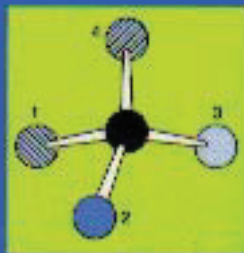


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Harper's Illustrated Biochemistry



Robert K. Murray
Daryl K. Granner
Peter A. Mayes
Victor W. Rodwell

twenty-
sixth
edition

a LANGE medical book

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twenty-sixth edition

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Preface

The authors and publisher are pleased to present the twenty-sixth edition of *Harper's Illustrated Biochemistry*. *Review of Physiological Chemistry* was first published in 1939 and revised in 1944, and it quickly gained a wide readership. In 1951, the third edition appeared with Harold A. Harper, University of California School of Medicine at San Francisco, as author. Dr. Harper remained the sole author until the ninth edition and co-authored eight subsequent editions. Peter Mayes and Victor Rodwell have been authors since the tenth edition, Daryl Granner since the twentieth edition, and Rob Murray since the twenty-first edition. Because of the increasing complexity of biochemical knowledge, they have added co-authors in recent editions.

Fred Keeley and Margaret Rand have each co-authored one chapter with Rob Murray for this and previous editions. Peter Kennelly joined as a co-author in the twenty-fifth edition, and in the present edition has co-authored with Victor Rodwell all of the chapters dealing with the structure and function of proteins and enzymes. The following additional co-authors are very warmly welcomed in this edition: Kathleen Botham has co-authored, with Peter Mayes, the chapters on bioenergetics, biologic oxidation, oxidative phosphorylation, and lipid metabolism. David Bender has co-authored, also with Peter Mayes, the chapters dealing with carbohydrate metabolism, nutrition, digestion, and vitamins and minerals. P. Anthony Weil has co-authored chapters dealing with various aspects of DNA, of RNA, and of gene expression with Daryl Granner. We are all very grateful to our co-authors for bringing their expertise and fresh perspectives to the text.

CHANGES IN THE TWENTY-SIXTH EDITION

A major goal of the authors continues to be to provide both medical and other students of the health sciences with a book that both describes the basics of biochemistry and is user-friendly and interesting. A second major ongoing goal is to reflect the most significant advances in biochemistry that are important to medicine. However, a third major goal of this edition was to achieve a substantial reduction in size, as feedback indicated that many readers prefer shorter texts.

To achieve this goal, all of the chapters were rigorously edited, involving their amalgamation, division, or deletion, and many were reduced to approximately one-half to two-thirds of their previous size. This has been effected without loss of crucial information but with gain in conciseness and clarity.

Despite the reduction in size, there are many new features in the twenty-sixth edition. These include:

- A new chapter on amino acids and peptides, which emphasizes the manner in which the properties of biologic peptides derive from the individual amino acids of which they are comprised.
- A new chapter on the primary structure of proteins, which provides coverage of both classic and newly emerging "proteomic" and "genomic" methods for identifying proteins. A new section on the application of mass spectrometry to the analysis of protein structure has been added, including comments on the identification of covalent modifications.
- The chapter on the mechanisms of action of enzymes has been revised to provide a comprehensive description of the various physical mechanisms by which enzymes carry out their catalytic functions.
- The chapters on integration of metabolism, nutrition, digestion and absorption, and vitamins and minerals have been completely re-written.
- Among important additions to the various chapters on metabolism are the following: update of the information on oxidative phosphorylation, including a description of the rotary ATP synthase; new insights into the role of GTP in gluconeogenesis; additional information on the regulation of acetyl-CoA carboxylase; new information on receptors involved in lipoprotein metabolism and reverse cholesterol transport; discussion of the role of leptin in fat storage; and new information on bile acid regulation, including the role of the farnesoid X receptor (FXR).
- The chapter on membrane biochemistry in the previous edition has been split into two, yielding two new chapters on the structure and function of membranes and intracellular traffic and sorting of proteins.
- Considerable new material has been added on RNA synthesis, protein synthesis, gene regulation, and various aspects of molecular genetics.
- Much of the material on individual endocrine glands present in the twenty-fifth edition has been replaced with new chapters dealing with the diversity of the endocrine system, with molecular mechanisms of hormone action, and with signal transduction.

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- The chapter on plasma proteins, immunoglobulins, and blood coagulation in the previous edition has been split into two new chapters on plasma proteins and immunoglobulins and on hemostasis and thrombosis.
- New information has been added in appropriate chapters on lipid rafts and caveolae, aquaporins, connexins, disorders due to mutations in genes encoding proteins involved in intracellular membrane transport, absorption of iron, and conformational diseases and pharmacogenomics.
- A new and final chapter on “The Human Genome Project” (HGP) has been added, which builds on the material covered in Chapters 35 through 40. Because of the impact of the results of the HGP on the future of biology and medicine, it appeared appropriate to conclude the text with a summary of its major findings and their implications for future work.
- As initiated in the previous edition, references to useful Web sites have been included in a brief Appendix at the end of the text.

ORGANIZATION OF THE BOOK

The text is divided into two introductory chapters (“Biochemistry & Medicine” and “Water & pH”) followed by six main sections.

Section I deals with the structures and functions of proteins and enzymes, the workhorses of the body. Because almost all of the reactions in cells are catalyzed by enzymes, it is vital to understand the properties of enzymes before considering other topics.

Section II explains how various cellular reactions either utilize or release energy, and it traces the pathways by which carbohydrates and lipids are synthesized and degraded. It also describes the many functions of these two classes of molecules.

Section III deals with the amino acids and their many fates and also describes certain key features of protein catabolism.

Section IV describes the structures and functions of the nucleotides and nucleic acids, and covers many major topics such as DNA replication and repair, RNA synthesis and modification, and protein synthesis. It also discusses new findings on how genes are regulated and presents the principles of recombinant DNA technology.

Section V deals with aspects of extracellular and intracellular communication. Topics covered include membrane structure and function, the molecular bases of the actions of hormones, and the key field of signal transduction.

Section VI consists of discussions of eleven special topics: nutrition, digestion, and absorption; vitamins and minerals; intracellular traffic and sorting of proteins; glycoproteins; the extracellular matrix; muscle and the cytoskeleton; plasma proteins and immunoglobulins; hemostasis and thrombosis; red and white blood cells; the metabolism of xenobiotics; and the Human Genome Project.

