THE NATURE OF ENZYMOLOGY

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CROOM HELM LONDON

Croom Helm Ltd, 2-10 St John's Road, London SW11

British Library Cataloguing in Publication Data

Foster, Ronald L
The nature of enzymology – (Croom Helm applied biology series).
1. Enzymes
I. Title
574.1'925 QP601

ISBN 0-85664-434-X
ISBN 0-85664-915-5 Pbk

Printed in Great Britain by offset lithography by
Billing & Sons Ltd, Guildford, London and Worcester

Acknowledgements

The author would like to thank the following who have given their permission to reproduce figures and tables.

Professor D. C. Phillips, Figures 1.2 and 1.4; Professor D. Blow, Figures 1.6 and 4.9; Dr J. E. Walker, Figure 1.7; Dr G. C. K. Roberts, Figure 1.8; Professor M. G. Rossman, Figures 4.11 and 4.12; Professor J. H. Wilkinson, Figures 6.3 and 6.4; Professor E. Katchalski-Katzir, Figure 7.3; Professor F. W. Schmidt, Figure 6.2; Professor K. K. Kannan, Figures 5.15 and 5.16.

Nature (London), Figures 1.2, 1.4, 1.7 and 4.9; Elsevier (Amsterdam), Table 5.11 and Appendix I; Academic Press (London and New York), Figures 1.6, 1.8, 4.11, 5.15, 5.16, 6.2 and 7.3; The Institute of Medical Laboratory Sciences, Figures 6.3 and 6.4; International Union of Biochemistry—International Union of Pure and Applied Chemistry (IUB-IUPAC), Table 5.11 and Appendix I.

Contents

1 The Character of Enzymes

Preface

The study and application of enzymes are profoundly influencing the development of biochemistry and related disciplines. X-ray crystallographic investigations have illuminated many structural features underlying their functions, their selectivities of action are beginning to be applied to the treatment of thrombo-embolic and neoplastic diseases and their high efficiencies are increasingly utilised to improve the quality and economic supply of naturally occurring molecules.

Although there are many advanced treatises dealing with these different aspects the majority have been written for specialists. This book has been designed to serve the following three purposes (a) to introduce undergraduates to the broad subject of enzymology by explaining the principal concepts underlying its modern ideas and applications, (b) to give an account for interested persons in other fields and (c) to present to both a picture of the extraordinary diversification which the subject has now attained.

The first half of the book is devoted to the general characteristics and properties of enzymes, placing emphasis on their structures and active site chemistry, and in the second half their important physiological roles and current applications in clinical medicine and industry are outlined. In all the chapters the object has been to bring to the attention of the reader as large a part of the field of enzymology as possible within a reasonable volume. Thus specific enzymes have been used as examples since there are far too many to describe each in detail (approximately 2,000 have been classified to date). For the same reason, more references, especially to key articles and reviews, are given than is perhaps usual in this type of book, but it is hoped that they will provide bridges to further more detailed and advanced study.

My thanks are due to many present colleagues, past teachers and questioning students who knowingly or unknowingly have helped the writing of this book. In particular I should like to thank Dr T. Wileman for his thorough reading of the manuscript, the publishers for their patience and above all, my wife, whose constancy and secretarial help ensured its fulfilment.

R. L. F.

Enzymes are the catalysts of living organisms. Virtually all the chemical reactions occurring in plants, microorganisms and animals proceed at a measurable rate as a direct consequence of enzymic catalysis. The phenotypes of these living systems are functions of the molecules synthesised enzymically and the morphologies, organisations and functions of their cells are effected through enzymic agency.

Many of the commonly encountered substances which make palatable man's existence are also the products of enzymic action. Cheese, for example, is produced from milk by the action of 'rennin' a protein digesting enzyme derived from the stomachs of weaning calves and everyday alcohol, ethanol, is one product of the consecutive action of twelve enzymes elaborated by yeast cells growing on glucose.

Enzyme catalysis is therefore one of the most important, ubiquitous but at the same time, enigmatic expressions of evolution.

Biological catalysis has been known for nearly 150 years. In 1837 Berzelius recognised that there were naturally occurring 'ferments' which promoted chemical reactions and which fulfilled the criteria of catalysis he had proposed a few years earlier. These ferments he classified as 'organised' or 'unorganised' depending on the presence or absence of intact cells. But although the origins of enzymology can be traced so far back in time, the first significant advances were made a century ago by W. Kühne through his investigations into the nature of trypsin catalysed reactions. Kühne is also responsible for introducing the name 'enzyme', transliterated from the classical Greek 'in yeast', to describe the naturally occurring catalysts present in unorganised ferments.

