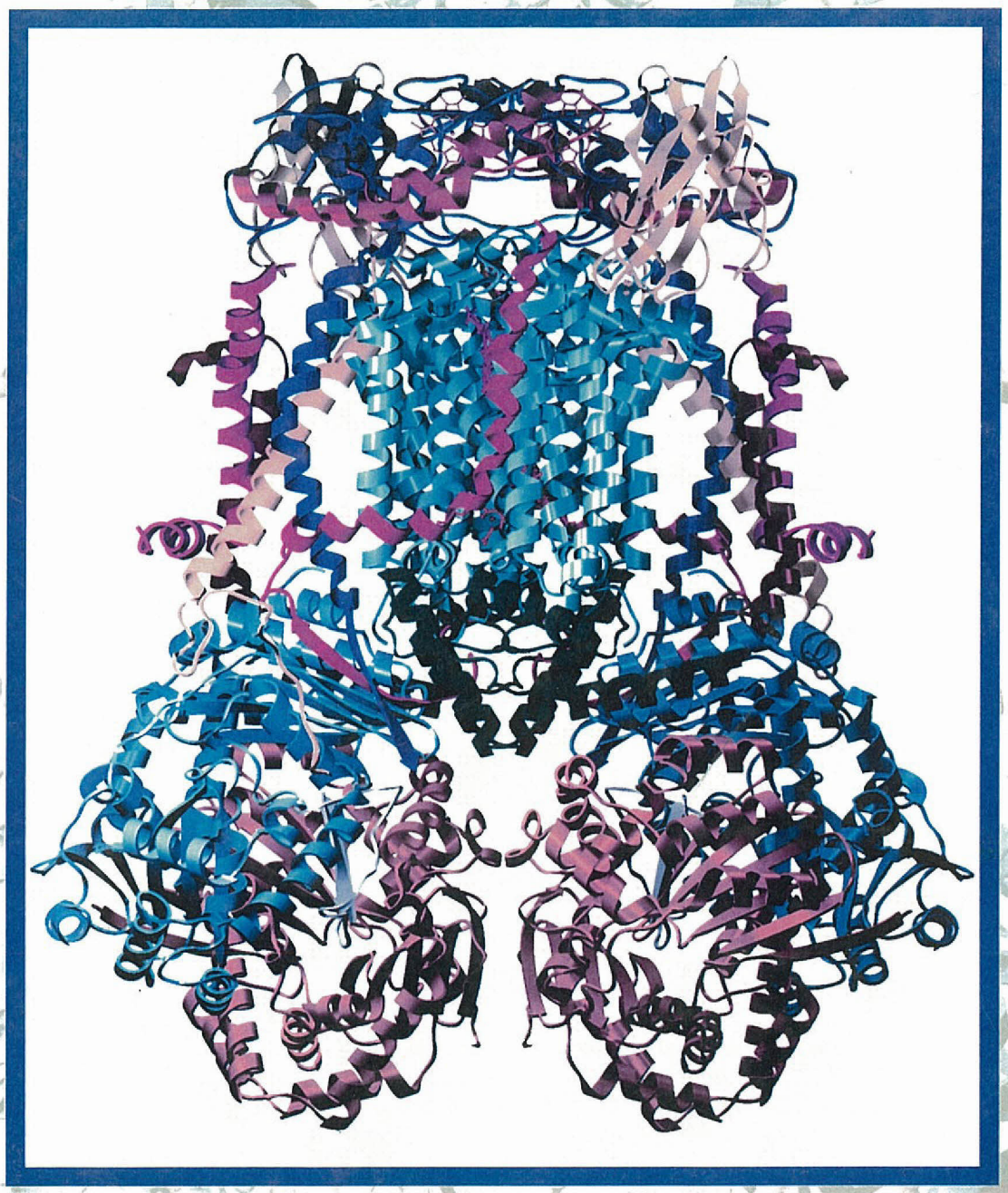

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"Now! Now!" cried the Queen. "Faster! Faster! . . . Now *here,* you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"

LEWIS CARROLL, *Alice's Adventures in Wonderland* (1865)

Chapter 14

Enzyme Kinetics

OUTLINE

"Alice and the Queen of Hearts," illustrated by John Tenniel, *The Nursery Alice.* (Mary Evans Picture Library, London)

Living organisms seethe with metabolic activity. Thousands of chemical reactions are proceeding very rapidly at any given instant within all living cells. Virtually all of these transformations are mediated by **enzymes,** proteins (and occasionally RNA) specialized to catalyze metabolic reactions. The substances transformed in these reactions are often organic compounds that show little tendency for reaction outside the cell. An excellent example is glucose, a sugar that can be stored indefinitely on the shelf with no deterioration. Most cells quickly oxidize glucose, producing carbon dioxide and water and releasing lots of energy:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 2870\ \text{kJ of energy}$$

(-2870 kJ/mol is the standard free energy change [$\Delta G^{\circ\prime}$] for the oxidation of glucose; see Chapter 3). In chemical terms, 2870 kJ is a large amount of energy, and glucose can be viewed as an energy-rich compound even though at ambi-

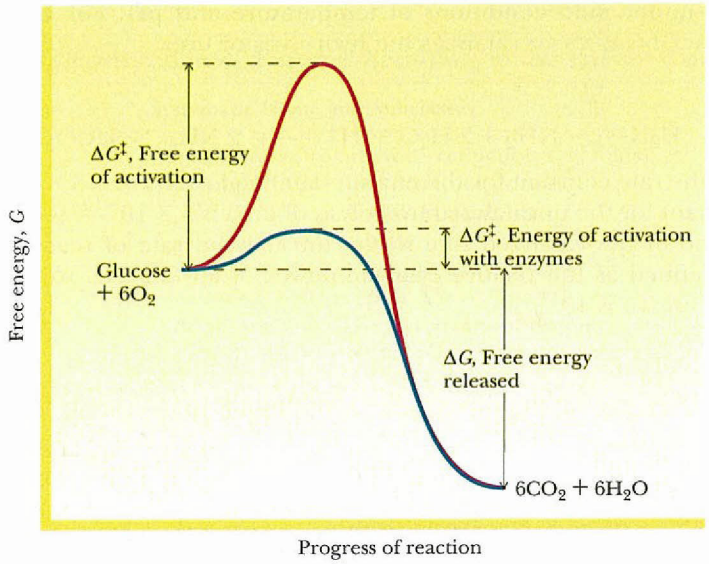


FIGURE 14.1 • Reaction profile showing large $\Delta G^{\ddagger}$ for glucose oxidation, free energy change of −2,870 kJ/mol; catalysts lower $\Delta G^{\ddagger}$, thereby accelerating rate.

ent temperature it is not readily reactive with oxygen outside of cells. Stated another way, glucose represents **thermodynamic potentiality:** its reaction with oxygen is strongly exergonic, but it just doesn't occur under normal conditions. On the other hand, enzymes can catalyze such thermodynamically favorable reactions so that they proceed at extraordinarily rapid rates (Figure 14.1). In glucose oxidation and countless other instances, enzymes provide cells with the ability to exert *kinetic control over thermodynamic potentiality.* That is, living systems use enzymes to accelerate and control the rates of vitally important biochemical reactions.

Enzymes Are the Agents of Metabolic Function

Acting in sequence, enzymes form metabolic pathways by which nutrient molecules are degraded, energy is released and converted into metabolically useful forms, and precursors are generated and transformed to create the literally thousands of distinctive biomolecules found in any living cell (Figure 14.2). Situated at key junctions of metabolic pathways are specialized **regulatory enzymes** capable of sensing the momentary metabolic needs of the cell and adjusting their catalytic rates accordingly. The responses of these enzymes ensure the harmonious integration of the diverse and often divergent metabolic activities of cells so that the living state is promoted and preserved.

14.1 • Enzymes—Catalytic Power, Specificity, and Regulation

Enzymes are characterized by three distinctive features: **catalytic power, specificity,** and **regulation.**

Catalytic Power

Enzymes display enormous catalytic power, accelerating reaction rates as much as 10^{16} over uncatalyzed levels, which is far greater than any synthetic catalysts can achieve, and enzymes accomplish these astounding feats in dilute aqueous

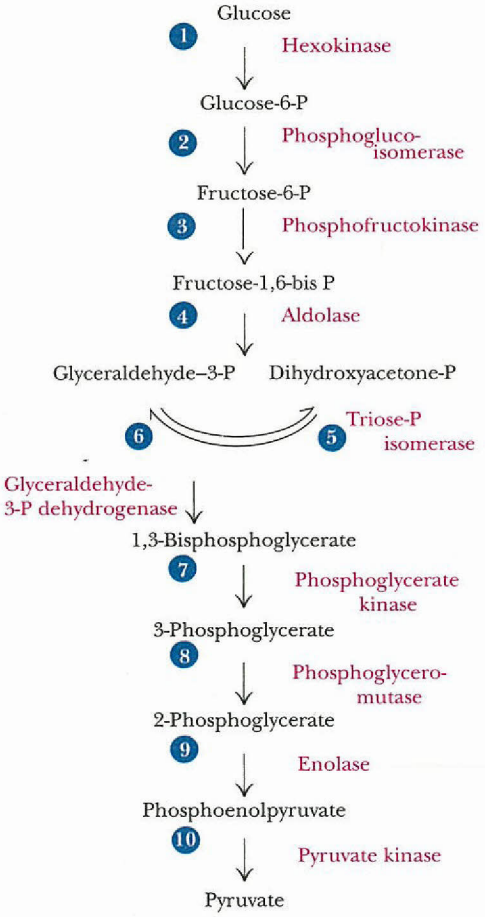


FIGURE 14.2 • The breakdown of glucose by *glycolysis* provides a prime example of a metabolic pathway. Ten enzymes mediate the reactions of glycolysis. Enzyme 4, *fructose 1,6, biphosphate aldolase,* catalyzes the C—C bond–breaking reaction in this pathway.

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}.

Specificity

A given enzyme is very selective, both in the substances with which it interacts and in the reaction that it catalyzes. The substances upon which an enzyme acts are traditionally called **substrates.** In an enzyme-catalyzed reaction, none of the substrate is diverted into nonproductive side-reactions, so no wasteful by-products are produced. It follows then that the products formed by a given enzyme are also very specific. This situation can be contrasted with your own experiences in the organic chemistry laboratory, where yields of 50% or even 30% are viewed as substantial accomplishments (Figure 14.3). The selective qualities of an enzyme are collectively recognized as its **specificity.** Intimate interaction between an enzyme and its substrates occurs through molecular recognition based on structural complementarity; such mutual recognition is the basis of specificity. The specific site on the enzyme where substrate binds and catalysis occurs is called the **active site.**

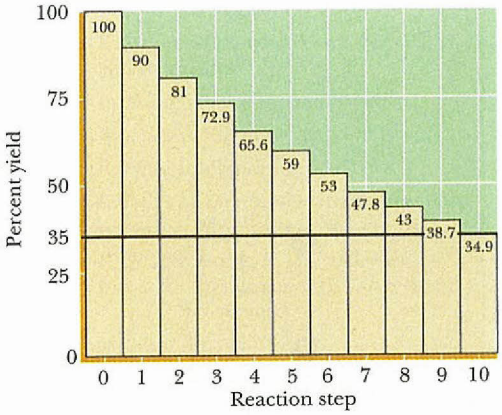


FIGURE 14.3 • A 90% yield over 10 steps, for example, in a metabolic pathway, gives an overall yield of 35%. Therefore, yields in biological reactions *must be substantially greater;* otherwise, unwanted by-products would accumulate to unacceptable levels.

Regulation

Regulation of enzyme activity is achieved in a variety of ways, ranging from controls over the amount of enzyme protein produced by the cell to more rapid, reversible interactions of the enzyme with metabolic inhibitors and activators. Chapter 15 is devoted to discussions of enzyme regulation. Because most enzymes are proteins, we can anticipate that the functional attributes of enzymes are due to the remarkable versatility found in protein structures.

Enzyme Nomenclature

Traditionally, enzymes often were named by adding the suffix *-ase* to the name of the substrate upon which they acted, as in *urease* for the urea-hydrolyzing enzyme or *phosphatase* for enzymes hydrolyzing phosphoryl groups from organic phosphate compounds. Other enzymes acquired names bearing little resemblance to their activity, such as the peroxide-decomposing enzyme *catalase* or the proteolytic enzymes (*proteases*) of the digestive tract, *trypsin* and *pepsin.* Because of the confusion that arose from these trivial designations, an International Commission on Enzymes was established in 1956 to create a systematic basis for enzyme nomenclature. Although common names for many enzymes remain in use, all enzymes now are classified and formally named according to the reaction they catalyze. Six classes of reactions are recognized (Table 14.1). Within each class are subclasses, and under each subclass are sub-subclasses within which individual enzymes are listed. Classes, subclasses, sub-subclasses, and individual entries are each numbered, so that a series of four numbers serves to specify a particular enzyme. A systematic name, descriptive

Table 14.1

Systematic Classification of Enzymes According to the Enzyme Commission

E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)
1.1	Acting on CH—OH group of donors
1.1.1	With NAD or NADP as acceptor
1.1.3	With O_2 as acceptor
1.2	Acting on the $\diagdown$C=O group of donors
1.2.3	With O_2 as $\diagup$acceptor
1.3	Acting on the CH—CH group of donors
1.3.1	With NAD or NADP as acceptor
2	*Transferases* (transfer of functional groups)
2.1	Transferring C-1 groups
2.1.1	Methyltransferases
2.1.2	Hydroxymethyltransferases and formyltransferases
2.1.3	Carboxyltransferases and carbamoyltransferases
2.2	Transferring aldehydic or ketonic residues
2.3	Acyltransferases
2.4	Glycosyltransferases
2.6	Transferring N-containing groups
2.6.1	Aminotransferases
2.7	Transferring P-containing groups
2.7.1	With an alcohol group as acceptor
3	*Hydrolases* (hydrolysis reactions)
3.1	Cleaving ester linkage
3.1.1	Carboxylic ester hydrolases
3.1.3	Phosphoric monoester hydrolases
3.1.4	Phosphoric diester hydrolases
4	*Lyases* (addition to double bonds)
4.1	C=C lyases
4.1.1	Carboxy lyases
4.1.2	Aldehyde lyases
4.2	C=O lyases
4.2.1	Hydrolases
4.3	C=N lyases
4.3.1	Ammonia-lyases
5	*Isomerases* (isomerization reactions)
5.1	Racemases and epimerases
5.1.3	Acting on carbohydrates
5.2	Cis-trans isomerases
6	*Ligases* (formation of bonds with ATP cleavage)
6.1	Forming C—O bonds
6.1.1	Amino acid–RNA ligases
6.2	Forming C—S bonds
6.3	Forming C—N bonds
6.4	Forming C—C bonds
6.4.1	Carboxylases

of the reaction, is also assigned to each entry. To illustrate, consider the enzyme that catalyzes this reaction:

$$\text{ATP} + \text{D-glucose} \longrightarrow \text{ADP} + \text{D-glucose-6-phosphate}$$

A phosphate group is transferred from ATP to the C-6-OH group of glucose, so the enzyme is a *transferase* (Class 2, Table 14.1). Subclass 7 of transferases is

enzymes transferring phosphorus-containing groups, and sub-subclass 1 covers those *phosphotransferases with an alcohol group as an acceptor.* Entry 2 in this sub-subclass is **ATP: D-glucose-6-phosphotransferase,** and its classification number is **2.7.1.2.** In use, this number is written preceded by the letters **E.C.,** denoting the Enzyme Commission. For example, entry 1 in the same sub-subclass is E.C.2.7.1.1, ATP: D-hexose-6-phosphotransferase, an ATP-dependent enzyme that transfers a phosphate to the 6-OH of hexoses (that is, it is nonspecific regarding its hexose acceptor). These designations can be cumbersome, so in everyday usage, trivial names are employed frequently. The glucose-specific enzyme, E.C.2.7.1.2, is called *glucokinase* and the nonspecific E.C.2.7.1.1 is known as *hexokinase. Kinase* is a trivial term for enzymes that are ATP-dependent phosphotransferases.

