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Principles of Biochemistry

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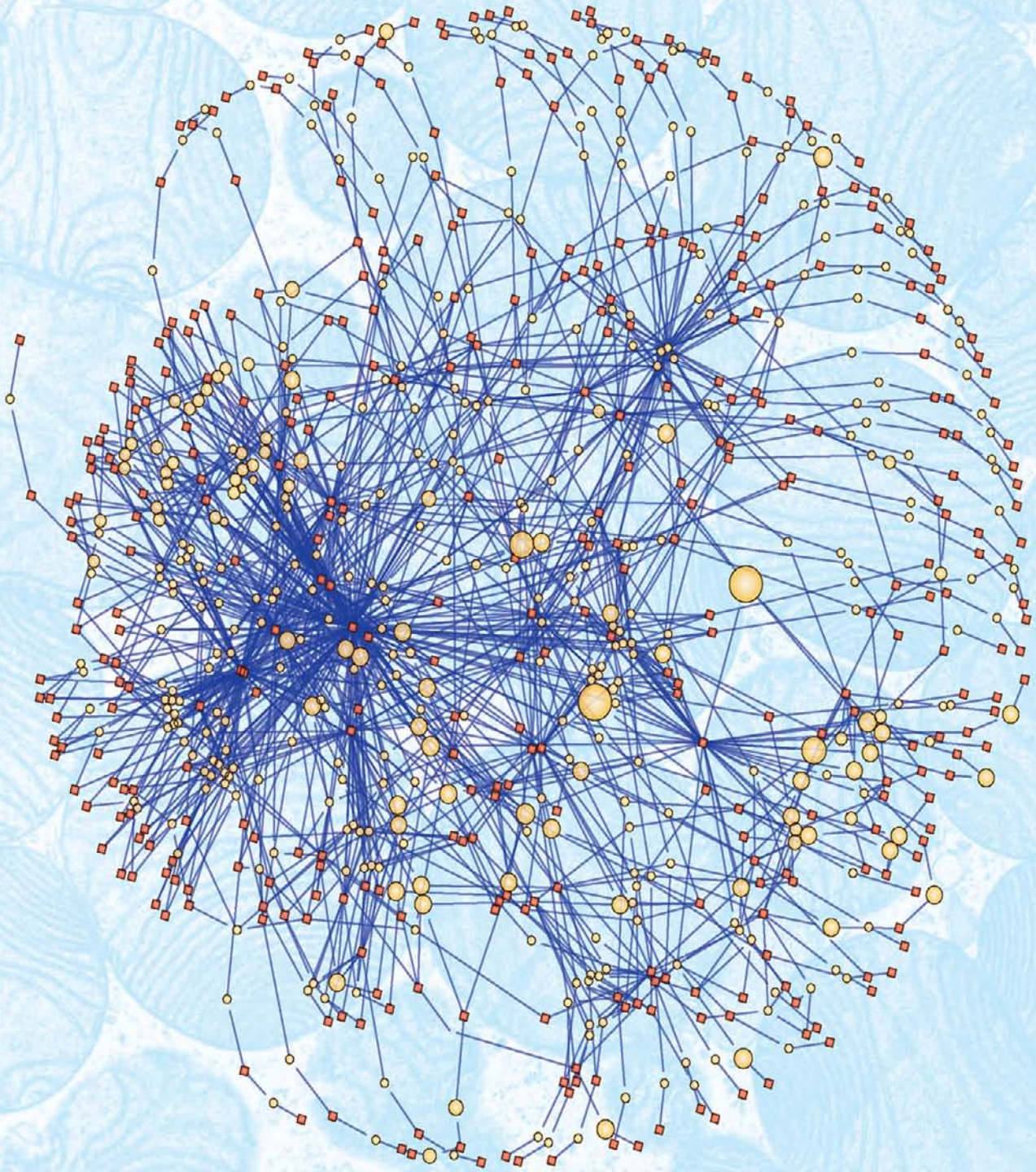


13

Bioenergetics and Biochemical Reaction Types

Living cells and organisms must perform work to stay alive, to grow, and to reproduce. The ability to harness energy and to channel it into biological work is a fundamental property of all living organisms; it must have been acquired very early in cellular evolution. Modern organisms carry out a remarkable variety of energy transductions, conversions of one form of energy to another. They use the chemical energy in fuels to bring about the synthesis of complex, highly ordered macromolecules from simple precursors. They also convert the chemical energy of fuels into concentration gradients and electrical gradients, into motion and heat, and, in a few organisms such as fireflies and deep-sea fish, into light. Photosynthetic organisms transduce light energy into all these other forms of energy.

The chemical mechanisms that underlie biological energy transductions have fascinated and challenged biologists for centuries. The French chemist Antoine Lavoisier recognized that animals somehow transform chemical fuels (foods) into heat and that this process of respiration is essential to life. He observed that

Antoine Lavoisier, 1743–1794

> . . . in general, respiration is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lighted lamp or candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves. . . . One may say that this analogy between combustion and respiration has not escaped the notice of the poets, or rather the philosophers of antiquity, and which they had expounded and interpreted. This fire stolen from heaven, this torch of Prometheus, does not only represent an ingenious and poetic idea, it is a faithful picture of the operations of nature, at least for animals that breathe; one may therefore say, with the ancients, that the torch of life lights itself at the moment the infant breathes for the first time, and it does not extinguish itself except at death.*

In the twentieth century, we began to understand much of the chemistry underlying that "torch of life." Biological energy transductions obey the same chemical and physical laws that govern all other natural processes. It is therefore essential for a student of biochemistry to understand these laws and how they apply to the flow of energy in the biosphere.

In this chapter we first review the laws of thermodynamics and the quantitative relationships among free energy, enthalpy, and entropy. We then review the common types of biochemical reactions that occur in living cells, reactions that harness, store, transfer, and release the energy taken up by organisms from their surroundings. Our focus then shifts to reactions that have special roles in biological energy exchanges, particularly those involving ATP. We finish by considering the importance of oxidation-reduction reactions in living cells, the energetics of biological electron transfers, and the electron carriers commonly employed as cofactors in these processes.

*From a memoir by Armand Seguin and Antoine Lavoisier, dated 1789, quoted in Lavoisier, A. (1862) *Oeuvres de Lavoisier*, Imprimerie Impériale, Paris.

13.1 Bioenergetics and Thermodynamics

Bioenergetics is the quantitative study of **energy transductions**—changes of one form of energy into another—that occur in living cells, and of the nature and function of the chemical processes underlying these transductions. Although many of the principles of thermodynamics have been introduced in earlier chapters and may be familiar to you, a review of the quantitative aspects of these principles is useful here.

Biological Energy Transformations Obey the Laws of Thermodynamics

Many quantitative observations made by physicists and chemists on the interconversion of different forms of energy led, in the nineteenth century, to the formulation of two fundamental laws of thermodynamics. The first law is the principle of the conservation of energy: *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.* The second law of thermodynamics, which can be stated in several forms, says that the universe always tends toward increasing disorder: *in all natural processes, the entropy of the universe increases.*

"Now, in the *second* law of thermodynamics . . . "

Living organisms consist of collections of molecules much more highly organized than the surrounding materials from which they are constructed, and organisms maintain and produce order, seemingly immune to the second law of thermodynamics. But living organisms do not violate the second law; they operate strictly within it. To discuss the application of the second law to biological systems, we must first define those systems and their surroundings.

The reacting system is the collection of matter that is undergoing a particular chemical or physical process; it may be an organism, a cell, or two reacting compounds. The reacting system and its surroundings together constitute the universe. In the laboratory, some chemical or physical processes can be carried out in isolated or closed systems, in which no material or energy is exchanged with the surroundings. Living cells and organisms, however, are open systems, exchanging both material and energy with their surroundings; living systems are never at equilibrium with their surroundings, and the constant transactions between system and surroundings explain how organisms can create order within themselves while operating within the second law of thermodynamics.

In Chapter 1 (p. 23) we defined three thermodynamic quantities that describe the energy changes occurring in a chemical reaction:

Gibbs free energy, ***G***, expresses the amount of an energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with the release of free energy (that is, when the system changes so as to possess less free energy), the free-energy change, ΔG, has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive.

Enthalpy, ***H***, 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; the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH.

Entropy, ***S***, is a quantitative expression for the randomness or disorder in a system (see Box 1–3). 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.

The units of ΔG and ΔH are joules/mole or calories/mole (recall that 1 cal = 4.184 J); units of entropy are joules/mole·Kelvin (J/mol·K) (Table 13–1).

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

$$\Delta G = \Delta H - T\,\Delta S \qquad (13\text{–}1)$$

in which ΔG is the change in Gibbs free energy of the reacting system, ΔH is the change in enthalpy of the system, T is the absolute temperature, and ΔS is the change in entropy of the system. By convention, ΔS has a positive sign when entropy increases and ΔH, as noted above, has a negative sign when heat is released by the system to its surroundings. Either of these conditions,

TABLE 13–1 Some Physical Constants and Units Used in Thermodynamics

Boltzmann constant, $\mathbf{k} = 1.381 \times 10^{-23}$ J/K
Avogadro's number, $N = 6.022 \times 10^{23}$ mol^{-1}
Faraday constant, $\mathcal{F} = 96{,}480$ J/V·mol
Gas constant, $R = 8.315$ J/mol·K
$(= 1.987$ cal/mol·K$)$
Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol·K (or cal/mol·K)
1 cal = 4.184 J
Units of absolute temperature, T, are Kelvin, K
25 °C = 298 K
At 25 °C, $RT = 2.478$ kJ/mol
$(= 0.592$ kcal/mol$)$

which are typical of energetically favorable processes, tend to make ΔG negative. In fact, ΔG of a spontaneously reacting system is always negative.

The second law of thermodynamics states that the entropy *of the universe* increases during all chemical and physical processes, but it does not require that the entropy increase take place *in the reacting system* itself. The order produced within cells as they grow and divide is more than compensated for by the disorder they create in their surroundings in the course of growth and division (see Box 1–3, case 2). In short, living organisms preserve their internal order by taking from the surroundings free energy in the form of nutrients or sunlight, and returning to their surroundings an equal amount of energy as heat and entropy.

Cells Require Sources of Free Energy

Cells are isothermal systems—they function at essentially constant temperature (and also function at constant pressure). Heat flow is not a source of energy for cells, because heat can do work only as it passes to a zone or object at a lower temperature. The energy that cells can and must use is free energy, described by the Gibbs free-energy function G, which allows prediction of the direction of chemical reactions, their exact equilibrium position, and the amount of work they can (in theory) perform at constant temperature and pressure. Heterotrophic cells acquire free energy from nutrient molecules, and photosynthetic cells acquire it from absorbed solar radiation. Both kinds of cells transform this free energy into ATP and other energy-rich compounds capable of providing energy for biological work at constant temperature.

Standard Free-Energy Change Is Directly Related to the Equilibrium Constant

The composition of a reacting system (a mixture of chemical reactants and products) 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 concentrations of reactants and products *at equilibrium* define the equilibrium constant, K_{eq} (p. 25). In the general reaction aA + bB $\rightleftharpoons$ cC + dD, where a, b, c, and d are the number of molecules of A, B, C, and D participating, the equilibrium constant is given by

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \qquad (13\text{–}2)$$

where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium.

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. 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 kilopascals (kPa), or 1 atm, the force driving the system toward equilibrium is defined as the standard free-energy change, $\Delta G°$. By this definition, the standard state for reactions that involve hydrogen ions is $[H^+] = 1$ M, or pH 0. Most biochemical reactions, however, occur in well-buffered aqueous solutions near pH 7; both the pH and the concentration of water (55.5 M) are essentially constant.

KEY CONVENTION: For convenience of calculations, biochemists define a standard state different from that used in chemistry and physics: in the biochemical standard state, $[H^+]$ is 10^{-7} M (pH 7) and $[H_2O]$ is 55.5 M. For reactions that involve Mg^{2+} (which include most of those with ATP as a reactant), $[Mg^{2+}]$ in solution is commonly taken to be constant at 1 mM. ■

Physical constants based on this biochemical standard state are called **standard transformed constants** and are written with a prime (such as $\Delta G'°$ and K'_{eq}) to distinguish them from the untransformed constants used by chemists and physicists. (Note that most other textbooks use the symbol $\Delta G°'$ rather than $\Delta G'°$. Our use of $\Delta G'°$, recommended by an international committee of chemists and biochemists, is intended to emphasize that the transformed free energy, $\Delta G'$, is the criterion for equilibrium.) For simplicity, we will hereafter refer to these transformed constants as **standard free-energy changes**.

KEY CONVENTION: In another simplifying convention used by biochemists, when H_2O, H^+, and/or Mg^{2+} are reactants or products, their concentrations are not included in equations such as Equation 13–2 but are instead incorporated into the constants K'_{eq} and $\Delta G'°$. ■

Just as K'_{eq} is a physical constant characteristic for each reaction, so too is $\Delta G'°$ a constant. As we noted in

Chapter 6, there is a simple relationship between K'_{eq} and $\Delta G'^{\circ}$:

$$\Delta G'^{\circ} = -RT \ln K'_{eq} \qquad (13\text{–}3)$$

The standard free-energy change of a chemical reaction is simply an alternative mathematical way of expressing its equilibrium constant. Table 13–2 shows the relationship between $\Delta G'^{\circ}$ and K'_{eq}. If the equilibrium constant for a given chemical reaction is 1.0, the standard free-energy change of that reaction is 0.0 (the natural logarithm of 1.0 is zero). If K'_{eq} of a reaction is greater than 1.0, its $\Delta G'^{\circ}$ is negative. If K'_{eq} is less than 1.0, $\Delta G'^{\circ}$ is positive. Because the relationship between $\Delta G'^{\circ}$ and K'_{eq} is exponential, relatively small changes in $\Delta G'^{\circ}$ correspond to large changes in K'_{eq}.

It may be helpful to think of the standard free-energy change in another way. $\Delta G'^{\circ}$ is the difference between the free-energy content of the products and the free-energy content of the reactants, under standard conditions. When $\Delta G'^{\circ}$ is negative, the products contain less free energy than the reactants and the reaction will proceed spontaneously under standard conditions; all chemical reactions tend to go in the direction that results in a decrease in the free energy of the system. A positive value of $\Delta G'^{\circ}$ means that the products of the reaction contain more free energy than the reactants, and this reaction will tend to go in the reverse direction if we start with 1.0 M concentrations of all components (standard conditions). Table 13–3 summarizes these points.

TABLE 13–2 Relationship between Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G'^{\circ}$ (kcal/mol)*
10^{3}	−17.1	−4.1
10^{2}	−11.4	−2.7
10^{1}	−5.7	−1.4
1	0.0	0.0
10^{-1}	5.7	1.4
10^{-2}	11.4	2.7
10^{-3}	17.1	4.1
10^{-4}	22.8	5.5
10^{-5}	28.5	6.8
10^{-6}	34.2	8.2

*Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists and nutritionists sometimes express $\Delta G'^{\circ}$ values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Tables 13–4 and 13–6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

TABLE 13–3 Relationships among K'_{eq}, $\Delta G'^{\circ}$, and the Direction of Chemical Reactions

When K'_{eq} is . . .	$\Delta G'^{\circ}$ is . . .	Starting with all components at 1 M, the reaction . . .
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

WORKED EXAMPLE 13–1 Calculation of $\Delta G'^{\circ}$

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

$$\text{Glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

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:

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

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, $\Delta G'^{\circ}$ has the same magnitude but the *opposite* sign.)

Table 13–4 gives the standard free-energy changes for some representative chemical reactions. Note that hydrolysis of simple esters, amides, peptides, and glycosides, as well as rearrangements and eliminations, proceed with relatively small standard free-energy changes, whereas hydrolysis of acid anhydrides is accompanied by relatively large decreases in standard free energy. The complete oxidation of organic compounds such as glucose or palmitate to CO_2 and H_2O, which in cells requires many steps, results in very large decreases in standard free energy. However, standard free-energy changes such as those in Table 13–4 indicate how much free energy is available from a reaction under *standard conditions*. To describe the energy released under the conditions existing in cells, an expression for the *actual* free-energy change is essential.

TABLE 13–4 Standard Free-Energy Changes of Some Chemical Reactions

Reaction type	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (kcal/mol)
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \longrightarrow$ 2 acetate	−91.1	−21.8
ATP + $H_2O \longrightarrow$ ADP + P_i	−30.5	−7.3
ATP + $H_2O \longrightarrow$ AMP + PP_i	−45.6	−10.9
PP_i + $H_2O \longrightarrow$ 2P_i	−19.2	−4.6
UDP-glucose + $H_2O \longrightarrow$ UMP + glucose 1-phosphate	−43.0	−10.3
Esters		
Ethyl acetate + $H_2O \longrightarrow$ ethanol + acetate	−19.6	−4.7
Glucose 6-phosphate + $H_2O \longrightarrow$ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + $H_2O \longrightarrow$ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + $H_2O \longrightarrow$ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + $H_2O \longrightarrow$ 2 glucose	−15.5	−3.7
Lactose + $H_2O \longrightarrow$ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose 1-phosphate $\longrightarrow$ glucose 6-phosphate	−7.3	−1.7
Fructose 6-phosphate $\longrightarrow$ glucose 6-phosphate	−1.7	−0.4
Elimination of water		
Malate $\longrightarrow$ fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \longrightarrow 6CO_2 + 6H_2O$	−2,840	−686
Palmitate + $23O_2 \longrightarrow 16CO_2 + 16H_2O$	−9,770	−2,338

Actual Free-Energy Changes Depend on Reactant and Product Concentrations

We must be careful to distinguish between two different quantities: the actual free-energy change, ΔG, and the standard free-energy change, $\Delta G'^\circ$. 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. The standard free-energy change tells us in which direction and how far a given reaction must go to reach equilibrium *when the initial concentration of each component is 1.0 M,* the pH is 7.0, the temperature is 25 °C, and the pressure is 101.3 kPa (1 atm). Thus $\Delta G'^\circ$ 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. Moreover, the ΔG of any reaction proceeding spontaneously toward its equilibrium is always negative, becomes less negative as the reaction proceeds, and is zero at the point of equilibrium, indicating that no more work can be done by the reaction.

ΔG and $\Delta G'^\circ$ for any reaction $aA + bB \rightleftharpoons cC + dD$ are related by the equation

$$\Delta G = \Delta G'^\circ + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b} \qquad (13\text{–}4)$$

in which the terms in red are those *actually prevailing* in the system under observation. 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 Equation 13–4 can be expressed as $\Delta G = \Delta G'^\circ + RT \ln Q$. As an example, let

us suppose that the reaction A + B $\rightleftharpoons$ C + D is taking place under the standard conditions of temperature (25 °C) and pressure (101.3 kPa) but that the concentrations of A, B, C, and D are *not* equal and none of the components is present at the standard concentration of 1.0 M. To determine the actual free-energy change, ΔG, under these nonstandard conditions of concentration as the reaction proceeds from left to right, we simply enter the *actual* concentrations of A, B, C, and D in Equation 13–4; the values of *R, T,* and $\Delta G'^\circ$ are the standard values. ΔG is negative and approaches zero as the reaction proceeds, because the actual concentrations of A and B decrease and the concentrations of C and D increase. Notice that when a reaction is at equilibrium—when there is no force driving the reaction in either direction and ΔG is zero—Equation 13–4 reduces to

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

or

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

which is the equation relating the standard free-energy change and equilibrium constant (Eqn 13–3).

The criterion for spontaneity of a reaction is the value of ΔG, not $\Delta G'^\circ$. A reaction with a positive $\Delta G'^\circ$ can go in the forward direction *if* ΔG *is negative.* This is possible if the term *RT* ln ([products]/[reactants]) in Equation 13–4 is negative and has a larger absolute value than $\Delta G'^\circ$. For example, the immediate removal of the products of a reaction can keep the ratio [products]/[reactants] well below 1, such that the term *RT* ln ([products]/[reactants]) has a large, negative value. $\Delta G'^\circ$ and ΔG are expressions of the *maximum* amount of free energy that a given reaction can *theoretically* deliver—an amount of energy that could be realized only if a perfectly efficient device were available to trap or harness it. Given that no such device is possible (some energy is always lost to entropy during any process), the amount of work done by the reaction at constant temperature and pressure is always less than the theoretical amount.

Another important point is that some thermodynamically favorable reactions (that is, reactions for which $\Delta G'^\circ$ is large and negative) do not occur at measurable rates. For example, combustion of firewood to CO_2 and H_2O is very favorable thermodynamically, but firewood remains stable for years because the activation energy (see Figs 6–2 and 6–3) for the combustion reaction is higher than the energy available at room temperature. If the necessary activation energy is provided (with a lighted match, for example), combustion will begin, converting the wood to the more stable products CO_2 and H_2O and releasing energy as heat and light. The heat released by this exothermic reaction provides the activation energy for combustion of neighboring regions of the firewood; the process is self-perpetuating.

In living cells, reactions that would be extremely slow *if uncatalyzed* are caused to proceed not by supplying additional heat but by lowering the activation energy through use of an enzyme. An enzyme provides an alternative reaction pathway with a lower activation energy than the uncatalyzed reaction, so that at room temperature a large fraction of the substrate molecules have enough thermal energy to overcome the activation barrier, and the reaction rate increases dramatically. *The free-energy change for a reaction is independent of the pathway by which the reaction occurs*; it depends only on the nature and concentration of the initial reactants and the final products. *Enzymes cannot, therefore, change equilibrium constants*; but they can and do increase the *rate* at which a reaction proceeds in the direction dictated by thermodynamics (see Section 6.2).

