Enzymes—Kinetics and Specificity

Living organisms seethe with metabolic activity. Thousands of chemical reactions are proceeding very rapidly at any 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:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} + 2870 \text{ kJ of energy}$$

(-2870 kJ/mol is the standard-state free energy change $\Delta G^\circ$ for the oxidation of glucose.) In chemical terms, 2870 kJ is a large amount of energy, and glucose can be viewed as an energy-rich compound even though at ambient temperature it is not readily reactive with oxygen outside of the cell. Stated another way, glucose represents thermodynamic potentiality: Its reaction with oxygen is strongly exergonic, but it doesn’t occur under normal conditions. On the other hand, enzymes can catalyze such thermodynamically favorable reactions, causing them to proceed at extraordinarily rapid rates (Figure 13.1). 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.

**FIGURE 13.1** Reaction profile showing the large $\Delta G^\circ$ for glucose oxidation. Enzymes lower $\Delta G^\circ$, thereby accelerating rate.
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 13.2). 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.

13.1 What Characteristic Features Define Enzymes?

Enzymes are remarkably versatile biochemical catalysts that have in common three distinctive features: catalytic power, specificity, and regulation.

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

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

$$\text{O}$$

$$\text{H}_2\text{N} \equiv \text{C} \equiv \text{NH}_2 + 2 \text{H}_2\text{O} + \text{H}^+ \rightarrow 2 \text{NH}_4^+ + \text{HCO}_3^-$$

At 20°C, the rate constant for the enzyme-catalyzed reaction is $3 \times 10^{11}$/sec; the rate constant for the uncatalyzed hydrolysis of urea is $3 \times 10^{-10}$/sec. Thus, $10^{21}$ 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 $10^{21}$.

Specificity Is the Term Used to Define the Selectivity of Enzymes for Their Substrates

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 13.3). 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.

Regulation of Enzyme Activity Ensures That the Rate of Metabolic Reactions Is Appropriate to Cellular Requirements

Regulation of enzyme activity is essential to the integration and regulation of metabolism. Enzyme regulation 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 this topic. Because most enzymes are proteins, we can

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**FIGURE 13.3** 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.
anticipate that the functional attributes of enzymes are due to the remarkable versatility found in protein structures.

**Enzyme Nomenclature Provides a Systematic Way of Naming Metabolic Reactions**

Traditionally, enzymes 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 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 13.1). 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 of the reaction, is also assigned to each entry. To illustrate, consider the enzyme that catalyzes this reaction:

\[
\text{ATP} + \text{d-glucose} \rightarrow \text{ADP} + \text{d-glucose-6-phosphate}
\]
A phosphate group is transferred from ATP to the C-6-OH group of glucose, so the enzyme is a transferase (class 2, Table 13.1). 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 commonly used. 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 and Cofactors Are Nonprotein Components Essential to Enzyme Activity**

Many enzymes carry out their catalytic function relying solely on their protein structure. Many others require nonprotein components, called cofactors (Table 13.2). Cofactors may be metal ions or organic molecules referred to as coenzymes. Coenzymes and cofactors provide proteins with chemically versatile functions not found in amino acid side chains. 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 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.

**TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated**

<table>
<thead>
<tr>
<th>Metal Ion and Some Enzymes That Require Them</th>
<th>Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups</th>
<th>Representative Enzymes Using Coenzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metal Ion</strong></td>
<td><strong>Coenzyme</strong></td>
<td><strong>Entity Transferred</strong></td>
</tr>
<tr>
<td>Fe³⁺ or Fe⁴⁺</td>
<td>Thiamine pyrophosphate (TPP)</td>
<td>Aldehydes</td>
</tr>
<tr>
<td></td>
<td>Flavin adenine dinucleotide (FAD)</td>
<td>Hydrogen atoms</td>
</tr>
<tr>
<td></td>
<td>Nicotinamide adenine dinucleotide</td>
<td>Hydride ion (⁻H⁻)</td>
</tr>
<tr>
<td></td>
<td>(NAD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coenzyme A (CoA)</td>
<td>Acyl groups</td>
</tr>
<tr>
<td></td>
<td>Pyridoxal phosphate (PLP)</td>
<td>Amino groups</td>
</tr>
<tr>
<td></td>
<td>5′-Deoxyadenosylcobalamin (vitamin B₁₂)</td>
<td>H atoms and alkyl groups</td>
</tr>
<tr>
<td></td>
<td>Biotin (biocytin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetrahydrofolate (THF)</td>
<td>CO₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other one-carbon groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>such as formyl and methyl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>groups</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arginase</td>
<td></td>
</tr>
<tr>
<td>K⁺ (also requires Mg²⁺)</td>
<td>Urease</td>
<td></td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>Nitril reductase</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>Glutathione peroxidase</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way?

Kinetics is the branch of science concerned with the rates of 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.

Chemical Kinetics Provides a Foundation for Exploring Enzyme 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:

\[ A \rightarrow P \]

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

\[ A \rightarrow I \rightarrow J \rightarrow P \]

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,

\[ v = \frac{d[P]}{dt} \]

or

\[ v = \frac{-d[A]}{dt} \]  \hspace{1cm} (13.1)

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

\[ v = \frac{-d[A]}{dt} = k[A] \]  \hspace{1cm} (13.2)

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 (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 13.4 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).
13.2 Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way?

Bimolecular Reactions Are Reactions Involving Two Reactant Molecules

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

\[ A + B \rightarrow P + Q \]

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:

\[
\frac{d[A]}{dt} = \frac{-d[B]}{dt} = \frac{d[P]}{dt} = \frac{d[Q]}{dt}
\]  

The rate law is

\[
v = k[A][B]
\]  

Since A and B must collide in order to react, the rate of their reaction will be 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 2 are rarely found (and greater than 3, never). (The likelihood of simultaneous collision of three molecules is very, very small.) 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 unimolecular or bimolecular elementary steps, and the overall rate obeys a simple first- or second-order rate law.

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.

Catalysts Lower the Free Energy of Activation for a Reaction

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\(\rightarrow\)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 13.5). 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 1 mol 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:

\[
k = A e^{-\Delta G^\ddagger/RT}
\]

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 kinetic energy of reactant molecules, and more reactant molecules will possess the energy to reach the transition state (Figure 13.5a). In effect, increasing the average energy of reactant molecules makes the energy difference between the average energy and the transition-state energy smaller. (Also note that the equation \( k = A e^{-\Delta G^\ddagger/RT} \) demonstrates that \( k \) increases as \( T \) increases.) 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 13.5b). 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: (1) They are regenerated after each reaction cycle (A→P), and therefore can be used over and over again; and
13.3 What Equations Define the 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 \( \text{A} \rightarrow \text{P} \), a plot of the reaction rate as a function of the concentration of \( \text{A} \) yields a straight line whose slope is \( k \) (Figure 13.6). The more \( \text{A} \) 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 13.7). At low concentrations of the substrate \( \text{S} \), \( v \) is proportional to \([\text{S}]\), as expected for a first-order reaction. However, \( v \) does not increase proportionally as \([\text{S}]\) increases, but instead begins to level off. At high \([\text{S}]\), \( v \) becomes virtually independent of \([\text{S}]\) and approaches a maximal limit. The value of \( v \) at this limit is written \( V_{\text{max}} \). Because rate is no longer dependent on \([\text{S}]\) at these high concentrations, the enzyme-catalyzed reaction is now obeying zero-order 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 \([\text{S}]\) 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 \( \text{S} \). Indeed, such curves were the initial clue that an enzyme interacts directly with its substrate by binding it.

The Substrate Binds at the Active Site of an Enzyme

An enzyme molecule is often (but not always) orders of magnitude larger than its substrate. In any case, its active site, that place on the enzyme where \( \text{S} \) binds, comprises only a portion of the overall enzyme structure. The conformation of the active site is structured to form a special pocket or cleft whose three-dimensional architecture is complementary to the structure of the substrate. The enzyme and the substrate molecules “recognize” each other through this structural complementarity. The substrate binds to the enzyme through relatively weak forces—H bonds, ionic bonds (salt bridges), and van der Waals interactions between sterically complementary clusters of atoms.
The Michaelis–Menten Equation Is the Fundamental Equation of Enzyme Kinetics

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:

\[
E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES
\]  
(13.6)

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

\[
k_{-1}[ES] = k_1[E][S]
\]  
(13.7)

and

\[
K_s = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}
\]  
(13.8)

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

\[
E + S \underset{k_{-2}}{\overset{k_2}{\rightarrow}} ES \rightarrow E + P
\]  
(13.9)

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

Assume That [ES] Remains Constant During an Enzymatic Reaction

The interpretations of Michaelis and Menten were refined and extended in 1925 by Briggs and Haldane, who assumed 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

\[
d[ES] \over dt = 0
\]  
(13.10)

That is, the change in concentration of ES with time, \( t \), is 0. Figure 13.8 illustrates the time course for formation of the ES complex and establishment of the steady-state condition.