Coenzymes

Many enzymes carry out their catalytic function relying solely on their protein structure. Many others require nonprotein components, called **cofactors** (Table 14.2). Cofactors may be metal ions or organic molecules referred to as **coenzymes.** Cofactors, because they are structurally less complex than proteins, tend to be stable to heat (incubation in a boiling water bath). Typically, proteins are denatured under such conditions. Many coenzymes are vitamins or contain vitamins as part of their structure. Usually coenzymes are actively involved in the catalytic reaction of the enzyme, often serving as intermediate carriers of functional groups in the conversion of substrates to products. In most cases, a coenzyme is firmly associated with its enzyme, perhaps even by covalent bonds, and it is difficult to

Table 14.2

Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		Representative Enzymes Using Coenzymes
Metal Ion	Enzyme	Coenzyme	Entity Transferred	
Fe^{2+} or Fe^{3+}	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion (H^-)	Alcohol dehydrogenase
Cu^{2+}	Cytochrome oxidase			
Zn^{2+}	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
Mg^{2+}	Hexokinase			
	Glucose-6-phosphatase	Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
Mn^{2+}	Arginase	Tetrahydrofolate (THF)	Other one-carbon groups	Thymidylate synthase
K^+	Pyruvate kinase (also requires Mg^{2+})			
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

separate the two. Such tightly bound coenzymes are referred to as **prosthetic groups** of the enzyme. The catalytically active complex of protein and prosthetic group is called the **holoenzyme.** The protein without the prosthetic group is called the **apoenzyme;** it is catalytically inactive.

14.2 • Introduction to Enzyme Kinetics

Kinetics is the branch of science concerned with the rates of chemical reactions. The study of **enzyme kinetics** addresses the biological roles of enzymatic catalysts and how they accomplish their remarkable feats. In enzyme kinetics, we seek to determine the maximum reaction velocity that the enzyme can attain and its binding affinities for substrates and inhibitors. Coupled with studies on the structure and chemistry of the enzyme, analysis of the enzymatic rate under different reaction conditions yields insights regarding the enzyme's mechanism of catalytic action. Such information is essential to an overall understanding of metabolism.

Significantly, this information can be exploited to control and manipulate the course of metabolic events. The science of pharmacology relies on such a strategy. **Pharmaceuticals,** or **drugs,** are often special inhibitors specifically targeted at a particular enzyme in order to overcome infection or to alleviate illness. A detailed knowledge of the enzyme's kinetics is indispensable to rational drug design and successful pharmacological intervention.

Review of Chemical Kinetics

Before beginning a quantitative treatment of enzyme kinetics, it will be fruitful to review briefly some basic principles of chemical kinetics. **Chemical kinetics** is the study of the rates of chemical reactions. Consider a reaction of overall stoichiometry

$$A \longrightarrow P$$

Although we treat this reaction as a simple, one-step conversion of A to P, it more likely occurs through a sequence of elementary reactions, each of which is a simple molecular process, as in

$$A \longrightarrow I \longrightarrow J \longrightarrow P$$

where I and J represent intermediates in the reaction. Precise description of all of the elementary reactions in a process is necessary to define the overall reaction mechanism for $A \rightarrow P$.

Let us assume that $A \rightarrow P$ *is* an elementary reaction and that it is spontaneous and essentially irreversible. Irreversibility is easily assumed if the rate of P conversion to A is very slow *or* the concentration of P (expressed as [P]) is negligible under the conditions chosen. The **velocity,** v, or **rate,** of the reaction $A \rightarrow P$ is the amount of P formed or the amount of A consumed per unit time, t. That is,

$$v = \frac{d[P]}{dt} \quad \text{or} \quad v = \frac{-d[A]}{dt} \tag{14.1}$$

The mathematical relationship between reaction rate and concentration of reactant(s) is the **rate law.** For this simple case, the rate law is

$$v = \frac{-d[A]}{dt} = k[A] \tag{14.2}$$

From this expression, it is obvious that the rate is proportional to the concentration of A, and k is the proportionality constant, or **rate constant.** k has the units of $(\text{time})^{-1}$, usually sec^{-1}. v is a function of [A] to the first power, or, in the terminology of kinetics, v is first-order with respect to A. For an elementary reaction, the **order** for any reactant is given by its exponent in the rate equation. The number of molecules that must simultaneously interact is defined as the **molecularity** of the reaction. Thus, the simple elementary reaction of $A \rightarrow P$ is a **first-order reaction.** Figure 14.4 portrays the course of a first-order reaction as a function of time. The rate of decay of a radioactive isotope, like ^{14}C or ^{32}P, is a first-order reaction, as is an intramolecular rearrangement, such as $A \rightarrow P$. Both are **unimolecular reactions** (the molecularity equals 1).

Bimolecular Reactions

Consider the more complex reaction, where two molecules must react to yield products:

$$A + B \longrightarrow P + Q$$

Assuming this reaction is an elementary reaction, its molecularity is 2; that is, it is a **bimolecular reaction.** The velocity of this reaction can be determined from the rate of disappearance of either A or B, or the rate of appearance of P or Q:

$$v = \frac{-d[A]}{dt} = \frac{-d[B]}{dt} = \frac{d[P]}{dt} = \frac{d[Q]}{dt} \tag{14.3}$$

The rate law is

$$v = k[A][B] \tag{14.4}$$

The rate is proportional to the concentrations of both A and B. Because it is proportional to the product of two concentration terms, the reaction is **second-order** overall, first-order with respect to A and first-order with respect to B. (Were the elementary reaction $2A \rightarrow P + Q$, the rate law would be $v = k[A]^2$, second-order overall and second-order with respect to A.) Second-order rate constants have the units of $(\text{concentration})^{-1}(\text{time})^{-1}$, as in $M^{-1}\text{sec}^{-1}$.

Molecularities greater than two are rarely found (and greater than three, never). When the overall stoichiometry of a reaction is greater than two (for example, as in $A + B + C \rightarrow$ or $2A + B \rightarrow$), the reaction almost always proceeds via uni- or bimolecular elementary steps, and the overall rate obeys a simple first- or second-order rate law.

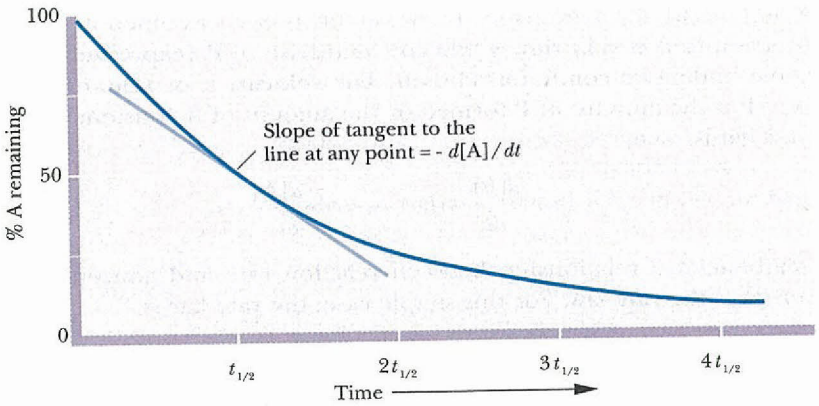


FIGURE 14.4 • Plot of the course of a first-order reaction. The half-time, $t_{1/2}$, is the time for one-half of the starting amount of A to disappear.

At this point, it may be useful to remind ourselves of an important caveat that is the first principle of kinetics: *Kinetics cannot prove a hypothetical mechanism.* Kinetic experiments can only rule out various alternative hypotheses because they don't fit the data. However, through thoughtful kinetic studies, a process of elimination of alternative hypotheses leads ever closer to the reality.

Free Energy of Activation and the Action of Catalysts

In a first-order chemical reaction, the conversion of A to P occurs because, at any given instant, a fraction of the A molecules has the energy necessary to achieve a reactive condition known as the **transition state.** In this state, the probability is very high that the particular rearrangement accompanying the A→P transition will occur. This transition state sits at the apex of the energy profile in the energy diagram describing the energetic relationship between A and P (Figure 14.5). The average free energy of A molecules defines the initial state and the average free energy of P molecules is the final state along the reaction coordinate. The rate of any chemical reaction is proportional to the concentration of reactant molecules (A in this case) having this transition-state energy. Obviously, the higher this energy is above the average energy, the smaller the fraction of molecules that will have this energy, and the slower the reaction will proceed. The height of this energy barrier is called the **free energy of activation, $\Delta G^{\ddagger}$.** Specifically, $\Delta G^{\ddagger}$ is the energy required to raise the average energy of one mole of reactant (at a given temperature) to the transition-state energy. The relationship between activation energy and the rate constant of the reaction, k, is given by the **Arrhenius equation:**

$$k = Ae^{-\Delta G^{\ddagger}/RT} \tag{14.5}$$

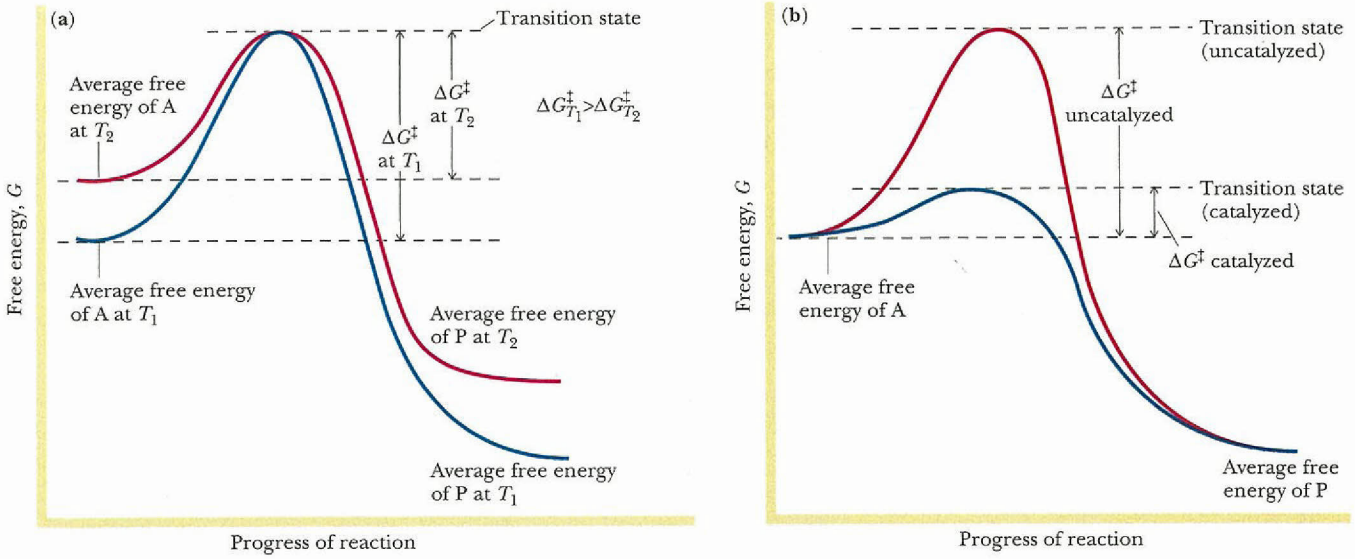


FIGURE 14.5 • Energy diagram for a chemical reaction (A→P) and the effects of (a) raising the temperature from T_1 to T_2 or (b) adding a catalyst. Raising the temperature raises the average energy of A molecules, which increases the population of A molecules having energies equal to the activation energy for the reaction, thereby increasing the reaction rate. In contrast, the average free energy of A molecules remains the same in uncatalyzed versus catalyzed reactions (conducted at the same temperature). The effect of the catalyst is to lower the free energy of activation for the reaction.

where A is a constant for a particular reaction (not to be confused with the reactant species, A, that we're discussing). Another way of writing this is $1/k = (1/A)\,e^{\Delta G^{\ddagger}/RT}$. That is, k is inversely proportional to $e^{\Delta G^{\ddagger}/RT}$. Therefore, if the energy of activation decreases, the reaction rate increases.

Decreasing $\Delta G^{\ddagger}$ Increases Reaction Rate

We are familiar with two general ways that rates of chemical reactions may be accelerated. First, the temperature can be raised. This will increase the average energy of reactant molecules, which in effect lowers the energy needed to reach the transition state (Figure 14.5a). The rates of many chemical reactions are doubled by a 10°C rise in temperature. Second, the rates of chemical reactions can also be accelerated by **catalysts.** Catalysts work by lowering the energy of activation rather than by raising the average energy of the reactants (Figure 14.5b). Catalysts accomplish this remarkable feat by combining transiently with the reactants in a way that promotes their entry into the reactive, transition-state condition. Two aspects of catalysts are worth noting: (a) they are regenerated after each reaction cycle (A → P), and so can be used over and over again; and (b) catalysts have *no* effect on the overall free energy change in the reaction, the free energy difference between A and P (Figure 14.5b).

