali to 2',3' cyclic diesters of the mononucleotides, compounds that cannot be formed from alkali-treated DNA because of the absence of a 2'- hydroxyl group.
- Location: In addition to nucleus, RNA is found in cytoplasm.
- Reverse Transcription**:** DNA forms RNA by transcription whereas the process by which RNA form DNA is called reverse transcription.
- **RNA Structural Hierarchy**
- RNA has no simple, structural hierarchy that serves as a reference point, as does the double helix for DNA.
- The three-dimensional structures of many RNAs, like those of proteins, are complex and unique.

Primary Structure of RNA

- It is defined as the number and sequence of ribonucleotides in the RNA chain.
- The sequence is complementary to the template strand of the gene from which it was transcribed.



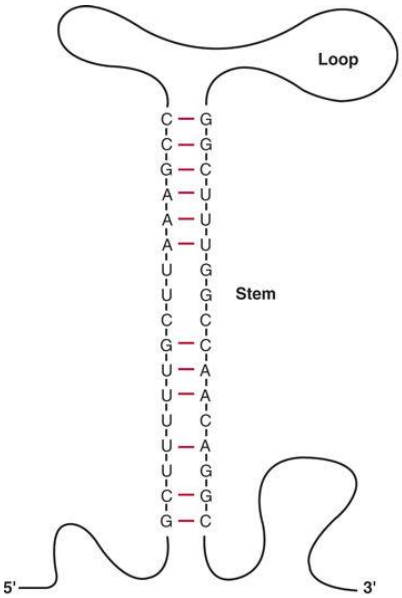
• The ribonucleotides are held together by 3´- 5´ phosphodiester bonds.
• 3´-OH group of one nucleotide is bound to 5´-PO4 of the other nucleotide and form a linear strand.



• The ribosyl moieties are attached to the nucleobases by *N*glycosidic bonds.
• Similar to DNA RNA polymer also has polarity.

Secondary Structure of RNA

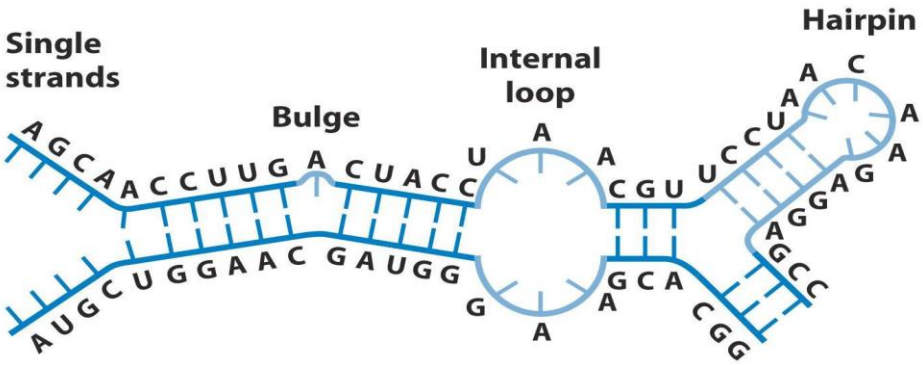
• Secondary structure involves coil formation of the polyribonucleotide chain.
• The coiled structures are stabilized by:
Hydrophobic interactions between purine and pyrimidine bases.
Intra-chain hydrogen bonds between G-C and A-U.



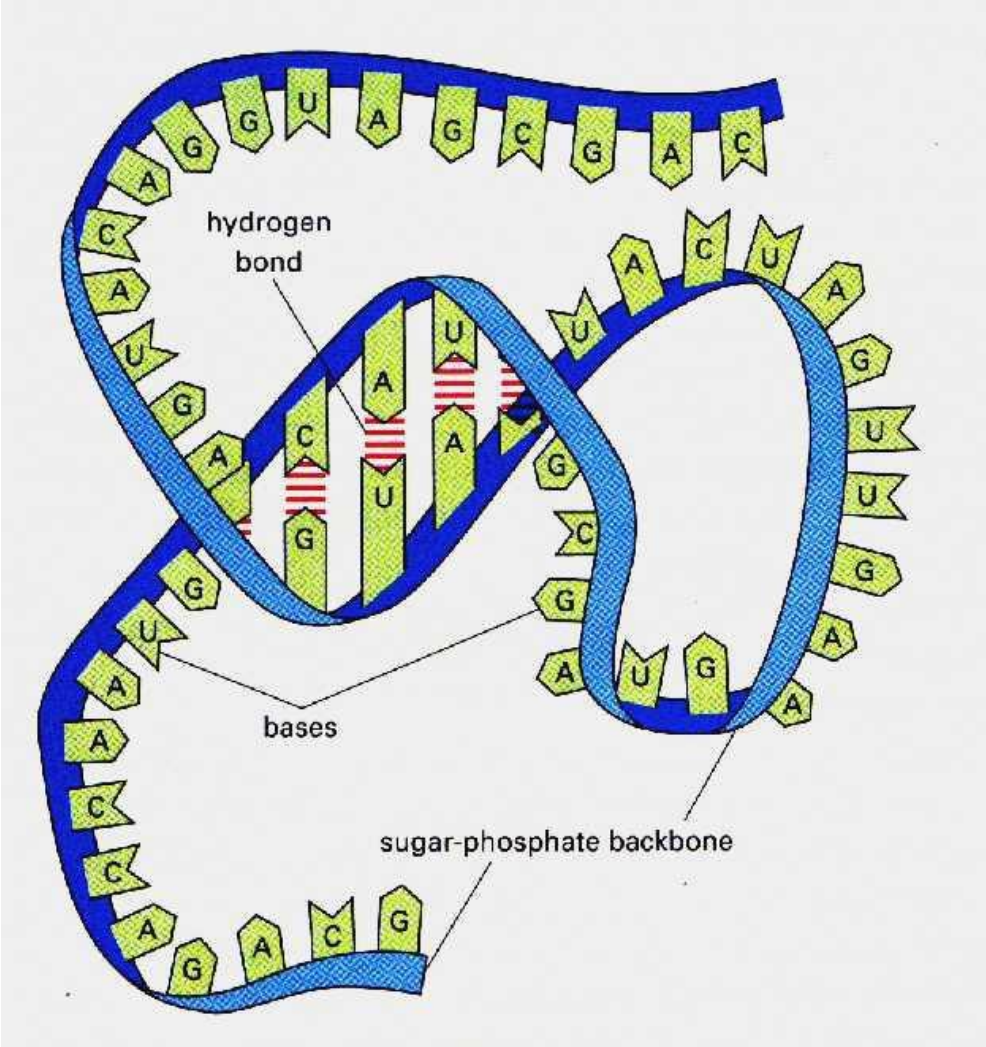
Note: Adenine pairs with Uracil in RNA

• Weak interactions, especially base stacking interactions.
• Where complementary sequences are present, the predominant double stranded structure is formed.

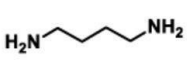
Common RNA secondary structures
• Internal loops: a short series of unpaired bases in a longer paired helix and
• bulges: regions in which one strand of a helix has "extra" inserted bases with no counterparts in the opposite strand

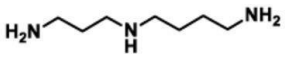


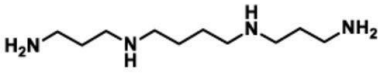
Tertiary Structure of RNA
• It is the folding of the molecule into three dimensional structure.
• cross-linking at various sites stabilized by hydrophobic and hydrogen bonds produces a compactly coiled globular structure.

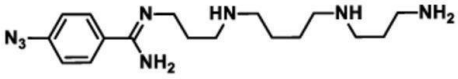


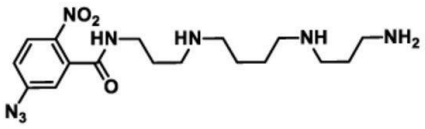
• The stacking of helices, together with specific helix–helix contacts or helix–loop interactions, lead to compact tertiary structure of the RNA assemblies, generally in the presence of divalent ions or polyamines

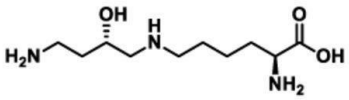

Putrescine

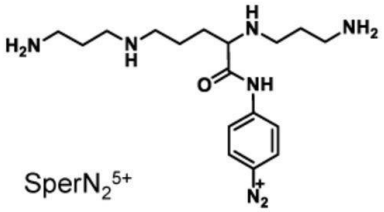

Spermidine

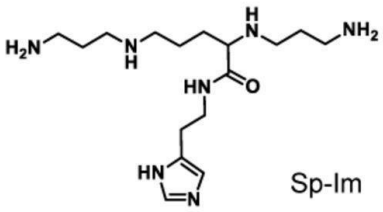

Spermine


ABA-spermine


ANB-spermine

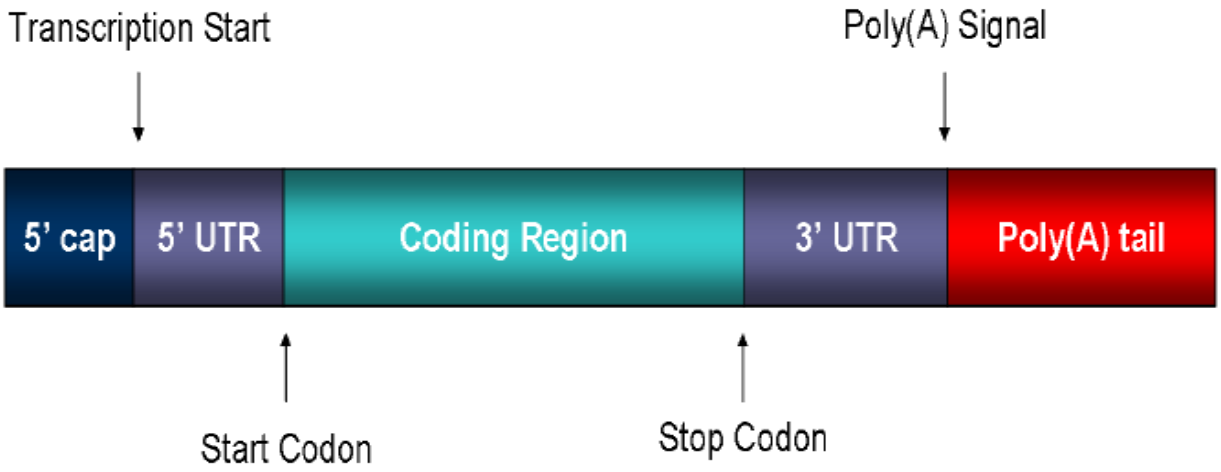

Hypusine


$SperN_2^{5+}$


Sp-Im

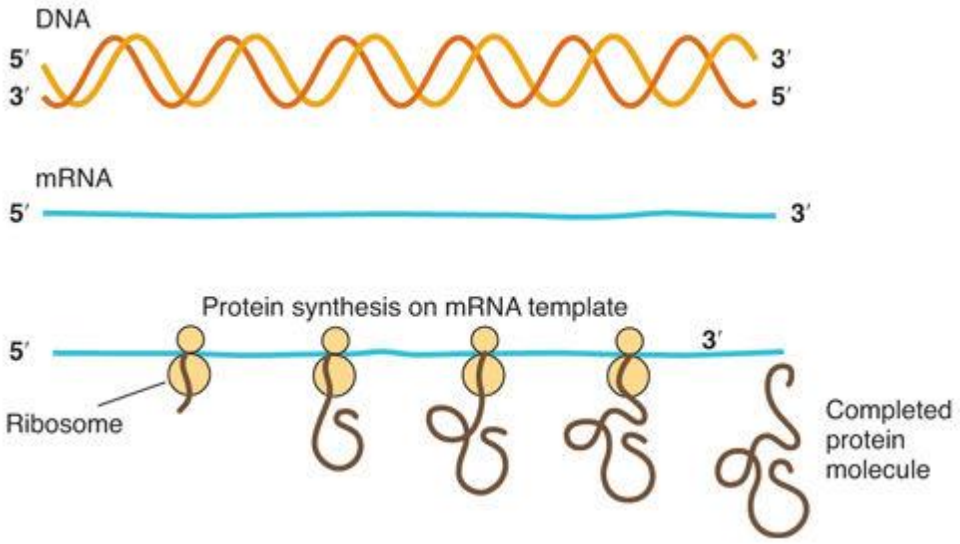
Messenger RNA (mRNA)

• This class is the most heterogeneous in
• Abundance
• Size (500-6000 nucleotides)
• base sequence • Stability
• mRNA comprise about 2–5% of total cellular RNA
• mRNA molecules are formed with the help of DNA template strand (3´-5´) during the process called transcription.
• In addition to the protein coding regions in the mature eukaryotic mRNA that can be translated,
• there are untranslated regions at its 5´ and 3´ ends
• Moreover, there is a 5´ cap and
• a poly A tail at 3´ end



Function of mRNA

• The members of this class function as messengers to convey the information in a gene to the protein synthesizing machinery.



• The mRNA carries genetic information from the nuclear DNA to the cytosol, where it is used as a template for protein synthesis.

Transfer RNA (tRNA)

• t RNA is the smallest of the three major species of RNA (4S).
• They are single stranded globular molecules.
• They remain largely in cytoplasm.
• They are generated by nuclear processing of a precursor molecule.
• tRNAs compose roughly 20% of total cellular RNA
• There are at least 20 species of tRNA molecules in every cell.
• Although each specific tRNA differs from the others in its sequence of nucleotides, the tRNA molecules as a class have many features in common

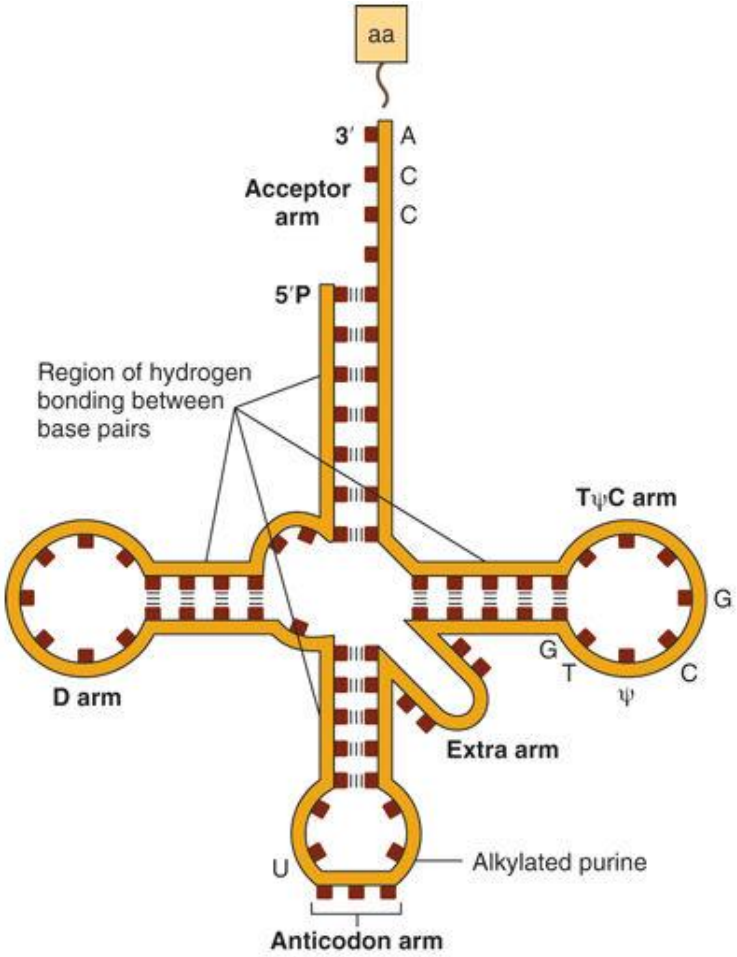
Primary structure

• t RNA molecules consist of 74-95 nucleotides in a particular sequence.
• The t RNA molecules contain not only the usual bases like adenine, guanine, cytosine, uracil but also contain **unusual bases.**
• These unusual bases(also called modified bases) include
• Dihydrouracil
• Pseudouridine
• Thymine.

Secondary Structure

Pseudouridine

• Each single stranded tRNA is folded extensively.
• Extensive intra chain base pairing which leads to a characteristic CLOVER-LEAF structure.



• These folds are stabilized by hydrogen bonds between complementary bases of the same strand.

Arms or loops of tRNA

• All tRNA molecules contain 4 main arms or loops.

• **1-Acceptor arm:** This is made up of unpaired sequences of cytosinecytosine-adenine (CCA) at the 3′end.

• The 3′OH group of adenine binds with the carboxylic group of a specific amino acid and carries it to ribosomes for protein synthesis.

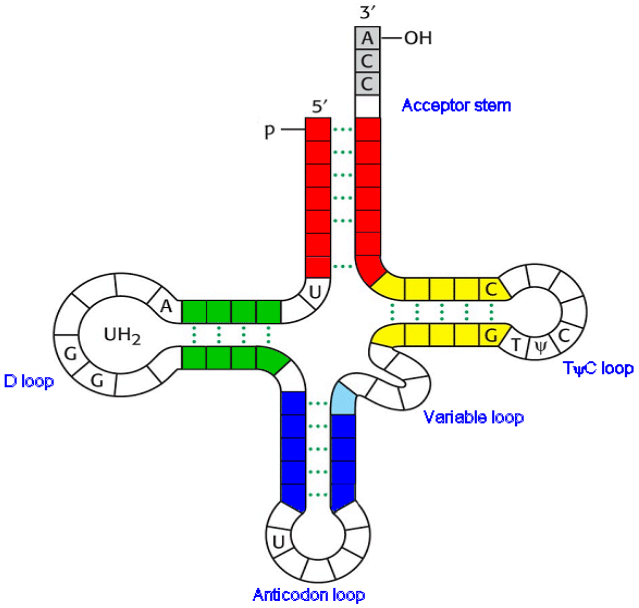
• **2-Anticodon arm:** It is in the form of a loop and carries specific sequences of three bases which constitute the anticodon.

• The bases of anticodon are bonded with three complementary bases of codon on mRNA.

• **3-D arm:** It contains the base dihydrouridine.

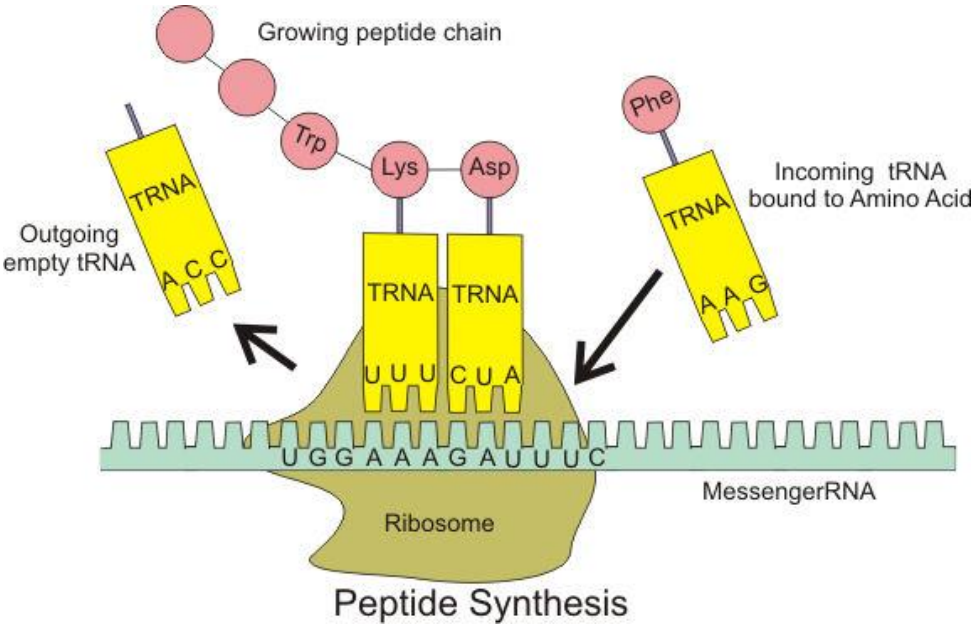
• **4-TΨC arm:** It contains thymine, pseudouridine and cytosine.

• The extra arm and the TΨC arms help to define a specific tRNA.



Function of tRNA

• The t RNA molecules serve as ADAPTERS for the translation of information in the sequence of nucleotides of the mRNA into specific amino acids.



• There is at least one (and often several) specific type of tRNA molecule for each of the amino acids commonly found in proteins.
• Each t RNA carries its specific amino acid to the site of protein synthesis.
• There it recognizes the genetic code word on mRNA (codon) and this specifies the addition of its amino acids to the growing peptide chain.
• All tRNA molecules contain 4 main arms or loops.

• **1-Acceptor arm:** This is made up of unpaired sequences of cytosinecytosine-adenine (CCA) at the 3´end.
• The 3´OH group of adenine binds with the carboxylic group of a specific amino acid and carries it to ribosomes for protein synthesis.

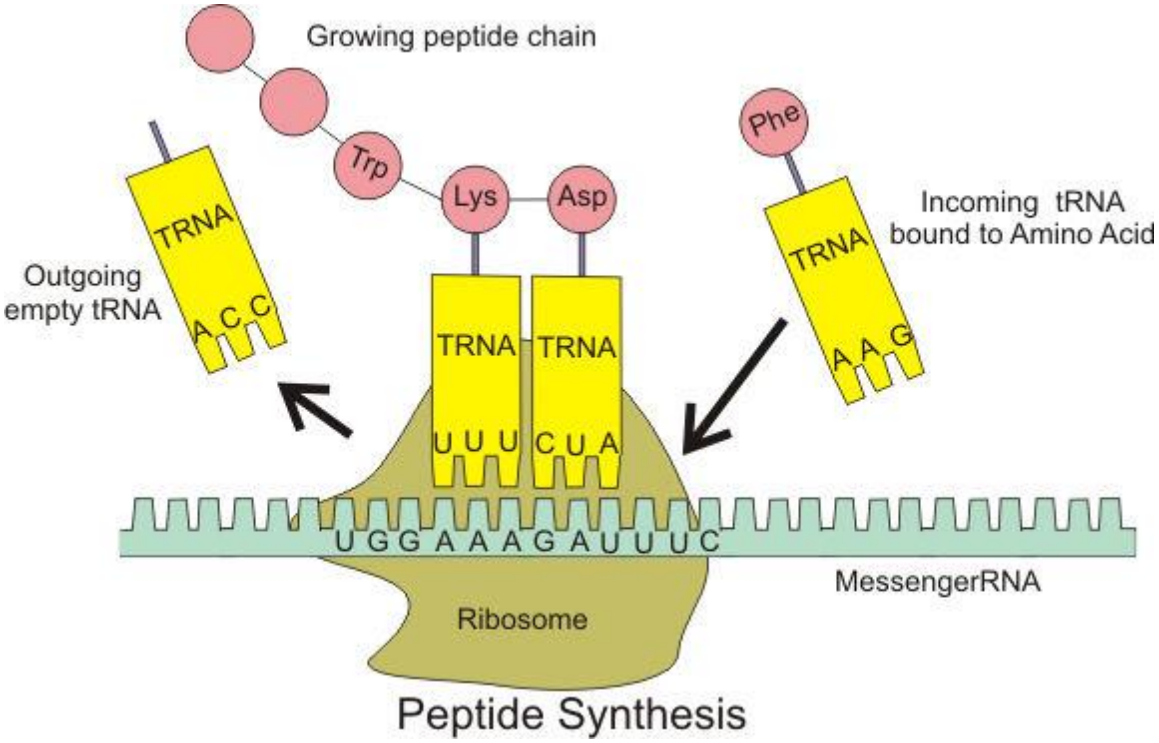
• **2-Anticodon arm:** It is in the form of a loop and carries specific sequences of three bases which constitute the anticodon.
• The bases of anticodon are bonded with three complementary bases of codon on mRNA.

• **3-D arm:** It contains the base dihydrouridine.

• **4-TΨC arm:** It contains thymine, pseudouridine and cytosine.
• The extra arm and the TΨC arms help to define a specific tRNA.

Function of tRNA

• The t RNA molecules serve as ADAPTERS for the translation of information in the sequence of nucleotides of the mRNA into specific amino acids.



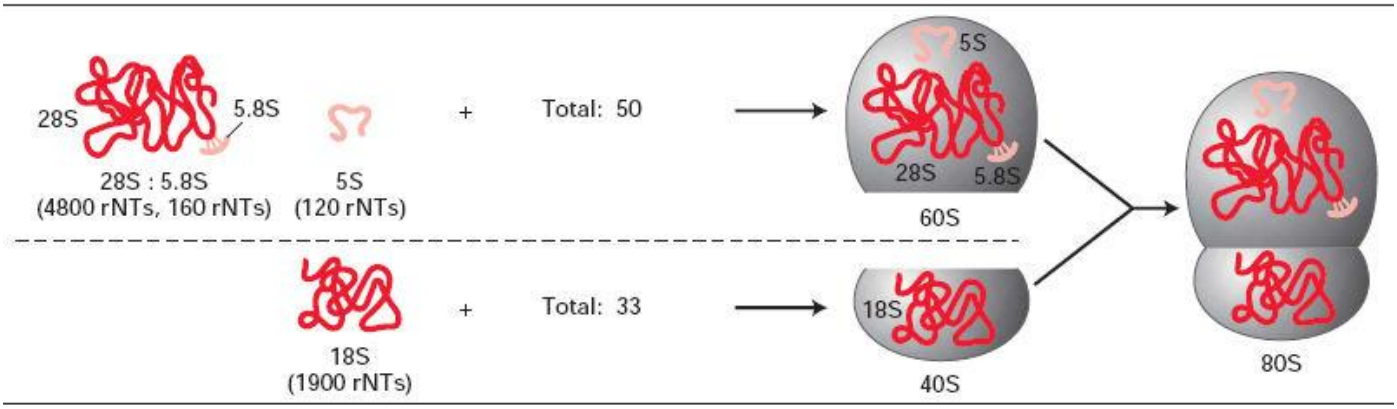
• There is at least one (and often several) specific type of tRNA molecule for each of the amino acids commonly found in proteins.
• Each t RNA carries its specific amino acid to the site of protein synthesis.
• There it recognizes the genetic code word on mRNA (codon) and this specifies the addition of its amino acids to the growing peptide chain.

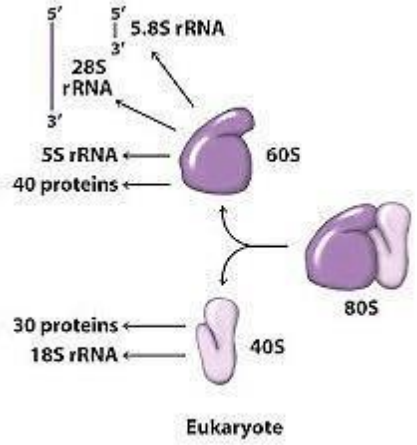
Ribosomal RNA (rRNA)

• found in association with several proteins as a component of ribosomes--- a cytoplasmic nucleoprotein structure that acts as the machinery for the synthesis of proteins from the mRNA template.
• RNAs make up 80% of the total RNA in the cell.
• The ribosomal subunits are defined according to their sedimentation velocity in **Svedberg units**.
• Svedberg unit is related to the molecular weight and shape of the compound.
• The bases in r RNA are mainly adenine, guanine, cytosine and uracil and a few pseudouridine.

Eukaryotic Ribosome

• The mammalian ribosome contains two major nucleoprotein subunits:
• a larger one with 60S
• a smaller one with 40S.
• The **60S** subunit contains
• a 5S rRNA
• a 5.8S rRNA
• a 28S rRNA
• more than 50 specific polypeptides.
• The **40S** subunit is smaller and contains
• a single 18S rRNA
• Approx. 30 distinct polypeptide chains.





• In eukaryotes, all of the ribosomal RNA molecules except the 5S rRNA, which is independently transcribed, are processed from a single 45S precursor RNA molecule in the nucleolus- packed with the specific ribosomal proteins.
• The rRNA are necessary for ribosomal assembly and play a key role in the binding of mRNA to ribosomes and its translation.
• In the cytoplasm, the ribosomes remain quite stable and capable of many translation cycles.

Other types of RNA

Small nuclear RNA (snRNA)

• Small nuclear RNA (snRNA) are large number of small stable
RNA species found in eukaryotic cells.
• Most of them are complexed with proteins to form ribonucleoproteins.
• They are distributed in the nucleus, in the cytoplasm or in both.
• They are significantly involved in rRNA and mRNA processing and gene regulation.

Large & Small Noncoding Regulatory RNAs

• One of the most exciting discoveries in the last decade of eukaryotic regulatory biology has been the identification and characterization of regulatory nonprotein coding RNAs (ncRNAs).

• NcRNAs exist in two general size classes,
• small consisting of microRNA (miRNAs) and silencing (siRNAs)
• Large consisting of long noncoding RNAs (lncRNAs)
• The small ncRNAs termed microRNA (miRNAs) and silencing (siRNAs) typically inhibit gene expression at the level of specific protein production by
• targeting mRNAs through one of several distinct mechanisms.
• Both siRNAs and miRNAs typically hybridize, via the formation of RNA–RNA hybridization to their targeted mRNAs

Long noncoding RNAs (lncRNAs).

• LncRNAs, which as their name implies, do not code for protein (ie, the mRNA encoding genes).
• ncRNAs make up a significant portion of eukaryotic transcription.
• ncRNAs play many roles ranging from contributing to structural aspects of chromatin to regulation of mRNA gene transcription by RNA polymerase II.

Enzymes

IUB Classification of Enzymes

• International Union of Biochemists (IUB) developed an unambiguous system of enzyme nomenclature in which each enzyme has a
• unique name and
• code number
• As an example, the formal systematic name of the enzyme (hexokinase) catalyzing the reaction
• ATP + D-glucose→ ADP + D-glucose-6 phosphate is
• ATP:glucose phosphotransferase,
• Its Enzyme Commission (E.C.) number is 2.7.1.1.
• (2) denotes the class name (transferase)
• (7) the subclass phosphotransferase
• (1) denotes a hydroxyl group as acceptor
• (1) D-glucose as the phosphoryl group acceptor.
• In the systematic naming system, enzymes are divided into six major classes each with numerous subgroups

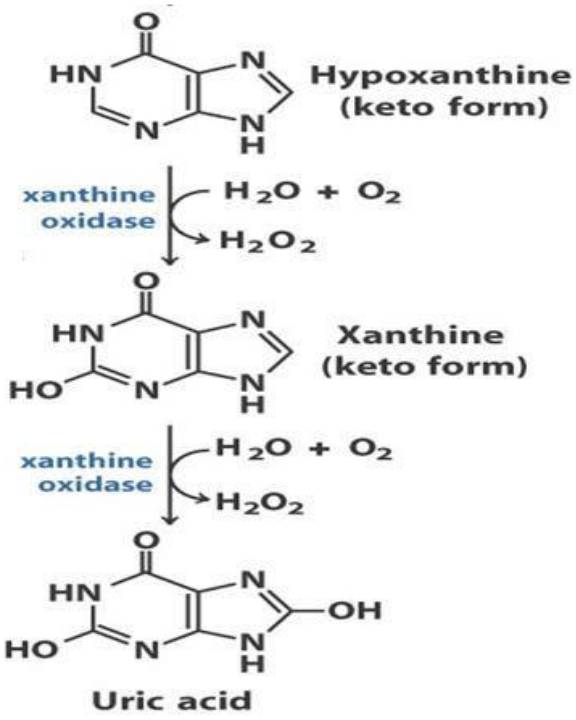
CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes

CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo Isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid Synthetase

1) Oxidoreductases

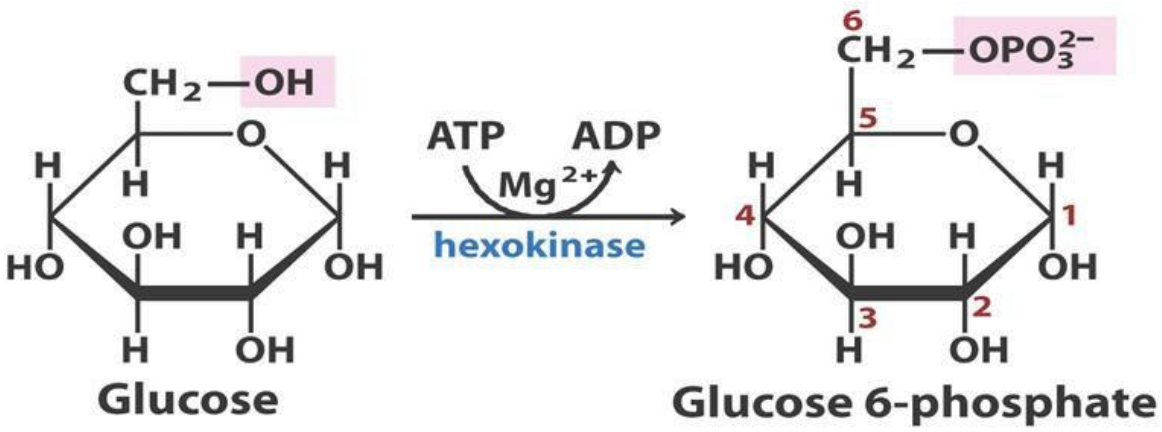
- catalyze oxidation reduction reactions
- further divided into four subgroups;
- Oxidase,
- Dehydrogenases,
- Hydroperoxidases
- Oxygenases.

- Two reactions both catalyzed by Xanthine oxidase are given
- Hypoxanthine → xanthine
- Xanthine → Uric acid



2) Transferases

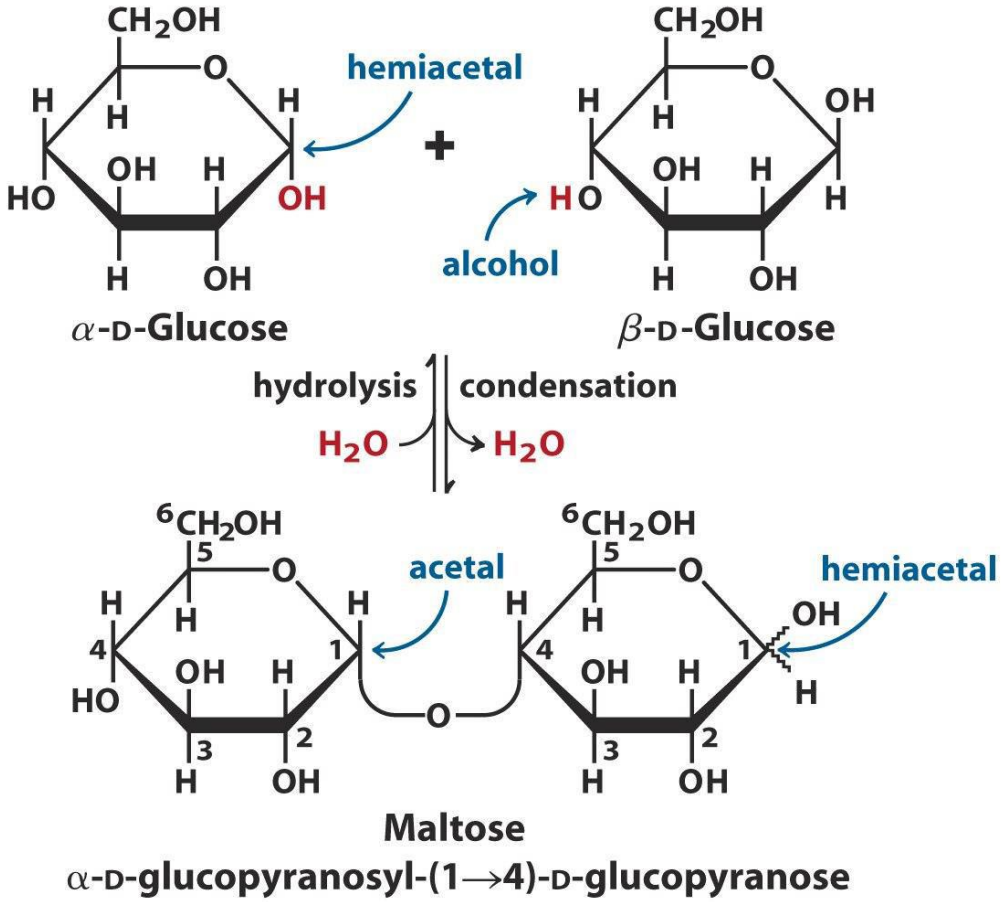
- These bring about a transfer of functional groups such as
- phosphate and amino group
- from one molecule to another molecule called donor and acceptor molecules respectively.
- The common examples of this group are
- Transminases
- Phosphotransfrases (Kinases)



• Hexokinase is a phosphotransferase which catalyze the transfer of phosphate groups.
• Glucose + ATP → Glucose 6-phosphate + ADP.

