substrate, including hydrogen bonds and hydrophobic and ionic interactions (Chapter 4). Formation of each weak interaction in the ES complex is accompanied by release of a small amount of free energy that stabilizes the interaction. The energy derived from enzyme-substrate interaction is called **binding energy**, ΔG_B. Its significance extends beyond a simple stabilization of the enzyme-substrate interaction. *Binding energy is a major source of free energy used by enzymes to lower the activation energies of reactions.*

Two fundamental and interrelated principles provide a general explanation for how enzymes use noncovalent binding energy:

1. Much of the catalytic power of enzymes is ultimately derived from the free energy released in forming many weak bonds and interactions between an enzyme and its substrate. This binding energy contributes to specificity as well as to catalysis.

2. Weak interactions are optimized in the reaction transition state; enzyme active sites are complementary not to the substrates per se but to the transition states through which substrates pass as they are converted to products during an enzymatic reaction.

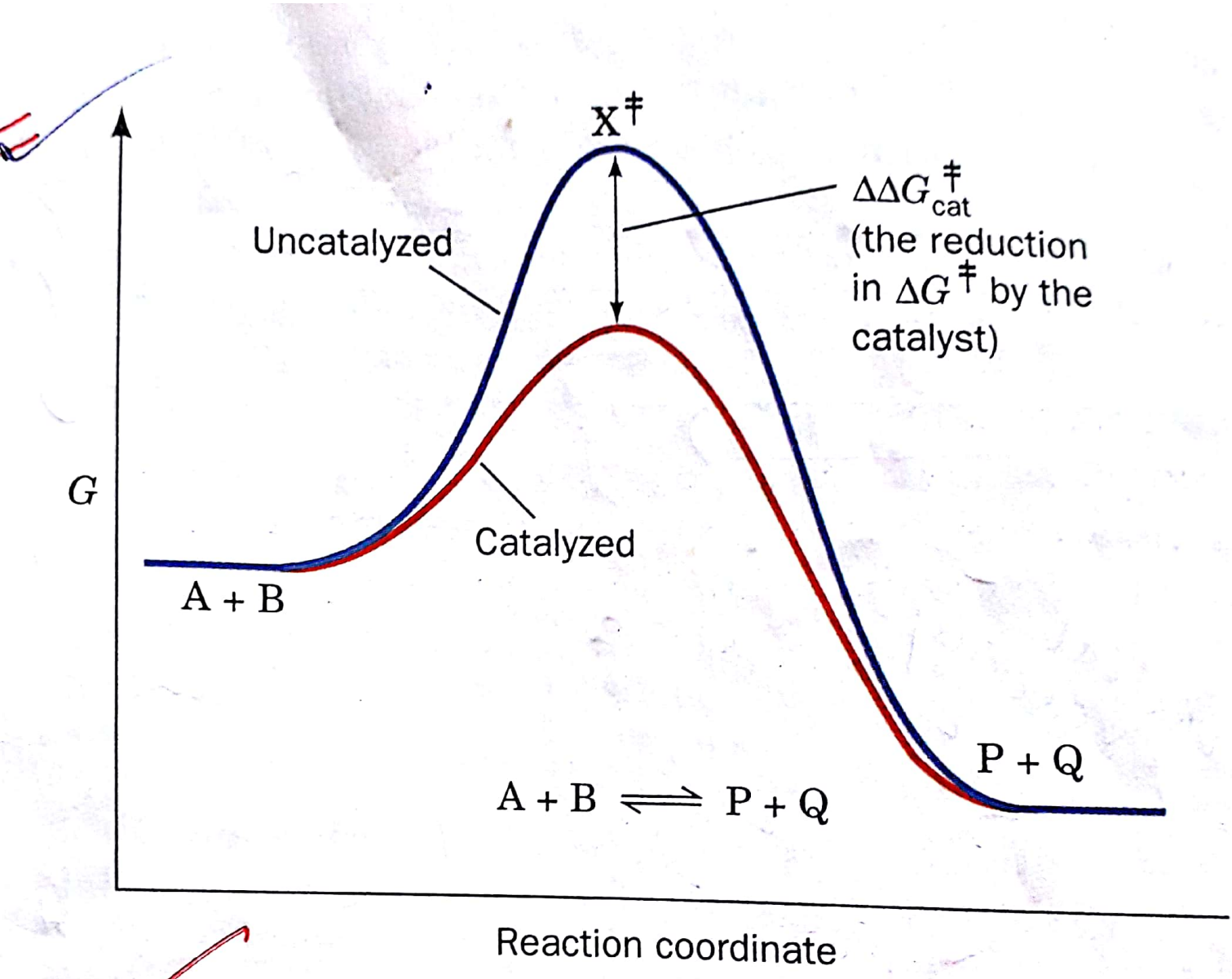
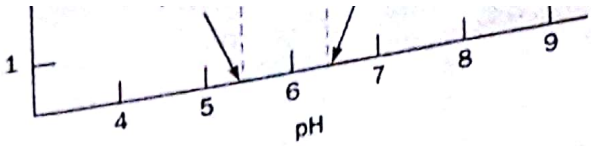
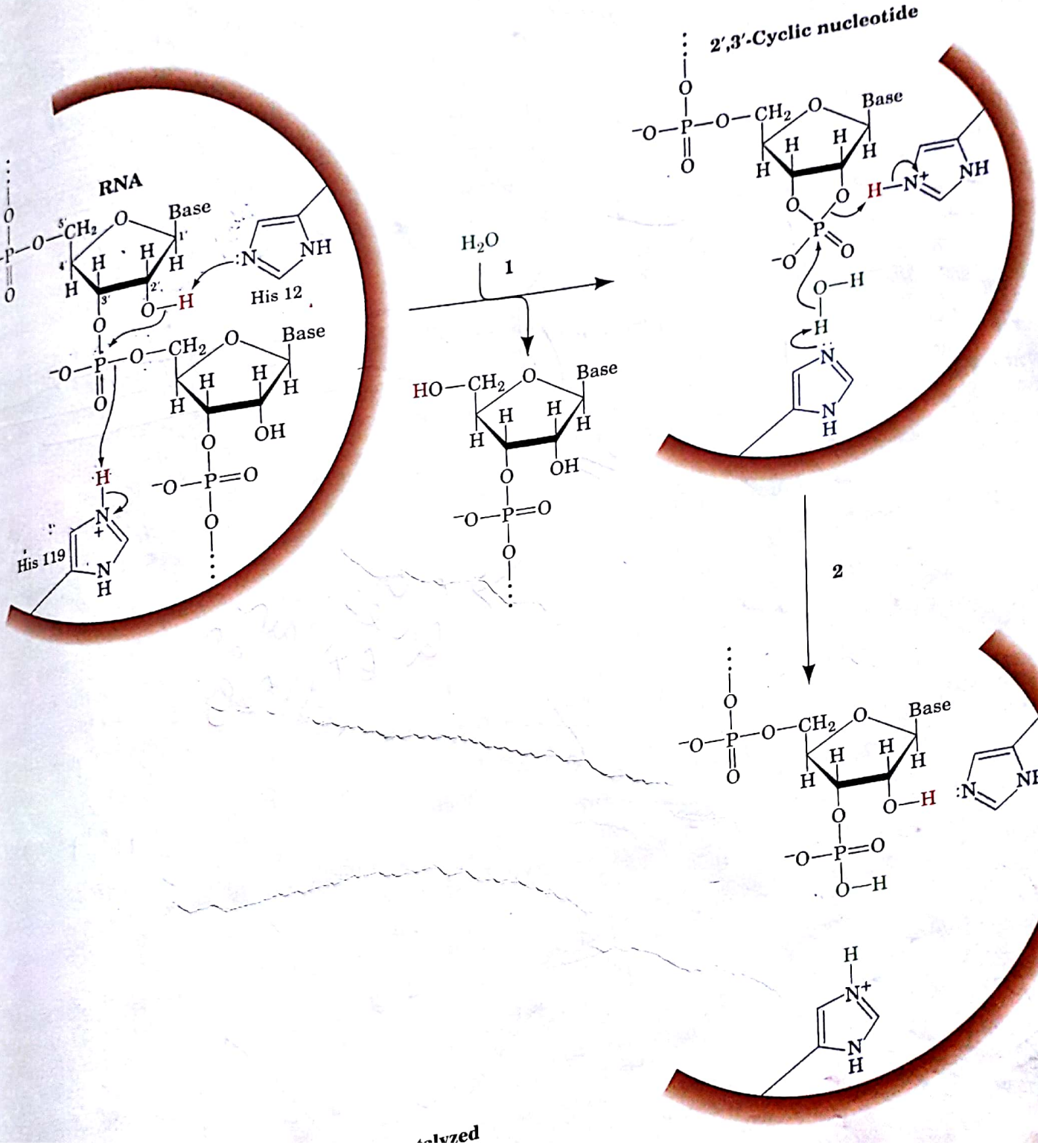


FIGURE 14-6 The effect of a catalyst on the transition state diagram of a reaction. Here $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{\text{uncat}} - \Delta G^{\ddagger}_{\text{cat}}$.

