enzymes exemplify this kind of control, which is called *proteolytic activation.* For example, trypsinogen is synthesized in the pancreas and is activated by peptide-bond cleavage in the small intestine to form the active enzyme trypsin (Figure 8-4). This type of control is also repeatedly used in the sequence of enzymatic reactions that lead to the clotting of blood. The enzymatically inactive precursors of proteolytic enzymes are called *zymogens.*

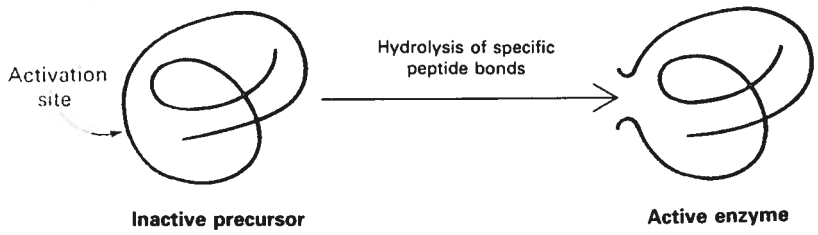


Figure 8-4
Activation of a zymogen by hydrolysis of specific peptide bonds.

ENZYMES TRANSFORM DIFFERENT FORMS OF ENERGY

In many biochemical reactions, *the energy of the reactants is converted with high efficiency into a different form.* For example, in photosynthesis, light energy is converted into chemical-bond energy. In mitochondria, the free energy contained in small molecules derived from food is converted into a different currency, the free energy of adenosine triphosphate (ATP). The chemical-bond energy of ATP is then utilized in many different ways. In muscle contraction, the energy of ATP is converted by myosin into mechanical energy. Membranes of cells and organelles contain pumps that utilize ATP to transport molecules and ions against chemical and electrical gradients. The molecular mechanisms of these energy-transducing enzymes are being unraveled. We will see in subsequent chapters how unidirectional cycles of discrete steps—binding, chemical transformation, and release—lead to the conversion of one form of energy into another.

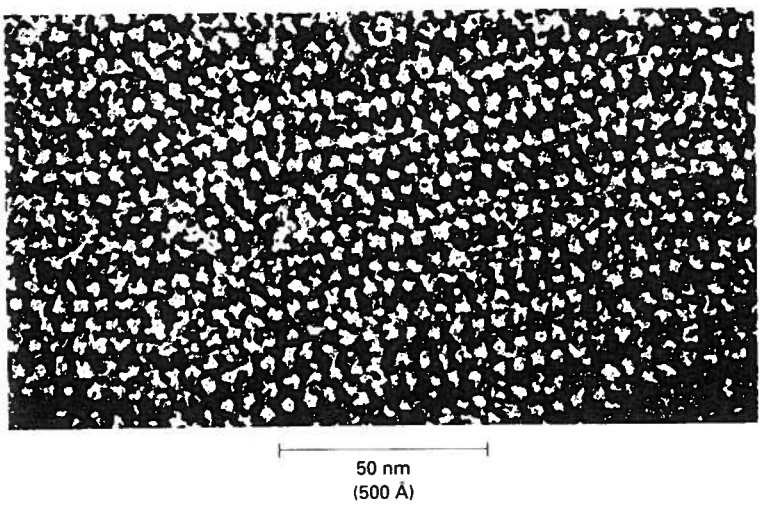


Figure 8-5
Electron micrograph of sodium-potassium pump molecules in a plasma membrane. These densely packed enzyme molecules catalyze the ATP-driven flux of Na^+ and K^+ out of and into cells. [Courtesy of Dr. Guido Zampighi.]

FREE ENERGY IS THE MOST USEFUL THERMODYNAMIC FUNCTION IN BIOCHEMISTRY

Let us review some key thermodynamic relations. In thermodynamics, a *system* is the matter within a defined region. The matter in the rest of the universe is called the *surroundings. The first law of thermodynamics states that the total energy of a system and its surroundings is a constant.* In other words, energy is conserved. The mathematical expression of the first law is

$$\Delta E = E_B - E_A = Q - W \qquad (1)$$

in which E_A is the energy of a system at the start of a process and E_B at the end of the process, Q is the heat absorbed by the system, and W is the work done by the system. An important feature of equation 1 is that *the change in energy of a system depends only on the initial and final states and not on the path of the transformation.*

The first law of thermodynamics cannot be used to predict whether a reaction can occur spontaneously. Some reactions do occur spontaneously even when ΔE is positive (the energy of the system increases). In such cases, the system absorbs heat from its surroundings. It is evident that a function different from ΔE is required. One such function is the *entropy* (S), which is a measure of the *degree of randomness or disorder of a system.* The entropy of a system increases (ΔS is positive) when it becomes more disordered (Figure 8-6). *The second law of thermodynamics states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases.*

$$(\Delta S_{system} + \Delta S_{surroundings}) > 0 \text{ for a spontaneous process} \qquad (2)$$

Note that the entropy of a system can decrease during a spontaneous process, provided that the entropy of the surroundings increases so that their sum is positive. For example, the formation of a highly ordered biological structure is thermodynamically feasible because the decrease in the entropy of such a system is more than offset by an increase in the entropy of its surroundings.

One difficulty in using entropy as a criterion of whether a biochemical process can occur spontaneously is that the entropy changes of chemical reactions are not readily measured. Furthermore, the criterion of spontaneity given in equation 2 requires that both the entropy change of the surroundings and that of the system of interest be known. These difficulties are obviated by using a different thermodynamic function called the *free energy,* which is denoted by the symbol G (or F, in the older literature). In 1878, Josiah Willard Gibbs created the free-energy function by combining the first and second laws of thermodynamics. The basic equation is

$$\Delta G = \Delta H - T\Delta S \qquad (3)$$

in which ΔG is the change in free energy of a system undergoing a transformation at constant pressure (P) and temperature (T), ΔH is the change in enthalpy (heat content) of this system, and ΔS is the change in entropy of this system. Note that the properties of the surroundings do not enter into this equation. The enthalpy change is given by

$$\Delta H = \Delta E + P\Delta V \qquad (4)$$

The volume change, ΔV, is small for nearly all biochemical reactions, and so ΔH is nearly equal to ΔE. Hence,

$$\Delta G \cong \Delta E - T\Delta S \qquad (5)$$

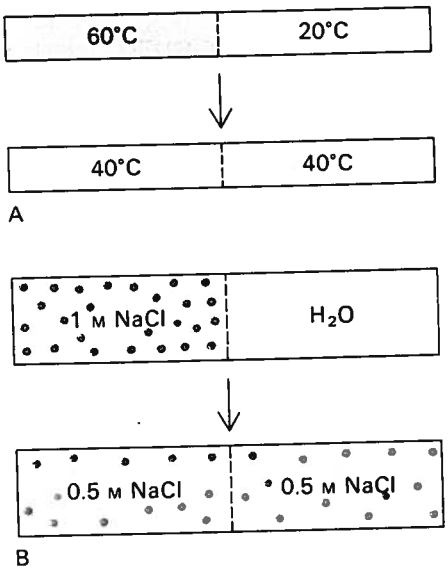


Figure 8-6
Processes that are driven by an increase in the entropy of a system: (A) diffusion of heat; and (B) diffusion of a solute.

Thus, the ΔG of a reaction depends both on the change in internal energy and on the change in entropy of the system.

The change in free energy (ΔG) of a reaction, in contrast with the change in internal energy (ΔE) of a reaction, is a valuable criterion of whether it can occur spontaneously:

1. *A reaction can occur spontaneously only if* ΔG *is negative.*

2. A system is at equilibrium and no net change can take place if ΔG is zero.

3. A reaction cannot occur spontaneously if ΔG is positive. An input of free energy is required to drive such a reaction.

Two additional points need to be emphasized here. First, the ΔG of a reaction depends only on the free energy of the products (the final state) minus that of the reactants (the initial state). *The* ΔG *of a reaction is independent of the path (or molecular mechanism) of the transformation.* The mechanism of a reaction has no effect on ΔG. For example, the ΔG for the oxidation of glucose to CO_2 and H_2O is the same whether it occurs by combustion in vitro or by a series of many enzyme-catalyzed steps in a cell. Second, *the* ΔG *provides no information about the rate of a reaction.* A negative ΔG indicates that a reaction can occur spontaneously, but it does not signify whether it will proceed at a perceptible rate. As will be discussed shortly (p. 188), the rate of a reaction depends on the *free energy of activation* ($\Delta G^{\ddagger}$), which is unrelated to ΔG.