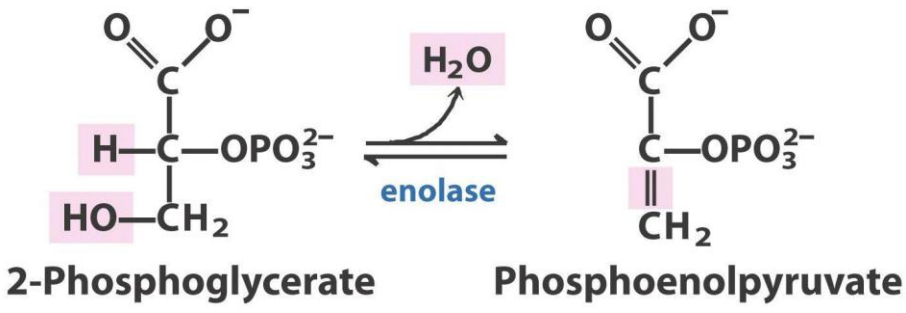
3) Hydrolases

• These enzymes catalyze hydrolysis, i.e.
• add water molecule to the substrate which is simultaneously decomposed; the functional group of substrate is transferred to water.
• Common example of hydrolases are:
• Protein hydrolyzing Enzymes (peptidases).
• Carbohydrases
• Lipid hydrolyzing enzymes e.g. Lipases and
• Phospholipases.

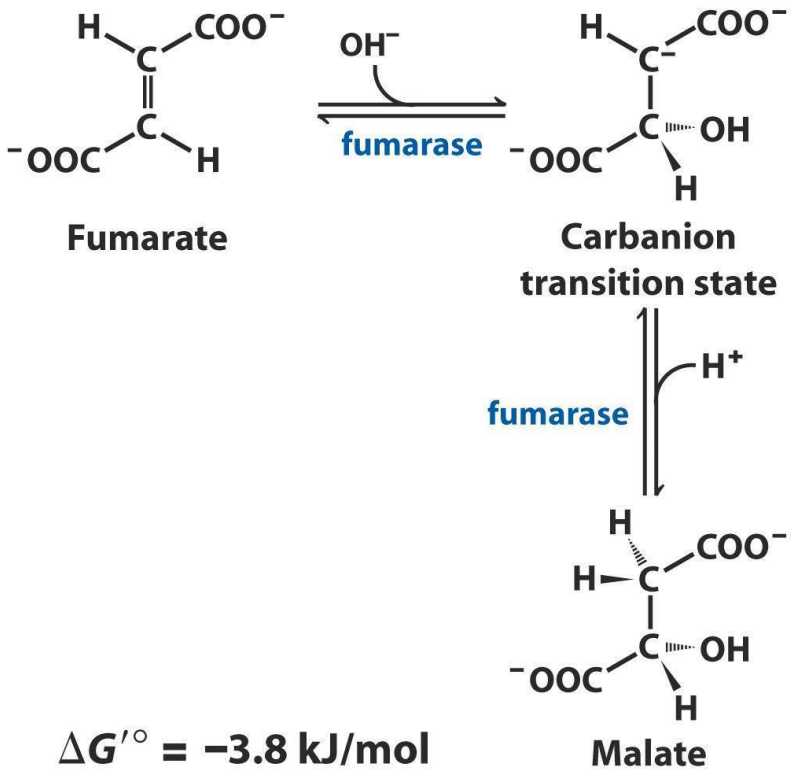


4) Lyases

• Theses enzymes catalyze the addition of
• NH_3,
• H_2O or
• CO_2 to double bonds or
• the removal of these groups leaving behind double bonds.



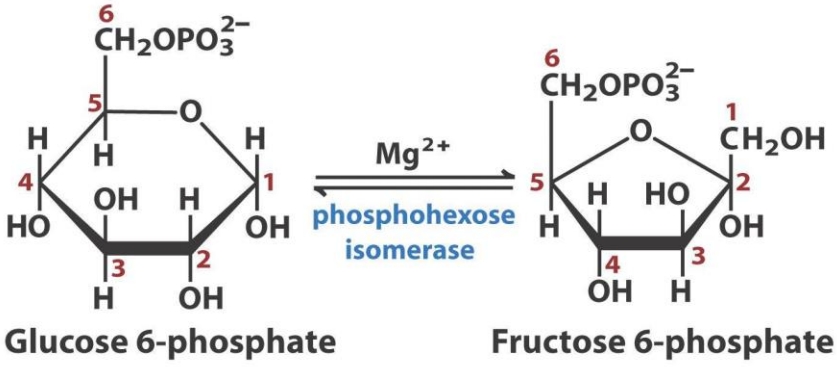
$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

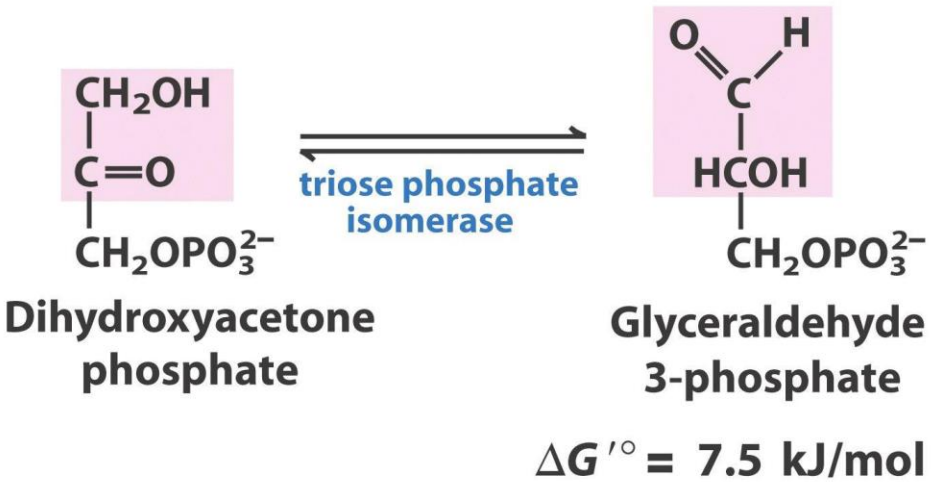


• Lyases are included in a separate class because they catalyze these reactions by means other than hydrolysis or oxidation.

5) Isomerases

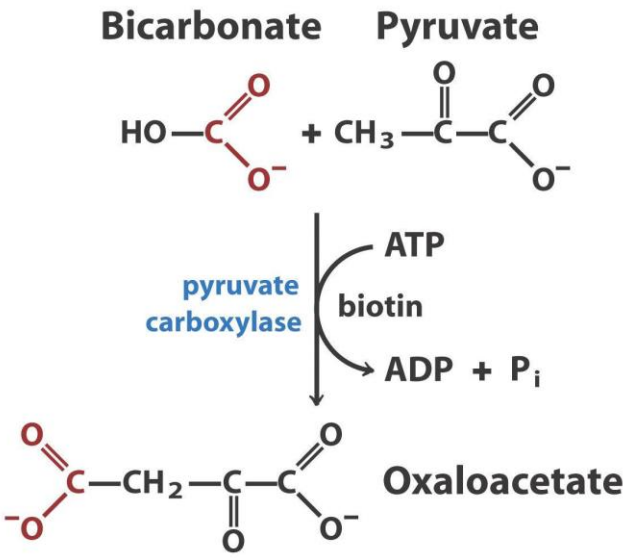
• These enzymes catalyze the structural change within a single molecule by the transfer of groups within it, resulting in the formation of an isomeric form of substrate.





6) Ligases

• These enzymes catalyze condensation reactions joining two molecules by forming
• C-O,
• C-S,
• C-N and
• C-C bonds.



• The energy for condensation is provided by cleavage of high energy phosphates, e.g. ATP, GTP etc.

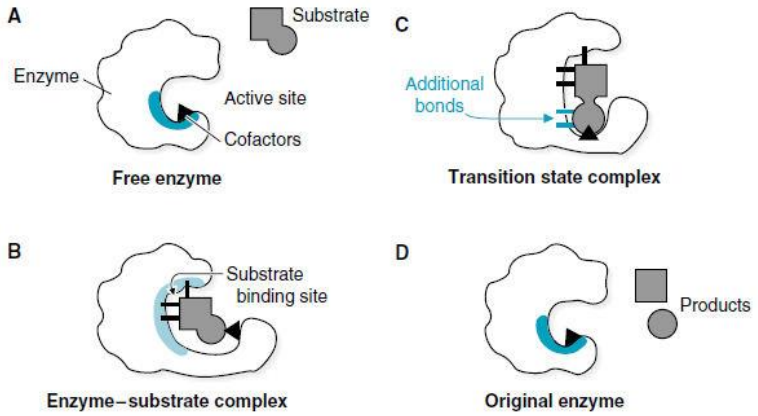
Ligand

• In biochemistry and pharmacology, a **ligand** (from the Latin *ligandum, binding*) is a substance (usually a small molecule),
• that forms a complex with a biomolecule to serve a biological purpose.
• In a narrower sense, it is a signal triggering molecule, binding to a site on a target protein
• A molecule bound reversibly by a protein is called a **ligand.**
• Ligands include substrates, inhibitors, activators, and neurotransmitters
• A ligand may be any kind of molecule, including another protein.
• A ligand binds at a site on the protein called the **binding site,**
• **binding site** is complementary to the ligand in
• size,
• shape,
• charge, and
• hydrophobic or
• hydrophilic character.
• Furthermore, the interaction is **specific**: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few.
• The binding of a protein and ligand is often coupled to a **conformational change** in the protein that makes the binding site more complementary to the ligand, permitting tighter binding called **induced fit.**
• A given protein may have **separate binding sites** for several different ligands.
• In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits.
• Interactions between **ligands** and proteins may be regulated, through interactions with additional ligands.
• These other ligands may cause conformational changes in the protein that affect the binding of the **first ligand**.

Mechanism of Enzyme Action

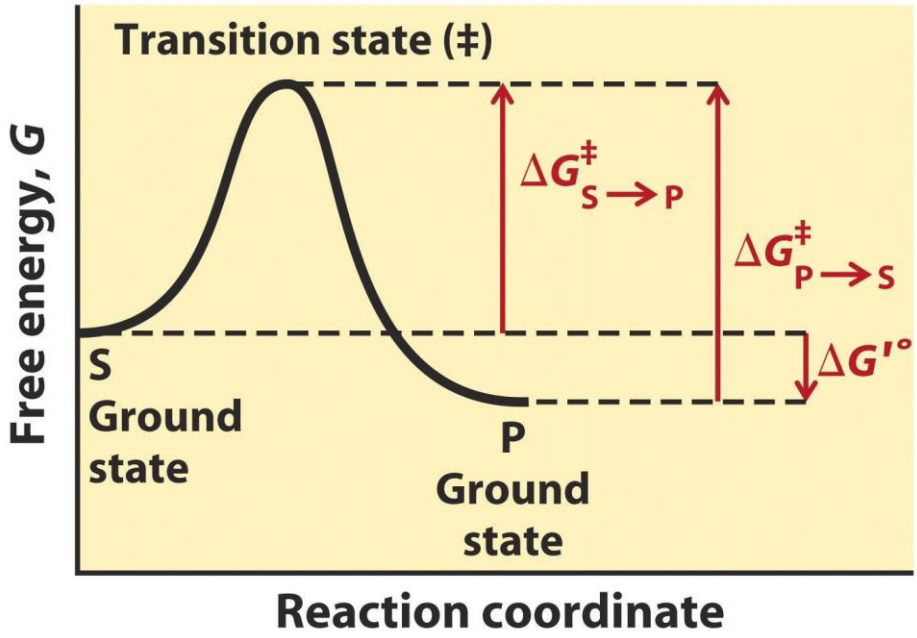
Enzymes bind and chemically transform other molecules— they catalyze reactions.
• The molecules acted upon by enzymes are called reaction **substrates** rather than ligands.
• Enzymes are highly effective catalysts, commonly enhancing reaction rates by a factor of 105 to 1017.

• The distinguishing feature of an enzyme catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site.**
• The surface of the active site is lined with **amino acid** residues with side chains that complement and bind the substrate and catalyze its chemical transformation.
• Often, the active site encloses a substrate, sequestering it completely from solution.
• The enzyme substrate complex, whose existence is central to the action of enzymes.
• Enzyme-catalyzed reactions have three basic steps:
• binding of substrate: **E+S↔ES**
• conversion of bound substrate to bound product: **ES ↔ EP**
• release of product : **EP ↔ E+P**



• To understand catalysis, we must first appreciate the important distinction between
• reaction equilibria and
• reaction rates.

Reaction Equilibria
• The function of a catalyst is to increase the rate of a reaction.
• Catalysts *do not affect reaction equilibria*
• Any reaction, such as S ↔ P, can be described by a reaction coordinate diagram
• The free energy of the system is plotted against the progress of the reaction **S → P**.
• A diagram of this kind is a description of the energy changes during the reaction.
• The horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P.



Activation energies, *G‡,*
• The activation energies, *G‡, for* the
• **S → P and P → S** reactions are indicated.
• *G'⁰ is the overall standard* free-energy change in the direction **S → P**

Free-energy change ΔG

• When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force
• the magnitude of which can be expressed as the free-energy change for the reaction, ΔG.

Standard free-energy change, ΔGo

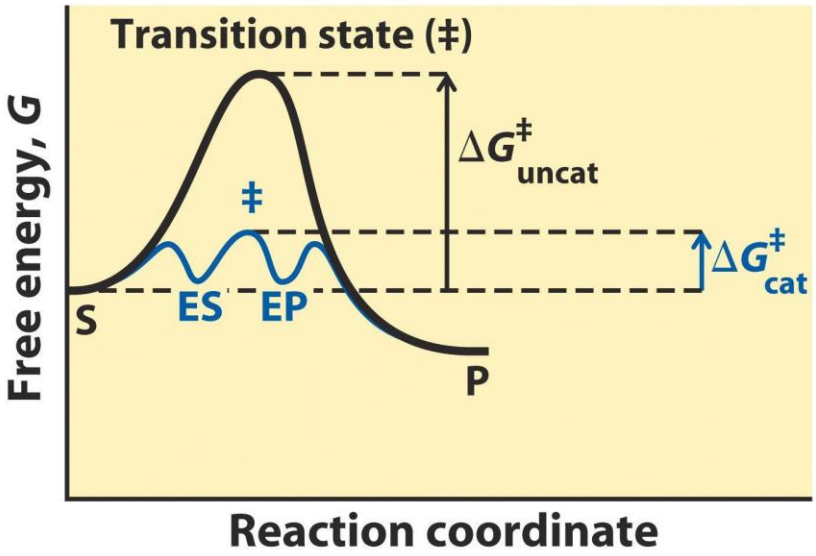
• Under standard conditions (298 K = 25 C)
• when reactants and products are initially present at 1 M concentrations or
• for gases, at partial pressures of 101.3 (kPa), or 1 atm
• the force driving the system toward equilibrium is defined as the standard free-energy change, Δ**Go**
• However, because the conditions in the body systems are different from standard conditions
• energy in biological systems is described in terms of free energy, G'^{0}.

Ground State & Transition State

• The starting point for either the forward or the reverse reaction is called the **ground state.**
• The equilibrium between S and P reflects the difference in the free energies of their ground states.
• The free energy of the ground state of P is **lower** than that of S
• So G'^{0} *for the* reaction is negative and the equilibrium favors P.
• The position and *direction* of equilibrium are *not affected by* any catalyst.
• But there is an **energy barrier** between S and P**:** The energy required for alignment of reacting groups
• formation of transient unstable charges
• bond rearrangements
• and other transformations
• This is illustrated by the energy "hill"
• To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level
• This is called the **transition state.**
• It is simply a fleeting molecular moment in which events such as
• bond breakage,
• bond formation, and
• charge development
• have proceeded to the precise point at which decay to either substrate or product is equally likely.
• The difference between the energy levels of the ground state and the transition state is the **activation energy, *G‡*.**
• At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way)
• A substance that modifies the transition state to lower the activation energy is termed a catalyst; a biological catalyst is termed an enzyme.

Activation energy Δ *G‡*.

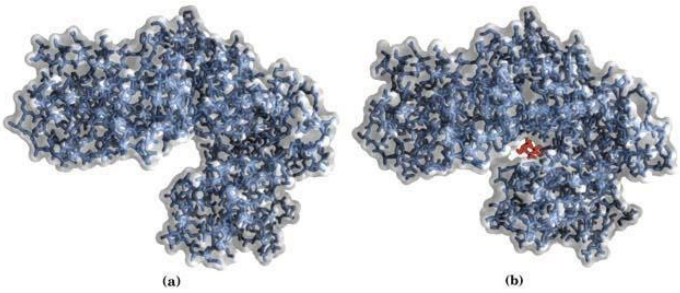
• The difference between the energy levels of the ground state and the transition state is the **activation energy Δ *G‡*.**
• The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction.
• Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier.
• Alternatively, the activation energy can be lowered by adding a catalyst.
• Catalysts enhance reaction rates by lowering activation energies.



• The role of enzymes is to accelerate the interconversion of S and P.
• i.e ***enzymes lower the energy of activation, Δ G‡, of a reaction*****.**
• The enzyme is not used up in the process, and the equilibrium point is unaffected.
• However, the reaction reaches equilibrium much faster when the appropriate enzyme is present,
• because the rate of the reaction is increased.

The Induced Fit Hypothesis

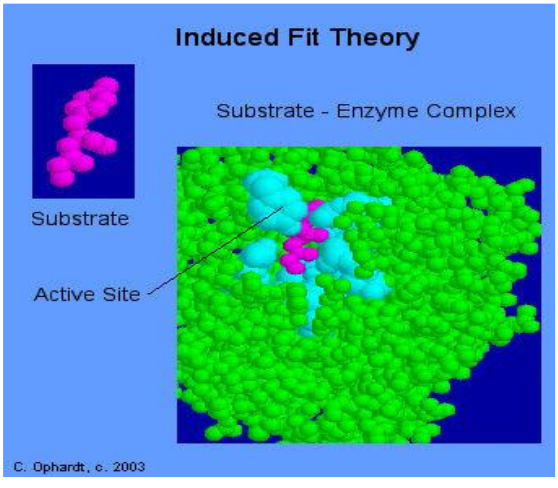
• Some proteins can change their shape (conformation)
• When a substrate combines with an enzyme, it induces a change in the enzyme's conformation
• This change in conformation when the substrate binds is induced by multiple weak interactions with the substrate.
• There may also be rearrangements of covalent bonds during an enzyme-catalyzed reaction.
• This conformational change is referred to as **induced fit.**



• Chemical reactions of many types take place between substrates and enzyme's functional groups (specific amino acid side chains, metal ions, and coenzymes)
• Induced fit serves to bring specific functional groups on the enzyme into the proper position to catalyze the reaction.

Induced Fit Theory

• Enzyme is not rigid, changes shape with substrate.
• The active site is also moulded into a precise conformation
• Making the chemical environment suitable for the reaction



Cofactors, Coenzymes and Prosthetic groups

• Some enzymes require no chemical groups for activity other than their amino acid residues.
• Whereas some enzymes require molecules other than proteins for enzymic activity.
• If the non-protein moiety is a metal ion such as Zn^{2+} or Fe^{2+}, it is called a cofactor.
• If it is a complex organic molecule or metallo-organic compound it is termed a coenzyme.

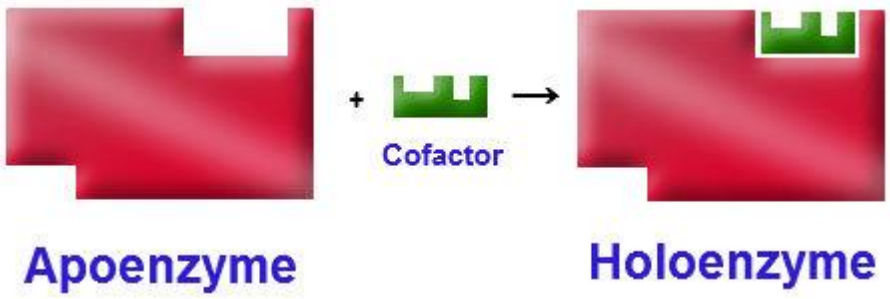
Some Coenzymes That Serve as Transient Carriers

Coenzyme	*Examples of chemical groups transferred*
Biocytin	CO_2
Coenzyme A	Acyl groups
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)

Some Inorganic Elements That Serve as Cofactors for Enzymes

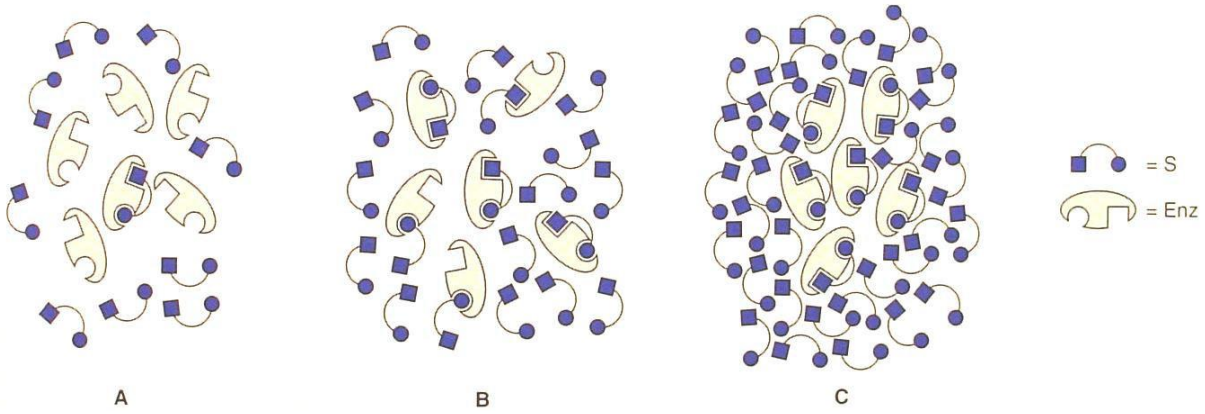
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase

• A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group.
• The term holoenzyme refers to the active enzyme with its non-protein component, whereas the enzyme without its non-protein moiety is termed an apoenzyme (apoprotein) and is inactive.



• Coenzymes serve as recyclable shuttles that transport many substrates from one point within the cell to another.
• The function of these shuttles is twofold.
• First, they stabilize species
• such as hydrogen atoms (FADH) or hydride ions (NADH)
• that are too reactive to persist for any significant time in the presence of the water or organic molecules that permeate cells.
• Second, they serve as an adaptor or handle that facilitates the
• recognition and binding of small chemical groups, such as acetate (coenzyme A) or glucose (UDP), by their target enzymes.

Reaction Rates and Order of Reactions
Reaction Velocity (v)
• The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time;
• Velocity is usually expressed as **µmol of product** formed **per minute**.
• The rate of an enzyme catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached- reflecting the **saturation** with substrate of **all available binding sites are occupied** on the enzyme molecules present.



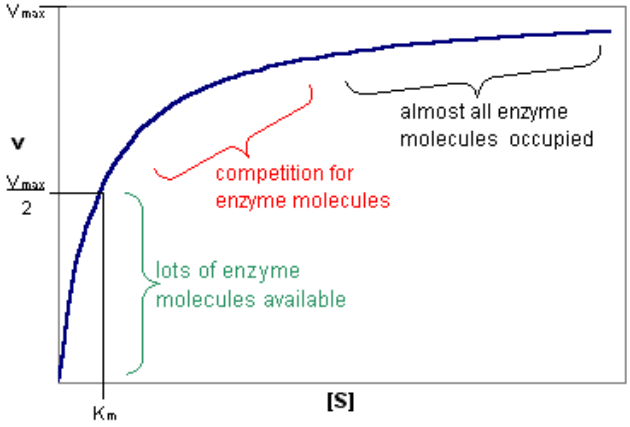
• An equilibrium such as S ↔ P is described by an **equilibrium constant, Keq**, or simply **K.**
• Under the standard conditions used to compare biochemical processes, an equilibrium constant is denoted ***K´eq*** (or ***K´*****).**
• The rate of any reaction is determined by the concentration of the reactant (or reactants) and by a **rate constant,** usually denoted by **k.**
• For the uni molecular reaction S → P, the rate (or velocity) of the reaction, V— representing the amount of S that reacts per unit time—is expressed by a **rate equation:** V = k[S].
• In this reaction, the rate depends only on the concentration of S.
• This is called a first order reaction.
• The factor k is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, and so forth).
• If a reaction rate depends on the concentration of two different compounds.
• or if the reaction is between two molecules of the same compound, the reaction is second order.
• The rate equation then becomes **$V = k[S_1][S_2]$**

Factors Affecting Enzymatic Activity

• Enzymes can be isolated from cells, and their properties studied in a test tube (that is, in vitro).
• Different enzymes show different responses to changes in;
• substrate concentration
• temperature, and
• pH.
• Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells (that is, in vivo).

Substrate Concentration

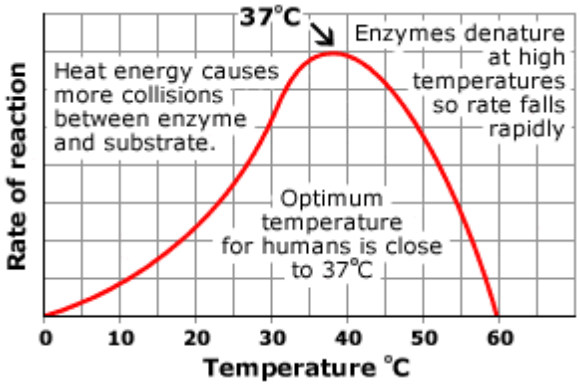
• The rate of an enzyme catalyzed reaction increases with substrate concentration until maximal velocity (Vmax) is reached.
• The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.



Temperature

• The reaction velocity increases with temperature until a peak velocity is reached.

• This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products.



• Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature induced denaturation of the enzyme.

Effect of pH

The pH optimum varies for different enzymes:

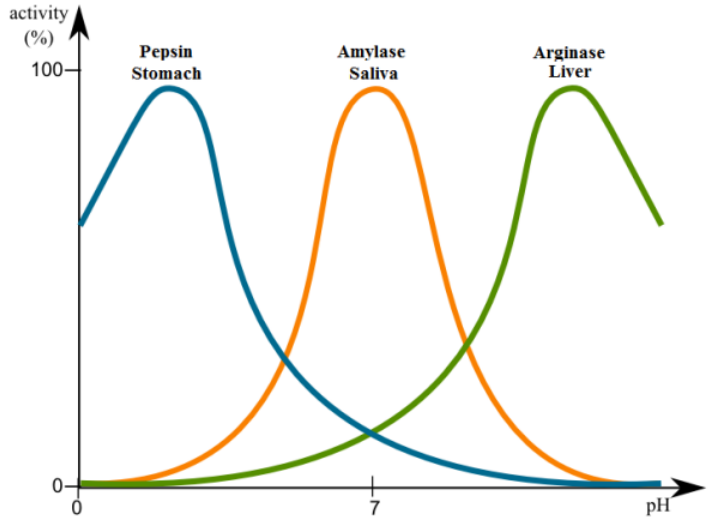
• The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the [H+] at which the enzyme functions in the body.

• For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2.

• Whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment

• Another examples is that there are two types of phosphatases in the body.

• The one that acts in the alkaline pH is called alkaline phosphatase and the other which acts at acidic pH is known as acid phosphatase.



Effect of pH on the ionization of the active site:

• The concentration of H+ affects reaction velocity in several ways.

• First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact.

• For example, catalytic activity may require that an amino group of the enzyme be in the protonated form (–NH3 +).

• At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

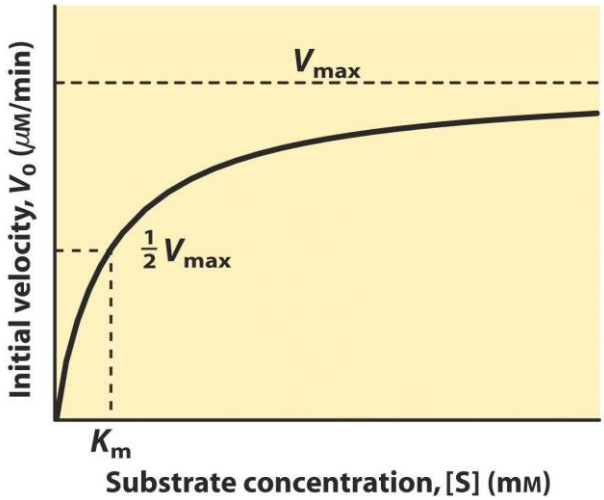
• Extremes of pH can also lead to denaturation of the enzyme.

Enzyme Kinetics

Substrate Concentration Affects the Rate of Enzyme-Catalyzed Reactions

$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$

• A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S].
• However, studying the effects of substrate concentration is complicated because substrate is converted to product and because of reversibility of reactions, e.g. conversion of product back to substrate.
• One simplifying approach in kinetics experiments is to measure the **initial rate (or initial velocity), designated V0,** when [S] is much greater than the concentration of enzyme, [E].
• The velocity (v) of a reaction is the rate of product formation
• Whereas V0 is the initial velocity and is measured as soon as the reactants and enzymes are mixed.
• At the start of a reaction, [S] is in large excess of [P].
• Thus the initial velocity of the reaction will be dependent on substrate concentration.
• At that time, the concentration of product is very small and, therefore, the rate of the back reaction from P to S can be ignored.



• Increase in substrate concentration increases V0 i.e.
• initial velocity is increased whenever a fixed concentration of enzyme is mixed with an increased concentration of substrate.

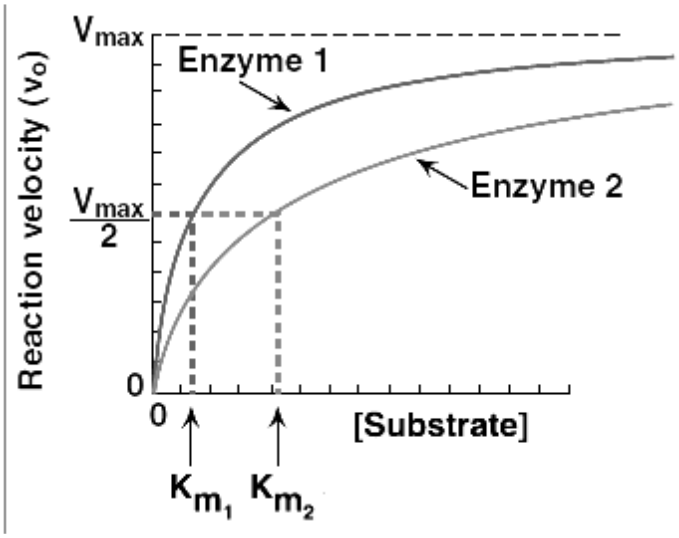
Maximal Velocity Vmax

• When initial velocity **(Vo)** is plotted against [S], a **hyperbolic curve** results, where Vmax represents the maximum reaction velocity.
• At relatively low concentrations of substrate, V_0 increases almost linearly.
• At higher substrate concentrations, *V_0 increases by smaller and smaller amounts in* response to increases in [S].
• This plateau-like *V_0 region is close to the* **maximal velocity, *Vmax.***
• *Vmax is extrapolated from the* plot, because *V_0 approaches but never quite reaches Vmax.*
• At this point in the reaction, if **[S] >> E**, all available enzyme is "saturated" with bound substrate, meaning only the **ES complex** is present.
• This dictates that further increasing the substrate concentration will not result in increased V0
• The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.
• This finite limit of Vmax is called saturation kinetics.
• Saturation kinetics is a characteristic property of all rate processes dependent on the binding of a ligand to a protein e.g. membrane transporter proteins.

Michaelis constant Km

• The substrate concentration at which V_0 is half maximal is Km, the Michaelis constant.
• Km reflects the affinity of the enzyme for the substrate.
• Small Km reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme i.e. to reach a velocity that is 1⁄2Vmax.

• A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.



a. Small Km: A numerically small (low) Km reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is 1⁄2 Vmax

b. Large Km: A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

• The velocity of an enzyme is most sensitive to changes in substrate concentration over a concentration range below its Km.
• At substrate concentrations less than 1⁄10th of the Km, a doubling of substrate concentration nearly doubles the velocity of the reaction
• At substrate concentrations 10 times the Km, doubling the substrate concentration has little effect on the velocity.
• A comparison between the isozymes of hexokinase illustrates the significance of the Km.
• Hexokinase catalyses the first step in glucose metabolism in most cells, the transfer of a phosphate from ATP to glucose to form glucose 6-phosphate.
• Hexokinase I, the isozyme in red blood cells has a low Km for glucose of approximately 0.05 mM- helpful in utilizing blood glucose even when the blood glucose concentration is very low.
• The isozyme of hexokinase, called glucokinase, which is found in the liver has a much higher Km of approximately 5 to 6 mM- helpful in storing large amounts of "excess" glucose as glycogen or converting it to fat after a carbohydrate meal.

Michaelis-Menten equation, the rate equation
• Leonor Michaelis and Maud Menten in 1913, proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.
• They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step: *k1* **E + S ↔ ES** *K-1*

• The ES complex then breaks down in a slower second step to yield the free enzyme (E) and the reaction product (P): *K2* **ES ↔ E + P** *K-2*
• Early in the reaction, the concentration of the product, **[P], is negligible**, and we make the simplifying assumption that the reverse reaction, P → S (described by *k-2*), *can be ignored*
• This assumption is not critical but it simplifies our task.
• The overall reaction then reduces to *k1 K2*
• **E + S ↔ ES → E + P** *K-I*

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P$$

E = Enzyme, S = Substrate, P = Product
ES = Enzyme-Substrate complex
k1 = rate constant for the forward reaction
k-1 = rate constant for the breakdown of the ES to substrate
k2 = rate constant for the formation of the products

• Because the slower second reaction must limit the rate of the overall reaction.
• The overall rate must be proportional to the concentration of the species that react in the second step, that is, ES.
• The maximum initial rate of the catalyzed reaction *(Vmax) is observed when virtually all the* enzyme is present as the ES complex and [E] is vanishingly small.
• Under these conditions, the enzyme is "saturated" with its substrate, so that further increases in [S] have no effect on rate.
• This condition exists when [S] is sufficiently high that essentially all the free enzyme has been converted to the ES form.
• After the ES complex breaks down to yield the product P, the enzyme is free to catalyze reaction of another molecule of substrate.

$$V_o = \frac{Vmax\,[S]}{Km + [S]}$$

Where;
• V_o = initial reaction velocity

• Vmax = maximal velocity
• Km = Michaelis constant = K1+K2 / K-1
• [S] = substrate Concentration

• This is the **Michaelis-Menten equation** statement of the quantitative relationship between the;
• initial velocity V_o,
• *the maximum velocity Vmax, and*
• *the initial substrate concentration [S], all related* through the Michaelis constant *Km*
• An important numerical relationship emerges from the Michaelis-Menten equation in the special case;
• when V_o *is exactly one-half*

$$Vmax\ V_0 = \frac{Vmax\,[S]}{Km + [S]}$$

$$\frac{Vmax}{2} = \frac{Vmax\,[S]}{km + [S]}$$

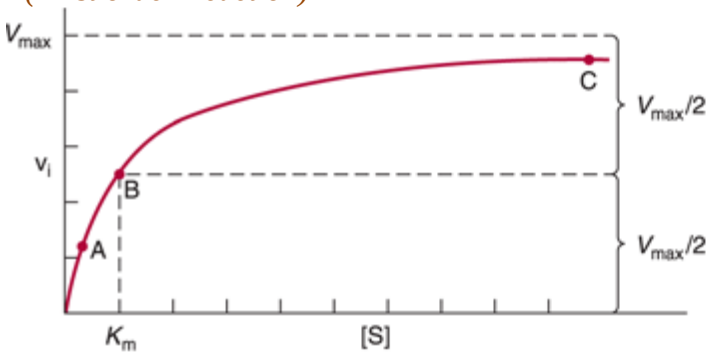
• On dividing by *Vmax, we obtain*
• 1/2 = [S] / Km + [S]
• Solving for *Km, we get*
• *Km + [S] = 2[S], or*
• *Km = [S], when* V_o = 1/2 Vmax
• This is a very useful, practical definition of *Km: Km is* equivalent to the substrate concentration at which V_o *is* one-half *Vmax*.
• The equation describes the kinetic behavior of a great many enzymes, and all enzymes that exhibit a hyperbolic dependence of *V0 on [S] are said to follow* ***Michaelis*-Menten kinetics.**
• The practical rule that *Km= [S] when V0 =1/2Vmax holds for all enzymes* that follow Michaelis-Menten kinetics.
• The most important exceptions to Michaelis-Menten kinetics are the regulatory enzymes.