Standard Free-Energy Changes Are Additive

In the case of two sequential chemical reactions, A $\rightleftharpoons$ B and B $\rightleftharpoons$ C, each reaction has its own equilibrium constant and each has its characteristic standard free-energy change, $\Delta G'^\circ_1$ and $\Delta G'^\circ_2$. As the two reactions are sequential, B cancels out to give the overall reaction A $\rightleftharpoons$ C, which has its own equilibrium constant and thus its own standard free-energy change, $\Delta G'^\circ_{total}$. *The* $\Delta G'^\circ$ *values of sequential chemical reactions are additive.* For the overall reaction A $\rightleftharpoons$ C, $\Delta G'^\circ_{total}$ is the sum of the individual standard free-energy changes, $\Delta G'^\circ_1$ and $\Delta G'^\circ_2$, of the two reactions: $\Delta G'^\circ_{total} = \Delta G'^\circ_1 + \Delta G'^\circ_2$.

(1)	A ⟶ B	$\Delta G'^\circ_1$
(2)	B ⟶ C	$\Delta G'^\circ_2$
Sum:	A ⟶ C	$\Delta G'^\circ_1 + \Delta G'^\circ_2$

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

$$\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^\circ = 13.8 \text{ kJ/mol}$$

The positive value of $\Delta G'^\circ$ predicts that under standard conditions the reaction will tend not to proceed spontaneously in the direction written. Another cellular reaction, the hydrolysis of ATP to ADP and P_i, is very exergonic:

$$ATP + H_2O \longrightarrow ADP + P_i \quad \Delta G'^\circ = -30.5 \text{ kJ/mol}$$

These two reactions share the common intermediates P_i and H_2O and may be expressed as sequential reactions:

(1)	Glucose + P_i ⟶ glucose 6-phosphate + H_2O
(2)	ATP + H_2O ⟶ ADP + P_i
Sum:	ATP + glucose ⟶ ADP + glucose 6-phosphate

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

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

The overall reaction is exergonic. In this case, energy stored in ATP is used to drive the synthesis of glucose 6-phosphate, even though its formation from glucose and inorganic phosphate (P_i) is endergonic. The *pathway* of glucose 6-phosphate formation from glucose by phosphoryl transfer from ATP is different from reactions (1) and (2) above, but the net result is the same as the sum of the two reactions. In thermodynamic calculations, all that matters is the state of the system at the beginning of the process and its state at the end; the route between the initial and final states is immaterial.

We have said that $\Delta G'^\circ$ is a way of expressing the equilibrium constant for a reaction. For reaction (1) above,

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

Notice that H_2O is not included in this expression, as its concentration (55.5 M) is assumed to remain unchanged by the reaction. The equilibrium constant for the hydrolysis of ATP is

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

The equilibrium constant for the two coupled reactions is

$$\begin{aligned} K'_{eq_3} &= \frac{[\text{glucose 6-phosphate}]\,[\text{ADP}]\,[P_i]}{[\text{glucose}]\,[P_i]\,[\text{ATP}]} \\ &= (K'_{eq_1})(K'_{eq_2}) = (3.9 \times 10^{-3}\ \text{M}^{-1})(2.0 \times 10^{5}\ \text{M}) \\ &= 7.8 \times 10^{2} \end{aligned}$$

This calculation illustrates an important point about 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*. By coupling ATP hydrolysis to glucose 6-phosphate synthesis, the K'_{eq} for formation of glucose 6-phosphate from glucose has been raised by a factor of about 2×10^5.

This common-intermediate strategy is employed by all living cells in the synthesis of metabolic intermediates and cellular components. Obviously, the strategy works only if compounds such as ATP are continuously available. In the following chapters we consider several of the most important cellular pathways for producing ATP.

SUMMARY 13.1 Bioenergetics and Thermodynamics

- Living cells constantly perform work. They require energy for maintaining their highly organized structures, synthesizing cellular components, generating electric currents, and many other processes.
- Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics.
- All chemical reactions are influenced by two forces: the tendency to achieve the most stable bonding state (for which enthalpy, H, is a useful expression) and the tendency to achieve the highest degree of randomness, expressed as entropy, S. The net driving force in a reaction is ΔG, the free-energy change, which represents the net effect of these two factors: $\Delta G = \Delta H - T\Delta S$.
- The standard transformed free-energy change, $\Delta G'^\circ$, is a physical constant that is characteristic for a given reaction and can be calculated from the equilibrium constant for the reaction: $\Delta G'^\circ = -RT \ln K'_{eq}$.
- The actual free-energy change, ΔG, is a variable that depends on $\Delta G'^\circ$ and on the concentrations of reactants and products: $\Delta G = \Delta G'^\circ + RT \ln$ ([products]/[reactants]).
- When ΔG is large and negative, the reaction tends to go in the forward direction; when ΔG is large and positive, the reaction tends to go in the reverse direction; and when $\Delta G = 0$, the system is at equilibrium.
- The free-energy change for a reaction is independent of the pathway by which the reaction occurs. 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.

13.2 Chemical Logic and Common Biochemical Reactions

The biological energy transductions we are concerned with in this book are chemical reactions. Cellular chemistry does not encompass every kind of reaction learned in a typical organic chemistry course. Which reactions take place in biological systems and which do not is determined by (1) their relevance to that particular metabolic system and (2) their rates. Both considerations play major roles in shaping the metabolic pathways we consider throughout the rest of the book. A relevant reaction is one that makes use of an available substrate and converts it to a useful product. However, even a potentially relevant reaction may not occur. Some chemical transformations are too slow (have activation energies that are too high) to contribute to living systems even with the aid of powerful enzyme catalysts. The reactions that do occur in cells represent a toolbox that evolution has used to construct metabolic pathways that circumvent the "impossible" reactions. Learning to

recognize the plausible reactions can be a great aid in developing a command of biochemistry.

Even so, the number of metabolic transformations taking place in a typical cell can seem overwhelming. Most cells have the capacity to carry out thousands of specific, enzyme-catalyzed reactions: for example, transformation of a simple nutrient such as glucose into amino acids, nucleotides, or lipids; extraction of energy from fuels by oxidation; and polymerization of monomeric subunits into macromolecules.

To study these reactions, some organization is essential. There are patterns within the chemistry of life; you do not need to learn every individual reaction to comprehend the molecular logic of biochemistry. Most of the reactions in living cells fall into one of five general categories: (1) reactions that make or break carbon–carbon bonds; (2) internal rearrangements, isomerizations, and eliminations; (3) free-radical reactions; (4) group transfers; and (5) oxidation-reductions. We discuss each of these in more detail below and refer to some examples of each type in later chapters. Note that the five reaction types are not mutually exclusive; for example, an isomerization reaction may involve a free-radical intermediate.

Before proceeding, however, we should review two basic chemical principles. First, a covalent bond consists of a shared pair of electrons, and the bond can be broken in two general ways **(Fig. 13–1)**. In **homolytic cleavage**, each atom leaves the bond as a **radical**, carrying one unpaired electron. In **heterolytic cleavage**,

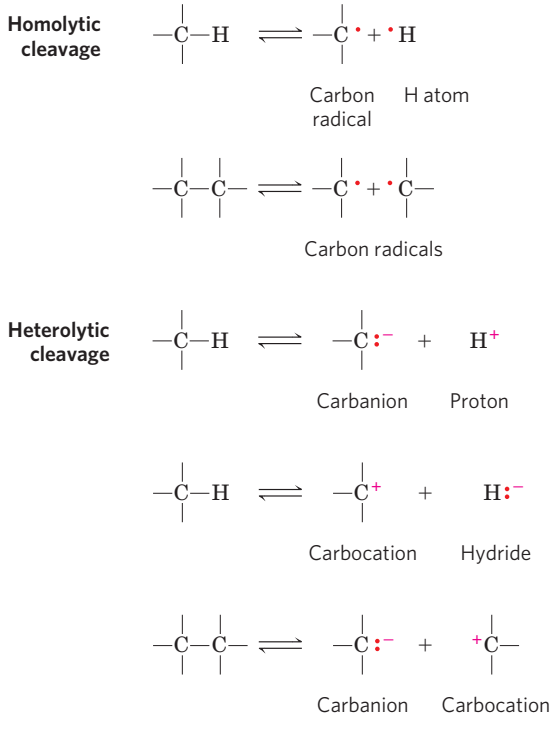


FIGURE 13–1 Two mechanisms for cleavage of a C—C or C—H bond. In a homolytic cleavage, each atom keeps one of the bonding electrons, resulting in the formation of carbon radicals (carbons having unpaired electrons) or uncharged hydrogen atoms. In a heterolytic cleavage, one of the atoms retains both bonding electrons. This can result in the formation of carbanions, carbocations, protons, or hydride ions.

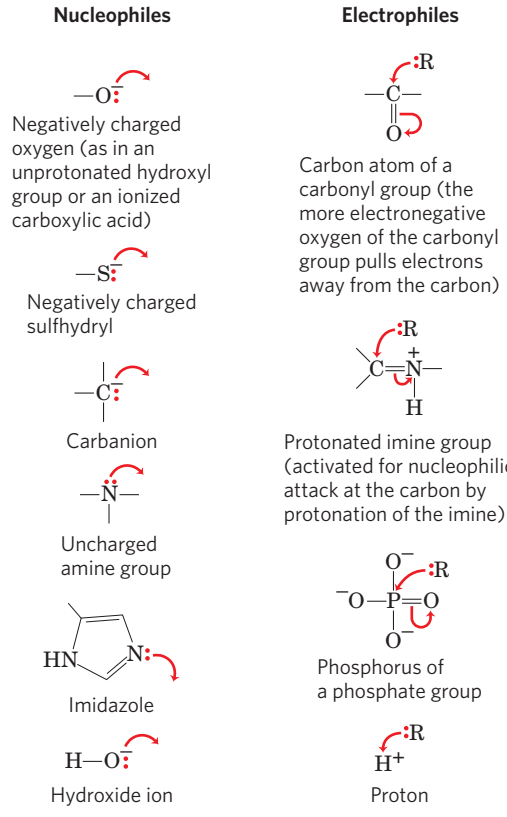


FIGURE 13–2 Common nucleophiles and electrophiles in biochemical reactions. Chemical reaction mechanisms, which trace the formation and breakage of covalent bonds, are communicated with dots and curved arrows, a convention known informally as "electron pushing." A covalent bond consists of a shared pair 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.

which is more common, one atom retains both bonding electrons. The species most often generated when C—C and C—H bonds are cleaved are illustrated in Figure 13–1. Carbanions, carbocations, and hydride ions are highly unstable; this instability shapes the chemistry of these ions, as we shall see.

The second basic principle is that many biochemical reactions involve interactions between **nucleophiles** (functional groups rich in and capable of donating electrons) and **electrophiles** (electron-deficient functional groups that seek electrons). Nucleophiles combine with and give up electrons to electrophiles. Common biological nucleophiles and electrophiles are shown in **Figure 13–2**. Note that a carbon atom can act as either a nucleophile or an electrophile, depending on which bonds and functional groups surround it.

Reactions That Make or Break Carbon–Carbon Bonds Heterolytic cleavage of a C—C bond yields a **carbanion** and a **carbocation** (Fig. 13–1). Conversely, the formation of a C—C bond involves the combination of a nucleophilic carbanion and an electrophilic carbocation. Carbanions

and carbocations are generally so unstable that their formation as reaction intermediates can be energetically inaccessible even with enzyme catalysts. For the purpose of cellular biochemistry they are impossible reactions—unless chemical assistance is provided in the form of functional groups containing electronegative atoms (O and N) that can alter the electronic structure of adjacent carbon atoms so as to stabilize and facilitate the formation of carbanion and carbocation intermediates.

Carbonyl groups are particularly important in the chemical transformations of metabolic pathways. The carbon of a carbonyl group has a partial positive charge due to the electron-withdrawing property of the carbonyl oxygen, and thus is an electrophilic carbon **(Fig. 13–3a)**. A carbonyl group can thus facilitate the formation of a carbanion on an adjoining carbon by delocalizing the carbanion's negative charge (Fig. 13–3b). An imine group (see Fig. 1–16) can serve a similar function (Fig. 13–3c). The capacity of carbonyl and imine groups to delocalize electrons can be further enhanced by a general acid catalyst or by a metal ion such as Mg^{2+} (Fig. 13–3d).

The importance of a carbonyl group is evident in three major classes of reactions in which C—C bonds are formed or broken **(Fig. 13–4)**: aldol condensations, Claisen ester condensations, and decarboxylations. In each type of reaction, a carbanion intermediate is stabilized by a carbonyl group, and in many cases another carbonyl provides the electrophile with which the nucleophilic carbanion reacts.

An **aldol condensation** is a common route to the formation of a C—C bond; the aldolase reaction, which converts a six-carbon compound to two three-carbon compounds in glycolysis, is an aldol condensation in reverse (see Fig. 14–6). In a **Claisen condensation**, the carbanion is stabilized by the carbonyl of an adjacent thioester; an example is the synthesis of citrate in the citric acid cycle (see Fig. 16–9). Decarboxylation also commonly involves the formation of a carbanion stabilized by a carbonyl group; the acetoacetate decarboxylase reaction that occurs in the formation of ketone bodies during fatty acid catabolism provides an example (see Fig. 17–19). Entire metabolic pathways are organized around the introduction of a carbonyl group in a particular location so that a nearby carbon–carbon bond can be formed or cleaved. In some reactions, an imine or a specialized cofactor such as pyridoxal phosphate plays the electron-withdrawing role of the carbonyl group.

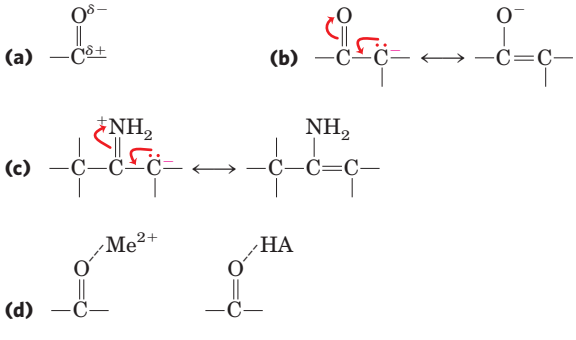


FIGURE 13–3 Chemical properties of carbonyl groups. (a) The carbon atom of a carbonyl group is an electrophile by virtue of the electron-withdrawing capacity of the electronegative oxygen atom, which results in a structure in which the carbon has a partial positive charge. **(b)** Within a molecule, delocalization of electrons into a carbonyl group stabilizes a carbanion on an adjacent carbon, facilitating its formation. **(c)** Imines function much like carbonyl groups in facilitating electron withdrawal. **(d)** Carbonyl groups do not always function alone; their capacity as electron sinks often is augmented by interaction with either a metal ion (Me^{2+}, such as Mg^{2+}) or a general acid (HA).

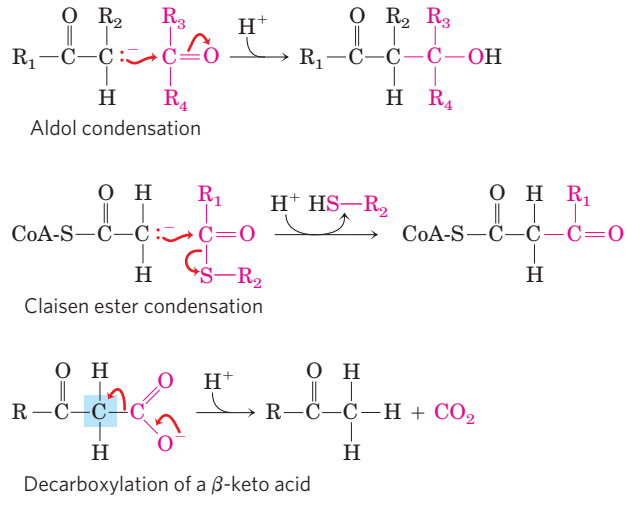


FIGURE 13–4 Some common reactions that form and break C—C bonds in biological systems. For both the aldol condensation and the Claisen condensation, a carbanion serves as nucleophile and the carbon of a carbonyl group serves as electrophile. The carbanion is stabilized in each case by another carbonyl at the adjoining carbon. In the decarboxylation reaction, a carbanion is formed on the carbon shaded blue as the CO_2 leaves. The reaction would not occur at an appreciable rate without the stabilizing effect of the carbonyl adjacent to the carbanion carbon. Wherever a carbanion is shown, a stabilizing resonance with the adjacent carbonyl, as shown in Figure 13–3b, is assumed. An imine (Fig. 13–3c) or other electron-withdrawing group (including certain enzymatic cofactors such as pyridoxal) can replace the carbonyl group in the stabilization of carbanions.

The carbocation intermediate occurring in some reactions that form or cleave C—C bonds is generated by the elimination of a very good leaving group, such as pyrophosphate (see Group Transfer Reactions below). An example is the prenyltransferase reaction **(Fig. 13–5)**, an early step in the pathway of cholesterol biosynthesis.

Internal Rearrangements, Isomerizations, and Eliminations

Another common type of cellular reaction is an intramolecular rearrangement in which redistribution of electrons results in alterations of many different types without a change in the overall oxidation state of the molecule. For example, different groups in a 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; or the positions of double bonds may be transposed. An example of an isomerization entailing oxidation-reduction is the formation of

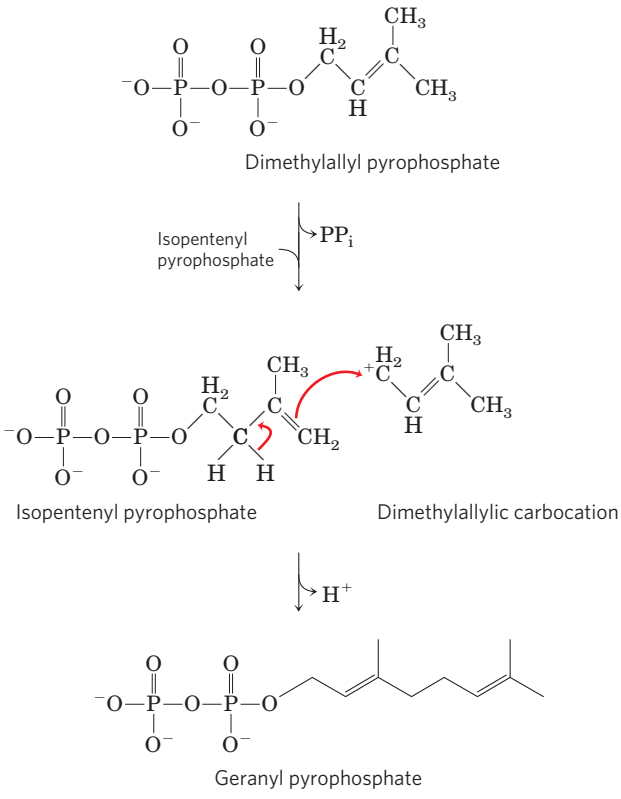
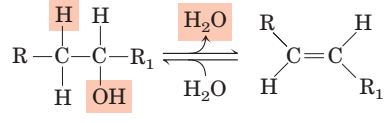


FIGURE 13–5 Carbocations in carbon–carbon bond formation. In one of the early steps in cholesterol biosynthesis, the enzyme prenyltransferase catalyzes condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate to form geranyl pyrophosphate (see Fig. 21-36). The reaction is initiated by elimination of pyrophosphate from the dimethylallyl pyrophosphate to generate a carbocation, stabilized by resonance with the adjacent C═C bond.

fructose 6-phosphate from glucose 6-phosphate in glycolysis **(Fig. 13–6)**; this reaction is discussed in detail in Chapter 14): C-1 is reduced (aldehyde to alcohol) and C-2 is oxidized (alcohol to ketone). Figure 13–6b shows the details of the electron movements in this type of isomerization. A cis-trans rearrangement is illustrated by the prolyl cis-trans isomerase reaction in the folding of certain proteins (see Fig. 4–8). A simple transposition of a C═C bond occurs during metabolism of oleic acid, a common fatty acid (see Fig. 17–10). Some spectacular examples of double-bond repositioning occur in the biosynthesis of cholesterol (see Fig. 21–33).

An example of an elimination reaction that does not affect overall oxidation state is the loss of water from an alcohol, resulting in the introduction of a C═C bond:



Similar reactions can result from eliminations in amines.