Assume That Velocity Measurements Are Made Immediately After Adding S

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 13.9 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

\[
\text{Total enzyme}, \ [E]_T = [E] + [ES]
\]  
(13.11)
where $[E]$ is free enzyme and $[ES]$ is the amount of enzyme in the enzyme-substrate complex. From Equation 13.9, the rate of $[ES]$ formation is

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

where

$$(13.12) [E_T] - [ES] = [E]$$

From Equation 13.9, the rate of $[ES]$ disappearance is

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

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

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

Rearranging gives

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

(13.14)

The Michaelis Constant, $K_m$, is defined as $(k_{-1} + k_2)/k_1$

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

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

(13.16)

Note from Equation 13.15 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. (Also, because the units of $k_{-1}$ and $k_2$ are in time$^{-1}$ and the units of $k_1$ are M$^{-1}$time$^{-1}$, it becomes obvious that the units of $K_m$ are M.) From Equation 13.15, we can write

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

(13.17)

which rearranges to

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

(13.18)

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

$$\nu = \frac{d[P]}{dt}$$

(13.19)

and for this reaction

$$\nu = k_2[ES]$$

(13.20)

Substituting the expression for $[ES]$ from Equation 13.18 into Equation 13.20 gives

$$\nu = \frac{k_2[ES][S]}{K_m + [S]}$$

(13.21)

The product $k_2[ES]$ has special meaning. When $[S]$ is high enough to saturate all of the enzyme, the velocity of the reaction, $\nu$, is maximal. At saturation, the amount of $[ES]$ complex is equal to the total enzyme concentration, $E_T$, its maximum possible value. From Equation 13.20, the initial velocity $\nu$ then equals $k_2[E_T] = V_{max}$. Written symbolically, when $[S] \gg [E_T]$ (and $K_m$), $[E_T] = [ES]$ and $\nu = V_{max}$. Therefore,

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

(13.22)
Substituting this relationship into the expression for \( v \) gives the Michaelis–Menten equation:

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

This equation says that the initial rate of an enzyme-catalyzed reaction, \( v \), is determined by two constants, \( K_m \) and \( V_{\text{max}} \), and the initial concentration of substrate.

**When \([S] = K_m, v = V_{\text{max}}/2\)**

We can provide an operational definition for the constant \( K_m \) by rearranging Equation 13.23 to give

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

Then, at \( v = V_{\text{max}}/2, K_m = [S] \). That is, \( K_m \) is defined by the substrate concentration that gives a velocity equal to one-half the maximal velocity. Table 13.3 gives the \( K_m \) values of some enzymes for their substrates.

**Plots of \( v \) Versus [S] Illustrate the Relationships Between \( V_{\text{max}}, K_m \), and Reaction Order**

The Michaelis–Menten equation (Equation 13.23) describes a curve known from analytical geometry as a *rectangular hyperbola*. In such curves, as [S] is increased, \( v \) approaches the limiting value, \( V_{\text{max}} \), in an asymptotic fashion. \( V_{\text{max}} \) can be approximated experimentally from a substrate saturation curve (Figure 13.7), and \( K_m \)

---

**TABLE 13.3 \( K_m \) Values for Some Enzymes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>( K_m ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase</td>
<td>CO₂</td>
<td>12</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>N-Benzoyltyrosinamide</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Acetyl-tryptophanamide</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>N-Formyltyrosinamide</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>N-Acetyltyrosinamide</td>
<td>32</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>Glucose</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>1.5</td>
</tr>
<tr>
<td>( \beta )-Galactosidase</td>
<td>Lactose</td>
<td>4</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>NH₄⁺</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>( \alpha )-Ketoglutarate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>NAD⁺</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>NADH</td>
<td>0.018</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Aspartate</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>( \alpha )-Ketoglutarate</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Oxaloacetate</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>4</td>
</tr>
<tr>
<td>Threonine deaminase</td>
<td>Threonine</td>
<td>5</td>
</tr>
<tr>
<td>Arginyl-tRNA synthetase</td>
<td>Arginine</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>tRNA(^{\text{Arg}})</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>0.3</td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>HCO₃⁻</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Pyruvate</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>Benzylpenicillin</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Hexa-N-acetylglucosamine</td>
<td>0.006</td>
</tr>
</tbody>
</table>
can be derived from \( V_{\text{max}}/2 \), so the two constants of the Michaelis–Menten equation can be obtained from plots of \( v \) versus \([S]\). Note, however, that actual estimation of \( V_{\text{max}} \), and consequently \( K_m \), is only approximate from such graphs. That is, according to Equation 13.23, to get \( v = 0.99 \ V_{\text{max}} \ [S] \) must equal 99 \( K_m \), a concentration that may be difficult to achieve in practice.

From Equation 13.23, when \([S] \gg K_m\), then \( v = V_{\text{max}} \). That is, \( v \) is no longer dependent on \([S]\), so the reaction is obeying zero-order kinetics. Also, when \([S] < K_m\), then \( v \approx (V_{\text{max}} / K_m)[S] \). That is, the rate, \( v \), approximately follows a first-order rate equation, \( v = k'[A] \), where \( k' = V_{\text{max}} / K_m \).

\( K_m \) and \( V_{\text{max}} \), 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 concentrations of all other substrates are held constant.
2. The reaction \( \text{ES} \rightarrow \text{E} + \text{P} \) is irreversible, or the experiment is limited to observing only initial velocities where \([P] = 0\).
3. \([S]_0 > [E_f] \) and \([E_f] \) is held constant.
4. All other variables that might influence the rate of the reaction (temperature, pH, ionic strength, and so on) are held constant.

### Turnover Number Defines the Activity of One Enzyme Molecule

The **turnover number** of an enzyme, \( k_{\text{cat}} \), is a measure of its maximal catalytic activity. \( k_{\text{cat}} \) 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 (Equation 13.9) under conditions of initial velocity measurements, \( k_{\text{cat}} = k_2 \). Provided the concentration of enzyme, \([E_f]\), in the reaction mixture is known, \( k_{\text{cat}} \) can be determined from \( V_{\text{max}} \). At saturating \([S]\), \( v = V_{\text{max}} = k_2 [E_f] \). Thus,

\[
k_2 = \frac{V_{\text{max}}}{[E_f]} = k_{\text{cat}} \quad (13.25)
\]

The term \( k_{\text{cat}} \) represents the kinetic efficiency of the enzyme. Table 13.4 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 \( \text{H}_2\text{O}_2 \) in 1 second! At the other end of the scale, lysozyme requires 2 seconds to cleave a glycosidic bond in its glycan substrate.

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** as the amount that catalyzes the formation of 1 micromole of product in 1 minute. (Because enzymes are very sensitive to factors such as pH, temperature, and ionic strength, the conditions of assay must be specified.) In the process of purifying enzymes from cellular sources, many extraneous proteins may be present. Then, the units of enzyme activity are expressed as enzyme units per mg protein, a term known as **specific activity** (see Table 5.1).

### The Ratio, \( k_{\text{cat}} / K_m \), Defines the Catalytic Efficiency of an Enzyme

Under physiological conditions, \([S]\) is seldom saturating and \( k_{\text{cat}} \) 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 using the following equations. As presented in Equation 13.23, if

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

\( [S] \) can be derived from \( V_{\text{max}}/2 \), so the two constants of the Michaelis–Menten equation can be obtained from plots of \( v \) versus \([S]\). Note, however, that actual estimation of \( V_{\text{max}} \), and consequently \( K_m \), is only approximate from such graphs. That is, according to Equation 13.23, to get \( v = 0.99 \ V_{\text{max}} \ [S] \) must equal 99 \( K_m \), a concentration that may be difficult to achieve in practice.