ACID-BASE CATALYSIS





Catalytic Power Is Defined as the Ratio of the Enzyme-Catalyzed Rate of a Reaction to the Uncatalyzed Rate

Enzymes display enormous catalytic power, accelerating reaction rates as much as 10^{21} over uncatalyzed levels, which is far greater than any synthetic catalysts can achieve, and enzymes accomplish these astounding feats in dilute aqueous solutions under mild conditions of temperature and pH. For example, the enzyme jack bean *urease* catalyzes the hydrolysis of urea:

$$H_2N{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}NH_2 + 2\,H_2O + H^+ \longrightarrow 2\,NH_4^+ + HCO_3^-$$

At 20°C, the rate constant for the enzyme-catalyzed reaction is 3×10^4/sec; the rate constant for the uncatalyzed hydrolysis of urea is 3×10^{-10}/sec. Thus, 10^{14} is the ratio of the catalyzed rate to the uncatalyzed rate of reaction. Such a ratio is defined as the relative **catalytic power** of an enzyme, so the catalytic power of urease is 10^{14}.

Enzymes Affect Reaction Rates, Not Equilibria

A simple enzymatic reaction might be written

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P \qquad (8\text{–}1)$$

where E, S, and P represent the enzyme, substrate, and product. ES and EP are transient complexes of the enzyme with the substrate and with the product.

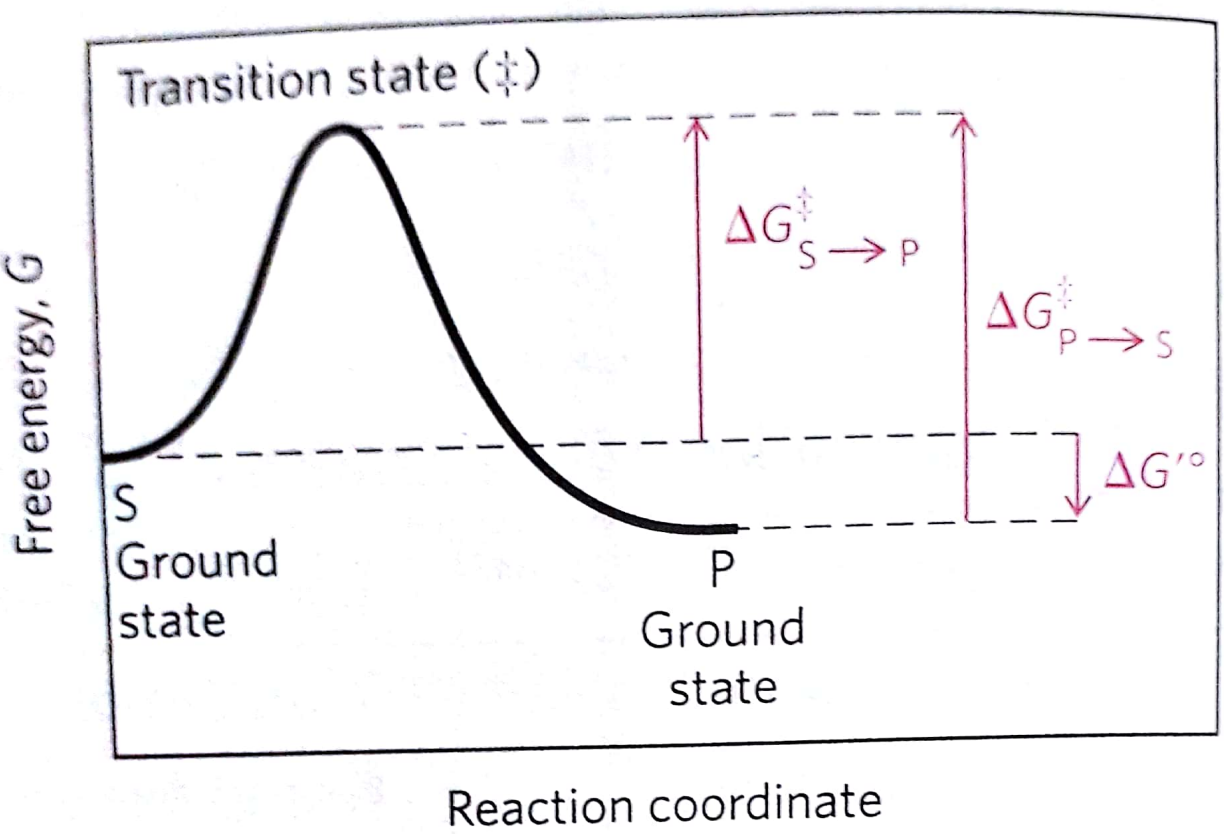
To understand catalysis, we must first appreciate the important distinction between reaction equilibria (discussed in Chapter 4) and reaction rates. The function of a catalyst is to increase the *rate* of a reaction. Catalysts do not affect reaction *equilibria*. Any reaction, such as $S \rightleftharpoons P$, can be described by a reaction coordinate diagram (Fig. 8–2), a picture of the energy changes during the reaction. As we noted in Chapters 1 and 3, energy in biological systems is described in terms of free energy, *G*. In the coordinate diagram, the free energy of the system is plotted against the progress of the reaction (reaction coordinate). The starting point for either the forward or the reverse reaction is called the **ground state,** the contribution to the free energy of the system by an average molecule (S or P) under a given set of conditions. To describe the free-energy changes for reactions, chemists define a standard set of conditions (temperature 298 K; partial pressure of each gas 1 atm or 101.3 kPa; concentration of each solute 1 M) and express the free-energy change for this reacting system as $\Delta G°$, the **standard free-energy change.** Because biochemical systems commonly involve H^+ concentrations far from 1 M, biochemists define a **biochemical standard free-energy change** $\Delta G'°$, the standard free-energy change *at pH 7.0,* which we will employ throughout the book. A more complete definition of $\Delta G'°$ is given in Chapter 14.

