ENZYMES

A Practical Introduction to Structure, Mechanism, and Data Analysis

SECOND EDITION

Robert A. Copeland

WILEY-VCH

A JOHN WILEY & SONS, INC., PUBLICATION

New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

ENZYMES

SECOND EDITION

ENZYMES

A Practical Introduction to Structure, Mechanism, and Data Analysis

SECOND EDITION

Robert A. Copeland

WILEY-VCH

A JOHN WILEY & SONS, INC., PUBLICATION

New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

This title is also available in print as ISBN 0-471-35929-7.

For more information about Wiley products, visit our web site at www.Wiley.com.

To Clyde Worthen
for teaching me all the important lessons:
arigato sensei.

And to Theodore (Doc) Janner
for stoking the fire.

CONTENTS

PREFACE

In the four years since the first edition of *Enzymes* was published, I have been delighted to learn of the wide acceptance of the book throughout the biochemical community, and particularly in the pharmaceutical community. During this time a number of colleagues have contacted me to express their views on the value of the text, and importantly to make suggestions for improvements to the content and presentation of some concepts. I have used the first edition as a teaching supplement for a course in which I lecture at the University of Pennsylvania School of Medicine. From my lecture experiences and from conversations with students, I have developed some new ideas for how to better explain some of the concepts in the text and have identified areas that deserve expanded coverage. Finally, while the first edition has become popular with students and industrial scientists, some of my academic colleagues have suggested a need for a more in-depth treatment of chemical mechanisms in enzymology.

In this second edition I have refined and expanded the coverage of many of the concepts in the text. To help the reader better understand some of the interactions between enzymes and their substrates and inhibitors, a new chapter on protein–ligand binding equilibria has been added (Chapter 4). The chapters on chemical mechanisms in enzyme catalysis (Chapter 6) and on experimental measures of enzyme activity (Chapter 7) have been expanded significantly. The discussions of enzyme inhibitors and multiple substrate reactions (Chapters 8 through 11) have been refined, and in some cases alternative treatments have been presented. In all of this, however, I have tried to maintain the introductory nature of the book. There are many excellent advanced texts on catalysis, enzyme mechanisms, and enzyme kinetics, but the level at which these are generally written is often intimidating to the beginner. Hence, as stated in the preface to the first edition, this book is intended to serve as a mechanism for those new to the field of enzymology to develop a reasonable understanding of the science and experimental methods, allowing them to competently begin laboratory studies with enzymes. I have continued to rely on extensive citations to more advanced texts and primary literature as a means for the interested reader to go beyond the treatments offered here and delve more deeply into specific areas of enzymology.

In developing this second edition I have had fruitful conversations and advice from a number of colleagues. In particular, I wish to thank Andy Stern, Ross Stein, Trevor Penning, Bill Pitts, John Blanchard, Dennis Murphy, and the members of the Chemical Enzymology Department at the DuPont Pharmaceuticals Company. As always, the love and support of my family has been most important in making this work possible.

ROBERT A. COPELAND
Wilmington, Delaware

ACKNOWLEDGMENTS

It is a great pleasure for me to thank the many friends and coworkers who have helped me in the preparation of this work. Many of the original lecture notes from which this text has developed were generated while I was teaching a course on biochemistry for first-year medical students at the University of Chicago, along with the late Howard S. Tager. Howard contributed greatly to my development as a teacher and writer. His untimely death was a great loss to many of us in the biomedical community; I dearly miss his guidance and friendship.

As described in the Preface, the notes on which this text is based were significantly expanded and reorganized to develop a course of enzymology for employees and students at the DuPont Merck Pharmaceutical Company. I am grateful for the many discussions with students during this course, which helped to refine the final presentation. I especially thank Diana Blessington for the original suggestion of a course of this nature. That a graduate-level course of this type could be presented within the structure of a for-profit pharmaceutical company speaks volumes for the insight and progressiveness of the management of DuPont Merck. I particularly thank James M. Trzaskos, Robert C. Newton, Ronald L. Magolda, and Pieter B. Timmermans for not only tolerating, but embracing this endeavor.