ACKNOWLEDGMENTS

The authors thank Janet Foltin for her thoroughly professional approach. Her constant interest and input have had a significant impact on the final structure of this text. We are again immensely grateful to Jim Ransom for his excellent editorial work; it has been a pleasure to work with an individual who constantly offered wise and informed alternatives to the sometimes primitive text transmitted by the authors. The superb editorial skills of Janene Matragrano Oransky and Harriet Lebowitz are warmly acknowledged, as is the excellent artwork of Charissa Baker and her colleagues. The authors are very grateful to Kathy Pitcoff for her thoughtful and meticulous work in preparing the Index. Suggestions from students and colleagues around the world have been most helpful in the formulation of this edition. We look forward to receiving similar input in the future.

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March 2003

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Enzymes: Kinetics

8

Victor W. Rodwell, PhD, & Peter J. Kennelly, PhD

BIOMEDICAL IMPORTANCE

Enzyme kinetics is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme-catalyzed reactions and the systematic study of factors that affect these rates. Kinetic analyses permit scientists to reconstruct the **number** and **order** of the individual steps by which enzymes transform substrates into products. The study of enzyme kinetics also represents the principal way to identify potential therapeutic agents that selectively enhance or inhibit the rates of specific enzyme-catalyzed processes. Together with site-directed mutagenesis and other techniques that probe protein structure, kinetic analysis can also reveal details of the catalytic mechanism. A complete, balanced set of enzyme activities is of fundamental importance for maintaining homeostasis. An understanding of enzyme kinetics thus is important for understanding how physiologic stresses such as anoxia, metabolic acidosis or alkalosis, toxins, and pharmacologic agents affect that balance.

CHEMICAL REACTIONS ARE DESCRIBED USING BALANCED EQUATIONS

A **balanced chemical equation** lists the initial chemical species (substrates) present and the new chemical species (products) formed for a particular chemical reaction, all in their correct proportions or **stoichiometry**. For example, balanced equation (1) below describes the reaction of one molecule each of substrates A and B to form one molecule each of products P and Q.



The double arrows indicate reversibility, an intrinsic property of all chemical reactions. Thus, for reaction (1), if A and B can form P and Q, then P and Q can also form A and B. Designation of a particular reactant as a “substrate” or “product” is therefore somewhat arbitrary since the products for a reaction written in one direction are the substrates for the reverse reaction. The term “products” is, however, often used to designate the reactants whose formation is thermodynamically favored. Reactions for which thermodynamic factors strongly favor formation of the products to which the arrow points often are represented with a single arrow as if they were “irreversible”:



Unidirectional arrows are also used to describe reactions in living cells where the products of reaction (2) are immediately consumed by a subsequent enzyme-catalyzed reaction. The rapid removal of product P or Q therefore precludes occurrence of the reverse reaction, rendering equation (2) **functionally irreversible under physiologic conditions**.

CHANGES IN FREE ENERGY DETERMINE THE DIRECTION & EQUILIBRIUM STATE OF CHEMICAL REACTIONS

The Gibbs free energy change ΔG (also called either the free energy or Gibbs energy) describes both the *direction* in which a chemical reaction will tend to proceed and the concentrations of reactants and products that will be present at equilibrium. ΔG for a chemical reaction equals the sum of the free energies of formation of the reaction products ΔG_p minus the sum of the free energies of formation of the substrates ΔG_s . ΔG^0 denotes the change in free energy that accompanies transition from the standard state, one-molar concentrations of substrates and products, to equilibrium. A more useful biochemical term is ΔG^0 , which defines ΔG^0 at a standard state of 10^{-7} M protons, pH 7.0 (Chapter 10). If the free energy of the products is lower than that of the substrates, the signs of ΔG^0 and ΔG^0 will be negative, indicating that the reaction as written is favored in the direction left to right. Such reactions are referred to as **spontaneous**. The **sign** and the **magnitude** of the free energy change determine how far the reaction will proceed. Equation (3)—

$$\Delta G^0 = -RT \ln K_{eq} \quad (3)$$

—illustrates the relationship between the equilibrium constant K_{eq} and ΔG^0 , where R is the gas constant (1.98 cal/mol/°K or 8.31 J/mol/°K) and T is the absolute temperature in degrees Kelvin. K_{eq} is equal to the product of the concentrations of the reaction products, each raised to the power of their stoichiometry, divided by the product of the substrates, each raised to the power of their stoichiometry.

For the reaction $A + B \rightarrow P + Q$ —

$$K_{eq} = \frac{[P][Q]}{[A][B]} \quad (4)$$

and for reaction (5)



$$K_{eq} = \frac{[P]}{[A]^2} \quad (6)$$

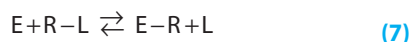
— ΔG^0 may be calculated from equation (3) if the concentrations of substrates and products present at equilibrium are known. If ΔG^0 is a negative number, K_{eq} will be greater than unity and the concentration of products at equilibrium will exceed that of substrates. If ΔG^0 is positive, K_{eq} will be less than unity and the formation of substrates will be favored.

Notice that, since ΔG^0 is a function exclusively of the initial and final states of the reacting species, it can provide information only about the *direction* and *equilibrium state* of the reaction. ΔG^0 is independent of the mechanism of the reaction and therefore provides no information concerning *rates* of reactions. Consequently—and as explained below—although a reaction may have a large negative ΔG^0 or $\Delta G^{0'}$, it may nevertheless take place at a negligible rate.

THE RATES OF REACTIONS ARE DETERMINED BY THEIR ACTIVATION ENERGY

Reactions Proceed via Transition States

The concept of the **transition state** is fundamental to understanding the chemical and thermodynamic basis of catalysis. Equation (7) depicts a displacement reaction in which an entering group E displaces a leaving group L, attached initially to R.



Midway through the displacement, the bond between R and L has weakened but has not yet been completely severed, and the new bond between E and R is as yet incompletely formed. This transient intermediate—in which neither free substrate nor product exists—is termed the **transition state**, $E \cdots R \cdots L$. Dotted lines represent the “partial” bonds that are undergoing formation and rupture.

Reaction (7) can be thought of as consisting of two “partial reactions,” the first corresponding to the formation (F) and the second to the subsequent decay (D) of the transition state intermediate. As for all reactions,

characteristic changes in free energy, ΔG_F , and ΔG_D are associated with each partial reaction.



For the overall reaction (10), ΔG is the sum of ΔG_F and ΔG_D . As for any equation of two terms, it is not possible to infer from ΔG either the sign or the magnitude of ΔG_F or ΔG_D .