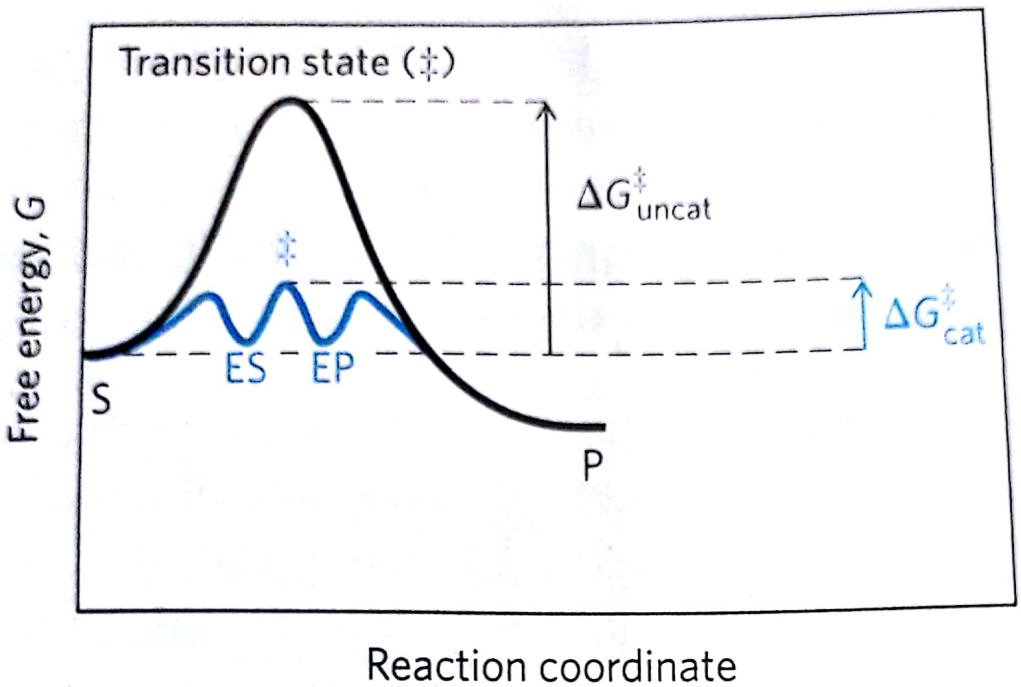
The equilibrium between S and P reflects the difference in the free energies of their ground states. In the example shown in Figure 8–2, the free energy of the ground state of P is lower than that of S, so $\Delta G'°$ for the reaction is negative and the equilibrium favors P. The position and direction of equilibrium are *not* affected by any catalyst.