14.3 • Kinetics of Enzyme-Catalyzed Reactions

Examination of the change in reaction velocity as the reactant concentration is varied is one of the primary measurements in kinetic analysis. Returning to A → P, a plot of the reaction rate as a function of the concentration of A yields a straight line whose slope is k (Figure 14.6). The more A that is available, the greater the rate of the reaction, v. Similar analyses of enzyme-catalyzed reactions involving only a single substrate yield remarkably different results (Figure 14.7). At low concentrations of the substrate S, v is proportional to [S], as expected for a first-order reaction. However, v does not increase proportionally as [S] increases, but instead begins to level off. At high [S], v becomes virtually independent of [S] and approaches a maximal limit. The value of v at this limit is written V_{max}. Because rate is no longer dependent on [S] at these high concentrations, the enzyme-catalyzed reaction is now obeying **zero-order**

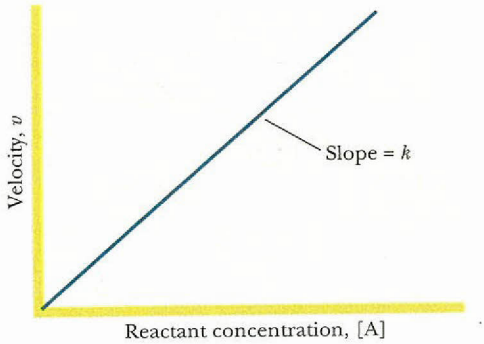


FIGURE 14.6 • A plot of v versus [A] for the unimolecular chemical reaction, A → P, yields a straight line having a slope equal to k.

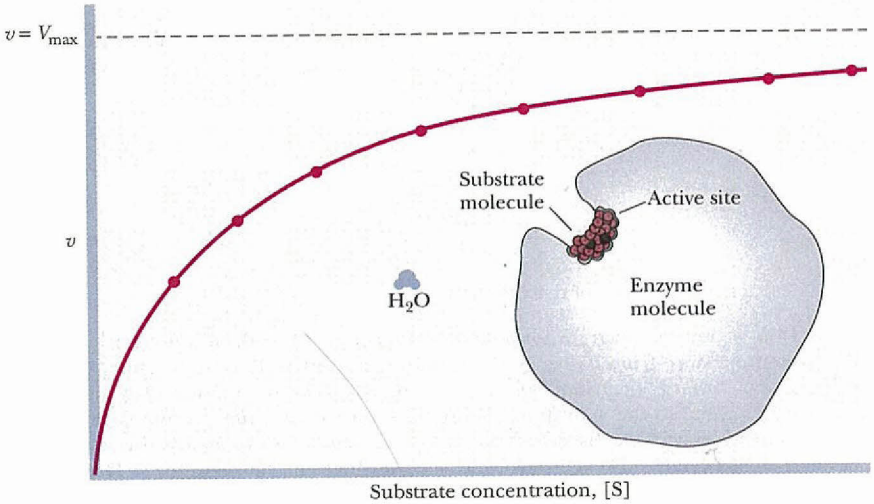


FIGURE 14.7 • Substrate saturation curve for an enzyme-catalyzed reaction. The amount of enzyme is constant, and the velocity of the reaction is determined at various substrate concentrations. The reaction rate, v, as a function of [S] is described by a rectangular hyperbola. At very high [S], $v = V_{max}$. That is, the velocity is limited only by conditions (temperature, pH, ionic strength) and by the amount of enzyme present; v becomes independent of [S]. Such a condition is termed *zero-order kinetics.* Under zero-order conditions, velocity is directly dependent on [enzyme]. The H_2O molecule provides a rough guide to scale. The substrate is bound at the active site of the enzyme.

kinetics; that is, the rate is independent of the reactant (substrate) concentration. This behavior is a **saturation effect:** when v shows no increase even though [S] is increased, the system is saturated with substrate. Such plots are called **substrate saturation curves.** The physical interpretation is that every enzyme molecule in the reaction mixture has its substrate-binding site occupied by S. Indeed, such curves were the initial clue that an enzyme interacts directly with its substrate by binding it.

The Michaelis–Menten Equation

Lenore Michaelis and Maud L. Menten proposed a general theory of enzyme action in 1913 consistent with observed enzyme kinetics. Their theory was based on the assumption that the enzyme, E, and its substrate, S, associate reversibly to form an enzyme-substrate complex, ES:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \tag{14.6}$$

This association/dissociation is assumed to be a rapid equilibrium, and K_s is the *enzyme:substrate dissociation constant.* At equilibrium,

$$k_{-1}[ES] = k_1[E][S] \tag{14.7}$$

and

$$K_S = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} \tag{14.8}$$

Product, P, is formed in a second step when ES breaks down to yield E + P.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \tag{14.9}$$

E is then free to interact with another molecule of S.

Steady-State Assumption

The interpretations of Michaelis and Menten were refined and extended in 1925 by Briggs and Haldane, by assuming the concentration of the enzyme–substrate complex ES quickly reaches a constant value in such a dynamic system. That is, ES is formed as rapidly from E + S as it disappears by its two possible fates: dissociation to regenerate E + S, and reaction to form E + P. This assumption is termed the **steady-state assumption** and is expressed as

$$\frac{d[ES]}{dt} = 0 \tag{14.10}$$

That is, the change in concentration of ES with time, t, is 0. Figure 14.8 illustrates the time course for formation of the ES complex and establishment of the steady-state condition.

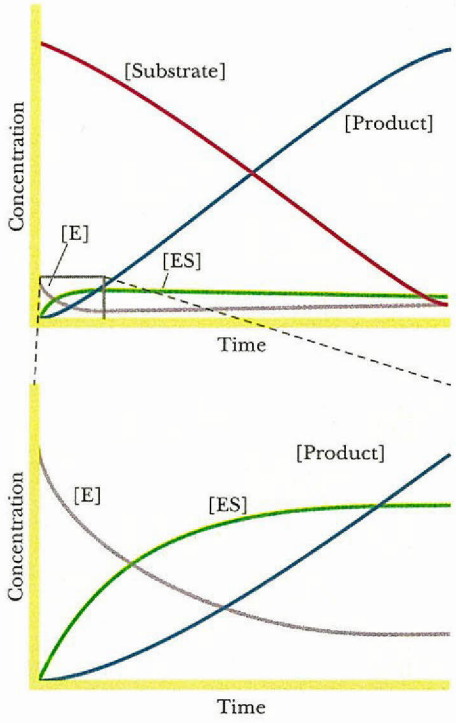


FIGURE 14.8 • Time course for the consumption of substrate, the formation of product, and the establishment of a steady-state level of the enzyme-substrate [ES] complex for a typical enzyme obeying the Michaelis–Menten, Briggs–Haldane models for enzyme kinetics. The early stage of the time course is shown in greater magnification in the bottom graph.

Initial Velocity Assumption

One other simplification will be advantageous. Because enzymes accelerate the rate of the reverse reaction as well as the forward reaction, it would be helpful to ignore any back reaction by which E + P might form ES. The velocity of this back reaction would be given by $v = k_{-2}[E][P]$. However, if we observe only the *initial velocity* for the reaction immediately after E and S are mixed in the absence of P, the rate of any back reaction is negligible because its rate will

be proportional to [P], and [P] is essentially 0. Given such simplification, we now analyze the system described by Equation (14.9) in order to describe the initial velocity v as a function of [S] and amount of enzyme.

The total amount of enzyme is fixed and is given by the formula

$$\text{Total enzyme, } [E_T] = [E] + [ES] \tag{14.11}$$

where [E] = free enzyme and [ES] = the amount of enzyme in the enzyme–substrate complex. From Equation (14.9), the rate of [ES] formation is

$$v_f = k_1([E_T] - [ES])[S]$$

where

$$[E_T] - [ES] = [E] \tag{14.12}$$

From Equation (14.9), the rate of [ES] disappearance is

$$v_d = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES] \tag{14.13}$$

At steady state, $d[ES]/dt = 0$, and therefore, $v_f = v_d$.
So,

$$k_1([E_T] - [ES])[S] = (k_{-1} + k_2)[ES] \tag{14.14}$$

Rearranging gives

$$\frac{([E_T] - [ES])[S]}{[ES]} = \frac{(k_{-1} + k_2)}{k_1} \tag{14.15}$$

The Michaelis Constant, K_m

The ratio of constants $(k_{-1} + k_2)/k_1$ is itself a constant and is defined as the **Michaelis constant, K_m**

$$K_m = \frac{(k_{-1} + k_2)}{k_1} \tag{14.16}$$

Note from (14.15) that K_m is given by the ratio of two concentrations (([E_T] − [ES]) and [S]) to one ([ES]), so K_m has the units of *molarity*. From Equation (14.15), we can write

$$\frac{([E_T] - [ES])[S]}{[ES]} = K_m \tag{14.17}$$

which rearranges to

$$[ES] = \frac{[E_T]\,[S]}{K_m + [S]} \tag{14.18}$$

Now, the most important parameter in the kinetics of any reaction is the **rate of product formation.** This rate is given by

$$v = \frac{d[P]}{dt} \tag{14.19}$$

and for this reaction

$$v = k_2[ES] \tag{14.20}$$

Substituting the expression for [ES] from Equation (14.18) into (14.20) gives

$$v = \frac{k_2[E_T]\,[S]}{K_m + [S]} \tag{14.21}$$

The product $k_2[E_T]$ has special meaning. When [S] is high enough to saturate all of the enzyme, the velocity of the reaction, v, is maximal. At saturation, the

amount of [ES] complex is equal to the total enzyme concentration, E_T, its maximum possible value. From Equation (14.20), the initial velocity v then equals $k_2[E_T] = V_{max}$. Written symbolically, when $[S] \gg [E_T]$ (and K_m), $[E_T] = [ES]$ and $v = V_{max}$. Therefore,

$$V_{max} = k_2[E_T] \tag{14.22}$$

Substituting this relationship into the expression for v gives the **Michaelis–Menten equation**

$$v = \frac{V_{max}[S]}{K_m + [S]} \tag{14.23}$$

This equation says that the rate of an enzyme-catalyzed reaction, v, at any moment is determined by two constants, K_m and V_{max}, *and* the concentration of substrate at that moment.

When $[S] = K_m$, $v = V_{max}/2$

We can provide an operational definition for the constant K_m by rearranging Equation (14.23) to give

$$K_m = [S]\left(\frac{V_{max}}{v} - 1\right) \tag{14.24}$$

Then, at $v = V_{max}/2$, $K_m = [S]$. That is, K_m is defined by the substrate concentration that gives a velocity equal to one-half the maximal velocity. Table 14.3 gives the K_m values of some enzymes for their substrates.

Relationships Between V_{max}, K_m, and Reaction Order

The Michaelis–Menten equation (14.23) describes a curve known from analytical geometry as a *rectangular hyperbola.*[1] In such curves, as [S] is increased, v approaches the limiting value, V_{max}, in an asymptotic fashion. V_{max} can be approximated experimentally from a substrate saturation curve (Figure 14.7), and K_m can be derived from $V_{max}/2$, so the two constants of the Michaelis–Menten equation can be obtained from plots of v versus [S]. Note, however, that actual estimation of V_{max}, and consequently K_m, is only approximate from such graphs. That is, according to Equation (14.23), in order to get $v = 0.99\ V_{max}$, [S] must equal 99 K_m, a concentration that may be difficult to achieve in practice.

From Equation (14.23), when $[S] \gg K_m$, then $v = V_{max}$. That is, v is no longer dependent on [S], so the reaction is obeying zero-order kinetics. Also, when $[S] < K_m$, then $v \approx (V_{max}/K_m)[S]$. That is, the rate, v, approximately follows a first-order rate equation, $v = k'[A]$, where $k' = V_{max}/K_m$.

K_m and V_{max}, once known explicitly, define the rate of the enzyme-catalyzed reaction, *provided:*

1. The reaction involves only one substrate, *or* if the reaction is multisubstrate, the concentration of only one substrate is varied while the concentration of all other substrates is held constant.
2. The reaction $ES \rightarrow E + P$ is irreversible, *or* the experiment is limited to observing only initial velocities where $[P] = 0$.
3. $[S]_0 > [E_T]$ and $[E_T]$ is held constant.
4. All other variables that might influence the rate of the reaction (temperature, pH, ionic strength, and so on) are constant.

[1] A proof that the Michaelis–Menten equation describes a rectangular hyperbola is given by Naqui, A., 1986. Where are the asymptotes of Michaelis–Menten? *Trends in Biochemical Sciences* 11:64–65.

Table 14.3

K_m Values for Some Enzymes

Enzyme	Substrate	K_m (m*M*)
Carbonic anhydrase	CO_2	12
Chymotrypsin	*N*-Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	N-Formyltyrosinamide	12
	N-Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
β-Galactosidase	Lactose	4
Glutamate dehydrogenase	NH_4^+	57
	Glutamate	0.12
	α-Ketoglutarate	2
	NAD^+	0.025
	NADH	0.018
Aspartate aminotransferase	Aspartate	0.9
	α-Ketoglutarate	0.1
	Oxaloacetate	0.04
	Glutamate	4
Threonine deaminase	Threonine	5
Arginyl-tRNA synthetase	Arginine	0.003
	$tRNA^{Arg}$	0.0004
	ATP	0.3
Pyruvate carboxylase	HCO_3^-	1.0
	Pyruvate	0.4
	ATP	0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa-*N*-acetylglucosamine	0.006

Enzyme Units

In many situations, the actual molar amount of the enzyme is not known. However, its amount can be expressed in terms of the activity observed. The International Commission on Enzymes defines **One International Unit** of enzyme as *the amount that catalyzes the formation of one micromole of product in one minute.* (Because enzymes are very sensitive to factors such as pH, temperature, and ionic strength, the conditions of assay must be specified.) Another definition for units of enzyme activity is the **katal.** One katal is *that amount of enzyme catalyzing the conversion of one mole of substrate to product in one second.* Thus, one katal equals 6×10^7 international units.

Turnover Number

The **turnover number** of an enzyme, k_{cat}, is a measure of its maximal catalytic activity. k_{cat} is defined as the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate. The turnover number is also referred to as the **molecular activity** of the enzyme. For the simple Michaelis–Menten reaction (14.9) under conditions of initial velocity measurements, $k_2 = k_{cat}$. Provided the concentration of

enzyme, $[E_T]$, in the reaction mixture is known, k_{cat} can be determined from V_{max}. At saturating [S], $v = V_{max} = k_2[E_T]$. Thus,

$$k_2 = \frac{V_{max}}{[E_T]} = k_{cat} \tag{14.25}$$

The term k_{cat} represents the kinetic efficiency of the enzyme. Table 14.4 lists turnover numbers for some representative enzymes. Catalase has the highest turnover number known; each molecule of this enzyme can degrade 40 million molecules of H_2O_2 in one second! At the other end of the scale, lysozyme requires 2 seconds to cleave a glycosidic bond in its glycan substrate.

Table 14.4

Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

k_{cat}/K_m

Under physiological conditions, [S] is seldom saturating, and k_{cat} itself is not particularly informative. That is, the *in vivo* ratio of $[S]/K_m$ usually falls in the range of 0.01 to 1.0, so active sites often are not filled with substrate. Nevertheless, we can derive a meaningful index of the efficiency of Michaelis–Menten-type enzymes under these conditions by employing the following equations. As presented in Equation (14.23), if

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

and $V_{max} = k_{cat}[E_T]$, then

$$v = \frac{k_{cat}[E_T][S]}{K_m + [S]} \tag{14.26}$$

When $[S] \ll K_m$, the concentration of free enzyme, [E], is approximately equal to $[E_T]$, so that

$$v = \left(\frac{k_{cat}}{K_m}\right)[E][S] \tag{14.27}$$

That is, k_{cat}/K_m is an *apparent second-order rate constant* for the reaction of E and S to form product. Because K_m is inversely proportional to the affinity of the enzyme for its substrate and k_{cat} is directly proportional to the kinetic efficiency of the enzyme, k_{cat}/K_m provides an index of the catalytic efficiency of an enzyme operating at substrate concentrations substantially below saturation amounts.