STANDARD FREE-ENERGY CHANGE OF A REACTION AND ITS RELATION TO THE EQUILIBRIUM CONSTANT

Consider the reaction

$$A + B \rightleftharpoons C + D$$

The ΔG of this reaction is given by

$$\Delta G = \Delta G^\circ + RT \log_e \frac{[C][D]}{[A][B]} \qquad (6)$$

in which ΔG° is the *standard free-energy change,* R is the gas constant, T is the absolute temperature, and [A], [B], [C], and [D] are the molar concentrations (more precisely, the activities) of the reactants. ΔG° is the free-energy change for this reaction under standard conditions—that is, when each of the reactants A, B, C, and D is present at a concentration of 1.0 M (for a gas, the standard state is usually chosen to be 1 atmosphere). Thus, the ΔG of a reaction depends on the *nature* of the reactants (expressed in the ΔG° term of equation 6) and on their *concentrations* (expressed in the logarithmic term of equation 6).

A convention has been adopted to simplify free-energy calculations for biochemical reactions. The standard state is defined as having a pH of 7. Consequently, when H^+ is a reactant, its activity has the value 1 (corresponding to a pH of 7) in equations 6 and 9. The activity of water also is taken to be 1 in these equations. The *standard free-energy change at pH 7,* denoted by the symbol $\Delta G^{\circ\prime}$, will be used throughout this book. The *kilocalorie* (abbreviated *kcal*) will be used as the unit of energy.

The relation between the standard free energy and the equilibrium constant of a reaction can be readily derived. At equilibrium, $\Delta G = 0$. Equation 6 then becomes

Units of energy—
A *calorie* (cal) is equivalent to the amount of heat required to raise the temperature of 1 gram of water from 14.5°C to 15.5°C.
A *kilocalorie* (kcal) is equal to 1000 cal.
A *joule* (J) is the amount of energy needed to apply a 1-newton force over a distance of 1 meter. A *kilojoule* (kJ) is equal to 1000 J.
1 kcal = 4.184 kJ

$$0 = \Delta G^{\circ\prime} + RT \log_e \frac{[C][D]}{[A][B]} \tag{7}$$

and so

$$\Delta G^{\circ\prime} = -RT \log_e \frac{[C][D]}{[A][B]} \tag{8}$$

The equilibrium constant under standard conditions, K'_{eq}, is defined as

$$K'_{eq} = \frac{[C][D]}{[A][B]} \tag{9}$$

Substituting equation 9 into equation 8 gives

$$\Delta G^{\circ\prime} = -RT \log_e K'_{eq} \tag{10}$$

$$\Delta G^{\circ\prime} = -2.303\, RT \log_{10} K'_{eq} \tag{11}$$

which can be rearranged to give

$$K'_{eq} = 10^{-\Delta G^{\circ\prime}/(2.303RT)} \tag{12}$$

Substituting $R = 1.987 \times 10^{-3}$ kcal mol^{-1} deg^{-1} and $T = 298$ K (corresponding to 25°C) gives

$$K'_{eq} = 10^{-\Delta G^{\circ\prime}/1.36} \tag{13}$$

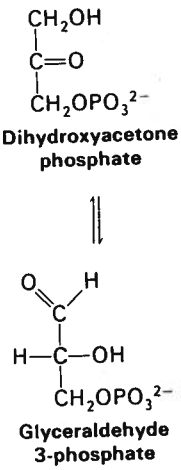
when $\Delta G^{\circ\prime}$ is expressed in kcal/mol. Thus, the standard free energy and the equilibrium constant of a reaction are related by a simple expression. For example, an equilibrium constant of 10 gives a standard free-energy change of −1.36 kcal/mol (−5.69 kJ/mol) at 25°C (Table 8-1).

Table 8-1
Relation between $\Delta G^{\circ\prime}$ and K'_{eq} (at 25°C)

K'_{eq}	$\Delta G^{\circ\prime}$	
	kcal/mol	*kJ/mol*
10^{-5}	6.82	28.53
10^{-4}	5.46	22.84
10^{-3}	4.09	17.11
10^{-2}	2.73	11.42
10^{-1}	1.36	5.69
1	0	0
10	−1.36	−5.69
10^{2}	−2.73	−11.42
10^{3}	−4.09	−17.11
10^{4}	−5.46	−22.84
10^{5}	−6.82	−28.53

Let us calculate $\Delta G^{\circ\prime}$ and ΔG for the isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate as an example. This reaction occurs in glycolysis (p. 488). At equilibrium, the ratio of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate is 0.0475 at 25°C (298 K) and pH 7. Hence, $K'_{eq} = 0.0475$. The standard free-energy change for this reaction is then calculated from equation 11

$$\begin{aligned}\Delta G^{\circ\prime} &= -2.303\, RT \log_{10} K'_{eq} \\ &= -2.303 \times 1.987 \times 10^{-3} \times 298 \times \log_{10}(0.0475) \\ &= +1.8 \text{ kcal/mol}\end{aligned}$$



Now let us calculate ΔG for this reaction when the initial concentration of dihydroxyacetone phosphate is 2×10^{-4} M and the initial concentration of glyceraldehyde 3-phosphate is 3×10^{-6} M. Substituting these values into equation 6 gives

$$\begin{aligned}\Delta G &= 1.8 \text{ kcal/mol} + 2.303\, RT \log_{10} \frac{3 \times 10^{-6}\text{ M}}{2 \times 10^{-4}\text{ M}} \\ &= 1.8 \text{ kcal/mol} - 2.5 \text{ kcal/mol} \\ &= -0.7 \text{ kcal/mol}\end{aligned}$$

This negative value for the ΔG indicates that the isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate can occur spontaneously when these species are present at the concentrations stated above. Note that ΔG for this reaction is negative although $\Delta G^{\circ\prime}$ is positive. *It is important to stress that whether the* ΔG *for a reaction is larger, smaller, or the same as* ΔG$^{\circ\prime}$ *depends on the concentrations of the reactants.* The criterion of spontaneity for a reaction is ΔG, not $\Delta G^{\circ\prime}$.

"I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substances and the products of reaction for these catalyzed processes. The attraction of the enzyme molecule for the activated complex would thus lead to a decrease in its energy and hence to a decrease in the energy of activation of the reaction and to an increase in the rate of reaction."

LINUS PAULING
Nature 161:707 (1948)

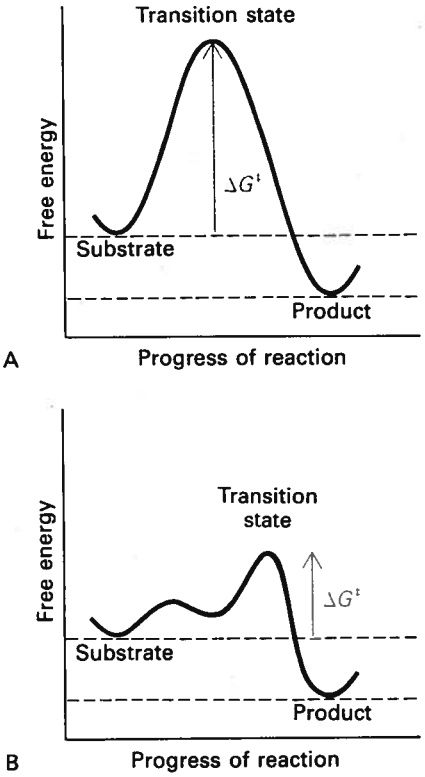


Figure 8-7
Enzymes accelerate reactions by decreasing $\Delta G^{\ddagger}$, the free energy of activation. The free-energy profiles of uncatalyzed (A) and catalyzed (B) reactions are compared.

ENZYMES CANNOT ALTER REACTION EQUILIBRIA

An enzyme is a catalyst, and consequently it cannot alter the equilibrium of a chemical reaction. This means that an enzyme accelerates the forward and reverse reaction by precisely the same factor. Consider the interconversion of A and B. Suppose that in the absence of enzyme the forward rate constant (k_F) is $10^{-4}\ s^{-1}$ and the reverse rate constant (k_R) is $10^{-6}\ s^{-1}$. The equilibrium constant K is given by the ratio of these rate constants:

$$A \underset{10^{-6}\ s^{-1}}{\overset{10^{-4}\ s^{-1}}{\rightleftharpoons}} B$$

$$K = \frac{[B]}{[A]} = \frac{k_F}{k_R} = \frac{10^{-4}}{10^{-6}} = 100$$

The equilibrium concentration of B is 100 times that of A, whether or not enzyme is present. However, it would take more than an hour to approach this equilibrium without enzyme, whereas equilibrium would be attained within a second in the presence of a suitable enzyme. *Enzymes accelerate the attainment of equilibria but do not shift their position.*

ENZYMES ACCELERATE REACTIONS BY STABILIZING TRANSITION STATES

A chemical reaction of substrate S to form product P goes through a *transition state* $S^{\ddagger}$ that has a higher free energy than either S or P.