Many reactions involve multiple transition states, each with an associated change in free energy. For these reactions, the overall ΔG represents the sum of *all* of the free energy changes associated with the formation and decay of *all* of the transition states. **Therefore, it is not possible to infer from the overall ΔG the number or type of transition states through which the reaction proceeds.** Stated another way: overall thermodynamics tells us nothing about kinetics.

ΔG_F Defines the Activation Energy

Regardless of the sign or magnitude of ΔG , ΔG_F for the overwhelming majority of chemical reactions has a positive sign. The formation of transition state intermediates therefore requires surmounting of energy barriers. For this reason, ΔG_F is often termed the **activation energy**, E_{act} , the energy required to surmount a given energy barrier. The ease—and hence the frequency—with which this barrier is overcome is inversely related to E_{act} . The thermodynamic parameters that determine how *fast* a reaction proceeds thus are the ΔG_F values for formation of the transition states through which the reaction proceeds. For a simple reaction, where $\propto$ means “proportionate to,”

$$\text{Rate} \propto e^{\frac{-E_{act}}{RT}} \quad (11)$$

The activation energy for the reaction proceeding in the opposite direction to that drawn is equal to $-\Delta G_D$.

NUMEROUS FACTORS AFFECT THE REACTION RATE

The **kinetic theory**—also called the **collision theory**—of chemical kinetics states that for two molecules to react they must (1) approach within bond-forming distance of one another, or “collide”; and (2) must possess sufficient kinetic energy to overcome the energy barrier for reaching the transition state. It therefore follows

that anything which increases the *frequency* or *energy* of collision between substrates will increase the rate of the reaction in which they participate.

Temperature

Raising the temperature increases the kinetic energy of molecules. As illustrated in Figure 8–1, the total number of molecules whose kinetic energy exceeds the energy barrier E_{act} (vertical bar) for formation of products increases from low (A), through intermediate (B), to high (C) temperatures. Increasing the kinetic energy of molecules also increases their motion and therefore the frequency with which they collide. This combination of more frequent and more highly energetic and productive collisions increases the reaction rate.

Reactant Concentration

The frequency with which molecules collide is directly proportionate to their concentrations. For two different molecules A and B, the frequency with which they collide will double if the concentration of either A or B is doubled. If the concentrations of both A and B are doubled, the probability of collision will increase fourfold.

For a chemical reaction proceeding at constant temperature that involves one molecule each of A and B,



the number of molecules that possess kinetic energy sufficient to overcome the activation energy barrier will be a constant. The number of collisions with sufficient energy to produce product P therefore will be directly proportionate to the number of collisions between A and B and thus to their molar concentrations, denoted by square brackets.

$$\text{Rate} \propto [A][B] \quad (13)$$

Similarly, for the reaction represented by

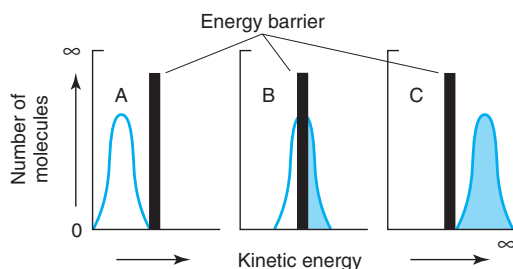


Figure 8–1. The energy barrier for chemical reactions.

which can also be written as



the corresponding rate expression is

$$\text{Rate} \propto [A][B][B] \quad (16)$$

or

$$\text{Rate} \propto [A][B]^2 \quad (17)$$

For the general case when n molecules of A react with m molecules of B,



the rate expression is

$$\text{Rate} \propto [A]^n [B]^m \quad (19)$$

Replacing the proportionality constant with an equal sign by introducing a proportionality or **rate constant** k characteristic of the reaction under study gives equations (20) and (21), in which the subscripts 1 and -1 refer to the rate constants for the forward and reverse reactions, respectively.

$$\text{Rate}_1 = k_1 [A]^n [B]^m \quad (20)$$

$$\text{Rate}_{-1} = k_{-1} [P] \quad (21)$$

K_{eq} Is a Ratio of Rate Constants

While all chemical reactions are to some extent reversible, at equilibrium the *overall* concentrations of reactants and products remain constant. At equilibrium, the rate of conversion of substrates to products therefore equals the rate at which products are converted to substrates.

$$\text{Rate}_1 = \text{Rate}_{-1} \quad (22)$$

Therefore,

$$k_1 [A]^n [B]^m = k_{-1} [P] \quad (23)$$

and

$$\frac{k_1}{k_{-1}} = \frac{[P]}{[A]^n [B]^m} \quad (24)$$

The ratio of k_1 to k_{-1} is termed the equilibrium constant, K_{eq} . The following important properties of a system at equilibrium must be kept in mind:

- (1) The equilibrium constant is a ratio of the reaction *rate constants* (not the reaction *rates*).

- (2) At equilibrium, the reaction *rates* (not the *rate constants*) of the forward and back reactions are equal.
- (3) Equilibrium is a *dynamic* state. Although there is no *net* change in the concentration of substrates or products, individual substrate and product molecules are continually being interconverted.
- (4) The numeric value of the equilibrium constant K_{eq} can be calculated either from the concentrations of substrates and products at equilibrium or from the ratio k_1/k_{-1} .

THE KINETICS OF ENZYMATIC CATALYSIS

Enzymes Lower the Activation Energy Barrier for a Reaction

All enzymes accelerate reaction rates by providing transition states with a lowered ΔG_F for formation of the transition states. However, they may differ in the way this is achieved. Where the mechanism or the sequence of chemical steps at the active site is essentially the same as those for the same reaction proceeding in the absence of a catalyst, **the environment of the active site lowers ΔG_F** by stabilizing the transition state intermediates. As discussed in Chapter 7, stabilization can involve (1) acid-base groups suitably positioned to transfer protons to or from the developing transition state intermediate, (2) suitably positioned charged groups or metal ions that stabilize developing charges, or (3) the imposition of steric strain on substrates so that their geometry approaches that of the transition state. HIV protease (Figure 7–6) illustrates catalysis by an enzyme that lowers the activation barrier by stabilizing a transition state intermediate.

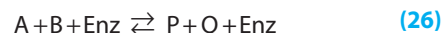
Catalysis by enzymes that proceeds via a *unique* reaction mechanism typically occurs when the transition state intermediate forms a covalent bond with the enzyme (**covalent catalysis**). The catalytic mechanism of the serine protease chymotrypsin (Figure 7–7) illustrates how an enzyme utilizes covalent catalysis to provide a unique reaction pathway.