An interesting point emerges if we restrict ourselves to the simple case where $k_{cat} = k_2$. Then

$$\frac{k_{cat}}{K_m} = \frac{k_1 k_2}{(k_{-1} + k_2)} \tag{14.28}$$

But k_1 must always be greater than or equal to $k_1k_2/(k_{-1} + k_2)$. That is, the reaction can go no faster than the rate at which E and S come together. Thus, k_1 sets the upper limit for k_{cat}/K_m. In other words, *the catalytic efficiency of an enzyme cannot exceed the diffusion-controlled rate of combination of E and S to form ES.* In H_2O, the rate constant for such diffusion is approximately $10^9/M \cdot$ sec. Those enzymes that are most efficient in their catalysis have k_{cat}/K_m ratios approaching this value. Their catalytic velocity is limited only by the rate at which they encounter S; enzymes this efficient have achieved so-called *catalytic perfection.* All E and S encounters lead to reaction because such "catalytically perfect" enzymes can channel S to the active site, regardless of where S hits E. Table 14.5 lists the kinetic parameters of several enzymes in this category. Note that k_{cat} and K_m both show a substantial range of variation in this table, even though their ratio falls around $10^8/M \cdot$ sec.

Table 14.5

Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($sec^{-1}\ M^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	0.012	8.3×10^7
	HCO_3^-	4×10^5	0.026	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^3	1.8×10^{-5}	2.4×10^8
β-Lactamase	Benzylpenicillin	2×10^3	2×10^{-5}	1×10^8

*K_m for glyceraldehyde-3-phosphate is calculated on the basis that only 3.8% of the substrate in solution is unhydrated and therefore reactive with the enzyme.

Adapted from Fersht, A. 1985. *Enzyme Structure and Mechanism*, 2nd ed. New York: W.H. Freeman & Co.

Linear Plots Can Be Derived from the Michaelis–Menten Equation

Because of the hyperbolic shape of v versus [S] plots, V_{max} can only be determined from an extrapolation of the asymptotic approach of v to some limiting value as [S] increases indefinitely (Figure 14.7); and K_m is derived from that value of [S] giving $v = V_{max}/2$. However, several rearrangements of the Michaelis–Menten equation transform it into a straight-line equation. The best known of these is the **Lineweaver–Burk double-reciprocal plot:**

Taking the reciprocal of both sides of the Michaelis–Menten equation, Equation (14.23), yields the equality

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}} \tag{14.29}$$

This conforms to $y = mx + b$ (the equation for a straight line), where $y = 1/v$; m, the slope, is K_m/V_{max}; $x = 1/[S]$; and $b = 1/V_{max}$. Plotting $1/v$ versus $1/[S]$ gives a straight line whose x-intercept is $-1/K_m$, whose y-intercept is $1/V_{max}$, and whose slope is K_m/V_{max} (Figure 14.9).

The **Hanes–Woolf plot** is another rearrangement of the Michaelis–Menten equation that yields a straight line:

Multiplying both sides of Equation (14.29) by [S] gives

$$\frac{[S]}{v} = [S]\left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{[S]}{V_{max}} = \frac{K_m}{V_{max}} + \frac{[S]}{V_{max}} \tag{14.30}$$

and

$$\frac{[S]}{v} = \left(\frac{1}{V_{max}}\right)[S] + \frac{K_m}{V_{max}} \tag{14.31}$$

Graphing $[S]/v$ versus [S] yields a straight line where the slope is $1/V_{max}$, the

An Example of the Effect of Amino Acid Substitutions on K_m and k_{cat}: Wild-Type and Mutant Forms of Human Sulfite Oxidase

Mammalian sulfite oxidase is the last enzyme in the pathway for degradation of sulfur-containing amino acids. Sulfite oxidase (SO) catalyzes the oxidation of sulfite (SO_3^{2-}) to sulfate (SO_4^{2-}), using the heme-containing protein, cytochrome *c*, as electron acceptor:

$$SO_3^{2-} + 2 \text{ cytochrome } c_{oxidized} + H_2O \rightleftharpoons SO_4^{2-} + 2 \text{ cytochrome } c_{reduced} + 2\ H^+$$

Isolated sulfite oxidase deficiency is a rare and often fatal genetic disorder in humans. The disease is characterized by severe neurological abnormalities, revealed as convulsions shortly after birth. R. M. Garrett and K. V. Rajagopalan at Duke University Medical Center have isolated the human cDNA for sulfite oxidase from the cells of normal (*wild-type*) and SO-deficient individuals. Expression of these SO cDNAs in transformed *Escherichia coli* cells allowed the isolation and kinetic analysis of wild-type and mutant forms of SO, including one (designated R160Q) in which the Arg at position 160 in the polypeptide chain is replaced by Gln. A genetically engineered version of SO (designated R160K) in which Lys replaces Arg^{160} was also studied.

Kinetic Constants for Wild-Type and Mutant Sulfite Oxidase

Enzyme	$K_m^{sulfite}$ (μM)	k_{cat}(sec^{-1})	k_{cat}/K_m ($10^6\ M^{-1}sec^{-1}$)
Wild-type	17	18	1.1
R160Q	1900	3	0.0016
R160K	360	5.5	0.015

Replacing R^{160} in sulfite oxidase by Q increases K_m, decreases k_{cat}, and markedly diminishes the catalytic efficiency (k_{cat}/K_m) of the enzyme. The R160K mutant enzyme has properties intermediate between wild-type and the R160Q mutant form. The substrate, SO_3^{2-}, is strongly anionic, and R^{160} is one of several Arg residues situated within the SO substrate-binding site. Positively charged side chains in the substrate-binding site facilitate SO_3^{2-} binding and catalysis, with Arg being optimal in this role.

y-intercept is K_m/V_{max}, and the *x*-intercept is $-K_m$, as shown in Figure 14.10. The common advantage of these plots is that they allow both K_m and V_{max} to be accurately estimated by extrapolation of straight lines rather than asymptotes. Computer fitting of v versus [S] data to the Michaelis–Menten equation is more commonly done than graphical plotting.

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

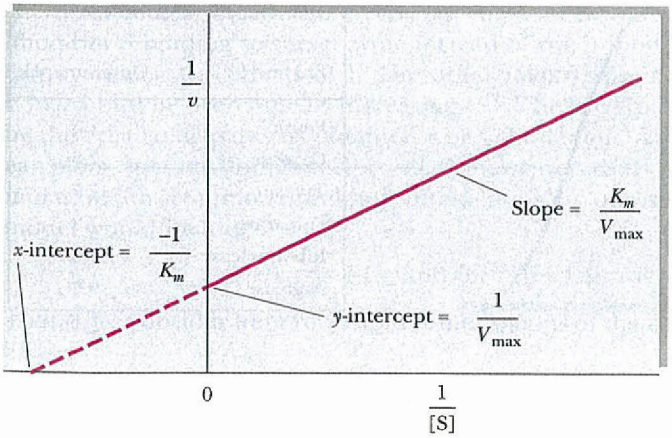


FIGURE 14.9 • The Lineweaver–Burk double-reciprocal plot, depicting extrapolations that allow the determination of the *x*- and *y*-intercepts and slope.

$$\frac{[S]}{v} = \left(\frac{1}{V_{max}}\right)[S] + \frac{K_m}{V_{max}}$$

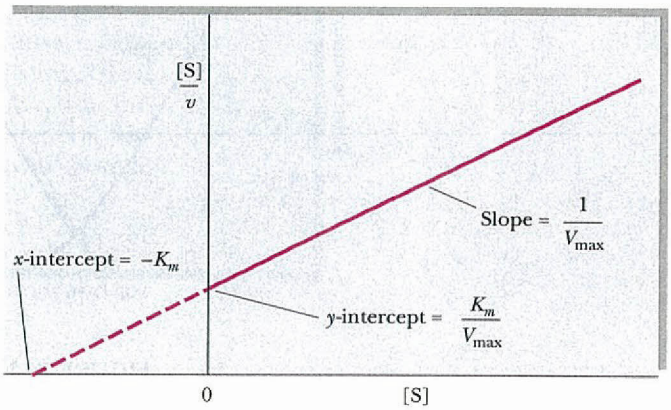


FIGURE 14.10 • A Hanes–Wolff plot of $[S]/v$ versus [S], another straight-line rearrangement of the Michaelis–Menten equation.

Departures from Linearity: A Hint of Regulation?

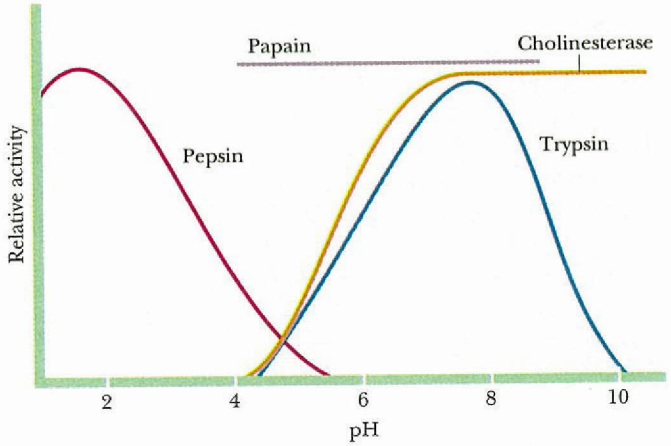
If the kinetics of the reaction disobey the Michaelis–Menten equation, the violation is revealed by a departure from linearity in these straight-line graphs. We shall see in the next chapter that such deviations from linearity are characteristic of the kinetics of regulatory enzymes known as **allosteric enzymes.** Such regulatory enzymes are very important in the overall control of metabolic pathways.

Effect of pH on Enzymatic Activity

Enzyme-substrate recognition and the catalytic events that ensue are greatly dependent on pH. An enzyme possesses an array of ionizable side chains and prosthetic groups that not only determine its secondary and tertiary structure but may also be intimately involved in its active site. Further, the substrate itself often has ionizing groups, and one or another of the ionic forms may preferentially interact with the enzyme. Enzymes in general are active only over a limited pH range and most have a particular pH at which their catalytic activity is optimal. These effects of pH may be due to effects on K_m or V_{max} or both. Figure 14.11 illustrates the relative activity of four enzymes as a function of pH. Although the pH optimum of an enzyme often reflects the pH of its normal environment, the optimum may not be precisely the same. This difference suggests that the pH-activity response of an enzyme may be a factor in the intracellular regulation of its activity.

Effect of Temperature on Enzymatic Activity

Like most chemical reactions, the rates of enzyme-catalyzed reactions generally increase with increasing temperature. However, at temperatures above 50° to 60°C, enzymes typically show a decline in activity (Figure 14.12). Two effects are operating here: (a) the characteristic increase in reaction rate with temperature, and (b) thermal denaturation of protein structure at higher tem-



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

FIGURE 14.11 • The pH activity profiles of four different enzymes. *Trypsin,* an intestinal protease, has a slightly alkaline pH optimum, whereas *pepsin,* a gastric protease, acts in the acidic confines of the stomach and has a pH optimum near 2. *Papain,* a protease found in papaya, is relatively insensitive to pHs between 4 and 8. *Cholinesterase* activity is pH-sensitive below pH 7 but not between pH 7 and 10. The cholinesterase pH activity profile suggests that an ionizable group with a pK' near 6 is essential to its activity. Might it be a histidine residue within the active site?

peratures. Most enzymatic reactions double in rate for every 10°C rise in temperature (that is, $Q_{10} = 2$, where Q_{10} is defined as *the ratio of activities at two temperatures 10° apart*) as long as the enzyme is stable and fully active. Some enzymes, those catalyzing reactions having very high activation energies, show proportionally greater Q_{10} values. The increasing rate with increasing temperature is ultimately offset by the instability of higher orders of protein structure at elevated temperatures, where the enzyme is inactivated. Not all enzymes are quite so thermally labile. For example, the enzymes of thermophilic bacteria (*thermophilic* = "heat-loving") found in geothermal springs retain full activity at temperatures in excess of 85°C.

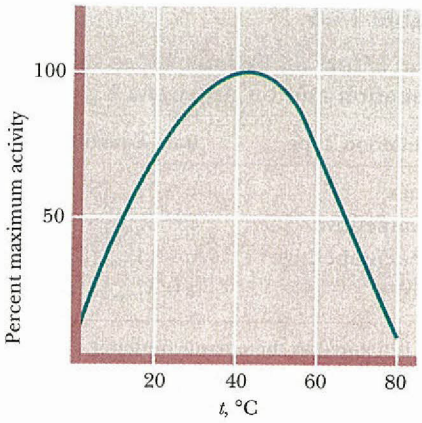


FIGURE 14.12 • The effect of temperature on enzyme activity. The relative activity of an enzymatic reaction as a function of temperature. The decrease in the activity above 50°C is due to thermal denaturation.

14.4 • Enzyme Inhibition

If the velocity of an enzymatic reaction is decreased or **inhibited,** the kinetics of the reaction obviously have been perturbed. Systematic perturbations are a basic tool of experimental scientists; much can be learned about the normal workings of any system by inducing changes in it and then observing the effects of the change. The study of enzyme inhibition has contributed significantly to our understanding of enzymes.

Reversible Versus Irreversible Inhibition

Enzyme inhibitors are classified in several ways. The inhibitor may interact either reversibly or irreversibly with the enzyme. **Reversible inhibitors** interact with the enzyme through noncovalent association/dissociation reactions. In contrast, **irreversible inhibitors** usually cause stable, covalent alterations in the enzyme. That is, the consequence of irreversible inhibition is a decrease in the concentration of active enzyme. The kinetics observed are consistent with this interpretation, as we shall see later.

Reversible Inhibition

Reversible inhibitors fall into two major categories: competitive and noncompetitive (although other more unusual and rare categories are known). **Competitive inhibitors** are characterized by the fact that the substrate and inhibitor compete for the same binding site on the enzyme, the so-called **active site** or **S-binding site.** Thus, increasing the concentration of S favors the likelihood of S binding to the enzyme instead of the inhibitor, I. That is, high [S] can overcome the effects of I. The other major type, noncompetitive inhibition, cannot be overcome by increasing [S]. The two types can be distinguished by the particular patterns obtained when the kinetic data are analyzed in linear plots, such as double-reciprocal (Lineweaver–Burk) plots. A general formulation for common inhibitor interactions in our simple enzyme kinetic model would include

$$\mathrm{E} + \mathrm{I} \rightleftharpoons \mathrm{EI} \quad \text{and/or} \quad \mathrm{I} + \mathrm{ES} \rightleftharpoons \mathrm{IES} \tag{14.32}$$

That is, we consider here reversible combinations of the inhibitor with E and/or ES.