$$\underset{\text{Substrate}}{S} \overset{K^{\ddagger}}{\rightleftharpoons} \underset{\text{Transition state}}{S^{\ddagger}} \xrightarrow{V} \underset{\text{Product}}{P}$$

The transition state is the most seldom occupied species along the reaction pathway because it has the highest free energy. The *Gibbs free energy of activation,* symbolized by $\Delta G^{\ddagger}$, is equal to the difference in free energy between the transition state and the substrate. The double dagger (‡) denotes a thermodynamic quantity of a transition state.

$$\Delta G^{\ddagger} = G_{S^{\ddagger}} - G_S$$

The reaction rate V is proportional to the concentration of $S^{\ddagger}$, which depends on $\Delta G^{\ddagger}$ because it is in equilibrium with S.

$$[S^{\ddagger}] = [S]e^{-\Delta G^{\ddagger}/RT}$$

$$V = \nu[S^{\ddagger}] = \frac{kT}{h}[S]e^{-\Delta G^{\ddagger}/RT}$$

In these equations, k is Boltzmann's constant and h is Planck's constant. The value of kT/h at 25°C is $6.2 \times 10^{12}\ s^{-1}$. Suppose that the free energy of activation is 6.82 kcal/mol. The ratio $[S^{\ddagger}]/[S]$ is then 10^{-5} (see Table 8-1); we have assumed that [S] = 1, and so the reaction rate V is $6.2 \times 10^{7}\ s^{-1}$. As Table 8-1 shows, a decrease of 1.36 kcal/mol in $\Delta G^{\ddagger}$ results in a tenfold faster V.

Enzymes accelerate reactions by decreasing $\Delta G^{\ddagger}$, *the activation barrier.* The combination of substrate and enzyme creates a new reaction pathway whose transition state energy is lower than that of the reaction in the absence of enzyme (Figure 8-7). *The essence of catalysis is specific binding of the transition state,* to be discussed later in this chapter and the next.

FORMATION OF AN ENZYME-SUBSTRATE COMPLEX IS THE FIRST STEP IN ENZYMATIC CATALYSIS

Much of the catalytic power of enzymes comes from their bringing substrates together in favorable orientations in *enzyme-substrate (ES)* complexes. The substrates are bound to a specific region of the enzyme called the *active site.* Most enzymes are highly selective in their binding of substrates. Indeed, the catalytic specificity of enzymes depends in part on the specificity of binding. Furthermore, the activities of some enzymes are controlled at this stage.

The existence of ES complexes has been shown in a variety of ways:

1. At a constant concentration of enzyme, the reaction rate increases with increasing substrate concentration until a maximal velocity is reached (Figure 8-8). In contrast, uncatalyzed reactions do not show this saturation effect. In 1913, Leonor Michaelis interpreted the *maximal velocity of an enzyme-catalyzed reaction* in terms of the formation of a discrete ES complex. At a sufficiently high substrate concentration, the catalytic sites are filled and so the reaction rate reaches a maximum. Though indirect, this is the most general evidence for the existence of ES complexes.

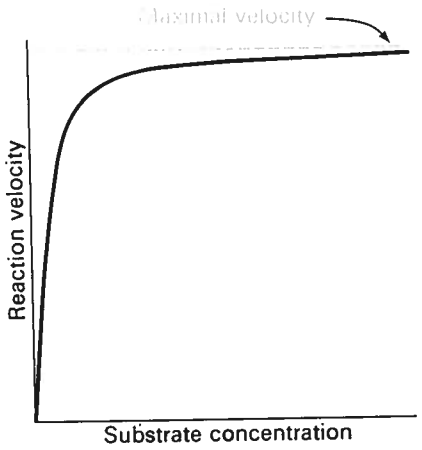


Figure 8-8
Velocity of an enzyme-catalyzed reaction as a function of the substrate concentration.

2. ES complexes have been directly visualized by *electron microscopy,* as in the micrograph of DNA polymerase I bound to its DNA template (Figure 8-9). *X-ray crystallography* has provided high-resolution images of substrates and substrate analogs bound to the active sites of many enzymes. In the next chapter, we shall take a close look at several of these complexes. Moreover, x-ray studies carried out at low temperatures (to slow reactions down) are providing revealing views of intermediates in enzymatic reactions. Also, light can be used to generate a substrate from a photolabile precursor that is bound to the active site in the crystal. Dynamic images of these nascent ES complexes can be obtained with fast pulses of x-rays.

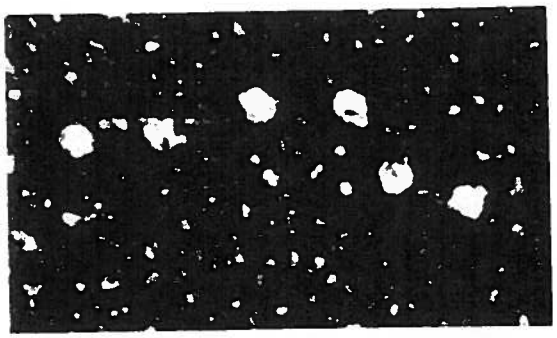


Figure 8-9
Electron micrograph of DNA polymerase I molecules (white spheres) bound to a threadlike synthetic DNA template. [Courtesy of Dr. Jack Griffith.]

3. The *spectroscopic characteristics* of many enzymes and substrates change upon formation of an ES complex just as the absorption spectrum of deoxyhemoglobin changes markedly when it binds oxygen or when it is oxidized to the ferric state, as described previously (see Figure 7-12, on p. 152). These changes are particularly striking if the enzyme contains a colored prosthetic group. Tryptophan synthetase, a bacterial enzyme that contains a pyridoxal phosphate prosthetic group, affords a nice illustration. This enzyme catalyzes the synthesis of L-tryptophan from L-serine and indole. The addition of L-serine to the enzyme produces a marked increase in the fluorescence of the pyridoxal phosphate group (Figure 8-10). The subsequent addition of indole, the second substrate, quenches

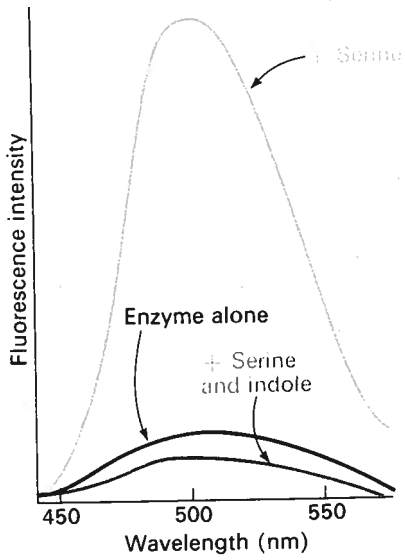


Figure 8-10
Fluorescence intensity of the pyridoxal phosphate group at the active site of tryptophan synthetase changes upon addition of serine and indole, the substrates.

this fluorescence to a level even lower than that of the enzyme alone. Thus, fluorescence spectroscopy reveals the existence of an enzyme-serine complex and of an enzyme-serine-indole complex. Other spectroscopic techniques, such as nuclear magnetic resonance and electron spin resonance, also are highly informative about ES interactions.

ACTIVE SITES OF ENZYMES HAVE SOME COMMON FEATURES

The active site of an enzyme is the region that binds the substrates (and the prosthetic group, if any) and contains the residues that directly participate in the making and breaking of bonds. These residues are called the *catalytic groups.* Although enzymes differ widely in structure, specificity, and mode of catalysis, a number of generalizations concerning their active sites can be stated:

1. *The active site takes up a relatively small part of the total volume of an enzyme.* Most of the amino acid residues in an enzyme are not in contact with the substrate. This raises the intriguing question of why enzymes are so big. Nearly all enzymes are made up of more than 100 amino acid residues, which gives them a mass greater than 10 kd and a diameter of more than 25 Å.

2. *The active site is a three-dimensional entity* formed by groups that come from different parts of the linear amino acid sequence—indeed, residues far apart in the linear sequence may interact more strongly than adjacent residues in the amino acid sequence, as has already been seen for myoglobin and hemoglobin. In lysozyme, an enzyme that will be discussed in more detail in the next chapter, the important groups in the active site are contributed by residues numbered 35, 52, 62, 63, and 101 in the linear sequence of 129 amino acids (Figure 8-11).