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\[
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\[
v = \frac{V_{\text{max}} [S]}{K_m + [S]}
\]
Chapter 13  Enzymes—Kinetics and Specificity

and \( V_{\text{max}} = k_{\text{cat}} [E] \), then

\[
v = \frac{k_{\text{cat}} [E] [S]}{K_m + [S]} \tag{13.26}
\]

When \( [S] \ll K_m \) the concentration of free enzyme, \([E]\), is approximately equal to \([E]_0\), so

\[
v = \left( \frac{k_{\text{cat}}}{K_m} \right) [E] [S] \tag{13.27}
\]

That is, \( k_{\text{cat}}/K_m \) 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 \( k_{\text{cat}} \) is directly proportional to the kinetic efficiency of the enzyme, \( k_{\text{cat}}/K_m \) 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 \( k_{\text{cat}} = k_2 \). Then

\[
\frac{k_{\text{cat}}}{K_m} = \frac{k_1 k_2}{k_1 + k_2} \tag{13.28}
\]

But \( k_1 \) must always be greater than or equal to \( k_1 k_2 / (k_1 + k_2) \). 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 \( k_{\text{cat}}/K_m \). 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 \( 10^8 / M \cdot \text{sec} \) for small substrates (for example, glyceraldehyde 3-P) and an order of magnitude smaller \( (\approx 10^6 / M \cdot \text{sec}) \) for substrates the size of nucleotides. Those enzymes that are most efficient in their catalysis have \( k_{\text{cat}}/K_m \) 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 13.5 lists the kinetic parameters of several enzymes in this category. Note that \( K_m \) and \( k_{\text{cat}} \) both show a substantial range of variation in this table, even though their ratio falls around \( 10^6 / M \cdot \text{sec} \).

**Linear Plots Can Be Derived from the Michaelis–Menten Equation**

Because of the hyperbolic shape of \( v \) versus \( [S] \) plots, \( V_{\text{max}} \) can be determined only from an extrapolation of the asymptotic approach of \( v \) to some limiting value as \( [S] \) increases indefinitely (Figure 13.7); and \( K_m \) is derived from that value of \( [S] \) giving

**TABLE 13.5 Enzymes Whose \( k_{\text{cat}}/K_m \) Approaches the Diffusion-Controlled Rate of Association with Substrate**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>( k_{\text{cat}} ) (sec(^{-1}))</th>
<th>( K_m ) (M)</th>
<th>( k_{\text{cat}}/K_m ) (M(^{-1}) sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>( 1.4 \times 10^4 )</td>
<td>( 9 \times 10^{-5} )</td>
<td>( 1.6 \times 10^9 )</td>
</tr>
<tr>
<td>Carboxic anhydrase</td>
<td>( CO_2 )</td>
<td>( 1.1 \times 10^7 )</td>
<td>( 0.013 )</td>
<td>( 8.3 \times 10^5 )</td>
</tr>
<tr>
<td>Catalase</td>
<td>( HCO_3^- )</td>
<td>( 4 \times 10^3 )</td>
<td>( 0.026 )</td>
<td>( 1.5 \times 10^7 )</td>
</tr>
<tr>
<td>Crotatase</td>
<td>Crotinyl-CoA</td>
<td>( 5.7 \times 10^6 )</td>
<td>( 2 \times 10^{-4} )</td>
<td>( 2.8 \times 10^9 )</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>( 800 )</td>
<td>( 5 \times 10^{-4} )</td>
<td>( 1.6 \times 10^6 )</td>
</tr>
<tr>
<td>Malate</td>
<td></td>
<td>( 900 )</td>
<td>( 2.5 \times 10^{-4} )</td>
<td>( 3.6 \times 10^5 )</td>
</tr>
<tr>
<td>Triosephosphate</td>
<td>Glyceroldehyde</td>
<td>( 4.3 \times 10^6 )</td>
<td>( 1.8 \times 10^{-4} )</td>
<td>( 2.4 \times 10^9 )</td>
</tr>
<tr>
<td>isomerase</td>
<td>3-phosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Lactamase</td>
<td>Benzylpenicilin</td>
<td>( 2 \times 10^6 )</td>
<td>( 2 \times 10^{-5} )</td>
<td>( 1 \times 10^8 )</td>
</tr>
</tbody>
</table>

*\( k_{\text{cat}} \) 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.

What Equations Define the Kinetics of Enzyme-Catalyzed Reactions?

13.3 What Equations Define the Kinetics of Enzyme-Catalyzed Reactions?

$v = V_{\text{max}}/2$. 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 13.23, yields the equality

\[
\frac{1}{v} = \left( \frac{K_m}{V_{\text{max}}} \right) \left( \frac{1}{[S]} \right) + \frac{1}{V_{\text{max}}} \tag{13.29}
\]

This conforms to \( y = mx + b \) (the equation for a straight line), where \( y = 1/v \); \( m \), the slope, is \( K_m/V_{\text{max}} \); \( x = 1/[S] \); and \( b = 1/V_{\text{max}} \). Plotting \( 1/v \) versus \( 1/[S] \) gives a straight line whose \( x \)-intercept is \( -1/K_m \), whose \( y \)-intercept is \( 1/V_{\text{max}} \), and whose slope is \( K_m/V_{\text{max}} \) (Figure 13.9).

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

Multiplying both sides of Equation 13.29 by \([S]\) gives

\[
\frac{[S]}{v} = [S] \left( \frac{K_m}{V_{\text{max}}} \right) \left( \frac{1}{[S]} \right) + [S] \left( \frac{1}{V_{\text{max}}} \right) = \frac{K_m}{V_{\text{max}}} + \frac{[S]}{V_{\text{max}}} \tag{13.30}
\]

and

\[
\frac{[S]}{v} = \frac{1}{V_{\text{max}}} [S] + \frac{K_m}{V_{\text{max}}} \tag{13.31}
\]

Graphing \( [S]/v \) versus \([S]\) yields a straight line where the slope is \( 1/V_{\text{max}} \), the \( y \)-intercept is \( K_m/V_{\text{max}} \), and the \( x \)-intercept is \( -K_m \) as shown in Figure 13.10. The Hanes–Woolf plot has the advantage of not overemphasizing the data obtained at low \([S]\), a fault inherent in the Lineweaver–Burk plot. The common advantage of these plots is that they allow both \( K_m \) and \( V_{\text{max}} \) 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.

Nonlinear Lineweaver–Burk or Hanes–Woolf Plots Are a Property of Regulatory Enzymes

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.
A DEEPER LOOK

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 ($\text{SO}_3^{2-}$) to sulfate ($\text{SO}_4^{2-}$), using the heme-containing protein, cytochrome $c$, as electron acceptor:

\[
\text{SO}_3^{2-} + 2 \text{cytochrome } c_{\text{oxidized}} + \text{H}_2\text{O} \rightleftharpoons \text{SO}_4^{2-} + 2 \text{cytochrome } c_{\text{reduced}} + 2 \text{H}^+
\]

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 Arg160 was also studied.

Replacing R160 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, $\text{SO}_3^{2-}$, is strongly anionic, and R160 is one of several Arg residues situated within the SO substrate-binding site. Positively charged side chains in the substrate-binding site facilitate $\text{SO}_3^{2-}$ binding and catalysis, with Arg being optimal in this role.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_m$ (µM)</th>
<th>$k_{cat}$ (sec$^{-1}$)</th>
<th>$k_{cat}/K_m$ (10$^6$ M$^{-1}$ sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>17</td>
<td>18</td>
<td>1.1</td>
</tr>
<tr>
<td>R160Q</td>
<td>1900</td>
<td>3</td>
<td>0.0016</td>
</tr>
<tr>
<td>R160K</td>
<td>360</td>
<td>5.5</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Enzymatic Activity Is Strongly Influenced by pH

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. Furthermore, 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 13.11 illustrates the relative activity of four enzymes as a function of pH. 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 activity-pH profile suggests that an ionizable group with a $pK_a$ near 6 is essential to its activity. Might this group be a histidine side chain within its active site? 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.

The Response of Enzymatic Activity to Temperature Is Complex

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 13.12). Two effects are operating here: (1) the characteristic increase in reaction rate with temperature and (2) thermal denaturation of protein structure at higher temperatures. 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 prokaryotes (thermophilic = “heat-loving”) found in geothermal springs retain full activity at temperatures in excess of 85°C.

13.4 What Can Be Learned from the Inhibition of Enzyme Activity?

If the velocity of an enzymatic reaction is decreased or inhibited by some agent, 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.

