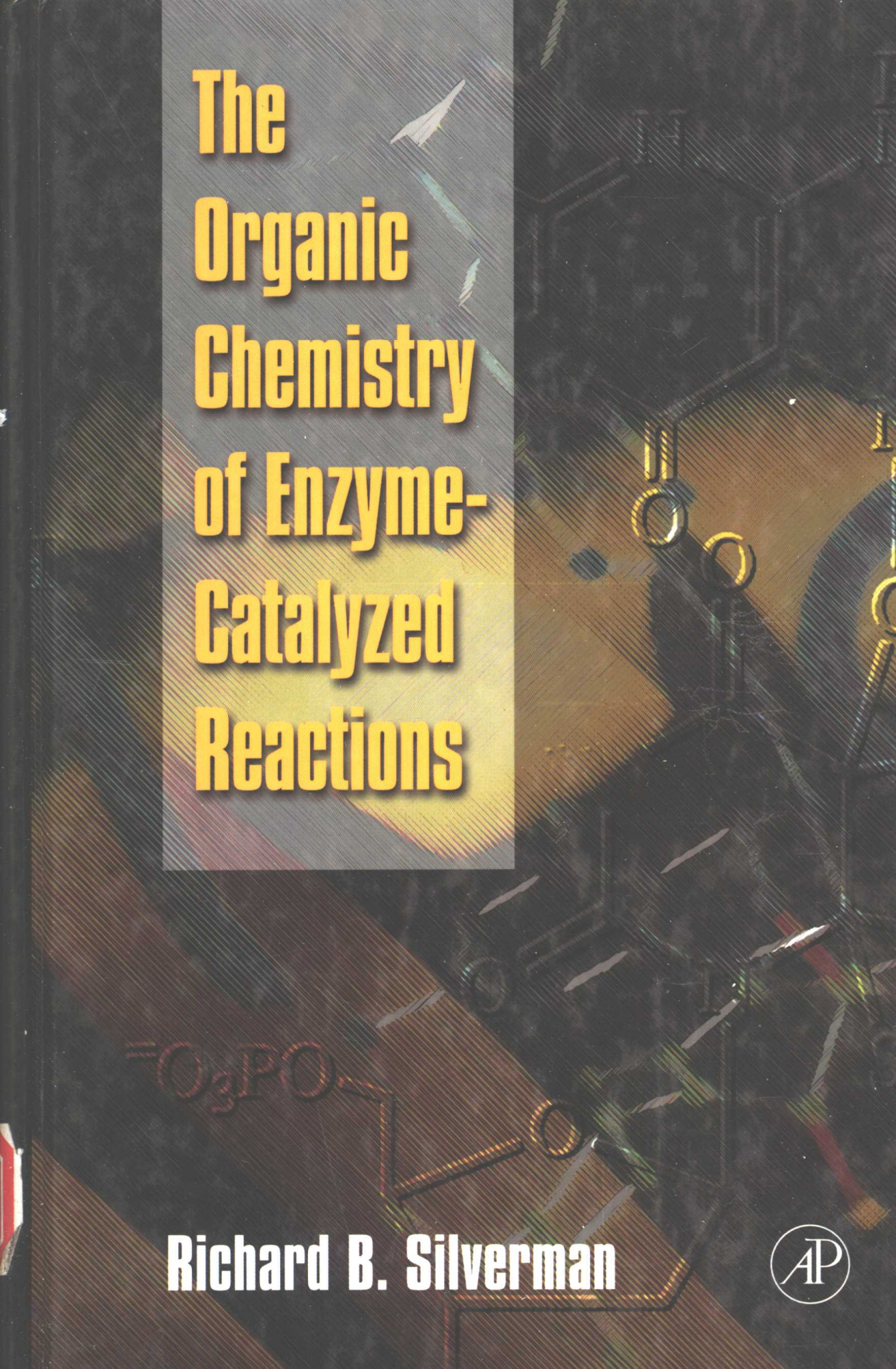
The Organic Chemistry of Enzyme-Catalyzed Reactions



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Preface

This is *not* your standard enzymology text. It actually serves two functions: it is an enzymology text for organic chemists and an organic chemistry text for enzymologists. It does not follow the usual traditions of biochemistry texts, which discuss such topics in enzymology as metabolic pathways, biosynthesis, protein synthesis and structure, and regulatory mechanisms. Instead, it seeks to give organic chemists an appreciation that enzymology is simply a biological application of physical organic chemistry, and to teach biochemists to view enzymology from the perspective of organic chemical mechanisms.