The Michaelis-Menton Equation at low [S]
• Interpreting Vmax and Km shows a simple graphical method for obtaining an approximate value for *Km.*
• This graph shows the kinetic parameters that define the limits of the curve at high and low [S].
• At low [S], *Km>>[S]* and the *[S]* term in the denominator of the Michaelis-Menten equation becomes insignificant.

• low [S]; Km >> [S]
• $V_0 = \frac{Vmax\,[S]}{km+[S]}$

• $V_0 = \frac{Vmax\,[S]}{km}$

• Since *V*max and *K*m are both constants, their ratio is a constant.
• In other words, when [S] is considerably below *K*m,
• *V0* is proportionate to k[S].
• The initial reaction velocity, *V0* , therefore is directly proportionate to [S].
• *V0* exhibits a linear dependence on [S], *as observed here.*
• *(First order Reaction)*



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition:* http://www.accessmedicine.com

• Therefore at concentrations below Km reaction rate is first order i.e. it is directly proportional to the concentration of the substrate.

The Michaelis-Menton Equation at high [S]
• At high [S] [S] >> K^m
• The term *K*m + [S] is essentially equal to [S].
• *The Km term in the denominator of the Michaelis-* Menten equation becomes insignificant
• Replacing *K*m + [S] with [S] reduces equation
• high [S] [S] >> Km

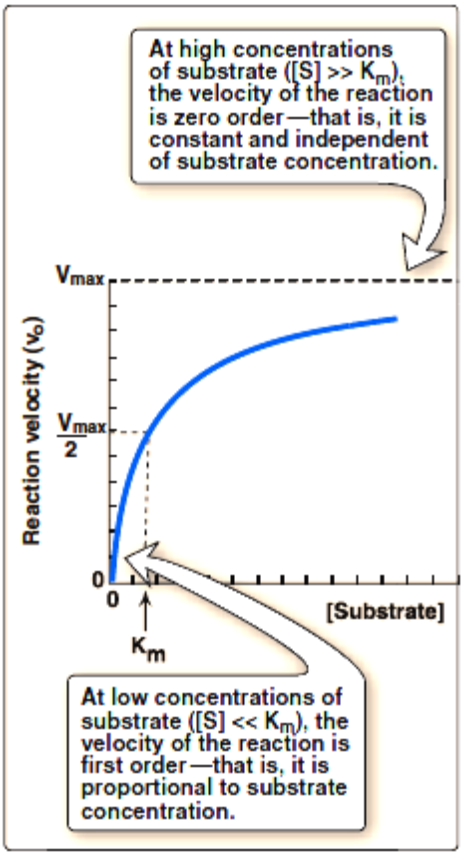
$V_0 = \frac{Vmax\,[S]}{k_m + [S]}$
ignoring k_m

$V_0 = \frac{Vmax\,[S]}{[S]}$

• *V0* = Vmax.
• *This is consistent with the plateau observed at high [S]. (Zero Order Reaction)*
• The rate of reaction is then independent of substrate concentration ***[S],*** and is said to be **zero order** with respect to substrate concentration.
• The Michaelis-Menten equation is therefore consistent with the observed dependence of *V0* on [S], and the shape of the curve is defined by the terms;
• *Vmax/Km at low [S] and*
• *Vmax at high [S].*

Order of Reaction
• When **[S]** is much less, then the velocity of the reaction is approximately proportional to the substrate concentration.
• The rate of reaction is then said to be first order with respect to substrate.
• When **[S]** is much greater than Km the velocity is constant and equal to Vmax.

• The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to Substrate concentration



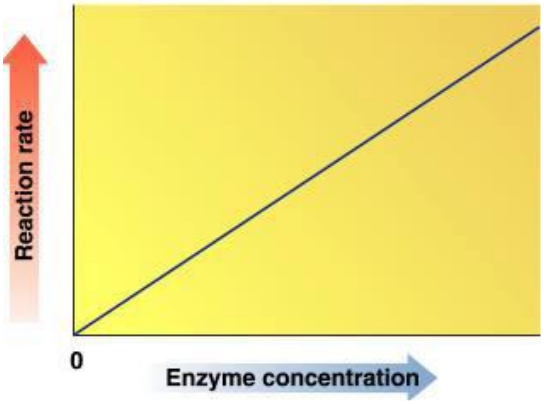
Reaction Orders with Respect to Substrate Concentration

Order	Rate Equation	Comments
Zero	Rate = k	Rate is independent of substrate concentration
First	First rate = k[S]	Rate is proportional to the first power of substrate concentration
Second	Rate = k[S1][S2]	Rate is proportional to the first power of each of two reactants

Relationship of velocity to enzyme concentration

• The rate of the reaction is directly proportional to the enzyme concentration.

• There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration).

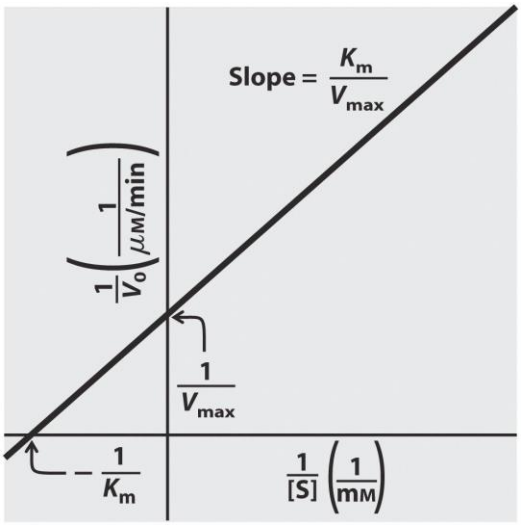


For example,

• if the enzyme concentration is halved, the initial rate of the reaction (V_0) as well as that of Vmax are reduced to one half that of the original.

Lineweaver-Burke plot

• The Michaelis-Menten equation can be algebraically transformed into **Lineweaver-Burke plot,** a *Double Reciprocal Plot*, that is useful in the practical determination of Km and Vmax.



• $V_0 = \frac{Vmax\,[S]}{Km + [S]}$

• Lineweaver-Burke transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

• $\frac{1}{V_0} = \frac{Km + [S]}{Vmax\,[S]}$

• $\frac{1}{V_0} = \frac{Km + [S]}{Vmax\,[S]}$

• $\frac{1}{V_0} = \frac{Km}{Vmax[S]} + \frac{[S]}{Vmax[S]}$

• $\frac{1}{V_0} = \frac{Km}{Vmax[S]} + \frac{1}{Vmax}$

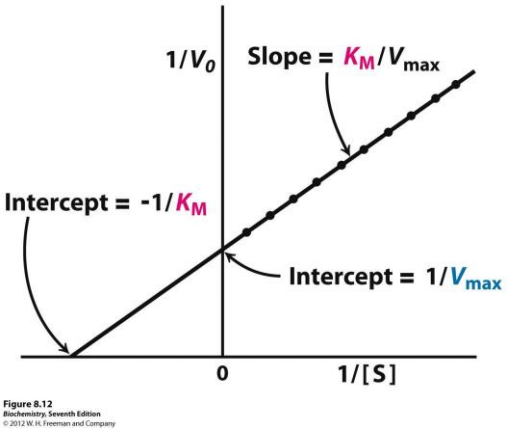
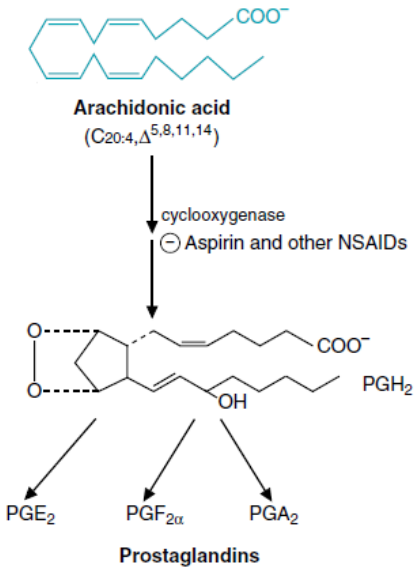


Figure 8.12
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

• Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of *Vmax*,
• *Which can only be approximated from* a simple plot of *V_0 versus [S]*.
• The double-reciprocal plot of enzyme reaction rates is very useful in distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition.

INHIBITION OF ENZYME ACTIVITY

• Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions.
• Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor.
• Enzymes catalyze virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known.



• Inhibitors can be classified on the basis of their site of action on the enzyme,
• on whether they chemically modify the enzyme, or on the kinetic parameters they influence.
• Two broad classes of enzyme inhibitors:

1. Reversible
2. Irreversible.

• In general, irreversible inhibitors bind to enzymes through covalent bonds.
• Reversible inhibitors typically bind to enzymes through non-covalent bonds

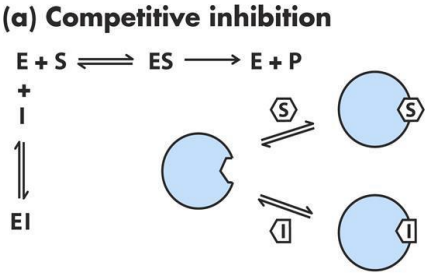
• The two most commonly encountered types of reversible inhibition are;
• competitive and
• noncompetitive.

• Competitive inhibitors resemble the substrate and compete for binding to the active site of the enzyme.
• Noncompetitive inhibitors do not bind at the active site. They bind either free enzyme at a site other than active site or the ES complex.

Competitive Inhibition

• This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy i.e. active site and, therefore, competes with the substrate for that site.

Competitive inhibitors bind to the enzyme's active site.



• Reversible inhibitors bind to enzymes through non covalent bonds.
• Dilution of the enzyme-inhibitor complex results in dissociation of the reversibly bound inhibitor, and recovery of enzyme activity.

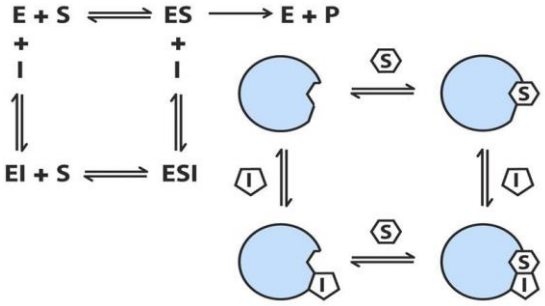
1. Effect on Vmax

• The effect of a competitive inhibitor is reversed by increasing [S].
• At a sufficiently high substrate concentration, the reaction velocity reaches the Vmax as observed in the absence of inhibitor.
• When [S] far exceeds [I], the probability that an inhibitor molecule will bind to the enzyme is minimized and the reaction exhibits a normal *Vmax*.
• Therefore, a competitive inhibitor does not decrease Vmax.

Noncompetitive Inhibition

• Inhibitors bind enzymes at sites distinct from the substrate-binding site and
• generally bear little or no structural resemblance to the substrate.

Noncompetitive inhibitors bind at a separate site, but may bind to either E or ES.



• Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.

1. Effect on Vmax

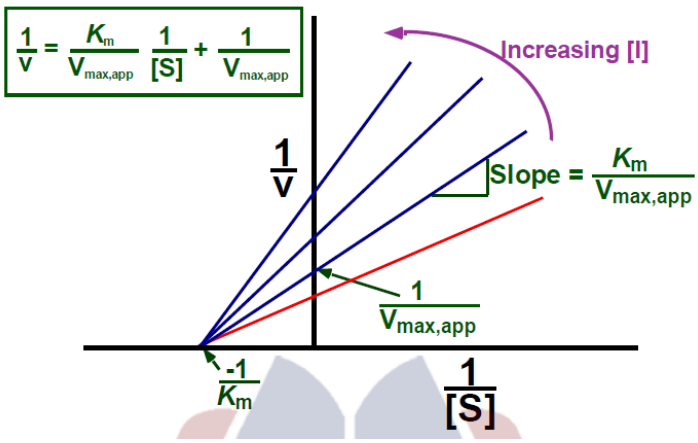
• the apparent Vmax changes, because the inhibitor is capable of preventing catalysis regardless of whether the substrate is bound to the enzyme.
• Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.
• Thus, noncompetitive inhibitors decrease the Vmax of the reaction.
• The noncompetitive inhibitor, in effect, lowers the concentration of the active enzyme and therefore decreases the Vmax of the enzyme.

2. Effect on Km

• Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme.
• Thus, the enzyme shows the same Km in the presence or absence of the noncompetitive inhibitor.

3. Effect on Lineweaver-Burk plot

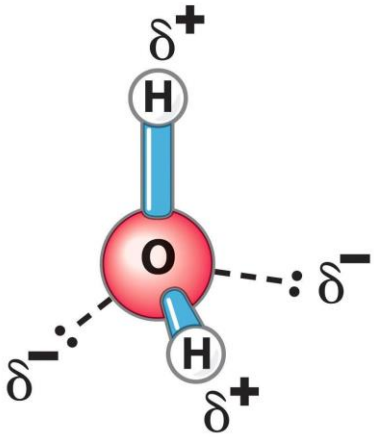
• Noncompetitive inhibition shows a characteristic Lineweaver-Burke plot
• the plots of the inhibited and uninhibited reactions intersect at a single point on the x-axis at Km
• Km is unchanged
• However, the inhibited and uninhibited reactions show; different y axis intercepts indicating the decrease in Vmax in the presence of the competitive inhibitor.



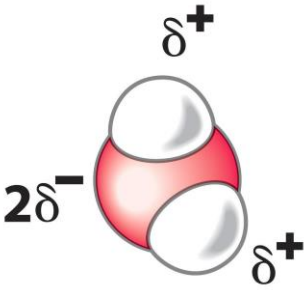
• While certain inhibitors exhibit characteristics of a mixture of competitive and noncompetitive inhibition, the evaluation of these inhibitors exceeds the scope of our level of study.

Water ,pH & Buffer Systems

Water

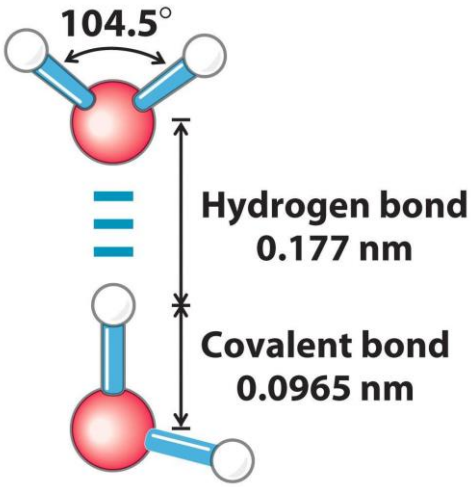


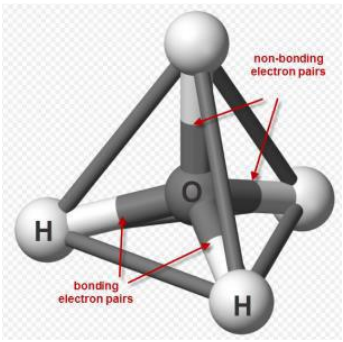
• Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms.
• The hydrogen bonding between water molecules and the slight tendency of water to ionize are of crucial importance to the structure and function of biomolecules
• **Hydrogen Bonding gives Water its Unusual Properties**
• Water has a higher melting point, boiling point, and heat of vaporization than most other common solvents.
• Each hydrogen atom of a water molecule shares an electron pair with the central oxygen atom
• The geometry of the molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom.



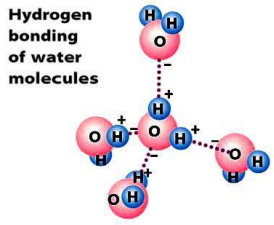
• The result of this unequal electron sharing is two electric dipoles in the water molecule
• Each hydrogen bears a partial positive charge (δ+), and the oxygen atom bears a partial negative charge equal to the sum of the two partial positives (2δ-).

• As a result, there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another, called a hydrogen bond.





• The nearly tetrahedral arrangement of the orbitals about the oxygen atom allows each water molecule to form hydrogen bonds with as many as four neighboring water molecules.



Ionization of Water

• Although many of the solvent properties of water can be explained in terms of the uncharged H2O molecule, the small degree of ionization of water into hydrogen ions (H+) and hydroxide ions (OH-) must also be taken into account

• Pure Water ls Slightly ionized

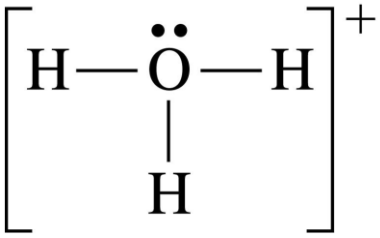
• Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium reaction

• $H2O \leftrightarrow H^+ + OH^-$

• Hydrogen ions formed in water are immediately hydrated to hydronium ions (H3O+)

• The ionization of water can be measured by its electrical conductivity

• Pure water carries electrical current as H3O+ migrates toward the cathode and OH toward the anode



The lonization Of Water ls Expressed By an Equilibrium Constant

• The degree of ionization of water at equilibrium is small

• At 250C only about two out of every 109 molecules in pure water are ionized at any instant

• The equilibrium constant for the reversible ionization of water is

$$K = \frac{[H^+] + [OH^-]}{[H2O\ \ ]}$$

• Since 1 mole (mol) of water weighs 18 g,

• 1 liter (L) (1000 g) of water contains 1000 ÷ 18 = 55.56 mol

• Pure water thus is 55.56 molar(M)

• Accordingly, we can substitute 55.5 M in the equilibrium constant expression to yield

• $Keq = [H^+][OH^-]/55.5$

• $Keq = [H^+][OH^-]/55.5$

• On rearranging, this becomes $(Keq)(55.5)= [H^+][OH^-]= Kw$

• Where Kw designates the product (55.5M)(Keq), the ion product of water at 250C

• The value for Keq, determined by electrical conductivity measurements of pure water, is 1.8 X 10-16 M at 250°C

• $Kw= (Keq)(55.5)= [H^+][OH^-]$

• $Kw = (1.8 \times 10^{-16}M)\ (55.5M)= [H^+][OH^-]$

• $Kw = 1.0 \times 10^{-14}\ M^2= [H^+][OH^-]$

• $Kw= [H^+][OH^-]= [H^+]^2 = [OH-]^2$

• Solving for$[H^+]$ gives:

$[H^+]= \sqrt{Kw} = \sqrt{}\ 10^{-14}\ M^2$

$[H^+]= 10^{-7}\ M$

• Thus the product [H+][OH-] in aqueous solutions at 250°C always equals 1 x 10-14 M2

• When there are exactly equal concentrations of H+ and OH- , as in pure water, the solution is said to be at neutral pH.

• As the ion product of water is constant, whenever [H+] is greater than 1 x 10-7 M, [OH-] must be less than 1 X 10-7 M, and vice versa.

WORKING EXAMPLES

• From the ion product of water we can calculate [H+]

• if we know [OH-], and vice versa

What is the concentration of H+ in a solution of 0.1 M NaOH?

Solution:

Kw= $[H^+][OH^-]$

• With [OH-] = 0.1 M, solving for [H+] gives

• $[H^+] = Kw/[OH^-]$

= 1 × 10-14M2/ 0.1M

= 10-14M2/0.1M

= 10-13M

What is the concentration of OH- in a solution with an H+ concentration of 1.3x10-4M?

• **Solution:**

• Kw= $[H^+][OH^-]$

• With [H+] = 1.3x 10-4M, solving for [OH-] gives

• [OH-] = Kw/[H+]

• = 1x10-14M2/ 1.3x 10-4M

• = **7.7 x 10-11**

The pH Scale

• Designates the H+ and OH- Concentrations

• The **pH** of a solution is defined as the logarithm to the base 10 of the reciprocal of the [H+], i. e, the negative logarithm of the [H+]

• pH = log 1/[H+]

• = -log[H+]

• The pH of water at 25°C, in which H+ and OH– ions are present in equal numbers, is 7.0

• pH = -log[1 x 10-7]= 7

• The symbol p denotes "negative logarithm of" For each pH unit less than 7.0, the [H+] is increased tenfold;

• for each pH unit above 7.0, it is decreased tenfold

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

What will be the pH of 0.1 M HCl?

• Assuming that being a strong acid HCl is completely dissociated, it's 0.1 M solution will contain 0.1 or 10^{-1} grams H^+ per litre

pH= - log [H^+]

pH = - log [10^{-1}]

= - [- 1]

= **1**

Weak Acids and Bases

• Each acid has a characteristic tendency to ionize in an aqueous solution
• The stronger the acid, the greater its tendency ionize i.e. to lose its proton
• This tendency is measured by an acid dissociation constant
• Weak Acids and Bases have Characteristic Acid Dissociation Constants
• HCl, H2SO4, and HNO3, commonly called strong acids, are fully ionized in aqueous solutions
• The strong bases NaOH and KOH are also completely ionized
• Of more interest is the behavior of weak acids and bases-those not completely ionized when dissolved in water.
• These are ubiquitous in biological systems and play important roles in metabolism and its regulation
• Acids may be defined as proton donors and bases as proton acceptors
• A proton donor and its corresponding proton acceptor make up a conjugate acid-base pair

Acetic acid
(CH3COOH), a proton donor, and the acetate anion (CH3COO-), the corresponding proton acceptor, constitute a conjugate acid-base pair, related by thereversible reaction:

CH3COOH ↔ CH3COO- + H+

• The tendency of any acid (HA) to lose a proton and form its conjugate base (A-) is defined by the acid dissociation constant (Ka) for the reversible
reaction

• HA ↔ H+ + A- **;**
• Ka = [H+][A-]/[HA]

• Stronger acids, have larger dissociation constants (Ka) i.e they ionize completely
• Weaker acids, have smaller dissociation constants (Ka) i.e. they ionize only partially.

pKa

• analogous to pH, pKa is defined by the equation
• **pKa = log 1/Ka**
= -logKa
• The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pKa.

Working with pKa

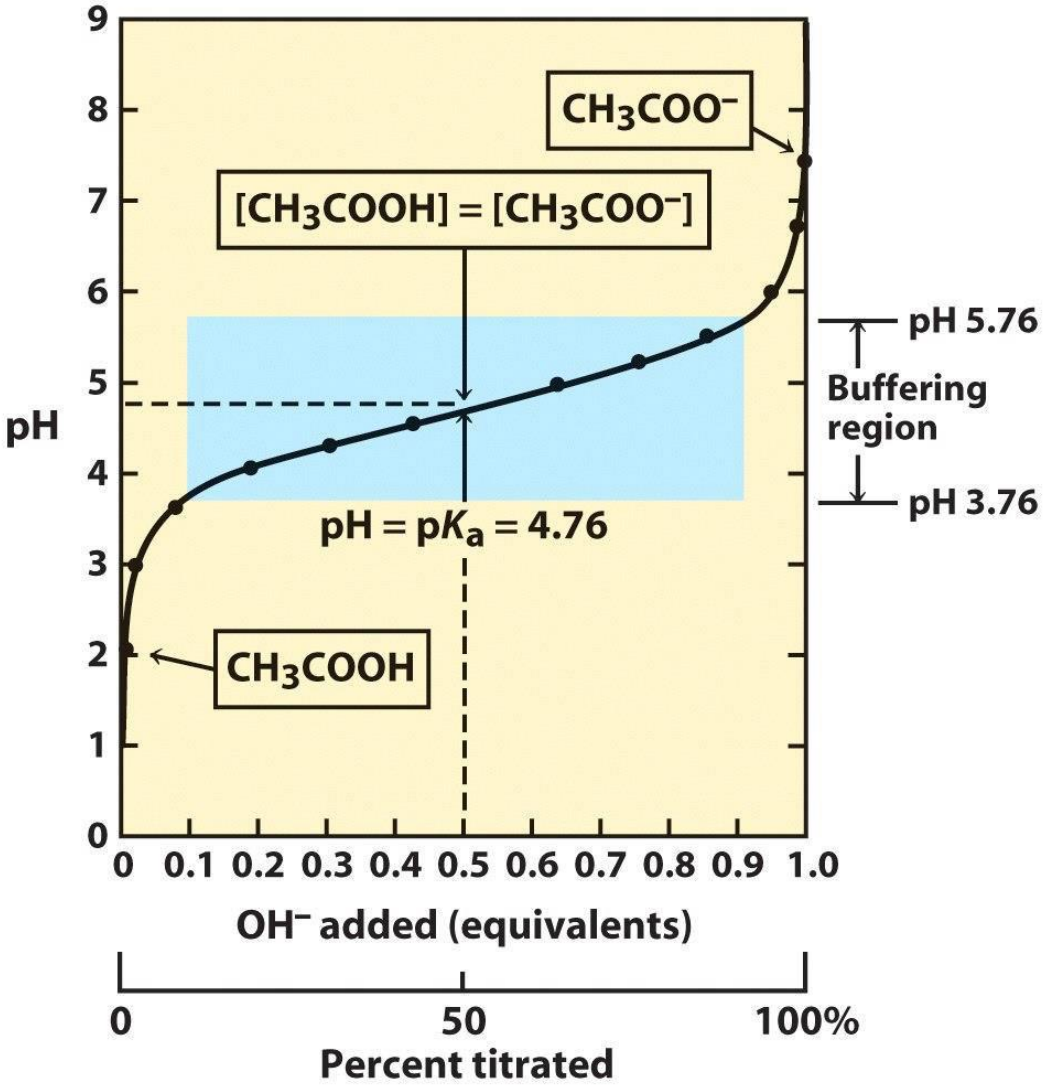
• Titration is used to determine the amountof an acid in a given solution
• A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration

• Consider the titration of a 0.1M solution of acetic acid with 0.1M NaOH at 250C
• Two reversible equilibria are involved in the process (here, for simplicity, acetic acid is denoted HAc)
H2O □□H+ + OH
HAc □□H+ + Ac-

• The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,
• Kw = [H+][OH-]

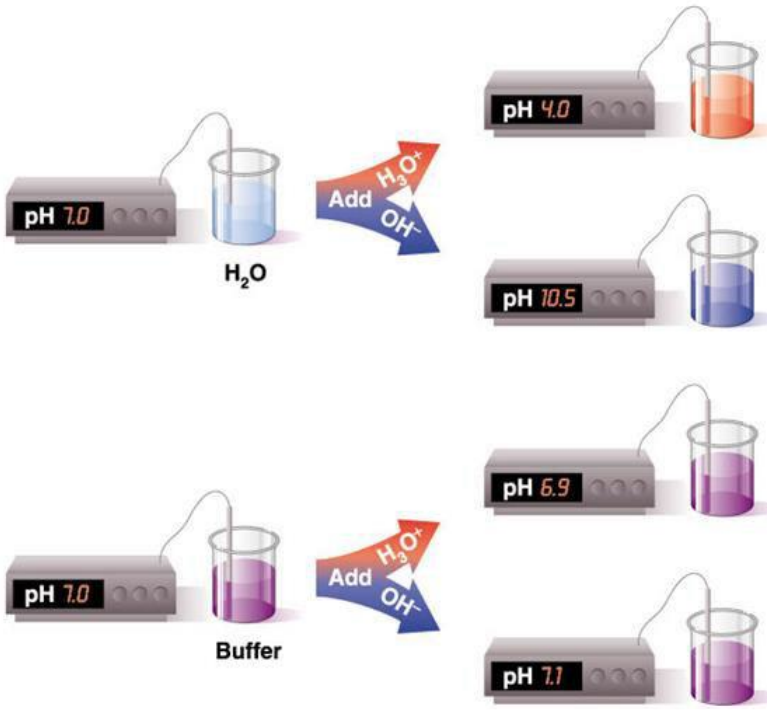
= 1 x 10-14 M2
• Ka = [H+][Ac-]/[HAc]
= 1.74 x 10^{-5} M

• At the beginning of the titration, the acetic acid is only slightly ionized
• As NaOH is gradually added, OH- combines with the free H+ in the solution to form H2O,
• As free H+ is removed, HAc dissociates further to satisfy its equilibrium constant



At the midpoint a very important relationship holds:
• The pH of the equimolar solution of acetic acid and acetate is exactly equal to the pKa of acetic acid (pKa = 4.76)
• At pKa of a weak acid, half of the acid is in dissociated form
• whereas other half is un-dissociated.
• Increasing the pH will result in an increased dissociation and vice versa.

Buffer Solutions
• Buffers are aqueous systems that tend to resist changes in pH when small amounts of acid (H+) or base (OH-) are added.
• A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor).



• As an example, a mixture of ;
• acetic acid and
• acetate ion,
• is a buffer system,

• The pH of the acetate buffer system does change slightly when a small amount of H+ or OH- is added, but this pH change is very small.
• compared with the pH change that would result if the same amount of H+ or OH were added to pure water.
• Each conjugate acid-base pair has a characteristic pH zone in which it is an effective buffer
• For example, the H2PO4-/HPO4 2- pair has a pKa of 6.86 and thus can serve as an effective buffer system between approximately pH 5.9 and pH 7.9

Citric Acid Cycle- (CAC)- 1

Citric Acid Cycle

• Also called Tricarboxylic Acid Cycle (TCA) or Krebs Cycle (after its discoverer, Hans Krebs) .
• Three names for the same thing.
• A series of chemical reactions used by all aerobic organisms to generate energy through the **OXIDATION** of acetyl CoA … derived from carbohydrates, fatty acids and proteins into carbondioxide, water and chemical energy in the form of **ATP**
• Conversion of pyruvate to activated acetate
• Cellular respiration and intermediates for biosynthesis.
• Conversion of **acetate to carbohydrate precursors in the glyoxylate** cycle.

Cellular Respiration

• For most eukaryotic cells and many bacteria, which live under aerobic conditions and oxidize their organic fuels to CO2 and H2O, **glycolysis is the first stage in the complete oxidation of glucose.**
• Rather than being reduced to lactate, ethanol, or some other fermentation product, the pyruvate produced by glycolysis is further oxidized to H2O and CO2. This aerobic phase of catabolism is called **respiration**.
• Biochemists and cell biologists, however, use the term in a narrower sense to refer to the molecular processes by which *cells* consume O2 and produce CO2— processes more precisely termed **cellular respiration**.
• Cellular respiration occurs in three major stages.
• In the first stage, organic fuel **molecules—glucose, fatty acids, and some amino acids** are oxidized to **yield two-carbon fragments** in the form of the **acetyl group of acetyl-coenzyme A (acetyl-CoA).**

• In the second stage, the **acetyl groups are fed into the citric acid cycle**, which enzymatically oxidizes them to CO2; the energy released is conserved in the reduced electron carriers NADH and FADH2.
• In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up **protons (H+) and electrons**. The electrons are transferred to **O2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.**

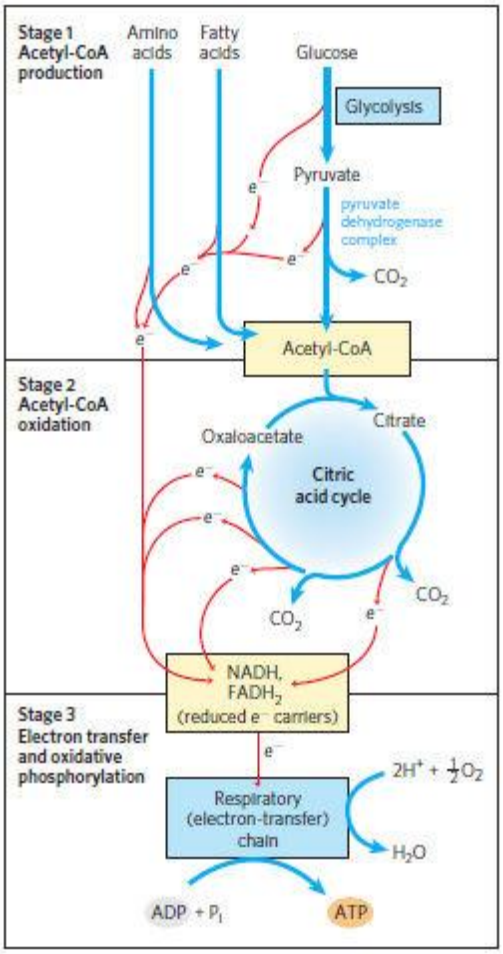


FIGURE 16–1 Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration.

• **Stage 1:** oxidation of fatty acids, glucose, and some amino acids yields acetyl-CoA.
• **Stage 2:** oxidation of acetyl groups in the citric acid cycle includes four steps in which electrons are abstracted.
• **Stage 3:** electrons carried by NADH and FADH2 are funneled into a chain of mitochondrial (or, in bacteria, plasma membrane–bound) electron carriers—the respiratory chain—ultimately reducing O2 to H2O. This electron flow drives the production of ATP.

Production of Acetyl-CoA

• Pyruvate, the product of glycolysis, is converted to acetyl-CoA
• acetyl-CoA is the starting material for the citric acid cycle
• **Enzyme: Pyruvate dehydrogenase complex (PDH complex)**
• The PDH complex is composed of multiple copies of three enzymes:
• Pyruvate dehydrogenase,
• E1, E2 and E3
• Mitochondria of eukaryotes and cytosol of bacteria

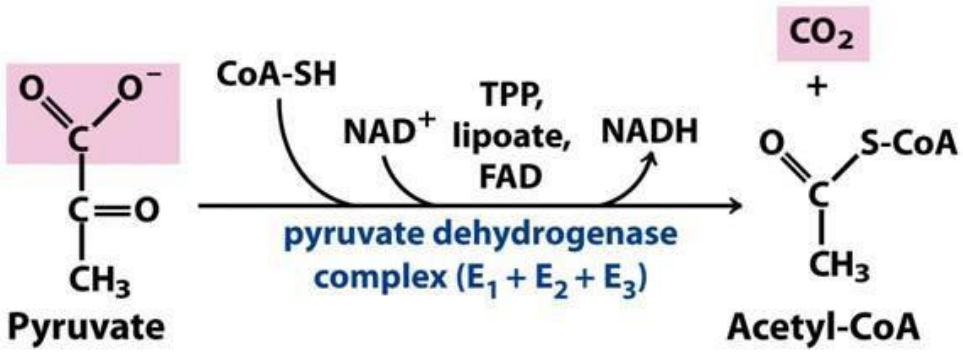
Pyruvate Is Oxidized to Acetyl-CoA and CO_2

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation**

• Irreversible oxidation process in which following things take place

1. the carboxyl group is removed from pyruvate as a molecule of CO2 and the two remaining carbons become the acetyl group of acetyl-CoA
2. The NADH formed in this reaction gives up a hydride ion (**:**H2) to the respiratory chain
3. NADH carries the two electrons to oxygen or, in anaerobic microorganisman alternative electron acceptor such as nitrate or sulfate.
4. The **transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.**

• The irreversibility of the PDH complex reaction has been demonstrated by **isotopic labeling experiments**: the complex cannot reattach radioactively labeled CO2 to acetyl-CoA to yield carboxyl-labeled pyruvate.



$\Delta G'^{\circ} = -33.4$ kJ/mol

Production of Acetyl-CoA-2

The Pyruvate Dehydrogenase Complex (PDH) Requires Five Coenzymes

• The process of conversion of Pyruvate to the acetyl group of acetyl-CoA requires the sequential action of ..