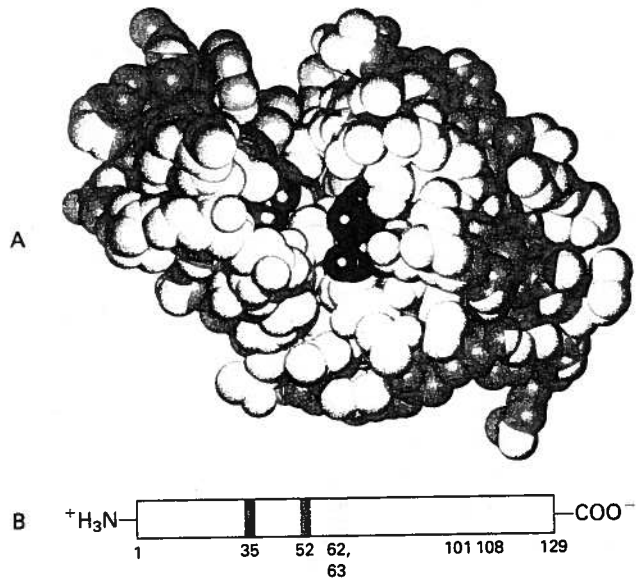


Figure 8-11
(A) Space-filling model of lysozyme. Two catalytically critical residues are shown in red and green. Several other residues in the active site are shown in yellow. (B) Schematic diagram of lysozyme showing that the active site is formed by residues that come from different segments of the polypeptide chain. [(A) Drawn from 6lyz. R. Diamond. *J. Mol. Biol.* 82(1974):371.]

3. *Substrates are bound to enzymes by multiple weak attractions.* ES complexes usually have equilibrium constants that range from 10^{-2} to 10^{-8} M, corresponding to free energies of interaction ranging from about −3 to −12 kcal/mol. The noncovalent interactions in ES complexes are much weaker than covalent bonds, which have energies between −50 and −110 kcal/mol. As was discussed in Chapter 1 (p. 7), reversible interac-

tions of biomolecules are mediated by electrostatic bonds, hydrogen bonds, van der Waals forces, and hydrophobic interactions. Van der Waals forces become significant in binding only when numerous substrate atoms can simultaneously come close to many enzyme atoms. Hence, the enzyme and substrate should have complementary shapes. The directional character of hydrogen bonds between enzyme and substrate often enforces a high degree of specificity.

4. *Active sites are clefts or crevices.* In all enzymes of known structure, substrate molecules are bound to a cleft or crevice. Water is usually excluded unless it is a reactant. The nonpolar character of much of the cleft enhances the binding of substrate. However, the cleft may also contain polar residues. It creates a microenvironment in which certain of these residues acquire special properties essential for catalysis. The internal positions of these polar residues are biologically crucial exceptions to the general rule that polar residues are exposed to water.

5. *The specificity of binding depends on the precisely defined arrangement of atoms in an active site.* To fit into the site, a substrate must have a matching shape. Emil Fischer's metaphor of the lock and key (Figure 8-13), expressed in 1890, has proved to be highly stimulating and fruitful. However, it is now evident that the shapes of the active sites of many enzymes are markedly modified by the binding of substrate, as was postulated by Daniel E. Koshland, Jr., in 1958. The active sites of these enzymes assume shapes that are complementary to that of the substrate only *after* the substrate is bound. This process of dynamic recognition is called *induced fit* (Figure 8-14).

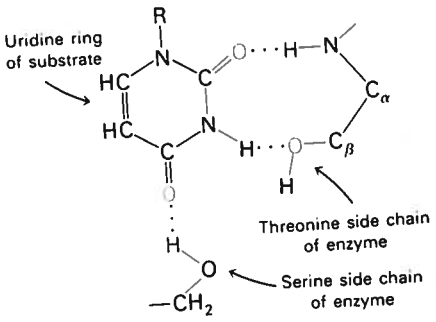


Figure 8-12
Hydrogen bond interactions in the binding of a uridine substrate to ribonuclease. [After F.M. Richards, H.W. Wyckoff, and N. Allewell. In *The Neurosciences: Second Study Program*, F.O. Schmidt, ed. (Rockefeller University Press, 1970), p. 970.]

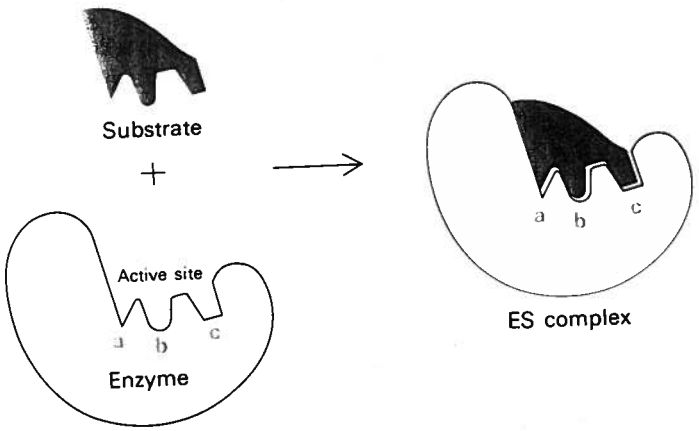


Figure 8-13
Lock-and-key model of the interaction of substrates and enzymes. The active site of the unbound enzyme is complementary in shape to that of the substrate.

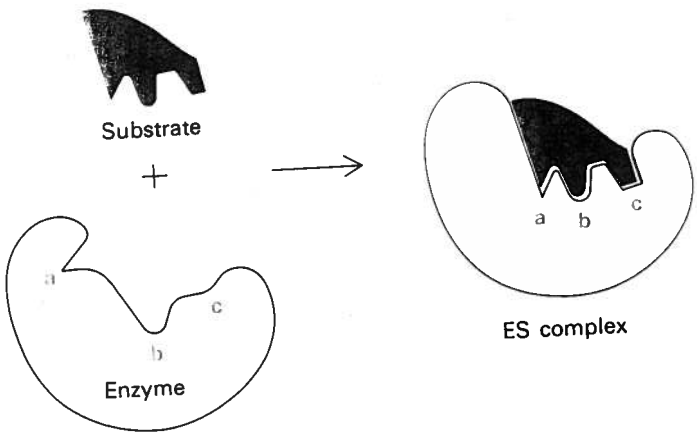


Figure 8-14
Induced-fit model of the interaction of substrates and enzymes. The enzyme changes shape upon binding substrate. The active site has a shape complementary to that of the substrate only after the substrate is bound.

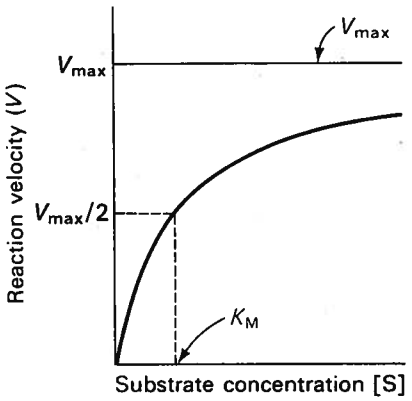


Figure 8-15
A plot of the reaction velocity V as a function of the substrate concentration [S] for an enzyme that obeys Michaelis-Menten kinetics (V_{max} is the maximal velocity and K_M is the Michaelis constant).

THE MICHAELIS-MENTEN MODEL ACCOUNTS FOR THE KINETIC PROPERTIES OF MANY ENZYMES

For many enzymes, the rate of catalysis V varies with the substrate concentration [S] in a manner shown in Figure 8-15. V is defined as the number of moles of product formed per second. At a fixed concentration of enzyme, V is almost linearly proportional to [S] when [S] is small. At high [S], V is nearly independent of [S]. In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. The critical feature in their treatment is that a specific ES complex is a necessary intermediate in catalysis. The model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \tag{14}$$

An enzyme E combines with S to form an ES complex, with a rate constant k_1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k_2, or it can proceed to form product P, with a rate constant k_3. It is assumed that almost none of the product reverts to the initial substrate, a condition that holds in the initial stage of a reaction before the concentration of product is appreciable.