Competitive Inhibition

Consider the following system

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad \mathrm{E} + \mathrm{I} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{EI} \tag{14.33}$$

Table 14.6

The Effect of Various Types of Inhibitors on the Michaelis–Menten Rate Equation and on Apparent K_m and Apparent V_{max}

Inhibition Type	Rate Equation	Apparent K_m	Apparent V_{max}
None	$v = V_{max}[S]/(K_m + [S])$	K_m	V_{max}
Competitive	$v = V_{max}[S]/([S] + K_m(1 + [I]/K_I))$	$K_m(1 + [I]/K_I)$	V_{max}
Noncompetitive	$v = (V_{max}[S]/(1 + [I]/K_I))/(K_m + [S])$	K_m	$V_{max}/(1 + [I]/K_I)$
Mixed	$v = V_{max}[S]/((1 + [I]/K_I)K_m + (1 + [I]/K_I'[S]))$	$K_m(1 + [I]/K_I)/(1 + [I]/K_I')$	$V_{max}/(1 + [I]/K_I')$

K_I is defined as the enzyme:inhibitor dissociation constant $K_I = [E][I]/[EI]$; K_I' is defined as the enzyme substrate complex : inhibitor dissociation constant $K_I' = [ES][I]/[ESI]$

where an inhibitor, I, binds *reversibly* to the enzyme at the same site as S. S-binding and I-binding are mutually exclusive, *competitive* processes. Formation of the ternary complex, EIS, where both S and I are bound, is physically impossible. This condition leads us to anticipate that S and I must share a high degree of structural similarity because they bind at the same site on the enzyme. Also notice that, in our model, EI does not react to give rise to E + P. That is, I is not changed by interaction with E. The rate of the product-forming reaction is $v = k_2[ES]$.

It is revealing to compare the equation for the uninhibited case, Equation (14.23) (the Michaelis–Menten equation) with Equation (14.43) for the rate of the enzymatic reaction in the presence of a fixed concentration of the competitive inhibitor, [I]

$$v = \frac{V_{max}[S]}{[S] + K_m}$$

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \dfrac{[I]}{K_I}\right)}$$

(see also Table 14.6). The K_m term in the denominator in the inhibited case is increased by the factor $(1 + [I]/K_I)$; thus, v is less in the presence of the inhibitor, as expected. Clearly, in the absence of I, the two equations are identical. Figure 14.13 shows a Lineweaver–Burk plot of competitive inhibition.

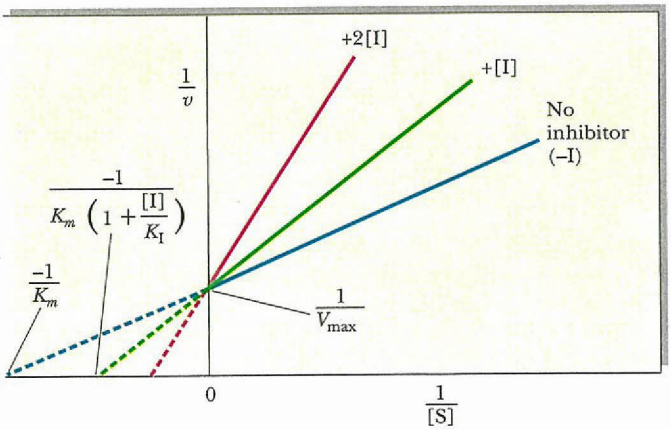


FIGURE 14.13 • Lineweaver–Burk plot of competitive inhibition, showing lines for no I, [I], and 2[I]. Note that when [S] is infinitely large $(1/[S] = 0)$, V_{max} is the same, whether I is present or not. In the presence of I, the negative x-intercept $= -1/K_m(1 + [I]/K_I)$.

A DEEPER LOOK

The Equations of Competitive Inhibition

Given the relationships between E, S, and I described previously and recalling the steady-state assumption that $d[ES]/dt = 0$, from Equations (14.14) and (14.16) we can write

$$[ES] = \frac{k_1[E][S]}{(k_2 + k_{-1})} = \frac{[E][S]}{K_m} \tag{14.34}$$

Assuming that $E + I \rightleftharpoons EI$ reaches rapid equilibrium, the rate of EI formation, $v_f' = k_3[E][I]$, and the rate of disappearance of EI, $v_d' = k_{-3}[EI]$, are equal. So,

$$k_3[E][I] = k_{-3}[EI] \tag{14.35}$$

Therefore,

$$[EI] = \left(\frac{k_3}{k_{-3}}\right)[E][I] \tag{14.36}$$

If we define K_I as k_{-3}/k_3, an *enzyme-inhibitor dissociation constant,* then

$$[EI] = \frac{[E][I]}{K_I} \tag{14.37}$$

knowing $[E_T] = [E] + [ES] + [EI]$.
Then

$$[E_T] = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_I} \tag{14.38}$$

Solving for [E] gives

$$[E] = \frac{K_I K_m[E_T]}{(K_I K_m + K_I[S] + K_m[I])} \tag{14.39}$$

Because the rate of product formation is given by $v = k_2[ES]$, from Equation (14.34) we have

$$v = \frac{k_2[E][S]}{K_m} \tag{14.40}$$

So,

$$v = \frac{(k_2 K_I[E_T][S])}{(K_I K_m + K_I[S] + K_m[I])} \tag{14.41}$$

Because $V_{max} = k_2[E_T]$,

$$v = \frac{V_{max}[S]}{K_m + [S] + \dfrac{K_m[I]}{K_I}} \tag{14.42}$$

or

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \dfrac{[I]}{K_I}\right)} \tag{14.43}$$

Several features of competitive inhibition are evident. First, at a given [I], v decreases ($1/v$ increases). When [S] becomes infinite, $v = V_{max}$ and is unaffected by I because all of the enzyme is in the ES form. Note that the value of the $-x$-intercept decreases as [I] increases. This $-x$-intercept is often termed the *apparent* K_m (or K_{mapp}) because it is the K_m apparent under these conditions. The diagnostic criterion for competitive inhibition is that V_{max} is unaffected by I; that is, all lines share a common y-intercept. This criterion is also the best experimental indication of binding at the same site by two substances. Competitive inhibitors resemble S structurally.

Succinate Dehydrogenase—A Classic Example of Competitive Inhibition

The enzyme *succinate dehydrogenase (SDH)* is competitively inhibited by malonate. Figure 14.14 shows the structures of succinate and malonate. The structural similarity between them is obvious and is the basis of malonate's ability to mimic succinate and bind at the active site of SDH. However, unlike succinate, which is oxidized by SDH to form fumarate, malonate cannot lose two hydrogens; consequently, it is unreactive.

Noncompetitive Inhibition

Noncompetitive inhibitors interact with both E and ES (or with S and ES, but this is a rare and specialized case). Obviously, then, the inhibitor is not binding to the same site as S, and the inhibition cannot be overcome by raising [S]. There are two types of noncompetitive inhibition: pure and mixed.

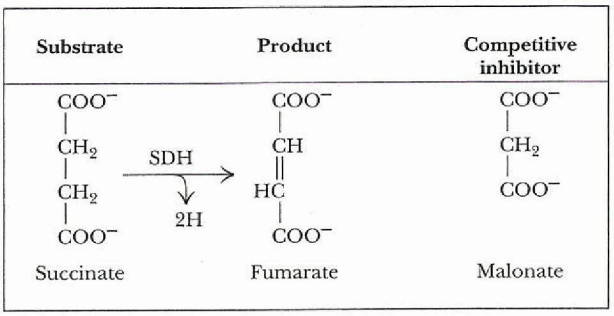


FIGURE 14.14 • Structures of succinate, the substrate of succinate dehydrogenase (SDH), and malonate, the competitive inhibitor. Fumarate (the product of SDH action on succinate) is also shown.

Pure Noncompetitive Inhibition

In this situation, the binding of I by E has no effect on the binding of S by E. That is, S and I bind at different sites on E, and binding of I does not affect binding of S. Consider the system

$$E + I \underset{}{\overset{K_I}{\rightleftharpoons}} EI \qquad ES + I \overset{K_I'}{\rightleftharpoons} IES \tag{14.44}$$

Pure noncompetitive inhibition occurs if $K_I = K_I'$. This situation is relatively uncommon; the Lineweaver–Burk plot for such an instance is given in Figure 14.15. Note that K_m is unchanged by I (the x-intercept remains the same, with or without I). Note also that V_{max} decreases. A similar pattern is seen if the amount of enzyme in the experiment is decreased. Thus, it is as if I lowered [E].

Mixed Noncompetitive Inhibition

In this situation, the binding of I by E influences the binding of S by E. Either the binding sites for I and S are near one another or conformational changes in E caused by I affect S binding. In this case, K_I and K_I', as defined previously, are not equal. Both K_m and V_{max} are altered by the presence of I, and K_m/V_{max} is not constant (Figure 14.16). This inhibitory pattern is commonly encountered. A reasonable explanation is that the inhibitor is binding at a site distinct from the active site, yet is influencing the binding of S at the active site.

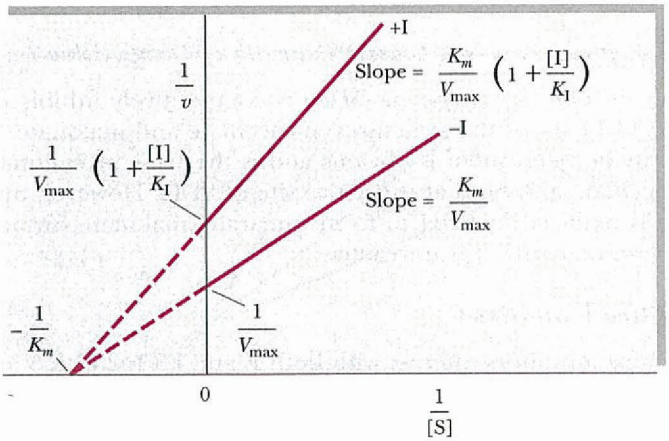


FIGURE 14.15 • Lineweaver–Burk plot of pure noncompetitive inhibition. Note that I does not alter K_m but that it decreases V_{max}. In the presence of I, the y-intercept is equal to $(1/V_{max})(1 + I/K_I)$.

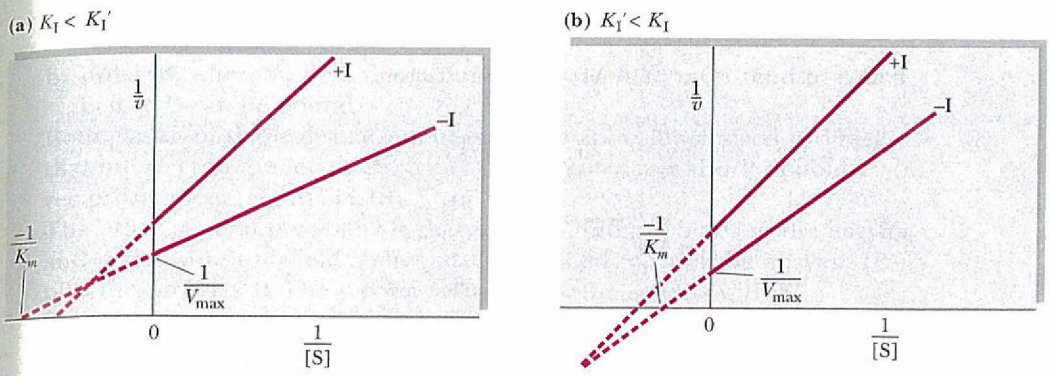


FIGURE 14.16 • Lineweaver–Burk plot of mixed noncompetitive inhibition. Note that both intercepts and the slope change in the presence of I. (a) When K_I is less than K_I'; (b) when K_I is greater than K_I'.

Presumably, these effects are transmitted via alterations in the protein's conformation. Table 14.6 includes the rate equations and apparent K_m and V_{max} values for both types of noncompetitive inhibition.

Irreversible Inhibition

If the inhibitor combines irreversibly with the enzyme—for example, by covalent attachment—the kinetic pattern seen is like that of noncompetitive inhibition, because the net effect is a loss of active enzyme. Usually, this type of inhibition can be distinguished from the noncompetitive, reversible inhibition case since the reaction of I with E (and/or ES) is not instantaneous. Instead, there is a *time-dependent decrease in enzymatic activity* as E + I → EI proceeds, and the rate of this inactivation can be followed. Also, unlike reversible inhibitions, dilution or dialysis of the enzyme : inhibitor solution does not dissociate the EI complex and restore enzyme activity.

Suicide Substrates—Mechanism-Based Enzyme Inactivators

Suicide substrates are inhibitory substrate analogs designed so that, via normal catalytic action of the enzyme, a very reactive group is generated. This reactive group then forms a covalent bond with a nearby functional group within the active site of the enzyme, thereby causing irreversible inhibition. Suicide substrates, also called *Trojan horse substrates,* are a type of **affinity label.** As substrate analogs, they bind with specificity and high affinity to the enzyme active site; in their reactive form, they become covalently bound to the enzyme. This covalent link effectively labels a particular functional group within the active site, identifying the group as a key player in the enzyme's catalytic cycle.

Penicillin—A Suicide Substrate

Several drugs in current medical use are mechanism-based enzyme inactivators. For example, the antibiotic **penicillin** exerts its effects by covalently reacting with an essential serine residue in the active site of *glycoprotein peptidase,* an enzyme that acts to cross-link the peptidoglycan chains during synthesis of bacterial cell walls (Figure 14.17). Once cell wall synthesis is blocked, the bacterial cells are very susceptible to rupture by osmotic lysis, and bacterial growth is halted.