Many colleagues and coworkers contributed suggestions and artwork for this text. I thank June Davis, Petra Marchand, Diane Lombardo, Robert Lombardo, John Giannaras, Jean Williams, Randi Dowling, Drew Van Dyk, Rob Bruckner, Bill Pitts, Carl Decicco, Pieter Stouten, Jim Meek, Bill DeGrado, Steve Betz, Hank George, Jim Wells, and Charles Craik for their contributions.

Finally, and most importantly, I wish to thank my wife, Nancy, and our children, Lindsey and Amanda, for their constant love, support, and encouragement, without which this work could not have been completed.

PREFACE TO THE FIRST EDITION

The latter half of this century has seen an unprecedented expansion in our knowledge and use of enzymes in a broad range of basic research and industrial applications. Enzymes are the catalytic cornerstones of metabolism, and as such are the focus of intense research within the biomedical community. Indeed enzymes remain the most common targets for therapeutic intervention within the pharmaceutical industry. Since ancient times enzymes also have played central roles in many manufacturing processes, such as in the production of wine, cheese, and breads. During the 1970s and 1980s much of the focus of the biochemical community shifted to the cloning and expression of proteins through the methods of molecular biology. Recently, some attention has shifted back to physicochemical characterization of these proteins, and their interactions with other macromolecules and small molecular weight ligands (e.g., substrates, activators, and inhibitors). Hence, there has been a resurgence of interest in the study of enzyme structures, kinetics, and mechanisms of catalysis.

The availability of up-to-date, introductory-level textbooks, however, has not kept up with the growing demand. I first became aware of this void while teaching introductory courses at the medical and graduate student level at the University of Chicago. I found that there were a number of excellent advanced texts that covered different aspects of enzymology with heavy emphasis on the theoretical basis for much of the science. The more introductory texts that I found were often quite dated and did not offer the blend of theoretical and practical information that I felt was most appropriate for a broad audience of students. I thus developed my own set of lecture notes for these courses, drawing material from a wide range of textbooks and primary literature.

In 1993, I left Chicago to focus my research on the utilization of basic enzymology and protein science for the development of therapeutic agents to combat human diseases. To pursue this goal I joined the scientific staff of the DuPont Merck Pharmaceutical Company. During my first year with this company, a group of associate scientists expressed to me their frustration at being unable to find a textbook on enzymology that met their needs for guidance in laboratory protocols and data analysis at an appropriate level and

at the same time provide them with some relevant background on the scientific basis of their experiments. These dedicated individuals asked if I would prepare and present a course on enzymology at this introductory level.

Using my lecture notes from Chicago as a foundation, I prepared an extensive set of notes and intended to present a year-long course to a small group of associate scientists in an informal, over-brown-bag-lunch fashion. After the lectures had been announced, however, I was shocked and delighted to find that more than 200 people were registered for this course! The makeup of the student body ranged from individuals with associate degrees in medical technology to chemists and molecular biologists who had doctorates. This convinced me that there was indeed a growing interest and need for a new introductory enzymology text that would attempt to balance the theoretical and practical aspects of enzymology in such a way as to fill the needs of graduate and medical students, as well as research scientists and technicians who are actively involved in enzyme studies.

The text that follows is based on the lecture notes for the enzymology course just described. It attempts to fill the practical needs I have articulated, while also giving a reasonable introduction to the theoretical basis for the laboratory methods and data analyses that are covered. I hope that this text will be of use to a broad range of scientists interested in enzymes. The material covered should be of direct use to those actively involved in enzyme research in academic, industrial, and government laboratories. It also should be useful as a primary text for senior undergraduate or first-year graduate course, in introductory enzymology. However, in teaching a subject as broad and dynamic as enzymology, I have never found a single text that would cover all of my students' needs; I doubt that the present text will be an exception. Thus, while I believe this text can serve as a useful foundation, I encourage faculty and students to supplement the material with additional readings from the literature cited at the end of each chapter, and the primary literature that is continuously expanding our view of enzymes and catalysis.