The text is organized according to organic reaction types so that the reader learns to think this way when looking at unknown enzyme systems. Each chapter represents a particular class of organic reactions catalyzed by different classes of enzymes, rather than the typical approach of standard biochemistry texts in which classes of enzymes are discussed and the reactions that they catalyze are mentioned as part of the characterization of the enzyme. This text also emphasizes the design of experiments to test enzyme mechanisms, so that the reader becomes familiar with approaches taken to elucidate new enzyme mechanisms. No attempt has been made to discuss each reference in detail; some experiments are cited and conclusions from these experiments are made. If more detail is desired, the original reference should be consulted.

There is no way that a text designed for a one-semester or even a one-year course could cover all of the enzymes that have been reported in the literature. In fact, that is not the purpose of this text, nor is it important to do so. I believe that what is most important is to be able to recognize and categorize an enzymatic reaction, to associate that reaction with a particular class of enzymes, and then to design experiments to test hypotheses regarding the mechanism for that enzyme-catalyzed reaction. Consequently, only representative enzymatic examples of each of the various reaction types are described here. Therefore, some of your favorite enzymes may not be included in this text, simply because I chose a different enzyme as an example for that particular reaction mechanism. This approach allows the instructor

to add other enzymes to the discussion of a particular reaction mechanism. If more in-depth knowledge of a particular enzyme system is desired, or if a full-year course is offered, then the literature references cited can be assigned for critical analysis. I have taken examples from both the current literature and the older literature so that readers can appreciate that clever experiments have been carried out for many years.

I must thank a succession of teachers for my excitement about enzyme-catalyzed reactions. As an organic chemistry graduate student at Harvard, I started with no interest at all in enzymes because I had the impression that they were magic boxes that somehow catalyzed reactions. My only passion was the synthesis of natural products. My mentor, David Dolphin, who had other interests as well, asked me, as a side project, to synthesize a deuterated compound that his "collaborator" needed for studying the mechanism of an enzyme-catalyzed reaction. Having no interest in enzymes, I synthesized the compound without asking its utility in this mechanistic study (something I now tell my students *never* to do). Not long after I began working on my main project, the synthesis of the antitumor antibiotic camptothecin, its first total synthesis was reported. By this time, David had cunningly convinced me that, because I had already synthesized the desired deuterated molecule for his collaborator, it would be easy to attach it to a cobalt complex, which his collaborator would then use in his mechanistic study. So, while working hard on the synthesis of camptothecin, I learned about making cobalt complexes and attached the ligand. It soon became apparent that camptothecin was the focus of no less than six other research groups, because all these groups published syntheses of this molecule by my second year in graduate school! Having no desire to be the seventh (or tenth?) person to synthesize camptothecin, I finally asked David what this cobalt complex was for and found out that it was to carry out a model study of a potential mechanism for the coenzyme B_{12}-dependent rearrangements. Although I had resisted the temptation to become interested in enzymology, my curiosity was piqued. It was not difficult to convince David that this new project sounded interesting, so he agreed to let me work on this project for my Ph.D. thesis. (Was there ever really a collaborator, or was this my introduction to the psychology of assistant professors?)

In my second year, I sat in on a general biochemistry course, which corroborated my suspicions that enzymes were black boxes, and I realized that organic chemists needed to enter this field to clarify the "mysteries" of enzymology. The fog about enzymes began to lift in my third year, when I was fortunate to sit in on a unique course in enzymology taught by a relatively young (and getting younger every year, from my perspective) visiting professor. It was in this class that I was shown the connection between the black box of enzyme-catalyzed reactions and organic chemical mechanisms. The excitement of the subject, the clarity of the exposition, and the wit of the professor changed my opinions about the science of enzymology and changed the direction of my career goals and my research interests. Thanks, Jeremy Knowles.

Not long after I finished this course, another great stroke of fate occurred; Bob Abeles gave a colloquium at Harvard. It was this colloquium, and my two-year post-

doctoral stint in his lab, that demonstrated the applications of the concepts in Jeremy's course and the value of organic chemistry to the study of enzyme-catalyzed reactions.