Enzymes May Be Inhibited Reversibly or Irreversibly

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 Inhibitors May Bind at the Active Site or at Some Other Site

Reversible inhibitors fall into three major categories: competitive, noncompetitive, and uncompetitive. 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 substrate-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 effects of the other major types, noncompetitive and uncompetitive inhibition, cannot be overcome by increasing [S]. The three 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

\[
E + I \rightleftharpoons EI \quad \text{and/or} \quad I + ES \rightleftharpoons IES
\]

(13.32)

Competitive Inhibition

Consider the following system:

\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_{-1}} E + P \quad E + I \xrightarrow{k_3} EI
\]

(13.33)

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, IES, 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 \(v = k_3[ES]\).

It is revealing to compare the equation for the uninhibited case, Equation 13.23 (the Michaelis–Menten equation) with Equation 13.43 for the rate of the enzymatic reaction in the presence of a fixed concentration of the competitive inhibitor, [I]

\[
v = \frac{V_{\text{max}}[S]}{K_v + [S]}
\]

(13.32)

(see also Table 13.6). The \(K_v\) term in the denominator of 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 13.13 shows a Lineweaver–Burk plot of competitive inhibition. Several features of competitive inhibition are evident. First, at a given [I], \(v\) decreases (1/v increases).

### Table 13.6 The Effect of Various Types of Inhibitors on the Michaelis–Menten Rate Equation and on Apparent \(K_v\) and Apparent \(V_{\text{max}}\)

<table>
<thead>
<tr>
<th>Inhibition Type</th>
<th>Rate Equation</th>
<th>Apparent (K_v)</th>
<th>Apparent (V_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>(v = V_{\text{max}}[S]/(K_v + [S]))</td>
<td>(K_v)</td>
<td>(V_{\text{max}})</td>
</tr>
<tr>
<td>Competitive</td>
<td>(v = V_{\text{max}}[S]/([S] + K_v(1 + [I]/K_i)))</td>
<td>(K_v(1 + [I]/K_i))</td>
<td>(V_{\text{max}})</td>
</tr>
<tr>
<td>Noncompetitive</td>
<td>(v = (V_{\text{max}}[S]/(1 + [I]/K_i))/K_v + [S]))</td>
<td>(K_v)</td>
<td>(V_{\text{max}}/(1 + [I]/K_i))</td>
</tr>
<tr>
<td>Mixed</td>
<td>(v = V_{\text{max}}[S]/((1 + [I]/K_v)K_v + (1 + [I]/K_i'[S])))</td>
<td>(K_v(1 + [I]/K_i)(1 + [I]/K_i'))</td>
<td>(V_{\text{max}}/(1 + [I]/K_i'))</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>(v = V_{\text{max}}[S]/(K_v + [S](1 + [I]/K_i')))</td>
<td>(K_i'/(1 + [I]/K_i'))</td>
<td>(V_{\text{max}}/(1 + [I]/K_i'))</td>
</tr>
</tbody>
</table>

\(K_v\) is defined as the enzyme-inhibitor dissociation constant \(K_v = [I]/[E][I]\); \(K_i'\) is defined as the enzyme-substrate complex-inhibitor dissociation constant \(K_i' = [S][I]/[E][I][S]\).
13.4 What Can Be Learned from the Inhibition of Enzyme Activity?

When \([S]\) becomes infinite, \(v = V_{\text{max}}\) 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 \(K_{\text{app}}\)) because it is the \(K_m\) apparent under these conditions. The diagnostic criterion for competitive inhibition is that \(V_{\text{max}}\) 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

**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 (13.14) and (13.16) we can write

\[
[ES] = \frac{k_1[E][S]}{(k_2 + k_{-1})} = \frac{[E][S]}{K_a} \tag{13.34}
\]

Assuming that \(E + I \rightleftharpoons EI\) reaches rapid equilibrium, the rate of EI formation, \(v' = k_1[E][I]\), and the rate of disappearance of EI, \(v_{-1}' = k_{-1}[EI]\), are equal. So,

\[
k_1[E][I] = k_{-1}[EI] \tag{13.35}
\]

Therefore,

\[
[EI] = \frac{k_3}{k_{-3}} [E][I] \tag{13.36}
\]

If we define \(K_i\) as \(k_{-3}/k_3\), an enzyme-inhibitor dissociation constant, then

\[
[ EI ] = \frac{[E][I]}{K_i} \tag{13.37}
\]

knowing \([EI] = [E] + [ES] + [EI]\). Then

\[
[E] = [E] + \frac{[E][S]}{K_a} + \frac{[E][I]}{K_i} \tag{13.38}
\]

Solving for \([E]\) gives

\[
[E] = \frac{K_iK_m[E_I]}{(K_a + K_i[S] + K_m[I])} \tag{13.39}
\]

Because the rate of product formation is given by \(v = k_2[ES]\), from Equation 13.34 we have

\[
v = \frac{k_2[E][S]}{K_a} \tag{13.40}
\]

So,

\[
v = \frac{(k_2K_a[S])}{(K_a + K_i[S] + K_m[I])} \tag{13.41}
\]

Because \(V_{\text{max}} = k_2[E_I]\),

\[
v = \frac{V_{\text{max}}[S]}{K_a + [S] + \frac{K_m[I]}{K_i}} \tag{13.42}
\]

or

\[
v = \frac{V_{\text{max}}[S]}{[S] + \frac{K_m[I]}{K_i}} \tag{13.43}
\]
13.14 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.

**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

\[
E + I \rightleftharpoons EI \quad \text{ES} \rightleftharpoons \text{IES}
\]

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 13.15. Note that \( K_{m} \) is unchanged by I (the x-intercept remains the same, with or without I). Note also that the apparent \( V_{\text{max}} \) 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 the apparent \( K_{m} \) and the apparent \( V_{\text{max}} \) are altered.
by the presence of I, and $K_m/V_{\text{max}}$ is not constant (Figure 13.16). 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. Presumably, these effects are transmitted via alterations in the protein’s conformation. Table 13.6 includes the rate equations and apparent $K_m$ and $V_{\text{max}}$ values for both types of noncompetitive inhibition.

**Uncompetitive Inhibition** Completing the set of inhibitory possibilities is uncompetitive inhibition. Unlike competitive inhibition (where I combines only with E) or noncompetitive inhibition (where I combines with E and ES), in uncompetitive inhibition, I combines only with ES.

$$\text{ES} + \text{I} \rightleftharpoons \text{IES}$$

(13.45)

Because IES does not lead to product formation, the observed rate constant for product formation, $k_2$, is uniquely affected. In simple Michaelis–Menten kinetics, $k_2$ is the only rate constant that is part of both $V_{\text{max}}$ and $K_m$. The pattern obtained in Lineweaver–Burk plots is a set of parallel lines (Figure 13.17). A clinically important example is the action of lithium in alleviating manic depression; Li$^+$ ions are uncompetitive inhibitors of myo-inositol monophosphatase. Some pesticides are also uncompetitive inhibitors, such as Roundup, an uncompetitive inhibitor of 3-enolpyruvylshikimate-5-P synthase, an enzyme essential to aromatic amino acid biosynthesis (see Chapter 25).

**Enzymes Also Can Be Inhibited in an Irreversible Manner**

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 because the reaction of I with E (and/or ES) is not instantaneous. Instead, there is a *time-dependent decrease in enzymatic activity* as $E + I\rightarrow EI$ 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
Chapter 13  Enzymes—Kinetics and Specificity

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.

![Lineweaver–Burk plot of uncompetitive inhibition](image)

FIGURE 13.17 Lineweaver–Burk plot of uncompetitive inhibition. Note that both intercepts change but the slope \( (K_m/V_{\text{max}}) \) remains constant in the presence of I.

Penicillin is an irreversible inhibitor of the enzyme glycopeptide transpeptidase, also known as glycoprotein peptidase, which catalyzes an essential step in bacterial cell wall synthesis.

![Penicillin](image)

FIGURE 13.18 Penicillin is an irreversible inhibitor of the enzyme glycopeptide transpeptidase, also known as glycoprotein peptidase, which catalyzes an essential step in bacterial cell wall synthesis.
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 glycopeptide transpeptidase, an enzyme that acts to crosslink the peptidoglycan chains during synthesis of bacterial cell walls (Figure 13.18). Penicillin consists of a thiazolidine ring fused to a β-lactam ring to which a variable R group 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 glycopeptide transpeptidase substrate.) The penicillinoyl–enzyme complex is catalytically inactive. Once cell wall synthesis is blocked, the bacterial cells are very susceptible to rupture by osmotic lysis and bacterial growth is halted.