Except for Fischer's attempt to explain the specificity of an enzyme for its substrate (reacting substance transformed during catalysis) by the analogy of complementarity between a lock and its key, little further progress was made until the turn of the present century. Considerable impetus to the development of enzymology was then provided by the mechanistic formulations of V. Henri, L. Michaelis and others, who rationalised the kinetic manifestations of enzymic catalysis by postulating the intermediate formation of an

active enzyme-substrate complex which reacts to form products and release active enzyme. This kinetic advance was followed soon afterwards in 1926 by the demonstration by Sumner that 'urease', an enzyme catalysing the hydrolysis of urea ($NH_2 \cdot CO \cdot NH_2$) to ammonia plus carbon dioxide, was a protein. By showing that an enzyme had a distinctive chemical identity and belonged to an established class of molecules, Sumner tolled the knell of the 'vitalistic force' and similar theories and paved the way for the laboratory handling and analysis of enzymes.

During the succeeding half century enzymology has developed apace (Table 1.1). As the steps comprising the major metabolic pathways, for example, glycolysis and the tricarboxylic acid cycle, were elucidated, knowledge grew of the multifarious reactions catalysed by enzymes, and as the biochemical events underlying the physiological processes of digestion, muscular contraction, endocrine function, coagulation and biosynthesis were unravelled, their important roles in the maintenance, control and integration of complex metabolic processes came to be recognised. Simultaneously during this fruitful period, kinetic frameworks to rationalise the observations of enzyme action and inhibition, and procedures specifically designed to analyse the structures of functionally sensitive proteins were developed. By the time the structural explorations culminated in the 1960s with the resolution of the three-dimensional structures of the oxygen transporting 'honorary enzymes', myoglobin and haemoglobin, and more especially with the resolution of the structure of the bacteriolytic enzyme, lysozyme, by Phillips *et al.*, the basic conceptual and molecular bases of enzymology had been laid down. This groundwork is covered in the first five chapters of this book.

More recently, attention has increasingly been directed to the applications of enzymes. Their high efficiencies make them potentially valuable as catalysts in manufacturing industry. For example, glucamylase and amylase (polysaccharide degrading enzymes) are gradually replacing strong acids in the depolymerisation of starch to glucose. Both enzymes can act on a wide variety of glucose polymers so making available potentially more supplies of the sweetener. In a more general industrial context, the other main advantages offerred by enzymic catalysis are the mild conditions needed for efficient operation. These requisites thus facilitate process design and maintenance. Their other remarkable capability, that is, specificity of action, is proving of benefit in clinical medicine.

Table 1.1: Chronology of Enzyme Studies

1833	Payen and Persoz. Alcohol precipitation of thermolabile 'diastase' from malt.
1835	Berzelius. Concept of *catalysis*.
1837	Berzelius. Recognition of biological catalysis.
1850	Wilhelmy. Quantitative evaluation of the rates of sucrose inversion.
1878	Kühne. Investigations of trypsin catalysed reactions and introduction of the word 'enzyme'.
1894–5	Fischer. 'Lock and key' simile of enzyme specificity.
1896–7	Bertrand. 'Coenzyme' or 'coferment' (now called cofactors).
1898	Duclaux. Nomenclature—substrate plus suffix 'ase'.
1901–3	Henri. General procedures for the derivation of kinetic rate laws: principle of the enzyme-substrate complex.
1906	Harden and Young. 'Cozymase' (NAD).
1913	Michaelis and Menten. Extension of the kinetic theory of enzyme catalysis.
1925	Briggs and Haldane. Derivation of enzyme rate equations using the steady-state approximation.
1926	Sumner. Crystallisation of urease—a 'protein' not a 'property'.
1930–3	Northrop and Kunitz. Crystallisation of proteolytic enzymes.
1937–9	Cori and Cori. Muscle phosphorylase.
1940	Beadle and Tatum. 'One gene-one enzyme' hypothesis.
1943	Chance. Spectrophotometric techniques.
1953	Koshland. Induced fit hypothesis.
1956	Umbarger, Yates and Pardee. Control of enzyme activity through feedback inhibition.
1956	Sutherland. Cyclic AMP, adenyl cyclase.
1956–8	Anfinsen. Amino acid sequence determines folding pattern and activity of ribonuclease.
1961	Jacob, Monod and Changeux. Allosterism.
1965	Phillips, Johnson and North. Three-dimensional structure of lysozyme obtained at 1.5 Å resolution.