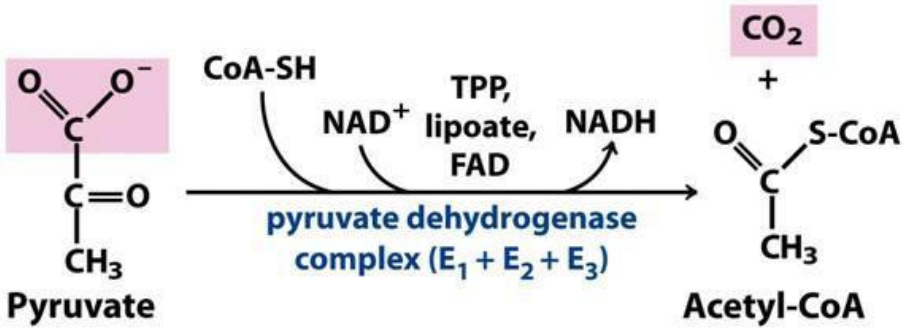
• **three different enzymes**

• **five different coenzymes or prosthetic groups**—

Thiamine pyrophosphate (TPP)
flavin adenine dinucleotide (FAD)
Coenzyme A (CoA, sometimes denoted CoA-SH, to emphasize the role of the OSH group)
Nicotinamide adenine dinucleotide (NAD)
Lipoate

• Four different vitamins required in human nutrition are vital components of this system:
thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA).

• FAD and NAD act as electron carriers ..TPP as the coenzyme of pyruvate decarboxylase



$\Delta G'^{\circ} = -33.4$ kJ/mol

• Five **coenzymes** for the PDH complex

• TPP, FAD, NAD, CoA, lipoate

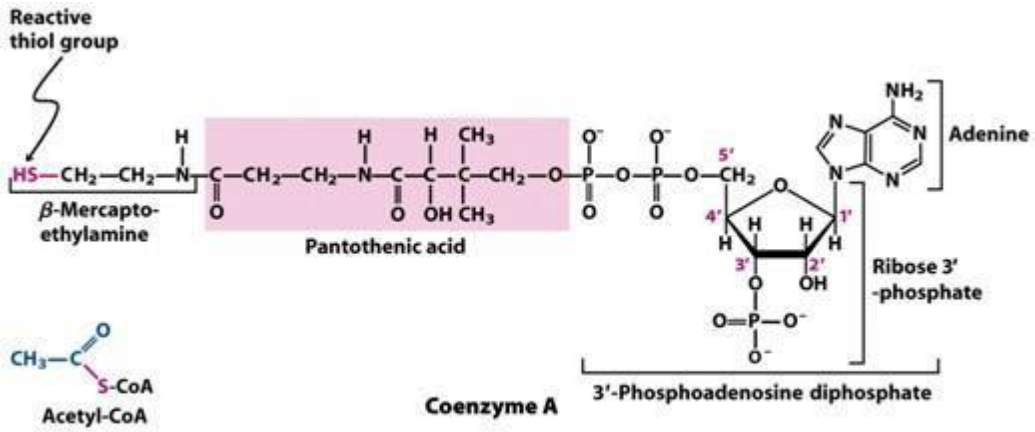


Figure 16-3
Lehninger Principles of Biochemistry, Fifth Edition

• Coenzyme A (CoA). A hydroxyl group of pantothenic acid is joined to a modified ADP moiety by a phosphate ester bond, and its carboxyl group is attached to b-mercaptoethylamine in amide linkage.
The hydroxyl group at the 3' position of the ADP moiety has a phosphoryl group not present in free ADP.
• The —SH group forms a thioester with acetate in acetyl-coenzyme A (acetyl-CoA)

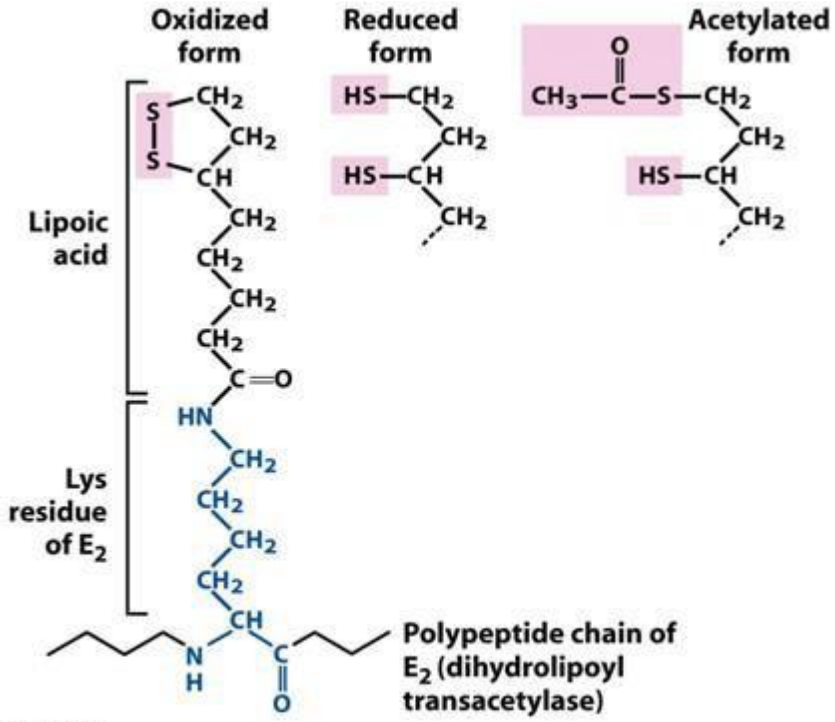


Figure 16-4
Lehninger Principles of Biochemistry, Fifth Edition

• **Lipoic acid (lipoate) in amide linkage with a Lys residue.**
• The lipoyllysyl moiety is the prosthetic group of **dihydrolipoyl transacetylase** (E2 of the PDH complex).
• The lipoyl group occurs in oxidized (disulfide) and reduced (dithiol) forms and acts as a carrier of both hydrogen and an acetyl (or other acyl) group.

Sulfhydryl (-SH group)

• A **sulfhydryl** is a functional **group** consisting of a sulfur bonded to a hydrogen atom. The **sulfhydryl group**, also called a **thiol**, is indicated in **chemistry** nomenclature by "**-thiol**" as a suffix and "mercapto-" or "sulfanyl" as a prefix.
• Thiols have great affinity for soft metals.

Acyl Group

• It contains a double bonded oxygen atom and an alkyl **group**. (R-C=O **group** is called **Acyl Group**)

Acetyl Group

• In organic chemistry, acetyl is a moiety, the acyl with chemical formula CH_3CO.

• (The **acetyl group** contains a methyl **group** single-bonded to a carbonyl).

The Pyruvate Dehydrogenase (PDH) Complex

The PDH Complex Consists of 3 Enzymes

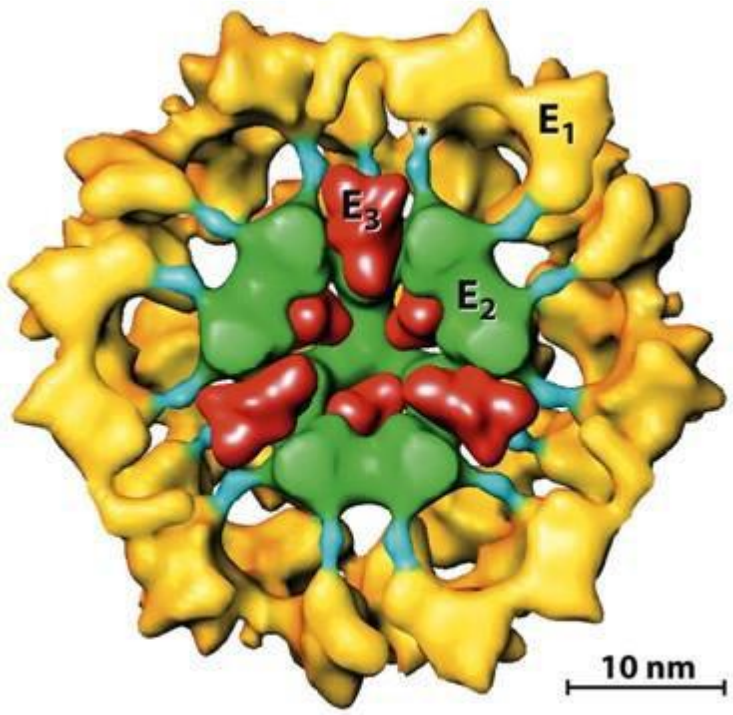
The PDH complex contains three enzymes—

1. Pyruvate dehydrogenase (E1)

2. dihydrolipoyl transacetylase (E2)

3. dihydrolipoyl dehydrogenase (E3)

The PDH complex isolated from mammals is about 50 nm in diameter—more than five times the size of an entire ribosome and big enough to be visualized with the electron microscope



• In the bovine enzyme, 60 identical copies of E2 form a pentagonal dodecahedron **(the core)** with a diameter of about 25 nm • The core of the *Escherichia coli* enzyme contains 24 copies of E2.

• E2 is the point of connection for the prosthetic group lipoate **(blue),** attached through an amide bond to the amino group of a Lys residue …reaches outward to touch the active sites of E1 molecules **(yellow)** arranged on the E2 core.

• The domains of E2 are separated by linkers, sequences of 20 to 30 amino acid residues, rich in Ala and Pro and interspersed with charged residues; these linkers tend to assume their extended forms, holding the three domains apart.

• Several E3 subunits **(red)** are also bound to the core, where the E2 can reach their active sites.

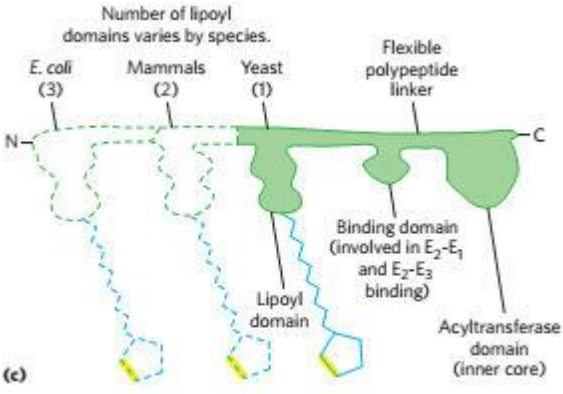
• An **asterisk** marks the site where a lipoyl group is attached to the lipoyl domain of E2.

• To make the structure clearer, about half of the complex has been cut away from the front.

• This model was prepared by Z. H. Zhou and colleagues (2001)

E2 has three functionally distinct domains

1. the amino-terminal ***lipoyl domain***, containing the lipoyl-Lys residue(s)
2. the central E1- and E3-*binding domain*
3. the innercore ***acyltransferase*** *domain,* which contains the acyltransferase active site.

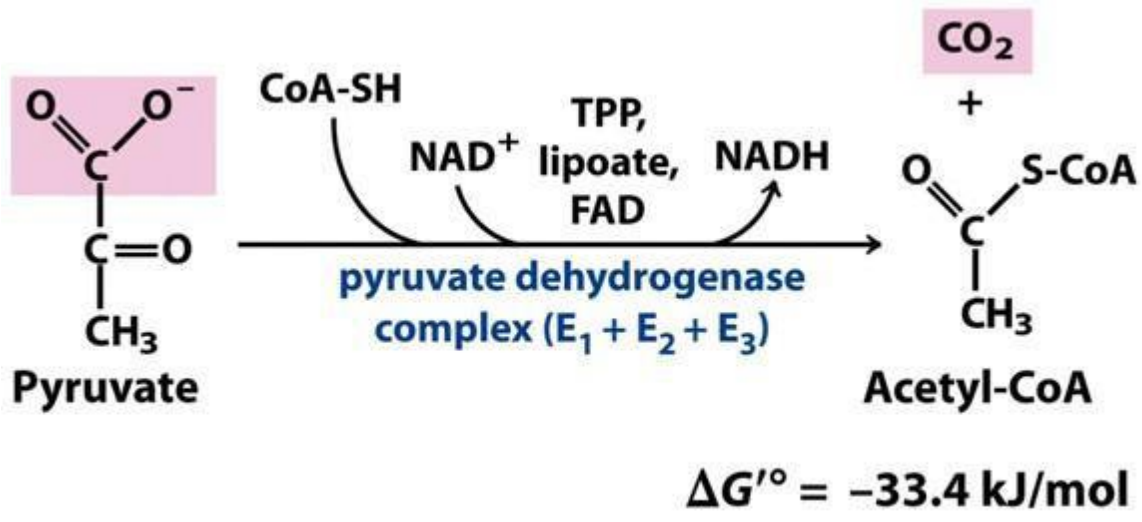


• The yeast PDH complex has a single lipoyl domain with a lipoate attached but the mammalian complex has two, and *E. coli* has three.
• The active site of E1 has bound TPP, and that of E3 has bound FAD.
• Also part of the complex are two regulatory proteins, a protein kinase and a phosphoprotein phosphatase,
• This basic E1–E2–E3 structure has been conserved during evolution and used in a number of similar metabolic reactions.
• The attachment of lipoate to the end of a Lys side chain in E2 produces a long, flexible arm that can move from the active site of E1 to the active sites of E2 and E3, a distance of 5 nm.

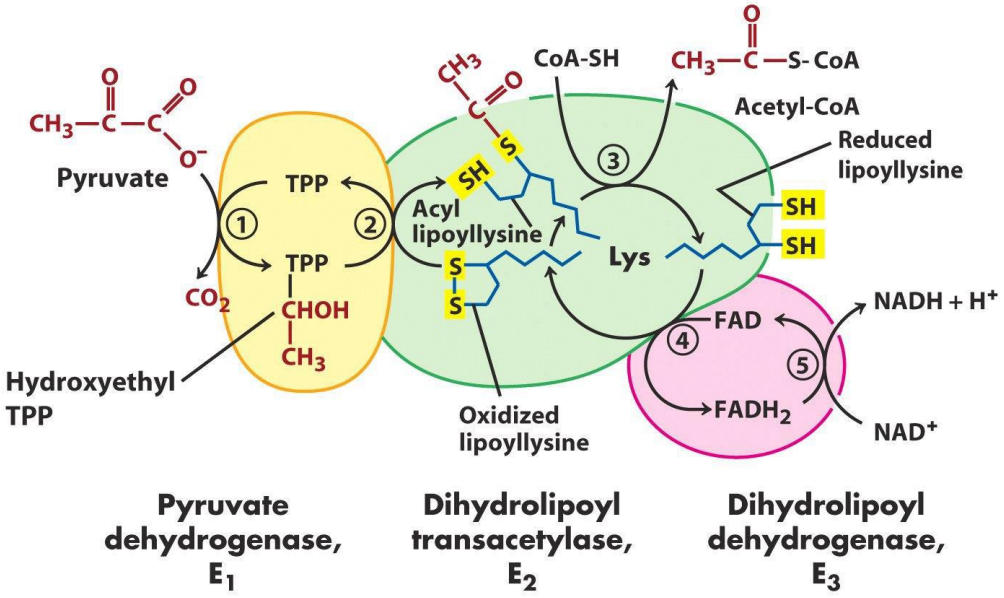
The Coenzymes and prosthetic groups of pyruvate dehydrogenase

Cofactor	Location	Function
Thiamine pyrophosphate	Bound to E1	Decarboxylates pyruvate
Lipoic acid	Covalently linked to a Lys on E2 (lipoamide)	Accepts hydroxyethyl carbanion from TPP
CoenzymeA	Substrate for E2	Accepts acetyl group from lipoamide
FAD (flavin)	Bound to E3	reduced by lipoamide
NADH	Substrate for E3	Reduced by FADH2

The Pyruvate Dehydrogenase (PDH) Complex-2



One Unit of PDH



The PDH Complex

• Pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate.

• **Step 1** is essentially identical to the reaction catalyzed by pyruvate decarboxylase.. C-1 of pyruvate is released as CO2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group.

• This first step is the slowest and therefore limits the rate of the overall reaction.

• It is also the point at which the PDH complex checks its substrate specificity.

• **Step 2** the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate).

• The two electrons removed in this reaction reduce the —S—S— of a lipoyl group on E2 to two thiol (—SH) groups.

• **Step 3** The acetyl group produced in this oxidation-reduction reaction is first transferred into thioester linkage (—SH group), then transesterified to CoA to form acetyl-CoA.

• Thus the energy of oxidation drives the formation of a high-energy thioester of acetate.

• **Steps 4 and 5** The remaining reactions catalyzed by the PDH complex (by E3) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of E2 to prepare the enzyme complex for another round of oxidation.

• The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD+.

Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex.

1. C-1 is released as CO2, C-2 is attached to TPP as a hydroxyethyl group
2. the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group to form the acetyl thioester of the reduced lipoyl group.
3. a transesterification to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group.
4. transfer of two hydrogen to the FAD (lipoyllysyl group gets oxidized)
5. the reduced FADH2 transfers a hydride ion to NAD+ ,forming NADH.

Substrate channeling

Substrate channeling is the passing of the intermediary metabolic product of one enzyme directly to another enzyme or active site without its release into solution.

• The five-reaction sequence is thus an example of **substrate**

channeling.

• **The most important component of PDH complex** are the swinging lipoyllysyl arms of E2, which accept from E1 the two electrons and the acetyl group derived from pyruvate, passing them to E3.

• All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex.

• The intermediates of the multistep sequence never leave the complex, and the local concentration of the substrate of E2 is kept very high.
• Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate.
• A similar tethering mechanism for the channeling of substrate between active sites is used in some other enzymes, with lipoate, biotin, or a CoA-like moiety serving as cofactors.

Acetyl-CoA enters Citric Acid Cycle

Citric Acid Cycle-8 enzymes

• **Acetyl CoA** enters TCA and undergoes oxidation.
• acetyl-CoA donates its acetyl group to the four-carbon compound **oxaloacetate** to form the six-carbon citrate.
• Citrate is then transformed into **isocitrate,** also a six-carbon molecule
• Isocitrate is dehydrogenated with loss of CO2 to yield the five-carbon compound **a-ketoglutarate** (also called oxoglutarate).
• *a*-Ketoglutarate undergoes loss of a second molecule of CO2 and yields the **four-carbon compound succinate.**
• Succinate is then enzymatically converted in three steps into the **four-carbon oxaloacetate**
• oxaloacetate is ready to react with another molecule of acetyl-CoA.
• In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO2 leave.
• one molecule of oxaloacetate (OAA) is used to form citrate and one molecule of oxaloacetate is regenerated. No net removal of oxaloacetate occurs
• **Four of the eight steps in this process are oxidations,** in which the energy of oxidation is very efficiently conserved in the form of the **reduced coenzymes NADH and FADH2**.
• In eukaryotes, cycle takes place in mitochondria - the site of most energy- yielding oxidative reactions and of the coupled synthesis of ATP
• In prokaryotes cycle takes place in the cytosol, plasma membrane plays a role analogues to that of inner mitrochondrial membrane in ATP synthesis

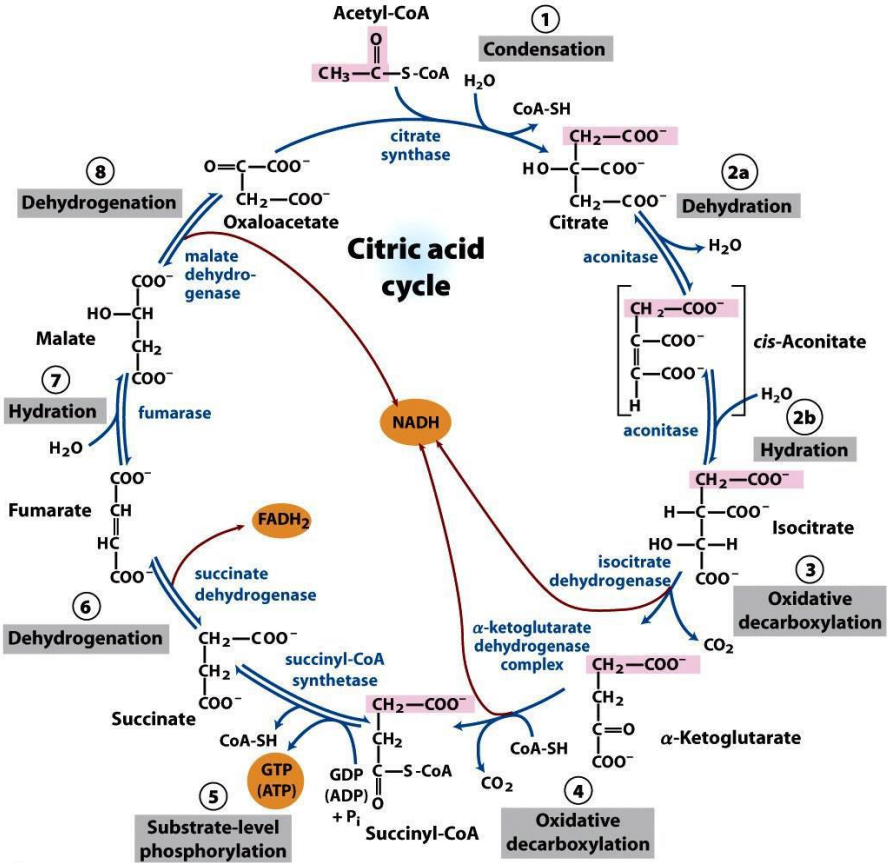


FIGURE 16-7 Reactions of the citric acid cycle. The carbon atoms shaded in pink are those derived from the acetate of acetyl-CoA in the first turn of the cycle; these are *not* the carbons released as CO2 in the first turn. Note that in **succinate and fumarate**, the two-carbon group derived from acetate can no longer be specifically denoted; because succinate and fumarate are symmetric molecules, C-1 and C-2 are indistinguishable from C-4 and C-3. The red arrows show where energy is conserved by electron transfer to FAD or NAD+, forming FADH2 or NADH + H+. Steps 1, 3, and 4 are essentially irreversible in the cell; all other steps are reversible. The product of step 5 may be either ATP or GTP, depending on which succinyl-CoA synthetase isozyme is the catalyst.

Products

• Products of the first turn of the cycle are one GTP (or ATP), three NADH, 1FADH2 and two CO2.
• Because two acetyl-CoA are produced from each GLUCOSE molecule, two cycles are required per glucose molecule.
• Therefore, at the end of two cycles, the products are: two GTP, six NADH, two $FADH_2$, and four CO2.

	Substrates	Products	Enzyme	Reaction type	Comment
1.	Oxaloacetate + Acetyl CoA + H2O	Citrate + CoA-SH	Citrate synthase	Aldol Condensation	irreversible, extends the 4C oxaloacetate to a 6C molecule
2.	Citrate	*cis*-Aconitate + H2O	Aconitase	Dehydration	reversible isomerization
3.	*cis*-Aconitate + H2O	Isocitrate		Hydration	
4.	Isocitrate + NAD+	Oxalosuccinate + NADH + H +	Isocitrate Dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
5.	Oxalosuccinate	α-Ketoglutarate + CO2		Decarboxylation	rate-limiting, irreversible stage, generates a 5C molecule
6.	α-Ketoglutarate + NAD+ + CoA-SH	Succinyl-CoA + NADH + H+ + CO2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)
7.	Succinyl-CoA + GDP + Pi	Succinate + CoA-SH + GTP	Succinyl-CoA Synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP,[12] generates 1 ATP or equivalent Condensation reaction of GDP + Pi and hydrolysis of Suc cinyl-CoA involve the H2O needed for balanced equation.
8.	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH2)	Succinate Dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→FADH2i n the first step of

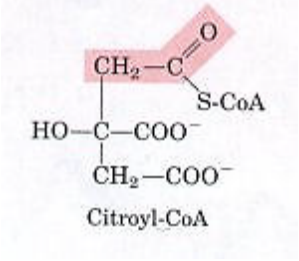
					the reaction) in the enzyme,[12] generates the equivalent of 1.5 ATP
9.	Fumarate + H2O	*L*-Malate	Fumarase	Hydration	Hydration of C-C double bond
10.	*L*-Malate + NAD+	Oxaloacetate + NADH + H+	Malate Dehydrogenase	Oxidation	reversible (in fact, equilibrium favors malate), generates NADH (eq uivalent of 2.5 ATP)
11.	Oxaloacetate + Acetyl CoA + H2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	This is the same as step 0 and restarts the cycle. The reaction is irreversible and extends the 4C oxaloacetate to a 6C molecule

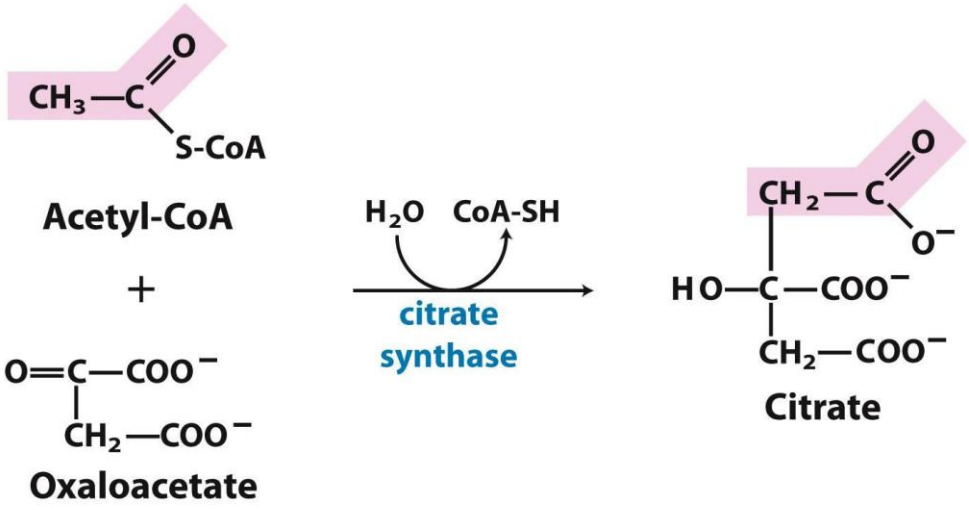
1.In each turn of the cycle, on acetyl group enters as acetyl-CoA and two Co2 leave; 1 OAA used and 1 OAA generated; NADH and FADH2, GTP or ATP
2.Four or five carbon intermediate serve as precursor of biomolecule
3.In eucaryotes, cycle takes place in mitochondria - the site of most energy yielding oxidative reactions and of the coupled synthesis of ATP
3. In prokaryotes. cycle are in the cytosol, plasma memb. plays a role analogues to that of inner mitrochondrial membrane in ATP synthesis

Steps of Citric Acid Cycle-1

1. Formation of Citrate (The Citrate Synthase Reaction)

• The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**
• The methyl carbon of the acetyl group is joined to the carbonyl group (C-2) of oxaloacetate.
• Citroyl-CoA is a transient intermediate formed on the active site of the enzyme …It rapidly undergoes hydrolysis to release CoA and citrate
• The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic.
• The CoA **liberated** in this reaction is recycled to participate in the oxidative decarboxylation of another molecule of pyruvate by the PDH complex.



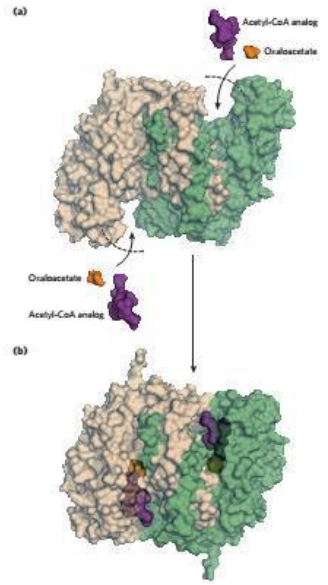


$$\Delta G'^{\circ} = -32.2\ \text{kJ/mol}$$

• Formation of citrate: the condensation of acetyl-CoA with oxaloacetate. -Methyl carbon of acetyl group is joined to the **carbonyl group** (C-2) of OAA
• The hydrolysis of high-energy thioester makes the forward reaction
• Citrate sythase: homodimeric enzyme; induce conformational change by OAA binding, creating binding site for acetyl-CoA; when citroyl- CoA has formed, another conformational change brings about thioester hydorlysis; ordered bisubstrate mechanism
• **The only cycle reaction with C-C bond formation**
• **Essentially irreversible process**

The Citrate Synthase

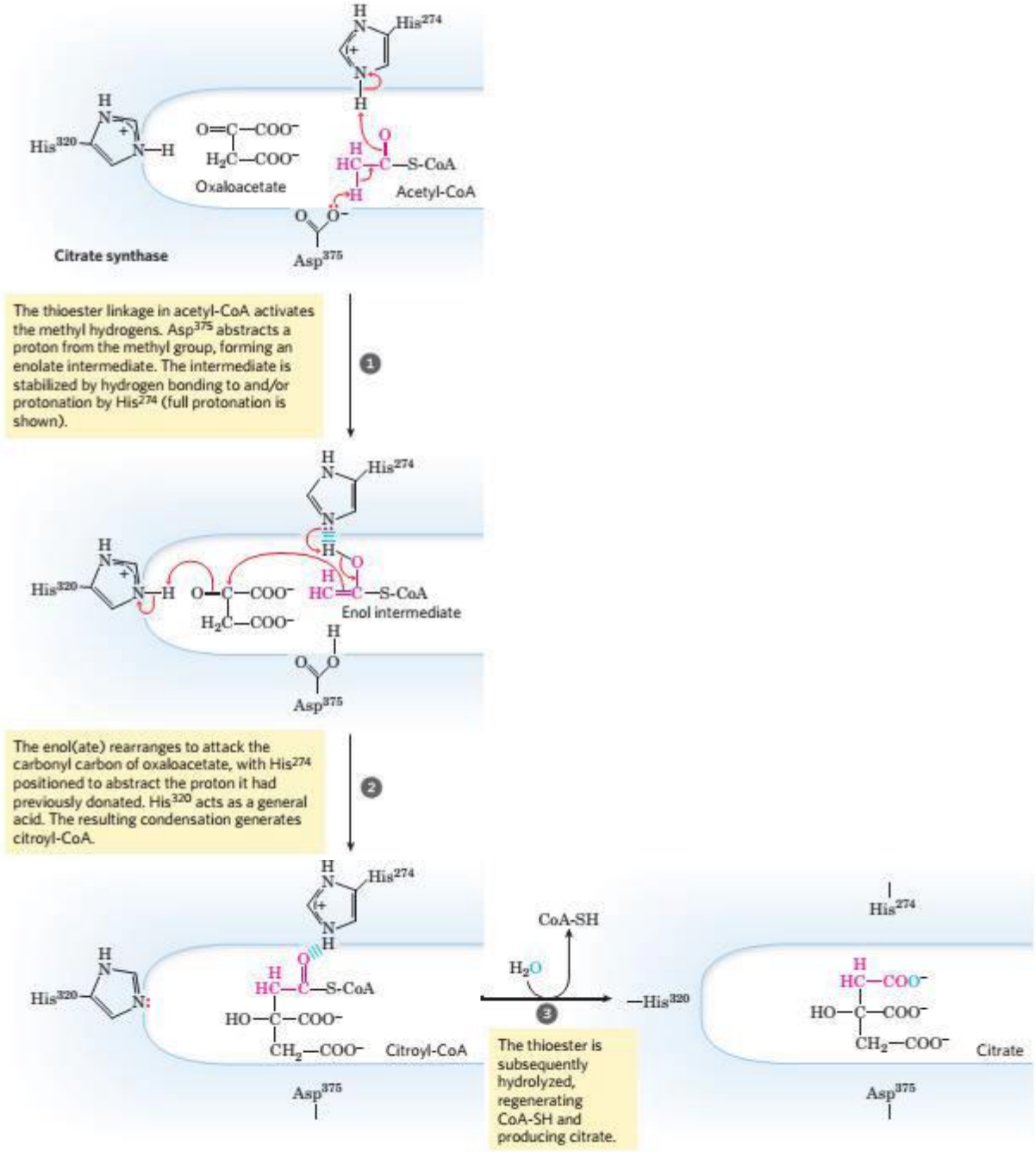
• Citrate synthase : the homodimeric enzyme
In these representations one subunit is colored tan and one green.
• Each subunit is a single polypeptide **with two domains, with the active site between them.**
1. large and rigid,
2. smaller and more flexible
a) Open form of the enzyme alone
b) closed form with bound oxaloacetate and a stable analog of acetyl-CoA
• The flexible domain of each subunit undergoes a large conformational change on binding oxaloacetate, creating a binding site for acetyl-CoA.



Structure of Citrate Synthase

Ordered bisubstrate mechanism of Citrate synthase.

• In the mammalian citrate synthase reaction, oxaloacetate binds first, in a strictly **ordered reaction sequence**.
• This binding triggers a conformation change that opens up the binding site for acetyl-CoA.
• Oxaloacetetate is specifically oriented in the active site of citrate synthase by interaction of its two carboxylates with two positively charged Arg residues



• Ordered bisubstrate mechanism of Citrate synthase.

• **Oxaloacetate, the first substrate** to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the **second substrate, acetyl-CoA**.
• When citroyl-CoA has formed in the enzyme active site..Another conformational change brings about thioester hydrolysis, releasing CoA-SH. This induced fit of the enzyme first to its substrate and then to its reaction intermediate decreases the likelihood of premature and unproductive cleavage of the thioester bond of acetyl-CoA.

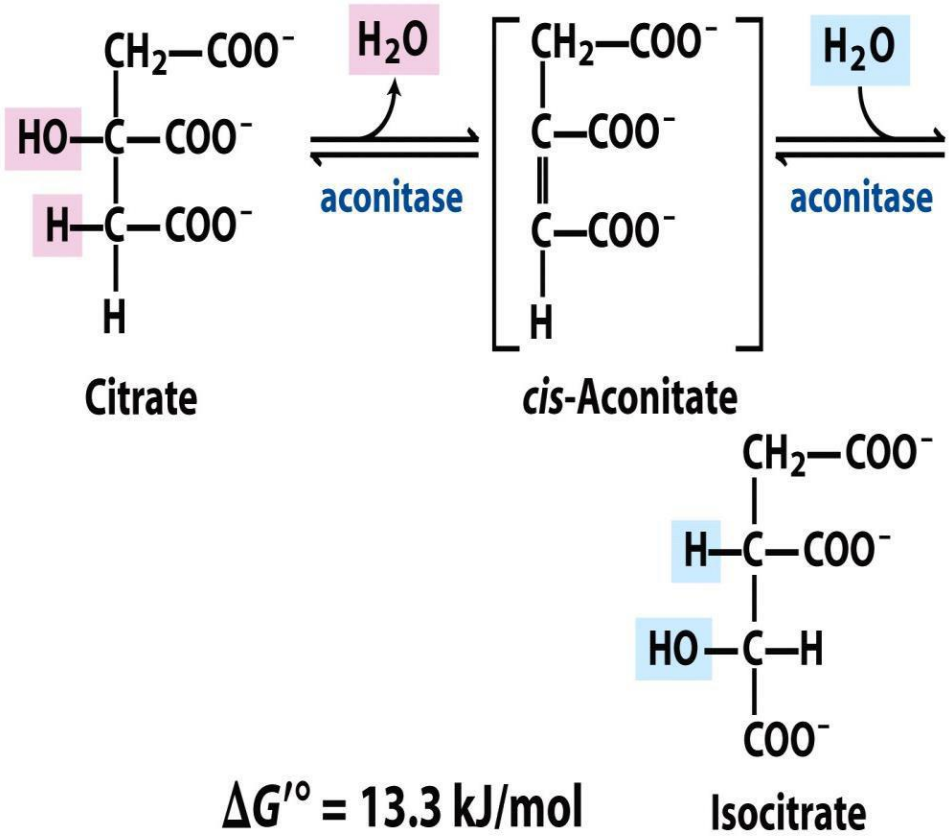
The Citrate Synthase mechanism

• **Oxaloacetate, the first substrate** to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the **second substrate, acetyl-CoA**.
• When citroyl-CoA has formed in the enzyme active site..Another conformational change brings about thioester hydrolysis, releasing CoA-SH. This induced fit of the enzyme first to its substrate and then to its reaction intermediate decreases the likelihood of premature and unproductive cleavage of the thioester bond of acetyl-CoA.
• Kinetic studies of the enzyme are consistent with this ordered bisubstrate mechanism The reaction catalyzed by citrate synthase is essentially a Claisen condensation involving a thioester (acetyl-CoA) and a ketone (oxaloacetate)

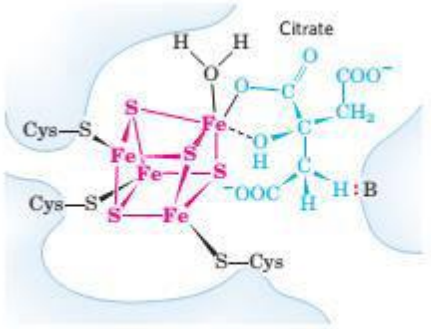
Steps of Citric Acid Cycle-2

2. Isomerization of Citrate by Aconitase

• The enzyme **aconitase** (**aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**
• Reaction Intermediate of the tricarboxylic acid is formed..known as ***cis*-aconitate**
• ***cis*-aconitate does not dissociate from the active site**
• **Aconitase can promote the reversible addition of H2O to the double bond** of enzyme-bound *cis*-aconitate in two different ways, **one leading to citrate and the other to isocitrate**
• Under physiological conditions in the cell.. Conc. Of isocitrate is very little , the reaction is pulled to the right because isocitrate is rapidly consumed in the next step of the cycle



• Aconitase contains an **iron sulfur center** which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H2O.
• In iron-depleted cells, aconitase loses its iron-sulfur center and acquires a new role in the regulation of iron homeostasis.
• **Aconitase is one of many enzymes known to "moonlight" in a second role (protein with 2 jobs)**



Iron-sulfur center in aconitase.

