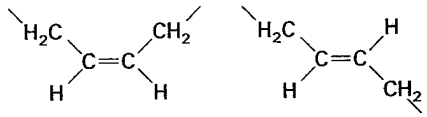
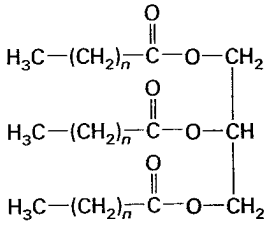
by mammals and must be supplied in their diet. Mammals can synthesize other common fatty acids. Two stereoisomeric configurations, cis and trans, are possible around each carbon-carbon double bond:



Cis **Trans**

A cis double bond introduces a rigid kink in the otherwise flexible straight chain of a fatty acid (Figure 2-21). In general, the unsaturated fatty acids in biological systems contain only cis double bonds. Saturated fatty acids without the kink can pack together more tightly and so have higher melting points than unsaturated fatty acids (saturated fatty acids are usually solid rather than liquid at room temperature). The trans fatty acids (popularly called "trans fats") found in partially hydrogenated margarine (used to make solid margarine sticks) and other food products are not natural, arising from the catalytic process used for hydrogenation. Saturated and trans fatty acids have similar physical properties, and their consumption, relative to the consumption of unsaturated fats, is associated with increased plasma cholesterol levels.

Fatty acids can be covalently attached to another molecule by a type of dehydration reaction called *esterification*, in which the OH from the carboxyl group of the fatty acid and a H from a hydroxyl group on the other molecule are lost. In the combined molecule formed by this reaction, the portion derived from the fatty acid is called an *acyl group*, or *fatty acyl group*. This is illustrated by the most common form of phospholipids, **phosphoglycerides**, with two acyl groups attached to two of the hydroxyl groups of glycerol (see Figure 2-20) and **triacylglycerols**, or **triglycerides**, which contain three acyl groups esterfied to glycerol:

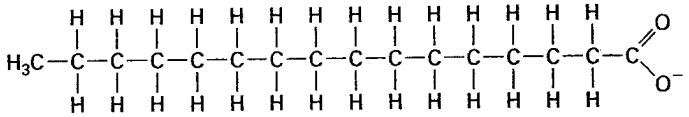


Triacylglycerol

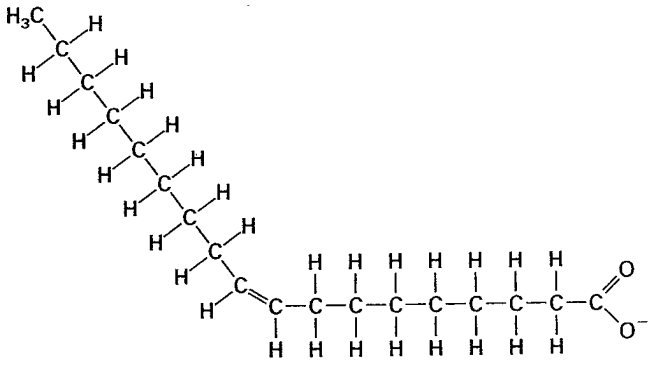
Fatty acyl groups also can be covalently linked to another fatty molecule, cholesterol, to form cholesteryl esters.

Triglycerides and cholesteryl esters are extremely water-insoluble compounds in which fatty acids and cholesterol are either stored or transported. Triglycerides are the storage form of fatty acids in the fat cells of adipose tissue and are the principal components of dietary fats. Cholesteryl esters and triglycerides are transported between tissues through the bloodstream in specialized carriers called lipoproteins (Chapter 14).

In phosphoglycerides, one hydroxyl group of the glycerol is esterified to phosphate while the other two normally are esterified to fatty acids. The simplest phospholipid, phosphatidic acid, contains only these components. In most phospholipids found in membranes, the phosphate group is also esterified to a hydroxyl group on another hydrophilic compound. In phosphatidylcholine, for example, choline is attached to the phosphate (see Figure 2-20). The negative charge on the phosphate as well as the charged or polar groups esterified to it can interact strongly with water. The phosphate and its associated esterified group, the "head" group of a phospholipid, is hydrophilic, whereas the fatty acyl chains, the "tails," are hydrophobic. (Other common phosphoglycerides and associated head groups are shown in Table 2-5.) Molecules such as phospholipids that have both hydrophobic and



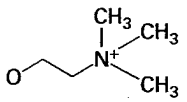
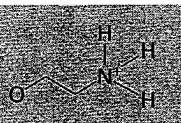
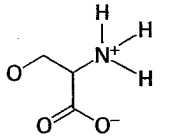
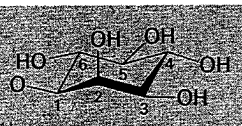
Palmitate
(ionized form of palmitic acid)



Oleate
(ionized form of oleic acid)

▲ **FIGURE 2-21 The effect of a double bond on the shape of fatty acids.** Shown are chemical structures of the ionized form of palmitic acid, a saturated fatty acid with 16 C atoms, and oleic acid, an unsaturated one with 18 C atoms. In saturated fatty acids, the hydrocarbon chain is often linear; the cis double bond in oleate creates a rigid kink in the hydrocarbon chain. [After L. Stryer, 1994, *Biochemistry*, 4th ed., W. H. Freeman and Company, p. 265.]

TABLE 2-5 Common Phosphoglycerides and Head Groups

COMMON PHOSPHOGLYCERIDES	HEAD GROUP
Phosphatidylcholine	 Choline
Phosphatidylethanolamine	 Ethanolamine
Phosphatidylserine	 Serine
Phosphatidylinositol	 Inositol

hydrophilic regions are called amphipathic. In Chapter 10, we will see how the amphipathic properties of phospholipids are responsible for the assembly of phospholipids into sheetlike bilayer biomembranes in which the fatty acyl tails point into the center of the sheet and the head groups point outward toward the aqueous environment (see Figure 2-13). ▮