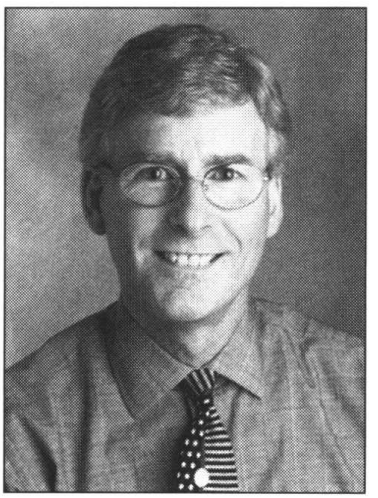
I am very grateful to those who unselfishly agreed to act as reviewers of this text. I selected four scientists, whom I considered to be the real experts in the general areas discussed, for each of the chapters; the editor's assistant, Linda Klinger (née McAleer), tried to get two of these to read each chapter. She was successful in all but three of the chapters, for which only one reviewer participated. Many thanks go to Vern Schramm (for two chapters), Dick Schowen, Frank Raushel, Ted Widlanski, Ben Liu, Paul Ortiz de Montellano, Paul Fitzpatrick, John Lipscomb, Mark Nelson, Richard Armstrong, Steve Withers, Ron Kluger, Marion O'Leary, George Kenyon, Ralph Pollack, Al Mildvan, Chris Whitman, Dennis Flint, Rob Phillips, Eileen Jaffe, Rowena Matthews, Jim Coward, Perry Frey, Bob Abeles, and John Blanchard. Your efforts are much appreciated.

As some of you may recognize, Chris Walsh's textbook *Enzymatic Reaction Mechanisms* (W. H. Freeman: San Francisco, 1979), played an important part in shaping my approach to presenting the intricacies of enzyme-catalyzed reactions. I thank Chris for getting the study of modern mechanistic enzymology off to a great pedagogical start.

For those of you who believe that a textbook should be a formal piece of writing, I apologize for the informality throughout this text; I wanted this book to be read as though I was talking to you about enzyme mechanisms.

Richard B. Silverman

About the Author



Professor Richard B. Silverman received his B.S. degree in chemistry from The Pennsylvania State University, his M.A. and Ph.D. degrees in organic chemistry from Harvard University, and he carried out postdoctoral research in enzymology under the guidance of Professor Robert H. Abeles at Brandeis University. He has been on the faculty of Northwestern University in the Department of Chemistry since 1976 and also in the Department of Biochemistry, Molecular Biology, and Cell Biology since 1986. Professor Silverman is a member of the Northwestern University Institute for Neuroscience, the Lurie Cancer Center, the Center for Biotechnology, and the Drug Discovery Program.

He was named a DuPont Young Faculty Fellow (1976), an Alfred P. Sloan Research Fellow (1981), a NIH Research Career Development Awardee (1982), a Fellow of the American Institute of Chemists (1985), and a Fellow of the American Association for the Advancement of Science (1990). In addition to having been chosen for the Northwestern University Faculty Honor Roll seven times, he was honored with the 1999 E. LeRoy Hall Award for Teaching Excellence. He is a member of the editorial boards of the *Journal of Medicinal Chemistry, Archives of Biochemistry and Biophysics,* the *Journal of Enzyme Inhibition,* and *Archiv der Pharmazie-Pharmaceutical and Medicinal Chemistry* and has co-chaired the Gordon Research Conference on Enzymes, Coenzymes, and Metabolic Pathways (1994). He has given numerous two- and three-day short courses at meetings and at companies on drug design and drug action and on enzyme mechanisms and inhibition.

Professor Silverman is the author or co-author of over 170 research publications in enzymology, medicinal chemistry, and organic chemistry and is the holder of 10 patents. He also has written two other textbooks: *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology* and *The Organic Chemistry of Drug Design and Drug Action.*

Contents

CHAPTER 1

Enzymes as Catalysts

I. WHAT ARE ENZYMES, AND HOW DO THEY WORK?

A. Historical

Segel[1] has given a fascinating historical perspective on the discovery of enzymes; some of the notable events are mentioned here. One of the earliest observations of enzyme activity was reported in 1783 by Spallanzani, who noted that the gastric juice of hawks liquefied meat. Although the digestive effects were not ascribed to enzymes per se, Spallanzani recognized that something in the hawk juice was capable of converting solid meat into a liquid. Over the next 50 years many other observations suggested the existence of enzymes, but the first "isolation" of an enzyme is credited to Payen and Persoz. In 1833 they added ethanol to an aqueous extract of malt and obtained a heat-labile precipitate that was utilized to hydrolyze starch to soluble sugar. The substance in this precipitate, which they called *diastase,* is now known as amylase. Schwann "isolated" the first enzyme from an animal source, pepsin, in 1834 by acid extraction of animal stomach wall. Berthelot obtained an alcohol precipitate from yeast in 1860, which converted sucrose to glucose and fructose; he concluded that there were many such ferments in yeast. In 1878 Kühne coined the name *enzyme,* which means "in yeast" to denote these ferments. It was Duclaux who proposed in 1898 that all enzymes should have the suffix "ase" so that a substance would be recognized as an enzyme from the name.