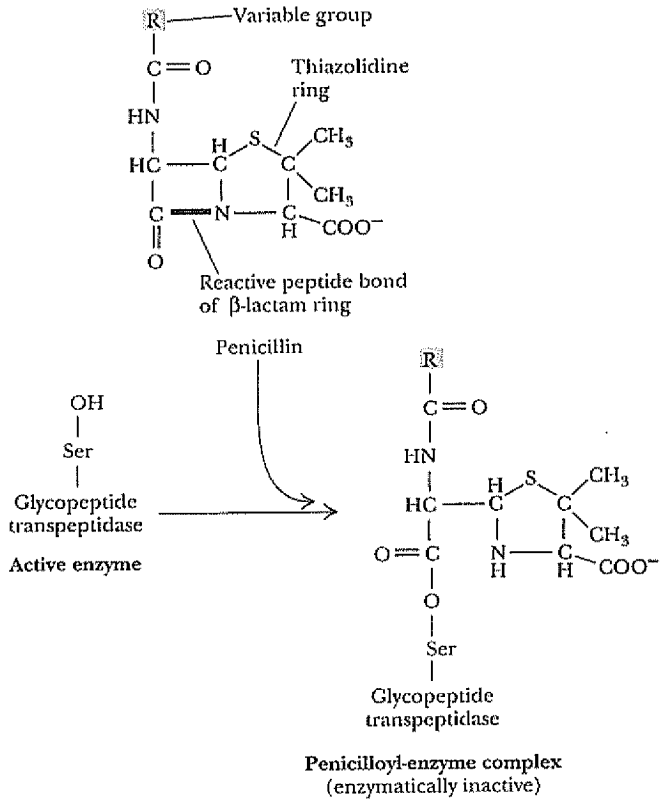


FIGURE 14.17 • Penicillin is an irreversible inhibitor of the enzyme *glycoprotein peptidase*, which catalyzes an essential step in bacterial cell wall synthesis. Penicillin consists of a thiazolidine ring fused to a β-lactam ring to which a variable group R is attached. A reactive peptide bond in the β-lactam ring covalently attaches to a serine residue in the active site of the glycopeptide transpeptidase. (The conformation of penicillin around its reactive peptide bond resembles the transition state of the normal glycoprotein peptidase substrate.) The penicilloyl–enzyme complex is catalytically inactive. The bond between the enzyme and penicillin is indefinitely stable; that is, penicillin binding is irreversible.

14.5 • Kinetics of Enzyme-Catalyzed Reactions Involving Two or More Substrates

Thus far, we have considered only the simple case of enzymes that act upon a single substrate, S. This situation is not common. Usually, enzymes catalyze reactions in which two (or even more) substrates take part.

Consider the case of an enzyme catalyzing a reaction involving two substrates, A and B, and yielding the products P and Q:

$$A + B \xrightarrow{\text{enzyme}} P + Q \qquad (14.45)$$

Such a reaction is termed a **bisubstrate reaction.** In general, bisubstrate reactions proceed by one of two possible routes:

1. Both A and B are bound to the enzyme and then reaction occurs to give P + Q:

$$E + A + B \longrightarrow AEB \longrightarrow PEQ \longrightarrow E + P + Q \qquad (14.46)$$

Reactions of this type are defined as **sequential** or **single-displacement reactions.** They can be either of two distinct classes:

a. **random,** where either A or B may bind to the enzyme first, followed by the other substrate, or
b. **ordered,** where A, designated the *leading substrate,* must bind to E first before B can be bound.

Both classes of single-displacement reactions are characterized by lines that intersect to the left of the $1/v$ axis in Lineweaver–Burk double-reciprocal plots (Figure 14.18).

2. The other general possibility is that one substrate, A, binds to the enzyme and reacts with it to yield a chemically modified form of the enzyme (E′) plus the product, P. The second substrate, B, then reacts with E′, regenerating E and forming the other product, Q.

$$\mathrm{E} + \mathrm{A} \longrightarrow \mathrm{EA} \longrightarrow \mathrm{E'P} \longrightarrow \mathrm{E'} \xrightarrow{\mathrm{B}} \mathrm{E'B} \longrightarrow \mathrm{EQ} \longrightarrow \mathrm{E} + \mathrm{Q} \qquad (14.47)$$

Reactions that fit this model are called **ping-pong** or **double-displacement reactions.** Two distinctive features of this mechanism are the obligatory formation of a modified enzyme intermediate, E′, and the pattern of parallel lines obtained in double-reciprocal plots (Figure 14.19).

Random, Single-Displacement Reactions

In this type of sequential reaction, all possible binary enzyme : substrate complexes (AE, EB, QE, EP) are formed rapidly and reversibly when the enzyme is added to a reaction mixture containing A, B, P, and Q:

$$\begin{array}{ccccc} \mathrm{A} + \mathrm{E} \rightleftharpoons \mathrm{AE} & & & & \mathrm{QE} \rightleftharpoons \mathrm{Q} + \mathrm{E} \\ & \searrow\!\!\nearrow & & \nearrow\!\!\searrow & \\ & & \mathrm{AEB} \rightleftharpoons \mathrm{QEP} & & \\ & \nearrow\!\!\searrow & & \searrow\!\!\nearrow & \\ \mathrm{E} + \mathrm{B} \rightleftharpoons \mathrm{EB} & & & & \mathrm{EP} \rightleftharpoons \mathrm{E} + \mathrm{P} \end{array} \qquad (14.48)$$

The rate-limiting step is the reaction AEB→QEP. It doesn't matter whether A or B binds first to E, or whether Q or P is released first from QEP. Sometimes,

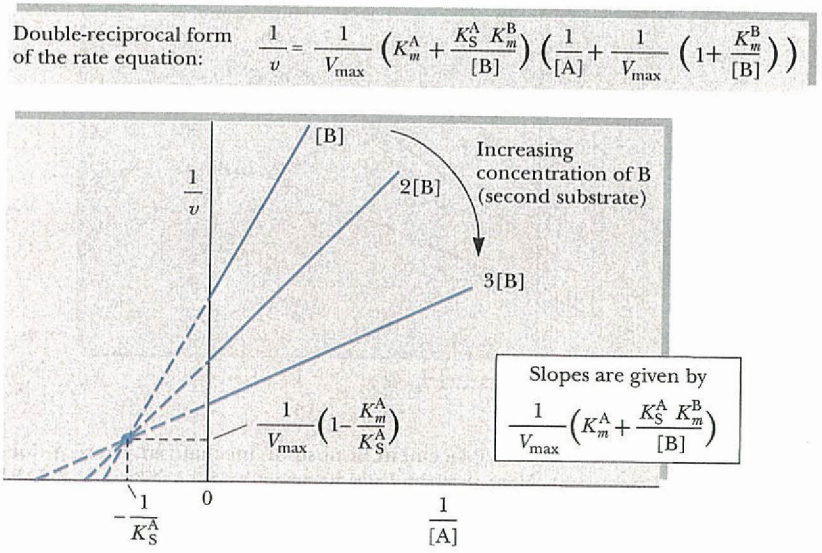


FIGURE 14.18 • Single-displacement bisubstrate mechanism. Double-reciprocal plots of the rates observed with different fixed concentrations of one substrate (B here) are graphed versus a series of concentrations of A. Note that, in these Lineweaver–Burk plots for single-displacement bisubstrate mechanisms, the lines intersect to the left of the $1/v$ axis.

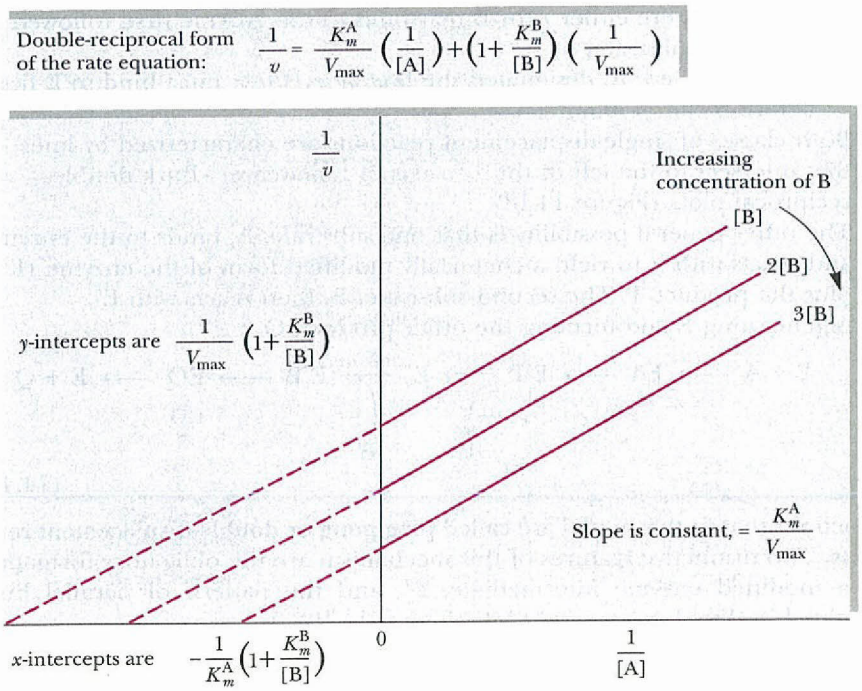


FIGURE 14.19 • Double-displacement (ping-pong) bisubstrate mechanisms are characterized by Lineweaver–Burk plots of parallel lines when double-reciprocal plots of the rates observed with different fixed concentrations of the second substrate, B, are graphed versus a series of concentrations of A.

reactions that follow this random order of addition of substrates to E can be distinguished mechanistically from reactions obeying an ordered, single-displacement mechanism, *if* A has *no* influence on the binding constant for B (and vice versa); that is, the mechanism is purely random. Then, the lines in a Lineweaver–Burk plot intersect at the 1/[A] axis (Figure 14.20).

Creatine Kinase Acts by a Random, Single-Displacement Mechanism

An example of a random, single-displacement mechanism is seen in the enzyme *creatine kinase,* a phosphoryl-transfer enzyme that uses ATP as a phosphoryl

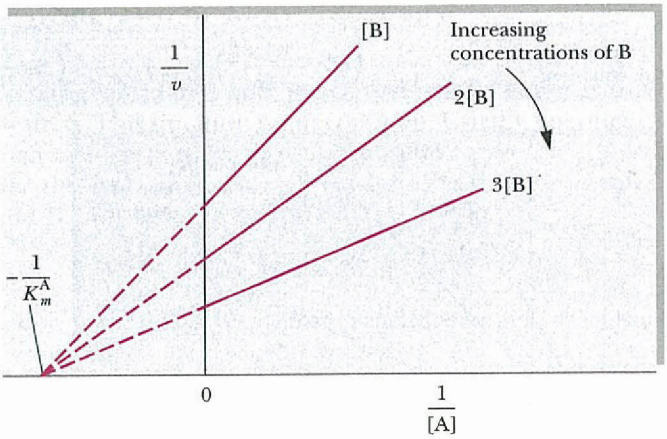
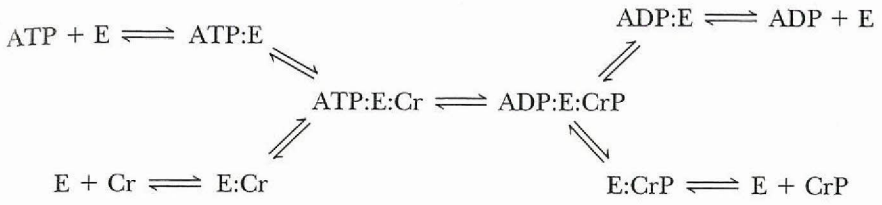
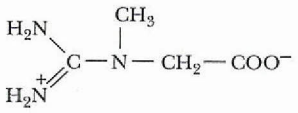


FIGURE 14.20 • Random, single-displacement bisubstrate mechanism where A does not affect B binding, and vice versa. Note that the lines intersect at the 1/[A] axis. (If [B] were varied in an experiment with several fixed concentrations of A, the lines would intersect at the 1/[B] axis in a $1/v$ versus 1/[B] plot.)

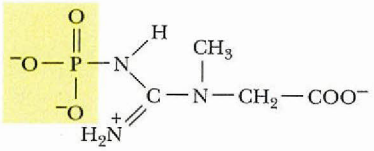
donor to form creatine phosphate (CrP) from creatine (Cr). Creatine-P is an important reservoir of phosphate-bond energy in muscle cells (Figure 14.21).



The overall direction of the reaction will be determined by the relative concentrations of ATP, ADP, Cr, and CrP and the equilibrium constant for the reaction. The enzyme can be considered to have two sites for substrate (or product) binding: an adenine nucleotide site, where ATP or ADP binds, and a creatine site, where Cr or CrP is bound. In such a mechanism, ATP and ADP compete for binding at their unique site, while Cr and CrP compete at the specific Cr-, CrP-binding site. Note that no modified enzyme form (E′), such as an E-PO_4 intermediate, appears here. The reaction is characterized by rapid and reversible binary ES complex formation, followed by addition of the remaining substrate, and the rate-determining reaction taking place within the ternary complex.



Creatine

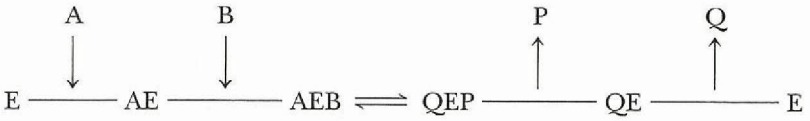


Creatine–P

FIGURE 14.21 • The structures of creatine and creatine phosphate, guanidinium compounds that are important in muscle energy metabolism.

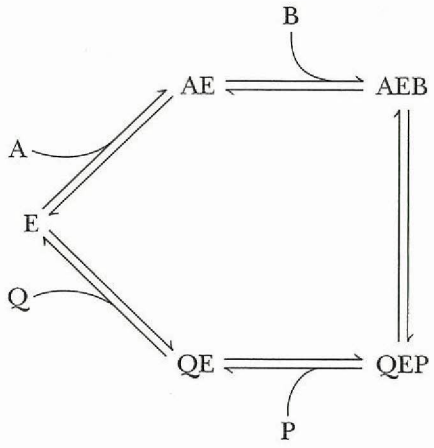
Ordered, Single-Displacement Reactions

In this case, the **leading substrate,** A (also called the **obligatory** or **compulsory substrate**), must bind first. Then the second substrate, B, binds. Strictly speaking, B cannot bind to free enzyme in the absence of A. Reaction between A and B occurs in the ternary complex, and is usually followed by an ordered release of the products of the reaction, P and Q. In the schemes below, Q is the product of A and is released last. One representation, suggested by W. W. Cleland, follows:



(14.49)

Another way of portraying this mechanism is as follows:



Note that A and Q are competitive for binding to the free enzyme, E, but not A and B (or Q and B).