In attempting to provide a balanced introduction to enzymes in a single, readable volume I have had to present some of the material in a rather cursory fashion; it is simply not possible, in a text of this format, to be comprehensive in such an expansive field as enzymology. I hope that the literature citations will at least pave the way for readers who wish to delve more deeply into particular areas. Overall, the intent of this book is to get people *started* in the laboratory and in their thinking about enzymes. It provides sufficient experimental and data handling methodologies to permit one to begin to design and perform experiments with enzymes, while at the same time providing a theoretical framework in which to understand the basis of the experimental work. Beyond this, if the book functions as a stepping-stone for the reader to move on to more comprehensive and in-depth treatments of enzymology, it will have served its purpose.

ROBERT A. COPELAND

Wilmington, Delaware

"All the mathematics in the world is no substitute for a reasonable amount of common sense."

W. W. Cleland

1

A BRIEF HISTORY OF ENZYMOLOGY

Life depends on a well-orchestrated series of chemical reactions. Many of these reactions, however, proceed too slowly on their own to sustain life. Hence nature has designed catalysts, which we now refer to as *enzymes*, to greatly accelerate the rates of these chemical reactions. The catalytic power of enzymes facilitates life processes in essentially all life-forms from viruses to man. Many enzymes retain their catalytic potential after extraction from the living organism, and it did not take long for mankind to recognize and exploit the catalytic power of enzyme for commercial purposes. In fact, the earliest known references to enzymes are from ancient texts dealing with the manufacture of cheeses, breads, and alcoholic beverages, and for the tenderizing of meats. Today enzymes continue to play key roles in many food and beverage manufacturing processes and are ingredients in numerous consumer products, such as laundry detergents (which dissolve protein-based stains with the help of proteolytic enzymes). Enzymes are also of fundamental interest in the health sciences, since many disease processes can be linked to the aberrant activities of one or a few enzymes. Hence, much of modern pharmaceutical research is based on the search for potent and specific inhibitors of these enzymes. The study of enzymes and the action of enzymes has thus fascinated scientists since the dawn of history, not only to satisfy erudite interest but also because of the utility of such knowledge for many practical needs of society. This brief chapter sets the stage for our studies of these remarkable catalysts by providing a historic background of the development of enzymology as a science. We shall see that while enzymes are today the focus of basic academic research, much of the early history of enzymology is linked to the practical application of enzyme activity in industry.

1.1 ENZYMES IN ANTIQUITY

The oldest known reference to the commercial use of enzymes comes from a description of wine making in the Codex of Hammurabi (ancient Babylon, circa 2100 B.C.). The use of microorganisms as enzyme sources for fermentation was widespread among ancient people. References to these processes can be found in writings not only from Babylon but also from the early civilizations of Rome, Greece, Egypt, China, India. Ancient texts also contain a number of references to the related process of vinegar production, which is based on the enzymatic conversion of alcohol to acetic acid. Vinegar, it appears, was a common staple of ancient life, being used not only for food storage and preparation but also for medicinal purposes.

Dairy products were another important food source in ancient societies. Because in those days fresh milk could not be stored for any reasonable length of time, the conversion of milk to cheese became a vital part of food production, making it possible for the farmer to bring his product to distant markets in an acceptable form. Cheese is prepared by curdling milk via the action of any of a number of enzymes. The substances most commonly used for this purpose in ancient times were ficin, obtained as an extract from fig trees, and rennin, as rennet, an extract of the lining of the fourth stomach of a multiple-stomach animal, such as a cow. A reference to the enzymatic activity of ficin can, in fact, be found in Homer's classic, the Iliad:

> As the juice of the fig tree curdles milk, and thickens it in a moment though it be liquid, even so instantly did Paeëon cure fierce Mars.

The philosopher Aristotle likewise wrote several times about the process of milk curdling and offered the following hypothesis for the action of rennet:

> Rennet is a sort of milk; it is formed in the stomach of young animals while still being suckled. Rennet is thus milk which contains fire, which comes from the heat of the animal while the milk is undergoing concoction.