A favorable equilibrium does not mean that the $S \rightarrow P$ conversion will occur at a detectable rate. The rate of a reaction is dependent on an entirely different parameter. There is an energy barrier between S and P, the energy required for alignment of reacting groups, formation of transient unstable charges, bond rearrangements, and other transformations required for the reaction to proceed in either direction. This is illustrated by the energy

"hill" in Figures 8–2 and 8–3. To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level. At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way). This is called the **transition state.** The transition state is not a chemical species with any significant stability and should not be confused with a reaction intermediate (such as ES or EP). It is simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decomposition to either substrate or product is equally likely. The difference between the energy levels of the ground state and the transition state is called the **activation energy** ($\Delta G^{\ddagger}$). The rate of a reaction reflects this activation energy; a higher activation energy corresponds to a slower reaction. Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst (Fig. 8–3). *Catalysts enhance reaction rates by lowering activation energies.*

Enzymes are no exception to the rule that catalysts do not affect





reac
inte
sev
det
vat
sim
po
pr
co
ha
al

time scale.

In practice, any reaction may have several steps involving the formation and decay of transient chemical species called **reaction intermediates.*** When the $S \rightleftharpoons P$ reaction is catalyzed by an enzyme, the ES and EP complexes are intermediates (Eqn 8–1); they occupy valleys in the reaction coordinate diagram (Fig. 8–3). When several steps occur in a reaction, the overall rate is determined by the step (or steps) with the highest activation

energy; this is called the **rate-limiting step.** In a simple case the rate-limiting step is the highest-energy point in the diagram for interconversion of S and P. In practice, the rate-limiting step can vary with reaction conditions, and for many enzymes several steps may have similar activation energies, which means they are all partially rate-limiting.

As described in Chapter 1, activation energies are energy barriers to chemical reactions; these barriers are crucial to life itself. The stability of a molecule increases with the height of its activation barrier. Without such energy barriers, complex macromolecules would revert spontaneously to much simpler molecular forms, and the complex and highly ordered structures and metabolic processes of cells could not exist. Enzymes have evolved to lower activation energies *selectively* for reactions that are needed for cell survival.

Reaction Rates and Equilibria Have Precise Thermodynamic Definitions

Reaction *equilibria* are inextricably linked to $\Delta G'^\circ$ and reaction *rates* are linked to $\Delta G^\ddagger$. A basic introduction to these thermodynamic relationships is the next step in understanding how enzymes work.

An equilibrium such as $S \rightleftharpoons P$ is described by an **equilibrium constant,** K_{eq} or simply K (Chapter 4). Under the standard conditions used to compare biochemical processes, an equilibrium constant is denoted K'_{eq} (or K'):

$$K'_{eq} = \frac{[P]}{[S]} \qquad (8\text{–}2)$$

From thermodynamics, the relationship between K'_{eq} and $\Delta G'^\circ$ can be described by the expression

$$\Delta G'^\circ = -RT \ln K'_{eq} \qquad (8\text{–}3)$$

where R is the gas constant, 8.315 J/mol · K, and T is the absolute temperature, 298 K (25 °C). Equation 8–3 is developed and discussed in more detail in Chapter 14. The important point here is that the equilibrium constant is directly related to the overall standard free-energy change for the reaction (Table 8–4). A large negative value for $\Delta G'^\circ$ reflects a favorable reaction equilibrium—but as already noted, this does not mean the reaction will proceed at a rapid rate.

Collision theory states that the rate of a chemical reaction is proportional to the number of collisions between reactant molecules. The more often reactant molecules collide, the more often they react with one another, and the faster the reaction rate. In reality, only a small fraction of the collisions are **effective collisions**. Effective collisions are those that result in a chemical reaction.

In order to produce an effective collision, reactant particles must possess some minimum amount of energy. This energy, used to initiate the reaction, is called the **activation energy**. For every sample of reactant particles there will be some that possess this amount of energy. The larger the sample, the greater the number of effective collisions, and the faster the rate of reaction. The number of particles possessing enough energy is dependent on the temperature of the reactants. If reactant particles do not possess the required activation energy when they collide, they bounce off each other without reacting.