The accuracy with which a disease state can be diagnosed often depends on the activity measurements of tissue specific enzymes. Once diagnosed the treatment of the pathological disorder may rely on the selective toxicity of chemotherapeutic agents. The differences between normal and abnormal or foreign cells are in many instances slight and one way in which they may be differentiated is to utilise the specificity particularly extant in enzymes. These more applied areas of enzymology are discussed in Chapters 6 and 7.

1.1 ENZYME STRUCTURE AND PROPERTIES

To date nearly two thousand enzymes have been catalogued, together catalysing all the known types of chemical reaction. Oxidation, reduction, hydrolysis, elimination, polymerisation, isomerisation and transfer are all catalysed in biological systems by enzymes that have evolved to respond to local physiological conditions. However, even though so many enzymes exist and catalyse a large variety of reactions, they do possess several characteristics in common.

All enzymes behave as catalysts, that is, only small quantities, relative to the concentrations of their substrates, are needed to considerably increase the rate of chemical reactions, while they themselves undergo no net change. In addition the total amount of substrate transformed per mass of enzyme is often very large. Like all true catalysts, an enzyme does not change the final equilibrium position of a reaction, which is thermodynamically determined, and only the *rate* of attainment of equilibrium of a feasible reaction is increased. The reaction rate may, of course, be unobservably slow in its absence.

A characteristic of considerable importance is that all enzymes are proteins. Sumner's first announcement to this effect was confirmed soon afterwards for several proteolytic enzymes by Northrop and Kunitz (Table 1.1). Therefore in addition to their catalytic properties, enzymes exhibit the chemical and physical behaviour of proteins: their electrolytic behaviours, solubilities, electrophoretic properties and chemical reactivities in addition to their catalytic activities depend on the L-α-amino acid sequence and peptide bonds constituting the protein molecule (see Table 1.2 and Figure 1.1).

Table 1.2: L-Amino Acid Side Chains (R) Commonly Found in Enzymes.

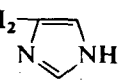
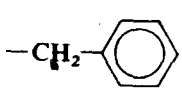
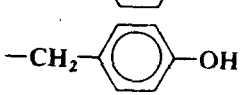
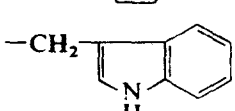
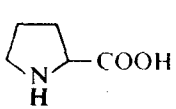
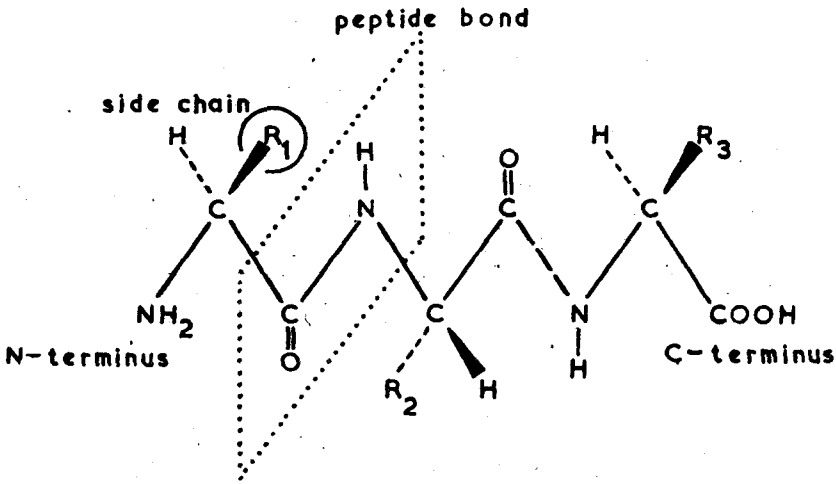
Aliphatic amino acids	*R*
1. Glycine (gly)	$-H$
2. Alanine (ala)	$-CH_3$
3. Valine (val)	$-CH(CH_3)_2$
4. Leucine (leu)	$-CH_2 \cdot CH(CH_3)_2$
5. Isoleucine (ile)	$-CH(CH_3) \cdot CH_2 \cdot CH_3$
Acidic amino acids	
6. Aspartic acid (asp)	$-CH_2 \cdot CO_2H$
7. Glutamic acid (glu)	$-CH_2 \cdot CH_2 \cdot CO_2H$
Acid amides	
8. Asparagine (asn)	$-CH_2 \cdot CO \cdot NH_2$
9. Glutamine (gln)	$-CH_2 \cdot CH_2 \cdot CO \cdot NH_2$
Basic amino acids	
10. Lysine (lys)	$-(CH_2)_4NH_2$
11. Histidine (his)	
12. Arginine (arg)	$-(CH_2)_3 \cdot NH \cdot C(:NH)NH_2$
Hydroxy amino acids	
13. Serine (ser)	$-CH_2 \cdot OH$
14. Threonine (thr)	$-CH(OH) \cdot CH_3$
Sulphur-containing amino acids	
15. Cysteine (cys)	$-CH_2 \cdot SH$
16. Cystine	$-CH_2 \cdot S \cdot S \cdot CH_2-$
17. Methionine (met)	$-(CH_2)_2 \cdot S \cdot CH_3$
Aromatic amino acids	
18. Phenylalanine (phe)	
19. Tyrosine (tyr)	
20. Tryptophan (try)	
Imino acid	
21. Proline (pro)	