• The iron-sulfur center is in red, the citrate molecule in blue. **Three Cys residues of the enzyme** bind three iron atoms

• the fourth iron is bound to one of the carboxyl groups of citrate and also interacts noncovalently with a hydroxyl group of citrate (dashed bond).

• A basic residue (**:**B) in the enzyme helps to position the citrate in the active site.

• The iron-sulfur center acts in both substrate binding and catalytic site.

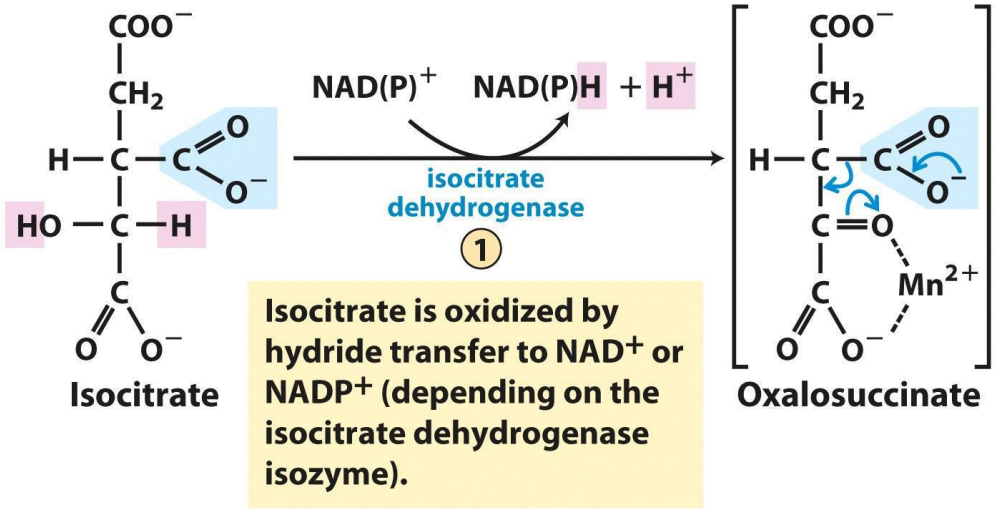
3. The Isocitrate Dehydrogenase Reaction

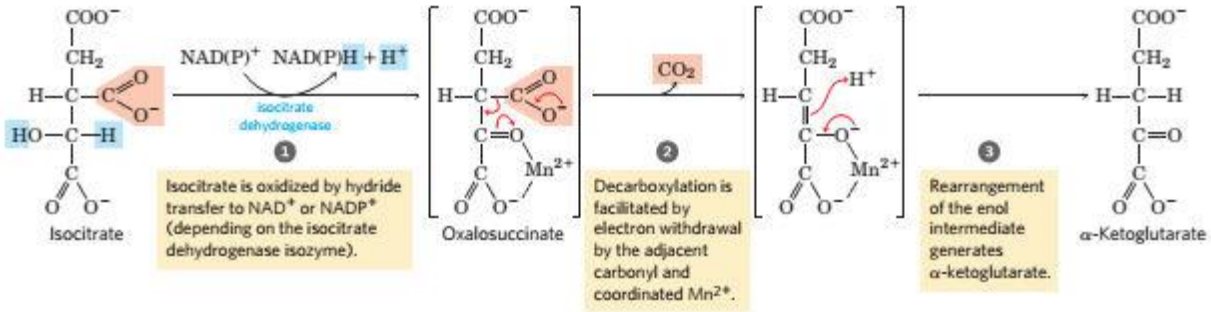
• Oxidation of Isocitrate to *a*-Ketoglutarate and CO2 In the next step

• **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form ***a*ketoglutarate**

• **Mn2+ in the active site interacts with the carbonyl group of the intermediate** oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to *a*-ketoglutarate.

• Mn2+ also stabilizes the enol formed transiently by decarboxylation.





• **Isocitrate dehydrogenase reaction.**

• **In this reaction, the substrate, isocitrate, loses one carbon by oxidative decarboxylation**

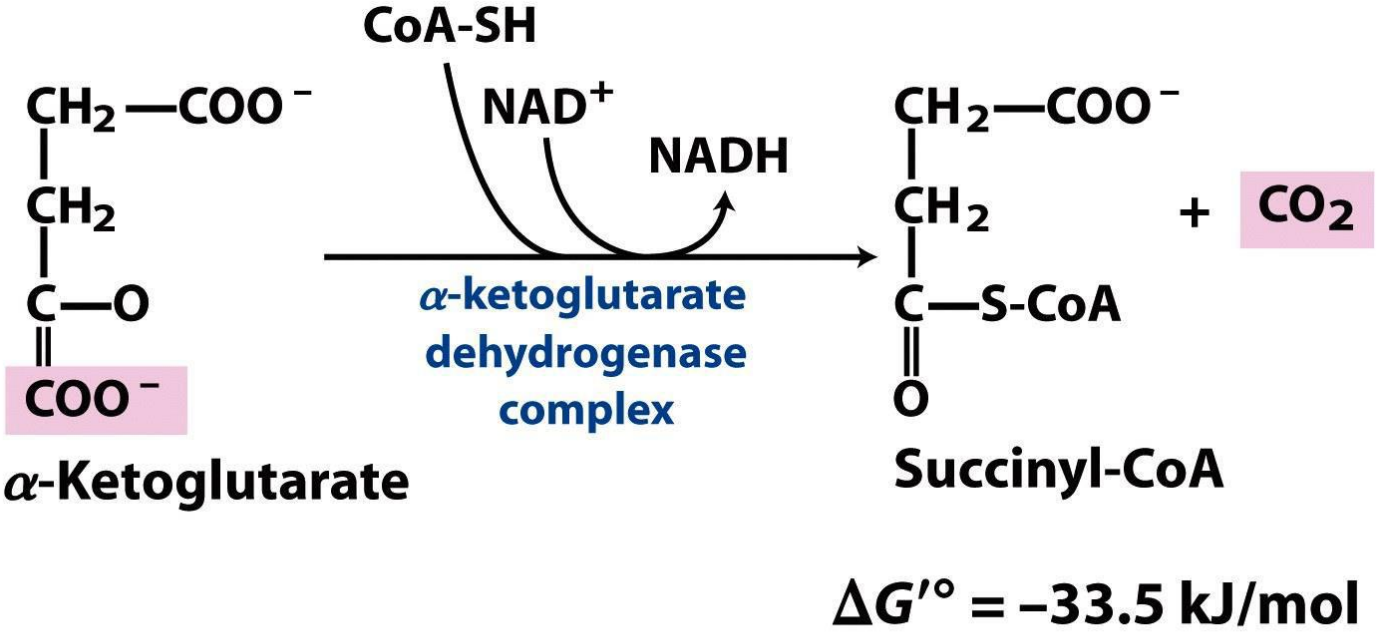
Two different forms of isocitrate dehydrogenase (Isozymes)

• There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD+ as electron acceptor and the other requiring NADP+.
• The overall reactions are otherwise identical
• In eukaryotic cells, the NAD-dependent enzyme occurs in the mitochondrial matrix and serves in the citric acid cycle.
• The main function of the NADP-dependent enzyme, found in both the mitochondrial matrix and the cytosol, may be the generation of NADPH, which is essential for reductive anabolic reactions

Steps of Citric Acid Cycle-3

4. Oxidation of α-ketoglutarate

• **The next step is another oxidative decarboxylation**
• Oxidation of *a*-Ketoglutarate to Succinyl-CoA and CO2
• a-ketoglutarate is converted to **succinyl-CoA** and CO2 by the action of the **a-ketoglutarate dehydrogenase complex**
• NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.
• The energy of oxidation of *a*-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA
• This reaction is somehow identical to the pyruvate dehydrogenase reaction and to the reaction sequence responsible for the breakdown of branched chain amino acids



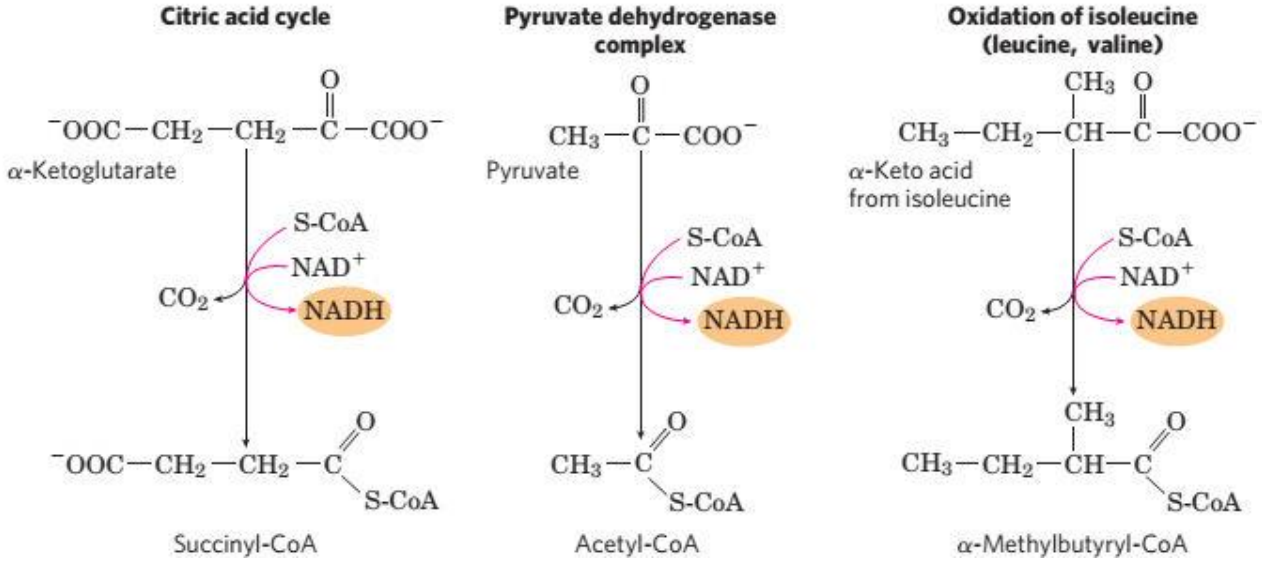
a-ketoglutarate dehydrogenase complex resembles PDH complex

• The a-ketoglutarate dehydrogenase complex closely resembles the PDH complex in both structure and function.
• It includes three enzymes, homologous to E1, E2, and E3 of the PDH complex, as well as enzyme-bound TPP, bound lipoate, FAD, NAD, and coenzyme A.
• Both complexes are certainly derived from a common evolutionary ancestor.
• **Although the E1 components of the two complexes are structurally similar, their amino acid sequences differ and, they have different binding specificities**
• E1 of the PDH complex binds pyruvate, and E1 of the *a*-ketoglutarate dehydrogenase complex binds *a*-ketoglutarate.
• The E2 components of the two complexes are also very similar, both having covalently bound lipoyl moieties.

• The subunits of E3 are identical in the two enzyme complexes.
• The enzymatic complex that degrades branched-chain *a*-keto acids catalyzes the same reaction sequence using the **same five cofactors**.
• This is a clear case of **divergent evolution**, in which the genes for an enzyme with one substrate specificity give rise, during evolution, to closely related enzymes with different substrate specificities but the same enzymatic mechanism.

A conserved mechanism for oxidative decarboxylation

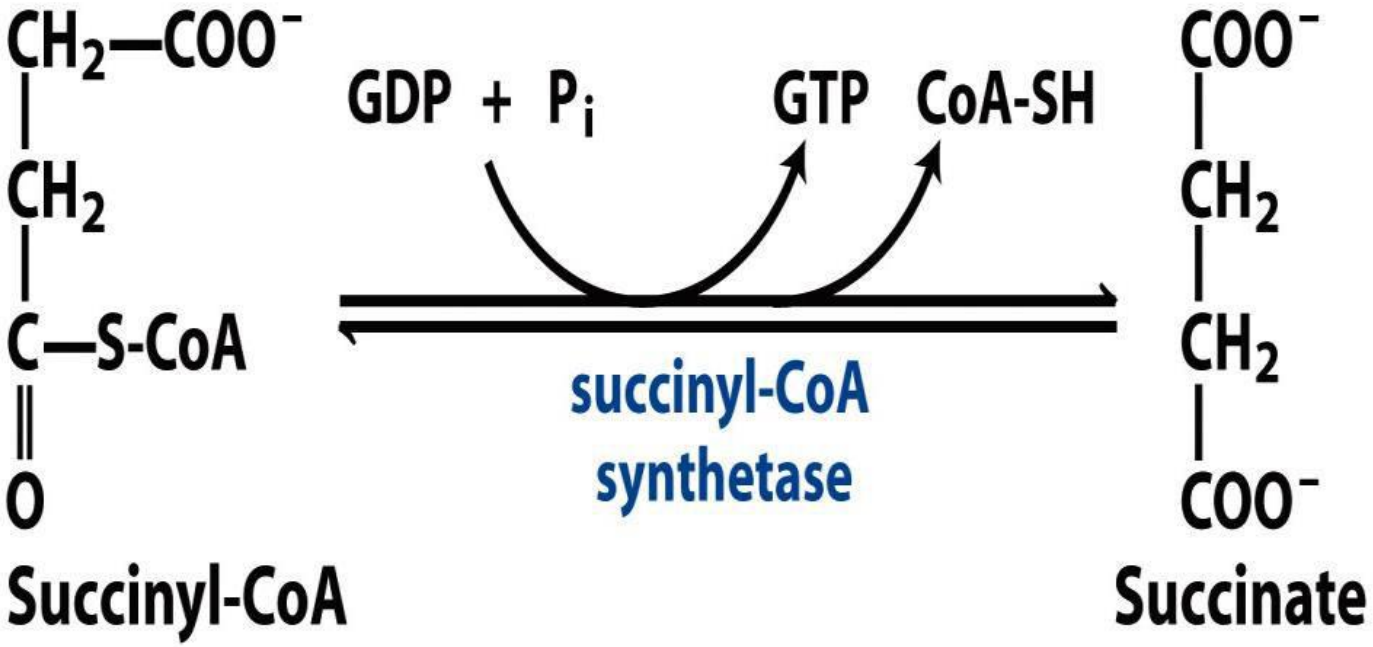
• The pathways shown use the same five cofactors (thiamine pyrophosphate, coenzyme A, lipoate, FAD, and NAD+)
• closely similar multienzyme complexes, and the same enzymatic mechanism to carry out oxidative decarboxylations of pyruvate (by the pyruvate dehydrogenase complex) a-ketoglutarate (in the citric acid cycle) and the carbon skeletons of the three branched-chain amino acids, isoleucine leucine, and valine



Steps of Citric Acid Cycle-4

5. Conversion of Succinyl-CoA to Succinate

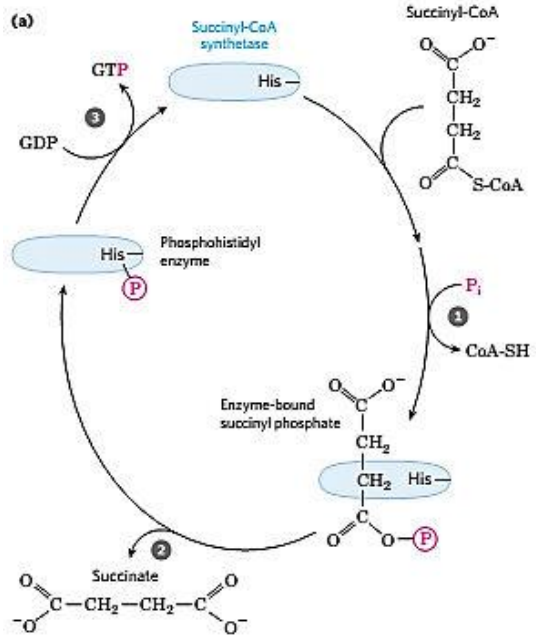
• **Succinyl-CoA** like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis (G= -36 kJ/mol).
• Energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net G= - 2.9 kJ/mol.
• The enzyme that catalyzes this reversible reaction is called **succinyl-CoA synthetase** or **succinic thiokinase**, names indicate the participation of a nucleoside triphosphate in the reaction



$\Delta G'^{\circ} = -2.9$ kJ/mol

The succinyl-CoA synthetase reaction.

(a) In step 1 a phosphoryl group replaces the CoA of succinyl- CoA bound to the enzyme, forming a high-energy acyl phosphate.
(b) In step 2 the succinyl phosphate donates its phosphoryl group to a His residue of the enzyme, forming a highenergy phosphohistidyl enzyme.
(c) In step 3 the phosphoryl group is transferred from the His residue to the terminal phosphate of GDP (or ADP), forming GTP (or ATP).



Nucleoside diphosphate kinase reaction:

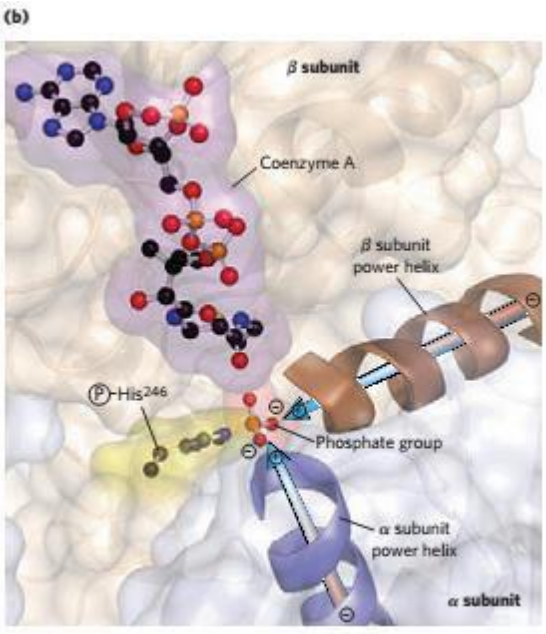
The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, reversible reaction catalyzed by **Nucleoside diphosphate kinase reaction**

• The enzyme has two subunits,

a subunit: **which has the P –His residue (His246) and the binding site for CoA**

• ***B subunit:*** **confers specificity for either ADP or GDP.**

• The active site is at the interface between subunits. The crystal structure of succinyl-CoA synthetase reveals two “power helices” (one from each subunit), oriented so that their electric dipoles situate partial positive charges close to the negatively charged P –His (Fig. 16–13b), stabilizing the phosphoenzyme intermediate.



Steps of Citric Acid Cycle-5

6. Oxidation of Succinate to Fumarate

Succinate Dehydrogenase reaction

• The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**

• In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane; in bacteria, to the plasma membrane.

• **The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD**

• Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration linked phosphorylation).

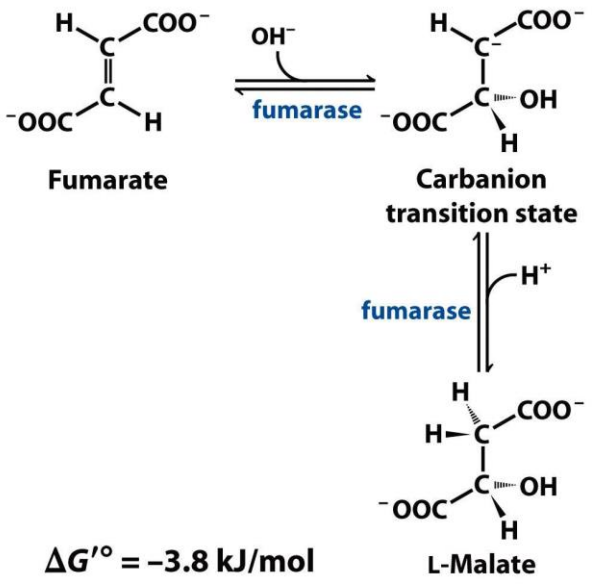
7. Hydration of Fumarate to Malate

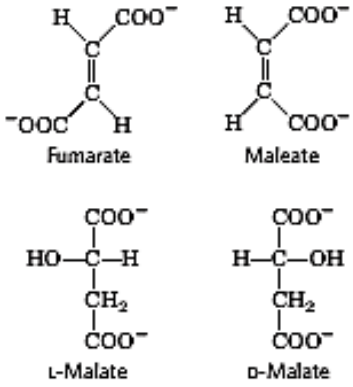
• The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase** (**fumarate hydratase**).

• The transition state in this reaction is a carbanion.

• This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).

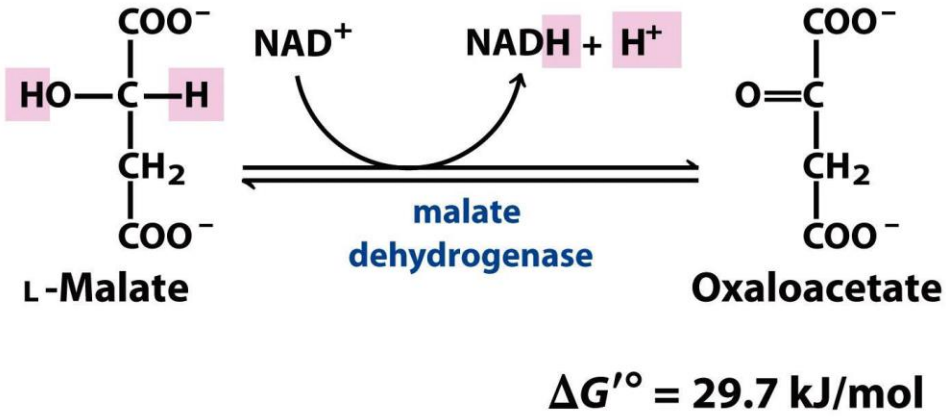
• In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.





8. Oxidation of Malate to Oxaloacetate

• Oxidation of Malate to Oxaloacetate Is the last reaction of the citric acid cycle
• NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate
• In intact cells oxaloacetate is continually removed by the **highly exergonic citrate synthase** reaction
• This keeps the concentration of oxaloacetate in the cell remains extremely low pulling **the malate dehydrogenase** reaction toward the formation of oxaloacetate.



$\Delta G'^{\circ} = 29.7$ kJ/mol

Interesting facts

• The individual reactions of the citric acid cycle were initially worked out in vitro, using minced muscle tissue
• Later, the pathway and its regulation were studied in vivo.
• By using radioactively labeled precursors such as [14C] pyruvate and [14C]acetate
• Carbon-14, **14C**, or radiocarbon, is a radioactive **isotope** of carbon with an atomic nucleus containing 6 protons and 8 neutrons.
• Researchers have traced the fate of individual carbon atoms through the citric acid cycle.

Direct and Indirect ATP Yield

TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

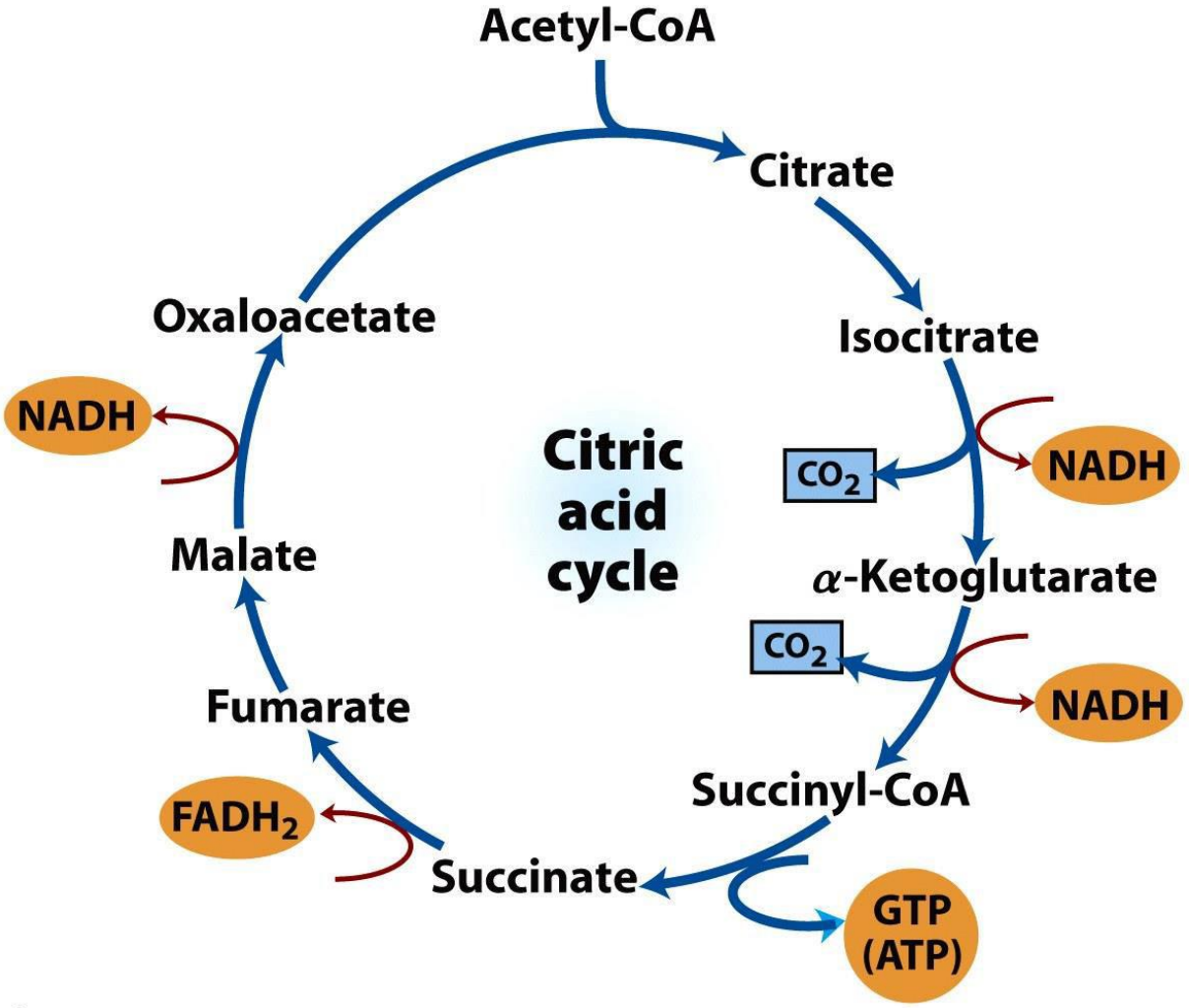
*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

Table 16-1

Lehninger Principles of Biochemistry, Fifth Edition

Products from One Turn of the Cycle

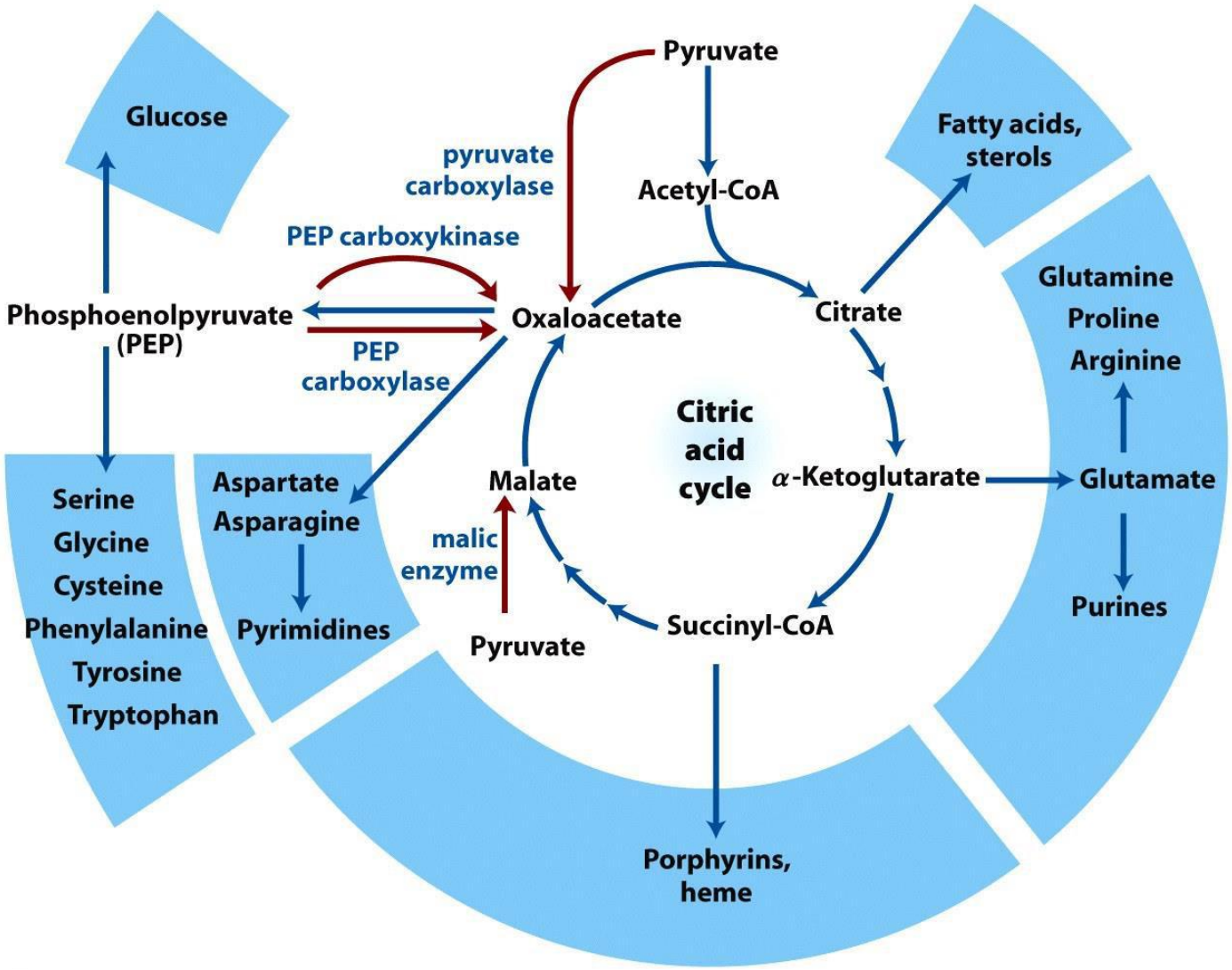


Citric Acid Cycle

Acetyl-CoA + 3NAD+ + FAD + GDP + Pi + 2 H_2O ⟶ 2CO_2 +3NADH + $FADH_2$ + GTP + CoA + 3H+

- Carbons of acetyl groups in acetyl-CoA are oxidized to CO_2
- Electrons from this process reduce NAD+ and FAD
- One GTP is formed per cycle, this can be converted to ATP
- Intermediates in the cycle are not depleted

Role of the Citric Acid Cycle in Anabolism



The amphibolic nature of Citric acid cycle:

This pathway is utilized for the both catabolic reactions to generate energy as well as for anabolic reactions to generate metabolic intermediates for biosynthesis.

• If the CAC intermediate are used for synthetic reactions, they are replenished by anaplerotic reactions in the cells (indicated by red colours).

Anaplerotic Reactions

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$Pyruvate + HCO_3^- + ATP \overset{\text{pyruvate carboxylase}}{\rightleftharpoons} oxaloacetate + ADP + P_i$	Liver, kidney
$Phosphoenolpyruvate + CO_2 + GDP \overset{\text{PEP carboxykinase}}{\rightleftharpoons} oxaloacetate + GTP$	Heart, skeletal muscle
$Phosphoenolpyruvate + HCO_3^- \overset{\text{PEP carboxylase}}{\rightleftharpoons} oxaloacetate + P_i$	Higher plants, yeast, bacteria
$Pyruvate + HCO_3^- + NAD(P)H \overset{\text{malic enzyme}}{\rightleftharpoons} malate + NAD(P)^+$	Widely distributed in eukaryotes and bacteria

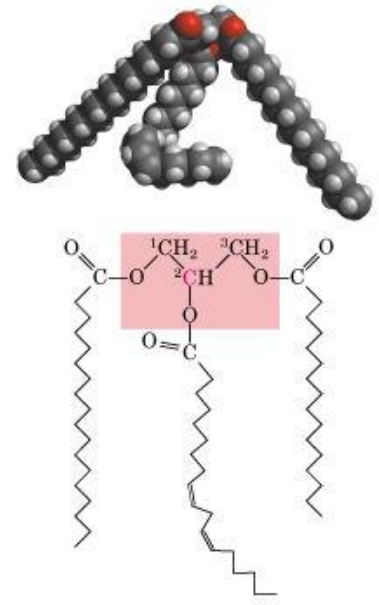
Table 16-2
Lehninger Principles of Biochemistry, Fifth Edition

Summary

In this chapter, we learned that:
• Citric acid cycle is an important catabolic process: it makes GTP, and reduced cofactors that could yield ATP
• Citric acid cycle plays important anabolic roles in the cell
• A large multi-subunit enzyme, pyruvate dehydrogenase complex, converts pyruvate into acetyl-CoA
• Several cofactors are involved in reactions that harness the energy from pyruvate
• The rules of organic chemistry help to rationalize reactions in the citric acid cycle

Fatty Acid Catabolism

Triacylglycerols (fats or triglycerides) Three fatty acids ester-linked to a glycerol molecule Used as energy storage molecules in eukaryotes Stored in adipocytes



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol

Advantages of using triacylglycerols for energy storage

1. Fats are highly reduced hydrocarbons with a large energy of oxidation.
2. Fats are insoluble molecules that aggregate into droplets. They are unsolvated and no storage mass is water.
3. Fats are chemically inert. They can be stored without fear of unfavorable reactions.

Disadvantages of triacylglycerols as energy storage

1. Fats must be emulsified before enzymes can digest them.
2. Fats are insoluble in the blood and must be carried in the blood as protein complexes.

Extraction of energy from fatty acids

Step 1. Oxidation of fatty acids to acetyl-CoA. This generates NADH and FADH2.

Step 2. Oxidation of acetyl-CoA to CO2 in the citric acid cycle. This generates NADH, FADH2 and GTP (ATP).

Step 3. Transfer of electrons from NADH and FADH2 to O2. This results in the synthesis of ATP

Anaplerotic reactions

• This pathway is utilized for the both catabolic reactions to generate energy as well as for anabolic reactions to generate metabolic intermediates for biosynthesis.

• As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by **anaplerotic reactions.**

• **Anaplerotic reactions :**

These are chemical **reactions** that form intermediates of a metabolic pathway.

• Most common anaplerotic reactions convert either **pyruvate or phosphoenolpyruvate to oxaloacetate or malate.**

• The most important anaplerotic reaction in mammalian liver and kidney is the reversible **carboxylation of pyruvate by CO2 to form oxaloacetate**, catalyzed by **pyruvate carboxylase**.

• When the citric acid cycle is deficient in oxaloacetate or any other intermediates, pyruvate is carboxylated to produce more oxaloacetate.