Another food staple throughout the ages is bread. The leavening of bread by yeast, which results from the enzymatic production of carbon dioxide, was well known and widely used in ancient times. The importance of this process to ancient society can hardly be overstated.

Meat tenderizing is another enzyme-based process that has been used since antiquity. Inhabitants of many Pacific islands have known for centuries that the juice of the papaya fruit will soften even the toughest meats. The active enzyme in this plant extract is a protease known as papain, which is used even today in commercial meat tenderizers. When the British Navy began exploring the Pacific islands in the 1700s, they encountered the use of the papaya fruit as a meat tenderizer and as a treatment for ringworm. Reports of these native uses of the papaya sparked a great deal of interest in eighteenth-century

Europe, and may, in part, have led to some of the more systematic studies of digestive enzymes that ensued soon after.

1.2 EARLY ENZYMOLOGY

While the ancients made much practical use of enzymatic activity, these early applications were based purely on empirical observations and folklore, rather than any systematic studies or appreciation for the chemical basis of the processes being utilized. In the eighteenth and nineteenth centuries scientists began to study the actions of enzymes in a more systematic fashion. The process of digestion seems to have been a popular subject of investigation during the years of the enlightenment. Wondering how predatory birds manage to digest meat without a gizzard, the famous French scientist Réaumur (1683–1757) performed some of the earliest studies on the digestion of buzzards. Réaumur designed a metal tube with a wire mesh at one end that would hold a small piece of meat immobilized, to protect it from the physical action of the stomach tissue. He found that when a tube containing meat was inserted into the stomach of a buzzard, the meat was digested within 24 hours. Thus he concluded that digestion must be a chemical rather than a merely physical process, since the meat in the tube had been digested by contact with the gastric juices (or, as he referred to them, "a solvent"). He tried the same experiment with a piece of bone and with a piece of a plant. He found that while meat was digested, and the bone was greatly softened by the action of the gastric juices, the plant material was impervious to the "solvent"; this was probably the first experimental demonstration of enzyme specificity.

Réaumur's work was expanded by Spallanzani (1729–1799), who showed that the digestion of meat encased in a metal tube took place in the stomachs of a wide variety of animals, including humans. Using his own gastric juices, Spallanzani was able to perform digestion experiments on pieces of meat in vitro (in the laboratory). These experiments illustrated some critical features of the active ingredient of gastric juices: by means of a control experiment in which meat treated with an equal volume of water did not undergo digestion Spallanzani demonstrated the presence of a specific active ingredient in gastric juices. He also showed that the process of digestion is temperature dependent, and that the time required for digestion is related to the amount of gastric juices applied to the meat. Finally, he demonstrated that the active ingredient in gastric juices is unstable outside the body; that is, its ability to digest meat wanes with storage time.

Today we recognize all the foregoing properties as common features of enzymatic reactions, but in Spallanzani's day these were novel and exciting findings. The same time period saw the discovery of enzyme activities in a large number of other biological systems. For example, a peroxidase from the horseradish was described, and the action of α-amylase in grain was observed. These early observations all pertained to materials — crude extract from plants or animals — that contained enzymatic activity.

During the latter part of the nineteenth century scientists began to attempt fractionations of these extracts to obtain the active ingredients in pure form. For example, in 1897 Bertrand partially purified the enzyme laccase from tree sap, and Buchner, using the "pressed juice" from rehydrated dried yeast, demonstrated that alcoholic fermentation could be performed in the absence of living yeast cells. Buchner's report contained the interesting observation that the activity of the pressed juice diminished within 5 days of storage at ice temperatures. However, if the juice was supplemented with cane sugar, the activity remained intact for up to 2 weeks in the ice box. This is probably the first report of a now well-known phenomenon — the stabilization of enzymes by substrate. It was also during this period that Kühne, studying catalysis in yeast extracts, first coined the term "enzyme" (the word derives from the medieval Greek word *enzymos*, which relates to the process of leavening bread).