NAD^+-Dependent Dehydrogenases Show Ordered Single-Displacement Mechanisms

Nicotinamide adenine dinucleotide (NAD^+)-dependent dehydrogenases are enzymes that typically behave according to the kinetic pattern just described. A general reaction of these dehydrogenases is

$$NAD^+ + BH_2 \rightleftharpoons NADH + H^+ + B$$

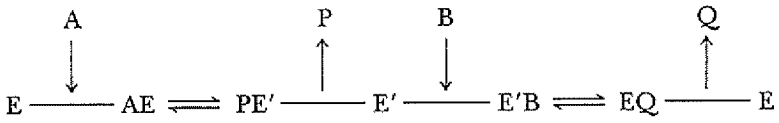
The leading substrate (A) is nicotinamide adenine dinucleotide (NAD^+), and NAD^+ and NADH (product Q) compete for a common site on E. A specific example is offered by *alcohol dehydrogenase (ADH):*

$$\underset{\text{(A)}}{NAD^+} + \underset{\substack{\text{ethanol}\\\text{(B)}}}{CH_3CH_2OH} \rightleftharpoons \underset{\text{(Q)}}{NADH} + H^+ + \underset{\substack{\text{acetaldehyde}\\\text{(P)}}}{CH_3CHO}$$

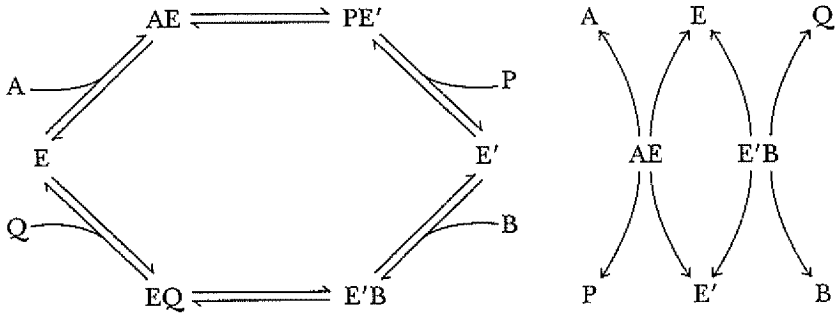
We can verify that this ordered mechanism is not random by demonstrating that no B (ethanol) is bound to E in the absence of A (NAD^+).

Double-Displacement (Ping-Pong) Reactions

Reactions conforming to this kinetic pattern are characterized by the fact that the product of the enzyme's reaction with A (called P in the following schemes) is released *prior* to reaction of the enzyme with the second substrate, B. As a result of this process, the enzyme, E, is converted to a modified form, E′, which then reacts with B to give the second product, Q, and regenerate the unmodified enzyme form, E:



or



Note that these schemes predict that A and Q compete for the free enzyme form, E, while B and P compete for the modified enzyme form, E′. A and Q do not bind to E′, nor do B and P combine with E.

Aminotransferases Show Double-Displacement Catalytic Mechanisms

One class of enzymes that follow a ping-pong-type mechanism are *aminotransferases* (previously known as transaminases). These enzymes catalyze the transfer of an amino group from an amino acid to an α–keto acid. The products are a new amino acid and the keto acid corresponding to the carbon skeleton of the amino donor:

$$\text{amino acid}_1 + \text{keto acid}_2 \longrightarrow \text{keto acid}_1 + \text{amino acid}_2$$

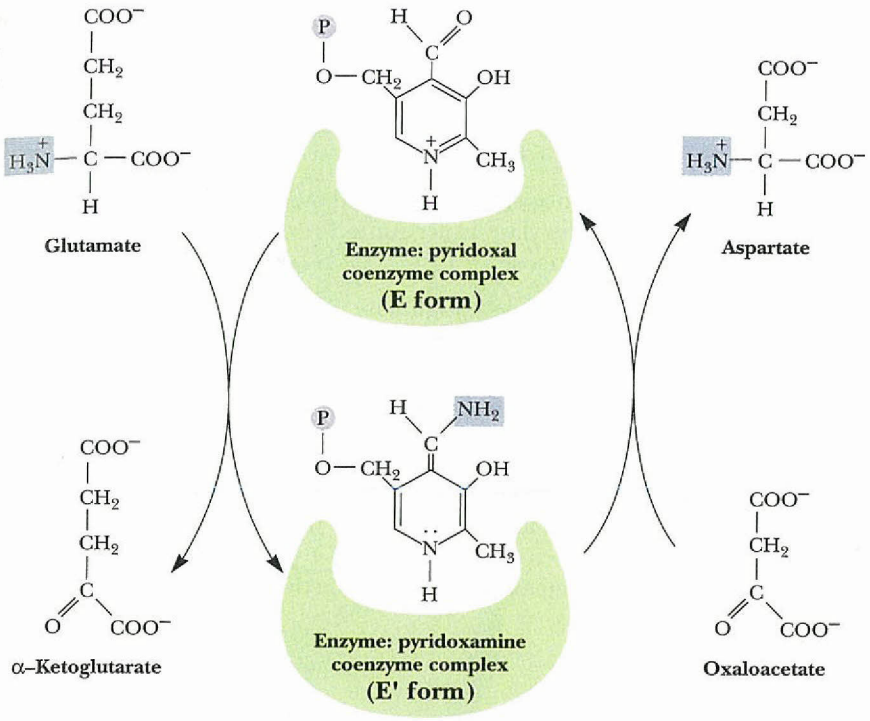


FIGURE 14.22 • *Glutamate : aspartate aminotransferase,* an enzyme conforming to a double-displacement bisubstrate mechanism. Glutamate : aspartate aminotransferase is a pyridoxal phosphate–dependent enzyme. The pyridoxal serves as the $—NH_2$ acceptor from glutamate to form pyridoxamine. Pyridoxamine is then the amino donor to oxaloacetate to form asparate and regenerate the pyridoxal coenzyme form. (The pyridoxamine : enzyme is the E′ form.)

A specific example would be *glutamate : aspartate aminotransferase.* Figure 14.22 depicts the scheme for this mechanism. Note that glutamate and aspartate are competitive for E, and that oxaloacetate and α-ketoglutarate compete for E′. In glutamate : aspartate aminotransferase, an enzyme-bound coenzyme, *pyridoxal phosphate* (a vitamin B_6 derivative), serves as the amino group acceptor/donor in the enzymatic reaction. The unmodified enzyme form, E, has the coenzyme in the aldehydic pyridoxal form, whereas the modified enzyme form, E′, is actually pyridoxamine phosphate (Figure 14.22). Not all enzymes displaying ping-pong–type mechanisms require coenzymes as carriers for the chemical substituent transferred in the reaction.

Diagnosis of Bisubstrate Mechanisms

Kineticists rely on a number of diagnostic tests for the assignment of a reaction mechanism to a specific enzyme. One is the graphic analysis of the kinetic patterns observed. It is usually easy to distinguish between single- and double-displacement reactions in this manner, and examining competitive effects between substrates aids in assigning reactions to random versus ordered patterns of S-binding. A second diagnostic test is to determine whether the enzyme catalyzes an exchange reaction. Consider as an example the two enzymes *sucrose phosphorylase* and *maltose phosphorylase.* Both catalyze the phosphorolysis of a disaccharide and both yield glucose-1-phosphate and a free hexose:

$$\text{sucrose} + P_i \rightleftharpoons \text{glucose-1-phosphate} + \text{fructose}$$

$$\text{maltose} + P_i \rightleftharpoons \text{glucose-1-phosphate} + \text{glucose}$$

Interestingly, in the absence of sucrose and fructose, sucrose phosphorylase will catalyze the exchange of inorganic phosphate, P_i, into glucose-1-phosphate. This reaction can be followed by using $^{32}P_i$ as a radioactive tracer and observing the appearance of ^{32}P into glucose-1-phosphate:

$$^{32}P_i + \text{G-1-P} \rightleftharpoons P_i + \text{G-1-}^{32}P$$

Maltose phosphorylase cannot carry out a similar reaction. The ^{32}P exchange reaction of sucrose phosphorylase is accounted for by a double-displacement mechanism where E′ = E-glucose:

$$\text{sucrose} + E \rightleftharpoons \text{E-glucose} + \text{fructose}$$

$$\text{E-glucose} + P_i \rightleftharpoons E + \text{glucose-1-phosphate}$$

Thus, in the presence of just $^{32}P_i$ and glucose-1-phosphate, sucrose phosphorylase still catalyzes the second reaction and radioactive P_i is incorporated into glucose-1-phosphate over time.

Maltose phosphorylase proceeds via a single-displacement reaction that necessarily requires the formation of a ternary maltose : E : P_i (or glucose : E : glucose-1-phosphate) complex for any reaction to occur. Exchange reactions are a characteristic of enzymes that obey double-displacement mechanisms at some point in their catalysis.

Multisubstrate Reactions

Thus far, we have considered enzyme-catalyzed reactions involving one or two substrates. How are the kinetics described in those cases in which more than two substrates participate in the reaction? An example might be the glycolytic enzyme *glyceraldehyde-3-phosphate dehydrogenase* (Chapter 19):

$$NAD^+ + \text{glyceraldehyde-3-P} + P_i \longrightarrow NADH + H^+ + \text{1,3-bisphosphoglycerate}$$

Many other multisubstrate examples abound in metabolism. In effect, these situations are managed by realizing that the interaction of the enzyme with its many substrates can be treated as a series of uni- or bisubstrate steps in a multistep reaction pathway. Thus, the complex mechanism of a multisubstrate reaction is resolved into a sequence of steps, each of which obeys the single- and double-displacement patterns just discussed.

14.6 • RNA and Antibody Molecules as Enzymes: Ribozymes and Abzymes

Catalytic RNA Molecules: Ribozymes

It was long assumed that all enzymes are proteins. However, in recent years, more and more instances of biological catalysis by RNA molecules have been discovered. These catalytic RNAs, or **ribozymes,** satisfy several enzymatic criteria: They are substrate-specific, they enhance the reaction rate, and they emerge from the reaction unchanged. For example, RNase P, an enzyme responsible for the formation of mature tRNA molecules from tRNA precursors, requires an RNA component as well as a protein subunit for its activity in the cell. *In vitro,* the protein alone is incapable of catalyzing the maturation reaction, but the RNA component by itself can carry out the reaction under appropriate conditions. In another case, in the ciliated protozoan *Tetrahymena,* formation of mature ribosomal RNA from a pre-rRNA precursor involves the removal of an internal RNA segment and the joining of the two ends in a process known as **splicing out.** The excision of this intervening internal sequence of RNA and

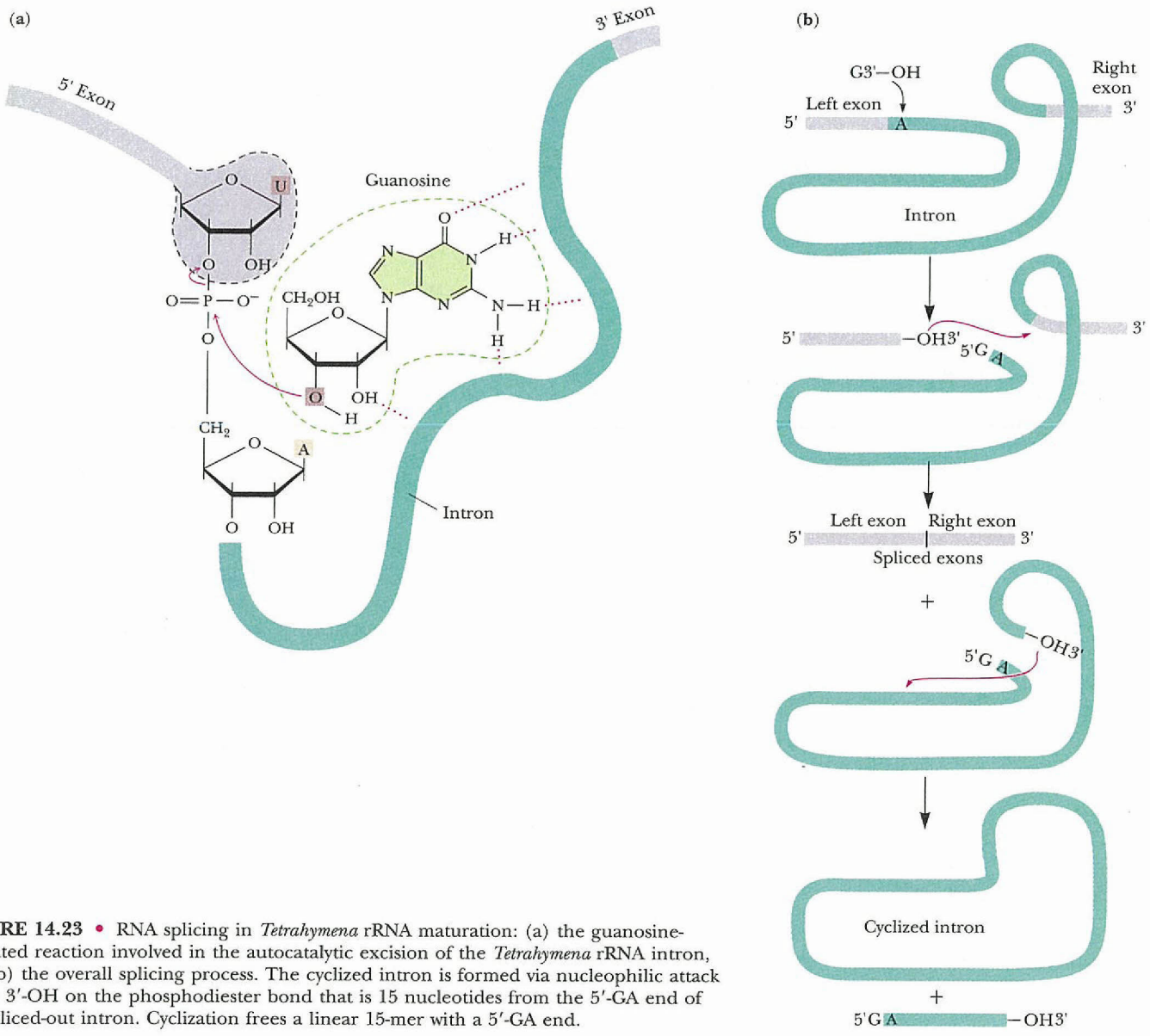


FIGURE 14.23 • RNA splicing in *Tetrahymena* rRNA maturation: (a) the guanosine-mediated reaction involved in the autocatalytic excision of the *Tetrahymena* rRNA intron, and (b) the overall splicing process. The cyclized intron is formed via nucleophilic attack of the 3′-OH on the phosphodiester bond that is 15 nucleotides from the 5′-GA end of the spliced-out intron. Cyclization frees a linear 15-mer with a 5′-GA end.

ligation of the ends is, remarkably, catalyzed by the intervening sequence of RNA itself, in the presence of Mg^{2+} and a free molecule of guanosine nucleoside or nucleotide (Figure 14.23). *In vivo,* the intervening sequence RNA probably acts only in splicing itself out; *in vitro,* however, it can act many times, turning over like a true enzyme.