We want an expression that relates the rate of catalysis to the concentrations of substrate and enzyme and the rates of the individual steps. Our starting point is that the catalytic rate is equal to the product of the concentration of the ES complex and k_3.

$$V = k_3[ES] \tag{15}$$

Now we need to express [ES] in terms of known quantities. The rates of formation and breakdown of ES are given by

$$\text{Rate of formation of ES} = k_1[E][S] \tag{16}$$

$$\text{Rate of breakdown of ES} = (k_2 + k_3)[ES] \tag{17}$$

We are interested in the catalytic rate under steady-state conditions. In a *steady state,* the concentrations of intermediates stay the same while the concentrations of starting materials and products are changing. This occurs when the rates of formation and breakdown of the ES complex are equal. On setting the right-hand sides of equations 16 and 17 equal,

$$k_1[E][S] = (k_2 + k_3)[ES] \tag{18}$$

By rearranging equation 18,

$$[ES] = \frac{[E][S]}{(k_2 + k_3)/k_1} \tag{19}$$

Equation 19 can be simplified by defining a new constant, K_M, called the *Michaelis constant,*

$$K_M = \frac{k_2 + k_3}{k_1} \tag{20}$$

and substituting it into equation 19, which then becomes

$$[ES] = \frac{[E][S]}{K_M} \tag{21}$$

Now let us examine the numerator of equation 21. The concentration of uncombined substrate [S] is very nearly equal to the total substrate concentration, provided that the concentration of enzyme is much lower than that of the substrate. The concentration of uncombined enzyme [E] is equal to the total enzyme concentration $[E_T]$ minus the concentration of the ES complex.

$$[E] = [E_T] - [ES] \tag{22}$$

On substituting this expression for [E] in equation 21,

$$[ES] = ([E_T] - [ES])[S]/K_M \tag{23}$$

Solving equation 23 for [ES] gives

$$[ES] = [E_T]\frac{[S]/K_M}{1 + [S]/K_M} \tag{24}$$

or

$$[ES] = [E_T]\frac{[S]}{[S] + K_M} \tag{25}$$

By substituting this expression for [ES] into equation 15, we get

$$V = k_3[E_T]\frac{[S]}{[S] + K_M} \tag{26}$$

The maximal rate V_{max} is attained when the catalytic sites on the enzyme are saturated with substrate—that is, when [S] is much greater than K_M—so that $[S]/([S] + K_M)$ approaches 1. Thus,

$$V_{max} = k_3[E_T] \tag{27}$$

Substituting equation 27 into equation 26 yields the *Michaelis-Menten equation.*

$$V = V_{max}\frac{[S]}{[S] + K_M} \tag{28}$$

This equation accounts for the kinetic data given in Figure 8-15. At very low substrate concentration, when [S] is much less than K_M, $V = [S]V_{max}/K_M$; that is, the rate is directly proportional to the substrate concentration. At high substrate concentration, when [S] is much greater than K_M, $V = V_{max}$; that is, the rate is maximal, independent of substrate concentration.

The meaning of K_M is evident from equation 28. When $[S] = K_M$, then $V = V_{max}/2$. Thus, K_M *is equal to the substrate concentration at which the reaction rate is half its maximal value.*

V_{max} AND K_M CAN BE DETERMINED BY VARYING THE SUBSTRATE CONCENTRATION

The Michaelis constant, K_M, and the maximal rate, V_{max}, can be readily derived from rates of catalysis measured at different substrate concentrations if an enzyme operates according to the simple scheme given in equation 14. It is convenient to transform the Michaelis-Menten equation into one that gives a straight-line plot. This can be done by taking the reciprocal of both sides of equation 28 to give

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} \tag{29}$$

A plot of $1/V$ versus $1/[S]$, called a *Lineweaver-Burk plot,* yields a straight line with an intercept of $1/V_{max}$ and a slope of K_M/V_{max} (Figure 8-16). Alternatively, K_M and V_{max} can be obtained by fitting the data to equation 28 using a computer program.

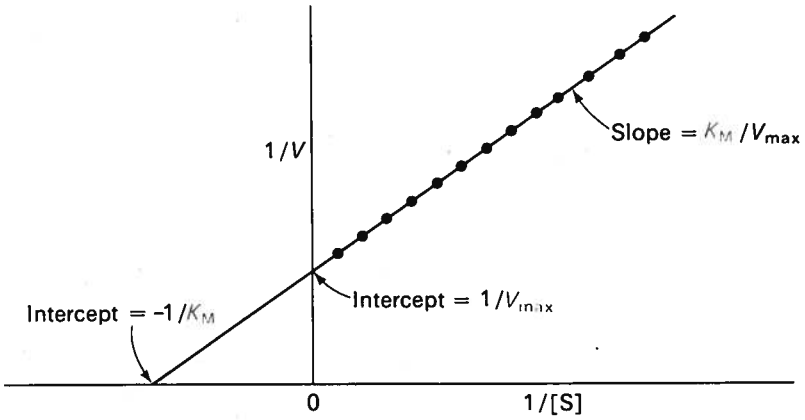


Figure 8-16
A double-reciprocal plot of enzyme kinetics: $1/V$ is plotted as a function of $1/[S]$. The slope is K_M/V_{max}, the intercept on the vertical axis is $1/V_{max}$, and the intercept on the horizontal axis is $-1/K_M$.

SIGNIFICANCE OF K_M AND V_{max} VALUES

The K_M values of enzymes range widely (Table 8-2). For most enzymes, K_M lies between 10^{-1} and 10^{-7} M. The K_M value for an enzyme depends on the particular substrate and also on environmental conditions such as pH, temperature, and ionic strength. The Michaelis constant K_M has two meanings. First, K_M is the concentration of substrate at which half the active sites are filled. Once the K_M is known, the fraction of sites filled f_{ES} at any substrate concentration can be calculated from

$$f_{ES} = \frac{V}{V_{max}} = \frac{[S]}{[S] + K_M} \tag{30}$$

Second, K_M is related to the rate constants of the individual steps in the catalytic scheme given in equation 14. In equation 20, K_M is defined as $(k_2 + k_3)/k_1$. Consider a limiting case in which k_2 is much greater than k_3. This means that dissociation of the ES complex to E and S is much more rapid than formation of E and product. Under these conditions $(k_2 \gg k_3)$,

Table 8-2
K_M values of some enzymes

Enzyme	*Substrate*	K_M *(μM)*
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

$$K_M = \frac{k_2}{k_1} \tag{31}$$

The dissociation constant of the ES complex is given by

$$K_{ES} = \frac{[E][S]}{[ES]} = \frac{k_2}{k_1} \tag{32}$$

In other words, K_M *is equal to the dissociation constant of the ES complex if* k_3 *is much smaller than* k_2. When this condition is met, K_M is a measure of the strength of the ES complex: a high K_M indicates weak binding; a low K_M indicates strong binding. It must be stressed that K_M indicates the affinity of the ES complex only when k_2 is much greater than k_3.

The *turnover number* of an enzyme is *the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.* It is equal to the kinetic constant k_3. The maximal rate V_{max} reveals the turnover number of an enzyme if the concentration of active sites $[E_T]$ is known, because

$$V_{max} = k_3[E_T] \tag{33}$$

For example, a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate. Hence, k_3 is $6 \times 10^5\ s^{-1}$. This turnover number is one of the largest known. Each round of catalysis occurs in a time equal to $1/k_3$, which is 1.7 μs for carbonic anhydrase. The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second (Table 8-3).

Table 8-3
Maximum turnover numbers of some enzymes

Enzyme	*Turnover number (per second)*
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

KINETIC PERFECTION IN ENZYMATIC CATALYSIS: THE k_{cat}/K_M CRITERION

When the substrate concentration is much greater than K_M, the rate of catalysis is equal to k_3, the turnover number, as described in the preceding section. However, most enzymes are not normally saturated with substrate. Under physiological conditions, the $[S]/K_M$ ratio is typically between 0.01 and 1.0. When $[S] \ll K_M$, the enzymatic rate is much less than k_3 because most of the active sites are unoccupied. Is there a number that characterizes the kinetics of an enzyme under these conditions? Indeed there is, as can be shown by combining equations 15 and 21 to give

$$V = \frac{k_3}{K_M}[E][S] \tag{34}$$

When $[S] \ll K_M$, the concentration of free enzyme, [E], is nearly equal to the total concentration of enzyme $[E_T]$, and so

$$V = \frac{k_3}{K_M}[S][E_T] \tag{35}$$

Thus, when $[S] \ll K_M$, the enzymatic velocity depends on the value of k_3/K_M and on [S].

Are there any physical limits on the value of k_3/K_M? Note that this ratio depends on k_1, k_2, and k_3, as can be shown by substituting for K_M.

$$k_3/K_M = \frac{k_3 k_1}{k_2 + k_3} < k_1 \tag{36}$$

Suppose that the rate of formation of product (k_3) is much faster than the rate of dissociation of the ES complex (k_2). The value of k_3/K_M then

approaches k_1. Thus the ultimate limit on the value of k_3/K_M is set by k_1, the rate of formation of the ES complex. *This rate cannot be faster than the diffusion-controlled encounter of an enzyme and its substrate.* Diffusion limits the value of k_1 so that it cannot be higher than between 10^8 and 10^9 M^{-1} s^{-1}. Hence, the upper limit on k_3/K_M is between 10^8 and 10^9 M^{-1} s^{-1}.