13.5 What Is the Kinetic Behavior of Enzymes Catalyzing Bimolecular Reactions?

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:

\[
A + B \rightarrow E \rightarrow PEQ \rightarrow E + P + Q
\]  

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:
   \[
   E + A + B \rightarrow AEB \rightarrow PEQ \rightarrow E + P + Q
   \]  

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/ν axis in Lineweaver–Burk plots where the rates observed with different fixed concentrations of one substrate (B) are graphed versus a series of concentrations of A (Figure 13.19).

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
Viagra—An Unexpected Outcome in a Program of Drug Design

Prior to the accumulation of detailed biochemical information on metabolism, enzymes, and receptors, drugs were fortuitous discoveries made by observant scientists; the discovery of penicillin as a bacteria-killing substance by Fleming is an example. Today, drug design is the rational application of scientific knowledge and principles to the development of pharmacologically active agents. A particular target for therapeutic intervention is identified (such as an enzyme or receptor involved in illness), and chemical analogues of its substrate or ligand are synthesized in hopes of finding an inhibitor (or activator) that will serve as a drug to treat the illness. Sometimes the outcome is unanticipated, as the story of Viagra (sildenafil citrate) reveals.

When the smooth muscle cells of blood vessels relax, blood flow increases and blood pressure drops. Such relaxation is the result of decreases in intracellular \([\text{Ca}^{2+}]\) (which in turn is triggered by nitric oxide, NO; see Chapter 32). Cyclic GMP (cGMP) is hydrolyzed by phosphodiesterases to form 5'-GMP, and the muscles contract again. Scientists at Pfizer reasoned that, if phosphodiesterase inhibitors could be found, they might be useful drugs to treat angina (chest pain due to inadequate blood flow to heart muscle) or hypertension (high blood pressure). The phosphodiesterase (PDE) prevalent in vascular muscle is PDE 5, one of at least nine different subtypes of PDE in human cells. The search was on for substances that inhibit PDE 5, but not the other prominent PDE types, and Viagra was found. Disappointinggly, Viagra showed no significant benefits for angina or hypertension, but some men in clinical trials reported penile erection. Apparently, Viagra led to an increase in [cGMP] in penile vascular tissue, allowing vascular muscle relaxation, improved blood flow, and erection. A drug was born.

In a more focused way, detailed structural data on enzymes, receptors, and the ligands that bind to them has led to rational drug design, in which computer modeling of enzyme-ligand interactions replaces much of the initial chemical synthesis and clinical pre-screening of potential therapeutic agents, saving much time and effort in drug development.

![Note the structural similarity between cGMP (left) and Viagra (right).](image)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>( E + A \rightarrow EA \rightarrow E'P \rightarrow E' \rightarrow E'B \rightarrow EQ \rightarrow E + Q )</th>
</tr>
</thead>
</table>

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 of the rates observed with different fixed concentrations of one substrate (B) versus a series of concentrations of A (see Figure 13.22).

The Conversion of AEB to PEQ Is the Rate-Limiting Step in Random, Single-Displacement Reactions

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

\[
A + E \leftrightarrow AE \leftrightarrow EP \leftrightarrow P + E
\]

\[
A + B \leftrightarrow EB \leftrightarrow QE \leftrightarrow E + Q \quad (13.49)
\]
The rate-limiting step is the reaction $AEB \rightarrow PEQ$. It doesn’t matter whether $A$ or $B$ binds first to $E$, or whether $Q$ or $P$ is released first from $QEP$. Sometimes, reactions that follow this random order of addition of substrates to $E$ can be distinguished from reactions obeying an ordered, single-displacement mechanism. If $A$ has no influence on the binding constant for $B$ (and vice versa) and the mechanism is purely random, the lines in a Lineweaver–Burk plot intersect at the $1/[A]$ axis (Figure 13.20).

**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 donor to form creatine phosphate ($CrP$) from creatine ($Cr$). Creatine-P is an important reservoir of phosphate-bond energy in muscle cells (Figure 13.21).

\[
\begin{align*}
ATP + E & \rightleftharpoons ATP:E \\
ADP:E & \rightleftharpoons ADP + E \\
ATP:E:Cr & \rightleftharpoons ADP:E:CrP \\
E + Cr & \rightleftharpoons E:Cr \\
E:CrP & \rightleftharpoons E + CrP
\end{align*}
\]

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.

**In an Ordered, Single-Displacement Reaction, the Leading Substrate Must Bind First**

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 $ATP$ from $E:CrP$ and $E:CrP$ to $E:Cr:ADP$.
the products of the reaction, P and Q. In the following schemes, P is the product of A and is released last. One representation, suggested by W. W. Cleland, follows:

\[
\begin{align*}
A & \rightarrow AE \\
B & \rightarrow AEB \\
Q & \rightarrow PE \\
P & \rightarrow E
\end{align*}
\]

Another way of portraying this mechanism is as follows:

\[
\begin{align*}
A & \rightarrow AE \\
B & \rightarrow AEB \\
E & \rightarrow PE \\
Q & \rightarrow PEQ
\end{align*}
\]

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

\[
\text{NAD}^{+} + \text{BH}_2 \rightarrow \text{NADH} + \text{H}^+ + \text{B}
\]

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

\[
\begin{align*}
\text{NAD}^{+} + \text{CH}_3\text{CH}_2\text{OH} & \rightarrow \text{NADH} + \text{H}^+ + \text{CH}_3\text{CHO} \\
\text{(A)} & \text{ethanol} \\
\text{(P)} & \text{acetaldehyde} \\
\text{(B)} & \text{(Q)}
\end{align*}
\]

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 Proceed Via Formation of a Covalently Modified Enzyme Intermediate**

Double-displacement reactions are characterized by a pattern of parallel lines when 1/\(v\) is plotted as a function of 1/[A] at different concentrations of B, the second substrate (Figure 13.22). 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:

\[
\begin{align*}
A & \rightarrow AE \\
P & \rightarrow PE' \\
B & \rightarrow E'E \\
Q & \rightarrow E'Q \\
\end{align*}
\]
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:

\[
\text{amino acid}_1 + \text{keto acid}_2 \rightarrow \text{keto acid}_1 + \text{amino acid}_2
\]

A specific example would be *glutamate:aspartate aminotransferase*. Figure 13.23 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 B₆ derivative), serves as the amino group acceptor/donor in the enzymatic reaction. The unmodified enzyme, E, has the coenzyme in the aldehydic pyridoxal form, whereas in the modified enzyme, E’, the coenzyme is actually pyridoxamine phosphate (Figure 13.23). Not all enzymes displaying ping-pong-type mechanisms require coenzymes as carriers for the chemical substituent transferred in the reaction.
Exchange Reactions Are One Way to Diagnose 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:

\[
\text{Sucrose} + \text{Pi} \rightleftharpoons \text{glucose-1-phosphate} + \text{fructose}
\]

\[
\text{Maltose} + \text{Pi} \rightleftharpoons \text{glucose-1-phosphate} + \text{glucose}
\]

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

\[
^{32}\text{P_i} + \text{G-1-P} \rightleftharpoons \text{P}_i + \text{G-1-}^{32}\text{P}
\]

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

\[
\text{Sucrose} + E \rightleftharpoons E\text{-glucose} + \text{fructose}
\]

\[
E\text{-glucose} + \text{P}_i \rightleftharpoons E + \text{glucose-1-phosphate}
\]

Thus, in the presence of just \(^{32}\text{Pi}\), and glucose-1-phosphate, sucrose phosphorylase still catalyzes the second reaction and radioactive \(\text{Pi}\) 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: \(E: \text{P}_i\) (or glucose: \(E:\text{glucose-1-phosphate}\) complex for any reaction to occur. Exchange reactions are a character-
istic of enzymes that obey double-displacement mechanisms at some point in their catalysis.

**Multisubstrate Reactions Can Also Occur in Cells**

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 (see Chapter 18):

\[
\text{NAD}^+ + \text{glyceraldehyde-3-P} + P_i \rightarrow \text{NADH} + H^+ + 1,3\text{-bisphosphoglycerate}
\]

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 unisubstrate 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.