Figure 1.1: Diagrammatic Representation of the Polypeptide Backbone of an Enzyme



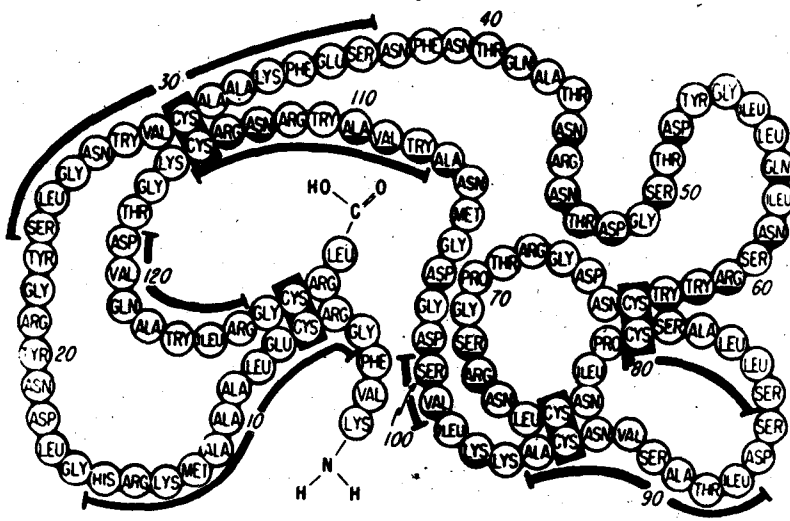
Being proteins, enzymes differ considerably from traditional chemical catalysts such as hydrogen ions, hydroxyl ions, heavy metals or metal oxides. Whereas these are most effective in organic solvents, at high temperatures or at extreme pH values, enzymes operate most efficiently under very mild conditions. Departure from homogeneous, aqueous solutions, physiological pH and temperature rapidly destroys their activities, but under normal conditions the rate increases achieved are rarely matched by their non-protein counterparts.

The linear chain of amino acid residues joined by peptide bonds, which constitutes a protein molecule has been called by Linderström Lang the *primary* structure. Localised folding of the primary structure is entitled *secondary* structure, and the overall folding of the molecule *tertiary* structure. To these Bernal later added *quaternary* structure to describe the agglomeration of several folded chains.

1.1.1 Primary and Secondary Structure

The amino acid sequence of hen's egg white lysozyme, the first enzyme to be successfully analysed three dimensionally,[1] illustrates

Figure 1.2: The Primary Structure of Lysozyme showing the Residues found in α-Helix Conformations (continuous lines) and those in the Apparent Binding Site (underlined)



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several features found in primary structures (see Figure 1.2). The order of amino acid residues is the same in all molecules from the same source and appears to be random with, as yet, no obvious predictability. No branching occurs, although many enzymes are intramolecularly crosslinked through the disulphide bridges of cystine. By this means widely separated sections along the chain may be covalently joined: for example residues 5 and 129 form a disulphide bond which brings together both ends of the lysozyme molecule. Present evidence indicates that few of the amino acids are superfluous and most are 'functional', i.e. most co-operatively determine the higher orders of structural organisation and hence catalytic activity.