• The enzymatic addition of a carboxyl group to pyruvate requires energy, supplied by ATP—required to attach a carboxyl group to pyruvate is about equal to the free energy available from ATP.

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

Table 16-2
Lehninger Principles of Biochemistry, Fifth Edition

• **Pyruvate carboxylase** is inactive in the absence of acetyl-CoA.

• Whenever acetyl-CoA, the fuel for the citric acid cycle, is present in excess, it stimulates the pyruvate carboxylase reaction to produce more oxaloacetate, enabling the cycle to use more acetylCoA in the citrate synthase reaction

• **Phosphoenolpyruvate (PEP) carboxylase** activated by the glycolytic intermediate fructose 1,6-bisphosphate, which accumulates when the citric acid cycle operates too slowly to process the pyruvate generated by glycolysis.

Glyoxylate cycle

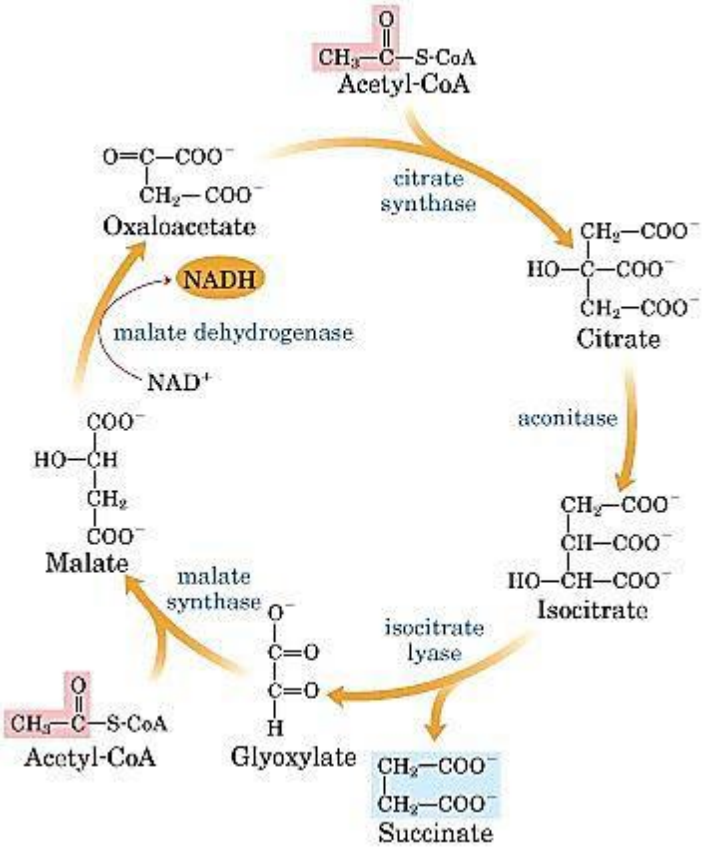
• The **glyoxylate cycle**, a variation of the tricarboxylic acid **cycle**, is an anabolic pathway occurring in plants, bacteria, protists, and fungi.

• The **glyoxylate cycle** relies on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.

• In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available

• Discovered by H. Kornberg and Neil Madsen in the lab. Of Hans Krebs.

• Gets name from product “glyoxylate”

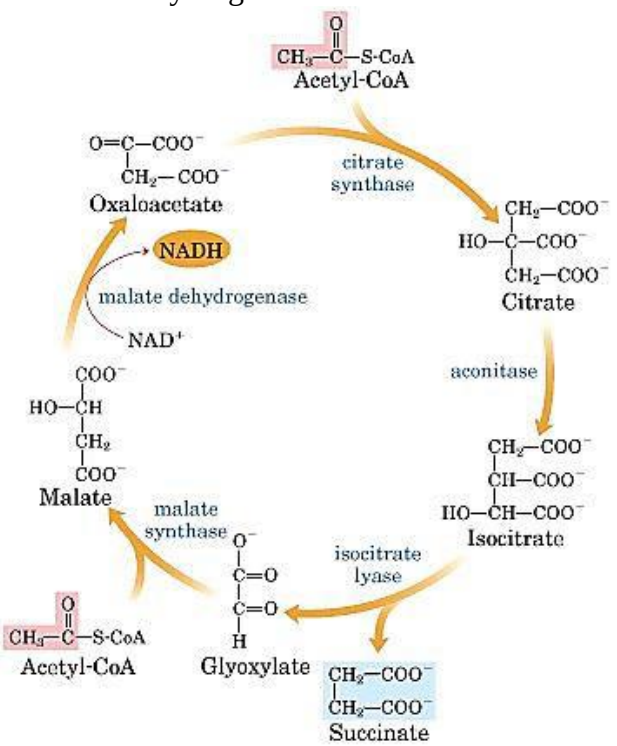


The two cycles differ in that **in the glyoxylate cycle, isocitrate is converted into glyoxylate and succinate by ICL** instead of into α-ketoglutarate.

Similarities with TCA cycle

• The glyoxylate cycle utilizes five of the eight enzymes associated with **the citric acid cycle:**

1. citrate synthase
2. Aconitase
3. Succinate dehydrogenase
4. Fumarase
5. malate dehydrogenase.



1. The citrate synthase, aconitase, and malate dehydrogenase of the glyoxylate cycle are isozymes of the citric acid cycle enzymes

2. Isocitrate lyase and malate synthase are unique to the glyoxylate cycle.

3. Notice that two acetyl groups (light red) enter the cycle and four carbons leave as succinate (blue).

1. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis.
2. In recent years, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals and suggests that animals encode alternative enzymes of the cycle that differ in function from known MS and ICL in non-metazoan species.

• This bypasses **the decarboxylation steps** that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose.
• Glyoxylate is subsequently combined **with acetyl-CoA to produce malate**, catalyzed by **malate synthase**
• **Malate is also formed in parallel from succinate by the action of succinate dehydrogenase and fumarase.**

Glyoxylate cycle-2

Steps of Glyoxylate cycle

1. Acetyl-CoA condenses with oxaloacetate to form citrate
2. citrate is converted to isocitrate, exactly as in the citric acid cycle.
3. Then the cleavage of isocitrate by **isocitrate lyase**, forming succinate and **glyoxylate** (not the breakdown of isocitrate by isocitrate dehydrogenase)
4. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by **malate synthase**
5. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle

• Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes.
• **The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids.**
• Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

Role in gluconeogenesis

• Fatty acids from lipids are commonly used as an energy source by vertebrates as fatty acids are degraded through beta oxidation into acetate molecules (used in acetyl coA).
• **This acetate**, bound to the active thiol group of coenzyme A, enters the Citric acid cycle (TCA or CCA cycle) where it is fully oxidized to carbon dioxide
• To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.
• This pathway thus allows cells to obtain energy from fat.
• The oxaloacetate can be converted into **Phosphoenolpyruvate**, which is the product of phosphoenol pyruvate carboxykinase, the first enzyme in gluconeogenesis.

Function in Plants

• In plants the glyoxylate cycle occurs in specialized peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. In plants, certain invertebrates, and some microorganisms (*E. coli* and yeast) acetate can serve 2 purposes:
1. as an energy-rich fuel
2. as a source of phosphoenolpyruvate for carbohydrate synthesis In these organisms, enzymes of the **glyoxylate cycle** catalyze conversion of acetate to succinate or other four-carbon intermediates of the citric acid cycle.
• Those enzymes common to the citric acid and glyoxylate cycles have two isozymes, one specific to mitochondria, the other to **glyoxysomes**.
• Glyoxysomes are not present in all plant tissues at all times.
• They develop in lipid-rich seeds during germination, before the developing plant acquires the ability to make glucose by photosynthesis.
• In addition to glyoxylate cycle enzymes, glyoxysomes contain all the enzymes needed for the degradation of the fatty acids stored in seed oils

Germinating seeds can therefore convert the carbon of stored lipids into glucose

• The glyoxylate cycle can also provide plants with another aspect of metabolic diversity.
• Acetyl-CoA formed from lipid breakdown is converted to succinate via the glyoxylate cycle, and the succinate is exported to mitochondria, where citric acid cycle enzymes transform it to malate.
• A cytosolic isozyme of malate dehydrogenase oxidizes malate to oxaloacetate, a precursor for gluconeogenesis.
• Germinating seeds can therefore convert the carbon of stored lipids into glucose.
• The four carbon succinate molecule can be transformed into a variety of carbohydrates through combinations of other metabolic processes; the plant can synthesize molecules using acetate as a source for carbon.
• The Acetyl CoA can also react with glyoxylate to produce some NADPH from NADP+, which is used to drive energy synthesis in the form of ATP later in the Electron Transport Chain.

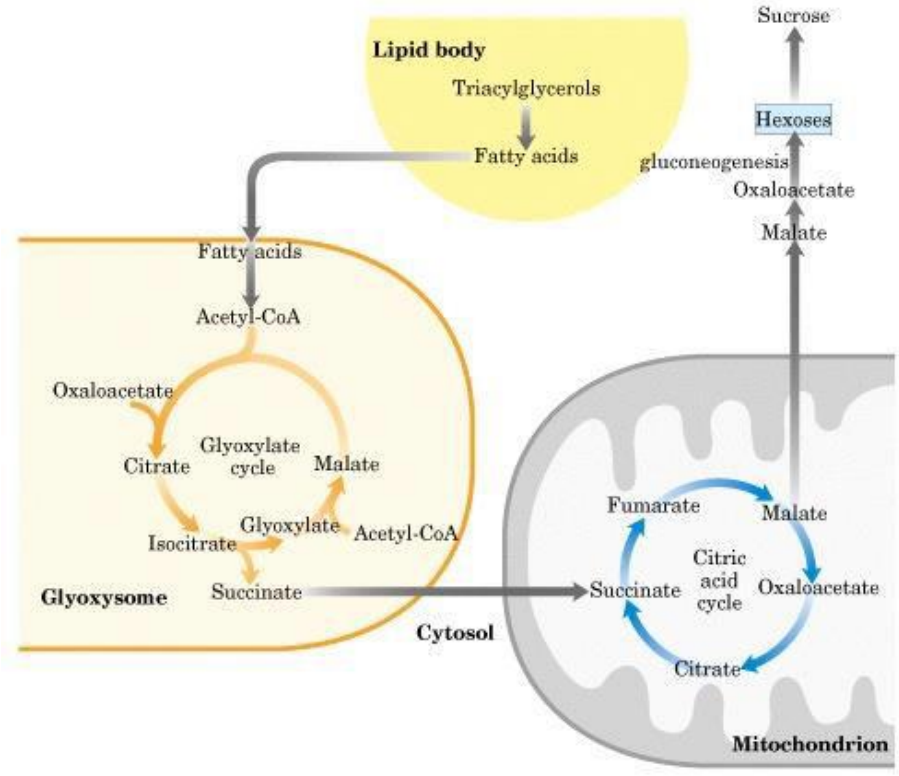
Glyoxylate cycle-3

Relationship between the glyoxylate and citric acid cycles

• The two initial steps of the glyoxylate cycle are identical to those in the citric acid cycle: *acetate* → *citrate* → *isocitrate*.
• The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO2.
• In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the *net* formation of succinate, oxaloacetate, and other cycle intermediates from acetyl- CoA.
• Oxaloacetate thus formed can be used to synthesize glucose via gluconeogenesis.

In germinating seeds, the enzymatic transformations of dicarboxylic and tricarboxylic acids occur in three intracellular compartments and there is a continuous interchange of metabolites among these compartments
1. Mitochondria
2. glyoxysomes
3. cytosol

Linkage to Gluconeogenesis in Plants



Relationship between the glyoxylate and citric acid cycles.
• The reactions of the glyoxylate cycle (in glyoxysomes) proceed simultaneously with, those of the citric acid cycle (in mitochondria) as intermediates pass between these compartments.
• The conversion of succinate to oxaloacetate is catalyzed by citric acid cycle enzymes.

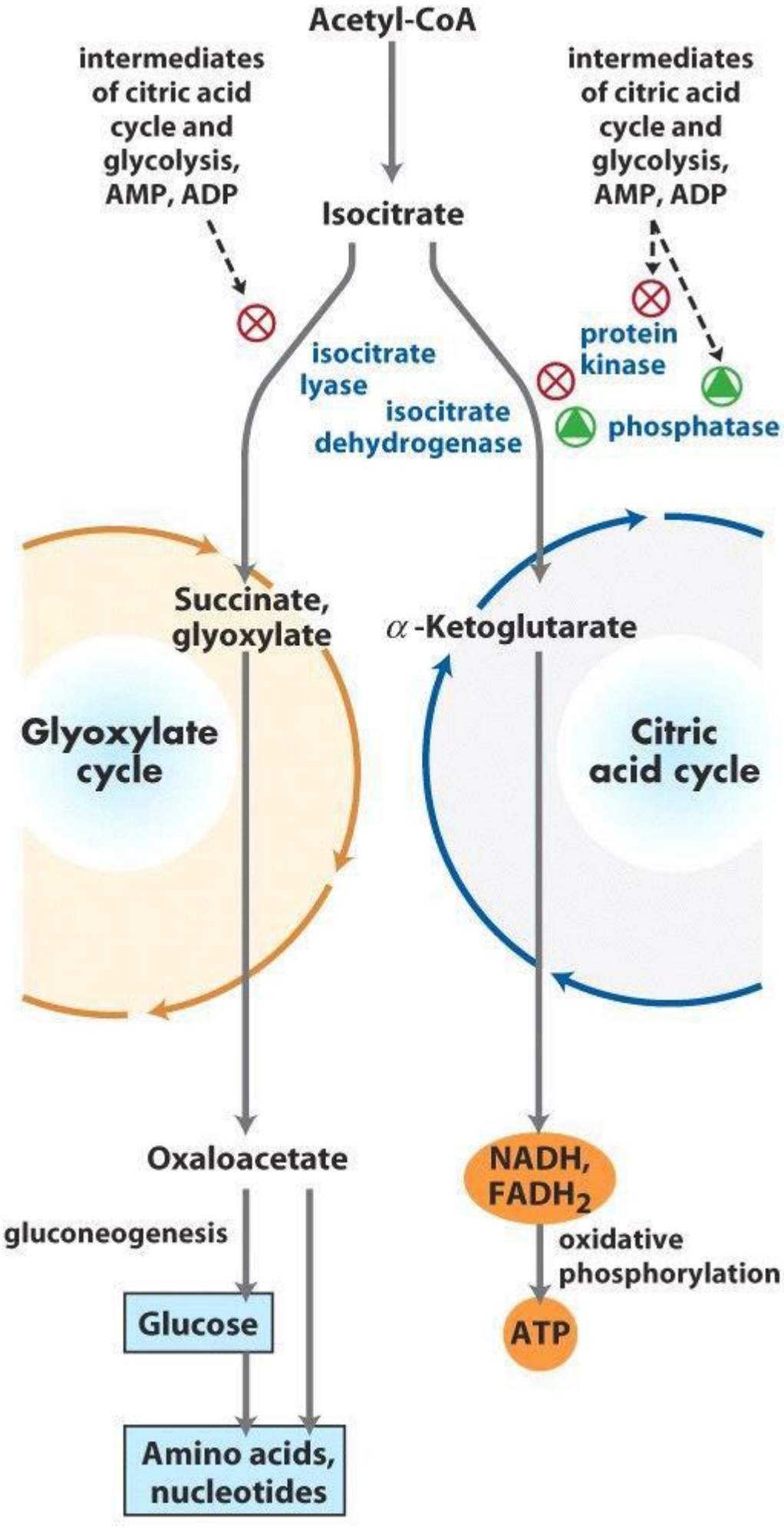
The carbon skeleton of oxaloacetate from the citric acid cycle (in the mitochondrion) is carried to the glyoxysome in the form of aspartate.
Aspartate is converted to oxaloacetate, which condenses with acetyl-CoA derived from fatty acid breakdown. The citrate thus formed is converted to isocitrate by aconitase, then split into glyoxylate and succinate by isocitrate lyase. The succinate returns to the mitochondrion, where it reenters the citric acid cycle and is transformed into malate, which enters the cytosol and is oxidized (by cytosolic malate dehydrogenase) to oxaloacetate. Oxaloacetate is converted via gluconeogenesis into hexoses and sucrose, which can be transported to the growing roots and shoot.
Four distinct pathways participate in these conversions:
1. fatty acid breakdown to acetyl-CoA (in glyoxysomes)
2. the glyoxylate cycle (in glyoxysomes)
3. the citric acid cycle (in mitochondria)
4. gluconeogenesis (in the cytosol)

Coordinated regulation of glyoxylate and citric acid cycles

• The central molecules between 2 cycles is isocitrate
• The **translocation of isocitrate** between the citric acid cycle and the glyoxylate cycle is controlled by the activity of **isocitrate dehydrogenase**, which is regulated by reversible phosphorylation.
• **A specific protein kinase phosphorylates and inactivates the dehydrogenase.** This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose.
• A phosphoprotein phosphatase removes the phosphoryl group from isocitrate dehydrogenase, **reactivating the enzyme**
• Reactivation of enzyme sends more isocitrate through the energy-yielding citric acid cycle
• The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.

Regulation
Linkage

Coordinated regulation of glyoxylate and citric acid cycles.
• Regulation of isocitrate dehydrogenase activity determines the partitioning of isocitrate between the glyoxylate and citric acid cycles.
• When the enzyme is inactivated by phosphorylation (by a specific protein kinase), isocitrate is directed into biosynthetic reactions via the glyoxylate cycle.
• When the enzyme is activated by dephosphorylation (by a specific phosphatase), isocitrate enters the citric acid cycle and ATP is produced.


Acetyl-CoA
intermediates of citric acid cycle and glycolysis, AMP, ADP
intermediates of citric acid cycle and glycolysis, AMP, ADP
Isocitrate
protein kinase
isocitrate lyase
isocitrate dehydrogenase
phosphatase
Succinate, glyoxylate
α-Ketoglutarate
Glyoxylate cycle
Citric acid cycle
Oxaloacetate
NADH, FADH2
gluconeogenesis
oxidative phosphorylation
Glucose
ATP
Amino acids, nucleotides

Fatty Acid Catabolism

Extraction of energy from fatty acids

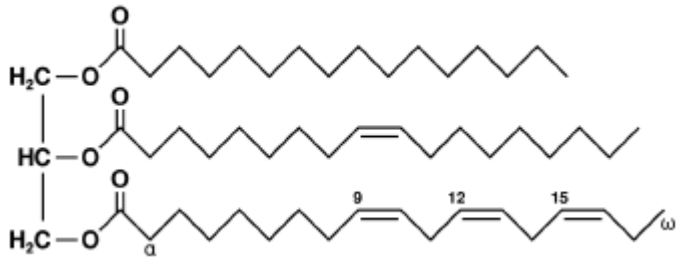
• Fatty acids yield the maximum amount of ATP on an energy per gram basis, when they are completely oxidized to CO2 and water by β-oxidation and the citric acid cycle.
• Fatty acids (mainly in the form of triglycerides) are the foremost storage form of fuel in most animals, and to a lesser extent in plants.

Beta oxidation

• The repetitive four-step process, **by which fatty acids are converted into acetyl-CoA** called ***Beta* oxidation.**
• To overcome the relative stability of the C—C bonds in a fatty acid, the carboxyl group at C-1 is activated by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group at the C-3, or *beta*, position-hence the name *beta* oxidation.

Triacylglycerols

• triesters of fatty acids and glycerol with 1 molecule of glycerol (3 –OH).
• Three fatty acids ester-linked to a glycerol molecule
• Each –OH bonded to –COO group of 1 fatty acid (forming ester)
• Triglycerides are the main constituents of body fat in humans and other animals, as well as vegetable fat
• Used as energy storage molecules in eukaryotes
• Stored in adipocytes



Example of an unsaturated fat triglyceride ($C_{55}H_{98}O_6$).
Left part: glycerol
Right part, from top to bottom: palmitic acid, oleic acid, alpha-linolenic acid.

Advantages of using triacylglycerols for energy Storage

1. Fats are highly reduced hydrocarbons with a large energy of complete oxidation (38 kJ/g) more than twice that for the same weight of carbohydrate or protein.
2. Fats are insoluble molecules that aggregate into droplets.
3. They are not dissolved due to which do not raise the osmolarity of the cytosol. (In storage polysaccharides, by contrast, water of solvation makes two-thirds of the overall weight of the stored molecules.)
4. Fats are chemically inert (triglycerides or neutral fats) that make them especially suitable as storage fuels so they can be stored without fear of unfavorable reactions.

Disadvantages of triacylglycerols as energy storage

1. The properties that make triacylglycerols good storage compounds present problems in their role as fuels.
2. Fats must be emulsified before enzymes can digest them.
3. Fats are insoluble in the blood and must be carried in the blood as protein complexes.

• **Emulsification**: The breakdown of fat globules in the duodenum into tiny droplets, which provides a larger surface area on which the enzyme pancreatic lipase can act to digest the fats into fatty acids and glycerol. **Emulsification** is assisted by the action of the bile salts.

Extraction of energy from fatty acids

• Complete oxidation of fatty acids to CO_2 and H_2O takes place in three stages:
• Step 1. Oxidation of fatty acids to acetyl-CoA. This generates NADH and $FADH_2$.
• Step 2. Oxidation of acetyl-CoA to CO_2 in the citric acid cycle. This generates NADH, $FADH_2$ and GTP (ATP).
• Step 3. Transfer of electrons from NADH and $FADH_2$ to O_2. This results in the synthesis of ATP.

Fatty Acid Catabolism-2

Digestion, Mobilization, and Transport of Fats

Cells can obtain fatty acid fuels from three sources:
1. Fats consumed in the diet
2. fats stored in cells as lipid droplets
3. fats synthesized in one organ for export to another

Some species use all three sources under various circumstances, others use one or two. Vertebrates, obtain fats in the diet, mobilize fats stored in specialized tissue (adipose tissue, consisting of cells called adipocytes), and, in the liver, convert excess dietary carbohydrates to fats for export to other tissues.

1. 40% or more of the daily energy requirement of humans in highly industrialized countries is supplied by **dietary triacylglycerols**
2. Triacylglycerols provide more than half the energy requirements of some organs, particularly the liver, heart, and resting skeletal muscle.
3. In hibernating animals and migrating birds, stored triacylglycerols are sole source of energy
4. Protists obtain fats by consuming organisms lower in the food chain, and some also store fats as cytosolic lipid droplets.
5. Vascular plants mobilize fats stored in **seeds during germination**, but do not otherwise depend on fats for energy.

Dietary Fats Are Absorbed in the Small Intestine

• **step 1** In vertebrates, before ingested triacylglycerols can be absorbed through the intestinal wall they must be converted from insoluble macroscopic fat particles to microscopic micelles (soluble) by bile salts, such as taurocholic acid.
step 2 Lipase action converts triacylglycerols to monoacylglycerols (monoglycerides) and diacylglycerols (diglycerides), free fatty acids, and glycerol.
• **step 3** These products of lipase action diffuse into the epithelial cells lining the intestinal surface (the intestinal mucosa).
• **step 4** In the intestinal mucosa they are reconverted to triacylglycerols and packed with dietary cholesterol and specific proteins into lipoprotein aggregates called **chylomicrons.**
• **step 5** The protein moieties of lipoproteins are recognized by receptors on cell surfaces. In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II), move from the intestinal mucosa into the lymphatic system, and then enter the blood, which carries them to muscle and adipose tissue
• **step 6** In the capillaries of these tissues, the extracellular enzyme **lipoprotein lipase**, activated by apoC-II, hydrolyzes triacylglycerols to fatty acids and glycerol.
• **step 7** fatty acids and glycerol are taken up by cells in the target tissues .
• **step 8** In muscle, the fatty acids are oxidized for energy; in adipose tissue, they are reesterified for storage as triacylglycerols.
• Bile salts are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of a fatty meal.
• Bile salts act as biological detergents, converting dietary fats into mixed micelles of bile salts and triacylglycerols
• Micelle formation makes lipid molecules accessible to the action of water-soluble lipases in the intestine.

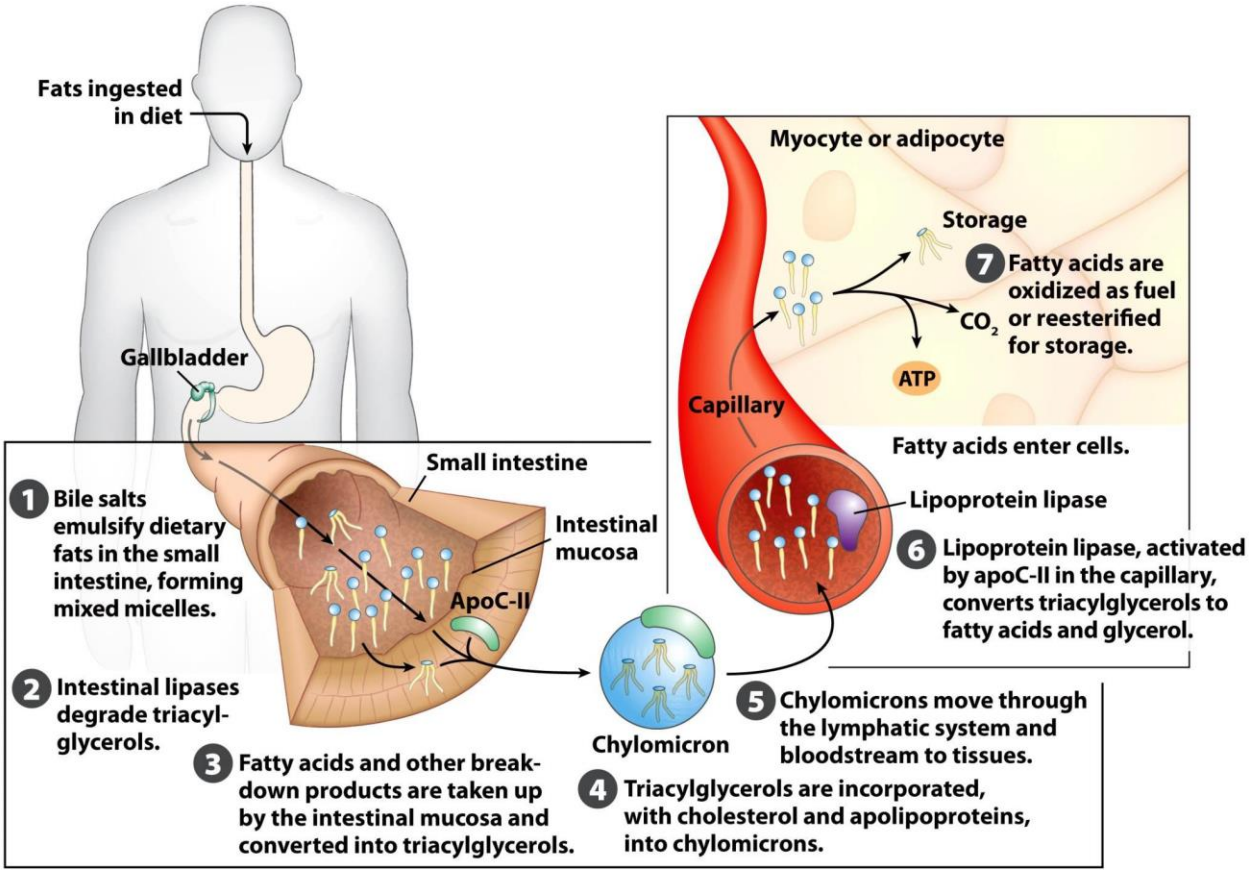


Figure 17-1
Lehninger Principles of Biochemistry, Sixth Edition

Molecular Structure of a Chylomicron

• The surface is a layer of phospholipids, with head groups facing the aqueous phase. Triacylglycerols sequestered in the interior (yellow) make up more than 80% of the mass. Several apolipoproteins that protrude from the surface (B-48, C-III, C-II) act as signals in the uptake and metabolism of chylomicron contents.
• The diameter of chylomicrons ranges from about 100 to 500 nm.

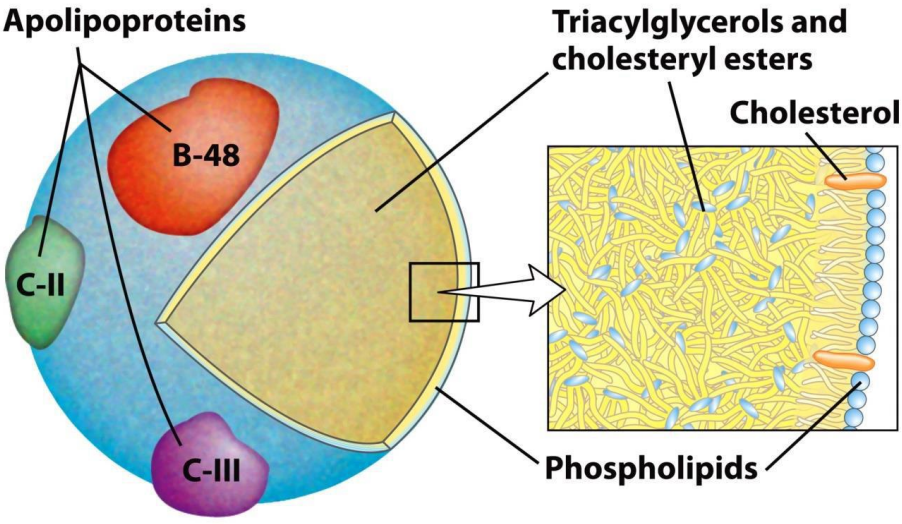


Figure 17-2
Lehninger Principles of Biochemistry, Sixth Edition

Types of Dietary Fats

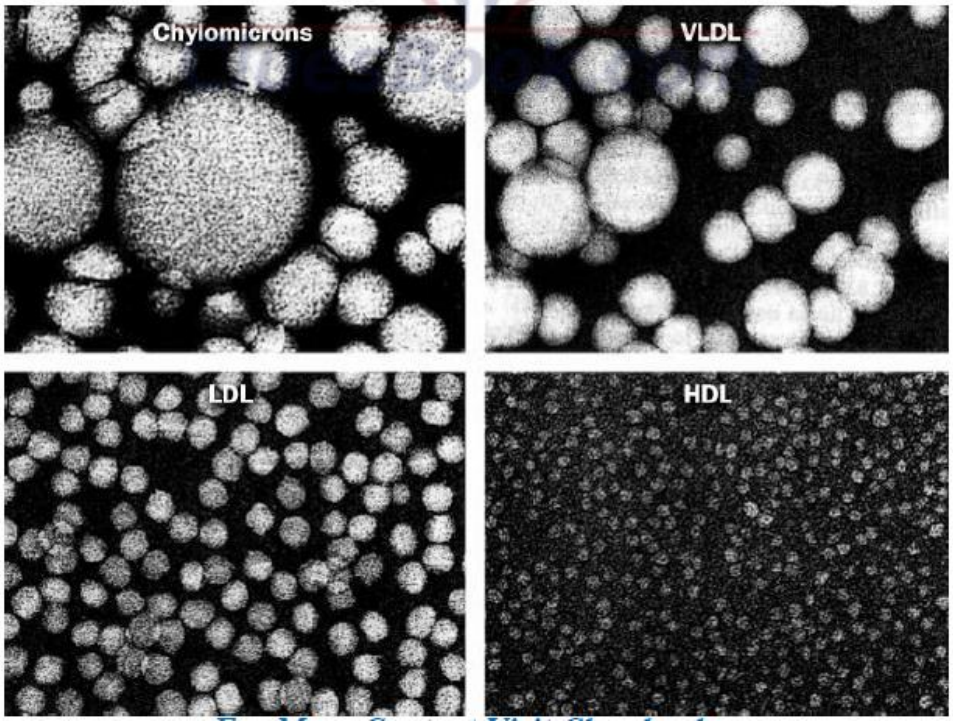
Apolipoproteins are lipid-binding proteins in the blood, responsible for the transport of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters between organs. Apolipoproteins ("apo" means "detached" or "separate," designating the protein in its lipid-free form) combine with lipids to form several classes of **lipoprotein** particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface. Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons

Lipoproteins are large complexes of lipids and proteins designed to transport lipids in the blood. The lipoproteins are classified by particle density.

Very-low-density lipoproteins (VLDL)

Very-high-density lipoproteins (VHDL)

The protein moieties of lipoproteins are recognized by receptors on cell surfaces. In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II)

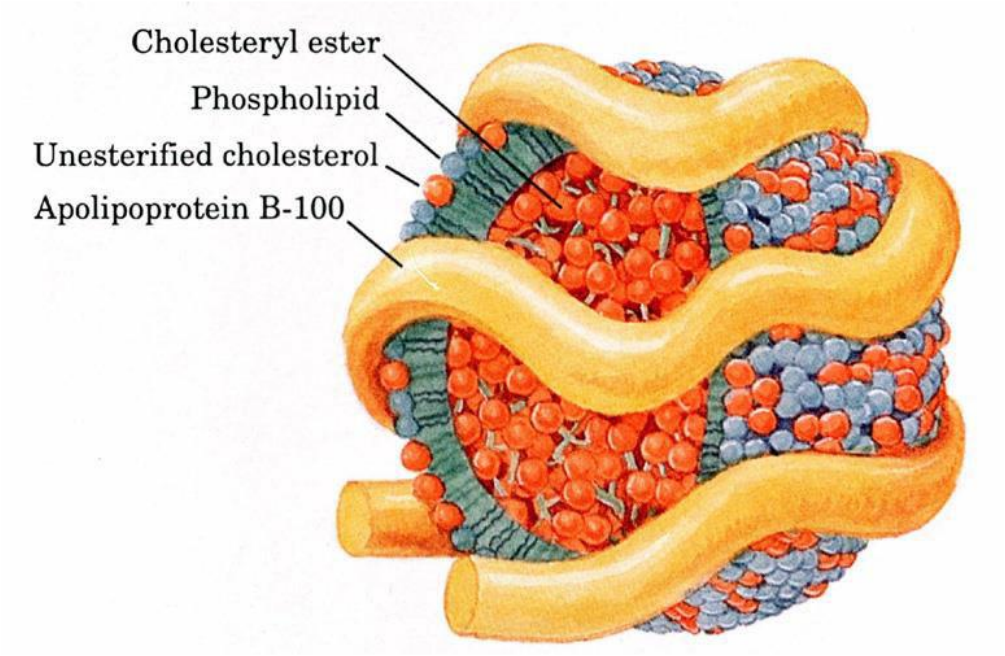


Dietry fats are absorbed in small intestine

1. Chylomicrons (transport dietary cholesterol to tissues)
2. Very low density lipoprotein (VLDL) transport cholesterol
3. Intermediate density lipoprotein (IDL) produced by the liver
4. Low density lipoprotein (LDL)

• 1st four transport cholesterol produced by the liver to tissues

5. High density lipoprotein (HDL) (transport cholesterol from tissue to liver)



Fatty Acid Catabolism-3

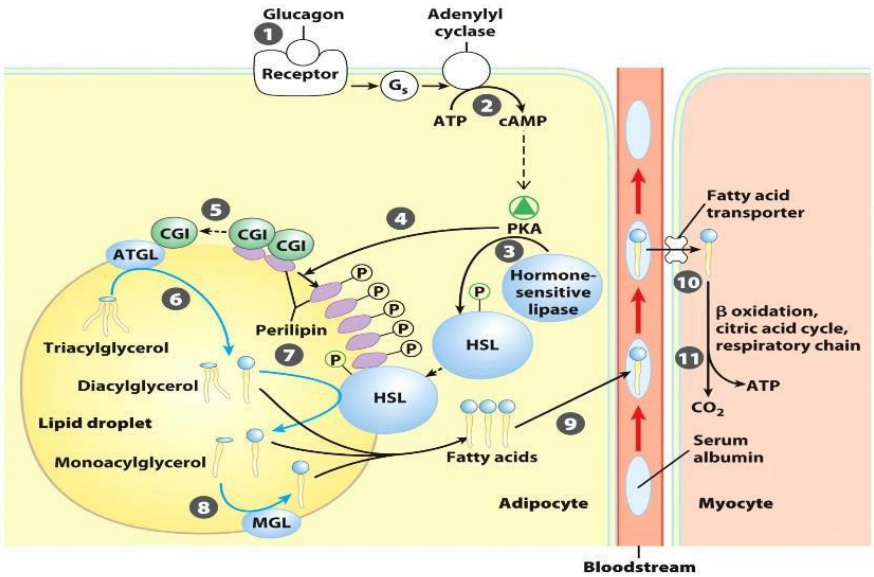
Hormones trigger metabolization of Triacylglycerols

1. Neutral lipids are stored in adipocytes (and in steroid synthesizing cells of the adrenal cortex, ovary, and testis) in the form of lipid droplets, with a core of sterol esters and triacylglycerols surrounded by a monolayer of phospholipids.
2. The surface of these droplets is coated with **perilipins**
3. Perilipins are a family of proteins that restrict access to lipid droplets, preventing untimely lipid mobilization.
4. When hormones signal the need for metabolic energy, triacylglycerols stored in adipose tissue are mobilized (brought out of storage) and transported to tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be oxidized for energy production.