Free-Radical Reactions Once thought to be rare, the homolytic cleavage of covalent bonds to generate free radicals has now been found in a wide range of biochemical processes. These include: isomerizations that make use of adenosylcobalamin (vitamin B_{12}) or *S*-adenosylmethionine, which are initiated with a 5′-deoxyadenosyl radical (see the methylmalonyl-CoA mutase reaction in Box 17–2); certain radical-initiated decarboxylation reactions **(Fig. 13–7)**; some reductase reactions, such as that catalyzed by ribonucleotide reductase (see Fig. 22–41);

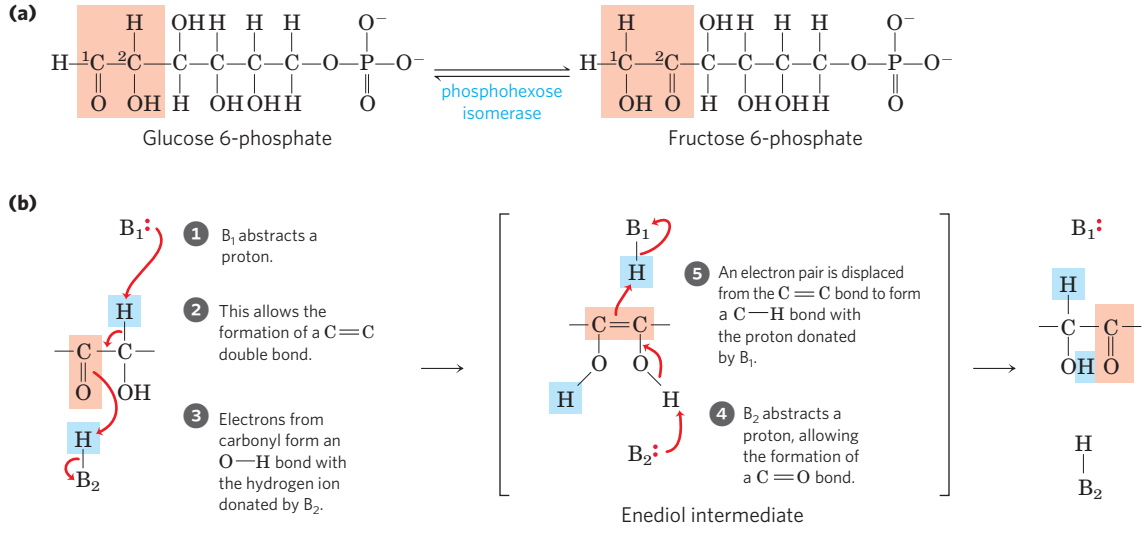


FIGURE 13–6 Isomerization and elimination reactions. (a) The conversion of glucose 6-phosphate to fructose 6-phosphate, a reaction of sugar metabolism catalyzed by phosphohexose isomerase. **(b)** This reaction proceeds through an enediol intermediate. Light red screens follow the path of oxidation from left to right. B_1 and B_2 are ionizable groups on the enzyme; they are capable of donating and accepting protons (acting as general acids or general bases) as the reaction proceeds.

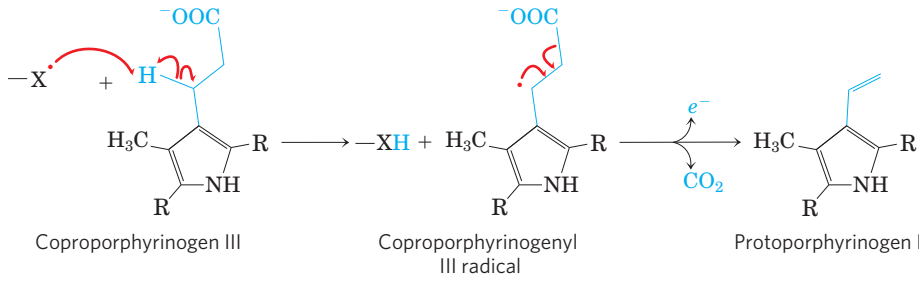
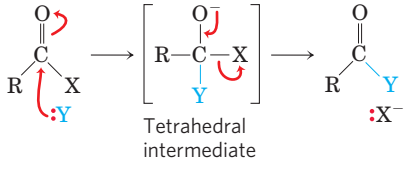


FIGURE 13–7 A free radical–initiated decarboxylation reaction. The biosynthesis of heme (see Fig. 22–26) in *Escherichia coli* includes a decarboxylation step in which propionyl side chains on the coproporphyrinogen III intermediate are converted to the vinyl side chains of protoporphyrinogen IX. When the bacteria are grown anaerobically the enzyme oxygen-independent coproporphyrinogen III oxidase, also called HemN protein, promotes decarboxylation via the free-radical mechanism shown here. The acceptor of the released electron is not known. For simplicity, only the relevant portions of the large coproporphyrinogen III and protoporphyrinogen molecules are shown; the entire structures are given in Figure 22–26. When *E. coli* are grown in the presence of oxygen, this reaction is an oxidative decarboxylation and is catalyzed by a different enzyme.

and some rearrangement reactions, such as that catalyzed by DNA photolyase (see Fig. 25–26).

Group Transfer Reactions The transfer of acyl, glycosyl, and phosphoryl groups from one nucleophile to another is common in living cells. Acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an acyl group to form a tetrahedral intermediate:



The chymotrypsin reaction is one example of acyl group transfer (see Fig. 6–22). Glycosyl group transfers involve nucleophilic substitution at C-1 of a sugar ring, which is the central atom of an acetal. In principle, the substitution could proceed by an S_N1 or S_N2 pathway, as described in Figure 6–28 for the enzyme lysozyme.

Phosphoryl group transfers play a special role in metabolic pathways, and these transfer reactions are discussed in detail in Section 13.3. A general theme in metabolism is the attachment of a good leaving group to a metabolic intermediate to "activate" the intermediate for subsequent reaction. Among the better leaving groups in nucleophilic substitution reactions are inorganic orthophosphate (the ionized form of H_3PO_4 at neutral pH, a mixture of $H_2PO_4^-$ and HPO_4^{2-}, commonly abbreviated P_i) and inorganic pyrophosphate ($P_2O_7^{4-}$, abbreviated PP_i); esters and anhydrides of phosphoric acid are effectively activated for reaction. Nucleophilic substitution is made more favorable by the attachment of a phosphoryl group to an otherwise poor leaving group such as —OH. Nucleophilic substitutions in which the phosphoryl group ($—PO_3^{2-}$) serves as a leaving group occur in hundreds of metabolic reactions.

Phosphorus can form five covalent bonds. The conventional representation of P_i **(Fig. 13–8a)**, with three P—O bonds and one P═O bond, is a convenient

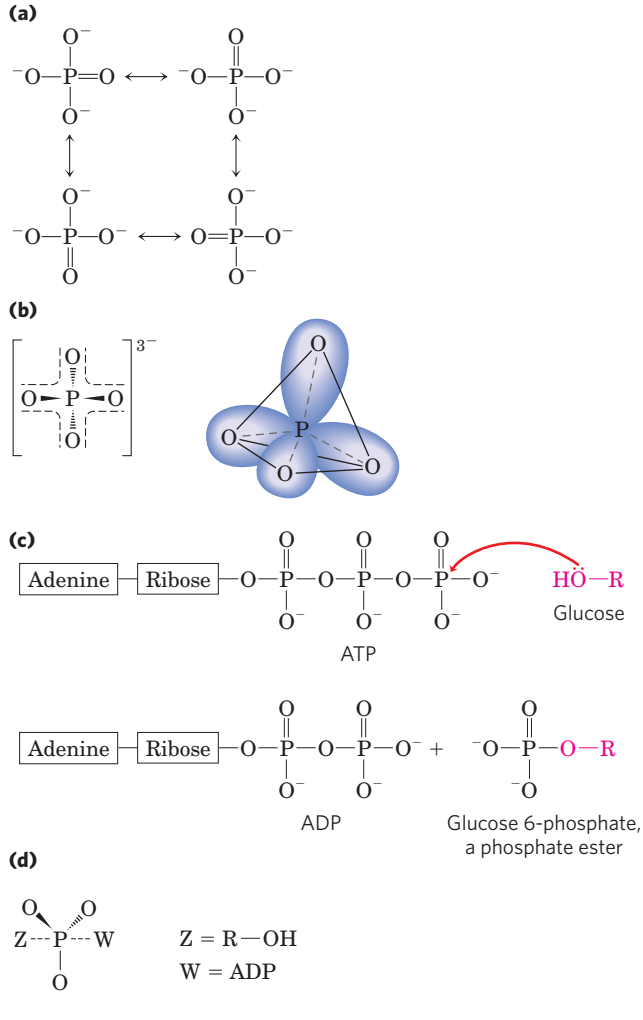


FIGURE 13–8 Phosphoryl group transfers: some of the participants. **(a)** In one (inadequate) representation of P_i, three oxygens are single-bonded to phosphorus, and the fourth is double-bonded, allowing the four different resonance structures shown here. **(b)** The resonance structures of P_i can be represented more accurately by showing all four phosphorus-oxygen bonds with some double-bond character; the hybrid orbitals so represented are arranged in a tetrahedron with P at its center. **(c)** When a nucleophile Z (in this case, the —OH on C-6 of glucose) attacks ATP, it displaces ADP (W). In this S_N2 reaction, a pentacovalent intermediate **(d)** forms transiently.

but inaccurate picture. In P_i, four equivalent phosphorus–oxygen bonds share some double-bond character, and the anion has a tetrahedral structure (Fig. 13–8b). 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 as an electrophile. In a great many metabolic reactions, a phosphoryl group ($—PO_3^{2-}$) is transferred from ATP to an alcohol, forming a phosphate ester (Fig. 13–8c), or to a carboxylic acid, forming a mixed anhydride. When a nucleophile attacks the electrophilic phosphorus atom in ATP, a relatively stable pentacovalent structure forms as a reaction intermediate (Fig. 13–8d). With departure of the leaving group (ADP), the transfer of a phosphoryl group is complete. The large family of enzymes that catalyze phosphoryl group transfers with ATP as donor are called **kinases** (Greek *kinein*, "to move"). Hexokinase, for example, "moves" a phosphoryl group from ATP to glucose.

Phosphoryl groups are not the only groups that activate molecules for reaction. Thioalcohols (thiols), in which the oxygen atom of an alcohol is replaced with a sulfur atom, are also good leaving groups. Thiols activate carboxylic acids by forming thioesters (thiol esters). In later chapters we discuss several reactions, including those catalyzed by the fatty acyl synthases in lipid synthesis (see Fig. 21–2), in which nucleophilic substitution at the carbonyl carbon of a thioester results in transfer of the acyl group to another moiety.

Oxidation-Reduction Reactions Carbon atoms can exist in five oxidation states, depending on the elements with which they share electrons **(Fig. 13–9)**, and transitions between these states are of crucial importance in metabolism (oxidation-reduction reactions are the topic of Section 13.4). In many biological oxidations, a compound loses two electrons and two hydrogen ions (that is, two hydrogen atoms); these reactions are commonly called dehydrogenations and the enzymes that catalyze them are called dehydrogenases **(Fig. 13–10)**. In some, but not all, biological oxidations, a carbon atom becomes covalently bonded to an oxygen atom. The enzymes that catalyze these oxidations are generally called oxidases or, if the oxygen atom is derived directly from molecular oxygen (O_2) oxygenases.

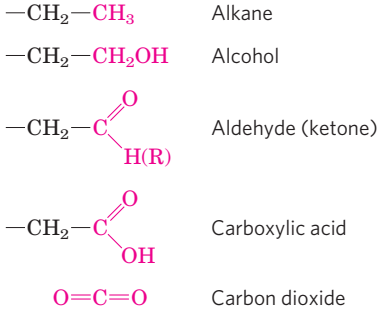


FIGURE 13–9 The oxidation levels of carbon in biomolecules. Each compound is formed by oxidation of the red carbon in the compound shown immediately above. Carbon dioxide is the most highly oxidized form of carbon found in living systems.

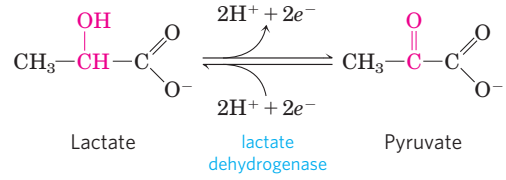


FIGURE 13–10 An oxidation-reduction reaction. Shown here is the oxidation of lactate to pyruvate. In this dehydrogenation, two electrons and two hydrogen ions (the equivalent of two hydrogen atoms) are removed from C-2 of lactate, an alcohol, to form pyruvate, a ketone. In cells the reaction is catalyzed by lactate dehydrogenase and the electrons are transferred to the cofactor nicotinamide adenine dinucleotide (NAD). This reaction is fully reversible; pyruvate can be reduced by electrons transferred from the cofactor.

Every oxidation must be accompanied by a reduction, in which an electron acceptor acquires the electrons removed by oxidation. Oxidation reactions generally release energy (think of camp fires: the compounds in wood are oxidized by oxygen molecules in the air). Most living cells obtain the energy needed for cellular work by oxidizing metabolic fuels such as carbohydrates or fat (photosynthetic organisms can also trap and use the energy of sunlight). The catabolic (energy-yielding) pathways described in Chapters 14 through 19 are oxidative reaction sequences that result in the transfer of electrons from fuel molecules, through a series of electron carriers, to oxygen. The high affinity of O_2 for electrons makes the overall electron-transfer process highly exergonic, providing the energy that drives ATP synthesis—the central goal of catabolism.

Many of the reactions within these five classes are facilitated by cofactors, in the form of coenzymes and metals (vitamin B_{12}, *S*-adenosylmethionine, folate, nicotinamide, and iron are some examples). Cofactors bind to enzymes—in some cases reversibly, in other cases almost irreversibly—and confer on them the capacity to promote a particular kind of chemistry (p. 190). Most cofactors participate in a narrow range of closely related reactions. In the following chapters, we will introduce and discuss each important cofactor at the point where we first encounter it. The cofactors provide another way to organize the study of biochemical processes, since the reactions facilitated by a given cofactor generally are mechanistically related.

Biochemical and Chemical Equations Are Not Identical

Biochemists write metabolic equations in a simplified way, and this is particularly evident for reactions involving ATP. Phosphorylated compounds can exist in several ionization states and, as we have noted, the different species can bind Mg^{2+}. For example, at pH 7 and 2 mM Mg^{2+}, ATP exists in the forms ATP^{4-}, $HATP^{3-}$, H_2ATP^{2-}, $MgHATP^{-}$, and Mg_2ATP. In thinking about the biological role of ATP, however, we are not always interested in all this detail, and so we consider ATP as an entity made up of a sum of species, and we write its hydrolysis as the biochemical equation

$$ATP + H_2O \longrightarrow ADP + P_i$$

where ATP, ADP, and P_i are sums of species. The corresponding standard transformed equilibrium constant, $K'_{eq} = [ADP][P_i]/[ATP]$, depends on the pH and the concentration of free Mg^{2+}. Note that H^+ and Mg^{2+} do not appear in the biochemical equation because they are held constant. Thus a biochemical equation does not necessarily balance H, Mg, or charge, although it does balance all other elements involved in the reaction (C, N, O, and P in the equation above).

We can write a chemical equation that *does* balance for all elements and for charge. For example, when ATP is hydrolyzed at a pH above 8.5 in the absence of Mg^{2+}, the chemical reaction is represented by

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$$

The corresponding equilibrium constant, $K'_{eq} = [ADP^{3-}][HPO_4^{2-}][H^+]/[ATP^{4-}]$, depends only on temperature, pressure, and ionic strength.

Both ways of writing a metabolic reaction have value in biochemistry. Chemical equations are needed when we want to account for all atoms and charges in a reaction, as when we are considering the mechanism of a chemical reaction. Biochemical equations are used to determine in which direction a reaction will proceed spontaneously, given a specified pH and $[Mg^{2+}]$, or to calculate the equilibrium constant of such a reaction.

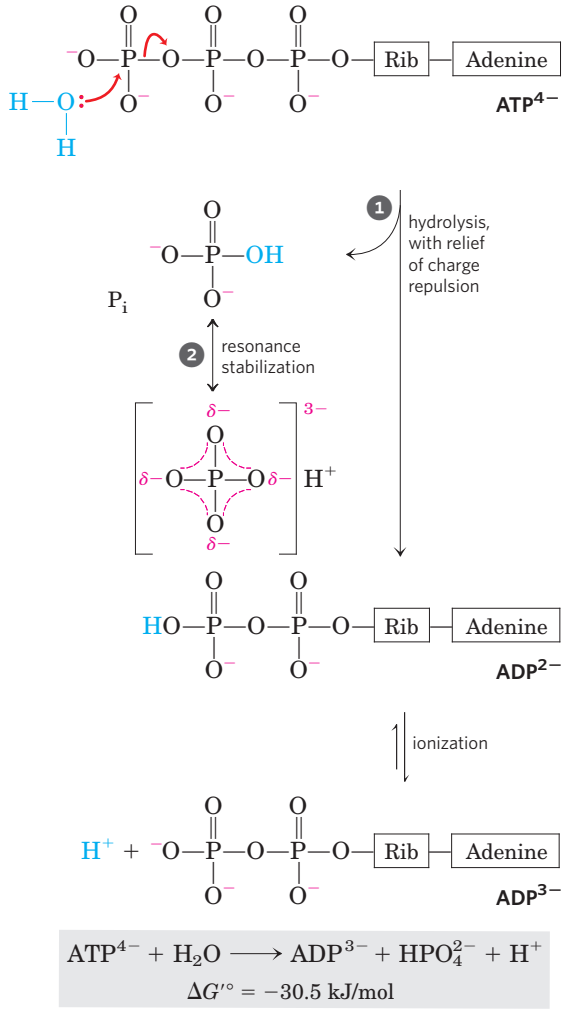
Throughout this book we use biochemical equations, unless the focus is on chemical mechanism, and we use values of $\Delta G'^{\circ}$ and K'_{eq} as determined at pH 7 and 1 mM Mg^{2+}.

SUMMARY 13.2 Chemical Logic and Common Biochemical Reactions

- Living systems make use of a large number of chemical reactions that can be classified into five general types.
- Carbonyl groups play a special role in reactions that form or cleave C—C bonds. Carbanion intermediates are common and are stabilized by adjacent carbonyl groups or, less often, by imines or certain cofactors.
- A redistribution of electrons can produce internal rearrangements, isomerizations, and eliminations. Such reactions include intramolecular oxidation-reduction, change in cis-trans arrangement at a double bond, and transposition of double bonds.
- Homolytic cleavage of covalent bonds to generate free radicals occurs in some pathways, such as in certain isomerization, decarboxylation, reductase, and rearrangement reactions.
- Phosphoryl transfer reactions are an especially important type of group transfer in cells, required for the activation of molecules for reactions that would otherwise be highly unfavorable.
- Oxidation-reduction reactions involve the loss or gain of electrons: one reactant gains electrons and is reduced, while the other loses electrons and is oxidized. Oxidation reactions generally release energy and are important in catabolism.

13.3 Phosphoryl Group Transfers and ATP

Having developed some fundamental principles of energy changes in chemical systems and reviewed the common classes of reactions, we can now examine the energy cycle in cells and the special role of ATP as the energy currency that links catabolism and anabolism (see Fig. 1–29). Heterotrophic cells obtain free energy in a chemical form by the catabolism of nutrient molecules, and they use that energy to make ATP from ADP and P_i. ATP then donates some of its chemical energy to endergonic processes such as the synthesis of metabolic intermediates and macromolecules from smaller precursors, the transport of substances across membranes against concentration gradients, and mechanical motion. This donation of energy from ATP generally involves the covalent participation of ATP in the reaction that is to be driven, with the eventual result that ATP is converted to ADP and P_i or, in some reactions, to AMP and 2 P_i. We discuss here the chemical basis for the large free-energy changes that accompany hydrolysis of ATP and other high-energy phosphate compounds, and we show that most cases of energy donation by ATP involve group transfer, not simple hydrolysis of ATP. To illustrate the range of energy transductions in which ATP provides the energy, we consider the synthesis of information-rich macromolecules, the transport of solutes across membranes, and motion produced by muscle contraction.



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$$
$$\Delta G'^\circ = -30.5 \text{ kJ/mol}$$

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.

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

Figure 13–11 summarizes the chemical basis for the relatively large, negative, standard free energy of hydrolysis of ATP. The hydrolytic cleavage of the terminal phosphoric acid anhydride (phosphoanhydride) bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP; the P_i released is stabilized by the formation of several resonance forms not possible in ATP.

The free-energy change for ATP hydrolysis is −30.5 kJ/mol under standard conditions, but the *actual* free energy of hydrolysis (ΔG) of ATP in living cells is very different: the cellular concentrations of ATP, ADP, and P_i are not identical and are much lower than the 1.0 M of standard conditions (Table 13–5). Furthermore, Mg^{2+} in the cytosol binds to ATP and ADP **(Fig. 13–12)**, and for most enzymatic reactions that involve ATP as phosphoryl group donor, the true substrate is $MgATP^{2-}$. The relevant $\Delta G'^\circ$ is therefore that for $MgATP^{2-}$ hydrolysis. We can calculate ΔG for ATP hydrolysis using data such as those in Table 13–5. The actual free energy of hydrolysis of ATP under intracellular conditions is often called its **phosphorylation potential**, ΔG_p.

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. 526.

†This value reflects total concentration; the true value for free ADP may be much lower (p. 519).

