Peter Friedrich

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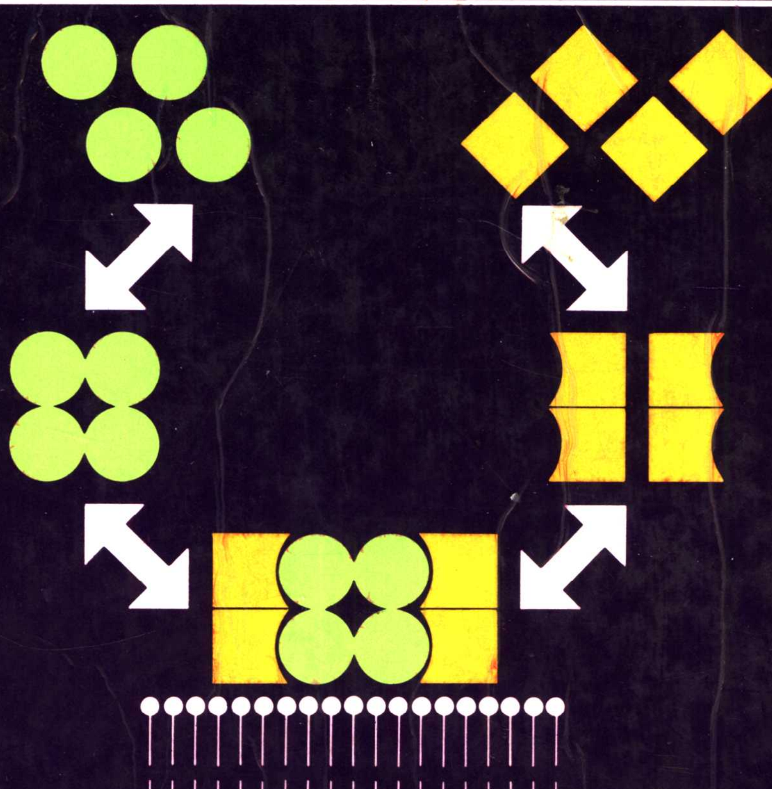
QUATERNARY STRUCTURE AND BEYOND



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BY

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PREFACE

Ever since I embarked on the writing of this book I have longed to come to the preface. This desire, of course, has been motivated by the circumstance that prefaces are written at the end, with the main bulk of work done, rather than by the inherent pleasures of preface-writing. In fact, these misplaced epilogues are in most cases utterly useless. They are superfluous to a good book, but do not help a poor one. Nevertheless, there are intermediate cases where some preliminary explanations may be handy. I entertain the hope that the present volume lives up to that standard.

This book reflects my scientific interest over the past ten years. Hence, it is a highly subjective treatise, particularly as regards the delimitation of subject matter. It may appear unusual to include the enzymes' quaternary structure in a monograph essentially aimed at their supramolecular organization or, the other way round, once preoccupied with subunit structure to trespass so far into the murky field of the "beyond". Perusal of the Contents may suggest to the reader that the author's meandering interest gave the book a backbone suffering from scoliosis.

Let me admit that, however arbitrary it may appear, the scope of the text was made such deliberately. Indeed, I believe it makes sense to start scrutinizing enzyme interactions at the quaternary level and then to proceed to higher orders of organization. In this way conceptually related phenomena can be placed in a common perspective. Numerous monographs, reviews and the like have already been written on the subunit structure of enzymes, but none of them really ventured to roam outside the safe walls of Fort Enzymology. However, structural and functional complementarities are not confined to individual enzyme molecules. To my mind, enzyme organization is a continuum and it is in this spirit that the present book was conceived.

Even if the above reasoning is acceptable, the selection of material was arbitrary of necessity. As stressed at too many points in this volume, I am afraid, my aim was to illustrate and not to give comprehensive lists of all observations for the various organizational modes. Obviously, more emphasis is given to areas and objects related to my own research work, perhaps at the expense of more meritorious sources. I apologize to all those who feel unfairly left out by my choices. There was certainly much more material available than I could cope with. Let the shortcomings and omissions of this book be a challenge to others to write their version of the story!

This book is intended, first of all, *not* for the specialist, who knows much more about the individual questions than the text offers, but rather for the general biochemical reader interested in enzyme organization. There is practically no mathematics involved. Instead, quite elementary things are described, so that students and biologists whose bread is not buttered with enzymes should also be able to join in. To facilitate further reading references have been amply given.