1.3 THE DEVELOPMENT OF MECHANISTIC ENZYMOLOGY

As enzymes became available in pure, or partially pure forms, scientists' attention turned to obtaining a better understanding of the details of the reaction mechanisms catalyzed by enzymes. The concept that enzymes form complexes with their substrate molecules was first articulated in the late nineteenth century. It is during this time period that Emil Fischer proposed the "lock and key" model for the stereochemical relationship between enzymes and their substrates; this model emerged as a result of a large body of experimental data on the stereospecificity of enzyme reactions. In the early twentieth century, experimental evidence for the formation of an enzyme–substrate complex as a reaction intermediate was reported. One of the earliest of these studies, reported by Brown in 1902, focused on the velocity of enzyme-catalyzed reactions. Brown made the insightful observation that unlike simple diffusion-limited chemical reactions, in enzyme-catalyzed reactions "it is quite conceivable… that the time elapsing during molecular union and transformation may be sufficiently prolonged to influence the general course of the action." Brown then went on to summarize the available data that supported the concept of formation of an enzyme–substrate complex:

> There is reason to believe that during inversion of cane sugar by invertase the sugar combines with the enzyme previous to inversion. C. O'Sullivan and Tompson… have shown that the activity of invertase in the presence of cane sugar survives a temperature which completely destroys it if cane sugar is not present, and regard this as indicating the existence of a combination of the enzyme and sugar molecules. Wurtz [1880] has shown that papain appears to form an insoluble compound with fibrin previous to hydrolysis. Moreover, the more recent conception of E. Fischer with regard to enzyme configuration and action, also implies some form of combination of enzyme and reacting substrate.

Observations like these set the stage for the derivation of enzyme rate equations, by mathematically modeling enzyme kinetics with the explicit

involvement of an intermediate enzyme–substrate complex. In 1903 Victor Henri published the first successful mathematical model for describing enzyme kinetics. In 1913, in a much more widely read paper, Michaelis and Menten expanded on the earlier work of Henri and rederived the enzyme rate equation that today bears their names. The Michaelis–Menten equation, or more correctly the Henri–Michaelis–Menten equation, is a cornerstone of much of the modern analysis of enzyme reaction mechanisms.

The question of how enzymes accelerate the rates of chemical reactions puzzled scientists until the development of transition state theory in the first half of the twentieth century. In 1948 the famous physical chemist Linus Pauling suggested that enzymatic rate enhancement was achieved by stabilization of the transition state of the chemical reaction by interaction with the enzyme active site. This hypothesis, which was widely accepted, is supported by the experimental observation that enzymes bind very tightly to molecules designed to mimic the structure of the transition state of the catalyzed reaction.

In the 1950s and 1960s scientists reexamined the question of how enzymes achieve substrate specificity in light of the need for transition state stabilization by the enzyme active site. New hypotheses, such as the "induced fit" model of Koshland emerged at this time to help rationalize the competing needs of substrate binding affinity and reaction rate enhancement by enzymes. During this time period, scientists struggled to understand the observation that metabolic enzyme activities can be regulated by small molecules other than the substrates or direct products of an enzyme. Studies showed that indirect interactions between distinct binding sites within an enzyme molecule could occur, even though these binding sites were quite distant from one another. In 1965 Monod, Wyman, and Changeux developed the theory of allosteric transitions to explain these observations. Thanks in large part to this landmark paper, we now know that many enzymes, and nonenzymatic ligand binding proteins, display allosteric regulation

1.4 STUDIES OF ENZYME STRUCTURE

One of the tenets of modern enzymology is that catalysis is intimately related to the molecular interactions that take place between a substrate molecule and components of the enzyme molecule, the exact nature and sequence of these interactions defining per se the catalytic mechanism. Hence, the application of physical methods to elucidate the structures of enzymes has had a rich history and continues to be of paramount importance today. Spectroscopic methods, x-ray crystallography, and more recently, multidimensional NMR methods have all provided a wealth of structural insights on which theories of enzyme mechanisms have been built. In the early part of the twentieth century, x-ray crystallography became the premier method for solving the structures of small molecules. In 1926 James Sumner published the first crystallization of an enzyme, urease (Figure 1.1). Sumner's paper was a landmark contribution, not