ENZYMES DO NOT AFFECT K_{eq}

Enzymes accelerate reaction rates by lowering the activation barrier ΔG_F . While they may undergo transient modification during the process of catalysis, enzymes emerge unchanged at the completion of the reaction. The presence of an enzyme therefore has no effect on ΔG^0 for the *overall* reaction, which is a function solely of the *initial and final states* of the reactants. Equation (25) shows the relationship between the equilibrium constant for a reaction and the standard free energy change for that reaction:

$$\Delta G^0 = -RT \ln K_{eq} \quad (25)$$

If we include the presence of the enzyme (E) in the calculation of the equilibrium constant for a reaction,



the expression for the equilibrium constant,

$$K_{eq} = \frac{[P][Q][\text{Enz}]}{[A][B][\text{Enz}]} \quad (27)$$

reduces to one identical to that for the reaction in the absence of the enzyme:

$$K_{eq} = \frac{[P][Q]}{[A][B]} \quad (28)$$

Enzymes therefore have no effect on K_{eq} .

MULTIPLE FACTORS AFFECT THE RATES OF ENZYME-CATALYZED REACTIONS

Temperature

Raising the temperature increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules. However, heat energy can also increase the kinetic energy of the enzyme to a point that exceeds the energy barrier for disrupting the noncovalent interactions that maintain the enzyme's three-dimensional structure. The polypeptide chain then begins to unfold, or **denature**, with an accompanying rapid loss of catalytic activity. The temperature range over which an enzyme maintains a stable, catalytically competent conformation depends upon—and typically moderately exceeds—the normal temperature of the cells in which it resides. Enzymes from humans generally exhibit stability at temperatures up to 45–55 °C. By contrast, enzymes from the thermophilic microorganisms that reside in volcanic hot springs or undersea hydrothermal vents may be stable up to or above 100 °C.

The **Q_{10} , or temperature coefficient**, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature. For the temperatures over which enzymes are stable, the rates of most biologic processes typically double for a 10 °C rise in temperature ($Q_{10} = 2$). Changes in the rates of enzyme-catalyzed reactions that accompany a rise or fall in body temperature constitute a prominent survival feature for “cold-blooded” life forms such as lizards or fish, whose body temperatures are dictated by the external environment. However, for mammals and other homeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.

Hydrogen Ion Concentration

The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration. Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9. The relationship of activity to hydrogen ion concentration (Figure 8–2) reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both. For enzymes whose mechanism involves acid-base catalysis, the residues involved must be in the appropriate state of protonation for the reaction to proceed. The binding and recognition of substrate molecules with dissociable groups also typically involves the formation of salt bridges with the enzyme. The most common charged groups are the negative carboxylate groups and the positively charged groups of protonated amines. Gain or loss of critical charged groups thus will adversely affect substrate binding and thus will retard or abolish catalysis.

ASSAYS OF ENZYME-CATALYZED REACTIONS TYPICALLY MEASURE THE INITIAL VELOCITY

Most measurements of the rates of enzyme-catalyzed reactions employ relatively short time periods, conditions that approximate **initial rate conditions**. Under these conditions, only traces of product accumulate, hence the rate of the reverse reaction is negligible. The **initial velocity** (v_i) of the reaction thus is essentially that of

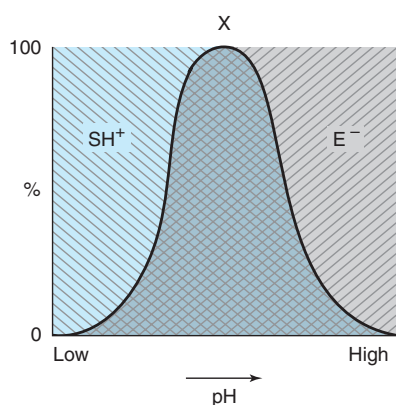


Figure 8–2. Effect of pH on enzyme activity. Consider, for example, a negatively charged enzyme (EH^-) that binds a positively charged substrate (SH^+). Shown is the proportion (%) of SH^+ [\\/] and of EH^- [///] as a function of pH. Only in the cross-hatched area do both the enzyme and the substrate bear an appropriate charge.

the rate of the forward reaction. Assays of enzyme activity almost always employ a large (10^3 – 10^7) molar excess of substrate over enzyme. Under these conditions, v_i is proportionate to the concentration of enzyme. Measuring the initial velocity therefore permits one to estimate the quantity of enzyme present in a biologic sample.

SUBSTRATE CONCENTRATION AFFECTS REACTION RATE

In what follows, enzyme reactions are treated as if they had only a single substrate and a single product. While most enzymes have more than one substrate, the principles discussed below apply with equal validity to enzymes with multiple substrates.

For a typical enzyme, as substrate concentration is increased, v_i increases until it reaches a maximum value $V_{\max}$ (Figure 8–3). When further increases in substrate concentration do not further increase v_i , the enzyme is said to be “saturated” with substrate. Note that the shape of the curve that relates activity to substrate concentration (Figure 8–3) is hyperbolic. At any given instant, only substrate molecules that are combined with the enzyme as an ES complex can be transformed into product. Second, the equilibrium constant for the formation of the enzyme-substrate complex is not infinitely large. Therefore, even when the substrate is present in excess (points A and B of Figure 8–4), only a fraction of the enzyme may be present as an ES complex. At points A or B, increasing or decreasing $[\text{S}]$ therefore will increase or decrease the number of ES complexes with a corresponding change in v_i . At point C (Figure 8–4), essentially all the enzyme is present as the ES complex. Since no free enzyme remains available for forming ES, further increases in $[\text{S}]$ cannot increase the rate of the reaction. Under these saturating conditions, v_i depends solely on—and thus is limited by—the rapidity with which free enzyme is released to combine with more substrate.

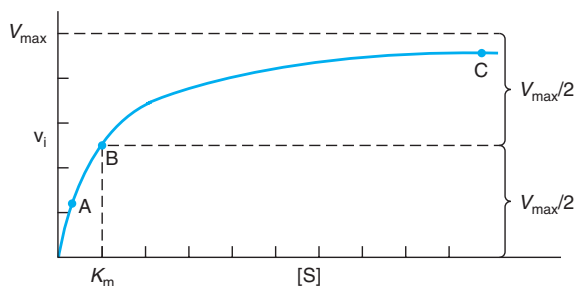


Figure 8–3. Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction.