• Two hormones are secreted in response to low blood glucose levels
• **Epinephrine and glucagon**
• **Epinephrine and glucagon** stimulate the enzyme **adenylyl cyclase** in the adipocyte plasma membrane (which produces the intracellular second messenger cyclic AMP)
• Cyclic AMP–dependent protein kinase (PKA) triggers changes that open the lipid droplet up to the action of three lipases, which act on tri-, di-, and monoacylglycerols, releasing fatty acids and glycerol.
• These **free fatty acids,** (**FFA**) pass from the adipocyte into the blood, bind to the blood protein **serum albumin** noncovalently, carried to tissues skeletal muscle, heart, and renal cortex
• **serum albumin** makes up about half of the total serum protein, binds as many as 10 fatty acids per protein monomer
• About 95% of the biologically available energy of triacylglycerols resides in their three long chain fatty acids; only 5% is contributed by the glycerol moiety
• The glycerol released by lipase action is phosphorylated by **glycerol kinase** and glycerol 3- phosphate is formed
• Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate.
• The glycolytic enzyme triose phosphate isomerase converts this compound to glyceraldehyde 3-phosphate, which is **oxidized via glycolysis.**

Mobilization of triacylglycerols stored in adipose tissue

1. When low levels of glucose in the blood trigger the release of glucagon, the hormone binds its receptor in the adipocyte membrane and thus stimulates adenylyl cyclase, via a G protein, to produce cAMP.
2. This activates PKA, which phosphorylates the hormone-sensitive lipase (HSL) And **perilipin molecules on the surface of the lipid droplet**.
3. Phosphorylation of perilipin causes dissociation of the protein CGI from perilipin.
4. CGI then associates with the enzyme adipose triacylglycerol lipase (ATGL), activating it.
5. Active ATGL converts triacylglycerols to diacylglycerols.
6. The phosphorylated perilipin associates with phosphorylated hormonesensitive lipase **HSL**, allowing it access to the surface of the lipid droplet, where it converts diacylglycerols to monoacylglycerols.
7. A third lipase, monoacylglycerol lipase (MGL) hydrolyzes monoacylglycerols.



Utilization of stored triacylglycerols

8. Fatty acids leave the adipocyte, bind serum albumin in the blood, and are carried in the blood; they are released from the albumin and enter a myocyte via a specific fatty acid transporter.
9. In the myocyte, fatty acids are oxidized to CO2
10. the energy of oxidation is conserved in ATP, which fuels muscle contraction and other **energy-requiring metabolism in the myocyte.**

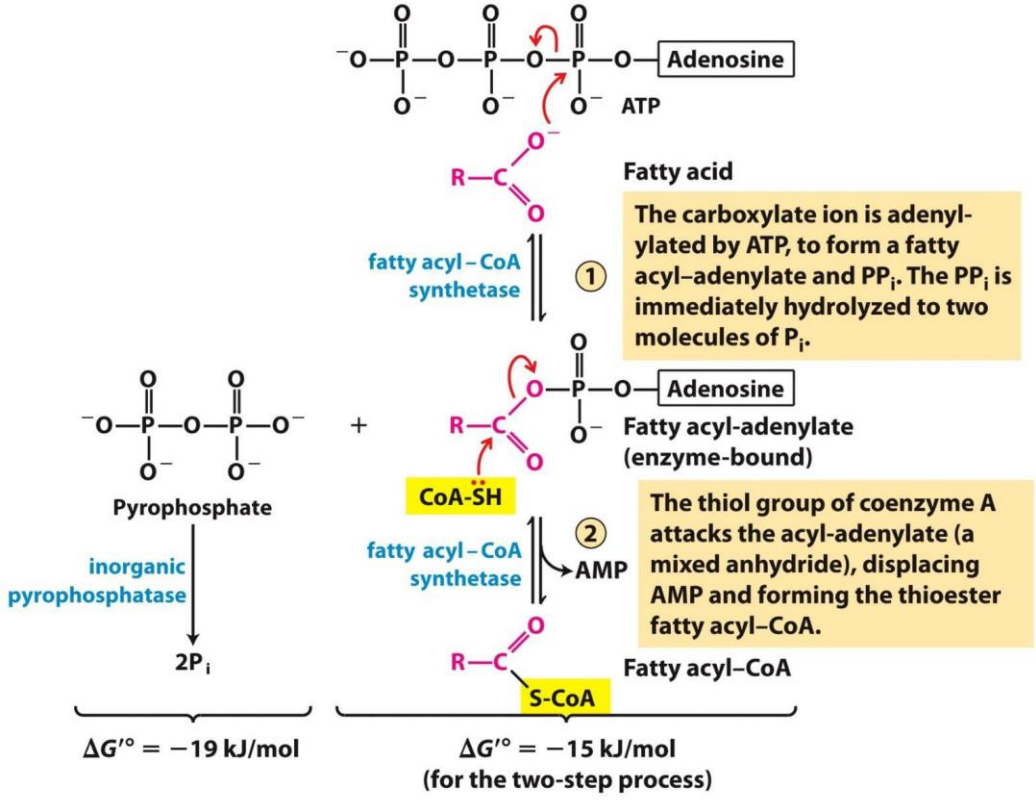
Fatty Acids Are Activated and Transported into Mitochondria

• The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger.
• The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of **membrane transporters.**
• The fatty acids with **chain lengths of 14 or more carbons**, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes.
• The fatty acids with chain lengths of 14 or more carbons must first undergo the three enzymatic reactions of the **carnitine shuttle**.
1. Esterification to CoA
2. Transesterification to carnitine followed by transport
3. Transesterification back to CoA

Three enzymatic reactions of the carnitine shuttle

The first reaction

• This is catalyzed by a family of isozymes (different isozymes specific for fatty acids having short, intermediate, or long carbon chains) present in the outer mitochondrial membrane, the **acyl- CoA synthetases**
• **Acyl–CoA synthetases** catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a **fatty acyl– CoA**, coupled to the cleavage of ATP to AMP and PPi. (free energy released by **cleavage of phosphoanhydride bonds in ATP can be coupled to the formation of a high-energy compound.)**
• The reaction occurs in two steps and involves **a fatty acyl-adenylate intermediate (Fatty acyl–CoAs)**
• **Fatty acyl–CoAs** like acetyl-CoA, are high-energy compounds; their hydrolysis to FFA and CoA has a large, negative standard free-energy change (*G*= -31 kJ/mol).
• **The formation of a fatty acyl–CoA is made more favorable by the hydrolysis of *two* high-energy bonds in ATP**; the pyrophosphate formed in the activation reaction is immediately hydrolyzed by inorganic pyrophosphatase which pulls the reaction in the direction of fatty acyl–CoA formation. The overall reaction is



Fatty Acyl–CoA Synthetase Mechanism

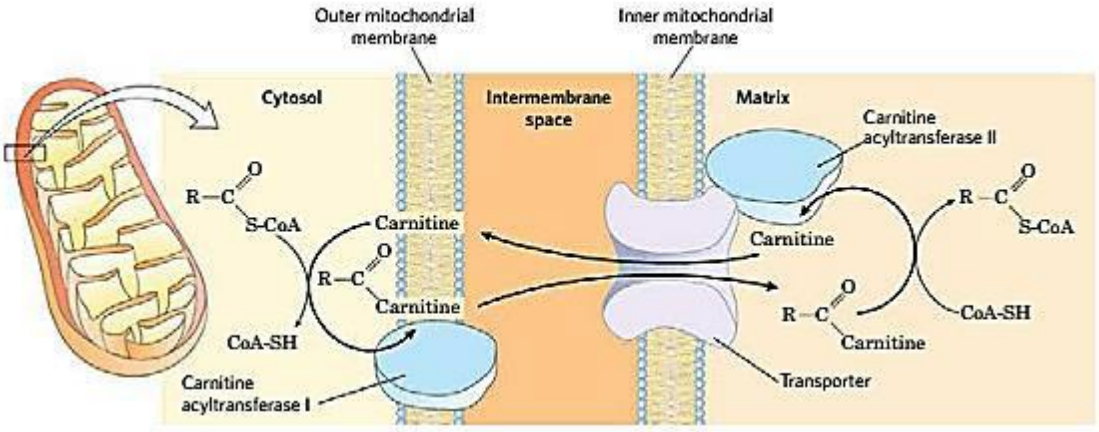
Conversion of a fatty acid to a fatty acyl–CoA.
The conversion is catalyzed by fatty **acyl–CoA synthetase** and inorganic pyrophosphatase.
Fatty acid activation by formation of the fatty acyl–CoA derivative occurs in two steps.
The overall reaction is highly exergonic.

The second reaction

• Fatty acyl–CoA esters formed at the cytosolic side of the outer mitochondrial membrane can be oxidized to produce ATP in the mitochondrion and or they can be used in the cytosol to synthesize membrane lipids.
• Fatty acids destined for mitochondrial oxidation are transiently attached to the hydroxyl group of **carnitine** to form fatty acyl–carnitine— the second reaction of the shuttle.
• This transesterification is catalyzed by **carnitine acyltransferase I,** in the outer membrane.
• So the carnitine ester is formed then moved across the outer membrane to the intermembrane space
• This passage into the intermembrane space (the space between the outer and inner membranes) occurs through large pores (formed by the protein porin) in the outer membrane.
• The fatty acyl–carnitine ester then enters the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter** of the inner mitochondrial membrane

Third (final step) of the carnitine shuttle

• The fatty acyl group is enzymatically transferred from carnitine to intramitochondrial coenzyme A by **carnitine acyltransferase II**.
• This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates fatty acyl–CoA and releases it, along with free carnitine, into the matrix .
• Carnitine reenters the intermembrane space via the acylcarnitine/carnitine transporter.



Fatty acid entry into mitochondria via the acyl-carnitine/ carnitine transporter.
After fatty acyl–carnitine is formed at the outer membrane or in the intermembrane space, it moves into the matrix by facilitated diffusion through the transporter in the inner membrane. In the matrix, the acyl group is transferred to mitochondrial coenzyme A, freeing carnitine to return to the intermembrane space through the same transporter.

This three-step process for transferring fatty acids into the mitochondrion—links two separate pools of coenzyme A and of fatty acyl–CoA, one in the cytosol, the other in mitochondria.
• These pools have different functions.
• **Coenzyme A in the mitochondrial matrix** is used in oxidative degradation of pyruvate, fatty acids, and some amino acids
• **Coenzyme A in cytosol..** Is conversely, is used in the biosynthesis of fatty acids
• Fatty acyl–CoA in the cytosolic pool can be used for membrane lipid synthesis or can be moved into the mitochondrial matrix for oxidation and ATP production.
• Conversion to the carnitine ester commits the fatty acyl moiety to the oxidative fate.
• **Rate limiting step for oxidation of fatty acids in mitochondria**
• The carnitine-mediated entry process is the rate limiting step for oxidation of fatty acids in mitochondria
• And is a regulation point.
• Once inside the mitochondrion, the fatty acyl–CoA is acted upon by a set of enzymes in the matrix.

Fatty Acid Catabolism-4

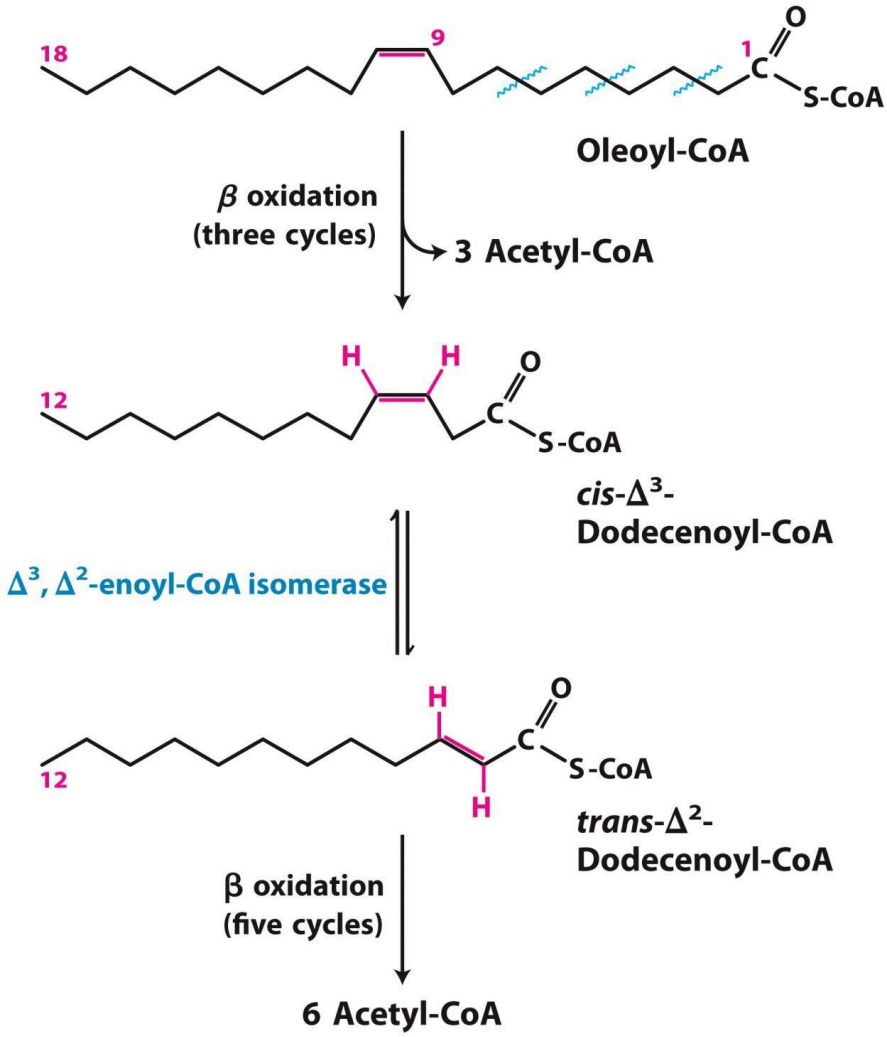
Oxidation of unsaturated fatty acids requires 2 additional steps

• The fatty acid oxidation sequence just described is typical when the incoming fatty acid is saturated (that is, has only single bonds in its carbon chain).

• These bonds are in the **cis configuration and cannot be acted upon by enoyl-CoA hydratase,** the enzyme catalyzing the addition of H2O to the trans double bond of the D2-enoyl-CoA generated during oxidation.

• Two auxiliary enzymes are needed for oxidation of the common unsaturated fatty acids:

1. an isomerase
2. a reductase



Beta oxidation of oleate 18:1Δ_9

Oxidation of Oleate (example of unsaturated fatty acids)

• Oleate is an abundant 18-carbon monounsaturated fatty acid **with a cis double bond between C-9 and C-10 (denoted Δ9).**

• In the first step of oxidation, oleate is converted to oleoyl-CoA and, like the saturated fatty acids, enters the mitochondrial matrix via the carnitine shuttle

• Oleoyl-CoA then undergoes three passes through the fatty acid oxidation cycle to yield three molecules of acetyl-CoA and the coenzyme A ester of a D3, 12-carbon unsaturated fatty acid, *cis*- Δ 3-dodecenoyl-CoA

• This product cannot serve as a substrate for enoyl-CoA hydratase, which acts only on trans double bonds.

• The auxiliary enzyme D3,D2-**enoyl-CoA isomerase** isomerizes the *cis*- D3-enoyl-CoA to the *trans*-D2-enoyl- CoA, which is converted **by enoyl- CoA hydratase** into the corresponding L-hydroxyacyl-CoA (*trans*-D2- dodecenoyl-CoA).

• This intermediate is now acted upon by the remaining enzymes of oxidation to yield acetyl-CoA and the coenzyme A ester of a 10-carbon saturated fatty acid, **decanoyl-CoA**.

• **decanoyl-CoA** undergoes four more passes through the –oxidation pathway to yield five more molecules of acetyl-CoA.

• Altogether, nine acetyl-CoAs are produced from one molecule of the 18- carbon oleate.

• The other auxiliary enzyme (a reductase) is required for oxidation of polyunsaturated fatty acids — for example, the 18-carbon linoleate, which has a ***cis*-D9,*cis*-D12 configuration.**
• Linoleoyl- CoA undergoes three passes through the –oxidation sequence to yield three molecules of acetyl-CoA and the **coenzyme A ester of a 12-carbon** unsaturated fatty acid with a ***cis*-D3,*cis*-D6 configuration**.
• This intermediate cannot be used by the enzymes of the **β**–oxidation pathway; its double bonds are in the wrong position.
• And have the wrong configuration (cis, not trans).
• However, the combined action of enoyl-CoA isomerase and **2,4- dienoyl-CoA reductase**, as shown in **Figure 17–11**, allows reentry of this intermediate into the –oxidation pathway and its degradation to six acetyl-CoAs.
• The overall result is conversion of linoleate to nine molecules of acetyl- CoA.

Auxiliary enzymes

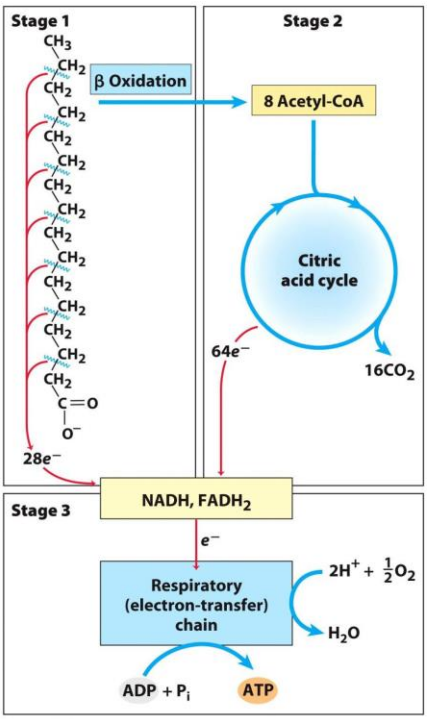
We investigated the enzyme activity of the blank in the spectrophotometric determination of the aminotransferase activities and aspartate aminotransferase activity. 6 lactate dehydrogenase and 3 malate dehydrogenase preparations from different manufactures and from different organs showed additional and contaminating activity. The additional activity depends upon the 2-oxoglutarate concentration. The contaminating activity is caused by alanine aminotransferase and aspartate aminotransferase in the auxiliary enzymes. We propose that exact definitions must be given for the auxiliary enzymes in the recommendations of standard determinations for enzyme activities.

Fatty Acid Catabolism-5

Stages for Oxidation of fatty acids

Oxidation of fatty acids

• The mitochondrial oxidation of fatty acids takes place in three stages
• **In First Sage** fatty acids undergo oxidative removal of successive two carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain.
• The **16-carbon palmitic acid** undergoes seven passes through the oxidative sequence
• In each pass two carbons are lost as acetyl-CoA.
• At the end of seven cycles the last two carbons of palmitate (originally C- 15 and C-16) remain as acetyl-CoA.
• The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules.
• Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H+) from the fatty acyl moiety by dehydrogenases.



Stages of fatty acid oxidation.
Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA. This process is called oxidation.
Stage 2: The acetyl groups are oxidized to CO_2 via the citric acid cycle.
Stage 3: Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

• **In the second stage** of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO2 in the citric acid cycle, which also takes place in the mitochondrial matrix.
• Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation.
• **Third stage Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.**
• This is due to production of reduced electron carriers NADH and FADH2 in first two stages of fatty acid oxidation

Fatty Acid Catabolism-6

Steps of *Beta* Oxidation

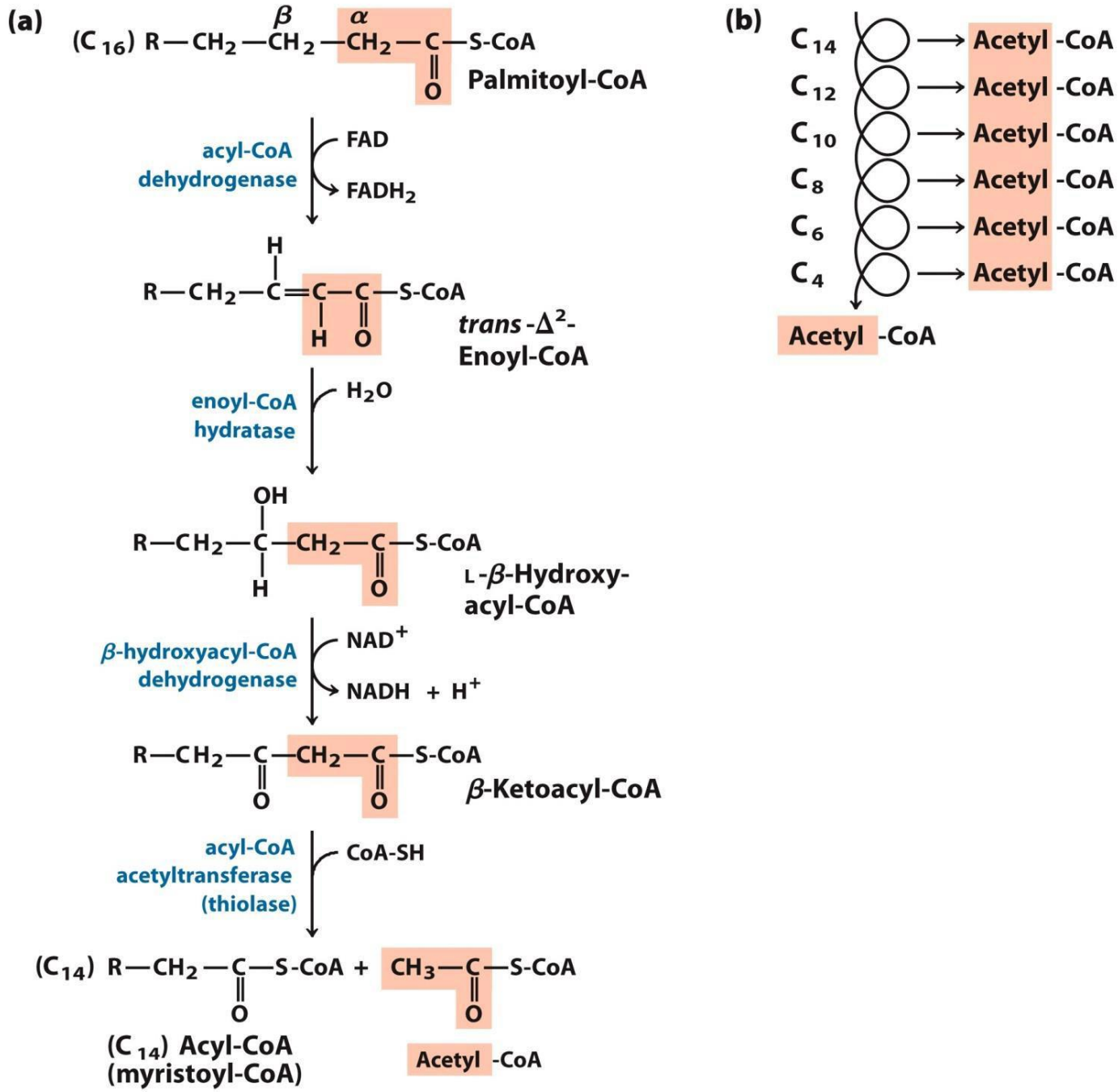
Beta - Oxidation of saturated fatty acids

• First, dehydrogenation of fatty acyl–CoA produces a double bond between the ***alpha*** and ***beta*** carbon atoms (C-2 and C-3), yielding a ***trans*-Δ2-enoyl-CoA** (the symbol Δ2 designates the position of the double bond).
• This first step is catalyzed by **acyl- CoA dehydrogenase**
• **Acyl- CoA dehydrogenase:**
• Three isozymes of each specific for a range of fatty acyl chain lengths. All three isozymes are flavoproteins with FAD as a prosthetic group.
a) Very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons
b) Medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons
c) Short-chain (SCAD), acting on fatty acids of 4 to 8 carbons.

• The electrons removed from the fatty acyl–CoA are transferred to FAD, the reduced form of the **dehydrogenase** immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the **electron transferring flavoprotein (ETF).**
• The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle in both reactions the enzyme is bound to the inner membrane
• A double bond is introduced into a carboxylic acid between ***alpha*** and ***beta*** **carbons**
• FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O_2, with the synthesis of about 1.5 ATP molecules per electron pair.

2nd and 3rd Steps: β-Oxidation of saturated fatty acids

• **Second Step:** In the second step of the beta-oxidation cycle water is added to the double bond of the *trans*-D2-enoyl-CoA to form the L-stereoisomer of beta**hydroxyacyl- CoA (3-hydroxyacyl-CoA)**.
• Enzyme: **Enoyl-CoA hydratase**, is analogous to the fumarase reaction in the citric acid cycle, in which H2O adds across an ***alpha*** and ***beta*** double bond
• **Third step:** In the third step, L-*beta*- hydroxyacyl-CoA is dehydrogenated to form beta**ketoacyl- CoA**, by the action of beta-**hydroxyacyl-CoA dehydrogenase**
• NAD+ is the electron acceptor.
• The NADH formed in the reaction donates its electrons to **NADH dehydrogenase**, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O_2.
• **Enzyme:** The reaction catalyzed by **beta-hydroxyacyl- CoA dehydrogenase** is closely analogous to the malate dehydrogenase reaction of the citric acid cycle



The beta -oxidation pathway.

(a) In each pass through this four-step sequence, one acetyl residue (shaded in pink) is removed in the form of acetyl-CoA from the carboxyl end of the fatty acyl chain— in this example palmitate (C16) is shown, which enters as palmitoyl-CoA.

(b) Six more passes through the pathway yield seven more molecules of acetyl-CoA, the seventh arising from the last two carbon atoms of the 16-carbon chain. Eight molecules of acetyl-CoA are formed in all.

4th Step: β-Oxidation of saturated fatty acids

• Last step is catalyzed by **acyl-CoA acetyltransferase**, called **thiolase**, which promotes reaction of *beta*-ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA.

• **Another product is the coenzyme A; thioester of the fatty acid, now shortened by two carbon atoms**

• **This reaction is called thiolysis,** by analogy with the process of hydrolysis, because the **β** -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.

• The thiolase reaction is a reverse Claisen condensation

• **The last three steps of this four-step sequence are catalyzed by either**
of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain.

• For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the **trifunctional protein (TFP)**. TFP is a heterooctamer of **α4β4 subunits.**

• Each subunit contains two activities, **the enoyl-CoA hydratase and the - hydroxyacyl-CoA dehydrogenase; the β subunits contain the thiolase activity.**
• When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

breakage of bonds in saturated fatty acids

• The single bond between methylene (—CH_2—) groups in fatty acids is relatively stable.
• During first three reactions of **β** -oxidation less stable C—C bonds are created, in which the carbon (C-2) is bonded to *two* carbonyl carbons (the **β** -ketoacyl-CoA intermediate).
• The ketone function on the carbon (C-3) makes it a good target for nucleophilic attack by the —SH of coenzyme A, catalyzed by thiolase.
• The acidity of **the α- hydrogen** and the resonance stabilization of the carbanion generated by the departure of this hydrogen make the terminal —CH_2—CO—S-CoA a good leaving group, facilitating breakage of the **α-β** bond.

Fatty Acid Catabolism-

The four steps are repeated to yield acetyl CoA

The four steps are repeated to yield acetyl CoA and ATP

• In one pass through the *B*-oxidation sequence, one molecule of acetyl- CoA, two pairs of electrons, and four protons (H+) are removed from the long-chain fatty acyl–CoA, shortening it by two carbon atoms.
• The equation for one pass, beginning with the coenzyme A ester of our example, palmitate, is oxidized as following equation:

$$\text{Palmitoyl-CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \longrightarrow$$
$$\text{myristoyl-CoA} + \text{acetyl-CoA} + \text{FADH}_2 + \text{NADH} + \text{H}^+$$

• Following removal of one acetyl-CoA unit from palmitoyl- CoA, the coenzyme A thioester of the shortened fatty acid (now the 14-carbon myristate) remains
• **The myristoyl- CoA** can now go through the four steps **β**–oxidation, exactly analogous to the first, to yield a second molecule of acetyl-CoA and **lauroyl-CoA,** the coenzyme A thioester of the **12-carbon laurate.**
• Altogether, seven passes through **the β-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight** molecules of acetyl-CoA • The overall equation is:

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow$$
$$8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{O}_2 + 28\text{P}_i + 28\text{ADP} \longrightarrow$$
$$8\ \text{acetyl-CoA} + 28\text{ATP} + 7\text{H}_2\text{O}$$

Beta oxidation in bears during hibernation

• Many animals depend on fat stores for energy during hibernation, during migratory periods, and in other situations involving metabolic adjustments.
• One of the most pronounced adjustments of fat metabolism occurs in hibernating grizzly bears.
• These animals remain in a continuous state of dormancy for periods as long as seven months.
• the bear maintains a body temperature of between 32 and 35C, close to the normal (nonhibernating) level
• Although expending about 25,000 kJ/day (6,000 kcal/day), the bear does not eat, drink, urinate, or defecate for months at a time.
• Fat oxidation yields sufficient energy for maintenance of body temperature, active synthesis of amino acids and proteins, and other energy-requiring activities, such as membrane transport.

• Fat oxidation also releases large amounts of water, which replenishes water lost in breathing.

• The glycerol released by degradation of triacylglycerols is converted into blood glucose by gluconeogenesis.
• Urea formed during breakdown of amino acids is reabsorbed in the kidneys and recycled**, the amino groups reused to make new amino acids for maintaining body proteins**.
• Bears store an enormous amount of body fat in preparation for their long sleep.
• An adult grizzly consumes about 38,000 kJ/day during the late spring and summer, but as winter approaches it feeds 20 hours a day, consuming up to 84,000 kJ daily.
• Large amounts of triacylglycerols are formed from the huge intake of carbohydrates during the fattening-up period.

Acetyl CoA produced by β-oxidation can enter Citric acid cycle

• **Acetyl CoA produced by β-oxidation can be** oxidized to CO_2 and H_2O by the citric acid cycle

$$8\ \text{Acetyl-CoA} + 16O_2 + 80P_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16CO_2 + 16H_2O \qquad (17\text{–}5)$$

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7O_2 + 28P_i + 28\text{ADP} \longrightarrow 8\ \text{acetyl-CoA} + 28\text{ATP} + 7H_2O$$

$$8\ \text{Acetyl-CoA} + 16O_2 + 80P_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16CO_2 + 16H_2O \qquad (17\text{–}5)$$

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 108\text{ATP} + 16CO_2 + 23H_2O$$

• The energetic cost of activating a fatty acid is equivalent to two ATP, and the net gain per molecule of palmitate is 106 ATP.

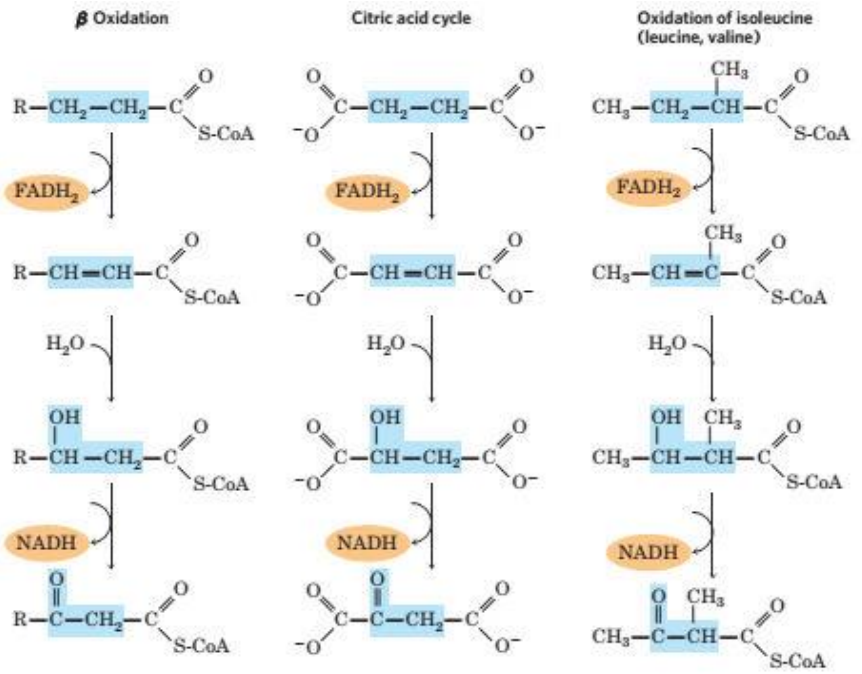
TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 526).

Table 17-1
Lehninger Principles of Biochemistry, Sixth Edition



A conserved reaction sequence to introduce a carbonyl function on the carbon β- to a carboxyl group. The β-oxidation pathway for fatty acyl–CoAs, the pathway from succinate to oxaloacetate in the citric acid cycle, and the pathway by which the deaminated carbon skeletons from isoleucine, leucine, and valine are oxidized as fuels, use the same reaction sequence.

Fatty Acid Catabolism

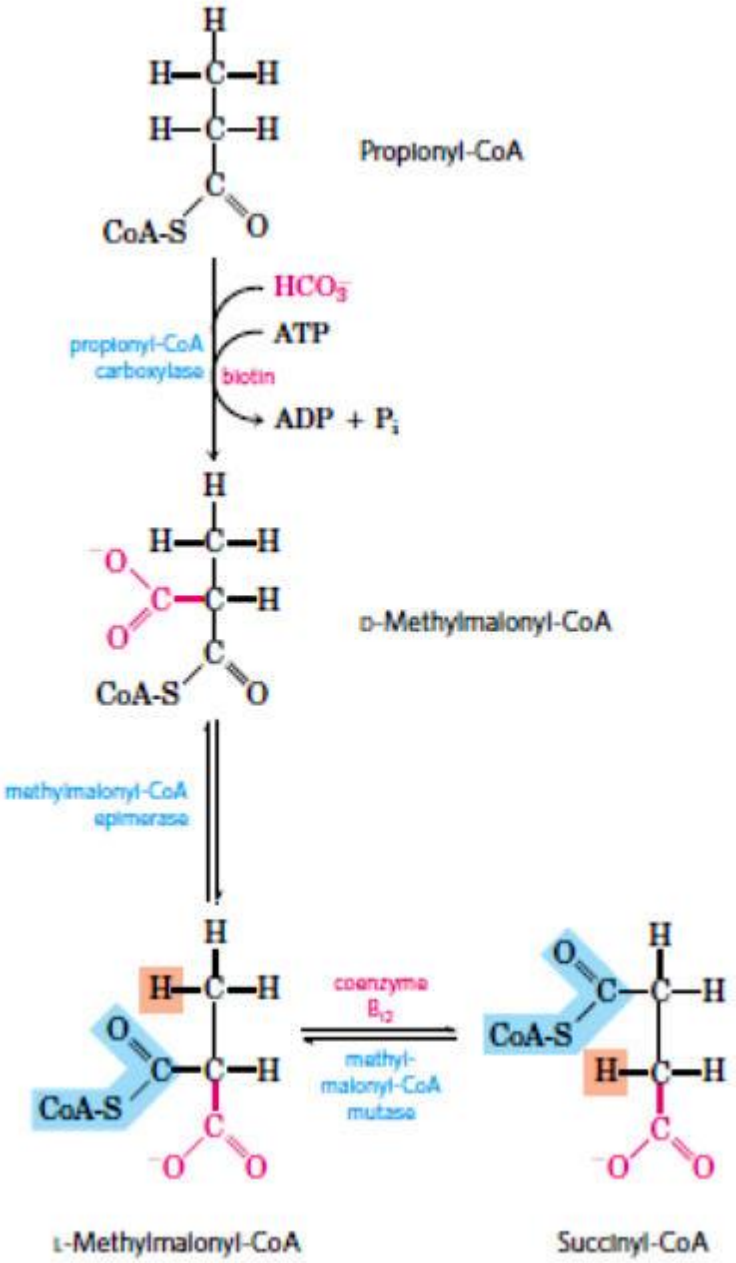
Complete Oxidation of Odd-numbered fatty acids requires 3 additional reactions

Oxidation of Odd-numbered fatty acids

• Fatty acids with an odd number of carbons are found in the lipids of many plants and marine organisms.
• Cattle and other ruminant animals form large amounts of the three-carbon **propionate** (CH3— CH2—COO2) during fermentation of carbohydrates in the rumen.
• The propionate is absorbed into the blood and oxidized by the liver and other tissues
• Small quantities of propionate are added as a mold inhibitor to some breads and cereals, thus entering the human diet.
• Long-chain odd-number fatty acids are oxidized in the same pathway as the even-number acids, … at the carboxyl end of the chain.
• the substrate for the last pass through the β-oxidation sequence is a fatty acyl–CoA with a five carbon fatty acid.