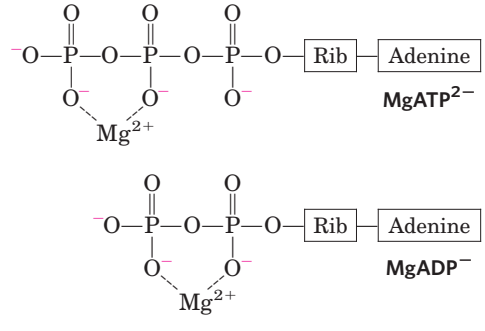


FIGURE 13–12 Mg^{2+} and ATP. Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

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

$$\begin{aligned}\Delta G_p &= -30.5 \text{ kJ/mol} + \left[(8.315 \text{ J/mol} \cdot \text{K})(310 \text{ K}) \ln \frac{(0.25 \times 10^{-3})(1.65 \times 10^{-3})}{(2.25 \times 10^{-3})}\right] \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol}) \ln 1.8 \times 10^{-4} \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol})(-8.6) \\ &= -30.5 \text{ kJ/mol} - 22 \text{ kJ/mol} \\ &= -52 \text{ kJ/mol}\end{aligned}$$

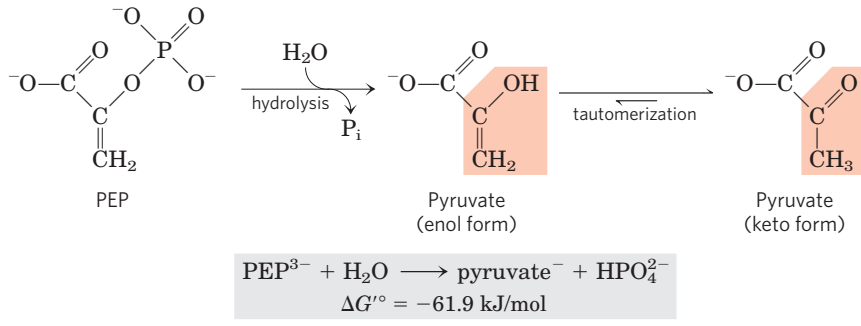
(Note that the final answer has been rounded to the correct number of significant figures (52.5 rounded to 52), following rules for rounding a number that ends in a 5 to the nearest even number.) Thus ΔG_p, the actual free-energy change for ATP hydrolysis in the intact erythrocyte (−52 kJ/mol), is much 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.

Because the concentrations of ATP, ADP, and P_i differ from one cell type to another, ΔG_p for ATP likewise differs among cells. Moreover, in any given cell, ΔG_p can vary from time to time, depending on the metabolic conditions and how they influence the concentrations of ATP, ADP, P_i, and H^+ (pH). We can calculate the actual free-energy change for any given metabolic reaction as it occurs in a cell, providing we know the concentrations of all the reactants and products and other factors (such as pH, temperature, and $[Mg^{2+}]$) that may affect the actual free-energy change.

To further complicate the issue, the *total* concentrations of ATP, ADP, P_i, and H^+ in a cell may be substantially higher than the *free* concentrations, which are the thermodynamically relevant values. The difference is due to tight binding of ATP, ADP, and P_i to cellular proteins. For example, the free [ADP] in resting muscle has been variously estimated at between 1 and 37 μM. Using the value 25 μM in Worked Example 13–2, we would get a ΔG_p of −64 kJ/mol. Calculation of the exact value of ΔG_p, however, is perhaps less instructive than the generalization we can make about actual free-energy changes: in vivo, the energy released by ATP hydrolysis is greater than the standard free-energy change, $\Delta G'^\circ$.

In the following discussions we use the $\Delta G'^\circ$ value for ATP hydrolysis because this allows comparison, on the same basis, with the energetics of other cellular reactions. Always keep in mind, however, that in living cells ΔG is the relevant quantity—for ATP hydrolysis and all other reactions—and may be quite different from $\Delta G'^\circ$.

Here we must make an important point about cellular ATP levels. We have shown (and will discuss further) how the chemical properties of ATP make it a suitable form of energy currency in cells. But it is not merely the molecule's intrinsic chemical properties that give it this ability to drive metabolic reactions and other energy-requiring processes. Even more important is that, in the course of evolution, there has been a very strong selective pressure for regulatory mechanisms that *hold cellular ATP concentrations far above the equilibrium concentrations* for the hydrolysis reaction. When the ATP level drops, not only does the *amount* of fuel decrease, but the fuel itself *loses its potency:* ΔG for its hydrolysis (that is, its phosphorylation potential, ΔG_p) is diminished. As our discussions of the metabolic pathways that produce and consume ATP will show, living cells have developed elaborate mechanisms—often at what might seem to us the expense of efficiency and common sense—to maintain high concentrations of ATP.



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

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.

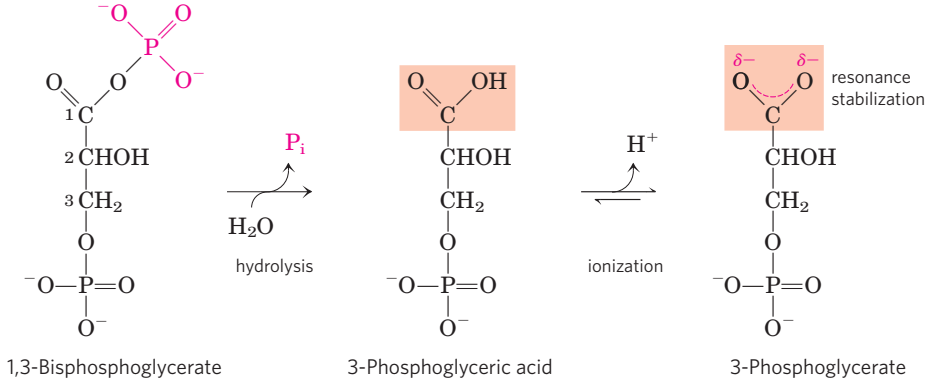
Other Phosphorylated Compounds and Thioesters Also Have Large Free Energies of Hydrolysis

Phosphoenolpyruvate (PEP; **Fig. 13–13**) contains a phosphate ester bond that undergoes hydrolysis to yield the enol form of pyruvate, and this direct product can tautomerize to the more stable keto form. Because the reactant (PEP) has only one form (enol) and the product (pyruvate) has two possible forms, the product is stabilized relative to the reactant. This is the greatest contributing factor to the high standard free energy of hydrolysis of phosphoenolpyruvate: $\Delta G'^\circ = -61.9$ kJ/mol.

Another three-carbon compound, 1,3-bisphosphoglycerate **(Fig. 13–14)**, contains an anhydride bond between the C-1 carboxyl group and a number that ends in a phosphoric acid. Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free-energy change ($\Delta G'^\circ = -49.3$ kJ/mol), which can, again, be explained in terms of the structure of reactant and products. When H_2O is added across the anhydride bond of 1,3-bisphosphoglycerate, one of the direct products, 3-phosphoglyceric acid, can lose a proton to give the carboxylate ion, 3-phosphoglycerate, which has two equally probable resonance forms (Fig. 13–14). Removal of the direct product (3-phosphoglyceric acid) and formation of the resonance-stabilized ion favor the forward reaction.

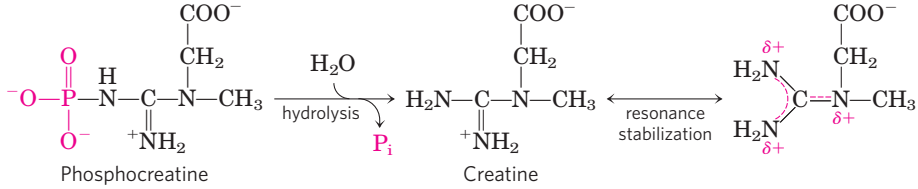
In phosphocreatine **(Fig. 13–15)**, the P—N bond can be hydrolyzed to generate free creatine and P_i. The release of P_i and the resonance stabilization of creatine favor the forward reaction. The standard free-energy change of phosphocreatine hydrolysis is again large, −43.0 kJ/mol.

In all these phosphate-releasing reactions, the several resonance forms available to P_i (Fig. 13–11) stabilize this product relative to the reactant, contributing to an already negative free-energy change. Table 13–6 lists



$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + HPO_4^{2-} + H^+$$
$$\Delta G'^\circ = -49.3 \text{ kJ/mol}$$

FIGURE 13–14 Hydrolysis of 1,3-bisphosphoglycerate. The direct product of hydrolysis is 3-phosphoglyceric acid, with an undissociated carboxylic acid. Its dissociation allows resonance structures that stabilize the product relative to the reactants. Resonance stabilization of P_i further contributes to the negative free-energy change.



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

FIGURE 13–15 Hydrolysis of phosphocreatine. Breakage of the P—N bond in phosphocreatine produces creatine, which is stabilized by formation of a resonance hybrid. The other product, P_i, is also resonance stabilized.

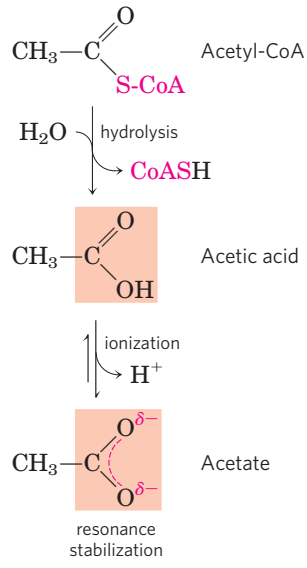
TABLE 13–6 Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

	$\Delta G'^\circ$	
	(kJ/mol)	(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.

the standard free energies of hydrolysis for some biologically important phosphorylated compounds.

Thioesters, in which a sulfur atom replaces the usual oxygen in the ester bond, also have large, negative, standard free energies of hydrolysis. Acetyl-coenzyme A, or acetyl-CoA **(Fig. 13–16)**, is one of many thioesters important in metabolism. The acyl group in these compounds is activated for transacylation, condensation, or oxidation-reduction reactions. Thioesters undergo much less resonance stabilization than do oxygen esters; consequently, the difference in free energy between the



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

FIGURE 13–16 Hydrolysis of acetyl-coenzyme A. Acetyl-CoA is a thioester with a large, negative, standard free energy of hydrolysis. Thioesters contain a sulfur atom in the position occupied by an oxygen atom in oxygen esters. The complete structure of coenzyme A (CoA, or CoASH) is shown in Figure 8–38.

reactant and its hydrolysis products, which *are* resonance-stabilized, is greater for thioesters than for comparable oxygen esters **(Fig. 13–17)**. In both cases, hydrolysis of the ester generates a carboxylic acid, which can ionize and assume several resonance forms. Together, these factors result in the large, negative $\Delta G'^\circ$ (−31.4 kJ/mol) for acetyl-CoA hydrolysis.

To summarize, 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, and thioesters; (3) the

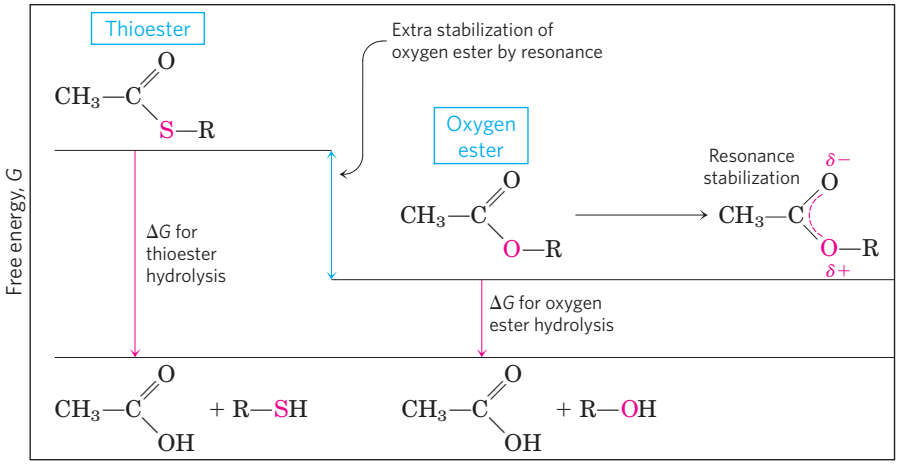


FIGURE 13–17 Free energy of hydrolysis for thioesters and oxygen esters. The *products* of both types of hydrolysis reaction have about the same free-energy content (G), but the thioester has a higher free-energy content than the oxygen ester. Orbital overlap between the O and C atoms allows resonance stabilization in oxygen esters; orbital overlap between S and C atoms is poorer and provides little resonance stabilization.

products are stabilized by isomerization (tautomerization), as for PEP; and/or (4) the products are stabilized by resonance, as for creatine released from phosphocreatine, carboxylate ion released from acyl phosphates and thioesters, and phosphate (P_i) released from anhydride or ester linkages.

ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

Throughout this book you will encounter reactions or processes for which ATP supplies energy, and the contribution of ATP to these reactions is commonly indicated as in **Figure 13–18a**, with a single arrow showing the conversion of ATP to ADP and P_i (or, in some cases, of ATP to AMP and pyrophosphate, PP_i). When written this way, these reactions of ATP seem to be simple hydrolysis reactions in which water displaces P_i (or PP_i), and one is tempted to say that an ATP-dependent reaction is "driven by the hydrolysis of ATP." This is *not* the case. ATP hydrolysis per se usually accomplishes nothing but the liberation of heat, which cannot drive a chemical process in an isothermal system. A single reaction arrow such as that in Figure 13–18a almost invariably represents a two-step process (Fig. 13–18b) in which part of the ATP molecule, a phosphoryl or pyrophosphoryl group or the adenylate moiety (AMP), is first transferred to a substrate molecule or to an amino acid residue in an enzyme, becoming covalently attached to the substrate or the enzyme and raising its free-energy content. Then, in a second step, the phosphate-containing moiety transferred in the first step is displaced, generating P_i, PP_i, or AMP. Thus ATP participates *covalently* in the enzyme-catalyzed reaction to which it contributes free energy.

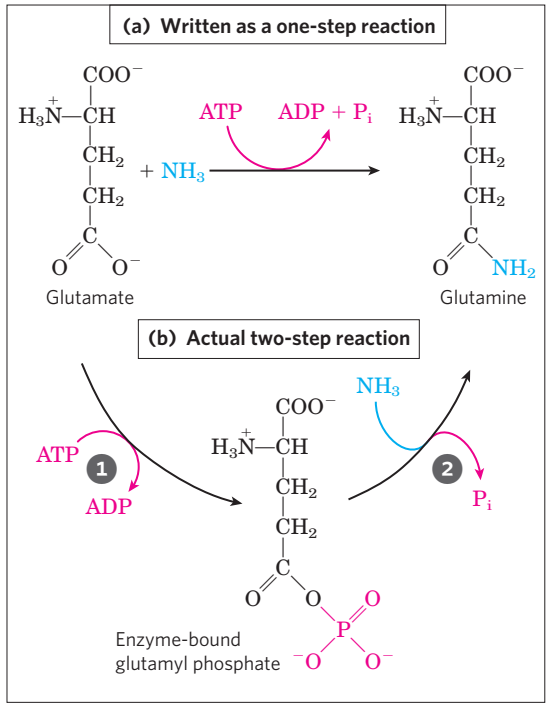


FIGURE 13–18 ATP hydrolysis in two steps. (a) The contribution of ATP to a reaction is often shown as a single step, but is almost always a two-step process. **(b)** Shown here is the reaction catalyzed by ATP-dependent glutamine synthetase. ❶ A phosphoryl group is transferred from ATP to glutamate, then ❷ the phosphoryl group is displaced by NH_3 and released as P_i.

Some processes *do* involve direct hydrolysis of ATP (or GTP), however. For example, noncovalent binding of ATP (or GTP), followed by its hydrolysis to ADP (or GDP) and P_i, can provide the energy to cycle some proteins between two conformations, producing mechanical motion. This occurs in muscle contraction (see Fig. 5–31), and in the movement of enzymes along DNA (see Fig. 25–31) or of ribosomes along messenger RNA (see Fig. 27–31). The energy-dependent reactions catalyzed by helicases, RecA protein, and some topoisomerases (Chapter 25) also involve direct hydrolysis of phosphoanhydride bonds. The AAA+ ATPases involved in DNA replication and other processes described in Chapter 25 use ATP hydrolysis to cycle associated proteins between active and inactive forms. GTP-binding proteins that act in signaling pathways directly hydrolyze GTP to drive conformational changes that terminate signals triggered by hormones or by other extracellular factors (Chapter 12).

The phosphate compounds found in living organisms can be divided somewhat arbitrarily into two groups, based on their standard free energies of hydrolysis **(Fig. 13–19)**. **"**High-energy" compounds have a $\Delta G'^\circ$ of hydrolysis more negative than −25 kJ/mol; "low-energy" compounds have a less negative $\Delta G'^\circ$. Based on this criterion, ATP, with a $\Delta G'^\circ$ of hydrolysis of −30.5 kJ/mol (−7.3 kcal/mol), is a high-energy compound; glucose 6-phosphate, with a $\Delta G'^\circ$ of hydrolysis of −13.8 kJ/mol (−3.3 kcal/mol), is a low-energy compound.

The term "high-energy phosphate bond," long used by biochemists 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. In fact, the breaking of all chemical bonds requires an *input* of energy. The free energy released by hydrolysis of phosphate compounds does not come from the specific bond that is broken; it results from the products of the reaction having a lower free-energy content than the reactants. For simplicity, we will sometimes use the term "high-energy phosphate compound" when referring to ATP or other phosphate compounds with a large, negative, standard free energy of hydrolysis.

As is evident from the additivity of free-energy changes of sequential reactions (see Section 13.1), any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative free energy of hydrolysis. For example, because cleavage of P_i from phosphoenolpyruvate releases more energy than is needed to drive the condensation of P_i with ADP, the

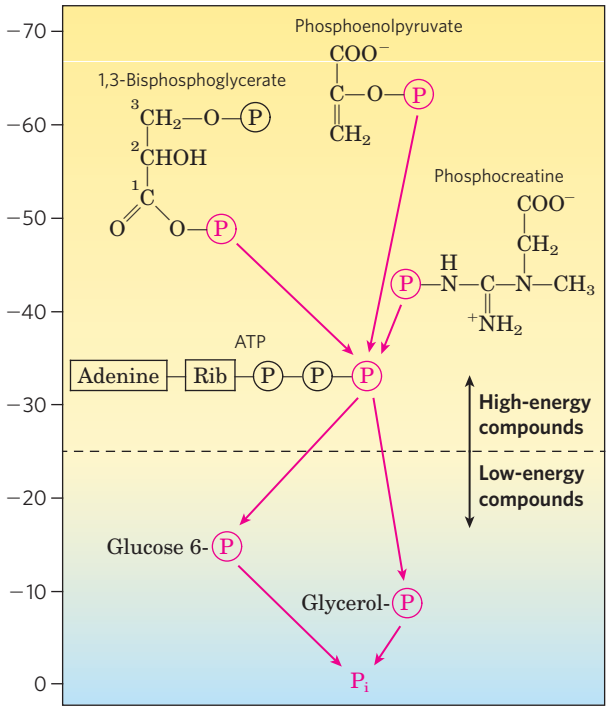


FIGURE 13–19 Ranking of biological phosphate compounds by standard free energies of hydrolysis. This shows the flow of phosphoryl groups, represented by Ⓟ, from high-energy phosphoryl group donors via ATP to acceptor molecules (such as glucose and glycerol) to form their low-energy phosphate derivatives. (The location of each compound's donor phosphoryl group along the scale approximately indicates the $\Delta G'°$ of hydrolysis.) This flow of phosphoryl groups, catalyzed by kinases, proceeds with an overall loss of free energy under intracellular conditions. Hydrolysis of low-energy phosphate compounds releases P_i, which has an even lower phosphoryl group transfer potential (as defined in the text).

direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible:

		$\Delta G'°$ (kJ/mol)
(1)	PEP + H_2O ⟶ pyruvate + P_i	−61.9
(2)	ADP + P_i ⟶ ATP + H_2O	+30.5
Sum:	PEP + ADP ⟶ pyruvate + ATP	−31.4

Notice that while the overall reaction is represented as the algebraic sum of the first two reactions, the overall reaction is actually a third, distinct reaction that does not involve P_i; PEP donates a *phosphoryl* group *directly* to ADP. We can describe phosphorylated compounds as having a high or low phosphoryl group transfer potential, on the basis of their standard free energies of hydrolysis (as listed in Table 13–6). The phosphoryl group transfer potential of PEP is very high, that of ATP is high, and that of glucose 6-phosphate is low (Fig. 13–19).

Much of catabolism is directed toward the synthesis of high-energy phosphate compounds, but their formation is not an end in itself; they are the means of activating a very wide variety of compounds for further chemical transformation. The transfer of a phosphoryl group to a compound effectively puts free energy into that compound, so that it has more free energy to give up during subsequent metabolic transformations. We described above how the synthesis of glucose 6-phosphate is accomplished by phosphoryl group transfer from ATP. In the next chapter we see how this phosphorylation of glucose activates, or "primes," the glucose for catabolic reactions that occur in nearly every living cell. Because of its intermediate position on the scale of group transfer potential, ATP can carry energy from high-energy phosphate compounds produced by catabolism to compounds such as glucose, converting them into more reactive species. ATP thus serves as the universal energy currency in all living cells.

One more chemical feature of ATP is crucial to its role in metabolism: although in aqueous solution ATP is thermodynamically unstable and is therefore a good phosphoryl group donor, it is *kinetically* stable. Because of the huge activation energies (200 to 400 kJ/mol) required for uncatalyzed cleavage of its phosphoanhydride bonds, ATP does not spontaneously donate phosphoryl groups to water or to the hundreds of other potential acceptors in the cell. Only when specific enzymes are present to lower the energy of activation does phosphoryl group transfer from ATP proceed. The cell is therefore able to regulate the disposition of the energy carried by ATP by regulating the various enzymes that act on it.