**13.6. How Can Enzymes Be So Specific?**

The extraordinary ability of an enzyme to catalyze only one particular reaction is a quality known as **specificity**. Specificity means an enzyme acts only on a specific substance, its substrate, invariably transforming it into a specific product. That is, an enzyme binds only certain compounds, and then, only a specific reaction ensues. Some enzymes show absolute specificity, catalyzing the transformation of only one specific substrate to yield a unique product. Other enzymes carry out a particular reaction but act on a class of compounds. For example, hexokinase (ATP:hexose-6-phosphotransferase) will carry out the ATP-dependent phosphorylation of a number of hexoses at the 6-position, including glucose. Specificity studies on enzymes entail an examination of the rates of the enzymatic reaction obtained with various structural analogs of the substrate. By determining which functional and structural groups within the substrate affect binding or catalysis, enzymologists can map the properties of the active site, analyzing questions such as: Can the active site accommodate sterically bulky groups? Are ionic interactions between E and S important? Are H bonds formed?

**The “Lock and Key” Hypothesis Was the First Explanation for Specificity**

Pioneering enzyme specificity studies at the turn of the 20th century by the great organic chemist Emil Fischer led to the notion of an enzyme resembling a “lock” and its particular substrate the “key.” This analogy captures the essence of the specificity that exists between an enzyme and its substrate, but enzymes are not rigid templates like locks.

**The “Induced Fit” Hypothesis Provides a More Accurate Description of Specificity**

Enzymes are highly flexible, conformationally dynamic molecules, and many of their remarkable properties, including substrate binding and catalysis, are due to their structural pliancy. Realization of the conformational flexibility of proteins led Daniel Koshland to hypothesize that the binding of a substrate by an enzyme is an interactive process. That is, the shape of the enzyme’s active site is actually modified upon binding S, in a process of dynamic recognition between enzyme and substrate aptly called **induced fit**. In essence, substrate binding alters the conformation of the protein, so that the protein and the substrate “fit” each other more precisely. The process is truly interactive in that the conformation of the substrate also changes as it adapts to the conformation of the enzyme.
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This idea also helps explain some of the mystery surrounding the enormous catalytic power of enzymes: In enzyme catalysis, precise orientation of catalytic residues comprising the active site is necessary for the reaction to occur; substrate binding induces this precise orientation by the changes it causes in the protein’s conformation.

“Induced Fit” Favors Formation of the Transition State

The catalytically active enzyme substrate complex is an interactive structure in which the enzyme causes the substrate to adopt a form that mimics the transition state of the reaction. Thus, a poor substrate would be one that was less effective in directing the formation of an optimally active enzyme:transition state conformation. This active conformation of the enzyme molecule is thought to be relatively unstable in the absence of substrate, and free enzyme thus reverts to a conformationally different state.

Specificity and Reactivity

Consider, for example, why hexokinase catalyzes the ATP-dependent phosphorylation of hexoses but not smaller phosphoryl-group acceptors such as glycerol, ethanol, or even water. Surely these smaller compounds are not sterically forbidden from approaching the active site of hexokinase (Figure 13.24). Indeed, water should penetrate the active site easily and serve as a highly effective phosphoryl-group acceptor. Accordingly, hexokinase should display high ATPase activity. It does not. Only the binding of hexoses induces hexokinase to assume its fully active conformation. The hexose-binding site of hexokinase is located between two protein domains. Binding of glucose in the active site induces a conformational change in hexokinase that causes the two domains to close upon one another, creating the catalytic site.

In Chapter 14, we explore in greater detail the factors that contribute to the remarkable catalytic power of enzymes and examine specific examples of enzyme reaction mechanisms.

13.7 Are All Enzymes Proteins?

RNA Molecules That Are Catalytic Have Been Termined “Ribozymes”

It was long assumed that all enzymes are proteins. However, several decades ago, instances of biological catalysis by RNA molecules were discovered. Catalytic RNAs, or ribozymes, satisfy several enzymatic criteria: They are substrate specific, they enhance the reaction rate, and they emerge from the reaction unchanged. Most ribozymes act
in RNA processing, cutting the phosphodiester backbone at specific sites and religating needed segments to form functional RNA strands while discarding extraneous pieces. For example, bacterial RNase P is a ribozyme involved in the formation of mature tRNA molecules from longer RNA transcripts. RNase P 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. As another example, the introns within some rRNAs and mRNAs are ribozymes that can catalyze their own excision from large RNA transcripts by a process known as self-splicing. For instance, 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. The excision of this intron and ligation of the exons is catalyzed by the intron itself, in the presence of Mg$^{2+}$ and a free molecule of guanosine nucleoside or nucleotide (Figure 13.25). 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.

**The Ribosome Is a Ribozyme** A particularly significant case of catalysis by RNA occurs in protein synthesis. The peptidyl transferase reaction, which is the reaction of peptide bond formation during protein synthesis, is catalyzed by the 23S rRNA of the 50S subunit of ribosomes (see Chapters 10 and 30). The substrates for the peptidyl transferase reaction are two tRNA molecules, one bearing the growing peptide chain (the peptidyl-tRNA$_{P}$) and the other bearing the next amino acid to be added.

![Figure 13.25](image-url) **FIGURE 13.25** 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.
Both the peptidyl chain and the amino acid are attached to their respective tRNAs via ester bonds to the O atom at the CCA-3’ ends of these tRNAs (see Figure 11.33). Base-pairing between these C residues in the two tRNAs and G residues in the 23S rRNA position the substrates for the reaction to occur (Figure 13.26). The two Cs at the peptidyl-tRNA CCA end pair with G2251 and G2252 of the 23S rRNA, and the last C (C75) at the 3’-end of the aminoacyl-tRNA pairs with G2553. The 3’-terminal A of the aminoacyl-tRNA interacts with G2583, and the terminal A of the peptidyl-tRNA binds to A2450. Addition of the incoming amino acid to the peptidyl chain occurs when the α-amino group of the aminoacyl-tRNA makes a nucleophilic attack on the carbonyl C of the peptidyl-tRNA. Specific 23S rRNA bases and ribose-OH groups facilitate this nucleophilic attack by favoring proton abstraction from the aminoacyl α-amino group (Figure 13.27). The products of this reaction are a one-residue-longer peptide chain attached to the tRNA and the “empty” tRNA.

The fact that RNA can catalyze such important reactions is experimental support for the idea that a primordial world dominated by RNA molecules existed before the evolution of DNA and proteins. Sidney Altman and Thomas R. Cech shared the 1989 Nobel Prize in Chemistry for their discovery of the catalytic properties of RNA.
Antibody Molecules Can Have Catalytic Activity

Antibodies are immunoglobulins, which, of course, are proteins. Catalytic antibodies are antibodies with catalytic activity (catalytic antibodies are also called abzymes, a word created by combining “Ab,” the abbreviation for antibody, with “enzyme.”) Like other antibodies, catalytic antibodies are elicited in an organism in response to immunological challenge by a foreign molecule called an antigen (see Chapter 28 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 in a reaction. The rationale is that a protein specific for binding the transition state 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 in the reactions they catalyze; see Chapter 14.)

One proof of this principle has been to prepare ester analogs by substituting a phosphorus atom for the carbon in the ester group (Figure 13.28). The phosphonate compound 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 13.2, reaction 4, and Chapter 18]) and the pyridoxal 5’-P-dependent aminotransferase reaction shown in Figure 13.23. This biotechnology offers the real possibility of creating specially tailored enzymes designed to carry out specific catalytic processes.

Catalytic antibodies apparently occur naturally. Autoimmune diseases are diseases that arise because an individual begins to produce antibodies against one of their own cellular constituents. Multiple sclerosis (MS), one such autoimmune disease, is characterized by gradual destruction of the myelin sheath surrounding neurons throughout the brain and spinal cord. Blood serum obtained from some MS patients contains antibodies capable of carrying out the proteolytic destruction of myelin basic protein (MBP). That is, these antibodies were MBP-destructive proteases. Similarly, hemophilia A is a blood-clotting disorder due to lack of the factor VIII, an essential protein for formation of a blood clot. Serum from some sufferers of hemophilia A contained antibodies with proteolytic activity against factor VIII. Thus, some antibodies may be proteases.

![Image of catalytic antibodies](A3_7.png)

**FIGURE 13.28** (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.
Chapter 13
Enzymes—Kinetics and Specificity

13.8 Is It Possible to Design an Enzyme to Catalyze Any Desired Reaction?

Enzymes have evolved to catalyze metabolic reactions with high selectivity, specificity, and rate enhancements. Given these remarkable attributes, it would be very desirable to have the ability to create designer enzymes individually tailored to catalyze any imaginable reaction, particularly those that might have practical uses in industrial chemistry, the pharmaceutical industry, or environmental remediation.