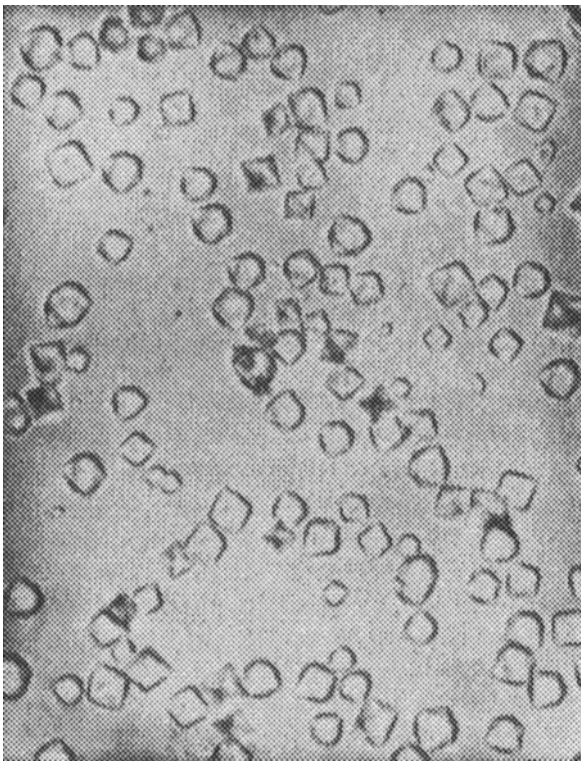

Figure 1.1 Photomicrograph of urease crystals (728 × magnification), the first reported crystals of an enzyme. [From J. B. Sumner, *J. Biol. Chem.* **69**, 435–441 (1926), with permission.]

only because it portended the successful application of x-ray diffraction for solving enzyme structures, but also because a detailed analysis allowed Sumner to show unequivocally that the crystals were composed of protein and that their dissolution in solvent led to enzymatic activity. These observations were very important to the development of the science of enzymology because they firmly established the protein composition of enzymes, a view that had not been widely accepted by Sumner's contemporaries.

Sumner's crystallization of urease opened a floodgate and was quickly followed by reports of numerous other enzyme crystals. Within 20 years of Sumner's first paper more than 130 enzyme crystals had been documented. It was not, however, until the late 1950s that protein structures began to be solved through x-ray crystallography. In 1957 Kendrew became the first to deduce from x-ray diffraction the entire three-dimensional structure of a protein, myoglobin. Soon after, the crystal structures of many proteins, including enzymes, were solved by these methods. Today, the structural

insights gained from x-ray crystallography and multidimensional NMR studies are commonly used to elucidate the mechanistic details of enzyme catalysis, and to design new ligands (substrate and inhibitor molecules) to bind at specific sites within the enzyme molecule.

The deduction of three-dimensional structures from x-ray diffraction or NMR methods depends on knowledge of the arrangement of amino acids along the polypeptide chain of the protein; this arrangement is known as the amino acid sequence. To determine the amino acid sequence of a protein, the component amino acids must be hydrolyzed in a sequential fashion from the polypeptide chain and identified by chemical or chromatographic analysis. Edman and coworkers developed a method for the sequential hydrolysis of amino acids from the N-terminus of a polypeptide chain. In 1957 Sanger reported the first complete amino acid sequence of a protein, the hormone insulin, utilizing the chemistry developed by Edman. In 1963 the first amino acid sequence of an enzyme, ribonuclease, was reported.

1.5 ENZYMOLOGY TODAY

Fundamental questions still remain regarding the detailed mechanisms of enzyme activity and its relationship to enzyme structure. The two most powerful tools that have been brought to bear on these questions in modern times are the continued development and use of biophysical probes of protein structure, and the application of molecular biological methods to enzymology. X-ray crystallography continues to be used routinely to solve the structures of enzymes and of enzyme–ligand complexes. In addition, new NMR methods and magnetization transfer methods make possible the assessment of the three-dimensional structures of small enzymes in solution, and the structure of ligands bound to enzymes, respectively.