This restriction also pertains to enzymes having more complex reaction pathways than that of equation 14. Their maximal catalytic rate when substrate is saturating, denoted by k_{cat}, depends on several rate constants rather than on k_3 alone. The pertinent parameter for these enzymes is k_{cat}/K_M. In fact, the k_{cat}/K_M ratios of the enzymes carbonic anhydrase, acetylcholinesterase, and triosephosphate isomerase are between 10^8 and 10^9 M^{-1} s^{-1}, which shows that they have attained *kinetic perfection. Their catalytic velocity is restricted only by the rate at which they encounter substrate in the solution.* Any further gain in catalytic rate can come only by decreasing the time for diffusion. Indeed, some series of enzymes are associated into organized assemblies (p. 517) so that the product of one enzyme is very rapidly found by the next enzyme. In effect, products are channeled from one enzyme to the next, much as in an assembly line. Thus, the limit imposed by the rate of diffusion in solution can be partly overcome by confining substrates and products in the limited volume of a multienzyme complex.

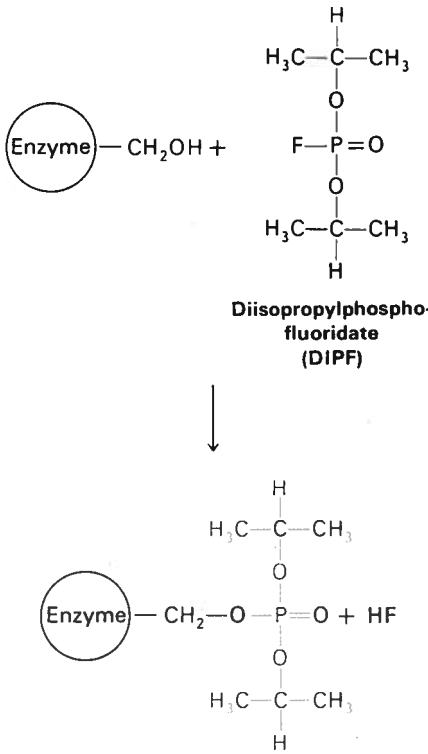


Figure 8-17
Inactivation of acetylcholinesterase by diisopropylphosphofluoridate (DIPF).

ENZYMES CAN BE INHIBITED BY SPECIFIC MOLECULES

The inhibition of enzymatic activity by specific small molecules and ions is important because it serves as a major control mechanism in biological systems. Also, many drugs and toxic agents act by inhibiting enzymes. Furthermore, inhibition can be a source of insight into the mechanism of enzyme action: residues critical for catalysis can often be identified by using specific inhibitors. The value of transition state analogs has already been discussed.

Enzyme inhibition can be either reversible or irreversible. An *irreversible inhibitor* dissociates very slowly from its target enzyme because it becomes very tightly bound to the enzyme, either covalently or noncovalently. The action of nerve gases on acetylcholinesterase, an enzyme that plays an important role in the transmission of nerve impulses, exemplifies irreversible inhibition. Diisopropylphosphofluoridate (DIPF), one of these agents, reacts with a critical serine residue at the active site to form an inactive diisopropylphosphoryl enzyme (Figure 8-17). Alkylating reagents, such as iodoacetamide, irreversibly inhibit the catalytic activity of some enzymes by modifying cysteine and other side chains (Figure 8-18).

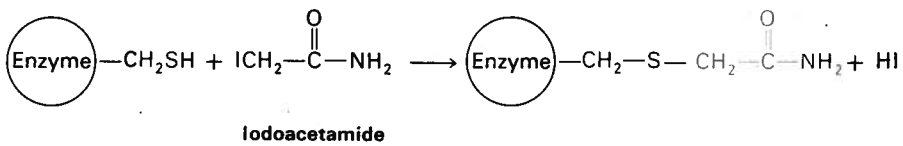


Figure 8-18
Inactivation of an enzyme with a critical cysteine residue by iodoacetamide.

Reversible inhibition, in contrast with irreversible inhibition, is characterized by a rapid dissociation of the enzyme-inhibitor complex. In *competitive inhibition,* the enzyme can bind substrate (forming an ES complex) or inhibitor (EI) but not both (ESI). Many competitive inhibitors resemble

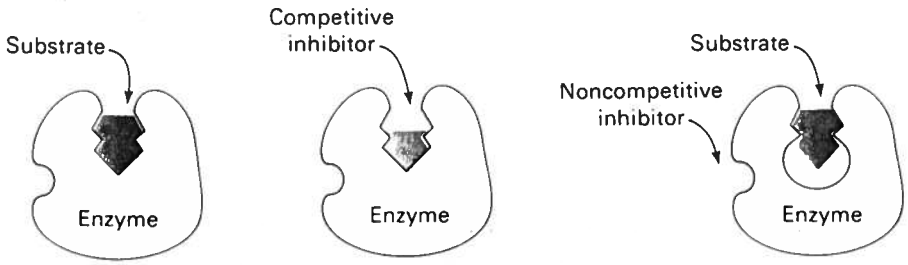
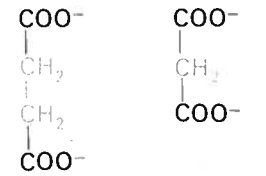
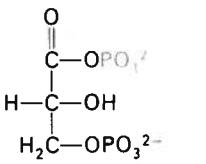
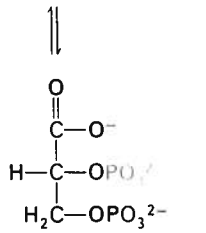


Figure 8-19
Distinction between a competitive and a noncompetitive inhibitor: (top) enzyme-substrate complex; (middle) a competitive inhibitor prevents the substrate from binding; (bottom) a noncompetitive inhibitor does not prevent the substrate from binding.







the substrate and bind to the active site of the enzyme (Figure 8-19). The substrate is thereby prevented from binding to the same active site. *A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate.* A classic example of competitive inhibition is the action of malonate on succinate dehydrogenase, an enzyme that removes two hydrogen atoms from succinate (p. 512). Malonate differs from succinate in having one rather than two methylene groups. A physiologically important example of competitive inhibition is found in the formation of 2,3-bisphosphoglycerate (BPG, p. 160) from 1,3-bisphosphoglycerate. Bisphosphoglycerate mutase, the enzyme catalyzing this isomerization, is competitively inhibited by even low levels of 2,3-bisphosphoglycerate. In fact, it is not uncommon for an enzyme to be competitively inhibited by its own product because of the product's structural resemblance to the substrate. Competitive inhibition can be overcome by increasing the concentration of substrate.

In *noncompetitive inhibition,* which is also reversible, the inhibitor and substrate can bind simultaneously to an enzyme molecule (Figure 8-19). Hence, their binding sites do not overlap. A noncompetitive inhibitor acts by decreasing the turnover number rather than by diminishing the proportion of enzyme molecules that are bound to substrate. Noncompetitive inhibition, in contrast with competitive inhibition, cannot be overcome by increasing the substrate concentration. A more complex pattern, called *mixed inhibition,* is produced when an inhibitor both affects the binding of substrate and alters the turnover number of the enzyme.

COMPETITIVE AND NONCOMPETITIVE INHIBITION ARE KINETICALLY DISTINGUISHABLE

Let us return to enzymes that exhibit Michaelis-Menten kinetics. Measurements of the rates of catalysis at different concentrations of substrate and inhibitor serve to distinguish between competitive and noncompetitive inhibition. In *competitive inhibition,* the intercept of the plot of $1/V$ versus $1/[S]$ is the same in the presence and absence of inhibitor, although the slope is different (Figure 8-20). This reflects the fact that V_{max} is not altered by a competitive inhibitor. *The hallmark of competitive inhibition is that it can be overcome by a sufficiently high concentration of substrate.* At a sufficiently high concentration, virtually all the active sites are filled by substrate, and the enzyme is fully operative. The increase in the slope of the $1/V$ versus $1/[S]$ plot indicates the strength of binding of competitive inhibitor. In the presence of a competitive inhibitor, equation 29 is replaced by

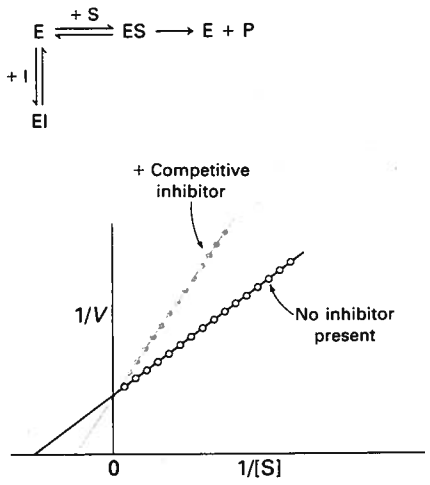


Figure 8-20
A double-reciprocal plot of enzyme kinetics in the presence (••••) and absence (○○○○) of a competitive inhibitor; V_{max} is unaltered, whereas K_M is increased.