Comparisons of the primary structures of enzymes performing similar functions have demonstrated extensive structural homologies in their sequences, particularly in the patterns of their non-polar residues.[2] For example, pancreatic juice contains five inactive

Figure 1.3: Sequence Homologies in the first 21 Residues of the B *Chains of Mammalian Serine Proteases* (CA, *bovine chymotrypsinogen* A; CB, *bovine chymotrypsinogen* B *and* E, *porcine elastase; chemically similar residues in any pair are in capitals*)

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
CA:	ILE	VAL	Asn	GLY	GLU	GLU	ALA	Val	Pro	GLY	SER	TRP	PRO	TRP	GLN	VAL	SER	LEU	GLN	Asp	LYS
CB:	ILE	VAL	Asn	GLY	GLU	ASP	ALA	Val	Pro	GLY	SER	TRP	PRO	TRP	GLN	VAL	SER	LEU	GLN	Asp	Ser
T:	ILE	VAL	GLY	GLY	Tyr	Thr	Cys	Gly	Ala	ASN	THR	Val	PRO	TYR	GLN	VAL	SER	Leu	ASN	–	–
E:	VAL	VAL	GLY	GLY	Thr	GLU	ALA	GLN	Arg	ASN	SER	TRP	PRO	Ser	GLN	ILE	SER	LEU	GLN	Tyr	ARG

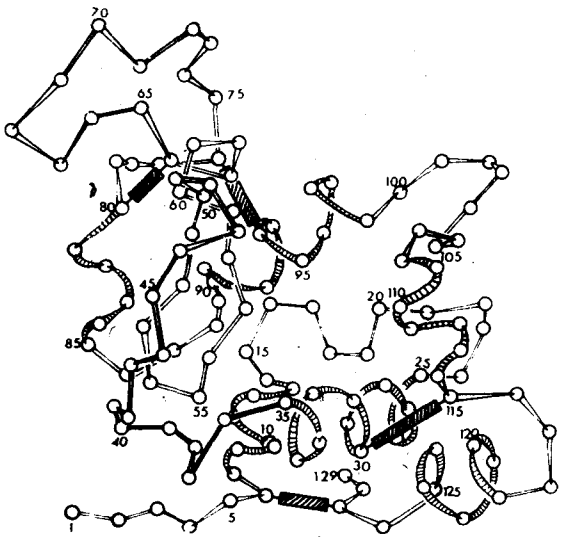
Source: Reference 2.

precursors (zymogens), chymotrypsinogens A, B and C, trypsinogen and proelastase, all of which are activated to the respective serine proteases* by proteolytic cleavage. Illustrative sections of their primary structures are delineated and compared in Figure 1.3. By arranging their amino acid sequences in this way to maximise the homologies, clusters of short homologous sequences, separated by oligopeptide regions of dissimilar residues are found. Overall the percentage residue identity between chymotrypsins A and B is about 78 percent, indicating the two are very closely related, more so than with the other pancreatic enzymes although with these there is still 40 percent residue identity and 50 percent chemical similarity. Calculations of the mutation values of amino acid pairs in CA versus CB and the chymotrypsins versus trypsin, indicate that the former pair possess a more recent evolutionary ancestor in common than do either with trypsin, i.e. the mammalian serine proteases appear to have undergone divergent evolution.

The overall folding of the amino acid sequences to give the functional enzyme appears to be unorganised (Figure 1.4), but on closer inspection, it can be seen that regions are organised into structures of definable symmetry. For example, residues 24–34 and 41–54 in lysozyme are folded into elements of secondary structure, α-helix and β-sheet respectively. These are named after the corresponding structures of the α- and β-keratins and were first predicted by Pauling and Corey using X-ray diffraction data obtained from crystalline short polypeptides. The peptide bond was found to be shorter by approximately 0.01 nm than the 0.14 nm of a single carbon-nitrogen bond, and to have more double bond character. Rotation

* Protein degrading enzymes each requiring an unmodified seryl residue in its active site for activity.

Figure 1.4: Schematic Drawing of the Main Chain Conformation of Hen's Egg White Lysozyme



Source: Reproduced with permission, from Reference 1.

around this bond is restricted at normal temperatures, causing the peptide bond and the two adjacent α-carbon atoms to lie in one plane, with the carbonyl oxygen and amino hydrogen in the energy minimum of a *trans* configuration, as shown in Figure 1.1. Rotation can then only occur around those bonds to the α-carbon atoms, and the possible types of stable structure which maximise the number of hydrogen bonds, are restricted to the α-helix and β-pleated sheet. In closer atomic detail these structures are shown in Figure 1.5.

1.1.2 Tertiary Structure

The overall folding of the polypeptide chain, which incorporates the organised secondary structure as well as random stretches, is entitled the tertiary structure (Figure 1.4). At present the most reliable and powerful method for determining the three-dimensional structure of a protein, and in combination with the primary sequence, for portraying the relative stereochemical positions of the atoms is X-ray crystallography.