ATP Donates Phosphoryl, Pyrophosphoryl, and Adenylyl Groups

The reactions of ATP are generally S_N2 nucleophilic displacements (see Section 13.2) in which the nucleophile may be, for example, the oxygen of an alcohol or carboxylate, or a nitrogen of creatine or of the side chain of arginine or histidine. Each of the three phosphates of ATP is susceptible to nucleophilic attack **(Fig. 13–20)**, and each position of attack yields a different type of product.

Nucleophilic attack by an alcohol on the γ phosphate (Fig. 13–20a) displaces ADP and produces a new phosphate ester. Studies with ^{18}O-labeled reactants have shown that the bridge oxygen in the new compound is

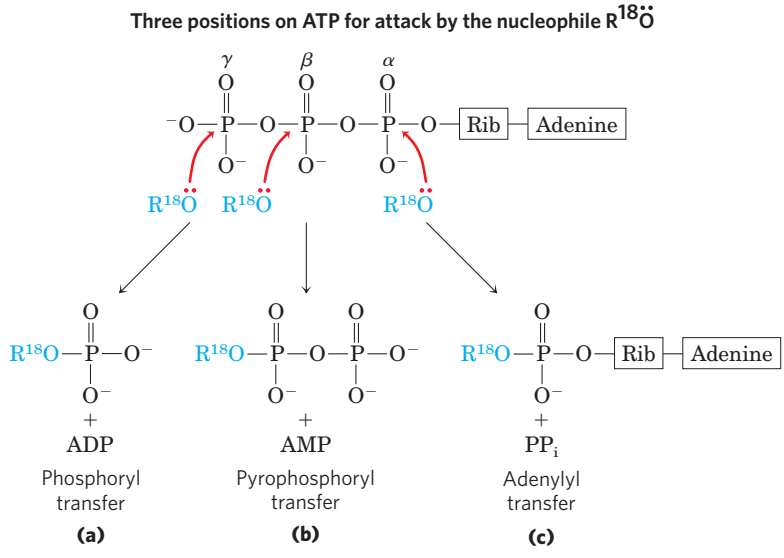


FIGURE 13–20 Nucleophilic displacement reactions of ATP. Any of the three P atoms (α, β, or γ) may serve as the electrophilic target for nucleophilic attack—in this case, by the labeled nucleophile R—^{18}O:. The nucleophile may be an alcohol (ROH), a carboxyl group ($RCOO^-$), or a phosphoanhydride (a nucleoside mono- or diphosphate, for example). **(a)** When the oxygen of the nucleophile attacks the γ position, the bridge oxygen of the product is labeled, indicating that the group transferred from ATP is a phosphoryl ($-PO_3^{2-}$), not a phosphate ($-OPO_3^{2-}$). **(b)** Attack on the β position displaces AMP and leads to the transfer of a pyrophosphoryl (not pyrophosphate) group to the nucleophile. **(c)** Attack on the α position displaces PP_i and transfers the adenylyl group to the nucleophile.

derived from the alcohol, not from ATP; the group transferred from ATP is therefore a phosphoryl ($-PO_3^{2-}$), not a phosphate ($-OPO_3^{2-}$). Phosphoryl group transfer from ATP to glutamate (Fig. 13–18) or to glucose (p. 219) involves attack at the γ position of the ATP molecule.

Attack at the β phosphate of ATP displaces AMP and transfers a pyrophosphoryl (not pyrophosphate) group to the attacking nucleophile (Fig. 13–20b). For example, the formation of 5-phosphoribosyl-1-pyrophosphate (p. 892), a key intermediate in nucleotide synthesis, results from attack of an —OH of the ribose on the β phosphate.

Nucleophilic attack at the α position of ATP displaces PP_i and transfers adenylate (5′-AMP) as an adenylyl group (Fig. 13–20c); the reaction is an **adenylylation** (a-den′-i-li-la′-shun, one of the most ungainly words in the biochemical language). Notice that hydrolysis of the α–β phosphoanhydride bond releases considerably more energy (~46 kJ/mol) than hydrolysis of the β–γ bond (~31 kJ/mol) (Table 13–6). Furthermore, the PP_i formed as a byproduct of the adenylylation is hydrolyzed to two P_i by the ubiquitous enzyme **inorganic pyrophosphatase**, releasing 19 kJ/mol and thereby providing a further energy "push" for the adenylylation reaction. In effect, both phosphoanhydride bonds of ATP are split in the overall reaction. Adenylylation reactions are therefore thermodynamically very favorable. When the energy of ATP is used to drive a particularly unfavorable metabolic reaction, adenylylation is often the mechanism of energy coupling. Fatty acid activation is a good example of this energy-coupling strategy.

The first step in the activation of a fatty acid—either for energy-yielding oxidation or for use in the synthesis of more complex lipids—is the formation of its thiol ester (see Fig. 17–5). The direct condensation of a fatty acid with coenzyme A is endergonic, but the formation of fatty acyl–CoA is made exergonic by stepwise removal of *two* phosphoryl groups from ATP. First, adenylate (AMP) is transferred from ATP to the carboxyl group of the fatty acid, forming a mixed anhydride (fatty acyl adenylate) and liberating PP_i. The thiol group of coenzyme A then displaces the adenylyl group and forms a thioester with the fatty acid. The sum of these two reactions is energetically equivalent to the exergonic hydrolysis of ATP to AMP and PP_i ($\Delta G'^\circ = -45.6$ kJ/mol) and the endergonic formation of fatty acyl–CoA ($\Delta G'^\circ = 31.4$ kJ/mol). The formation of fatty acyl–CoA is made energetically favorable by hydrolysis of the PP_i by inorganic pyrophosphatase. Thus, in the activation of a fatty acid, both phosphoanhydride bonds of ATP are broken. The resulting $\Delta G'^\circ$ is the sum of the $\Delta G'^\circ$ values for the breakage of these bonds, or -45.6 kJ/mol + (-19.2) kJ/mol:

$$ATP + 2H_2O \longrightarrow AMP + 2P_i \qquad \Delta G'^\circ = -64.8 \text{ kJ/mol}$$

The activation of amino acids before their polymerization into proteins (see Fig. 27–19) is accomplished by an analogous set of reactions in which a transfer RNA molecule takes the place of coenzyme A. An interesting use of the cleavage of ATP to AMP and PP_i occurs in the firefly, which uses ATP as an energy source to produce light flashes (Box 13–1).

Assembly of Informational Macromolecules Requires Energy

When simple precursors are assembled into high molecular weight polymers with defined sequences (DNA, RNA, proteins), as described in detail in Part III, energy is required both for the condensation of monomeric units and for the creation of *ordered* sequences. The precursors for DNA and RNA synthesis are nucleoside triphosphates, and polymerization is accompanied by cleavage of the phosphoanhydride linkage between the α and β phosphates, with the release of PP_i (Fig. 13–20). 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

BOX 13–1 Firefly Flashes: Glowing Reports of ATP

Bioluminescence requires considerable amounts of energy. In the firefly, ATP is used in a set of reactions that converts chemical energy into light energy. In the 1950s, from many thousands of fireflies collected by children in and around Baltimore, 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 (Fig. 1). 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. The color of the light flash differs with the firefly species and seems to be determined by differences in the structure of the luciferase. 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} 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 (see Fig. 9–25).

The firefly, a beetle of the *Lampyridae* family.

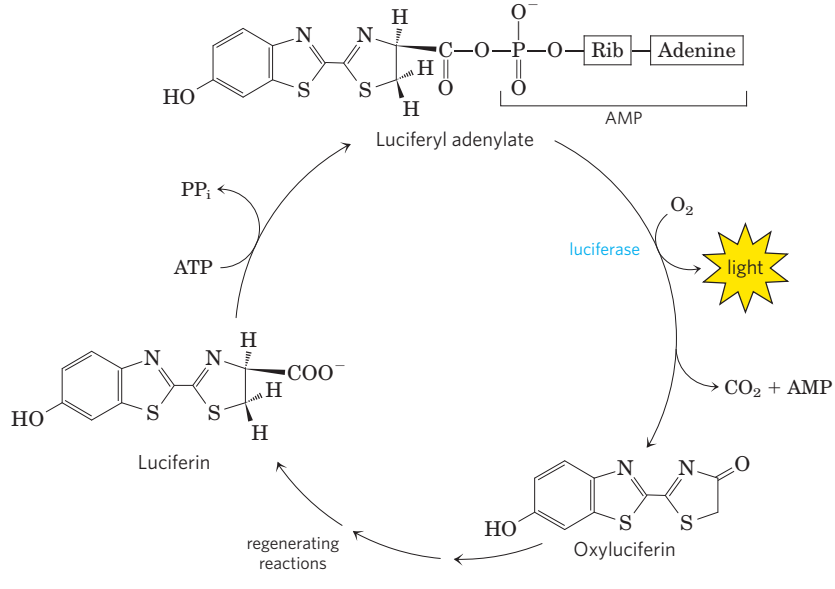


FIGURE 1 Important components in the firefly bioluminescence cycle.

of UMP) for DNA synthesis. As noted above, the activation of amino acids for protein synthesis involves the donation of adenylyl groups from ATP, and we shall see in Chapter 27 that several steps of protein synthesis on the ribosome are also accompanied by GTP hydrolysis. In all these cases, the exergonic breakdown of a nucleoside triphosphate is coupled to the endergonic process of synthesizing a polymer of a specific sequence.

ATP Energizes Active Transport and Muscle Contraction

ATP can supply the energy for transporting an ion or a molecule across a membrane into another aqueous compartment where its concentration is higher (see Fig. 11–38). Transport processes are major consumers of energy; in human kidney and brain, for example, as much as two-thirds of the energy consumed at rest is used to pump Na^+ and K^+ across plasma membranes via the Na^+K^+ ATPase. The transport of Na^+ and K^+ is driven by cyclic phosphorylation and dephosphorylation of the transporter protein, with ATP as the phosphoryl group donor. Na^+-dependent phosphorylation of the Na^+K^+ ATPase forces a change in the protein's conformation, and K^+-dependent dephosphorylation favors return to the original conformation. Each cycle in the transport process results in the conversion of ATP to ADP and P_i, and it is the free-energy change of ATP hydrolysis that drives the cyclic changes in protein conformation that result in the electrogenic pumping of Na^+ and K^+. Note that in this case ATP interacts covalently by phosphoryl group transfer to the enzyme, not the substrate.

In the contractile system of skeletal muscle cells, myosin and actin are specialized to transduce the chemical energy of ATP into motion (see Fig. 5–31). ATP binds tightly but noncovalently to one conformation of

myosin, holding the protein in that conformation. When myosin catalyzes the hydrolysis of its bound ATP, the ADP and P_i dissociate from the protein, allowing it to relax into a second conformation until another molecule of ATP binds. The binding and subsequent hydrolysis of ATP (by myosin ATPase) provide the energy that forces cyclic changes in the conformation of the myosin head. The change in conformation of many individual myosin molecules results in the sliding of myosin fibrils along actin filaments (see Fig. 5–30), which translates into macroscopic contraction of the muscle fiber. As we noted earlier, this production of mechanical motion at the expense of ATP is one of the few cases in which ATP hydrolysis per se, rather than group transfer from ATP, is the source of the chemical energy in a coupled process.

Transphosphorylations between Nucleotides Occur in All Cell Types

Although we have focused on ATP as the cell's energy currency and donor of phosphoryl groups, all other nucleoside triphosphates (GTP, UTP, and CTP) and all deoxynucleoside triphosphates (dATP, dGTP, dTTP, and dCTP) are energetically equivalent to ATP. The standard free-energy changes associated with hydrolysis of their phosphoanhydride linkages are very nearly identical with those shown in Table 13–6 for ATP. In preparation for their various biological roles, these other nucleotides are generated and maintained 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, and, in photosynthetic cells, photophosphorylation. Several enzymes then carry phosphoryl groups from ATP to the other nucleotides. **Nucleoside diphosphate kinase**, found in all cells, catalyzes the reaction

$$\text{ATP} + \text{NDP (or dNDP)} \overset{Mg^{2+}}{\rightleftharpoons} \text{ADP} + \text{NTP (or dNTP)} \qquad \Delta G'^{\circ} \approx 0$$

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. The enzyme actually catalyzes a two-step phosphoryl group transfer, which is a classic case of a double-displacement (Ping-Pong) mechanism (**Fig. 13–21**; see also Fig. 6–13b). First, phosphoryl group transfer from ATP to an active-site His residue produces a phosphoenzyme intermediate; then the phosphoryl group is transferred from the Ⓟ–His residue to an NDP acceptor. Because the enzyme is nonspecific for the base in the NDP and works equally well on dNDPs and NDPs, it can synthesize all NTPs and dNTPs, given the corresponding NDPs and a supply of ATP.

Phosphoryl group transfers from ATP result in an accumulation of ADP; for example, when muscle is contracting vigorously, ADP accumulates and interferes with ATP-dependent contraction. During periods of intense demand for ATP, the cell lowers the ADP concentration, and at the same time replenishes ATP, by the action of **adenylate kinase**:

$$2\text{ADP} \overset{Mg^{2+}}{\rightleftharpoons} \text{ATP} + \text{AMP} \qquad \Delta G'^{\circ} \approx 0$$

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 pathways such as these, energy conserved in the catabolic production of ATP is used to supply the cell with all required NTPs and dNTPs.

Phosphocreatine (PCr; Fig. 13–15), also called creatine phosphate, serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The PCr concentration in skeletal muscle is approximately 30 mM, nearly 10 times the concentration of ATP, and in other tissues such as smooth muscle, brain, and kidney [PCr] is 5 to 10 mM. The enzyme **creatine kinase** catalyzes the reversible reaction

$$\text{ADP} + \text{PCr} \overset{Mg^{2+}}{\rightleftharpoons} \text{ATP} + \text{Cr} \qquad \Delta G'^{\circ} = -12.5 \text{ kJ/mol}$$

When a sudden demand for energy depletes ATP, the PCr reservoir is used to replenish ATP at a rate considerably faster than ATP can be synthesized by catabolic pathways. When the demand for energy slackens, ATP produced by catabolism is used to replenish the PCr

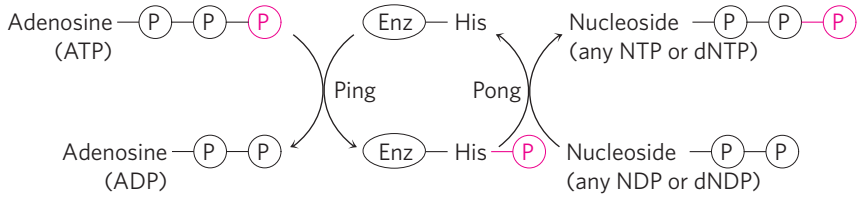
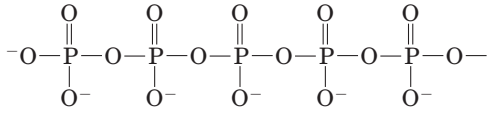


FIGURE 13–21 Ping-Pong mechanism of nucleoside diphosphate kinase. The enzyme binds its first substrate (ATP in our example), and a phosphoryl group is transferred to the side chain of a His residue. ADP departs, and another nucleoside (or deoxynucleoside) diphosphate replaces it, and this is converted to the corresponding triphosphate by transfer of the phosphoryl group from the phosphohistidine residue.

reservoir by reversal of the creatine kinase reaction (see Box 23–2). Organisms in the lower phyla employ other PCr-like molecules (collectively called **phosphagens**) as phosphoryl reservoirs.

Inorganic Polyphosphate Is a Potential Phosphoryl Group Donor

Inorganic polyphosphate, polyP (or $(polyP)_n$, where n is the number of orthophosphate residues), is a linear polymer composed of many tens or hundreds of P_i residues linked through phosphoanhydride bonds. This polymer, present in all organisms, may accumulate to high levels in some cells. In yeast, for example, the amount of polyP that accumulates in the vacuoles would represent, if distributed uniformly throughout the cell, a concentration of 200 mM! (Compare this with the concentrations of other phosphoryl group donors listed in Table 13–5.)



Inorganic polyphosphate (polyP)

One potential role for polyP is to serve as a phosphagen, a reservoir of phosphoryl groups that can be used to generate ATP, as creatine phosphate is used in muscle. PolyP has about the same phosphoryl group transfer potential as PP_i. The shortest polyphosphate, PP_i ($n = 2$), can serve as the energy source for active transport of H^+ across the vacuolar membrane in plant cells. For at least one form of the enzyme phosphofructokinase in plants, PP_i is the phosphoryl group donor, a role played by ATP in animals and microbes (p. 550). The finding of high concentrations of polyP in volcanic condensates and steam vents suggests that it could have served as an energy source in prebiotic and early cellular evolution.

In bacteria, the enzyme **polyphosphate kinase-1** (PPK-1) catalyzes the reversible reaction

$$ATP + (polyP)_n \overset{Mg^{2+}}{\rightleftharpoons} ADP + (polyP)_{n+1}$$

$$\Delta G'^\circ = -20 \text{ kJ/mol}$$

by a mechanism involving an enzyme-bound Ⓟ–His intermediate (recall the mechanism of nucleoside diphosphate kinase, described in Fig. 13–21). A second enzyme, **polyphosphate kinase-2** (PPK-2), catalyzes the reversible synthesis of GTP (or ATP) from polyphosphate and GDP (or ADP):

$$GDP + (polyP)_{n+1} \overset{Mn^{2+}}{\rightleftharpoons} GTP + (polyP)_n$$

PPK-2 is believed to act primarily in the direction of GTP and ATP synthesis, and PPK-1 in the direction of polyphosphate synthesis. PPK-1 and PPK-2 are present in a wide variety of bacteria, including many pathogenic species.

In bacteria, elevated levels of polyP have been shown to promote expression of genes involved in adaptation of the organism to conditions of starvation or other threats to survival. In *Escherichia coli*, for example, polyP accumulates when cells are starved for amino acids or P_i, and this accumulation confers a survival advantage. Deletion of the genes for polyphosphate kinases diminishes the ability of certain pathogenic bacteria to invade animal tissues. The enzymes may therefore prove to be suitable targets in the development of new antimicrobial drugs.

No yeast gene encodes a PPK-like protein, but four genes—unrelated to bacterial PPK genes—are necessary for the synthesis of polyphosphate. The mechanism for polyphosphate synthesis in eukaryotes seems to be quite different from that in bacteria.

SUMMARY 13.3 Phosphoryl Group Transfers and ATP

- ATP is the chemical link between catabolism and anabolism. It is the energy currency of the living cell. The exergonic conversion of ATP to ADP and P_i, or to AMP and PP_i, is coupled to many endergonic reactions and processes.
- Direct hydrolysis of ATP is the source of energy in some processes driven by conformational changes, but in general it is not ATP hydrolysis but the transfer of a phosphoryl, pyrophosphoryl, or adenylyl group from ATP to a substrate or enzyme that couples the energy of ATP breakdown to endergonic transformations of substrates.
- Through these group transfer reactions, ATP provides the energy for anabolic reactions, including the synthesis of informational macromolecules, and for the transport of molecules and ions across membranes against concentration gradients and electrical potential gradients.
- To maintain its high group transfer potential, ATP concentration must be held far above the equilibrium concentration by energy-yielding reactions of catabolism.
- Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphoryl group transfer potential. Thioesters also have high free energies of hydrolysis.
- Inorganic polyphosphate, present in all cells, may serve as a reservoir of phosphoryl groups with high group transfer potential.

13.4 Biological Oxidation-Reduction Reactions

The transfer of phosphoryl groups is a central feature of metabolism. Equally important is another kind of transfer, electron transfer in oxidation-reduction reactions. These reactions involve the loss of electrons by one chemical species, which is thereby oxidized, and the gain of electrons by another, which is reduced. The flow of electrons in oxidation-reduction reactions is responsible, directly or indirectly, for all work done by living organisms. In nonphotosynthetic organisms, the sources of electrons are reduced compounds (foods); in photosynthetic organisms, the initial electron donor is a chemical species excited by the absorption of light. The path of electron flow in metabolism is complex. Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalyzed reactions. The carriers in turn donate electrons to acceptors with higher electron affinities, with the release of energy. Cells contain a variety of molecular energy transducers, which convert the energy of electron flow into useful work.

We begin by discussing how work can be accomplished by an electromotive force (emf), then consider the theoretical and experimental basis for measuring energy changes in oxidation reactions in terms of emf and the relationship between this force, expressed in volts, and the free-energy change, expressed in joules. We conclude by describing the structures and oxidation-reduction chemistry of the most common of the specialized electron carriers, which you will encounter repeatedly in later chapters.

The Flow of Electrons Can Do Biological Work

Every time we use a motor, an electric light or heater, or a spark to ignite gasoline in a car engine, we use the flow of electrons to accomplish work. In the circuit that powers a motor, the source of electrons can be a battery containing two chemical species that differ in affinity for electrons. Electrical wires provide a pathway for electron flow from the chemical species at one pole of the battery, through the motor, to the chemical species at the other pole of the battery. Because the two chemical species differ in their affinity for electrons, electrons flow spontaneously through the circuit, driven by a force proportional to the difference in electron affinity, the **electromotive force**, **emf**. The emf (typically a few volts) can accomplish work if an appropriate energy transducer—in this case a motor—is placed in the circuit. The motor can be coupled to a variety of mechanical devices to do useful work.