To this end, several approaches have been taken to create a desired enzyme de novo (de novo: literally “new”; colloquially “from scratch.” In biochemistry, the synthesis of some end product from simpler precursors.) Most approaches begin with a known enzyme and then engineer it by using in vitro mutagenesis (see Chapter 12) to replace active-site residues with a new set that might catalyze the desired reaction. This strategy has the advantage that the known protein structure provides a stable scaffold into which a new catalytic site can be introduced. As pointed out in Chapter 6, despite the extremely large number of possible amino acid sequences for a polypeptide chain, a folded protein adopts one of a rather limited set of core protein structures. Yet proteins have an extraordinary range of functional possibilities. So, this approach is rational. A second, more difficult, approach attempts the completely new design of a protein with the desired structure and activity. Often, this approach relies on in silico methods, where the folded protein structure and the spatial and reactive properties of its putative active site are modeled, refined, and optimized via computer. Although this approach has fewer limitations in terms of size and shape of substrates, it brings other complications, such as protein folding and stability, to the problem, to say nothing of the difficulties of going from the computer model (in silico) to a real enzyme in a cellular environment (in vivo).

Enzymes have shown adaptability over the course of evolution. New enzyme functions have appeared time and time again, as mutation and selection according to Darwinian principles operate on existing enzymes. Some enzyme designers have coupled natural evolutionary processes with rational design using in vitro mutagenesis. Expression of mutated versions of the gene encoding the enzyme in bacteria, followed by rounds of selection for bacteria producing an enzyme with even better catalytic properties, takes advantage of naturally occurring processes to drive further mutation and selection for an optimal enzyme. This dual approach is whimsically referred to as semirational design because it relies on the rational substitution of certain amino acids with new ones in the active site, followed by directed evolution (selection for bacteria expressing more efficient versions of the enzyme).

An example of active-site engineering is the site-directed mutation of an epoxide hydrolase to change its range of substrate selection so that it now acts on chlorinated epoxides (Figure 13.29). Degradation of chlorinated epoxides is a major problem in the removal of toxic pollutants from water resources. Mutation of a bacterial epoxide hydrolase at three active-site residues (F108, I219, and C248) and se-

![Figure 13.29](image-url)

**Figure 13.29** cis-1,2-Dichloroethylene (DCE) is an industrial solvent that poses hazards to human health; DCE occurs as a pollutant in water sources. Bacterial metabolism of DCE to form cis-1,2-dichloroepoxyethane (step 1) occurs readily, but enzymatic degradation of the epoxide to glyoxal and chloride ions (step 2) is limited. Microbial detoxification of DCE in ground water requires enzymes for both steps 1 and 2. Genetic engineering of an epoxide hydrolase to create an enzyme capable of using cis-1,2-dichloroepoxyethane as a substrate is a practical example of de novo enzyme design.
lecion in bacteria for enhanced chlorinated epoxide hydrolase activity yielded an F108L, I219L, C248I mutant enzyme that catalyzed the conversion of cis-dichloroepoxyxethane to CI ions and glyoxal with a dramatically increased $V_{\text{max}}/K_m$ ratio.

SUMMARY

Living systems use enzymes to accelerate and control the rates of vitally important biochemical reactions. Enzymes provide kinetic control over thermodynamic potentiality: Reactions occur in a timeframe suitable to the metabolic requirements of cells. Enzymes are the agents of metabolic function.

13.1 What Characteristic Features Define Enzymes? Enzymes can be characterized in terms of three prominent features: catalytic power, specificity, and regulation. The site on the enzyme where substrate binds and catalysis occurs is called the active site. Regulation of enzyme activity is essential to the integration and regulation of metabolism.

13.2 Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way? Enzyme kinetics can determine the maximum reaction velocity that the enzyme can attain, its binding affinities for substrates and inhibitors, and the mechanism by which it accomplishes its catalysis. The kinetics of simple chemical reactions provides a foundation for exploring enzyme kinetics. Enzymes, like other catalysts, act by lowering the free energy of activation for a reaction.

13.3 What Equations Define the Kinetics of Enzyme-Catalyzed Reactions? A plot of the velocity of an enzyme-catalyzed reaction versus the concentration of the substrate $S$ is called a substrate saturation curve. The Michaelis-Menten equation is derived by assuming that $E$ combines with $S$ to form $ES$ and then $ES$ reacts to yield $P$ reach a steady-state condition where $[ES]$ is essentially constant. The Michaelis-Menten equation says that the initial rate of an enzyme reaction, $v$, is determined by two constants, $K_m$ and $V_{\text{max}}$, and the initial concentration of substrate. The turnover number of an enzyme, $k_{\text{cat}}$, is a measure of its maximal catalytic activity (the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate). The ratio $k_{\text{cat}}/K_m$ defines the catalytic efficiency of an enzyme. This ratio, $k_{\text{cat}}/K_m$, cannot exceed the diffusion-controlled rate of combination of $E$ and $S$ to form ES.

Several rearrangements of the Michaelis-Menten equation transform it into a straight-line equation, a better form for experimental determination of the constants $K_m$ and $V_{\text{max}}$ and for detection of regulatory properties of enzymes.

13.4 What Can Be Learned from the Inhibition of Enzyme Activity? Inhibition studies on enzymes have contributed significantly to our understanding of enzymes. Inhibitors may interact either reversibly or irreversibly with an enzyme. Reversible inhibitors bind to the enzyme through noncovalent association/dissociation reactions. Irreversible inhibitors typically form stable, covalent bonds with the enzyme. Reversible inhibitors may bind at the active site of the enzyme (competitive inhibition) or at some other site on the enzyme (noncompetitive inhibition). Uncompetitive inhibitors bind only to the ES complex.

13.5 What Is the Kinetic Behavior of Enzymes Catalyzing Bimolecular Reactions? Usually, enzymes catalyze reactions in which two (or even more) substrates take part, so the reaction is bimolecular. Several possibilities arise. In single-displacement reactions, both substrates, $A$ and $B$, are bound before reaction occurs. In double-displacement (or ping-pong) reactions, one substrate ($A$) is bound and reaction occurs to yield product $P$ and a modified enzyme form, $E'$. The second substrate ($B$) then binds to $E'$, and reaction occurs to yield product $Q$ and $E$, the unmodified form of enzyme. Graphical methods can be used to distinguish these possibilities. Exchange reactions are another way to diagnose bisubstrate mechanisms.

13.6 How Can Enzymes Be So Specific? Early enzyme specificity studies by Emil Fischer led to the hypothesis that an enzyme resembles a “lock” and its particular substrate the “key.” However, enzymes are not rigid templates like locks. Koshland noted that the conformation of an enzyme is dynamic and hypothesized that the interaction of $E$ with $S$ is also dynamic. The enzyme’s active site is actually modified upon binding $S$, in a process of dynamic recognition between enzyme and substrate called induced fit. Hexokinase provides a good illustration of the relationship between substrate binding, induced fit, and catalysis.

13.7 Are All Enzymes Proteins? Not all enzymes are proteins. Catalytic RNA molecules (“ribozymes”) play important cellular roles in RNA processing and protein synthesis, among other things. Catalytic RNAs give support to the idea that a primordial world dominated by RNA molecules existed before the evolution of DNA and proteins. Antibodies that have catalytic activity (“abzymes”) can be elicited in an organism in response to immunological challenge with an analog of the transition state for a reaction. Such antibodies are catalytic because they bind the transition state of a reaction and promote entry of the normal substrate into the reactive, transition-state conformation.

13.8 Is It Possible to Design an Enzyme to Catalyze Any Desired Reaction? Several approaches have been taken to create designer enzymes individually tailored to catalyze any imaginable reaction. One rational approach is to begin with a known enzyme and then engineer it using in vitro mutagenesis to replace active-site residues with a new set that might catalyze the desired reaction. A second, more difficult approach uses computer modeling to design a protein with the desired structure and activity. A third approach is to couple natural evolution- ary processes with rational design using in vitro mutagenesis. Expression of mutated versions of the gene encoding the enzyme in bacteria is followed by selection for bacteria producing an enzyme with even better catalytic properties. This dual approach is sometimes called semirational design, because it relies on the rational substitution of certain amino acids with new ones in the active site, followed by directed evolution. Active-site engineering and site-directed mutation have been used to modify an epoxide hydrolase so that it now acts on chlorinated epoxides, substances that are serious pollutants in water resources.