KEY CONCEPTS OF SECTION 2.2

Chemical Building Blocks of Cells

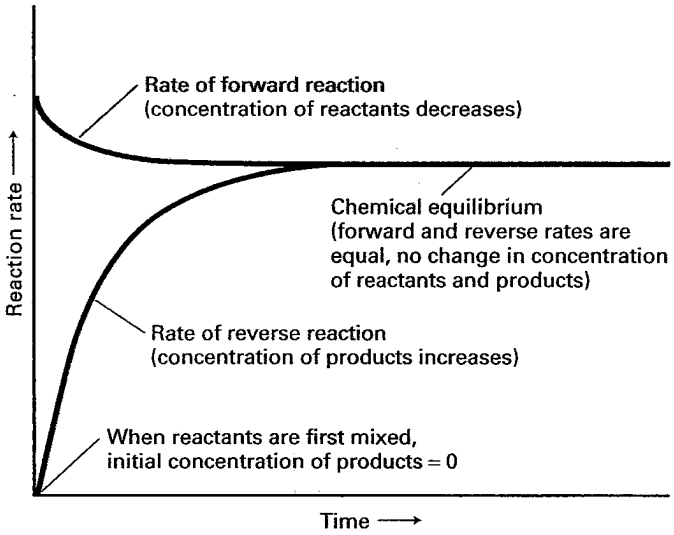
- Three major biopolymers formed by polymerization reactions (net dehydration) of basic chemical building blocks are present in cells: proteins, composed of amino acids linked by peptide bonds; nucleic acids, composed of nucleotides linked by phosphodiester bonds; and polysaccharides, composed of monosaccharides (sugars) linked by glycosidic bonds (see Figure 2-13). Phospholipids, the fourth major chemical building block, assemble noncovalently into biomembranes.
- Differences in the size, shape, charge, hydrophobicity, and reactivity of the side chains of the 20 common amino acids determine the chemical and structural properties of proteins (see Figure 2-14).
- The bases in the nucleotides composing DNA and RNA are carbon- and nitrogen-containing rings attached to a pentose sugar. They form two groups: the purines—adenine (A) and guanine (G)—and the pyrimidines—cytosine (C), thymine (T), and uracil (U) (see Figure 2-17). A, G, T, and C are in DNA, and A, G, U, and C are in RNA.
- Glucose and other hexoses can exist in three forms: an open-chain linear structure, a six-member (pyranose) ring, and a five-member (furanose) ring (see Figure 2-18). In biological systems, the pyranose form of D-glucose predominates.
- Glycosidic bonds are formed between either the α or the β anomer of one sugar and a hydroxyl group on another sugar, leading to formation of disaccharides and other polysaccharides (see Figure 2-19).
- Phospholipids are amphipathic molecules with a hydrophobic tail (often two fatty acyl chains) connected by a small organic molecule (often glycerol) to a hydrophilic head (see Figure 2-20). The long hydrocarbon chain of a fatty acid may contain no carbon-carbon double bond (saturated) or one or more double bonds (unsaturated); a cis double bond bends the chain.

2.3 Chemical Equilibrium

We now shift our discussion to chemical reactions in which bonds, primarily covalent bonds in *reactant* chemicals, are broken and new bonds are formed to generate reaction *products*. At any one time, several hundred different kinds of chemical reactions are occurring simultaneously in every cell, and many chemicals can, in principle, undergo multiple chemical reactions. Both the *extent* to which reactions can proceed and the *rate* at which they take place determine the chemical composition of cells.

When reactants first mix together—before any products have been formed—their rate of reaction to form products (forward reaction) is determined in part by their initial concentrations, which determine the likelihood of reactants bumping into one another and reacting (Figure 2-22). As the reaction products accumulate, the concentration of each reactant decreases and so does the forward reaction rate. Meanwhile, some of the product molecules begin to participate in the reverse reaction, which re-forms the reactants (the ability of a reaction to go "backward" is called *microscopic reversibility*). This reverse reaction is slow at first but speeds up as the concentration of product increases. Eventually, the rates of the forward and reverse reactions become equal, so that the concentrations of reactants and products stop changing. The system is then said to be in **chemical equilibrium** (plural: *equilibria*).

At equilibrium, the ratio of products to reactants, called the **equilibrium constant,** is a fixed value that is independent of the rate at which the reaction occurs. The rate of a chemical reaction can be increased by a **catalyst,** which accelerates the chemical transformation (making and breaking of covalent bonds) but is not permanently changed during a reaction (see Section 2.4). In this section, we discuss several aspects of chemical equilibria; in the next section, we examine energy changes during reactions and their relationship to equilibria.



▲ FIGURE 2-22 Time dependence of the rates of a chemical reaction. The forward and reverse rates of a reaction depend in part on the initial concentrations of reactants and products. The net forward reaction rate slows as the concentration of reactants decreases, whereas the net reverse reaction rate increases as the concentration of products increases. At equilibrium, the rates of the forward and reverse reactions are equal and the concentrations of reactants and products remain constant.

Equilibrium Constants Reflect the Extent of a Chemical Reaction

The equilibrium constant K_{eq} depends on the nature of the reactants and products, the temperature, and the pressure (particularly in reactions involving gases). Under standard physical conditions (25 °C and 1 atm pressure for biological systems), the K_{eq} is always the same for a given reaction, whether or not a catalyst is present.

For the general reaction with three reactants and three products

$$aA + bB + cC \rightleftharpoons zZ + yY + xX \qquad (2\text{-}1)$$

where capital letters represent particular molecules or atoms and lowercase letters represent the number of each in the reaction formula, the equilibrium constant is given by

$$K_{eq} = \frac{[X]^x[Y]^y[Z]^z}{[A]^a[B]^b[C]^c} \qquad (2\text{-}2)$$

where brackets denote the concentrations of the molecules at equilibrium. The rate of the forward reaction (left to right in Equation (2-1) is

$$\text{Rate}_{\text{forward}} = k_f[A]^a[B]^b[C]^c$$

where k_f is the **rate constant** for the forward reaction. Similarly, the rate of the reverse reaction (right to left in Equation 2-1) is

$$\text{Rate}_{\text{reverse}} = k_r[X]^x[Y]^y[Z]^z$$

where k_r is the rate constant for the reverse reaction. At equilibrium the forward and reverse rates are equal, so $\text{Rate}_{\text{forward}}/\text{Rate}_{\text{reverse}} = 1$. By rearranging these equations, we can express the equilibrium constant as the ratio of the rate constants

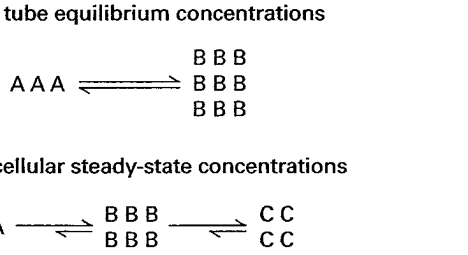
$$K_{eq} = \frac{k_f}{k_r} \qquad (2\text{-}3)$$

Chemical Reactions in Cells Are at Steady State

Under appropriate conditions and given sufficient time, individual biochemical reactions carried out in a test tube eventually will reach equilibrium. Within cells, however, many reactions are linked in pathways in which a product of one reaction serves as a reactant in another or is pumped out of the cell. In this more complex situation, when the rate of formation of a substance is equal to the rate of its consumption, the concentration of the substance remains constant, and the system of linked reactions for producing and consuming that substance is said to be in a **steady state** (Figure 2-23). One consequence of such linked reactions is that they prevent the accumulation of excess intermediates, protecting cells from the harmful effects of intermediates that have the potential of being toxic at high concentrations.