The application of Laue diffraction with synchrotron radiation sources holds the promise of allowing scientists to determine the structures of reaction intermediates during enzyme turnover, hence to develop detailed pictures of the individual steps in enzyme catalysis. Other biophysical methods, such as optical (e.g., circular dichroism, UV–visible, fluorescence) and vibrational (e.g., infrared, Raman) spectroscopies, have likewise been applied to questions of enzyme structure and reactivity in solution. Technical advances in many of these spectroscopic methods have made them extremely powerful and accessible tools for the enzymologist. Furthermore, the tools of molecular biology have allowed scientists to clone and express enzymes in foreign host organisms with great efficiency. Enzymes that had never before been isolated have been identified and characterized by molecular cloning. Overexpression of enzymes in prokaryotic hosts has allowed the purification and characterization of enzymes that are available only in minute amounts from their natural sources. This has been a tremendous advance for protein science in general.

The tools of molecular biology also allow investigators to manipulate the

amino acid sequence of an enzyme at will. The use of site-directed mutagenesis (in which one amino acid residue is substituted for another) and deletional mutagenesis (in which sections of the polypeptide chain of a protein are eliminated) have allowed enzymologists to pinpoint the chemical groups that participate in ligand binding and in specific chemical steps during enzyme catalysis.

The study of enzymes remains of great importance to the scientific community and to society in general. We continue to utilize enzymes in many industrial applications. Moreover enzymes are still in use in their traditional roles in food and beverage manufacturing. In modern times, the role of enzymes in consumer products and in chemical manufacturing has expanded greatly. Enzymes are used today in such varied applications as stereospecific chemical synthesis, laundry detergents, and cleaning kits for contact lenses.

Perhaps one of the most exciting fields of modern enzymology is the application of enzyme inhibitors as drugs in human and veterinary medicine. Many of the drugs that are commonly used today function by inhibiting specific enzymes that are associated with the disease process. Aspirin, for example, one of the most widely used drugs in the world, elicits its anti-inflammatory efficacy by acting as an inhibitor of the enzyme prostaglandin synthase. As illustrated in Table 1.1, enzymes take part in a wide range of human pathophysiologies, and many specific enzyme inhibitors have been developed to combat their activities, thus acting as therapeutic agents. Several of the inhibitors listed in Table 1.1 are the result of the combined use of biophysical methods for assessing enzyme structure and classical pharmacology in what is commonly referred to as rational or structure-based drug design. This approach uses the structural information obtained from x-ray crystallography or NMR spectroscopy to determine the topology of the enzyme active site. Next, model building is performed to design molecules that would fit well into this active site pocket. These molecules are then synthesized and tested as inhibitors. Several iterations of this procedure often lead to extremely potent inhibitors of the target enzyme.

The list in Table 1.1 will continue to grow as our understanding of disease state physiology increases. There remain thousands of enzymes involved in human physiology that have yet to be isolated or characterized. As more and more disease-related enzymes are discovered and characterized, new inhibitors will need to be designed to arrest the actions of these catalysts, in the continuing effort to fulfill unmet human medical needs.

1.6 SUMMARY

We have seen in this chapter that the science of enzymology has a long and rich history. From phenomenological observations, enzymology has grown to a quantitative molecular science. For the rest of this book we shall view enzymes from a chemical prospective, attempting to understand the actions of