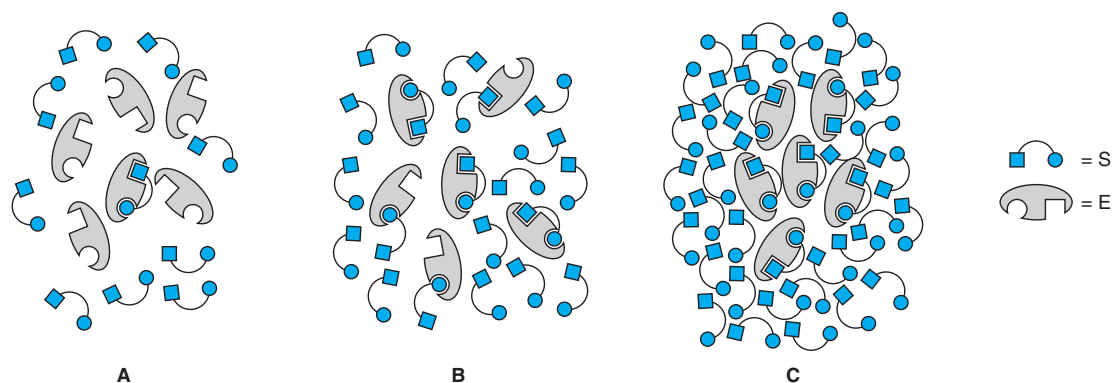


Figure 8-4. Representation of an enzyme at low (A), at high (C), and at a substrate concentration equal to K_m (B). Points A, B, and C correspond to those points in Figure 8-3.

THE MICHAELIS-MENTEN & HILL EQUATIONS MODEL THE EFFECTS OF SUBSTRATE CONCENTRATION

The Michaelis-Menten Equation

The Michaelis-Menten equation (29) illustrates in mathematical terms the relationship between initial reaction velocity v_i and substrate concentration $[S]$, shown graphically in Figure 8-3.

$$v_i = \frac{V_{\max}[S]}{K_m + [S]} \quad (29)$$

The Michaelis constant K_m is the substrate concentration at which v_i is half the maximal velocity ($V_{\max}/2$) attainable at a particular concentration of enzyme. K_m thus has the dimensions of substrate concentration. The dependence of initial reaction velocity on $[S]$ and K_m may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

(1) When $[S]$ is much less than K_m (point A in Figures 8-3 and 8-4), the term $K_m + [S]$ is essentially equal to K_m . Replacing $K_m + [S]$ with K_m reduces equation (29) to

$$v_i = \frac{V_{\max}[S]}{K_m + [S]} \quad v_i \approx \frac{V_{\max}[S]}{K_m} \approx \left(\frac{V_{\max}}{K_m} \right) [S] \quad (30)$$

where $\approx$ means “approximately equal to.” Since $V_{\max}$ and K_m are both constants, their ratio is a constant. In other words, when $[S]$ is considerably below K_m , $v_i \propto k[S]$. The initial reaction velocity therefore is directly proportionate to $[S]$.

(2) When $[S]$ is much greater than K_m (point C in Figures 8-3 and 8-4), the term $K_m + [S]$ is essentially

equal to $[S]$. Replacing $K_m + [S]$ with $[S]$ reduces equation (29) to

$$v_i = \frac{V_{\max}[S]}{K_m + [S]} \quad v_i \approx \frac{V_{\max}[S]}{[S]} \approx V_{\max} \quad (31)$$

Thus, when $[S]$ greatly exceeds K_m , the reaction velocity is maximal ($V_{\max}$) and unaffected by further increases in substrate concentration.

(3) When $[S] = K_m$ (point B in Figures 8-3 and 8-4).

$$v_i = \frac{V_{\max}[S]}{K_m + [S]} = \frac{V_{\max}[S]}{2[S]} = \frac{V_{\max}}{2} \quad (32)$$

Equation (32) states that when $[S]$ equals K_m , the initial velocity is half-maximal. Equation (32) also reveals that K_m is—and may be determined experimentally from—the substrate concentration at which the initial velocity is half-maximal.

A Linear Form of the Michaelis-Menten Equation Is Used to Determine K_m & $V_{\max}$

The direct measurement of the numeric value of $V_{\max}$ and therefore the calculation of K_m often requires impractically high concentrations of substrate to achieve saturating conditions. A linear form of the Michaelis-Menten equation circumvents this difficulty and permits $V_{\max}$ and K_m to be extrapolated from initial velocity data obtained at less than saturating concentrations of substrate. Starting with equation (29),

$$v_i = \frac{V_{\max}[S]}{K_m + [S]} \quad (29)$$

invert

$$\frac{1}{v_i} = \frac{K_m + [S]}{V_{\max}[S]} \quad (33)$$

factor

$$\frac{1}{v_i} = \frac{K_m}{V_{\max}[S]} + \frac{[S]}{V_{\max}[S]} \quad (34)$$

and simplify

$$\frac{1}{v_i} = \left(\frac{K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{1}{V_{\max}} \quad (35)$$

Equation (35) is the equation for a straight line, $y = ax + b$, where $y = 1/v_i$ and $x = 1/[S]$. A plot of $1/v_i$ as y as a function of $1/[S]$ as x therefore gives a straight line whose y intercept is $1/V_{\max}$ and whose slope is $K_m/V_{\max}$. Such a plot is called a **double reciprocal** or **Lineweaver-Burk plot** (Figure 8–5). Setting the y term of equation (36) equal to zero and solving for x reveals that the x intercept is $-1/K_m$.

$$0 = ax + b; \text{ therefore, } x = \frac{-b}{a} = \frac{-1}{K_m} \quad (36)$$

K_m is thus most easily calculated from the x intercept.

K_m May Approximate a Binding Constant

The affinity of an enzyme for its substrate is the inverse of the dissociation constant K_d for dissociation of the enzyme substrate complex ES.



$$K_d = \frac{k_{-1}}{k_1} \quad (38)$$

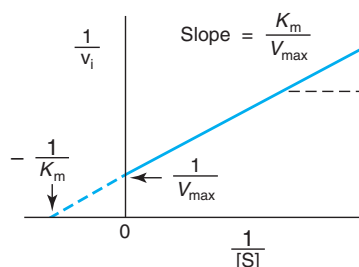


Figure 8–5. Double reciprocal or Lineweaver-Burk plot of $1/v_i$ versus $1/[S]$ used to evaluate K_m and $V_{\max}$.