Enzymes are, in general, natural proteins that catalyze chemical reactions; RNA also can catalyze reactions.[1a] The first enzyme recognized as a protein was jack bean urease, which was crystallized in 1926 by Sumner[2] and was shown to catalyze the hydrolysis of urea to CO_2 and NH_3. It took almost 70 years more, however, before Andrew Karplus obtained its crystal structure (for the enzyme from *Klebsiella aerogenes*).[3] As it turns out, urease is one of the few nickel-containing enzymes now

known. By the 1950s hundreds of enzymes had been discovered, and many were purified to homogeneity and crystallized. In 1960 Hirs, Moore, and Stein[4] were the first to sequence an enzyme, namely, ribonuclease A, having only 124 amino acids (molecular weight 13,680). This was an elegant piece of work, and William H. Stein and Stanford Moore shared the Nobel Prize in chemistry in 1972 for the methodology of protein sequencing that was developed to determine the ribonuclease A sequence. Ribonuclease A also was the target of the first chemical synthesis of an enzyme; two research groups independently reported its synthesis in 1969.[5]

Enzymes can have molecular weights of several thousand to several million, yet catalyze transformations on molecules as small as carbon dioxide or nitrogen. Carbonic anhydrase from human erythrocytes, for example, has a molecular weight of about 31,000 and each enzyme molecule can catalyze the hydration of 1,400,000 molecules of CO_2 to H_2CO_3 per second! This is almost 10^8 times faster than the uncatalyzed reaction.

In general, enzymes function by lowering transition-state energies and energetic intermediates and by raising the ground-state energy. The transition state for an enzyme-catalyzed reaction, as in the case of a chemical reaction, is a high-energy state having a lifetime of about 10^{-13}, the time for one bond vibration.[6] No spectroscopic method available can detect a transition-state structure.

At least 21 different hypotheses for how enzymes catalyze reactions have been proposed.[7] The one common link between all these proposals, however, is that an enzyme-catalyzed reaction always is initiated by the formation of an *enzyme–substrate (or E·S) complex,* from which the catalysis takes place. The concept of an enzyme–substrate complex was originally proposed independently in 1902 by Brown[8] and Henri;[9] this idea extends the 1894 *lock-and-key hypothesis* in which Fischer[10] proposed that an enzyme is the lock into which the substrate (the key) fits. This interaction of the enzyme and substrate would account for the high degree of specificity of enzymes, but the lock-and-key hypothesis does not rationalize certain observed phenomena. For example, compounds whose structures are related to that of the substrate, but have *less* bulky substituents, often fail to be substrates, even though they should have fit into the enzyme. Some compounds with *more* bulky substituents are observed to bind *more* tightly to the enzyme than does the substrate. If the lock-and-key hypothesis were correct, one would think a more bulky compound would not fit into the lock. Some enzymes that catalyze reactions between two substrates do not bind one substrate until the other one is already bound to the enzyme. These curiosities led Koshland[11] in 1958 to propose the *induced-fit hypothesis,* namely, that when a substrate begins to bind to an enzyme, interactions of various groups on the substrate with particular enzyme functional groups are initiated, and these mutual interactions induce a *conformational change* in the enzyme. This results in a change of the enzyme from a low catalytic form to a high catalytic form by destabilizing the enzyme and/or by inducing proper alignment of the groups involved in catalysis. The conformational change could serve as a basis for substrate specificity. Compounds resembling the substrate except with smaller or larger substituents may bind to the enzyme but may not induce the conformational change

necessary for catalysis. Also, different substrates may induce nonidentical forms of the activated enzyme. On the basis of *site-directed mutagenesis* studies (site-directed mutagenesis means that an amino acid residue in the enzyme is genetically changed to a different amino acid), Post and Ray[12] showed that a unique form of an enzyme is not required for efficient catalysis of a reaction.

In the case of bimolecular systems, the binding of the first substrate may induce the conformational change that exposes the binding site for the second substrate, and, consequently, this would account for an enzyme-catalyzed reaction that only occurs when the substrates bind in a particular order. Unlike the lock-and-key hypothesis, which implies a rigid active site, the induced-fit hypothesis requires a flexible active site to accommodate different binding modes and conformational changes in the enzyme. Actually, Pauling[13] stated the concept of a flexible active site earlier, hypothesizing that an enzyme is a flexible template that is most complementary to substrates at the transition state rather than at the ground state. This flexible model is consistent with many observations regarding enzyme action.