PROBLEMS

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

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

3. For a Michaelis-Menten reaction, $k_1 = 7 \times 10^7 / \text{M} \cdot \text{sec}$, $k_2 = 1 \times 10^7 / \text{sec}$, and $k_2 = 2 \times 10^4 / \text{sec}$. What are the values of $K_m$ and
For each example, indicate how
a. a pure noncompetitive inhibitor is added.
b. half as much enzyme is used.
c. a competitive inhibitor is added.
d. a pure noncompetitive inhibitor is added.
e. an uncompetitive inhibitor is added.

Graph these data as Lineweaver-Burk plots and use your answers to a and b.

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

\[ v = \frac{V_{\text{max}} [A][B]}{(K_d K_s + K_a [B] + K_B A + [A][B])} \]

Write the Lineweaver–Burk (double-reciprocal) equivalent of this equation and from it calculate algebraic expressions for the following:

a. The slope
b. The y-intercepts
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

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.

8. 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 to half of its normal value if the \( K_a \) values of canine ADH for ethanol and methanol are 1 millimolar and 10 millimolar, respectively? (The \( K_e \) for ethanol in its role as competitive inhibitor of methanol oxidation by ADH is the same as its \( K_a \).) 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.

9. Measurement of the rate constants for a simple enzymatic reaction obeying Michaelis–Menten kinetics gave the following results:

\[ k_1 = 2 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1} \]
\[ k_{-1} = 1 \times 10^5 \text{ sec}^{-1} \]
\[ k_2 = 5 \times 10^3 \text{ sec}^{-1} \]

a. What is \( K_a \), the dissociation constant for the enzyme–substrate complex?
b. What is \( K_a \) for this enzyme?
c. What is \( k_{cat} \) (the turnover number) for this enzyme?
d. What is the catalytic efficiency (\( k_{cat}/K_a \)) for this enzyme?
e. Does this enzyme approach “kinetic perfection”? (That is, does \( k_{cat}/K_a \) approach the diffusion-controlled rate of enzyme association with substrate?)
f. If a kinetic measurement was made using 2 nanomoles of enzyme per mL and saturating amounts of substrate, what would the \( V_{max} \) equal?
g. Again, using 2 nanomoles of enzyme per mL of reaction mixture, what concentration of substrate would give \( v = 0.75 V_{max} \)?
h. If a kinetic measurement was made using 4 nanomoles of enzyme per mL and saturating amounts of substrate, what would the \( V_{max} \) equal? What would \( K_a \) equal under these conditions?

10. Triose phosphate isomerase catalyzes the conversion of glyceraldehyde-3-phosphate to dihydroxyacetone phosphate.

Glyceraldehyde-3-P \( \rightleftharpoons \) dihydroxyacetone-P

The \( K_a \) of this enzyme for its substrate glyceraldehyde-3-phosphate is 1.8 \( \times \) 10^{-5} M. When [glyceraldehydes-3-phosphate] = 30 \( \mu \)M, the rate of the reaction, \( v \), was 82.5 \( \mu \)mol mL^{-1} sec^{-1}.

a. What is \( V_{max} \) for this enzyme?
b. Assuming 3 nanomoles per mL of enzyme was used in this experiment, what was \( k_{cat} \) for this enzyme?
c. What is the catalytic efficiency (\( k_{cat}/K_a \)) for triose phosphate isomerase?
d. Does the value of \( k_{cat}/K_a \) reveal whether triose phosphate isomerase approaches “catalytic perfection”?
e. What determines the ultimate speed limit of an enzyme-catalyzed reaction? That is, what is that which imposes the physical limit on kinetic perfection?

11. The citric acid cycle enzyme fumarase catalyzes the conversion of fumarate to form malate.

Fumarate + H\(_2\)O \( \rightleftharpoons \) malate

The turnover number, \( k_{cat} \), for fumarase is 800/sec. The \( K_a \) of fumarase for its substrate fumarate is 5 \( \mu \)M.

a. In an experiment using 2 nanomole/L of fumarase, what is \( V_{max} \)?
b. The cellular concentration of fumarate is 47.5 \( \mu \)M. What is \( v \) when [fumarate] = 47.5 \( \mu \)M?
c. What is the catalytic efficiency of fumarase?
d. Does fumarase approach “catalytic perfection”?
12. Carbonic anhydrase catalyzes the hydration of CO₂:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \]

The \( K_a \) of carbonic anhydrase for CO₂ is 12 mM. Carbonic anhydrase gave an initial velocity \( v_i = 4.5 \mu\text{mol}\) H₂CO₃formed/mL·sec when \([\text{CO}_2]=36\text{mM}\).

a. What is \( V_{max} \) for this enzyme?

b. Assuming 5 pmol/mL (\( 5 \times 10^{-11} \text{ moles/mL} \)) of enzyme were used in this experiment, what is \( k_m \) for this enzyme?

c. What is the catalytic efficiency of this enzyme?

d. Does carbonic anhydrase approach “catalytic perfection”?

13. Acetylcholinesterase catalyzes the hydrolysis of the neurotransmitter acetylcholine:

\[ \text{Acetylcholine} + \text{H}_2\text{O} \rightarrow \text{acetate} + \text{choline} \]

The \( K_m \) of acetylcholinesterase for its substrate acetylcholine is \( 9 \times 10^{-6} \text{M} \). In a reaction mixture containing 5 nanomoles/mL of acetylcholinesterase and 150 \( \mu\text{M} \) acetylcholine, a velocity \( v = 40 \mu\text{mol} \cdot \text{mol}^{-1} \cdot \text{sec}^{-1} \) was observed for the acetylcholinesterase reaction.

a. Calculate \( V_{max} \) for this amount of enzyme.

b. Calculate \( k_m \) for acetylcholinesterase.

c. Calculate the catalytic efficiency (\( k_{cat}/K_m \)) for acetylcholinesterase.

d. Does acetylcholinesterase approach “catalytic perfection”?

14. The enzyme catalase catalyzes the decomposition of hydrogen peroxide:

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

The turnover number (\( k_{cat} \)) for catalase is 40,000,000 sec\(^{-1}\). The \( K_m \) of catalase for its substrate \( \text{H}_2\text{O}_2 \) is 0.11 M.

a. In an experiment using 3 nanomole/L of catalase, what is \( V_{max} \)?

b. What is \( v \) when \([\text{H}_2\text{O}_2]=0.75\text{M}\)?

c. What is the catalytic efficiency of humarase?

d. Does catalase approach “catalytic perfection”?

15. Equation 13.9 presents the simple Michaelis–Menten situation where the reaction is considered to be irreversible (\( [P] \) is negligible). Many enzymatic reactions are reversible, and \( P \) does accumulate.

a. Derive an equation for \( v \); the rate of the enzyme-catalyzed reaction \( S \rightarrow P \) in terms of a modified Michaelis–Menten model that incorporates the reverse reaction that will occur in the presence of product, \( P \).

b. Solve this modified Michaelis–Menten equation for the special situation when \( v = 0 \) (that is, \( S \rightarrow \text{P} \) is at equilibrium, or in other words, \( K_m = [P]/[S] \)).

(c. B. S. Haldane first described this reversible Michaelis–Menten modification, and his expression for \( K_m \) in terms of the modified M-M equation is known as the Haldane relationship.)

Preparing for the MCAT Exam


a. The \( K_m \) of enzyme A for its substrate \( S \) is \( K_2=1\text{mM} \). Enzyme A also acts on substrate \( T \) and its \( K_3=10\text{mM} \). Is \( S \) or \( T \) the preferred substrate for enzyme \( A \)?

b. The rate constant \( k_3 \) with substrate \( S \) is \( 2 \times 10^9 \text{sec}^{-1} \); with substrate \( T \), \( k_3 = 4 \times 10^9 \text{sec}^{-1} \). Does enzyme A use substrate \( S \) or substrate \( T \) with greater catalytic efficiency?

17. Use Figure 13.12 to answer the following questions.

a. Is the enzyme whose temperature versus activity profile is shown in Figure 13.12 likely to be from an animal or a plant? Why?

b. What do you think the temperature versus activity profile for an enzyme from a thermophilic prokaryote growing in an 80°F pool of water would resemble?

### Further Reading

**Enzymes in General**


**Catalytic Power**


**General Reviews of Enzyme Kinetics**


**Graphical and Statistical Analysis of Kinetic Data**


**Effect of Active Site Amino Acid Substitutions on \( k_{cat}/K_m \)**


**Enzymes and Rational Drug Design**


**Enzyme Inhibition**


Catalytic RNA

Catalytic Antibodies