Stated another way, the smaller the tendency of the enzyme and its substrate to *dissociate*, the *greater* the affinity of the enzyme for its substrate. While the Michaelis constant K_m often approximates the dissociation constant K_d , this is by no means always the case. For a typical enzyme-catalyzed reaction,



the value of $[S]$ that gives $v_i = V_{\max}/2$ is

$$[S] = \frac{k_{-1} + k_2}{k_1} = K_m \quad (40)$$

When $k_{-1} \gg k_2$, then

$$k_{-1} + k_2 \approx k_{-1} \quad (41)$$

and

$$[S] \approx \frac{k_{-1}}{k_1} \approx K_d \quad (42)$$

Hence, $1/K_m$ only approximates $1/K_d$ under conditions where the association and dissociation of the ES complex is rapid relative to the rate-limiting step in catalysis. For the many enzyme-catalyzed reactions for which $k_{-1} + k_2$ is *not* approximately equal to k_{-1} , $1/K_m$ will underestimate $1/K_d$.

The Hill Equation Describes the Behavior of Enzymes That Exhibit Cooperative Binding of Substrate

While most enzymes display the simple **saturation kinetics** depicted in Figure 8–3 and are adequately described by the Michaelis-Menten expression, some enzymes bind their substrates in a cooperative fashion analogous to the binding of oxygen by hemoglobin (Chapter 6). Cooperative behavior may be encountered for multimeric enzymes that bind substrate at multiple sites. For enzymes that display positive cooperativity in binding substrate, the shape of the curve that relates changes in v_i to changes in $[S]$ is sigmoidal (Figure 8–6). Neither the Michaelis-Menten expression nor its derived double-reciprocal plots can be used to evaluate cooperative saturation kinetics. Enzymologists therefore employ a graphic representation of the **Hill equation** originally derived to describe the cooperative binding of O_2 by hemoglobin. Equation (43) represents the Hill equation arranged in a form that predicts a straight line, where k' is a complex constant.

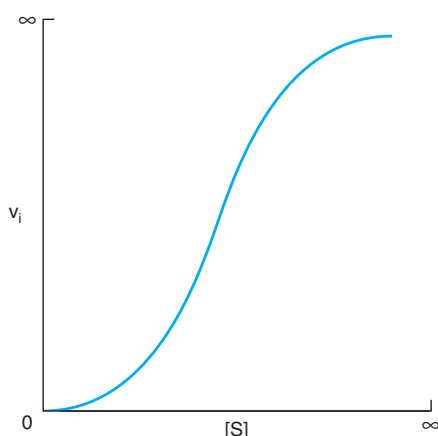


Figure 8-6. Representation of sigmoid substrate saturation kinetics.

$$\frac{\log v_i}{V_{\max} - v_i} = n \log[S] - \log k' \quad (43)$$

Equation (43) states that when $[S]$ is low relative to k' , the initial reaction velocity increases as the n th power of $[S]$.

A graph of $\log v_i/(V_{\max} - v_i)$ versus $\log[S]$ gives a straight line (Figure 8-7), where the slope of the line n is the **Hill coefficient**, an empirical parameter whose value is a function of the number, kind, and strength of the interactions of the multiple substrate-binding sites on the enzyme. When $n = 1$, all binding sites behave independently, and simple Michaelis-Menten kinetic behavior is observed. If n is greater than 1, the enzyme is said to exhibit positive cooperativity. Binding of the

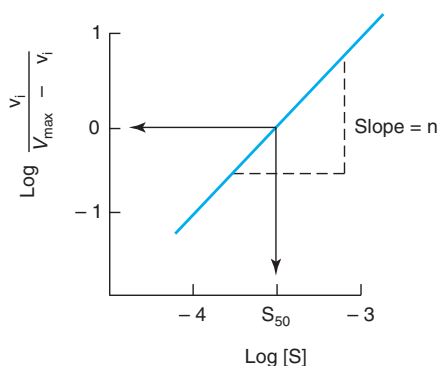


Figure 8-7. A graphic representation of a linear form of the Hill equation is used to evaluate S_{50} , the substrate concentration that produces half-maximal velocity, and the degree of cooperativity n .

first substrate molecule then enhances the affinity of the enzyme for binding additional substrate. The greater the value for n , the higher the degree of cooperativity and the more sigmoidal will be the plot of v_i versus $[S]$. A perpendicular dropped from the point where the y term $\log v_i/(V_{\max} - v_i)$ is zero intersects the x axis at a substrate concentration termed S_{50} , the substrate concentration that results in half-maximal velocity. S_{50} thus is analogous to the P_{50} for oxygen binding to hemoglobin (Chapter 6).

KINETIC ANALYSIS DISTINGUISHES COMPETITIVE FROM NONCOMPETITIVE INHIBITION

Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for study of the mechanism of enzyme action. Inhibitors can be classified based upon their site of action on the enzyme, on whether or not they chemically modify the enzyme, or on the kinetic parameters they influence. Kinetically, we distinguish two classes of inhibitors based upon whether raising the substrate concentration does or does not overcome the inhibition.

Competitive Inhibitors Typically Resemble Substrates

The effects of competitive inhibitors can be overcome by raising the concentration of the substrate. Most frequently, in competitive inhibition the inhibitor, **I**, binds to the substrate-binding portion of the active site and blocks access by the substrate. The structures of most classic competitive inhibitors therefore tend to resemble the structures of a substrate and thus are termed **substrate analogs**. Inhibition of the enzyme succinate dehydrogenase by malonate illustrates competitive inhibition by a substrate analog. Succinate dehydrogenase catalyzes the removal of one hydrogen atom from each of the two methylene carbons of succinate (Figure 8-8). Both succinate and its structural analog malonate ($^-OOC-CH_2-COO^-$) can bind to the active site of succinate dehydrogenase, forming an ES or an EI complex, respectively. However, since malonate contains

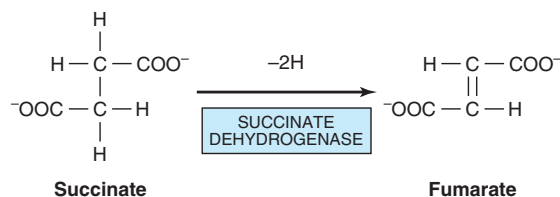
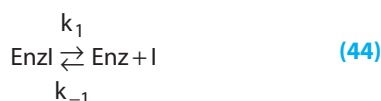


Figure 8-8. The succinate dehydrogenase reaction.