Protein-Free 50S Ribosomal Subunits Catalyze Peptide Bond Formation In Vitro

Perhaps the most significant case of catalysis by RNA occurs in protein synthesis. Harry F. Noller and his colleagues have found that the **peptidyl transferase reaction,** which is the reaction of peptide bond formation during protein synthesis (Figure 14.24), can be catalyzed by 50S ribosomal subunits (see Chapter 12) from which virtually all of the protein has been removed. These

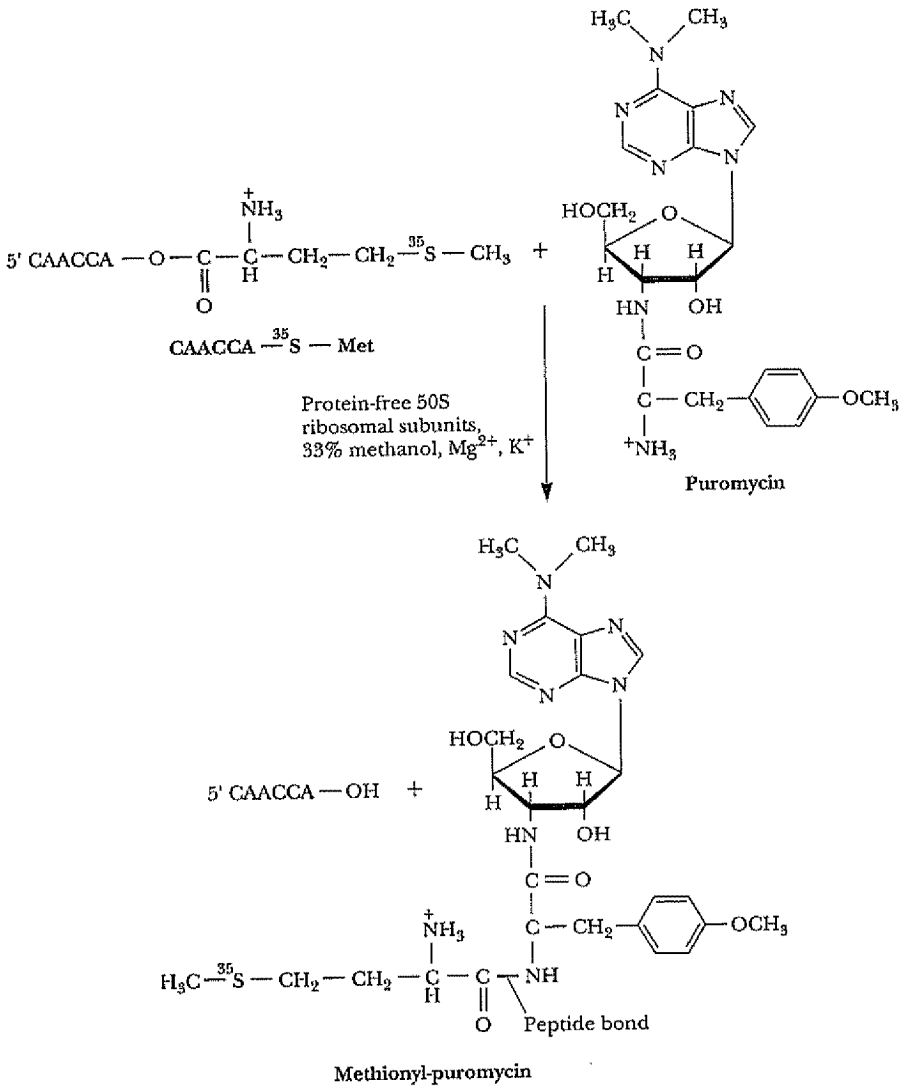


FIGURE 14.24 • Protein-free 50S ribosomal subunits have peptidyl transferase activity. Peptidyl transferase is the name of the enzymatic function that catalyzes peptide bond formation. The presence of this activity in protein-free 50S ribosomal subunits was demonstrated using a model assay for peptide bond formation in which an aminoacyl-tRNA analog (a short RNA oligonucleotide of sequence CAACCA carrying ^{35}S-labeled methionine attached at its 3′-OH end) served as the peptidyl donor and puromycin (another aminoacyl-tRNA analog) served as the peptidyl acceptor. Activity was measured by monitoring the formation of ^{35}S-labeled methioninyl-puromycin.

experiments imply that just the 23S rRNA by itself is capable of catalyzing peptide bond formation. Also, the laboratory of Thomas Cech has created a synthetic 196-nucleotide-long ribozyme capable of performing the peptidyl transferase reaction.

Several features of these "RNA enzymes," or **ribozymes,** lead to the realization that their biological efficiency does not challenge that achieved by proteins. First, RNA enzymes often do not fulfill the criterion of catalysis *in vivo* because they act only once in intramolecular events such as self-splicing. Second, the catalytic rates achieved by RNA enzymes *in vivo* and *in vitro* are

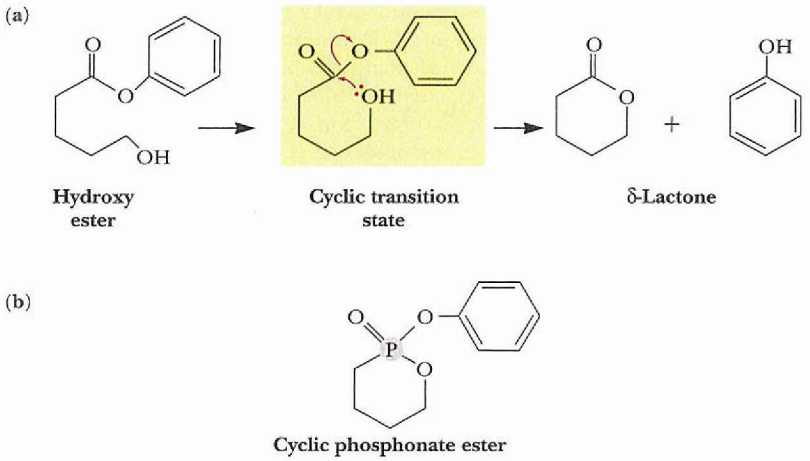


FIGURE 14.25 • Catalytic antibodies are designed to specifically bind the transition-state intermediate in a chemical reaction. (a) The intramolecular hydrolysis of a hydroxy ester to yield as products a δ-lactone and the alcohol phenol. Note the cyclic transition state. (b) The cyclic phosphonate ester analog of the cyclic transition state. Antibodies raised against this phosphonate ester act as *enzymes:* they are catalysts that markedly accelerate the rate of ester hydrolysis.

significantly enhanced by the participation of protein subunits. Nevertheless, the fact that RNA can catalyze certain reactions is experimental support for the idea that a primordial world dominated by RNA molecules existed before the evolution of DNA and proteins.

Catalytic Antibodies: Abzymes

Antibodies are *immunoglobulins,* which, of course, are proteins. Like other antibodies, **catalytic antibodies,** so-called **abzymes,** are elicited in an organism in response to immunological challenge by a foreign molecule called an **antigen** (see Chapter 29 for discussions on the molecular basis of immunology). In this case, however, the antigen is purposefully engineered to be *an analog of the transition-state intermediate in a reaction.* The rationale is that a protein specific for binding the transition-state intermediate of a reaction will promote entry of the normal reactant into the reactive, transition-state conformation. Thus, a catalytic antibody facilitates, or catalyzes, a reaction by forcing the conformation of its substrate in the direction of its transition state. (A prominent explanation for the remarkable catalytic power of conventional enzymes is their great affinity for the transition-state intermediates in the reactions they catalyze; see Chapter 16.)

One strategy has been to prepare ester analogs by substituting a phosphorus atom for the carbon in the ester group (Figure 14.25). The phosphocompound mimics the natural transition state of ester hydrolysis, and antibodies elicited against these analogs act like enzymes in accelerating the rate of ester hydrolysis as much as 1000-fold. Abzymes have been developed for a number of other classes of reactions, including C—C bond formation via aldol condensation (the reverse of the aldolase reaction [see Figure 14.2, reaction 4 and Chapter 19]) and the pyridoxal 5′-P-dependent aminotransferase reaction shown in Figure 14.22. In this latter instance, N^{α}-(5′-phosphopyridoxyl)-lysine (Figure 14.26a) coupled to a carrier protein served as the antigen. An antibody raised against this antigen catalyzed the conversion of D-alanine and pyridoxal 5′-P to pyruvate and pyridoxamine 5′-P (Figure 14.26b). This biotechnology offers the real possibility of creating "**designer enzymes,**" specially tailored enzymes designed to carry out specific catalytic processes.

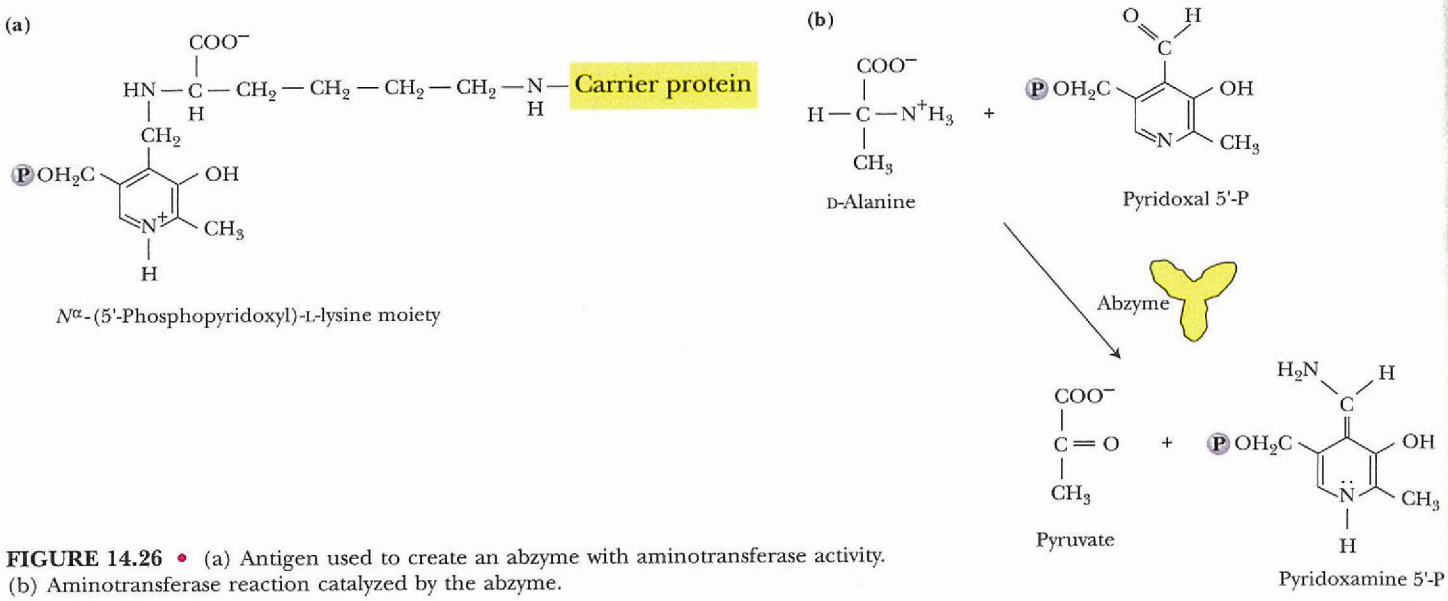


FIGURE 14.26 • (a) Antigen used to create an abzyme with aminotransferase activity. (b) Aminotransferase reaction catalyzed by the abzyme.

PROBLEMS

1. According to the Michaelis–Menten equation, what is the v/V_{max} ratio when [S] = 4 K_m?

2. If V_{max} = 100 μmol/mL sec and K_m = 2 mM, what is the velocity of the reaction when [S] = 20 mM?

3. For a Michaelis–Menten reaction, $k_1 = 7\times10^7/M\cdot$ sec, $k_{-1} = 1\times10^3$/sec, and $k_2 = 2\times10^4$/sec. What are the values of K_S and K_m? Does substrate binding approach equilibrium or does it behave more like a steady-state system?

4. The following kinetic data were obtained for an enzyme in the absence of any inhibitor (1), and in the presence of two different inhibitors (2) and (3) at 5 mM concentration. Assume $[E_T]$ is the same in each experiment.

[S] (mM)	(1) v(μmol/mL sec)	(2) v(μmol/mL sec)	(3) v(μmol/mL sec)
1	12	4.3	5.5
2	20	8	9
4	29	14	13
8	35	21	16
12	40	26	18

a. Determine V_{max} and K_m for the enzyme.

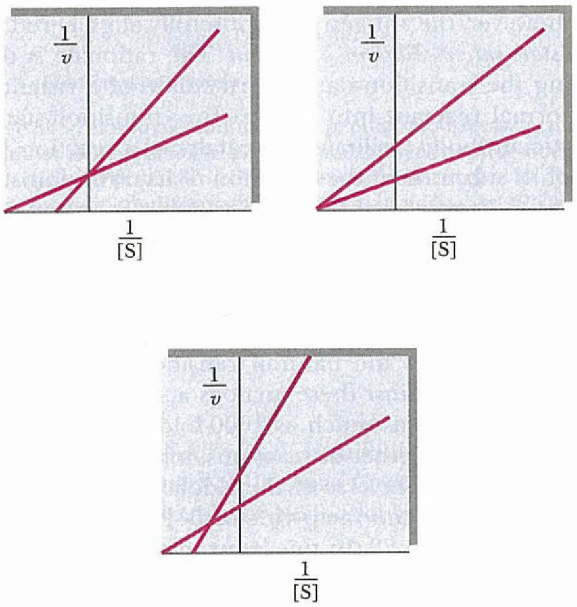
b. Determine the type of inhibition and the K_I for each inhibitor.

5. The general rate equation for an ordered, single-displacement reaction where A is the leading substrate is

$$v = \frac{V_{max}[A][B]}{(K_S^A K_m^B + K_m^A[B] + K_m^B[A] + [A][B])}$$

Write the Lineweaver–Burk (double-reciprocal) equivalent of this equation, and from it calculate algebraic expressions for (a) the slope; (b) the y-intercepts; and (c) the horizontal and vertical coordinates of the point of intersection when $1/v$ is plotted versus $1/[B]$ at various *fixed* concentrations of A.

6. The following graphical patterns obtained from kinetic experiments have several possible interpretations depending on the nature of the experiment and the variables being plotted. Give at least two possibilities for each.



7. Liver alcohol dehydrogenase (ADH) is relatively nonspecific and will oxidize ethanol or other alcohols, including methanol. Methanol oxidation yields formaldehyde, which is quite toxic, causing, among other things, blindness. Mistaking it for the cheap

wine he usually prefers, my dog Clancy ingested about 50 mL of windshield washer fluid (a solution 50% in methanol.) Knowing that methanol would be excreted eventually by Clancy's kidneys if its oxidation could be blocked, and realizing that, in terms of methanol oxidation by ADH, ethanol would act as a competitive inhibitor, I decided to offer Clancy some wine. How much of Clancy's favorite vintage (12% ethanol) must he consume in order to lower the activity of his ADH on methanol to 5% of its normal value if the K_m values of canine ADH for ethanol and methanol are 1 millimolar and 10 millimolar, respectively? (The K_I for ethanol in its role as competitive inhibitor of methanol oxidation by ADH is the same as its K_m.) Both the methanol and ethanol will quickly distribute throughout Clancy's body fluids, which amount to about 15 L. Assume the densities of 50% methanol and the wine are both 0.9 g/mL.

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