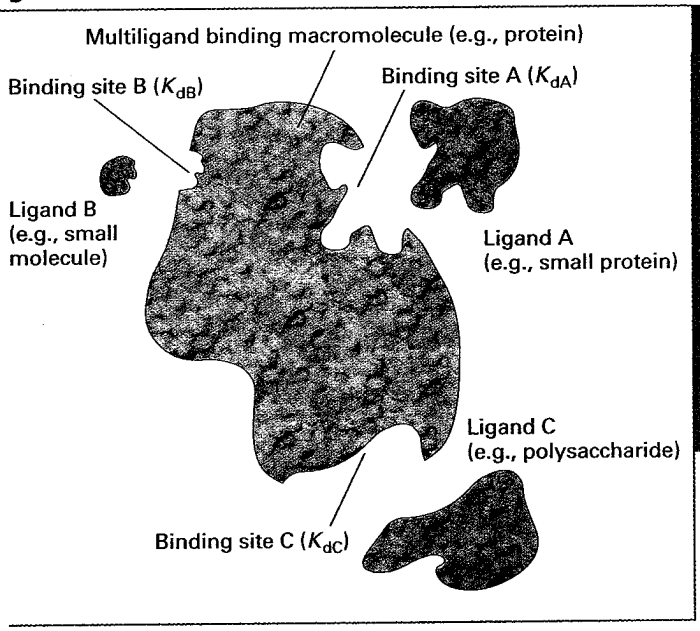
Dissociation Constants of Binding Reactions Reflect the Affinity of Interacting Molecules

The concept of equilibrium also applies to the binding of one molecule to another. Many important cellular processes depend on such binding "reactions," which involve the making and breaking of various noncovalent interactions rather than covalent bonds, as discussed above. A common example is the binding of a **ligand** (e.g., the hormone insulin or adrenaline) to its **receptor** on the surface of a cell forming a multimolecular assembly, or complex, that triggers a biological response. Another example is the binding of a protein to a specific sequence of base pairs in a molecule of DNA, which frequently causes the expression of a nearby gene to increase or decrease (Chapter 7). If the equilibrium constant for a binding reaction is



▲ FIGURE 2-23 Comparison of reactions at equilibrium and steady state. (a) In the test tube, a biochemical reaction (A → B) eventually will reach equilibrium, in which the rates of the forward and reverse reactions are equal (as indicated by the reaction arrows of equal length). (b) In metabolic pathways within cells, the product B commonly would be consumed, in this example by conversion to C. A pathway of linked reactions is at steady state when the rate of formation of the intermediates (e.g., B) equals their rate of consumption. As indicated by the unequal length of the arrows, the individual reversible reactions constituting a metabolic pathway do not reach equilibrium. Moreover, the concentrations of the intermediates at steady state can differ from what they would be at equilibrium.

Podcast: Macromolecules Can Bind Multiple Ligands



▶ **FIGURE 2-24 Macromolecules can have distinct binding sites for multiple ligands.** A large macromolecule (e.g., a protein, blue) with three distinct binding sites (A–C) is shown; each binding site exhibits molecular complementarity to three different binding partners (ligands A–C) with distinct dissociation constants (K_{dA-C}).

known, the intracellular stability of the resulting complex can be predicted. To illustrate the general approach for determining the concentration of noncovalently associated complexes, we will calculate the extent to which a protein (P) is bound to DNA (D) forming a protein-DNA complex (PD):

$$P + D \rightleftharpoons PD$$

Most commonly, binding reactions are described in terms of the **dissociation constant** K_d, which is the reciprocal of the equilibrium constant. For this binding reaction, the dissociation constant is given by

$$K_d = \frac{[P][D]}{[PD]} \qquad (2\text{-}4)$$

Typical reactions in which a protein binds to a specific DNA sequence have a K_d of 10^{-10} M, where M symbolizes molarity, or moles per liter (mol/L). To relate the magnitude of this dissociation constant to the intracellular ratio of bound to unbound DNA, let's consider the simple example of a bacterial cell having a volume of 1.5×10^{-15} L and containing 1 molecule of DNA and 10 molecules of the DNA-binding protein P. In this case, given a K_d of 10^{-10} M, 99 percent of the time this specific DNA sequence will have a molecule of protein bound to it and 1 percent of the time it will not, even though the cell contains only 10 molecules of the protein! Clearly, P and D bind very tightly (have a high affinity), as reflected by the low value of the dissociation constant for their binding reaction. For protein-protein and protein-DNA binding, K_d values of $\leq 10^{-9}$ M (nanomolar) are considered to be tight, ~10^{-6} M (micromolar) modestly tight, and ~10^{-3} M (millimolar) relatively weak.

The large size of biological macromolecules, such as proteins, can result in the availability of multiple surfaces for complementary intermolecular interactions (Figure 2-24). As a consequence, many macromolecules have the capacity to bind several other molecules simultaneously. In some cases, these binding reactions are independent, with their own distinct K_d values that are constant. In other cases, binding of a molecule at one site on a macromolecule can change the three-dimensional shape of a distant site, thus altering the binding interactions of that distant site with some other molecule. This is an important mechanism by which one molecule can alter (regulate) the activity of a second molecule (e.g., a protein) by changing its capacity to interact with a third molecule. We examine this regulatory mechanism in more detail in Chapter 3.

Biological Fluids Have Characteristic pH Values

The solvent inside cells and in all extracellular fluids is water. An important characteristic of any aqueous solution is the concentration of positively charged hydrogen ions (H^+) and negatively charged hydroxyl ions (OH^-). Because these ions are the dissociation products of H_2O, they are constituents of all living systems, and they are liberated by many reactions that take place between organic molecules within cells. These ions also can be transported into or out of cells, as when highly acidic gastric juice is secreted by cells lining the walls of the stomach.

When a water molecule dissociates, one of its polar H—O bonds breaks. The resulting hydrogen ion, often referred to as a **proton**, has a short lifetime as a free ion and quickly combines with a water molecule to form a hydronium ion (H_3O^+). For convenience, however, we refer to the concentration of hydrogen ions in a solution, $[H^+]$, even though this really represents the concentration of hydronium ions, $[H_3O^+]$. Dissociation of H_2O generates one OH^- ion along with each H^+. The dissociation of water is a reversible reaction:

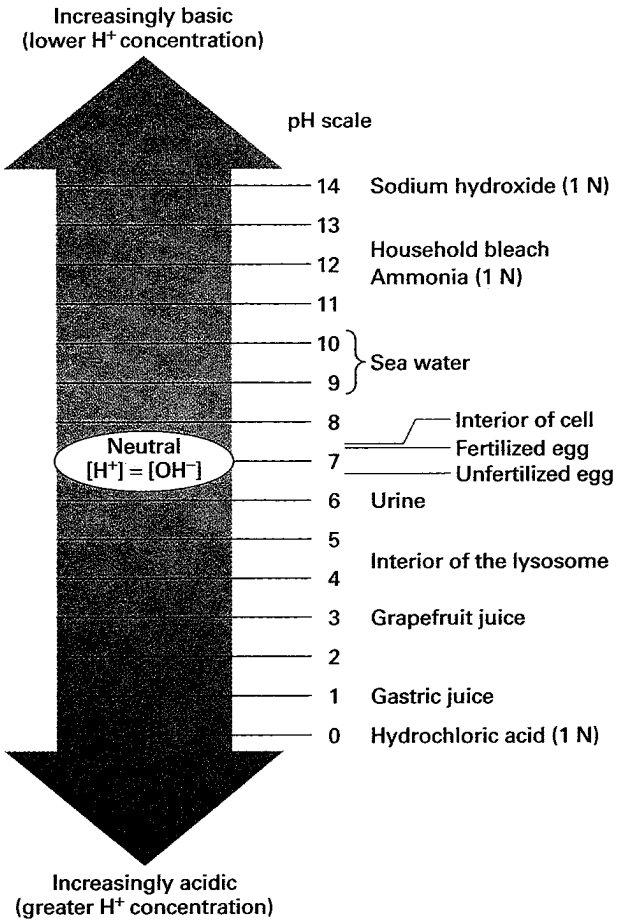
$$H_2O \rightleftharpoons H^+ + OH^-$$

At 25 °C, $[H^+][OH^-] = 10^{-14}$ M^2, so that in pure water, $[H^+] = [OH^-] = 10^{-7}$ M.

The concentration of hydrogen ions in a solution is expressed conventionally as its **pH**, defined as the negative log of the hydrogen ion concentration. The pH of pure water at 25 °C is 7:

$$pH = -\log[H^+] = \log\frac{1}{[H^+]} = \log\frac{1}{10^{-7}} = 7$$

It is important to keep in mind that a 1 unit difference in pH represents a tenfold difference in the concentration of protons. On the pH scale, 7.0 is considered neutral: pH values below 7.0 indicate acidic solutions (higher $[H^+]$), and values above 7.0 indicate basic (alkaline) solutions (Figure 2-25). For instance, gastric juice, which is rich in hydrochloric acid



▲ **FIGURE 2-25 pH values of common solutions.** The pH of an aqueous solution is the negative log of the hydrogen ion concentration. The pH values for most intracellular and extracellular biological fluids are near 7 and are carefully regulated to permit the proper functioning of cells, organelles, and cellular secretions.

(HCl), has a pH of about 1. Its $[H^+]$ is roughly a millionfold greater than that of cytoplasm with a pH of about 7.2.

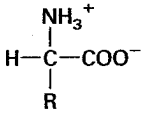
Although the cytosol of cells normally has a pH of about 7.2, the pH is much lower (about 4.5) in the interior of lysosomes, one type of organelle in eukaryotic cells (Chapter 9). The many degradative enzymes within lysosomes function optimally in an acidic environment, whereas their action is inhibited in the near neutral environment of the cytoplasm. This illustrates that maintenance of a specific pH is essential for proper functioning of some cellular structures. On the other hand, dramatic shifts in cellular pH may play an important role in controlling cellular activity. For example, the pH of the cytoplasm of an unfertilized egg of the sea urchin, an aquatic animal, is 6.6. Within 1 minute of fertilization, however, the pH rises to 7.2; that is, the $[H^+]$ decreases to about one-fourth its original value, a change necessary for subsequent growth and division of the egg.

Hydrogen Ions Are Released by Acids and Taken Up by Bases

In general, an **acid** is any molecule, ion, or chemical group that tends to release a hydrogen ion (H^+), such as hydrochloric acid (HCl) or the carboxyl group (—COOH), which tends to dissociate to form the negatively charged carboxylate ion ($—COO^-$). Likewise, a **base** is any molecule, ion, or chemical group that readily combines with a H^+, such as the hydroxyl ion (OH^-); ammonia (NH_3), which forms an ammonium ion (NH_4^+); or the amino group ($—NH_2$).

When acid is added to an aqueous solution, the $[H^+]$ increases (the pH goes down). Conversely, when a base is added to a solution, the $[H^+]$ decreases (the pH goes up). Because $[H^+][OH^-] = 10^{-14}M^2$, any increase in $[H^+]$ is coupled with a commensurate decrease in $[OH^-]$ and vice versa.

Many biological molecules contain both acidic and basic groups. For example, in neutral solutions (pH = 7.0), many amino acids exist predominantly in the doubly ionized form, in which the carboxyl group has lost a proton and the amino group has accepted one:



where R represents the uncharged side chain. Such a molecule, containing an equal number of positive and negative ions, is called a *zwitterion*. Zwitterions, having no net charge, are neutral. At extreme pH values, only one of these two ionizable groups of an amino acid will be charged.

The dissociation reaction for an acid (or acid group in a larger molecule) HA can be written as $HA \rightleftharpoons H^+ + A^-$. The equilibrium constant for this reaction, denoted K_a (the subscript *a* stands for "acid"), is defined as $K_a = [H^+][A^-]/[HA]$. Taking the logarithm of both sides and rearranging the result yields a very useful relation between the equilibrium constant and pH:

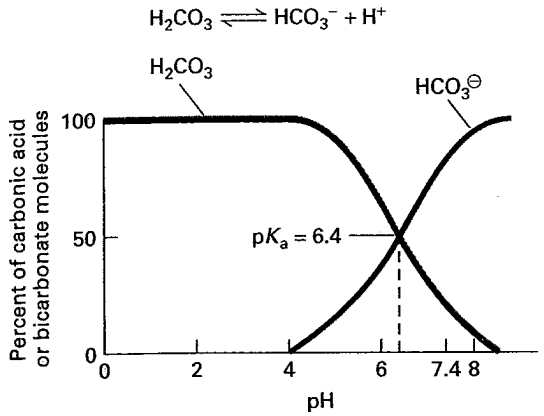
$$\text{pH} = \text{p}K_a + \log \frac{[A^-]}{[HA]} \qquad (2\text{-}5)$$

where $\text{p}K_a$ equals $-\log K_a$.