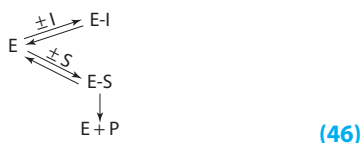
only one methylene carbon, it cannot undergo dehydrogenation. The formation and dissociation of the EI complex is a dynamic process described by



for which the equilibrium constant K_i is

$$K_i = \frac{[\text{Enz}][\text{I}]}{[\text{EnzI}]} = \frac{k_1}{k_{-1}} \quad (45)$$

In effect, a **competitive inhibitor acts by decreasing the number of free enzyme molecules available to bind substrate, ie, to form ES, and thus eventually to form product**, as described below:



A competitive inhibitor and substrate exert reciprocal effects on the concentration of the EI and ES complexes. Since binding substrate removes free enzyme available to combine with inhibitor, increasing the $[\text{S}]$ decreases the concentration of the EI complex and raises the reaction velocity. The extent to which $[\text{S}]$ must be increased to completely overcome the inhibition depends upon the concentration of inhibitor present, its affinity for the enzyme K_i , and the K_m of the enzyme for its substrate.

Double Reciprocal Plots Facilitate the Evaluation of Inhibitors

Double reciprocal plots distinguish between competitive and noncompetitive inhibitors and simplify evaluation of inhibition constants K_i . v_i is determined at several substrate concentrations both in the presence and in the absence of inhibitor. For classic competitive inhibition, the lines that connect the experimental data points meet at the y axis (Figure 8–9). Since the y intercept is equal to $1/V_{\max}$, this pattern indicates that **when $1/[\text{S}]$ approaches 0, v_i is independent of the presence of inhibitor**. Note, however, that the intercept on the x axis does vary with *inhibitor* concentration—and that since $-1/K_m'$ is smaller than $1/K_m$, K_m' (the “apparent K_m ”) becomes larger in the presence of increasing concentrations of inhibitor. Thus, **a competitive inhibitor has no effect on $V_{\max}$ but raises K_m' , the apparent K_m for the substrate**.

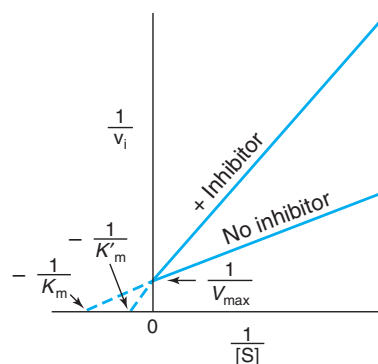


Figure 8–9. Lineweaver-Burk plot of competitive inhibition. Note the complete relief of inhibition at high $[\text{S}]$ (ie, low $1/[\text{S}]$).

For simple competitive inhibition, the intercept on the x axis is

$$x = \frac{-1}{K_m} \left(1 + \frac{[\text{I}]}{K_i} \right) \quad (47)$$

Once K_m has been determined in the absence of inhibitor, K_i can be calculated from equation (47). K_i values are used to compare different inhibitors of the same enzyme. The lower the value for K_i , the more effective the inhibitor. For example, the statin drugs that act as competitive inhibitors of HMG-CoA reductase (Chapter 26) have K_i values several orders of magnitude lower than the K_m for the substrate HMG-CoA.

Simple Noncompetitive Inhibitors Lower $V_{\max}$ but Do Not Affect K_m

In noncompetitive inhibition, binding of the inhibitor does not affect binding of substrate. Formation of both EI and EIS complexes is therefore possible. However, while the enzyme-inhibitor complex can still bind substrate, its efficiency at transforming substrate to product, reflected by $V_{\max}$, is decreased. Noncompetitive inhibitors bind enzymes at sites distinct from the substrate-binding site and generally bear little or no structural resemblance to the substrate.

For simple noncompetitive inhibition, E and EI possess identical affinity for substrate, and the EIS complex generates product at a negligible rate (Figure 8–10). More complex noncompetitive inhibition occurs when binding of the inhibitor *does* affect the apparent affinity of the enzyme for substrate, causing the lines to intercept in either the third or fourth quadrants of a double reciprocal plot (not shown).

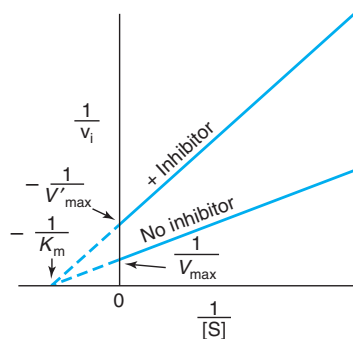


Figure 8-10. Lineweaver-Burk plot for simple non-competitive inhibition.

Irreversible Inhibitors “Poison” Enzymes

In the above examples, the inhibitors form a dissociable, dynamic complex with the enzyme. Fully active enzyme can therefore be recovered simply by removing the inhibitor from the surrounding medium. However, a variety of other inhibitors act irreversibly by chemically modifying the enzyme. These modifications generally involve making or breaking covalent bonds with aminoacyl residues essential for substrate binding, catalysis, or maintenance of the enzyme’s functional conformation. Since these covalent changes are relatively stable, an enzyme that has been “poisoned” by an irreversible inhibitor remains inhibited even after removal of the remaining inhibitor from the surrounding medium.

MOST ENZYME-CATALYZED REACTIONS INVOLVE TWO OR MORE SUBSTRATES

While many enzymes have a single substrate, many others have two—and sometimes more than two—substrates and products. The fundamental principles discussed above, while illustrated for single-substrate enzymes, apply also to multisubstrate enzymes. The mathematical expressions used to evaluate multisubstrate reactions are, however, complex. While detailed kinetic analysis of multisubstrate reactions exceeds the scope of this chapter, two-substrate, two-product reactions (termed “Bi-Bi” reactions) are considered below.