Oxidation of propionyl-CoA

• The acetyl-CoA can be oxidized in the citric acid cycle, but propionyl-CoA enters a different pathway having three enzymes.
• Propionyl-CoA is first carboxylated to form the D stereoisomer of **methylmalonyl-CoA** by **propionyl-CoA carboxylase**, which contains the cofactor biotin. In this enzymatic reaction, as in the pyruvate carboxylase reaction (see Fig. 16–17), CO2 (or its hydrated ion, HCO) is activated by attachment to biotin before its transfer to the substrate, in this case the propionate moiety.
• Formation of the carboxybiotin intermediate requires energy, which is provided by ATP.
• The D-methylmalonyl-CoA thus formed is enzymatically epimerized to its L stereoisomer by **methylmalonyl- CoA epimerase.**
• The L-methylmalonyl-CoA then undergoes an intramolecular rearrangement to form succinyl-CoA, which can enter the citric acid cycle.
• This rearrangement is catalyzed by **methylmalonyl- CoA mutase**, which requires as its coenzyme **5'-deoxyadenosylcobalamin**, or **coenzyme B12**, which is derived from vitamin B12 (cobalamin).



Oxidation of propionyl-CoA produced by oxidation of odd-number fatty acids.
The sequence involves the carboxylation of propionyl-CoA to D-methylmalonyl-CoA and conversion of the latter to succinyl-CoA. This conversion requires epimerization of D- to Lmethylmalonyl- CoA, followed by a reaction in which substituents on adjacent carbon atoms exchange positions

Fatty Acid Catabolism- 72

Transcription Factors Turn on the Synthesis of Proteins for Lipid Catabolism

Transcription Factors:

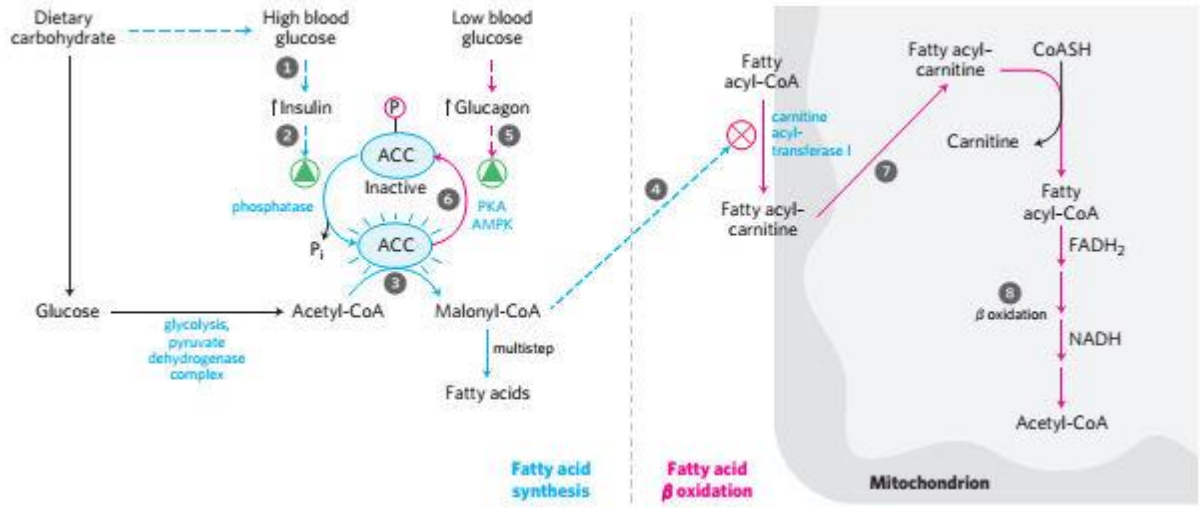
*a **transcription factor** (TF) (or sequence-specific DNA-binding **factor**) is a protein that controls the rate of **transcription** of particular genes; gene expression*

• Transcriptional regulation can change the number of molecules of the enzymes of fatty acid oxidation on a longer time scale

• The **PPAR** family of nuclear receptors are transcription factors that affect many metabolic processes in response to a variety of fatty acid–like ligands. (They were originally recognized as *p*eroxisome *p*roliferator-*a*ctivated *r*eceptors, then were found to function more broadly.)

• PPAR acts in muscle, adipose tissue, and liver to turn on a set of genes essential for fatty acid oxidation, including the fatty acid transporter, carnitine acyltransferases I and II, fatty acyl–CoA dehydrogenases for short, medium, long, and very long acyl chains, and related enzymes.

• This response is triggered when a cell or organism has an increased demand for energy from fat catabolism, **such as during a fast between meals or under conditions of longer-term starvation.**
• **Glucagon, released in response to low blood glucose**, can act through cAMP and the transcription factor CREB to turn on certain genes for lipid catabolism.



When the diet provides a ready source of carbohydrate as fuel, oxidation of fatty acids is unnecessary and is therefore downregulated. Two enzymes are key to the coordination of fatty acid metabolism: **acetyl-CoA carboxylase (ACC), the first enzyme in the synthesis of fatty acids, and carnitine acyltransferase I,** which limits the transport of fatty acids into the mitochondrial matrix for oxidation Ingestion of a high-carbohydrate meal raises the blood glucose level and thus
1. triggers the release of insulin.
2. Insulin dependent protein phosphatase dephosphorylates ACC, activating it.
3. ACC catalyzes the formation of malonyl-CoA (the first intermediate of fatty acid synthesis), and
4. malonyl-CoA inhibits carnitine acyltransferase I, thereby preventing fatty acid entry into the mitochondrial matrix. When blood glucose levels drop between meals,
5. Glucagon release activates cAMP-dependent protein kinase **(PKA)**
6. PKA phosphorylates and inactivates ACC.
7. The concentration of malonylCoA falls, the inhibition of fatty acid entry into mitochondria is relieved, and fatty acids enter the mitochondrial matrix
8. become the major fuel. Because glucagon also triggers the mobilization of fatty acids in adipose tissue, a supply of fatty acids begins arriving in the blood.

• This response is triggered when a cell or organism has an increased demand for energy from fat catabolism, **such as during a fast between meals or under conditions of longer-term starvation.**
• **Glucagon, released in response to low blood glucose**, can act through cAMP and the transcription factor CREB to turn on certain genes for lipid catabolism.
• Another situation that is accompanied by major changes in the expression of the enzymes of fatty acid oxidation is the **transition from fetal to neonatal metabolism in the heart.**
• In the fetus the **principal fuels are glucose and lactate,** but **in the neonatal heart, fatty acids are the main fuel.**
• At the time of this transition, PPAR is activated and in turn activates the genes essential for fatty acid metabolism.
• The major site of fatty acid oxidation, at rest and during exercise, is skeletal muscle.
• Endurance training increases PPAR expression in muscle, leading to increased levels of fatty acid–oxidizing enzymes and increased oxidative capacity of the muscle.

Genetic Defects in Fatty Acyl–CoA Dehydrogenases Cause Serious Disease

• Stored triacylglycerols are typically the chief source of energy for muscle contraction, and an inability to oxidize fatty acids from triacylglycerols has serious consequences for health.
• The most common genetic defect in fatty acid catabolism in U.S. and northern European populations is due to a mutation in the gene encoding the **medium-chain acyl-CoA dehydrogenase (MCAD)**.

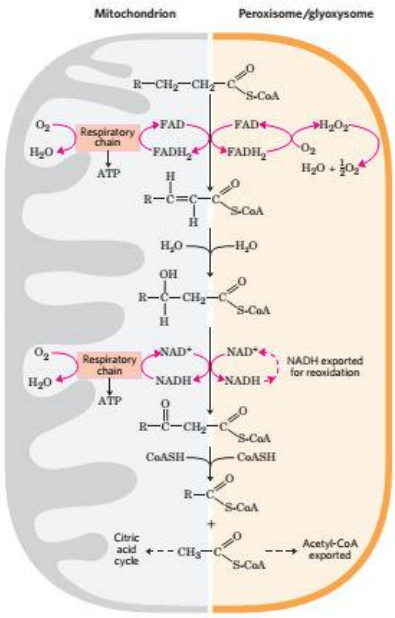
• Among northern Europeans, the frequency of carriers (individuals with this recessive mutation on one of the two homologous chromosomes) is about 1 in 40, and about 1 individual in 10,000 has the disease—that is, has two copies of the **mutant MCAD allele and is unable to oxidize fatty acids of 6 to 12 carbons.**
• **Symptoms:** The disease is characterized by recurring episodes of a syndrome that includes fat accumulation in the liver, **high blood levels of octanoic acid (8:0), low blood glucose (hypoglycemia), sleepiness, vomiting, and coma**.
• **Diagnosis :** The pattern of organic acids in the urine helps in the diagnosis of this disease: the urine commonly contains high levels of 6-carbon to 10-carbon dicarboxylic acids (produced by oxidation) and low levels of urinary ketone bodies (we discuss oxidation below and ketone bodies in Section 17.3).
• Although individuals may have no symptoms between episodes, the episodes are very serious; mortality from this disease is 25% to 60% in early childhood.
• If the genetic defect is detected shortly after birth, the infant can be started on a low-fat, high-carbohydrate diet.
• With early detection and careful management of the diet—including avoiding long intervals between meals, to prevent the body from turning to its fat reserves for energy—the prognosis for these individuals is good.
More than 20 other human genetic defects in fatty acid transport or oxidation have been documented, most much less common than the defect in MCAD.
• One of the most severe disorders results from loss of the **long-chain *Beta*-hydroxyacyl-CoA** dehydrogenase activity of the **trifunctional protein, TFP.**
• Other disorders include defects in the ***alpha*** or ***Beta*** **subunits** that affect all three activities of TFP and cause serious heart disease and abnormal skeletal muscle.

Transcription Factors:
a **transcription factor** (TF) (or sequence-specific DNA-binding **factor**) is a protein that controls the rate of **transcription** of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence.

Fatty Acid Catabolism- 73

Peroxisomes Also Carry Out β-Oxidation

• The mitochondrial matrix is the major site of fatty acid oxidation in animal cells, but in certain cells other compartments also contain enzymes capable of oxidizing fatty acids to acetyl-CoA, by a pathway not identical to that in mitochondria.
• In plant cells, the major site of *beta* oxidation is not mitochondria but peroxisomes. In **peroxisomes**, membrane-enclosed organelles of animal and plant cells, the intermediates for oxidation of fatty acids are coenzyme A derivatives, and the process consists of four steps, as in mitochondrial *beta* oxidation
(1) Dehydrogenation
(2) addition of water to the resulting double bond,
(3) oxidation of the *beta*-hydroxyacyl-CoA to a ketone, and
(4) thiolytic cleavage by coenzyme A. (The identical reactions also occur in glyoxysomes)



• One difference between the peroxisomal and mitochondrial pathways is in the chemistry of the first step.
• In peroxisomes, the flavoprotein acyl-CoA oxidase that introduces the double bond passes electrons directly to O2, producing H2O2 (hydrogen peroxide). (Thus the name peroxisomes.)
• This strong and potentially damaging oxidant is immediately cleaved to H_2O and O_2 by **catalase**.
• Recall that in mitochondria, the electrons removed in the first oxidation step pass through the respiratory chain to O_2 to produce H_2O, and this process is accompanied by ATP synthesis.
• In peroxisomes, the energy released in the first oxidative step of fatty acid breakdown is not conserved as ATP, but is dissipated as heat.
• A second important difference between mitochondrial and peroxisomal *Beta* oxidation in mammals is in the specificity for fatty acyl–CoAs; the peroxisomal system is much more active on very-long-chain fatty acids such as hexacosanoic acid (26:0) and on branched chain fatty acids such as phytanic acid and **pristanic acid**
• These less-common fatty acids are obtained in the diet from dairy products, the fat of ruminant animals, meat, and fish. Their catabolism in the peroxisome involves several auxiliary enzymes unique to this organelle.
• The inability to oxidize these compounds is responsible for several serious human diseases.
• Individuals with **Zellweger syndrome** are unable to make peroxisomes and therefore lack all the metabolism unique to that organelle.
• In **X-linked adrenoleukodystrophy (XALD)**, peroxisomes fail to oxidize very-long-chain fatty acids, apparently for lack of a functional transporter for these fatty acids in the peroxisomal membrane.
• Both defects lead to accumulation in the blood of very-long-chain fatty acids, especially 26:0.
• XALD affects young boys before the age of 10 years, causing loss of vision, behavioral disturbances, and death within a few years.
• **In mammals, high concentrations of fats in the diet result in increased synthesis of the enzymes of peroxisomal *Beta* oxidation in the liver.**
• Liver peroxisomes do not contain the enzymes of the citric acid cycle and cannot catalyze the oxidation of acetyl-CoA to CO2. Instead, longchain or branched fatty acids are catabolized to shorter-chain products, such as hexanoyl-CoA, which are exported to mitochondria and completely oxidized.

Fatty Acid Catabolism-74

The *beta*-Oxidation Enzymes of Different Organelles Have Diverged during Evolution

Glyoxysomes

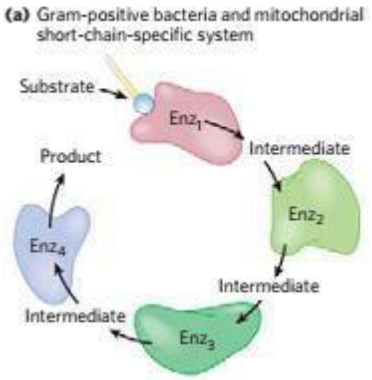
• Plant peroxisomes and glyoxysomes are similar in structure and function
• **Glyoxysomes** are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi.
• Seeds that contain fats and oils include corn, soybean, sunflower, peanut and pumpkin.

Plant Peroxisomes and Glyoxysomes Use Acetyl-CoA as a Biosynthetic Precursor

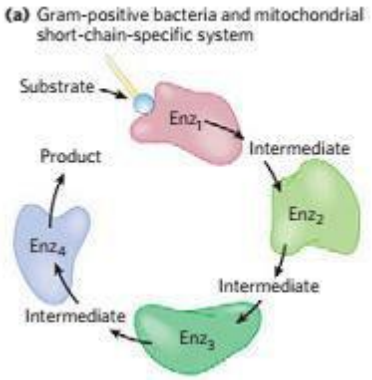
• In plants, fatty acid oxidation does not occur primarily in mitochondria but in the peroxisomes of leaf tissue and in the glyoxysomes of germinating seeds.
• The biological role of oxidation in these organelles is to use stored lipids primarily to provide biosynthetic precursors, not energy. During seed germination, stored triacylglycerols are converted into glucose, sucrose, and a wide variety of essential metabolites.
• Fatty acids released from the triacylglycerols are first activated to their coenzyme A derivatives and oxidized in glyoxysomes by the same four-step process that takes place in peroxisomes.
• The acetyl-CoA produced is converted via the glyoxylate cycle to four-carbon precursors for gluconeogenesis
• Glyoxysomes, like peroxisomes, contain high concentrations of catalase, which converts the H_2O_2 produced by oxidation to H2O and O2.
• Enzymes found in the **glyoxysome** catalyze the formation of acetyl CoA from fatty acids stored within germinating seeds.
• Although the beta-oxidation reactions in mitochondria are essentially the same as those in peroxisomes and glyoxysomes, the enzymes (isozymes) differ significantly between the two types of organelles.

• The differences reflect an evolutionary divergence that occurred very early, with the separation of gram-positive and gram-negative bacteria
• **In mitochondria, the four -oxidation enzymes that act on short-chain fatty acyl– CoAs are separate,** soluble proteins similar in structure to the analogous enzymes of gram-positive bacteria
• The gram-negative bacteria have four activities in three soluble subunits, and the eukaryotic enzyme system that acts on long-chain fatty acids—the trifunctional protein, **TFP—has three enzyme activities in two subunits that are membrane-associated**

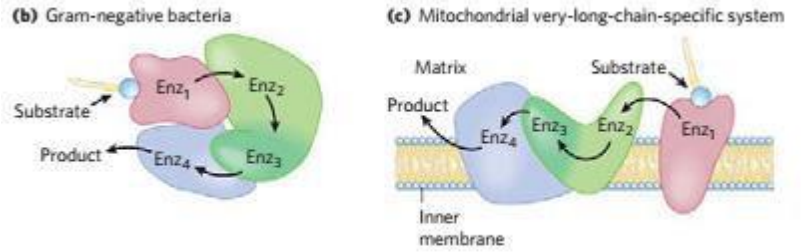
• The *beta*-oxidation enzymes of plant peroxisomes and glyoxysomes, form a complex of proteins, one of which contains four enzymatic activities in a single polypeptide
• The first enzyme, acyl-CoA oxidase, is a single polypeptide chain; the **multifunctional protein (MFP)** contains the second and third enzyme activities (enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase) as well as two auxiliary activities needed for the oxidation of unsaturated fatty acids (D-3- hydroxyacyl-CoA epimerase and D3,D2-enoyl-CoA isomerase); the fourth enzyme, thiolase, is a separate, soluble polypeptide.
• Interesting thing is that the enzymes that catalyze the synthesis of fatty acids are also organized differently in bacteria and eukaryotes; in bacteria, the seven enzymes needed for fatty acid synthesis are separate polypeptides, but in mammals, all seven activities are part of a single, huge polypeptide chain.
• One advantage to the cell in having several enzymes of the same pathway encoded in a single polypeptide chain is that this solves the problem of regulating the synthesis of enzymes that must interact functionally; regulation of the expression of *one* gene ensures production of the same number of active sites for all enzymes in the path.
• When each enzyme activity is on a separate polypeptide, some mechanism is required to coordinate the synthesis of all the gene products.
• The *disadvantage* of having several activities on the same polypeptide is that the longer the polypeptide chain, the greater is the probability of a mistake in its synthesis: a single incorrect amino acid in the chain may make all the enzyme activities in that chain useless.



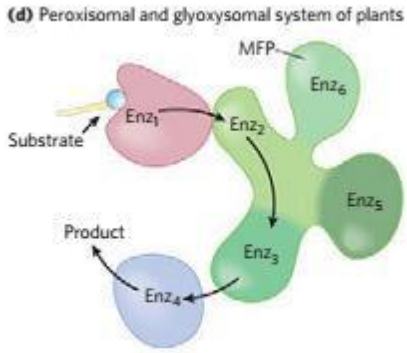
The enzymes of oxidation.
In the picture Shown on next slides, are the different subunit
structures of the enzymes of *Beta* oxidation in gram-positive and gram-negative bacteria, mitochondria, and plant peroxisomes and glyoxysomes.
Enz1 is acyl-CoA dehydrogenase; Enz2, enoyl-CoA hydratase;
Enz3, L- *Beta* -hydroxyacyl-CoA dehydrogenase; Enz4, thiolase;
Enz5, D-3-hydroxyacyl-CoA epimerase, and Enz6, D3,D2-enoyl- CoA isomerase.



(a) The four enzymes of *beta* oxidation in gram-positive bacteria are separate, soluble entities, as are those of the short-chain-specific system of mitochondria



(b) In gram negative bacteria, the four enzyme activities reside in three polypeptides; Enz 2 and Enz 3 are parts of a single polypeptide chain.
(c) The very-long-chain-specific system of mitochondria is also composed of three polypeptides, one of which includes the activities of Enz2 and Enz3; in this case, the system is bound to the inner mitochondrial membrane.



(d) In the peroxisomal and glyoxysomal *beta* oxidation systems of plants, Enz 1 and Enz4 are separate polypeptides, but Enz2 and Enz3, as well as two auxiliary enzymes (Enz5 and Enz6), are part of a single polypeptide chain: the multifunctional protein, MFP.

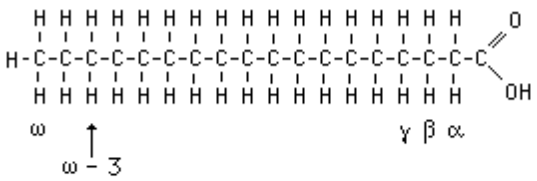
Isozymes

• In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants, of enzymes.
• Isozymes represent enzymes from different genes that process or catalyse the same reaction

Fatty Acid Catabolism-75

The *Omega*-Oxidation of Fatty Acids Occurs in the Endoplasmic Reticulum

• **Omega oxidation** (ω-**oxidation**) is a process of fatty acid metabolism in some species of animals.
• The carbon atom closest to the carboxyl group is the alpha carbon, the next carbon is the beta carbon and so on.
• It is an alternative pathway to beta **oxidation** that, instead of involving the β carbon, involves the **oxidation** of the ω carbon (the carbon most distant from the carboxyl group of the fatty acid).
• In vertebrates, the enzymes for ω oxidation are located in the smooth ER of liver and kidney cells, instead of in the mitochondria where β oxidation occurs.



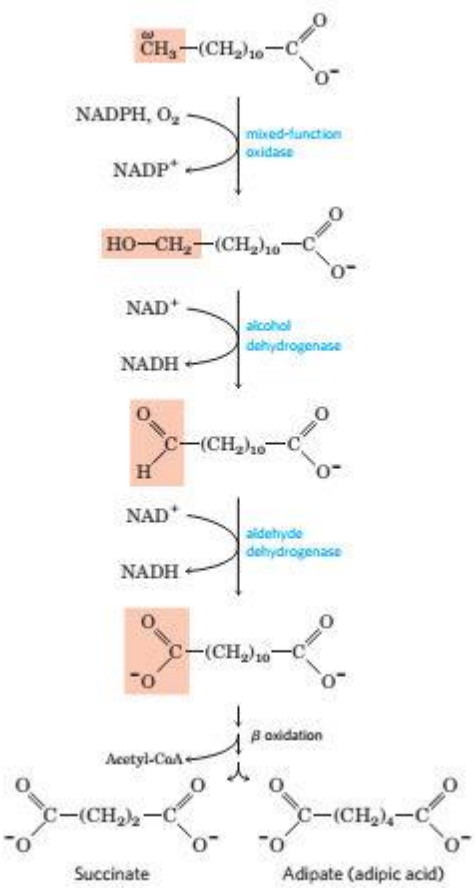
Use of Greek letters to designate carbons

The carbon next to the -COOH group is designated α; the next one is β, and so forth. The most distant carbon is designated ω. Sometimes carbon atoms close to the ω carbon are designated in relation to it. *E.g.*, the third from the end is ω - 3 (omega minus 3).

• Although mitochondrial ***Beta*** oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of **the (omega) carbon—the carbon most distant from the carboxyl group.**

• The enzymes unique to ***Omega*** **oxidation are located (in vertebrates) in the endoplasmic reticulum of liver and** kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms.

• In mammals ***Omega*** **-** oxidation is normally a minor pathway for fatty acid degradation, but when ***beta-*** oxidation is defective (because of mutation or a carnitine deficiency) it becomes more important.



1. The first step introduces a hydroxyl group onto the ***Omega*** **carbon ..** Hydroxylation**.** The oxygen for this group comes from molecular oxygen (O2) in a complex reaction that involves **cytochrome P450** and the electron donor NADPH. Reactions of this type are catalyzed by **mixed-function oxidases**
2. Oxidation of the hydroxyl group to an aldehyde by **alcohol dehydrogenase**
3. The third step is the Oxidation of the aldehyde group to a carboxylic acid by NAD+. The product of this step is a fatty acid with a carboxyl group at each end, by **aldehyde dehydrogenase**

• At this point, either end can be attached to coenzyme A, and the molecule can enter the mitochondrion and undergo oxidation by the normal route.

• In each pass through beta -oxidation pathway, **the "double-ended" fatty acid yields final products dicarboxylic acids such as succinic acid, which can enter the citric acid cycle, and adipic acid.**

Adipic acid

• **Adipic acid** or hexanedioic **acid (6 carbon chain)** is the organic compound with the formula $(CH_2)_4(COOH)_2$. From an industrial perspective, it is the most important dicarboxylic **acid**

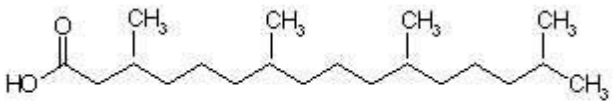
• About 2.5 billion kilograms of this white crystalline powder are produced annually, mainly as a precursor for the production of nylon.

Fatty Acid Catabolism- 76

Phytanic Acid Undergoes alpha-Oxidation in Peroxisomes

Alpha-Oxidation

• **α-Oxidation** is important in the catabolism of branchedchain fatty acids.
• **Takes place at alpha carbon atom in peroxisomes**
• **Phytanic acid:**
• **Phytanic acid** (or **3,7,11,15-tetramethyl hexadecanoic acid**-C20H40O2) is a branched chain fatty acid that humans can obtain through the consumption of dairy products, ruminant animal fats, and certain fish
• Unlike most fatty acids, phytanic acid cannot be metabolized by β-oxidation.
• Phytanic acid has a **methyl-substituted *beta*- carbon** and therefore cannot undergo ***beta*-** oxidation.
• These branched fatty acids are **catabolized in peroxisomes** of animal cells by ***Alpha*-oxidation.**



PHYTANIC ACID

Steps of *Alpha*-Oxidation

1. Phytanic acid is first attached to **CoA** to form **phytanoyl CoA**
2. Phytanoyl CoA is oxidized by **phytanoyl CoA hydroxylase** in a process using Fe2+ and O2, to yield 2-hydroxyphytanoyl-CoA.
3. 2-hydroxyphytanoyl-CoA is cleaved to form pristanal and **formyl-CoA** (in turn later broken down into formate and eventually CO2).
4. Pristanal is oxidized by **aldehyde dehydrogenase** to form **pristanic acid** (which can then undergo **beta-Oxidation.**
• Notice that ***beta*-** oxidation of pristanic acid releases propionyl- CoA, not acetyl-CoA.
• Propionyl-CoA is further catabolized as in case of odd numbered fatty acids
• **alpha carbon was decarboxylated to form an aldehyde, that is now having a carbon chain shorter of 1 carbon but has no functional group on beta carbon**
• **Now it's oxidized at beta carbon and process continues in same way**

Refsum disease

Refsum disease:

• A disorder resulting from a genetic defect in phytanoyl-CoA hydroxylase, leads to very high blood levels of phytanic acid and severe neurological problems, including blindness and deafness.
• Phytanic acid accumulates in patients suffering from a peroxisome biogenesis disorder, which includes Zellweger Syndrome, Neonatal Adrenoleukodystrophy, and Infantile Refsum Disease.
• Formic acid and not CO_2 is the primary reaction product of phytanic acid oxidation. The amount of formic acid was shown to be about 9-fold higher as compared to CO_2.

Origin of Phytanic Acid

• Phytol is the alcohol moiety of the chlorophyll molecule and, as an integral part of chlorophyll, it is abundantly present in plants and even in the marine environment
• Phytol can be released from chlorophyll by the action of bacteria present in the rumen of ruminant animals.
• The resulting free **phytol** can be converted to phytanic acid in the rumen of animals

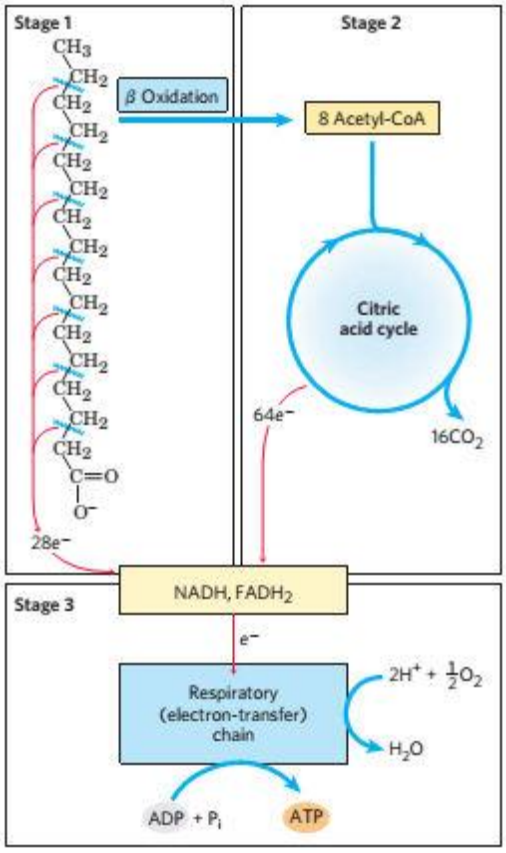
Fatty Acid Catabolism- 77

Ketone Bodies

• In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the "ketone bodies,"

• **These include acetone**, **acetoacetate**, and **D-*beta*hydroxybutyrate,** for export to other tissues**.**

• **The term "bodies" is a historical artifact; the term is occasionally applied to insoluble particles, but these compounds are soluble in blood and urine.**



• **Acetone**, produced in smaller quantities than the other ketone bodies, is exhaled.

• **Acetoacetate** and **D-*beta*- hydroxybutyrate** are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex.

• The brain, which preferentially uses glucose as fuel, can adapt to the use of **acetoacetate** or **D-*beta*-hydroxybutyrate** under starvation conditions, when glucose is unavailable.

• The production and export of ketone bodies from the liver to extrahepatic tissues allows continued oxidation of fatty acids in the liver when acetyl CoA is not being oxidized in the citric acid cycle.

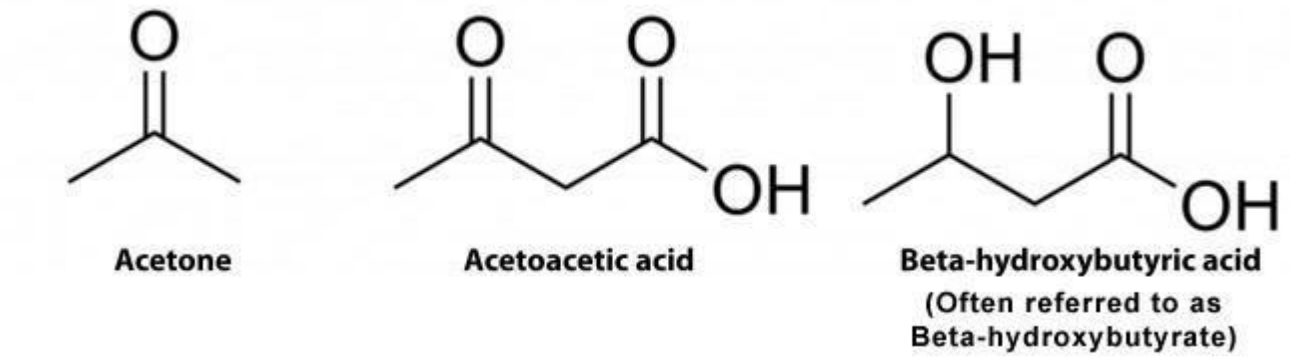
Ketone Bodies, Formed in the Liver, Are Exported to Other Organs as Fuel

• The first step in the formation of acetoacetate, occurring in the liver, is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase; this is simply the reversal of the last step of oxidation of fatty acids.

• The acetoacetyl-CoA then condenses with acetyl-CoA to form ***Beta*-hydroxy-*Beta*methylglutaryl- CoA (HMG-CoA), which is cleaved to free acetoacetate and acetyl-CoA.**

• **The acetoacetate is reversibly reduced by D-*Beta*-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-*Beta*-hydroxybutyrate.**

• **This enzyme is specific for the D stereoisomer; it does not act on L *Beta*-hydroxyacyl-CoAs and is not to be confused with L-*Beta*-hydroxyacyl-CoA dehydrogenase of the *Beta*-oxidation pathway.**



Ketone Bodies in healthy condition

• In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of **acetoacetate decarboxylase.**

• **Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic.**

• **Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.**

• In extrahepatic tissues, D-***Beta***-hydroxybutyrate is oxidized to acetoacetate by D-***Beta***hydroxybutyrate dehydrogenase.

• **The acetoacetate is activated to its coenzyme A ester** by transfer of CoA from succinylCoA, an intermediate of the citric acid cycle (see Fig.16–7), in a reaction catalyzed by ***Beta*-ketoacyl-CoA transferase, also called thiophorase.**

• **The acetoacetylCoA is then cleaved by thiolase to yield two acetylCoAs, which enter the citric acid cycle.**

• **Thus the ketone bodies are used as fuels in all tissues except liver, which lacks thiophorase.**

The liver is therefore a producer of ketone bodies for the other tissues, but not a consumer.

The production and export of ketone bodies by the liver allows continued oxidation of fatty acids with only minimal oxidation of acetyl-CoA.

• When intermediates of the citric acid cycle are being siphoned off for glucose synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slows— and so does acetyl-CoA oxidation.

• Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl-CoA, *Beta*-oxidation slows for want of the free coenzyme.

• The production and export of ketone bodies frees coenzyme A, allowing continued fatty acid oxidation.

Ketone Bodies Are Overproduced in Diabetes and during Starvation

• Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid cycle intermediates, diverting acetyl-CoA to ketone body production.

• In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat.

• Under these conditions, levels of malonyl-CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to **acetylCoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis**.

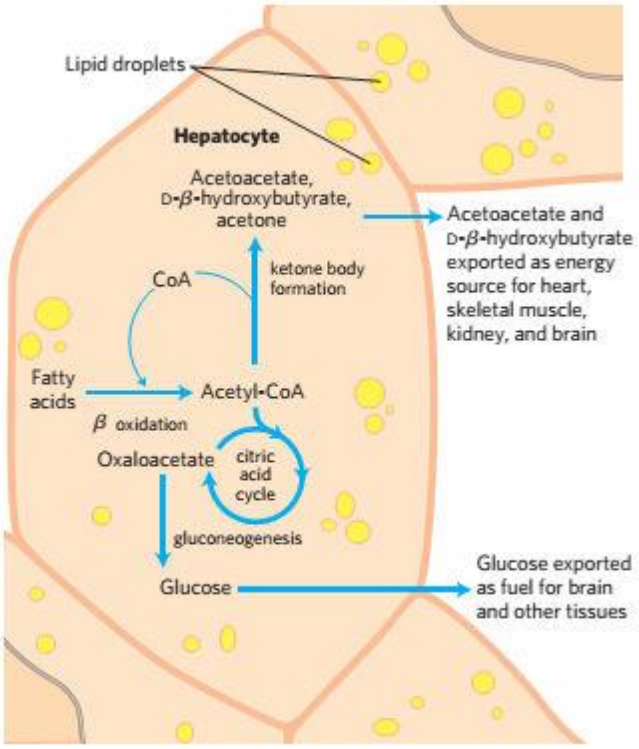
• The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them.

• The increased blood levels of acetoacetate and **D-*bet*Ahydroxybutyrate** lower the blood pH, causing the condition known as **acidosis**.

• Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of individuals with untreated diabetes can reach extraordinary levels—a blood concentration of 90 mg/100 mL

(compared with a normal level of ,3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of #125 mg/24 hr). This condition is called **ketosis**.

• Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis).



Ketone body formation and export from the liver.

Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate. The released coenzyme A allows continued *Beta* oxidation of fatty acids.