Table 1.1 Examples of enzyme inhibitors as potential drugs

Inhibitor/Drug	Disease/Condition	Enzyme Target
Acetazolamide	Glaucoma	Carbonic anhydrase
Acyclovir	Herpes	Viral DNA polymerase
Allopurinol	Gout	Xanthine oxidase
Argatroban	Coagulation	Thrombin
Aspirin, ibuprofen, DuP697	Inflammation, pain, fever	Prostaglandin synthase
β-Lactam antibiotics	Bacterial infections	D-Ala-D-Ala transpeptidase
Brequinar	Organ transplantation	Dihydroorotate dehydrogenase
Candoxatril	Hypertension, congestive heart failure	Atriopeptidase
Captopril	Hypertension	Angiotensin-converting enzyme
Clavulanate	Bacterial resistance	β-Lactamase
Cyclosporin	Organ transplantation	Cyclophilin/calcineurin
DuP450	AIDS	HIV protease
Enoximone	Congestive heart failure ischemia	cAMP phosphodiesterase
Finazteride	Benign prostate hyperplasia	Testosterone-5-α-reductase
FK-506	Organ transplantation, autoimmune disease	FK-506 binding protein
Fluorouracyl	Cancer	Thymidilate synthase
3-Fluorovinylglycine	Bacterial infection	Alanine racemase
(2-Furyl)-acryloyl-Gly-Phe-Phe	Lung elastin degradation in cystic fibrosis	*Pseudomonas* elastase
ICI-200,808	Emphysema	Neutrophil elastase
Lovastatin	High cholesterol	HMG CoA reductase
Ly-256548	Inflammation	Phospholipase A_2
Methotrexate	Cancer	Dihydrofolate reductase
Nitecapone	Parkinson's disease	Catechol-*O*-methyltransferase
Norfloxacin	Urinary tract infections	DNA gyrase
Omeprazole	Peptic ulcers	H^+, K^+-ATPase
PALA	Cancer	Aspartate transcarbamoylase
PD-116124	Metabolism of antineoplastic drugs	Purine nucleoside phosphorylase
Phenelzine	Depression	Brain monoamine oxidase
Ro 42-5892	Hypertension	Renin
Sorbinil	Diabetic retinopathy	Aldose reductase
SQ-29072	Hypertension, congestive heart failure, analgesia	Enkephalinase
Sulfamethoxazole	Bacterial infection, malaria	Dihydropteorate synthase
Testolactone	Hormone-dependent tumors	Aromatase
Threo-5-fluoro-L-dihydroorotate	Cancer	Dihydroorotase
Trimethoprim	Bacterial infection	Dihydrofolate reductase
WIN 51711	Common cold	Rhinovirus coat protein
Zidovudine	AIDS	HIV reverse transcriptase
Zileuton	Allergy	5-Lipoxygenase

Source: Adapted and expanded from M. A. Navia and M. A. Murcko, *Curr. Opin. Struct. Biol.* **2**, 202–210 (1992).

these proteins in the common language of chemical and physical forces. While the vital importance of enzymes in biology cannot be overstated, the understanding of their structures and functions remains a problem of chemistry.

REFERENCES AND FURTHER READING

Rather than providing an exhaustive list of primary references for this historical chapter, I refer the reader to a few modern texts that have done an excellent job of presenting a more detailed and comprehensive treatment of the history of enzymology. Not only do these books provide good descriptions of the history of science and the men and women who made that history, but they are also quite entertaining and inspiring reading—enjoy them!

Friedmann, H. C., Ed. (1981) *Enzymes*, Hutchinson Ross, Stroudsburg, PA. [This book is part of the series "Benchmark Papers in Biochemistry." In it, Friedmann has compiled reprints of many of the most influential publications in enzymology from the eighteenth through twentieth centuries, along with insightful commentaries on these papers and their importance in the development of the science.]

Judson, H. F. (1980) *The Eighth Day of Creation*, Simon & Schuster, New York. [This extremely entertaining book chronicles the history of molecular biology, including protein science and enzymology, in the twentieth century.]

Kornberg, A. (1989) *For the Love of Enzymes. The Odyssey of a Biochemist*, Harvard University Press, Cambridge, MA. [An autobiographical look at the career of a Nobel Prize–winning biochemist.]

Werth, B. (1994) *The Billion Dollar Molecule*, Simon & Schuster, New York. [An interesting, if biased, look at the modern science of structure-based drug design.]