Enzyme Structure and Mechanism

Second Edition

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Preface

We are now entering a new golden age of enzymology. Just as x-ray protein crystallography transformed the 1960s and 1970s, so recombinant DNA technology is changing the whole perspective in the 1980s. The cloning of enzyme genes for overproduction has facilitated the study of well-known enzymes and has enabled previously inaccessible enzymes to be characterized. Even more exciting are the prospects opened up by our ability to tailor the structure and activity of enzymes by manipulation of their genes. Specific amino acid residues may be changed by site-directed mutagenesis. Even whole genomes may be synthesized and their gene products expressed.

The second edition contains a chapter on protein engineering (Chapter 14) to summarize these developments. Two further new chapters (8 and 9) have been added to cover the recent advances in stereochemical methods and the continued progress in the design of highly specific irreversible inhibitors. Elsewhere, the book has been generally brought up to date, and certain chapters have been reorganized. Chapter 1, in particular, has been expanded to include discussion of the dynamic aspects of protein structure and recent ideas on protein evolution. Also, the chapter on allosteric proteins (Chapters 10) has been expanded to include specific examples of enzymes that regulate metabolic pathways.

I am indebted to several colleagues for their useful comments, especially those of Sir John Cornforth on Chapter 8.

A. F.
London, 1984

Preface to the First Edition

During the past two decades the advances in x-ray crystallography, transient kinetics, and the study of chemical catalysis have revolutionized our ideas on enzyme catalysis and mechanism. It is the intention of this text to provide a brief account of these developments for senior undergraduate students and postgraduates who have attended courses in chemistry and biochemistry. The philosophical and theoretical aspects of this book center upon how the interactions of an enzyme with its substrates lead to enzyme catalysis and specificity, and upon the relationship between structure and mechanism. The experimental approaches emphasized are those involving the direct study of enzymes as molecules. As such, there is a strong emphasis on pre-steady state kinetics where enzymes are handled in substrate quantities and enzyme-bound intermediates observed directly. The steady state kinetics of multisubstrate enzymes and the detailed chemistry of coenzymes and cofactors are discussed only in a cursory manner.

There have been two guiding rules in the preparation of this book. The first is to discuss general principles and ideas using specific enzymes as examples. [Although to avoid overloading the more theoretical chapters on kinetics, most of the illustrative examples are presented in a separate chapter (7).] The second is to stick closely to examples where hard evidence is available and to avoid speculation and woolly evidence. In consequence, the discussion of detailed chemical mechanisms is generally restricted to enzymes whose tertiary structures have been solved by x-ray crystallography. Similarly, the discussion of the theoretical aspects of allosteric proteins is very much restricted to hemoglobin because it is the only example where good (or any) evidence is available on the nature of the interactions of positive cooperativity.

The references cited tend to be those of the most recent reviews or papers where more extensive bibliographies are given, and also those of the original papers in order to maintain a historical perspective. Illustrative examples have been taken were possible from the files of the MRC Laboratory of Molecular Biology because of their ready availability and uniform quality of presentation. In this context I must thank Annette Lenton both for the illustations she has prepared especially for this book and also for those prepared for other members of the laboratory whose files I have shamelessly raided.

I am particularly indebted to W. P. Jencks, H. B. F. Dixon, H. Gutfreund, K. F. Tipton, and R. S. Mulvey for their critical comments on the entire manuscript, and also M. F. Perutz and D. M. Blow for their comments on individual chapters. I wish to thank The Royal Society, the American Chemical Society, the Cornell University Press, Academic Press, John Wiley, and Alan R. Liss for permission to reproduce illustrations.

A. F.
Cambridge 1977

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The three-dimensional structure of enzymes

In 1930, J. B. S. Haldane wrote a book on enzymes that is still worth reading today.[1] The most striking feature of this book is that so much was then known about the properties and action of enzymes, yet so little was known about the enzymes themselves: the question of whether or not enzymes are proteins was still the subject of raging controversy. The knowledge was so one-sided because there was no means of studying enzymes directly. All the information had been deduced indirectly from the effects of enzymes on their substrates. Nevertheless, the foundations of modern steady state kinetics had been laid in a little over thirty years, the first cell-free enzyme extract having been prepared by E. Büchner in 1897.

In order to proceed further, it was necessary to isolate purified enzymes in *substrate* quantities and examine them directly. This was accomplished in 1926, when J. B. Sumner crystallized urease from jack bean extracts. Soon afterwards (1930–36), J. H. Northrop and M. Kunitz crystallized pepsin, trypsin, and chymotrypsin. This provided the material to prove finally that enzymes are proteins, and to allow the development of the techniques of modern protein chemistry: the sequencing of proteins pioneered by F. Sanger; the solution of the three-dimensional structure of proteins pioneered by M. F. Perutz and J. C. Kendrew; and the use of rapid-reaction kinetics, which had been initiated by F. J. W. Roughton in 1923.

The major part of the present book deals with the direct study of enzymes in substrate quantities, taking up the story from where Haldane was forced to stop. This first chapter discusses the general features of the most significant advance in our knowledge of enzymes since then—their three-dimensional structure, from the basics of the peptide bond and the various elements of protein folding to macromolecular assemblies. The chapter also describes the evolution of protein structure and function, and considers the dynamic aspects of proteins. To set the scene for later