Let me finally say a word about the scientific credit of the book's content. I think, in this respect it is more heterogeneous than the average. It discusses in brief solid text-book facts while, on the other hand, it reports about the frontiers of research in a highly uncertain and deceptive field. The author is tantalized to know how many and which of the present trends will stand the test of time. Already now, I suffer from "journalophobia", a mental state characterized by avoidance of current journal issues in the fear of finding yet more new facts. For this reason readers are warned not to take a fundamentalist attitude towards what is written here, especially when it comes to provocative new ideas, but rather to follow up developments since the completion of the manuscript. Even in this scientometric world of ours, the impact of this book should not be quantitated by the number of workers it has led astray.

With these premonitions advanced, I wish the reader a useful journey through the land of quaternary enzyme structure — and beyond.

Peter Friedrich

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I wish to thank all colleagues who gave permission to reproduce their figures in this book; several of them also called my attention to recent developments. Their names are indicated in the legends to the figures. I am particularly obliged to Drs Pál Elődi, Tamás Keleti and John Londesborough for their thorough criticism of the manuscript. John Londesborough gave me invaluable help also by improving the English of the text. Whatever faults, scientific or linguistic, remain in the book, they lie entirely with me. I thank my coworkers Drs Magda Solti, János Hajdú, Ferenc Bartha, and all members of the Institute of Enzymology (directed by Brunó F. Straub) of the Hungarian Academy of Sciences who contributed to the scientific atmosphere in which this book was conceived. I am sincerely indebted to Mrs Katalin Radnai, Mrs Ágnes Külföldi, Mrs Ágnes Csurgó and Mrs Szilvia Kövécs for their devoted technical assistance. I remember with gratitude the helpful librarians and the peaceful guestroom of the Biological Research Centre at Szeged. Last but not least, I thank my family for tolerating my whims, while writing, with patience and love.

LIST OF ABBREVIATIONS

ADH	alcohol dehydrogenase
ADP	adenosine diphosphate
ALD	aldolase
AMP	adenosine-5'-phosphate
ATCase	aspartate carbamoyl transferase
ATP	adenosine triphosphate
B-3-P	band-3-protein of the erythrocyte membrane
BPG	bis-phosphoglycerate
cAMP	cyclic adenosine-3',5'-monophosphate
cGMP	cyclic guanosine-3',5'-monophosphate
CoA-SH	coenzyme A
CoQ and $CoQH_2$	oxidized and reduced forms of coenzyme Q (ubiquinone), respectively
DAHP	3-deoxy-D-arabinoheptulosonate-7-phosphate
DCCD	dicyclohexyl carbodiimide
DEAE	diethylaminoethyl-
DHQ	5-dehydroquinate
DHS	5-dehydroshikimate
EGTA	ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
FAD	flavin adenine dinucleotide
FBP	fructose-1,6-bisphosphate
GAP	D-glyceraldehyde-3-phosphate
GAPD	D-glyceraldehyde-3-phosphate dehydrogenase
GDP	guanosine diphosphate
GTP	guanosine triphosphate
Hb	haemoglobin
IMP	inosine monophosphate
LDH	lactate dehydrogenase
MAO	monoamine oxidase
NAD and NADH	nicotinamide adenine dinucleotide, oxidized and reduced forms, respectively
NADP and NADPH	nicotinamide adenine dinucleotide phosphate, oxidized and reduced forms, respectively
NMR	nuclear magnetic resonance
OMP	orotidine-5'-phosphate
OSCP	oligomycin sensitivity conferring protein
–P	phosphoryl group
P_i	inorganic phosphate

PDC	pyruvate dehydrogenase complex
PFK	phosphofructokinase
PGK	3-phosphoglycerate kinase
SDS	sodium dodecyl sulphate
Tris	tris(hydroxymethyl)amino methane
UDP	uridyl diphosphate

CONTENTS

CHAPTER 1

THE HIERARCHY OF ENZYME STRUCTURES

"Synopsis of previous chapters: there are no previous chapters."