Living cells have an analogous biological "circuit," with a relatively reduced compound such as glucose as the source of electrons. As glucose is enzymatically oxidized, the released electrons flow spontaneously through a series of electron-carrier intermediates to another chemical species, such as O_2. This electron flow is exergonic, because O_2 has a higher affinity for electrons than do the 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, for example, membrane-bound enzymes couple electron flow to the production of a transmembrane pH difference and a transmembrane electrical potential, accomplishing 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 P_i as protons flow spontaneously across the membrane. Similarly, membrane-localized enzymes in *E. coli* convert emf to proton-motive force, which is then used to power flagellar motion. The principles of electrochemistry that govern energy changes in the macroscopic circuit with a motor and battery apply with equal validity to the molecular processes accompanying electron flow in living cells.

Oxidation-Reductions Can Be Described as Half-Reactions

Although oxidation and reduction must occur together, it is convenient when describing electron transfers to consider the two halves of an oxidation-reduction reaction separately. For example, the oxidation of ferrous ion by cupric ion,

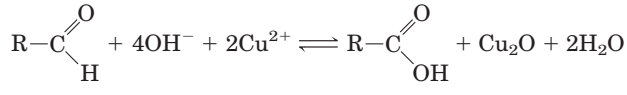
$$Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$$

can be described in terms of two half-reactions:

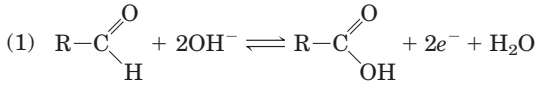
$$(1) \quad Fe^{2+} \rightleftharpoons Fe^{3+} + e^{-}$$
$$(2) \quad Cu^{2+} + e^{-} \rightleftharpoons Cu^{+}$$

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. Recall from Chapter 2 that in acid-base reactions we can write a general equation: proton donor $\rightleftharpoons$ H^+ + proton acceptor. In redox reactions we can write a similar general equation: electron donor (reductant) $\rightleftharpoons$ e^- + electron acceptor (oxidant). 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 electron transfers in the oxidation-reduction reactions of organic compounds are not fundamentally different from those of inorganic species. Consider the oxidation of a reducing sugar (an aldehyde or ketone) by cupric ion:



This overall reaction can be expressed as two half-reactions:



(1) $R-C(=O)-H + 2OH^- \rightleftharpoons R-C(=O)-OH + 2e^- + H_2O$

(2) $2Cu^{2+} + 2e^- + 2OH^- \rightleftharpoons Cu_2O + H_2O$

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.

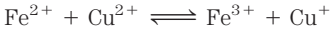
Biological Oxidations Often Involve Dehydrogenation

The carbon in living cells exists in a range of oxidation states **(Fig. 13–22)**. When a carbon atom shares an electron pair with another atom (typically H, C, S, N, or O), the sharing is unequal in favor of the more electronegative atom. The order of increasing electronegativity is H < C < S < N < O. In oversimplified but useful terms, the more electronegative atom "owns" the bonding electrons it shares with another atom. For example, in methane (CH_4), carbon is more electronegative than the four hydrogens bonded to it, and the C atom therefore "owns" all eight bonding electrons (Fig. 13–22). In ethane, the electrons in the C—C bond are shared equally, so each C atom "owns" only seven of its eight bonding electrons. In ethanol, C-1 is less electronegative than the oxygen to which it is bonded, and the O atom therefore "owns" both electrons of the C—O bond, leaving C-1 with only five bonding electrons. With each formal loss of "owned" electrons, the carbon atom has undergone oxidation—even when no oxygen is involved, as in the conversion of an alkane (—CH_2—CH_2—) to an alkene (—CH=CH—). In this case, oxidation (loss of electrons) is coincident with the loss of hydrogen. In biological systems, as we noted earlier in the chapter, oxidation is often synonymous with **dehydrogenation** and many enzymes that catalyze oxidation reactions are **dehydrogenases**. Notice that the more reduced compounds in Figure 13–22 (top) are richer in hydrogen than in oxygen, whereas the more oxidized compounds (bottom) have more oxygen and less hydrogen.

Not all biological oxidation-reduction reactions involve carbon. For example, in the conversion of molecular nitrogen to ammonia, $6H^+ + 6e^- + N_2 \rightarrow 2NH_3$, the nitrogen atoms are reduced.

Electrons are transferred from one molecule (electron donor) to another (electron acceptor) in one of four ways:

1. Directly as *electrons*. For example, the Fe^{2+}/Fe^{3+} redox pair can transfer an electron to the Cu^+/Cu^{2+} redox pair:



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

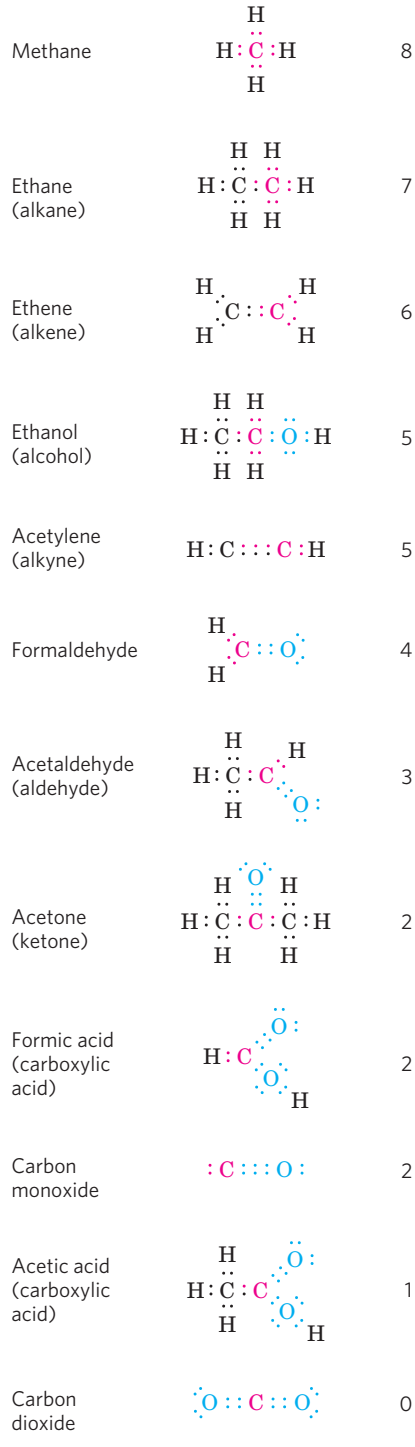


FIGURE 13–22 Different levels of oxidation of carbon compounds in the biosphere. To approximate the level of oxidation of these compounds, focus on the red carbon atom and its bonding electrons. When this carbon is bonded to the less electronegative H atom, both bonding electrons (red) are assigned to the carbon. When carbon is bonded to another carbon, bonding electrons are shared equally, so 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.

2. As *hydrogen atoms.* Recall that a hydrogen atom consists of a proton (H^+) and a single electron (e^-). In this case we can write the general equation

$$AH_2 \rightleftharpoons A + 2e^- + 2H^+$$

where AH_2 is the hydrogen/electron donor. (Do not mistake the above reaction for an acid dissociation, which involves a proton and no electron.) AH_2 and A together constitute a conjugate redox pair (A/AH_2), which can reduce another compound B (or redox pair, B/BH_2) by transfer of hydrogen atoms:

$$AH_2 + B \rightleftharpoons A + BH_2$$

3. As a *hydride ion* ($:H^-$), which has two electrons. This occurs in the case of NAD-linked dehydrogenases, described below.

4. Through direct *combination with oxygen.* In this case, oxygen combines with an organic reductant and is covalently incorporated in the product, as in the oxidation of a hydrocarbon to an alcohol:

$$R{-}CH_3 + \tfrac{1}{2}O_2 \longrightarrow R{-}CH_2{-}OH$$

The hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

All four types of electron transfer occur in cells. The neutral term **reducing equivalent** is commonly used to designate a single electron equivalent participating in an oxidation-reduction reaction, no matter whether this equivalent is an electron per se or part of a hydrogen atom or a hydride ion, or whether the electron transfer takes place in a reaction with oxygen to yield an oxygenated product. Because biological fuel molecules are usually enzymatically dehydrogenated to lose *two* reducing equivalents at a time, and because each oxygen atom can accept two reducing equivalents, biochemists by convention regard the unit of biological oxidations as two reducing equivalents passing from substrate to oxygen.

Reduction Potentials Measure Affinity for Electrons

When two conjugate redox pairs are together in solution, electron transfer from the electron donor of one pair to the electron acceptor of the other may proceed spontaneously. The tendency for such a reaction depends on the relative affinity of the electron acceptor of each redox pair for electrons. The **standard reduction potential**, $\boldsymbol{E}^\circ$, a measure (in volts) of this affinity, can be determined in an experiment such as that described in **Figure 13–23**. Electrochemists have chosen as a standard of reference the half-reaction

$$H^+ + e^- \longrightarrow \tfrac{1}{2}H_2$$

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

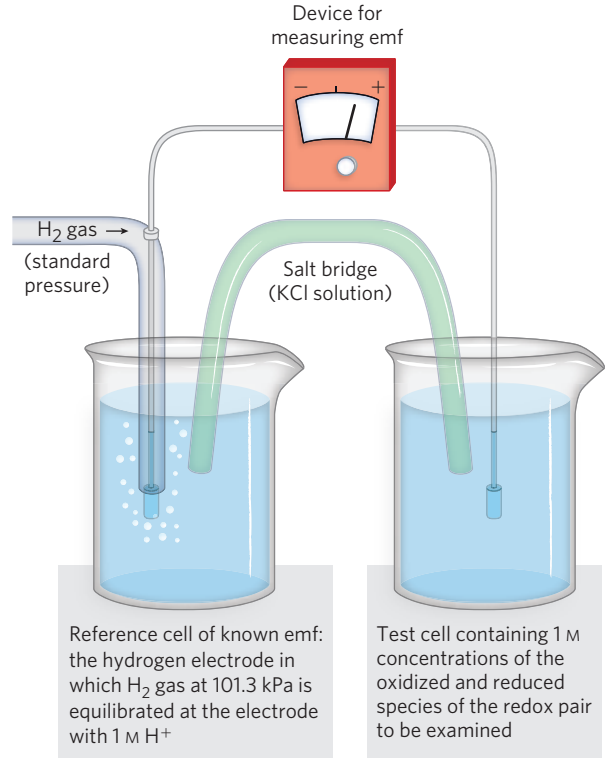


FIGURE 13–23 Measurement of the standard reduction potential (E'°) of a redox pair. Electrons flow from the test electrode to the reference electrode, or vice versa. The ultimate reference half-cell is the hydrogen electrode, as shown here, at pH 0. The electromotive force (emf) of this electrode is designated 0.00 V. At pH 7 in the test cell (and 25 °C), E'° for the hydrogen electrode is −0.414 V. The direction of electron flow depends on the relative electron "pressure" or potential of the two cells. A salt bridge containing a saturated KCl solution provides a path for counter-ion movement between the test cell and the reference cell. From the observed emf and the known emf of the reference cell, the experimenter can find the emf of the test cell containing the redox pair. The cell that gains electrons has, by convention, the more positive reduction potential.

When this hydrogen electrode is connected through an external circuit to another half-cell in which an oxidized species and its corresponding reduced species are present at standard concentrations (25 °C, each solute at 1 M, each gas at 101.3 kPa), electrons tend to flow through the external circuit from the half-cell of lower E° to the half-cell of higher E°. By convention, a half-cell that takes electrons from the standard hydrogen cell is assigned a positive value of E°, and one that donates electrons to the hydrogen cell, a negative value. When any two half-cells are connected, that with the larger (more positive) E° will get reduced; it has the greater reduction potential.

The reduction potential of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations. About a century ago, Walther Nernst derived an equation that

relates standard reduction potential ($E°$) to the actual reduction potential (E) at any concentration of oxidized and reduced species in a living cell:

$$E = E° + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{–}5)$$

where R and T have their usual meanings, n is the number of electrons transferred per molecule, and $\mathcal{F}$ is the Faraday constant (Table 13–1). At 298 K (25 °C), this expression reduces to

$$E = E° + \frac{0.026\text{V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{–}6)$$

KEY CONVENTION: Many half-reactions of interest to biochemists involve protons. As in the definition of $\Delta G'°$, biochemists define the standard state for oxidation-reduction reactions as pH 7 and express a standard transformed reduction potential, $E'°$, the standard reduction potential at pH 7 and 25 °C. By convention, $\Delta E'°$ for any redox reaction is given as $E'°$ of the electron acceptor minus $E'°$ of the electron donor. ■

The standard reduction potentials given in Table 13–7 and used throughout this book are values for $E'°$ and are therefore valid only for systems at neutral pH. Each value represents the potential difference when the conjugate redox pair, at 1 M concentrations, 25 °C, and pH 7, is connected with the standard (pH 0) hydrogen electrode. Notice in Table 13–7 that when the conjugate pair $2H^+/H_2$ at pH 7 is connected with the standard hydrogen electrode (pH 0), electrons tend to flow from the pH 7 cell to the standard (pH 0) cell; the measured $E'°$ for the $2H^+/H_2$ pair is −0.414 V.

Standard Reduction Potentials Can Be Used to Calculate Free-Energy Change

Why are reduction potentials so useful to the biochemist? When E values have been determined for any two half-cells, relative to the standard hydrogen electrode, we also know their reduction potentials relative to each other. We can then predict the direction in which electrons will tend to flow when the two half-cells are connected through an external circuit or when components of both half-cells are present in the same solution. Electrons tend to flow to the half-cell with the more positive E, and the strength of that tendency is proportional to ΔE, the difference in reduction potential. The energy made available by this spontaneous electron flow (the free-energy change, ΔG, for the oxidation-reduction reaction) is proportional to ΔE:

$$\Delta G = -n\mathcal{F}\Delta E \quad \text{or} \quad \Delta G'° = -n\mathcal{F}\,\Delta E'° \qquad (13\text{–}7)$$

where n is the number of electrons transferred in the reaction. With this equation we can calculate the actual free-energy change for any oxidation-reduction reaction from the values of $E'°$ in a table of reduction potentials (Table 13–7) and the concentrations of reacting species.

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^- ⟶ 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^- ⟶ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a (Fe^{3+}) + e^- ⟶ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + e^- ⟶ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- ⟶ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + e^- ⟶ cytochrome b (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol + H_2	0.045
Fumarate^{2-} + $2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (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 + $2H^+ + 2e^-$ ⟶ 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 + $2H^+ + 2e^-$ ⟶ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ ⟶ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

Source: Data mostly from Loach, R.A. (1976) in *Handbook of Biochemistry and Molecular Biology,* 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data,* Vol. 1, pp. 122–130, CRC Press, Boca Raton, FL.

* This is the value for free FAD; FAD bound to a specific flavoprotein (e.g., succinate dehydrogenase) has a different $E'°$ that depends on its protein environment.

WORKED EXAMPLE 13–3 **Calculation of $\Delta G'^\circ$ and ΔG of a Redox Reaction**

Calculate the standard free-energy change, $\Delta G'^\circ$, for the reaction in which acetaldehyde is reduced by the biological electron carrier NADH:

$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \longrightarrow \text{ethanol} + \text{NAD}^+$$

Then calculate the *actual* free-energy change, ΔG, when [acetaldehyde] and [NADH] are 1.00 M, and [ethanol] and [NAD^+] are 0.100 M. The relevant half-reactions and their E'° values are:

(1) $\text{Acetaldehyde} + 2\text{H}^+ + 2e^- \longrightarrow \text{ethanol}$ $\quad E'^\circ = -0.197$ V

(2) $\text{NAD}^+ + 2\text{H}^+ + 2e^- \longrightarrow \text{NADH} + \text{H}^+$ $\quad E'^\circ = -0.320$ V

Remember that, by convention, $\Delta E'^\circ$ is E'° of the electron acceptor minus E'° of the electron donor.

Solution: Because acetaldehyde is accepting electrons ($n = 2$) from NADH, $\Delta E'^\circ = -0.197 \text{ V} - (-0.320 \text{ V}) = 0.123$ V. Therefore,

$$\Delta G'^\circ = -n\mathcal{F}\Delta E'^\circ = -2(96.5 \text{ kJ/V} \cdot \text{mol})(0.123 \text{ V}) = -23.7 \text{ kJ/mol}$$

This is the free-energy change for the oxidation-reduction reaction at 25 °C and pH 7, when acetaldehyde, ethanol, NAD^+, and NADH are all present at 1.00 M concentrations.

To calculate ΔG when [acetaldehyde] and [NADH] are 1.00 M, and [ethanol] and [NAD^+] are 0.100 M, we can use Equation 13–4 and the standard free-energy change we calculated above:

$$\begin{aligned}\Delta G &= \Delta G'^\circ + RT \ln \frac{[\text{ethanol}][\text{NAD}^+]}{[\text{acetaldehyde}][\text{NADH}]} \\ &= -23.7 \text{ kJ/mol} + (8.315 \text{ J/mol·K})(298 \text{ K}) \ln \frac{(0.100 \text{ M})(0.100 \text{ M})}{(1.00 \text{ M})(1.00 \text{ M})} \\ &= -23.7 \text{ kJ/mol} + (2.48 \text{ J/mol}) \ln 0.01 \\ &= -35.1 \text{ kJ/mol}\end{aligned}$$

This is the actual free-energy change at the specified concentrations of the redox pairs.

Cellular Oxidation of Glucose to Carbon Dioxide Requires Specialized Electron Carriers

The principles of oxidation-reduction energetics described above apply to the many metabolic reactions that involve electron transfers. For example, in many organisms, the oxidation of glucose supplies energy for the production of ATP. The complete oxidation of glucose:

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

has a $\Delta G'^\circ$ of −2,840 kJ/mol. This is a much larger release of free energy than is required for ATP synthesis in cells (50 to 60 kJ/mol; see Worked Example 13–2). Cells convert glucose to CO_2 not in a single, high-energy-releasing reaction but rather in a series of controlled reactions, some of which are oxidations. The free energy released in these oxidation steps is of the same order of magnitude as that required for ATP synthesis from ADP, with some energy to spare. Electrons removed in these oxidation steps are transferred to coenzymes specialized for carrying electrons, such as NAD^+ and FAD (described below).

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

The multitude of enzymes that catalyze cellular oxidations channel electrons from their hundreds of different substrates into just a few types of universal electron carriers. The reduction of these carriers in catabolic processes results in the conservation of free energy released by substrate oxidation. NAD, NADP, FMN, and FAD are water-soluble coenzymes that undergo reversible oxidation and reduction in many of the electron-transfer reactions of metabolism. The nucleotides NAD and NADP move readily from one enzyme to another; the flavin nucleotides FMN and FAD are usually very tightly bound to the enzymes, called flavoproteins, for which they serve as prosthetic groups. Lipid-soluble quinones such as ubiquinone and plastoquinone act as electron carriers and proton donors in the nonaqueous environment of membranes. Iron-sulfur proteins and cytochromes, which have tightly bound prosthetic groups that undergo reversible oxidation and reduction, also serve as electron carriers in many oxidation-reduction reactions. Some of these proteins are water-soluble, but others are peripheral or integral membrane proteins (see Fig. 11–7).

We conclude this chapter by describing some chemical features of nucleotide coenzymes and some of the enzymes (dehydrogenases and flavoproteins) that use them. The oxidation-reduction chemistry of quinones, iron-sulfur proteins, and cytochromes is discussed in Chapter 19.

NADH and NADPH Act with Dehydrogenases as Soluble Electron Carriers

Nicotinamide adenine dinucleotide (NAD; NAD^+ in its oxidized form) and its close analog nicotinamide adenine dinucleotide phosphate (NADP; $NADP^+$ when oxidized) are composed of two nucleotides joined through their phosphate groups by a phosphoanhydride bond **(Fig. 13–24a)**. Because the nicotinamide ring resembles pyridine, these compounds are sometimes called **pyridine nucleotides**. The vitamin niacin is the source of the nicotinamide moiety in nicotinamide nucleotides.