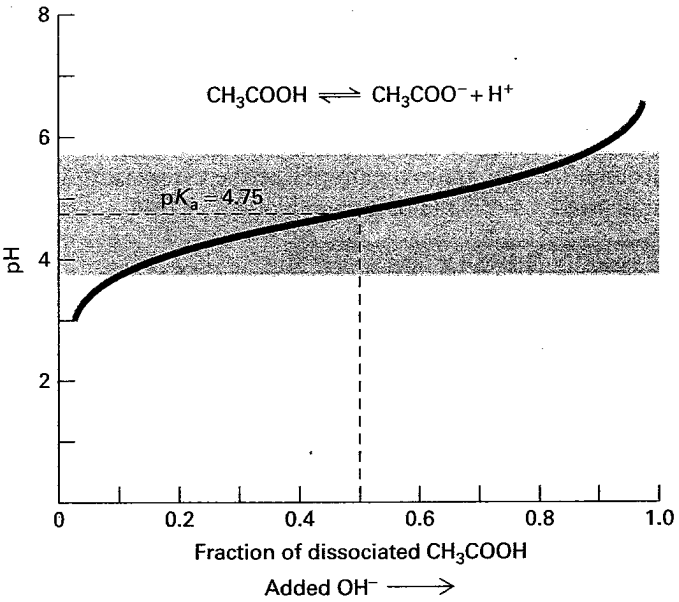
From this expression, commonly known as the *Henderson-Hasselbalch equation*, it can be seen that the $\text{p}K_a$ of any acid is equal to the pH at which half the molecules are dissociated and half are neutral (undissociated). This is because when $[A^-] = [HA]$, then $\log([A^-]/[HA]) = 0$, and thus $\text{p}K_a = \text{pH}$. The Henderson-Hasselbalch equation allows us to calculate the degree of dissociation of an acid if both the pH of the solution and the $\text{p}K_a$ of the acid are known. Experimentally, by measuring the $[A^-]$ and $[HA]$ as a function of the solution's pH, one can calculate the $\text{p}K_a$ of the acid and thus the equilibrium constant K_a for the dissociation reaction (Figure 2-26).

Buffers Maintain the pH of Intracellular and Extracellular Fluids

A growing cell must maintain a constant pH in the cytoplasm of about 7.2–7.4 despite the metabolic production of many acids, such as lactic acid and carbon dioxide; the latter reacts with water to form carbonic acid (H_2CO_3). Cells have a reservoir of weak bases and weak acids, called **buffers**, which ensure that the cell's pH remains relatively constant



▲ **FIGURE 2-26 The relationship between pH, p*K*a, and the dissociation of an acid.** As the pH of a solution of carbonic acid rises from 0 to 8.5, the percentage of the compound in the undissociated, or un-ionized, form (H_2CO_3) decreases from 100 percent and that of the ionized form increases from 0 percent. When the pH (6.4) is equal to the acid's p*K*a, half of the carbonic acid has ionized. When the pH rises to above 8, virtually all of the acid has ionized to the bicarbonate form (HCO_3^-).



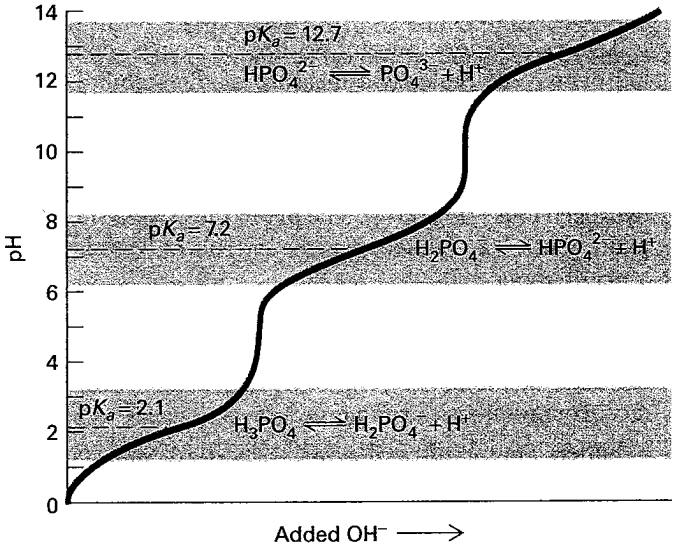
▲ **FIGURE 2-27 The titration curve of the buffer acetic acid (CH_3COOH).** The pK_a for the dissociation of acetic acid to hydrogen and acetate ions is 4.75. At this pH, half the acid molecules are dissociated. Because pH is measured on a logarithmic scale, the solution changes from 91 percent CH_3COOH at pH 3.75 to 9 percent CH_3COOH at pH 5.75. The acid has maximum buffering capacity in this pH range.

despite small fluctuations in the amounts of H^+ or OH^- being generated by metabolism or by the uptake or secretion of molecules and ions by the cell. Buffers do this by "soaking up" excess H^+ or OH^- when these ions are added to the cell or are produced by metabolism.

If additional acid (or base) is added to a buffered solution whose pH is equal to the pK_a of the buffer ($[HA] = [A^-]$), the pH of the solution changes, but it changes less than it would if the buffer had not been present. This is because protons released by the added acid are taken up by the ionized form of the buffer (A^-); likewise, hydroxyl ions generated by the addition of base are neutralized by protons released by the undissociated buffer (HA). The capacity of a substance to release hydrogen ions or take them up depends partly on the extent to which the substance has already taken up or released protons, which in turn depends on the pH of the solution relative to the pK_a of the substance. The ability of a buffer to minimize changes in pH, its *buffering capacity*, depends on the concentration of the buffer and the relationship between its pK_a value and the pH, which is expressed by the Henderson-Hasselbalch equation.

The titration curve for acetic acid shown in Figure 2-27 illustrates the effect of pH on the fraction of molecules in the un-ionized (HA) and ionized forms (A^-). At one pH unit below the pK_a of an acid, 91 percent of the molecules are in the HA form; at one pH unit above the pK_a, 91 percent are in the A^- form. At pH values more than one unit above or below the pK_a, the buffering capacity of weak acids and bases declines rapidly. In other words, the addition of the same number of moles of acid to a solution containing a mixture of HA and A^- that is at a pH near the pK_a will cause less of a pH change than it would if the HA and A^- were not present or if the pH were far from the pK_a value.

All biological systems contain one or more buffers. Phosphate ions, the ionized forms of phosphoric acid, are present in considerable quantities in cells and are an important factor in maintaining, or buffering, the pH of the cytoplasm. Phosphoric acid (H_3PO_4) has three protons that are capable of dissociating, but they do not dissociate simultaneously. Loss of each proton can be described by a discrete dissociation reaction and pK_a, as shown in Figure 2-28. The titration curve



▲ **FIGURE 2-28 The titration curve of phosphoric acid (H_3PO_4), a common buffer in biological systems.** This biologically ubiquitous molecule has three hydrogen atoms that dissociate at different pH values; thus phosphoric acid has three pK_a values, as noted on the graph. The shaded areas denote the pH ranges—within one pH unit of the three pK_a values—where the buffering capacity of phosphoric acid is high. In these regions, the addition of acid (or base) will cause relatively small changes in the pH.

for phosphoric acid shows that the pK_a for the dissociation of the second proton is 7.2. Thus at pH 7.2, about 50 percent of cellular phosphate is $H_2PO_4^-$ and about 50 percent is HPO_4^{2-} according to the Henderson-Hasselbalch equation. For this reason, phosphate is an excellent buffer at pH values around 7.2, the approximate pH of the cytoplasm of cells, and at pH 7.4, the pH of human blood.