Stephen Leacock: Gertrude the Governess or Simple Seventeen (1911)

Any treatise on enzyme organization should be based on a thorough consideration of the properties of protein structure. The pertinent knowledge, the content of unwritten previous chapters to this book, calls for a monograph in its own right. Fortunately, there are excellent such monographs at our disposal (e.g. [515, 1072]) where the reader can get a sure foothold on protein chemistry. On the following pages only a bird's-eye-view will be given of this vast field, just to serve as an introduction to our subject matter.

STRUCTURE OF MONOMERIC ENZYMES

Enzymes share the property with other globular proteins that they are linear polymers of amino acids folded in an irregular but highly specific manner to form fairly compact three-dimensional structures. One polypeptide chain usually gives rise to one globular unit. The simplest enzymes, such as lysozyme, trypsin or ribonuclease, consist of a single polypeptide chain or at least are derived from one chain. The latter case is exemplified by chymotrypsin, which is synthesized as single-chain chymotrypsinogen and then converted to α-chymotrypsin through several proteolytic fissions. The α-chymotrypsin molecule finally consists of three unlike polypeptides in one globular entity.

The structural organization within the above monomeric enzymes and other globular proteins has distinct levels. According to the classical categorization, the *primary structure* is the sequence of amino acid residues in the polypeptide chain. The *secondary structure* comprises various ordered arrangements of the polypeptide backbone, such as α-helices, reverse turns and β-pleated sheets. The term *tertiary structure* denotes the overall three-dimensional conformation of the polypeptide chain. More recently, as a result of the accumulation of data obtained by X-ray crystallography, two further levels of organization emerged between the secondary and tertiary ones. *Supersecondary structures* are aggregates of secondary structures apparently preferred in several proteins for reasons of thermodynamic or kinetic stability. Examples of them found in globular proteins are the β×β-unit (two parallel strands of a β-sheet connected by segment x), the Rossmann-fold (a βαβαβ-unit, i.e. two α-helical segments intercalated between three parallel β-strands), and the β-meander (a sheet of three antiparallel β-strands). *Structural domains* are somewhat vaguely defined entities separated by clefts in the overall structure and usually built up of a continuous part of the polypeptide chain. A globular protein consists of one or more structural domains. Different domains as a rule have different functions, while the same type of domain may be found in various enzymes (e.g. the NAD-binding domain of dehydrogenases). Sometimes the same domain occurs more than once even in a monomeric protein, conferring an element of quasi-symmetry on the structure. Such domain linkage might have been produced by gene duplication, whereas the splicing of different domains is assumed to have occurred through gene fusion. It appears that enzymes, and other globular proteins too, have been constructed on a *modular basis:* the various elements needed for biological function were joined to give a single polypeptide chain.

Detailed treatments of the above structural principles can be found in advanced texts on proteins [515, 1072].

ENZYME STRUCTURES BEYOND THE MONOMERIC STAGE

Most enzymes, however, are neither single-chain proteins nor multichain ones in the sense that chymotrypsin is. Rather, they are composed of two or more polypeptides each folded into a separate spheroidal particle (Fig. 1.1). Since the whole enzyme molecule as it is prepared and characterized physicochemically is regarded as *the* (structural) *unit,* the component folded polypeptide chains have been named *subunits.* If the subunits are identical, the protein is an *oligomer,* whose subunits are called *protomers.* An enzyme may be composed of unlike subunits as well, the different subunits (not protomers!) having different functions. If only one type of subunit is involved in catalysis whereas the other(s) fulfil non-catalytic functions (e.g. regulatory), we speak of a *complex enzyme.* On the other hand, if the unlike subunits carry out different, in most cases linked, catalytic reactions, we deal with a *multienzyme complex.* A special case of the juxtaposition of two or more enzymes is when the component enzymes share a common polypeptide chain. This arrangement, termed *multienzyme conjugate* [1307], is akin to the organization of a monomeric enzyme into different structural domains. The distinctive feature is that in multienzyme conjugates there is a separate active site on each "domain" catalysing different chemical reactions; in fact, such conjugates are sometimes resolvable into the component enzymes by mild proteolysis.