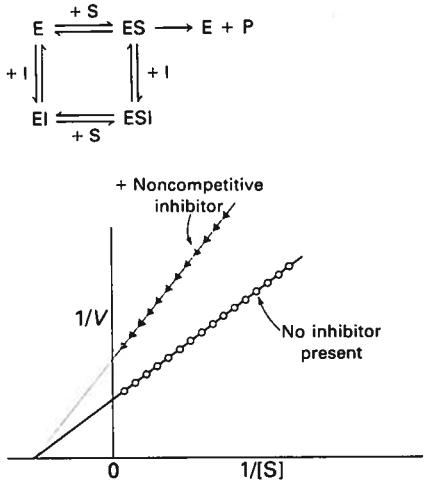


Figure 8-21
A double-reciprocal plot of enzyme kinetics in the presence (-◂-◂-◂-◂-) and absence (-○-○-○-○-) of a noncompetitive inhibitor; K_M is unaltered by the noncompetitive inhibitor, whereas V_{max} is decreased.

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\left(\frac{1}{[S]}\right) \tag{37}$$

in which [I] is the concentration of inhibitor and K_i is the dissociation constant of the enzyme-inhibitor complex.

$$E + I \rightleftharpoons EI$$

$$K_i = \frac{[E][I]}{[EI]} \tag{38}$$

In other words, the slope of the plot is increased by the factor $(1 + [I]/K_i)$ in the presence of a competitive inhibitor. Consider an enzyme with a K_M of 10^{-4} M. In the absence of inhibitor, $V = V_{max}/2$ when $[S] = 10^{-4}$ M. In the presence of 2×10^{-3} M competitive inhibitor that is bound to the enzyme with a K_i of 10^{-3} M, the apparent K_M will be 3×10^{-4} M. Substitution of these values into equation 29 gives $V = V_{max}/4$.

In *noncompetitive inhibition* (Figure 8-21), V_{max} is decreased to V^I_{max}, and so the intercept on the vertical axis is increased. The new slope, which is equal to K_M/V^I_{max}, is larger by the same factor. In contrast with V_{max}, K_M is not affected by this kind of inhibition. *Noncompetitive inhibition cannot be overcome by increasing the substrate concentration.* The maximal velocity in the presence of a noncompetitive inhibitor, V^I_{max}, is given by

$$V^I_{max} = \frac{V_{max}}{1 + [I]/K_i} \tag{39}$$

ALLOSTERIC ENZYMES DO NOT OBEY MICHAELIS-MENTEN KINETICS

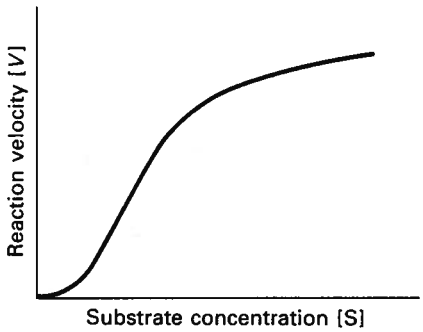


Figure 8-22
Sigmoidal dependence of reaction velocity on substrate concentration for an allosteric enzyme.

The Michaelis-Menten model has greatly affected the development of enzyme chemistry. Its virtues are simplicity and broad applicability. However, the kinetic properties of many enzymes cannot be accounted for by the Michaelis-Menten model. An important group consists of the *allosteric enzymes,* which often display sigmoidal plots (Figure 8-22) of the reaction velocity V versus substrate concentration [S], rather than the hyperbolic plots predicted by the Michaelis-Menten equation (equation 28). Recall that the oxygen-binding curve of myoglobin is hyperbolic, whereas that of hemoglobin is sigmoidal. The binding of enzymes to substrates is analogous. In allosteric enzymes, the binding of substrate to one active site can affect the properties of other active sites in the same enzyme molecule. A possible outcome of this interaction between subunits is that the binding of substrate becomes cooperative, which would give a sigmoidal plot of V versus [S]. A Hill plot (p. 159) for such an enzyme would have a slope greater than 1.0. In addition, the activity of an allosteric enzyme may be altered by regulatory molecules that are bound to sites other than the catalytic sites, just as oxygen binding to hemoglobin is affected by BPG, H^+, and CO_2.

Consider an allosteric enzyme consisting of two identical subunits, each containing an active site (Figure 8-23A). The T (tense) form has low affinity and the R (relaxed) form has high affinity for substrate. Two limiting models for the allosteric process were presented in the previous chapter (p. 166). In the simple sequential model, the binding of substrate to one of the subunits induces a T → R transition in that subunit but not in the other (Figure 8-23A). The affinity of the other subunit for substrate is increased because the subunit interface has been altered by the binding

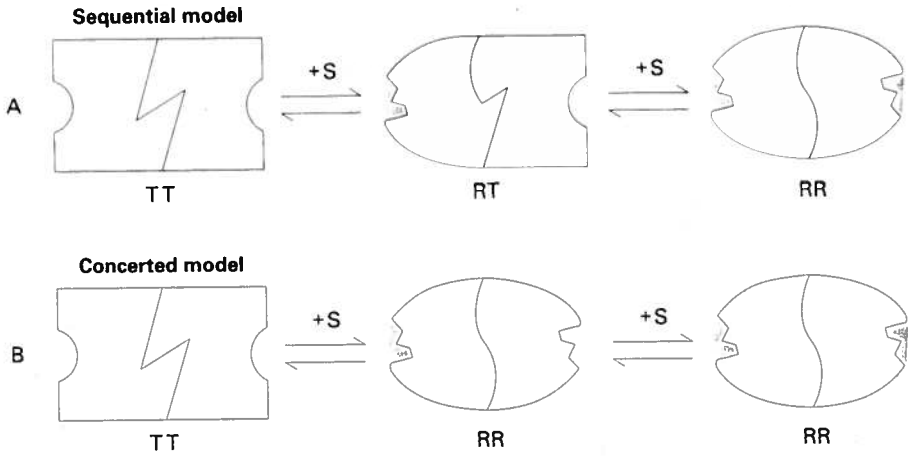


Figure 8-23
Comparison of allosteric models.
(A) Simple sequential model.
(B) Concerted model.

of the first substrate molecule. In the concerted model, the binding of substrate to one of the subunits increases the probability that both switch from the T to the R form (Figure 8-23B). Symmetry is conserved in the concerted model but not in the sequential model.

The effects of allosteric activators and inhibitors can be accounted for quite simply by the concerted model. An allosteric inhibitor binds preferentially to the T form, whereas an allosteric activator binds preferentially to the R form (Figure 8-24). Consequently, *an allosteric inhibitor shifts the R→ T conformational equilibrium toward T, whereas an allosteric activator shifts it toward R.* The result is that an allosteric activator increases the binding of substrate to the enzyme, whereas an allosteric inhibitor decreases substrate binding.

It is noteworthy that most allosteric interactions alter f_{ES} (the fraction of enzyme molecules containing bound substrate, also termed Y) rather than V_{max}. For such enzymes, the dependence of f_{ES} on [S] is sigmoidal rather than hyperbolic (Figure 8-25). Allosteric activators shift this curve to the left (to higher saturation), whereas allosteric inhibitors shift it to the right (to lower saturation).

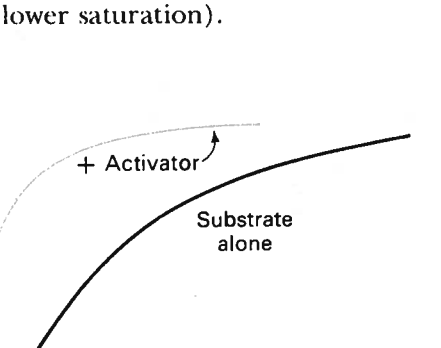


Figure 8-24
In the concerted model, an allosteric inhibitor (represented by a hexagon) stabilizes the T state, whereas an allosteric activator (represented by a triangle) stabilizes the R state.

Figure 8-25
Dependence of f_{ES}, the fraction of catalytic sites containing bound substrate, on the substrate concentration for an allosteric enzyme. An allosteric activator shifts the curve to the left, whereas an allosteric inhibitor shifts it to the right by changing L_0 (the equilibrium constant that gives the ratio of T to R forms in the absence of substrate). The activator decreases L_0 from 10^4 to 10^3, whereas the inhibitor increases L_0 to 10^5.