KEY CONCEPTS OF SECTION 2.3

Chemical Equilibrium

- A chemical reaction is at equilibrium when the rate of the forward reaction is equal to the rate of the reverse reaction (no net change in the concentration of the reactants or products).
- The equilibrium constant K_{eq} of a reaction reflects the ratio of products to reactants at equilibrium and thus is a measure of the extent of the reaction and the relative stabilities of the reactants and products.
- The K_{eq} depends on the temperature, pressure, and chemical properties of the reactants and products but is independent of the reaction rate and of the initial concentrations of reactants and products.
- For any reaction, the equilibrium constant K_{eq} equals the ratio of the forward rate constant to the reverse rate constant (k_f/k_r). The rates of conversion of reactants to products and vice versa depend on the rate constants and the concentrations of the reactants or products.
- Within cells, the linked reactions in metabolic pathways generally are at steady state, not equilibrium, at which rate of formation of the intermediates equals their rate of consumption (see Figure 2-23) and thus the concentrations of the intermediates are not changing.
- The dissociation constant K_d for the noncovalent binding of two molecules is a measure of the stability of the complex formed between the molecules (e.g., ligand-receptor or protein-DNA complexes).
- The pH is the negative logarithm of the concentration of hydrogen ions (–log [H+]). The pH of the cytoplasm is normally about 7.2–7.4, whereas the interior of lysosomes has a pH of about 4.5.
- Acids release protons (H^+) and bases bind them. In biological molecules, the carboxyl and phosphate groups are the most common acidic groups; the amino group is the most common basic group.
- Buffers are mixtures of a weak acid (HA) and its corresponding base form (A^-), which minimize the change in pH of a solution when acid or alkali is added. Biological systems use various buffers to maintain their pH within a very narrow range.

2.4 Biochemical Energetics

The production of energy, its storage, and its use are central to the economy of the cell. Energy may be defined as the ability to do work, a concept applicable to automobile engines and electric power plants in our day-to-day world and to cellular engines in the biological world. The energy associated with chemical bonds can be harnessed to support chemical work and the physical movements of cells.

Several Forms of Energy Are Important in Biological Systems

There are two principal forms of energy: kinetic and potential. **Kinetic energy** is the energy of movement—the motion of molecules, for example. The second form of energy, **potential energy,** or stored energy, is particularly important in the study of biological or chemical systems.

Thermal energy, or heat, is a form of kinetic energy—the energy of the motion of molecules. For heat to do work, it must flow from a region of higher temperature—where the average speed of molecular motion is greater—to one of lower temperature. Although differences in temperature can exist between the internal and external environments of cells, these thermal gradients do not usually serve as the source of energy for cellular activities. The thermal energy in warm-blooded animals, which have evolved a mechanism for thermoregulation, is used chiefly to maintain constant organismic temperatures. This is an important function because the rates of many cellular activities are temperature-dependent. For example, cooling mammalian cells from their normal body temperature of 37 °C to 4 °C can virtually "freeze" or stop many cellular processes (e.g., intracellular membrane movements).

Radiant energy is the kinetic energy of photons, or waves of light, and is critical to biology. Radiant energy can be converted to thermal energy, for instance when light is absorbed by molecules and the energy is converted to molecular motion. Radiant energy absorbed by molecules can also change the electronic structure of the molecules, moving electrons into higher-energy states (orbitals), whence it can later be recovered to perform work. For example, during photosynthesis, light energy absorbed by specialized molecules (e.g., chlorophyll) is subsequently converted into the energy of chemical bonds (Chapter 12).

Mechanical energy, a major form of kinetic energy in biology, usually results from the conversion of stored chemical energy. For example, changes in the lengths of cytoskeletal filaments generate forces that push or pull on membranes and organelles (Chapters 17 and 18).

Electric energy—the energy of moving electrons or other charged particles—is yet another major form of kinetic energy.

Several forms of potential energy are biologically significant. Central to biology is **chemical potential energy,** the energy stored in the bonds connecting atoms in molecules. Indeed, most of the biochemical reactions described in this book involve the making or breaking of at least one covalent chemical bond. We recognize this energy when chemicals undergo energy-releasing reactions. For example, the high potential energy in the covalent bonds of glucose can be released by controlled enzymatic combustion in cells (Chapter 12). This energy is harnessed by the cell to do many kinds of work.

A second biologically important form of potential energy is the energy in a **concentration gradient.** When the concentration

of a substance on one side of a barrier, such as a membrane, is different from that on the other side, a concentration gradient exists. All cells form concentration gradients between their interior and the external fluids by selectively exchanging nutrients, waste products, and ions with their surroundings. Also, organelles within cells (e.g., mitochondria, lysosomes) frequently contain different concentrations of ions and other molecules; the concentration of protons within a lysosome, as we saw in the last section, is about 500 times that of the cytoplasm.

A third form of potential energy in cells is an **electric potential**—the energy of charge separation. For instance, there is a gradient of electric charge of ≈200,000 volts per cm across the plasma membrane of virtually all cells. We discuss how concentration gradients and the potential difference across cell membranes are generated and maintained in Chapter 11 and how they are converted to chemical potential energy in Chapter 12.

Cells Can Transform One Type of Energy into Another

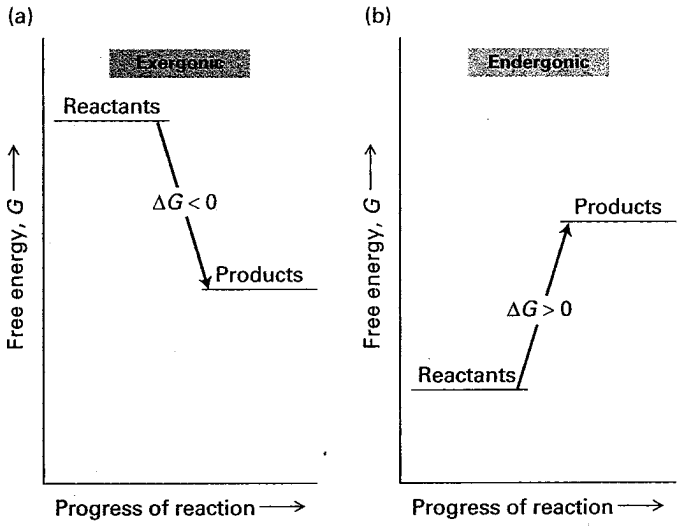
According to the first law of thermodynamics, energy is neither created nor destroyed but can be converted from one form to another. (In nuclear reactions, mass is converted to energy, but this is irrelevant to biological systems.) In photosynthesis, for example, the radiant energy of light is transformed into the chemical potential energy of the covalent bonds between the atoms in a sucrose or starch molecule. In muscles and nerves, chemical potential energy stored in covalent bonds is transformed, respectively, into the kinetic energy of muscle contraction and the electric energy of nerve transmission. In all cells, potential energy, released by breaking certain chemical bonds, is used to generate potential energy in the form of concentration and electric potential gradients. Similarly, energy stored in chemical concentration gradients or electric potential gradients is used to synthesize chemical bonds or to transport molecules from one side of a membrane to another to generate a concentration gradient. The latter process occurs during the transport of nutrients such as glucose into certain cells and transport of many waste products out of cells.