The enzyme structures discussed so far have the property in common that they are free to move about without breaking down the organizational level. This is not so with *scaffolded enzyme arrays* where the topology of enzymes is maintained by

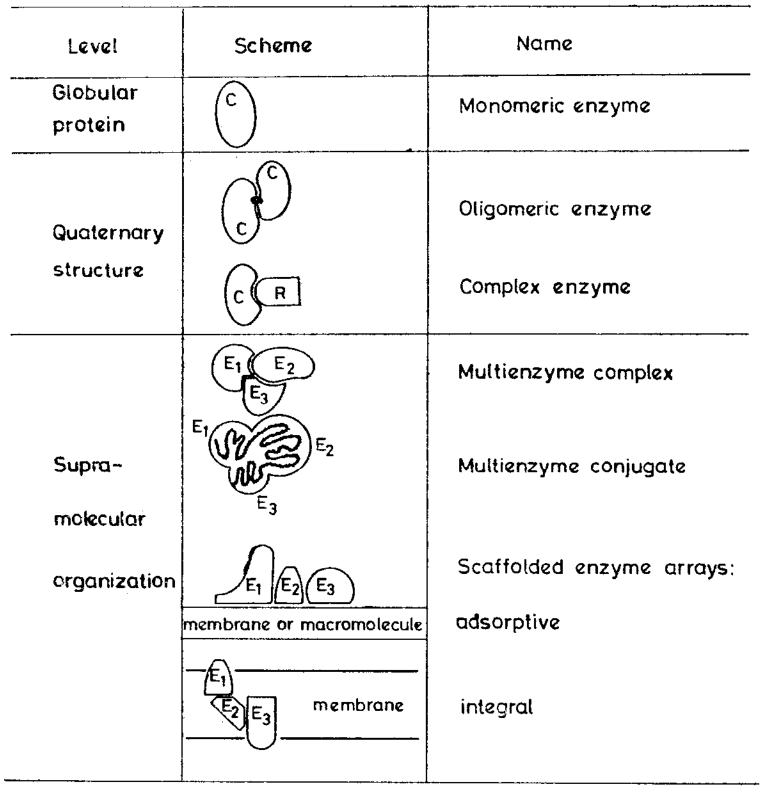

Fig. 1.1. Structural organizational levels of enzymes. Individual contours correspond to globular entities, usually composed of not more than one polypeptide chain. C and R denote catalytic and regulatory subunits, respectively. E_1, E_2 and E_3 are enzymes catalysing three consecutive steps in a pathway. In the multienzyme conjugate the continuity of polypeptide chain over $E_1 \rightarrow E_3$ is indicated.

some kind of support or matrix. One may distinguish *adsorptive arrays* in which enzymes are bound to the surface of a membrane or another macromolecule, and *integral arrays* incorporated into the lipid bilayer of a membrane. Adsorptive arrays are considered to be more dynamic structures than integral ones: the former may readily decompose or exist in a state of equilibrium between free (randomized) and bound (ordered) enzymes, whereas the latter are fairly stable, subject mainly to lateral sliding in the plane of membrane.

THE CONCEPTUAL FRAMEWORK OF PROTEIN–PROTEIN INTERACTIONS

In the previous section we have classified the various forms of quaternary structure and supramolecular organization of enzymes. It must be added that the terminology adopted is not universally accepted, let alone adhered to. There is some inconsistency in the literature as regards nomenclature that is not merely a semantic issue but has both practical and conceptual roots. For example, tryptophan synthetase, a simple and popular archetype of a multienzyme complex, is usually described as having α and β *subunits* and a *quaternary structure* $\alpha_2\beta_2$. In contrast, no one would describe the complex of, say, two glycolytic enzymes as "quaternary structure". The reason for this usage is that tryptophan synthetase is isolated as a complex, whereas glycolytic enzymes are typical individual proteins, most of them well-established oligomers. However, the association of two different catalytic proteins to promote their combined reaction is the same, distinct, level of enzyme organization, whatever the stability of the complex, which may vary greatly even among tryptophan synthetases from different sources.

Quaternary structure and most forms of supramolecular organization have the common feature that they are based on protein–protein interactions, in which definite areas on the protein surface recognize each other when forming specific aggregates. Instructions, "messages", may be transmitted through the *contact surfaces* by molecular movements recognized as conformational changes. If we look from this viewpoint, there is no difference in principle between enzyme–enzyme interactions in a complex, subunit interactions in an oligomer, or even the interaction of structural domains within a monomeric enzyme. All are subject to the stipulations discussed in the next chapter.