In 1930 Haldane[14] suggested that an enzyme–substrate (E·S) complex requires additional activation energy prior to enzyme catalysis, and this energy may be derived from substrate strain energy on the enzyme. Transition-state theory, developed by Eyring,[15] is the basis for the mentioned hypothesis of Pauling. According to this hypothesis, the substrate does not bind most effectively in the E·S complex; as the reaction proceeds, the enzyme conforms to the transition-state structure, leading to the tightest interactions (increased binding energy) with the transition-state structure.[16] This increased binding, known as *transition-state stabilization,* results in rate enhancement. Schowen has suggested[17] that all the mentioned 21 hypotheses of enzyme catalysis (as well as other correct hypotheses) are just alternative expressions of transition-state stabilization.

The E·S complex forms by the binding of the substrate to a small cavity in the enzyme known as the *active site.* Only a dozen or so amino acid residues may make up the active site, and of these only two or three may be involved directly in substrate binding and/or catalysis. Because all the catalysis takes place in the active site of the enzyme, you may wonder why it is necessary for enzymes to be so large. There are several hypotheses regarding the function of the remainder of the enzyme. One suggestion[18] is that the most effective binding of the substrate to the enzyme (the largest binding energy) results from close packing of the atoms within the protein; possibly, the remainder of the enzyme outside the active site is required to maintain the integrity of the active site for effective catalysis. The protein may also serve the function of channeling the substrate into the active site. Storm and Koshland[19] suggested that the active site aligns the orbitals of substrates and catalytic groups on the enzyme optimally for conversion to the transition-state structure. This hypothesis is termed *orbital steering.* Evidence to support the concept of orbital steering was obtained by structural modification studies with isocitrate dehydrogenase.[20] Small modifications were made to the structures of the *cofactors* (organic molecules or metal ions required for catalysis) for this enzyme, which led to a slight misalignment of the bound cofactors. Because the substrate must react with

one of the cofactors during catalysis, misalignment of the cofactor would translate into a perturbed reaction trajectory that should affect the catalytic power of the enzyme. In fact, the reaction with the modified cofactors resulted in large decreases in the reaction rate (factors of one-thousandth to one-hundred-thousandth the rate) with only small changes in the orientation of the substrates, as evidenced by X-ray crystallographic analyses of the active isocitrate dehydrogenase complexes. It appears, then, that small changes in the reaction trajectory, by misalignment of the reacting orbitals, can result in a major change in catalysis.

Enzyme catalysis is characterized by two features: *specificity* and *rate acceleration*. The active site contains moieties, namely, amino acid residues and, in the case of some enzymes, cofactors, that are responsible for these properties of an enzyme. As mentioned, a *cofactor,* also called a *coenzyme,* is an organic molecule or metal ion that binds to the active site, in some cases covalently and in others noncovalently, and is essential for the catalytic action of those enzymes that require cofactors. We will discuss various cofactors throughout the text for those enzyme-catalyzed reactions that require one or more cofactors.

B. Specificity of Enzyme-Catalyzed Reactions

1. *Enzyme Kinetics (Definitions only—See Appendix I for Derivations)*

Two types of specificity of enzymes must be considered: specificity of binding and specificity of reaction. As mentioned, enzyme catalysis is initiated by a prior interaction between the enzyme and the substrate, known as the *E·S complex* or *Michaelis complex* (Scheme 1.1). The driving force for the interactions of substrates with enzymes is the low-energy state of the E·S complex resulting from the covalent and noncovalent interactions (discussed later). The term k_1, sometimes referred to as k_{on}, is the rate constant for formation of the E·S complex, which depends on the concentrations of the substrate and enzyme; k_{-1}, also called k_{off}, is the rate constant for the breakdown of the complex, which depends on the concentration of the E·S complex and other forces (by the way, Cleland has proposed the utilize of odd-numbered subscripts for forward rate constants and even-numbered subscripts for reverse rate constants to avoid typos that omit minus signs; this seems quite sensible, but I have not adopted this usage here). The stability of the E·S complex is related to the affinity of the substrate for the enzyme, which is measured by its K_s, the dissociation constant for the E·S complex. When $k_2 << k_{-1}$, we refer to the term k_2

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot S \overset{k_2}{\rightleftharpoons} E \cdot P \rightleftharpoons E + P$$

$$K_s = \frac{k_{-1}}{k_1}$$

SCHEME 1.1 Generalized enzyme-catalyzed reaction.