Because all forms of energy are interconvertible, they can be expressed in the same units of measurement. Although the standard unit of energy is the joule, biochemists have traditionally used an alternative unit, the calorie (1 joule = 0.239 calorie). Throughout this book, we use the kilocalorie to measure energy changes (1 kcal = 1000 cal).

The Change in Free Energy Determines the Direction of a Chemical Reaction

Because biological systems are generally held at constant temperature and pressure, it is possible to predict the direction of a chemical reaction from the change in the **free energy** G, named after J. W. Gibbs, who showed that "all systems change in such a way that free energy [G] is minimized." In the case of a chemical reaction, reactants $\rightleftharpoons$ products, the free-energy change ΔG is given by

$$\Delta G = G_{\text{products}} - G_{\text{reactants}}$$



▲ **FIGURE 2-29 Changes in the free energy (ΔG) of exergonic and endergonic reactions.** (a) In exergonic reactions, the free energy of the products is lower than that of the reactants. Consequently, these reactions occur spontaneously and energy is released as the reactions proceed. (b) In endergonic reactions, the free energy of the products is greater than that of the reactants and these reactions do not occur spontaneously. An external source of energy must be supplied if the reactants are to be converted into products.

The relation of ΔG to the direction of any chemical reaction can be summarized in three statements:

- If ΔG is negative, the forward reaction will tend to occur spontaneously and energy usually will be released as the reaction takes place (**exergonic reaction**) (Figure 2-29).
- If ΔG is positive, the forward reaction will not occur spontaneously: energy will have to be added to the system in order to force the reactants to become products (**endergonic reaction**).
- If ΔG is zero, both forward and reverse reactions occur at equal rates and there will be no spontaneous conversion of reactants to products (or vice versa); the system is at equilibrium.

By convention, the standard free-energy change of a reaction $\Delta G^{\circ\prime}$ is the value of the change in free energy under the conditions of 298 K (25 °C), 1 atm pressure, pH 7.0 (as in pure water), and initial concentrations of 1 M for all reactants and products except protons, which are kept at 10^{-7} M (pH 7.0). Most biological reactions differ from standard conditions, particularly in the concentrations of reactants, which are normally less than 1 M.

The free energy of a chemical system can be defined as $G = H - TS$, where H is the bond energy, or **enthalpy,** of the system; T is its temperature in degrees Kelvin (K); and S is the **entropy,** a measure of its randomness or disorder. If temperature remains constant, a reaction proceeds spontaneously only if the free-energy change ΔG in the following equation is negative:

$$\Delta G = \Delta H - T\,\Delta S \qquad (2\text{-}6)$$

In an **exothermic** reaction, the products contain less bond energy than the reactants, the liberated energy is usually converted to heat (the energy of molecular motion), and ΔH is negative. In an **endothermic** reaction, the products contain more bond energy than the reactants, heat is absorbed during the reaction, and ΔH is positive. The combined effects of the changes in the enthalpy and entropy determine if the ΔG for a reaction is positive or negative. An exothermic reaction ($\Delta H < 0$) in which entropy increases ($\Delta S > 0$) occurs spontaneously ($\Delta G < 0$). An endothermic reaction ($\Delta H > 0$) will occur spontaneously if ΔS increases enough so that the $T\,\Delta S$ term can overcome the positive ΔH.

Many biological reactions lead to an increase in order and thus a decrease in entropy ($\Delta S < 0$). An obvious example is the reaction that links amino acids to form a protein. A solution of protein molecules has a lower entropy than does a solution of the same amino acids unlinked because the free movement of any amino acid in a protein is restricted when it is bound into a long chain. Often cells compensate for decreases in entropy by "coupling" such synthetic, entropy-lowering reactions with independent reactions that have a very highly negative ΔG (see below). In this way cells can convert sources of energy in their environment into the building of highly organized structures and metabolic pathways that are essential for life.

The actual change in free energy ΔG during a reaction is influenced by temperature, pressure, and the initial concentrations of reactants and products and usually differs from $\Delta G^{\circ\prime}$. Most biological reactions—like others that take place in aqueous solutions—also are affected by the pH of the solution. We can estimate free-energy changes for different temperatures and initial concentrations using the equation

$$\Delta G = \Delta G^{\circ\prime} + RT \ln Q = \Delta G^{\circ\prime} + RT \ln \frac{[\text{products}]}{[\text{reactants}]} \quad (2\text{-}7)$$

where R is the gas constant of 1.987 cal/(degree·mol), T is the temperature (in degrees Kelvin), and Q is the *initial* ratio of products to reactants. For a reaction A + B $\rightleftharpoons$ C, in which two molecules combine to form a third, Q in Equation 2-7 equals [C]/[A][B]. In this case, an increase in the initial concentration of either [A] or [B] will result in a larger negative value for ΔG and thus drive the reaction toward more formation of C.

Regardless of the $\Delta G^{\circ\prime}$ for a particular biochemical reaction, it will proceed spontaneously within cells only if ΔG is negative, given the intracellular concentrations of reactants and products. For example, the conversion of glyceraldehyde 3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP), two intermediates in the breakdown of glucose,

$$\text{G3P} \rightleftharpoons \text{DHAP}$$

has a $\Delta G^{\circ\prime}$ of −1840 cal/mol. If the initial concentrations of G3P and DHAP are equal, then $\Delta G = \Delta G^{\circ\prime}$ because $RT \ln 1 = 0$; in this situation, the reversible reaction G3P $\rightleftharpoons$ DHAP will proceed spontaneously in the direction of DHAP formation until equilibrium is reached. However, if the initial [DHAP] is 0.1 M and the initial [G3P] is 0.001 M, with other conditions standard, then Q in Equation 2-7 equals 0.1/0.001 = 100, giving a ΔG of +887 cal/mol. Under these conditions, the reaction will proceed in the direction of formation of G3P.

The ΔG for a reaction is independent of the reaction rate. Indeed, under usual physiological conditions, few if any of the biochemical reactions needed to sustain life would occur without some mechanism for increasing reaction rates. As we describe below and in more detail in Chapter 3, the rates of reactions in biological systems are usually determined by the activity of **enzymes**, the protein catalysts that accelerate the formation of products from reactants without altering the value of ΔG.

The $\Delta G^{\circ\prime}$ of a Reaction Can Be Calculated from Its K_{eq}

A chemical mixture at equilibrium is in a stable state of minimal free energy. For a system at equilibrium ($\Delta G = 0$, $Q = K_{eq}$), we can write

$$\Delta G^{\circ\prime} = -2.3RT \log K_{eq} = -1362 \log K_{eq} \quad (2\text{-}8)$$

under standard conditions (note the change to base 10 logarithms). Thus if we determine the concentrations of reactants and products at equilibrium (i.e., the K_{eq}), we can calculate the value of $\Delta G^{\circ\prime}$. For example, the K_{eq} for the interconversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate (G3P $\rightleftharpoons$ DHAP) is 22.2 under standard conditions. Substituting this value into Equation 2-8, we can easily calculate the $\Delta G^{\circ\prime}$ for this reaction as −1840 cal/mol.