Both coenzymes undergo reversible reduction of the nicotinamide ring (Fig. 13–24). As a substrate molecule undergoes oxidation (dehydrogenation), giving up two hydrogen atoms, the oxidized form of the nucleotide

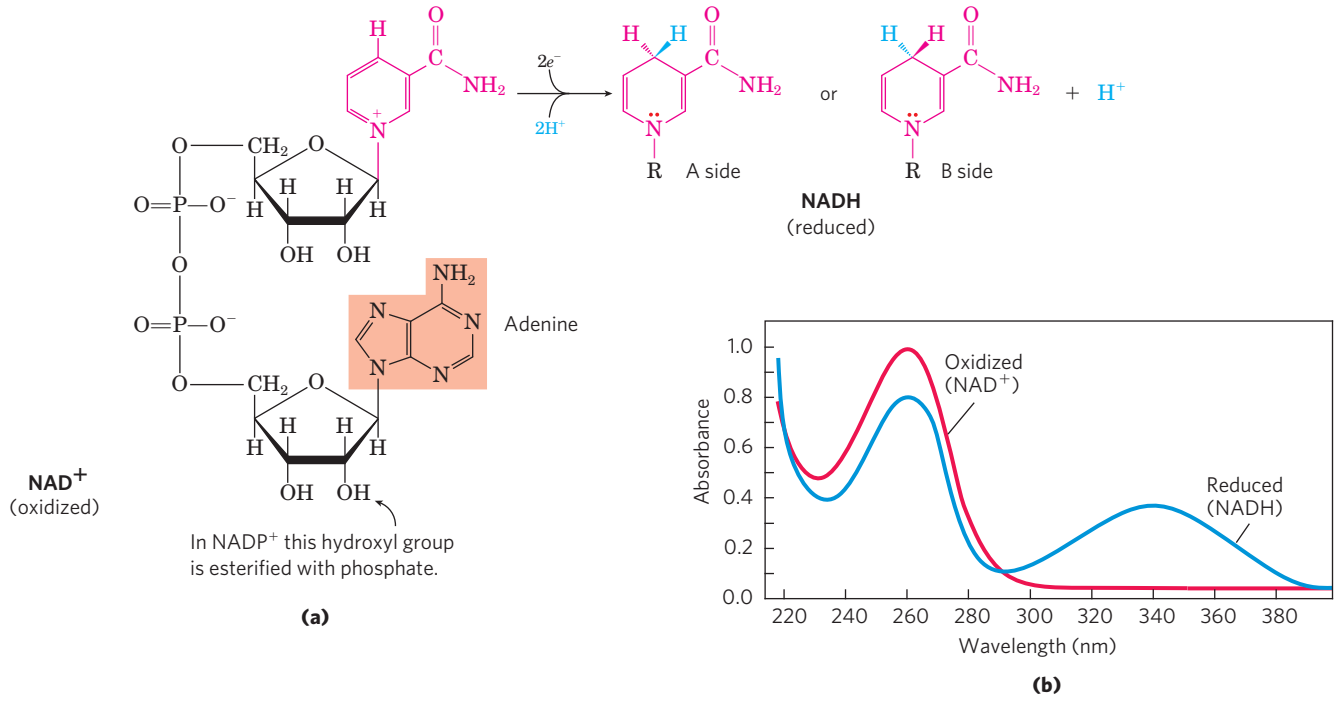


FIGURE 13–24 NAD and NADP. (a) Nicotinamide adenine dinucleotide, NAD^+, and its phosphorylated analog $NADP^+$ undergo reduction to NADH and NADPH, accepting a hydride ion (two electrons and one proton) from an oxidizable substrate. The hydride ion is added to either the front (the A side) or the back (the B side) of the planar nicotinamide ring (see Table 13-8). **(b)** The UV absorption spectra of NAD^+ and NADH. Reduction of the nicotinamide ring produces a new, broad absorption band with a maximum at 340 nm. The production of NADH during an enzyme-catalyzed reaction can be conveniently followed by observing the appearance of the absorbance at 340 nm (molar extinction coefficient $\varepsilon_{340} = 6{,}200\ \text{M}^{-1}\,\text{cm}^{-1}$).

(NAD^+ or $NADP^+$) accepts a hydride ion ($:H^-$, 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

$$NAD^+ + 2e^- + 2H^+ \longrightarrow NADH + H^+$$
$$NADP^+ + 2e^- + 2H^+ \longrightarrow NADPH + H^+$$

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 (Fig. 13–24b); this difference in absorption is used by biochemists to assay reactions involving these coenzymes. Note that the plus sign in the abbreviations NAD^+ and $NADP^+$ does *not* indicate the net charge on these molecules (in fact, both are negatively charged); rather, it indicates that the nicotinamide ring is in its oxidized form, with a positive charge on the nitrogen atom. In the abbreviations NADH and NADPH, the "H" denotes the added hydride ion. To refer to these nucleotides without specifying their oxidation state, we use NAD and NADP.

The total concentration of NAD^+ + NADH in most tissues is about 10^{-5} M; that of $NADP^+$ + NADPH is about 10^{-6} M. In many cells and tissues, the ratio of NAD^+ (oxidized) to NADH (reduced) is high, favoring hydride transfer from a substrate *to* NAD^+ to form NADH. By contrast, NADPH is generally present at a higher concentration than $NADP^+$, favoring hydride transfer *from* NADPH to a substrate. This reflects the specialized metabolic roles of the two coenzymes: NAD^+ generally functions in oxidations—usually as part of a catabolic reaction; NADPH is the usual coenzyme in reductions—nearly always as part of an anabolic reaction. A few enzymes can use either coenzyme, but most show a strong preference for one over the other. Also, the processes in which these two cofactors function are segregated in eukaryotic cells: for example, oxidations of fuels such as pyruvate, fatty acids, and α-keto acids derived from amino acids occur in the mitochondrial matrix, whereas reductive biosynthetic processes such as fatty acid synthesis take place in the cytosol. This functional and spatial specialization allows a cell to maintain two distinct pools of electron carriers, with two distinct functions.

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. The general reactions are

$$AH_2 + NAD^+ \longrightarrow A + NADH + H^+$$
$$A + NADPH + H^+ \longrightarrow AH_2 + NADP^+$$

where AH_2 is the reduced substrate and A the oxidized substrate. The general name for an enzyme of this type is **oxidoreductase**; they are also commonly called dehydrogenases. For example, alcohol dehydrogenase catalyzes the first step in the catabolism of ethanol, in which ethanol is oxidized to acetaldehyde:

$$\underset{\text{Ethanol}}{CH_3CH_2OH} + NAD^+ \longrightarrow \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH + H^+$$

Notice that one of the carbon atoms in ethanol has lost a hydrogen; the compound has been oxidized from an alcohol to an aldehyde (refer again to Fig. 13–22 for the oxidation states of carbon).

When NAD^+ or $NADP^+$ is reduced, the hydride ion could in principle be transferred to either side of the nicotinamide ring: the front (A side) or the back (B side), as represented in Figure 13–24a. Studies with isotopically labeled substrates have shown that a given enzyme catalyzes either an A-type or a B-type transfer, but not both. For example, yeast alcohol dehydrogenase and lactate dehydrogenase of vertebrate heart transfer a hydride ion to (or remove a hydride ion from) the A side of the nicotinamide ring; they are classed as type A dehydrogenases to distinguish them from another group of enzymes that transfer a hydride ion to (or remove a hydride ion from) the B side of the nicotinamide ring (Table 13–8). The specificity for one side or another can be very striking; lactate dehydrogenase, for example, prefers the A side over the B side by a factor of 5×10^7! The basis for this preference lies in the exact positioning of the enzyme groups involved in hydrogen bonding with the $—CONH_2$ group of the nicotinamide.

Most dehydrogenases that use NAD or NADP bind the cofactor in a conserved protein domain called the Rossmann fold (named for Michael Rossmann, who deduced the structure of lactate dehydrogenase and first described this structural motif). The Rossmann fold typically consists of a six-stranded parallel β sheet and four associated α helices **(Fig. 13–25)**.

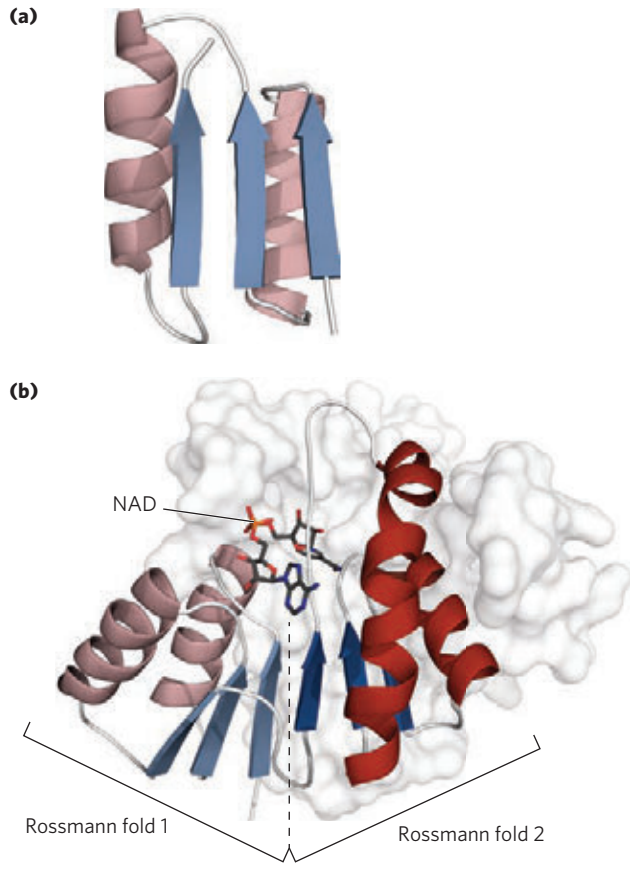


FIGURE 13–25 The Rossmann fold. This structural motif is found in the NAD-binding site of many dehydrogenases. **(a)** It consists of a pair of structurally similar motifs (only one of which is shown here), each having three parallel β sheets and two α helices (β-α-β-α-β). **(b)** The nucleotide-binding domain of the enzyme lactate dehydrogenase (derived from PDB ID 3LDH) with NAD (ball-and-stick structure) bound in an extended conformation through hydrogen bonds and salt bridges to the paired β-α-β-α-β motifs of the Rossmann fold (shades of red and blue).

The association between a dehydrogenase and NAD or NADP is relatively loose; the coenzyme readily diffuses from one enzyme to another, acting as a water-soluble

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

carrier of electrons from one metabolite to another. For example, in the production of alcohol during fermentation of glucose by yeast cells, a hydride ion is removed from glyceraldehyde 3-phosphate by one enzyme (glyceraldehyde 3-phosphate dehydrogenase, a type B enzyme) and transferred to NAD^+. The NADH produced then leaves the enzyme surface and diffuses to another enzyme (alcohol dehydrogenase, a type A enzyme), which transfers a hydride ion to acetaldehyde, producing ethanol:

(1) Glyceraldehyde 3-phosphate + NAD^+ ⟶ 3-phosphoglycerate + NADH + H^+

(2) Acetaldehyde + NADH + H^+ ⟶ ethanol + NAD^+

Sum: Glyceraldehyde 3-phosphate + acetaldehyde ⟶ 3-phosphoglycerate + ethanol

Notice that in the overall reaction there is no net production or consumption of NAD^+ or NADH; the coenzymes function catalytically and are recycled repeatedly without a net change in the concentration of NAD^+ + NADH.

Dietary Deficiency of Niacin, the Vitamin Form of NAD and NADP, Causes Pellagra

As we noted in Chapter 6, and will discuss further in the chapters to follow, most coenzymes are derived from the substances we call vitamins. The pyridine-like rings of NAD and NADP are derived from the vitamin **niacin** (nicotinic acid; **Fig. 13–26**), which is synthesized from tryptophan. Humans generally cannot synthesize sufficient quantities of niacin, and this is especially so for individuals with diets low in tryptophan (maize, for example, has a low tryptophan content). Niacin deficiency, which affects all the NAD(P)-dependent dehydrogenases, causes the serious human disease pellagra (Italian for "rough skin") and a related disease in dogs, blacktongue. These diseases are characterized by the "three Ds": dermatitis, diarrhea, and dementia, followed in many cases by death. A century ago, pellagra was a common human disease; in the southern United States, where maize was a dietary staple, about 100,000 people were afflicted and about 10,000 died as a result of this disease between 1912 and 1916. In 1920 Joseph Goldberger showed pellagra to be caused by a dietary insufficiency, and in 1937 Frank Strong, D. Wayne Woolley, and Conrad Elvehjem identified niacin as the curative agent for blacktongue. Supplementation of the human diet with this inexpensive compound has eradicated pellagra in the populations of the developed world, with one significant exception: people with alcoholism, or who drink excessive amounts of alcohol. In these individuals, intestinal absorption of niacin is much reduced, and caloric needs are often met with distilled spirits that are virtually devoid of vitamins, including niacin. In some parts of the world, including the Deccan Plateau in India, pellagra still occurs in the general population, especially among people living in poverty. ■

Frank Strong, 1908–1993

D. Wayne Woolley, 1914–1966

Conrad Elvehjem, 1901–1962

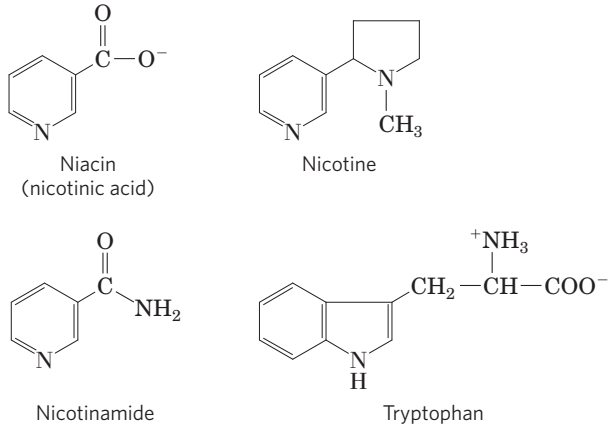


FIGURE 13–26 Niacin (nicotinic acid) and its derivative nicotinamide. The biosynthetic precursor of these compounds is tryptophan. In the laboratory, nicotinic acid was first produced by oxidation of the natural product nicotine—thus the name. Both nicotinic acid and nicotinamide cure pellagra, but nicotine (from cigarettes or elsewhere) has no curative activity.

Flavin Nucleotides Are Tightly Bound in Flavoproteins

Flavoproteins (Table 13–9) are enzymes that catalyze oxidation-reduction reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme **(Fig. 13–27)**. These coenzymes, the **flavin nucleotides**, are derived from the vitamin riboflavin. The fused ring structure of flavin nucleotides (the 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. The fully reduced forms are abbreviated $FADH_2$ and $FMNH_2$. When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), the semiquinone form of the isoalloxazine ring is produced, abbreviated $FADH^•$ and $FMNH^•$. Because flavin nucleotides have a slightly different chemical specialty from that of the nicotinamide

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

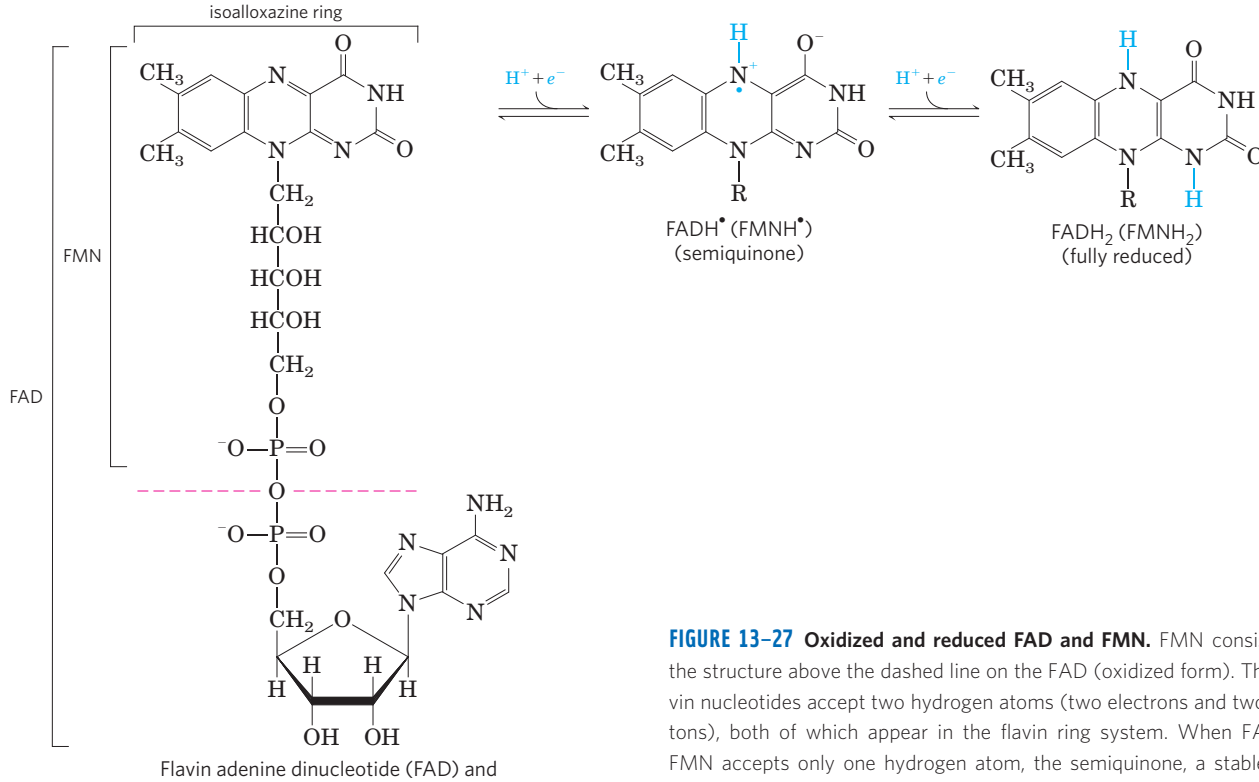
Enzyme	Flavin nucleotide	Text page(s)
Acyl-CoA dehydrogenase	FAD	673
Dihydrolipoyl dehydrogenase	FAD	637
Succinate dehydrogenase	FAD	646
Glycerol 3-phosphate dehydrogenase	FAD	759
Thioredoxin reductase	FAD	917
NADH dehydrogenase (Complex I)	FMN	738–739
Glycolate oxidase	FMN	813

coenzymes—the ability to participate in either one- or two-electron transfers—flavoproteins are involved in a greater diversity of reactions than the NAD(P)-linked dehydrogenases.

Like the nicotinamide coenzymes (Fig. 13–24), the flavin nucleotides undergo a shift in a major absorption band on reduction (again, useful to biochemists who want to monitor reactions involving these coenzymes). Flavoproteins that are fully reduced (two electrons accepted) generally have an absorption maximum near 360 nm. When partially reduced (one electron), they acquire another absorption maximum at about 450 nm; when fully oxidized, the flavin has maxima at 370 and 440 nm.

The flavin nucleotide in most flavoproteins is bound rather tightly to the protein, and in some enzymes, such as succinate dehydrogenase, it is bound covalently. Such tightly bound coenzymes are properly called prosthetic groups. They do not transfer electrons by diffusing from one enzyme to another; rather, they provide a means by which the flavoprotein can temporarily hold electrons while it catalyzes electron transfer from a reduced substrate to an electron acceptor. One important feature of the flavoproteins is the variability in the standard reduction potential ($E'°$) of the bound flavin nucleotide. Tight association between the enzyme and prosthetic group confers on the flavin ring a reduction potential typical of that particular flavoprotein, sometimes quite different from the reduction potential of the free flavin nucleotide. FAD bound to succinate dehydrogenase, for example, has an $E'°$ close to 0.0 V, compared with −0.219 V for free FAD; $E'°$ for other flavoproteins ranges from −0.40 V to + 0.06 V. Flavoproteins are often very complex; some have, in addition to a flavin nucleotide, tightly bound inorganic ions (iron or molybdenum, for example) capable of participating in electron transfers.

Certain flavoproteins act in a quite different role, as light receptors. **Cryptochromes** are a family of flavoproteins, widely distributed in the eukaryotic phyla, that 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). The cryptochromes are homologs of another family of flavoproteins, the photolyases. Found in both bacteria and eukaryotes, **photolyases** use the energy of absorbed light to repair chemical defects in DNA.



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

FIGURE 13–27 Oxidized and reduced FAD and FMN. 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.

We examine the function of flavoproteins as electron carriers in Chapter 19, when we consider their roles in oxidative phosphorylation (in mitochondria) and photophosphorylation (in chloroplasts), and we describe the photolyase reactions in Chapter 25.

SUMMARY 13.4 Biological Oxidation-Reduction Reactions

- In many organisms, a central energy-conserving process is the stepwise oxidation of glucose to CO_2, in which some of the energy of oxidation is conserved in ATP as electrons are passed to O_2.
- Biological oxidation-reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, $E'°$.
- When two electrochemical half-cells, each containing the components of a half-reaction, are connected, electrons tend to flow to the half-cell with the higher reduction potential. The strength of this tendency is proportional to the difference between the two reduction potentials (ΔE) and is a function of the concentrations of oxidized and reduced species.
- The standard free-energy change for an oxidation-reduction reaction is directly proportional to the difference in standard reduction potentials of the two half-cells: $\Delta G'° = -n\,\mathcal{F}\,\Delta E'°$.
- Many biological oxidation reactions are dehydrogenations in which one or two hydrogen atoms ($H^+ + e^-$) are transferred from a substrate to a hydrogen acceptor. Oxidation-reduction reactions in living cells involve specialized electron carriers.
- NAD and NADP are the freely diffusible coenzymes of many dehydrogenases. Both NAD^+ and $NADP^+$ accept two electrons and one proton.
- FAD and FMN, the flavin nucleotides, serve as tightly bound prosthetic groups of flavoproteins. They can accept either one or two electrons and one or two protons. Flavoproteins also serve as light receptors in cryptochromes and photolyases.