Sequential or Single Displacement Reactions

In **sequential reactions**, both substrates must combine with the enzyme to form a ternary complex before catalysis can proceed (Figure 8-11, top). Sequential reactions are sometimes referred to as single displacement

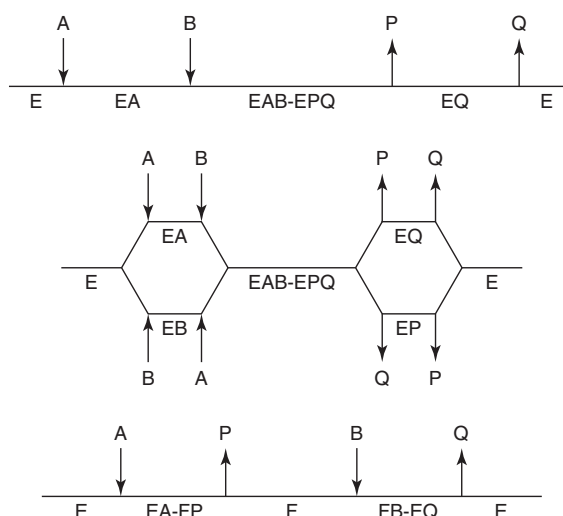


Figure 8-11. Representations of three classes of Bi-Bi reaction mechanisms. Horizontal lines represent the enzyme. Arrows indicate the addition of substrates and departure of products. **Top:** An ordered Bi-Bi reaction, characteristic of many NAD(P)H-dependent oxidoreductases. **Center:** A random Bi-Bi reaction, characteristic of many kinases and some dehydrogenases. **Bottom:** A ping-pong reaction, characteristic of aminotransferases and **serine proteases**.

reactions because the group undergoing transfer is usually passed directly, in a single step, from one substrate to the other. Sequential Bi-Bi reactions can be further distinguished based on whether the two substrates add in a **random** or in a **compulsory** order. For random-order reactions, either substrate A or substrate B may combine first with the enzyme to form an EA or an EB complex (Figure 8-11, center). For compulsory-order reactions, A must first combine with E before B can combine with the EA complex. One explanation for a compulsory-order mechanism is that the addition of A induces a conformational change in the enzyme that aligns residues which recognize and bind B.

Ping-Pong Reactions

The term “**ping-pong**” applies to mechanisms in which one or more products are released from the enzyme before all the substrates have been added. Ping-pong reactions involve covalent catalysis and a transient, modified form of the enzyme (Figure 7-4). Ping-pong Bi-Bi reactions are **double displacement reactions**. The group undergoing transfer is first displaced from substrate A by the enzyme to form product

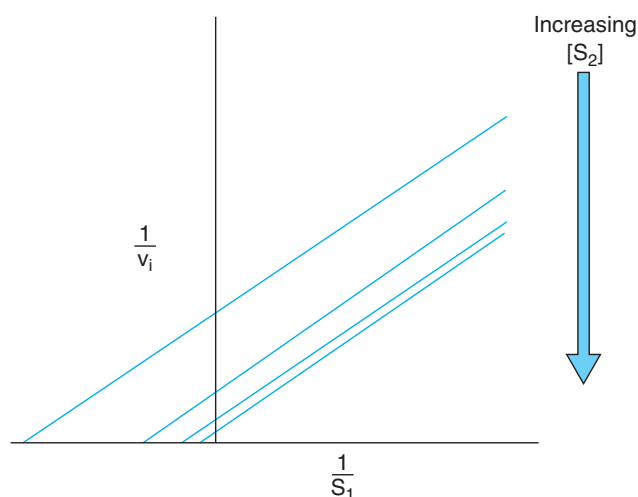


Figure 8-12. Lineweaver-Burk plot for a two-substrate ping-pong reaction. An increase in concentration of one substrate (S_1) while that of the other substrate (S_2) is maintained constant changes both the x and y intercepts, but not the slope.

P and a modified form of the enzyme (F). The subsequent group transfer from F to the second substrate B, forming product Q and regenerating E, constitutes the second displacement (Figure 8-11, bottom).

Most Bi-Bi Reactions Conform to Michaelis-Menten Kinetics

Most Bi-Bi reactions conform to a somewhat more complex form of Michaelis-Menten kinetics in which $V_{\max}$ refers to the reaction rate attained when both substrates are present at saturating levels. Each substrate has its own characteristic K_m value which corresponds to the concentration that yields half-maximal velocity when the second substrate is present at saturating levels. As for single-substrate reactions, double-reciprocal plots can be used to determine $V_{\max}$ and K_m . v_i is measured as a function of the concentration of one substrate (the variable substrate) while the concentration of the other substrate (the fixed substrate) is maintained constant. If the lines obtained for several fixed-substrate concentrations are plotted on the same graph, it is possible to distinguish between a ping-pong enzyme, which yields parallel lines, and a sequential mechanism, which yields a pattern of intersecting lines (Figure 8-12).

Product inhibition studies are used to complement kinetic analyses and to distinguish between ordered and random Bi-Bi reactions. For example, in a random-order Bi-Bi reaction, each product will be a competitive inhibitor regardless of which substrate is designated the variable substrate. However, for a sequential mechanism (Figure 8-11, bottom), only product Q will give the pattern indicative of competitive inhibition when A is the variable substrate, while only product P will produce this pattern with B as the variable substrate. The

other combinations of product inhibitor and variable substrate will produce forms of complex noncompetitive inhibition.

SUMMARY

- The study of enzyme kinetics—the factors that affect the rates of enzyme-catalyzed reactions—reveals the individual steps by which enzymes transform substrates into products.
- ΔG , the overall change in free energy for a reaction, is independent of reaction mechanism and provides no information concerning *rates* of reactions.
- Enzymes do not affect K_{eq} . K_{eq} , a ratio of reaction *rate constants*, may be calculated from the concentrations of substrates and products at equilibrium or from the ratio k_1/k_{-1} .
- Reactions proceed via transition states in which ΔG_F is the activation energy. Temperature, hydrogen ion concentration, enzyme concentration, substrate concentration, and inhibitors all affect the rates of enzyme-catalyzed reactions.
- A measurement of the rate of an enzyme-catalyzed reaction generally employs initial rate conditions, for which the essential absence of product precludes the reverse reaction.
- A linear form of the Michaelis-Menten equation simplifies determination of K_m and $V_{\max}$.
- A linear form of the Hill equation is used to evaluate the cooperative substrate-binding kinetics exhibited by some multimeric enzymes. The slope n , the Hill coefficient, reflects the number, nature, and strength of the interactions of the substrate-binding sites. A

value of n greater than 1 indicates positive cooperativity.

- The effects of competitive inhibitors, which typically resemble substrates, are overcome by raising the concentration of the substrate. Noncompetitive inhibitors lower $V_{\max}$ but do not affect K_m .
- Substrates may add in a random order (either substrate may combine first with the enzyme) or in a compulsory order (substrate A must bind before substrate B).
- In ping-pong reactions, one or more products are released from the enzyme before all the substrates have added.

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