TRANSITION STATE ANALOGS ARE POTENT INHIBITORS OF ENZYMES

We turn now to compounds that provide intimate views of the catalytic process itself. Linus Pauling proposed in 1946 that compounds resembling the transition state of a catalyzed reaction should be very effective inhibitors of enzymes. These mimics are called *transition state analogs.* The

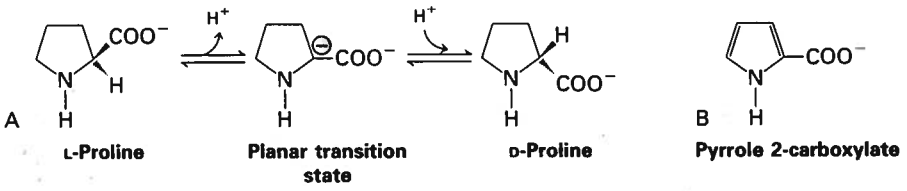


Figure 8-26
(A) The isomerization of L-proline to D-proline by proline racemase, a bacterial enzyme, proceeds through a planar transition state in which the α carbon is trigonal rather than tetrahedral. (B) Pyrrole 2-carboxylate is a transition state analog because it has a planar geometry.

inhibition of proline racemase is an instructive example. *The racemization of proline proceeds through a transition state in which the tetrahedral α carbon atom has become trigonal by loss of a proton* (Figure 8-26). In the trigonal form, all three bonds are in the same plane; C_α also carries a net negative charge. This symmetric carbanion can be reprotonated on one side to give the L isomer or on the other side to give the D isomer. This picture is supported by the finding that pyrrole 2-carboxylate binds to the racemase 160 times as tightly as does proline. *The α carbon atom of this inhibitor, like that of the transition state, is trigonal.* An analog that also carries a negative charge on C_α would be expected to bind even more tightly, but it has not been feasible to synthesize a stable compound of this kind. In general, highly potent and specific inhibitors of enzymes can be produced by synthesizing compounds that more closely resemble the transition state than the substrate itself. The inhibitory power of transition state analogs underscores the essence of catalysis: *selective binding of the transition state.*

CATALYTIC ANTIBODIES CAN BE FORMED BY USING TRANSITION STATE ANALOGS AS IMMUNOGENS

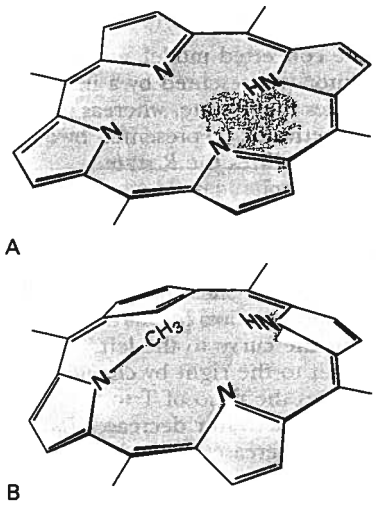


Figure 8-27
(A) The insertion of a metal ion into a porphyrin proceeds through a transition state in which the porphyrin is bent. (B) *N*-methylmesoporphyrin, a bent porphyrin, is an effective immunogen because it resembles the transition state of the reaction.

In 1969, William Jencks proposed that antibodies specific for the transition state of a chemical reaction should have catalytic power. This incisive prediction was realized in 1986 when the laboratories of Richard Lerner and Peter Schultz found that *catalytic antibodies could be produced by using transition state analogs as immunogens.* The preparation of an antibody that catalyzes the insertion of a metal ion into a porphyrin nicely illustrates this experimental approach. Ferrochelatase, the final enzyme in the biosynthetic pathway for the production of heme, catalyzes the insertion of Fe^{2+} into protoporphyrin IX. The nearly planar porphyrin must be bent for iron to enter (Figure 8-27A).

What kind of immunogen might elicit the production of an antibody that would catalyze this metallation? The clue came from studies showing that *N*-methylprotoporphyrin is a potent inhibitor of ferrochelatase. This compound resembles the transition state because N-*alkylation forces the porphyrin to be bent.* Moreover, it was known that *N*-alkylporphyrins chelate metal ions 10^4 times faster than their unalkylated counterparts. Bending increases the exposure of the pyrrole nitrogen lone pairs of electrons to solvent, which facilitates chelation.

Indeed, an effective catalyst was produced using an *N*-alkylporphyrin as the immunogen (Figure 8-27B). *The resulting antibody distorted a planar porphyrin to facilitate the entry of a metal ion.* Eighty porphyrin molecules were metallated per hour per antibody molecule, a rate only tenfold less than that of ferrochelatase. The uncatalyzed reaction was 2500 times as

slow as the antibody-catalyzed reaction. Antibodies catalyzing many other kinds of chemical reactions—exemplified by ester and amide hydrolysis, amide bond formation, transesterification, photoinduced cleavage, photoinduced dimerization, decarboxylation, and oxidization—have been produced using similar strategies. *The power of transition state analogs is now evident: (1) They provide insight into catalytic mechanisms, (2) they can serve as potent and specific inhibitors of enzymes, and (3) they can be used as immunogens to generate a wide range of novel catalysts.*

"If complementarity between the active site and the transition state contributes significantly to enzymatic catalysis, it should be possible to synthesize an enzyme by constructing such an active site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining sites of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrates to resemble the transition state."

W.P. JENCKS
Catalysis in Chemistry and Enzymology (McGraw-Hill, 1969), p. 288.

PENICILLIN IRREVERSIBLY INACTIVATES A KEY ENZYME IN BACTERIAL CELL WALL SYNTHESIS

Penicillin was discovered by Alexander Fleming in 1928, when he observed by chance that bacterial growth was inhibited by a contaminating mold (*Penicillium*). Fleming was encouraged to find that an extract from the mold was not toxic when injected into animals. However, on trying to concentrate and purify the antibiotic, he found that "penicillin is easily destroyed, and to all intents and purposes we failed. We were bacteriologists—not chemists—and our relatively simple procedures were unavailing." Ten years later, Howard Florey, a pathologist, and Ernst Chain, a biochemist, carried out an incisive series of studies that led to the isolation, chemical characterization, and clinical use of this antibiotic.

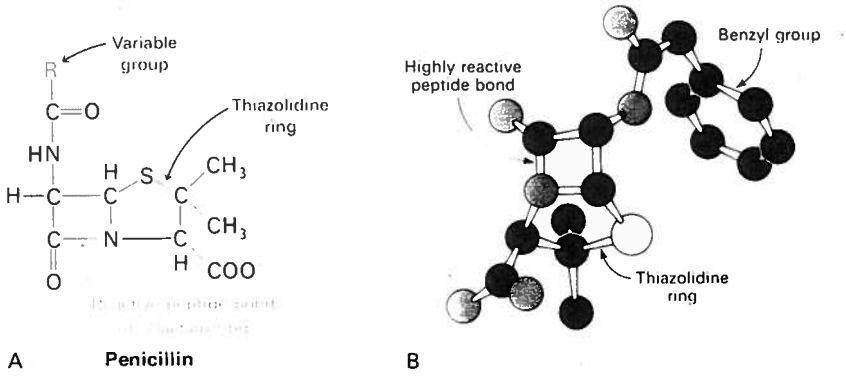


Figure 8-28
(A) Structural formula of penicillin and (B) model of benzyl penicillin. The reactive site of penicillin is the peptide bond of its β-lactam ring.

Penicillin consists of a thiazolidine ring fused to a *β-lactam* ring, to which a variable R group is attached by a peptide bond. In benzyl penicillin, for example, R is a benzyl group (Figure 8-28). This structure can undergo a variety of rearrangements, which accounts for the instability first encountered by Fleming. In particular, the β-lactam ring is very labile. Indeed, this property is closely tied to the antibiotic action of penicillin, as will be evident shortly.

How does penicillin inhibit bacterial growth? In 1957, Joshua Lederberg showed that bacteria ordinarily susceptible to penicillin could be grown in its presence if a hypertonic medium were used. The organisms obtained in this way, called *protoplasts*, are devoid of a cell wall and consequently lyse when transferred to a normal medium. Hence, it was inferred that penicillin interferes with the synthesis of the bacterial cell wall. The cell wall macromolecule, called a *peptidoglycan*, consists of linear polysaccharide chains that are cross-linked by short peptides (Figure 8-29). The

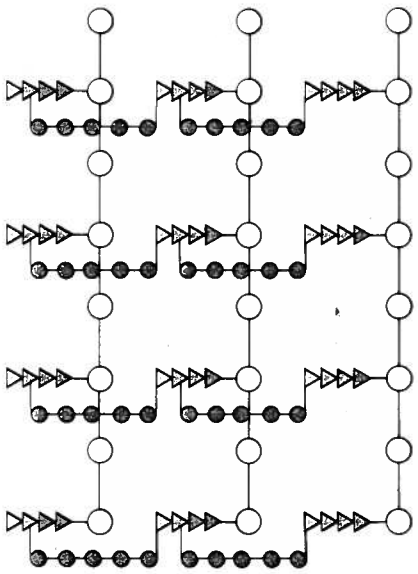


Figure 8-29
Schematic diagram of the peptidoglycan in *Staphylococcus aureus*. The sugars are shown in yellow, the tetrapeptides in red, and the pentaglycine bridges in blue. The cell wall is a single, enormous bag-shaped macromolecule because of extensive cross-linking.