Key Terms

Terms in bold are defined in the glossary.

autotroph 501
heterotroph 501
metabolism 502
metabolic pathways 502
metabolite 502
intermediary metabolism 502
catabolism 502
anabolism 502
standard transformed constants 507
homolytic cleavage 512
radical 512
heterolytic cleavage 512
nucleophile 512
electrophile 512
carbanion 512
carbocation 512
aldol condensation 513
Claisen condensation 513
kinases 516
phosphorylation potential (ΔG_p) 518
thioester 521
adenylylation 524
inorganic pyrophosphatase 524
nucleoside diphosphate kinase 526
adenylate kinase 526
creatine kinase 526
phosphagens 527
polyphosphate kinase-1, kinase-2 527
electromotive force (emf) 528
conjugate redox pair 528
dehydrogenation 529
dehydrogenases 529
reducing equivalent 530
standard reduction potential ($E'°$) 530
pyridine nucleotide 532
oxidoreductase 534
flavoprotein 535
flavin nucleotides 535
cryptochrome 536
photolyase 536

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Problems

1. Entropy Changes during Egg Development Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single cell to a complex organism. Discuss this irreversible process in terms of the entropy changes in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings.

2. Calculation of $\Delta G'^{\circ}$ from an Equilibrium Constant Calculate the standard free-energy change for each of the following metabolically important enzyme-catalyzed reactions, using the equilibrium constants given for the reactions at 25 °C and pH 7.0.

(a) Glutamate + oxaloacetate $\overset{\text{aspartate aminotransferase}}{\rightleftharpoons}$ aspartate + α-ketoglutarate $\quad K'_{eq} = 6.8$

(b) Dihydroxyacetone phosphate $\overset{\text{triose phosphate isomerase}}{\rightleftharpoons}$ glyceraldehyde 3-phosphate $\quad K'_{eq} = 0.0475$

(c) Fructose 6-phosphate + ATP $\overset{\text{phosphofructokinase}}{\rightleftharpoons}$ fructose 1,6-bisphosphate + ADP $\quad K'_{eq} = 254$

3. Calculation of the Equilibrium Constant from $\Delta G'^\circ$ Calculate the equilibrium constant K'_{eq} for each of the following reactions at pH 7.0 and 25 °C, using the $\Delta G'^\circ$ values in Table 13–4.

(a) Glucose 6-phosphate + H_2O $\overset{\text{glucose 6-phosphatase}}{\rightleftharpoons}$ glucose + P_i

(b) Lactose + H_2O $\overset{\beta\text{-galactosidase}}{\rightleftharpoons}$ glucose + galactose

(c) Malate $\overset{\text{fumarase}}{\rightleftharpoons}$ fumarate + H_2O

4. Experimental Determination of K'_{eq} and $\Delta G'^\circ$ If a 0.1 M solution of glucose 1-phosphate at 25 °C is incubated with a catalytic amount of phosphoglucomutase, the glucose 1-phosphate is transformed to glucose 6-phosphate. At equilibrium, the concentrations of the reaction components are

Glucose 1-phosphate $\rightleftharpoons$ glucose 6-phosphate

4.5×10^{-3} M $\qquad$ 9.6×10^{-2} M

Calculate K'_{eq} and $\Delta G'^\circ$ for this reaction.

5. Experimental Determination of $\Delta G'^\circ$ for ATP Hydrolysis A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^\circ$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

Glucose 6-phosphate + H_2O ⟶ glucose + P_i $\quad K'_{eq} = 270$

ATP + glucose ⟶ ADP + glucose 6-phosphate $\quad K'_{eq} = 890$

Using this information for equilibrium constants determined at 25 °C, calculate the standard free energy of hydrolysis of ATP.

6. Difference between $\Delta G'^\circ$ and ΔG Consider the following interconversion, which occurs in glycolysis (Chapter 14):

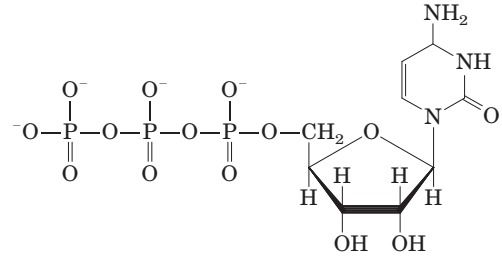
Fructose 6-phosphate $\rightleftharpoons$ glucose 6-phosphate $\quad K'_{eq} = 1.97$

(a) What is $\Delta G'^\circ$ for the reaction (K'_{eq} measured at 25 °C)?

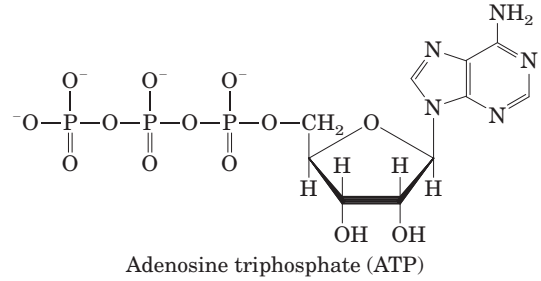
(b) If the concentration of fructose 6-phosphate is adjusted to 1.5 M and that of glucose 6-phosphate is adjusted to 0.50 M, what is ΔG?

(c) Why are $\Delta G'^\circ$ and ΔG different?

7. Free Energy of Hydrolysis of CTP Compare the structure of the nucleoside triphosphate CTP with the structure of ATP.



Cytidine triphosphate (CTP)



Adenosine triphosphate (ATP)

Now predict the K'_{eq} and $\Delta G'^\circ$ for the following reaction:

ATP + CDP ⟶ ADP + CTP

8. Dependence of ΔG on pH The free energy released by the hydrolysis of ATP under standard conditions is −30.5 kJ/mol. If ATP is hydrolyzed under standard conditions except at pH 5.0, is more or less free energy released? Explain. Use the Living Graph to explore this relationship.

9. The $\Delta G'^\circ$ for Coupled Reactions Glucose 1-phosphate is converted into fructose 6-phosphate in two successive reactions:

Glucose 1-phosphate ⟶ glucose 6-phosphate

Glucose 6-phosphate ⟶ fructose 6-phosphate

Using the $\Delta G'^\circ$ values in Table 13–4, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions:

Glucose 1-phosphate ⟶ fructose 6-phosphate

10. Effect of [ATP]/[ADP] Ratio on Free Energy of Hydrolysis of ATP Using Equation 13–4, plot ΔG against ln Q (mass-action ratio) at 25 °C for the concentrations of ATP, ADP, and P_i in the table below. $\Delta G'^\circ$ for the reaction is −30.5 kJ/mol. Use the resulting plot to explain why metabolism is regulated to keep the ratio [ATP]/[ADP] high.

	Concentration (mM)				
ATP	5	3	1	0.2	5
ADP	0.2	2.2	4.2	5.0	25
P_i	10	12.1	14.1	14.9	10

11. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling The phosphorylation of glucose to glucose 6-phosphate is the initial step in the

catabolism of glucose. The direct phosphorylation of glucose by P_i is described by the equation

$$\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^\circ = 13.8 \text{ kJ/mol}$$

(a) Calculate the equilibrium constant for the above reaction at 37 °C. In the rat hepatocyte the physiological concentrations of glucose and P_i are maintained at approximately 4.8 mM. What is the equilibrium concentration of glucose 6-phosphate obtained by the direct phosphorylation of glucose by P_i? Does this reaction represent a reasonable metabolic step for the catabolism of glucose? Explain.

(b) In principle, at least, one way to increase the concentration of glucose 6-phosphate is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of glucose and P_i. Assuming a fixed concentration of P_i at 4.8 mM, how high would the intracellular concentration of glucose have to be to give an equilibrium concentration of glucose 6-phosphate of 250 μM (the normal physiological concentration)? Would this route be physiologically reasonable, given that the maximum solubility of glucose is less than 1 M?

(c) The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is used to phosphorylate glucose:

(1) $\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^\circ = 13.8 \text{ kJ/mol}$

(2) $\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \qquad \Delta G'^\circ = -30.5 \text{ kJ/mol}$

Sum: $\text{Glucose} + \text{ATP} \longrightarrow \text{glucose 6-phosphate} + \text{ADP}$

Calculate K'_{eq} at 37 °C for the overall reaction. For the ATP-dependent phosphorylation of glucose, what concentration of glucose is needed to achieve a 250 μM intracellular concentration of glucose 6-phosphate when the concentrations of ATP and ADP are 3.38 mM and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose in the cell? Explain.

(d) Although coupling ATP hydrolysis to glucose phosphorylation makes thermodynamic sense, we have not yet specified how this coupling is to take place. Given that coupling requires a common intermediate, one conceivable route is to use ATP hydrolysis to raise the intracellular concentration of P_i and thus drive the unfavorable phosphorylation of glucose by P_i. Is this a reasonable route? (Think about the solubility products of metabolic intermediates.)

(e) The ATP-coupled phosphorylation of glucose is catalyzed in hepatocytes by the enzyme glucokinase. This enzyme binds ATP and glucose to form a glucose-ATP-enzyme complex, and the phosphoryl group is transferred directly from ATP to glucose. Explain the advantages of this route.

12. Calculations of $\Delta G'^\circ$ for ATP-Coupled Reactions From data in Table 13–6 calculate the $\Delta G'^\circ$ value for the following reactions

(a) $\text{Phosphocreatine} + \text{ADP} \longrightarrow \text{creatine} + \text{ATP}$

(b) $\text{ATP} + \text{fructose} \longrightarrow \text{ADP} + \text{fructose 6-phosphate}$

13. Coupling ATP Cleavage to an Unfavorable Reaction To explore the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation $X \rightarrow Y$, for which $\Delta G'^\circ = 20.0$ kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and P_i. The overall reaction is

$$X + \text{ATP} + H_2O \longrightarrow Y + \text{ADP} + P_i$$

Calculate [Y]/[X] for this reaction at equilibrium. Assume that the temperature is 25 °C and the equilibrium concentrations of ATP, ADP, and P_i are 1 M.

(c) We know that [ATP], [ADP], and [P_i] are *not* 1 M under physiological conditions. Calculate [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [P_i] are those found in rat myocytes (Table 13–5).

14. Calculations of ΔG at Physiological Concentrations Calculate the actual, physiological ΔG for the reaction

$$\text{Phosphocreatine} + \text{ADP} \longrightarrow \text{creatine} + \text{ATP}$$

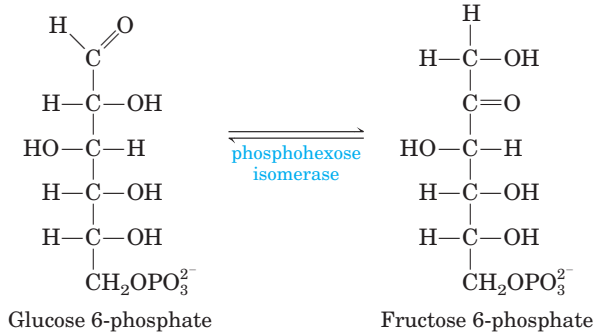
at 37 °C, as it occurs in the cytosol of neurons, with phosphocreatine at 4.7 mM, creatine at 1.0 mM, ADP at 0.73 mM, and ATP at 2.6 mM.

15. Free Energy Required for ATP Synthesis under Physiological Conditions In the cytosol of rat hepatocytes, the temperature is 37 °C and the mass-action ratio, Q, is

$$\frac{[\text{ATP}]}{[\text{ADP}][P_i]} = 5.33 \times 10^2 \text{ M}^{-1}$$

Calculate the free energy required to synthesize ATP in a rat hepatocyte.

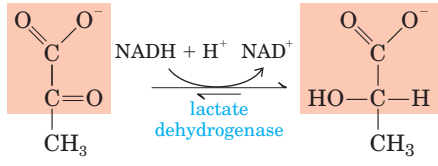
16. Chemical Logic In the glycolytic pathway, a six-carbon sugar (fructose 1,6-bisphosphate) is cleaved to form two three-carbon sugars, which undergo further metabolism (see Fig. 14–6). In this pathway, an isomerization of glucose 6-phosphate to fructose 6-phosphate (shown below) occurs two steps before the cleavage reaction (the intervening step is phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate (p. 549)).



Glucose 6-phosphate Fructose 6-phosphate

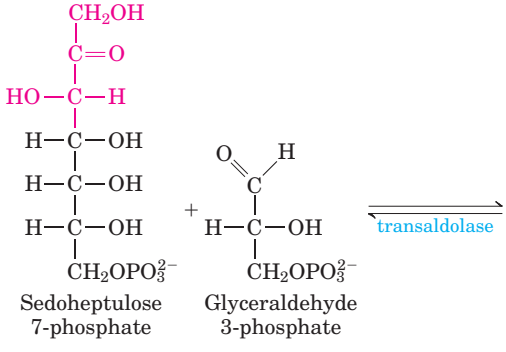
What does the isomerization step accomplish from a chemical perspective? (Hint: Consider what might happen if the C—C bond cleavage were to proceed without the preceding isomerization.)

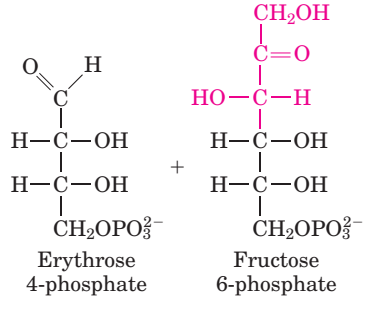
17. Enzymatic Reaction Mechanisms I Lactate dehydrogenase is one of the many enzymes that require NADH as coenzyme. It catalyzes the conversion of pyruvate to lactate:



Draw the mechanism of this reaction (show electron-pushing arrows). (Hint: This is a common reaction throughout metabolism; the mechanism is similar to that catalyzed by other dehydrogenases that use NADH, such as alcohol dehydrogenase.)

18. Enzymatic Reaction Mechanisms II Biochemical reactions often look more complex than they really are. In the pentose phosphate pathway (Chapter 14), sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate react to form erythrose 4-phosphate and fructose 6-phosphate in a reaction catalyzed by transaldolase.





Draw a mechanism for this reaction (show electron-pushing arrows). (Hint: Take another look at aldol condensations, then consider the name of this enzyme.)

19. Daily ATP Utilization by Human Adults

(a) A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i when the reactants and products are at 1 M concentrations and the temperature is 25 °C (standard state). Because the actual physiological concentrations of ATP, ADP, and P_i are not 1 M, and the temperature is 37 °C, the free energy required to synthesize ATP under physiological conditions is different from $\Delta G'°$. Calculate the free energy required to synthesize ATP in the human hepatocyte when the physiological concentrations of ATP, ADP, and P_i are 3.5, 1.50, and 5.0 mM, respectively.

(b) A 68 kg (150 lb) adult requires a caloric intake of 2,000 kcal (8,360 kJ) of food per day (24 hours). The food is metabolized and the free energy is used to synthesize ATP, which then provides energy for the body's daily chemical and mechanical work. Assuming that the efficiency of converting food energy into ATP is 50%, calculate the weight of ATP used by a human adult in 24 hours. What percentage of the body weight does this represent?

(c) Although adults synthesize large amounts of ATP daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

20. Rates of Turnover of γ and β Phosphates of ATP If a small amount of ATP labeled with radioactive phosphorus in the terminal position, [γ-^{32}P]ATP, is added to a yeast extract, about half of the ^{32}P activity is found in P_i within a few minutes, but the concentration of ATP remains unchanged. Explain. If the same experiment is carried out using ATP labeled with ^{32}P in the central position, [β-^{32}P]ATP, the ^{32}P does not appear in P_i within such a short time. Why?

21. Cleavage of ATP to AMP and PP_i during Metabolism Synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process:

$$\text{Acetate} + \text{CoA} + \text{ATP} \longrightarrow \text{acetyl-CoA} + \text{AMP} + PP_i$$

(a) The $\Delta G'°$ for hydrolysis of acetyl-CoA to acetate and CoA is −32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is −30.5 kJ/mol. Calculate $\Delta G'°$ for the ATP-dependent synthesis of acetyl-CoA.

(b) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA? Explain.

22. Energy for H^+ Pumping The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions from the cytosol (pH 7.0) into the stomach, contributing to the acidity of gastric juice (pH 1.0). Calculate the free energy required to transport 1 mol of hydrogen ions through these pumps. (Hint: See Chapter 11.) Assume a temperature of 37 °C.

23. Standard Reduction Potentials The standard reduction potential, $E'°$, of any redox pair is defined for the half-cell reaction:

$$\text{Oxidizing agent} + n \text{ electrons} \longrightarrow \text{reducing agent}$$

The $E'°$ values for the NAD^+/NADH and pyruvate/lactate conjugate redox pairs are −0.32 V and −0.19 V, respectively.

(a) Which redox pair has the greater tendency to lose electrons? Explain.

(b) Which pair is the stronger oxidizing agent? Explain.

(c) Beginning with 1 M concentrations of each reactant and product at pH 7 and 25 °C, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change ($\Delta G'°$) for the conversion of pyruvate to lactate?

(e) What is the equilibrium constant (K'_{eq}) for this reaction?

24. Energy Span of the Respiratory Chain Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate $\Delta E'°$ for the net reaction of mitochondrial electron transfer. Use $E'°$ values from Table 13–7.

(b) Calculate $\Delta G'°$ for this reaction.

(c) How many ATP molecules can *theoretically* be generated by this reaction if the free energy of ATP synthesis under cellular conditions is 52 kJ/mol?

25. Dependence of Electromotive Force on Concentrations Calculate the electromotive force (in volts) registered by an electrode immersed in a solution containing the following mixtures of NAD^+ and NADH at pH 7.0 and 25 °C, with reference to a half-cell of $E'°$ 0.00 V.

(a) 1.0 mM NAD^+ and 10 mM NADH

(b) 1.0 mM NAD^+ and 1.0 mM NADH

(c) 10 mM NAD^+ and 1.0 mM NADH

26. Electron Affinity of Compounds List the following in order of increasing tendency to accept electrons: (a) α-ketoglutarate + CO_2 (yielding isocitrate); (b) oxaloacetate; (c) O_2; (d) $NADP^+$.

27. Direction of Oxidation-Reduction Reactions Which of the following reactions would you expect to proceed in the direction shown, under standard conditions, in the presence of the appropriate enzymes?

(a) Malate + NAD^+ $\longrightarrow$ oxaloacetate + NADH + H^+

(b) Acetoacetate + NADH + H^+ $\longrightarrow$ β-hydroxybutyrate + NAD^+

(c) Pyruvate + NADH + H^+ $\longrightarrow$ lactate + NAD^+

(d) Pyruvate + β-hydroxybutyrate $\longrightarrow$ lactate + acetoacetate

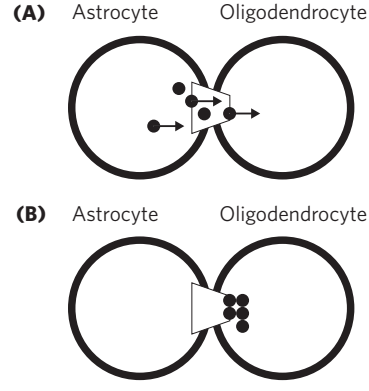
(e) Malate + pyruvate $\longrightarrow$ oxaloacetate + lactate

(f) Acetaldehyde + succinate $\longrightarrow$ ethanol + fumarate

Data Analysis Problem

28. Thermodynamics Can Be Tricky Thermodynamics is a challenging area of study and one with many opportunities for confusion. An interesting example is found in an article by Robinson, Hampson, Munro, and Vaney, published in *Science* in 1993. Robinson and colleagues studied the movement of small molecules between neighboring cells of the nervous system through cell-to-cell channels (gap junctions). They found that the dyes Lucifer yellow (a small, negatively charged molecule) and biocytin (a small zwitterionic molecule) moved in only one direction between two particular types of glia (nonneuronal cells of the nervous system). Dye injected into astrocytes would rapidly pass into adjacent astrocytes, oligodendrocytes, or Müller cells, but dye injected into oligodendrocytes or Müller cells passed slowly if at all into astrocytes. All of these cell types are connected by gap junctions.

Although it was not a central point of their article, the authors presented a molecular model for how this unidirectional transport might occur, as shown in their Figure 3:



The figure legend reads: "Model of the unidirectional diffusion of dye between coupled oligodendrocytes and astrocytes, based on differences in connection pore diameter. Like a fish in a fish trap, dye molecules (black circles) can pass from an astrocyte to an oligodendrocyte (A) but not back in the other direction (B)."

Although this article clearly passed review at a well-respected journal, several letters to the editor (1994) followed, showing that Robinson and coauthors' model violated the second law of thermodynamics.

(a) Explain how the model violates the second law. Hint: Consider what would happen to the entropy of the system if one started with equal concentrations of dye in the astrocyte and oligodendrocyte connected by the "fish trap" type of gap junctions.

(b) Explain why this model cannot work for small molecules, although it may allow one to catch fish.

(c) Explain why a fish trap *does* work for fish.

(d) Provide two plausible mechanisms for the unidirectional transport of dye molecules between the cells that do not violate the second law of thermodynamics.

References

Letters to the editor. (1994) *Science* **265**, 1017–1019.

Robinson, S.R., Hampson, E.C.G.M., Munro, M.N., & Vaney, D.I. (1993) Unidirectional coupling of gap junctions between neuroglia. *Science* **262**, 1072–1074.