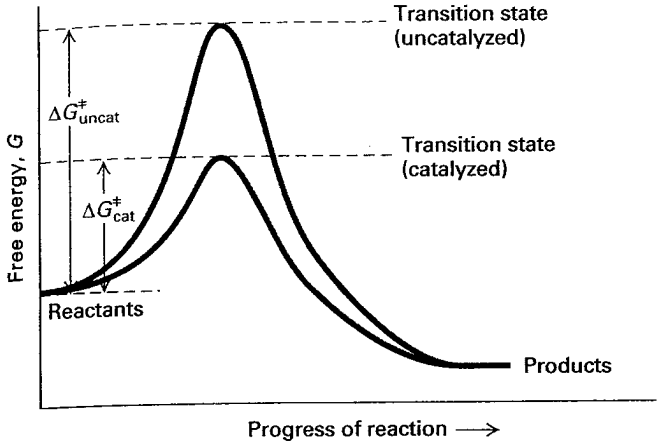
By rearranging Equation 2-8 and taking the antilogarithm, we obtain

$$K_{eq} = 10^{-(\Delta G^{\circ\prime}/2.3RT)} \quad (2\text{-}9)$$

From this expression, it is clear that if $\Delta G^{\circ\prime}$ is negative, the exponent will be positive and hence K_{eq} will be greater than 1. Therefore at equilibrium there will be more products than reactants; in other words, the formation of products from reactants is favored. Conversely, if $\Delta G^{\circ\prime}$ is positive, the exponent will be negative and K_{eq} will be less than 1.

The Rate of a Reaction Depends on the Activation Energy Necessary to Energize the Reactants into a Transition State

As a chemical reaction proceeds, reactants approach each other; some bonds begin to form while others begin to break. One way to think of the state of the molecules during this transition is that there are strains in the electronic configurations of the atoms and their bonds. In order for the collection of atoms to move from the relatively stable state of the reactants to this intermediate state during the reaction, an introduction of energy is necessary. This is illustrated in the reaction energy diagram in Figure 2-30. Thus the collection of atoms is transiently in a higher-energy state at some point during the course of the reaction. The state during a chemical reaction at which the system is at its highest energy level is called the **transition state** or **transition-**



▲ **FIGURE 2-30 Activation energy of uncatalyzed and catalyzed chemical reactions.** This hypothetical reaction pathway (blue) depicts the changes in free energy *G* as a reaction proceeds. A reaction will take place spontaneously if the free energy (*G*) of the products is less than that of the reactants ($\Delta G < 0$). However, all chemical reactions proceed through one (shown here) or more high-energy transition states, and the rate of a reaction is inversely proportional to the activation energy ($\Delta G^{\ddagger}$), which is the difference in free energy between the reactants and the transition state. In a catalyzed reaction (red), the free energies of the reactants and products are unchanged but the free energy of the transition state is lowered, thus increasing the velocity of the reaction.

state intermediate. The energy needed to excite the reactants to this higher-energy state is called the **activation energy** of the reaction. The activation energy is usually represented by $\Delta G^{\ddagger}$, analogous to the representation of the change in Gibbs free energy (ΔG) already discussed. From the transition state, the collection of atoms can either release energy as the reaction products are formed or release energy as the atoms go "backward" and re-form the original reactants. The velocity (V) at which products are generated from reactants during the reaction under a given set of conditions (temperature, pressure, reactant concentrations) will depend on the concentration of material in the transition state, which in turn will depend on the activation energy and the characteristic rate constant (ν) at which the transition state is converted to products. The higher the activation energy, the lower the fraction of reactants that reach the transition state and the slower the overall rate of the reaction. The relationship between the concentration of reactants, ν, and V is

$$V = \nu\,[\text{reactants}] \times 10^{-(\Delta G^{\ddagger}/2.3RT)}$$

From this equation, we can see that lowering the activation energy—that is, decreasing the free energy of the transition state $\Delta G^{\ddagger}$—leads to an acceleration of the overall reaction rate V. A reduction in $\Delta G^{\ddagger}$ of 1.36 kcal/mol leads to a tenfold increase in the rate of the reaction, whereas a 2.72 kcal/mol reduction increases the rate 100-fold. Thus relatively small changes in $\Delta G^{\ddagger}$ can lead to large changes in the overall rate of the reaction.

Catalysts such as enzymes (Chapter 3) accelerate reaction rates by lowering the relative energy of the transition state and so the activation energy (see Figure 2-30). The relative energies of reactants and products will determine if a reaction is thermodynamically favorable (negative ΔG), whereas the activation energy will determine how rapidly products form (reaction kinetics). Thermodynamically favorable reactions will not occur if the activation energies are too high.

Life Depends on the Coupling of Unfavorable Chemical Reactions with Energetically Favorable Reactions

Many processes in cells are energetically unfavorable ($\Delta G > 0$) and will not proceed spontaneously. Examples include the synthesis of DNA from nucleotides and transport of a substance across the plasma membrane from a lower to a higher concentration. Cells can carry out an energy-requiring, or endergonic, reaction ($\Delta G_1 > 0$) by coupling it to an energy-releasing, or exergonic, reaction ($\Delta G_2 < 0$) if the sum of the two reactions has an overall net negative ΔG.

Suppose, for example, that the reaction A $\rightleftharpoons$ B + X has a ΔG of +5 kcal/mol and that the reaction X $\rightleftharpoons$ Y + Z has a ΔG of −10 kcal/mol:

$$\begin{array}{lll} (1)\ \ A \rightleftharpoons B + X & \Delta G = +5 \text{ kcal/mol} \\ (2)\ \ X \rightleftharpoons Y + Z & \Delta G = -10 \text{ kcal/mol} \\ \text{Sum:}\ \ A \rightleftharpoons B + Y + Z & \Delta G^{\circ\prime} = -5 \text{ kcal/mol} \end{array}$$

In the absence of the second reaction, there would be much more A than B at equilibrium. However, because the conversion of X to Y + Z is such a favorable reaction, it will pull the first process toward the formation of B and the consumption of A. Energetically unfavorable reactions in cells often are coupled to the energy-releasing hydrolysis of ATP, as we discuss next.

Hydrolysis of ATP Releases Substantial Free Energy and Drives Many Cellular Processes

In almost all organisms, **adenosine triphosphate**, or **ATP**, is the most important molecule for capturing, transiently storing, and subsequently transferring energy to perform work (e.g., biosynthesis, mechanical motion). The useful energy in an ATP molecule is contained in **phosphoanhydride bonds**, which are covalent bonds formed from the condensation of two molecules of phosphate by the loss of water: