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# Organized Multienzyme Systems

## Catalytic Properties

Edited by

G. Rickey Welch

ACADEMIC PRESS

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**G. Rickey Welch**

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# Preface

When we picture cell metabolism we usually think of *multienzyme sequences*, rather than of individual enzymes. Although the study of specific isolated enzyme activities has carried us far in elaborating the kinetic/catalytic basis of the metabolic machinery, to understand the "whole" demands that we integrate the individual enzyme with its metabolically sequential neighbors and with the cellular infrastructure. There is now compelling evidence, both empirical and theoretical, that most (if not all) of intermediary metabolism takes place in organized states. The mode of organization of the component enzymes may entail one or more of the following: (i) protein–protein complexes, (ii) association on (or in) a membrane, and (iii) attachment to fibrous cytoskeletal elements. In some cases the organization is strong, such that the system can be extracted from cells and studied by standard analytical–enzymological methods. For others, however, the interaction is weak (or transient) and readily disrupted by extraction, dilution, etc., in which case special analytical techniques and indirect methods must be used.

Importantly, these organized multienzyme systems have exhibited unique forms of kinetic/catalytic facilitation, unlike the counterpart systems free in bulk solution. Studies with such designs are pointing us to a more realistic appreciation of the nature (and role) of the *microenvironment* in enzyme action. Indeed, microenvironmental factors dictate the very kinetics of enzyme systems, as regards influence on coupled reaction–diffusion flows as well as effects (mediated by protein–protein or protein–matrix interaction) on the intrinsic catalytic properties of the individual enzymes. Continued exploration of structure/function relationships in these organized regimes will be vital to the gradual, dialectical course of constructing a "cellular biochemistry," as well as to applied uses in enzyme technology.

Contributions to our understanding of the kinetic/catalytic properties of organized enzyme systems have come from two sectors. First, we have studies on multienzyme clusters isolated from living cells (or reconstructed with individual components isolated therefrom). These are "Nature's own" designs, a knowledge of which is foremost to biochemists. Second, there is the work on artificially immobilized enzymes. From the biological perspective these systems have served a significant role, as stable "macroscopic" models wherein organizational and

microenvironmental parameters can be defined and manipulated more discerningly. And, of course, the immobilized schemes have had an impact in areas of applied biochemistry.

Our purpose in this volume is to review current work in a number of areas concerning enzyme organization, largely from the biological angle. We include both immobilized and naturally occurring systems, although the heavier emphasis is on the latter. Chapter 1 provides an examination of the nature and function of enzyme organization in perhaps the most well-studied cellular organelle, the mitochondrion. Just as structuralization of metabolism permeates the cytoplasm, so is the case for the *milieu intèrieur* of organelles as well. Chapter 2 is a discourse on structural/functional coupling of the components in energy-transducing membrane systems. Exciting new advances are being made in the elucidation of these bioenergetic designs, giving us a more "local" view of protonmotivated ATP synthesis. Chapter 3 is a discussion of "dynamic compartmentation" in soluble multienzyme systems, with particular emphasis on glycolysis. Such transiently interacting designs may constitute the most prevalent form of organization for cytosolic metabolic processes. Chapter 4 is a detailed review of allosteric enzyme systems. The goal is to define the difference between the behavior of an isolated globular protein and that of a corresponding subunit in a polymeric system. Chapter 5 is a treatment of allosterism in reversibly adsorptive enzyme systems. There is increasing evidence that many metabolic enzymes can partition between particulate structures and the cytosol, depending on physiological conditions. Chapter 6 relates model studies with specific immobilized multienzyme sequences, as regards the analysis of microenvironmental effects. Both biological and applied implications are discussed. Chapter 7 gives a detailed mathematical exposition on the kinetic analysis of multienzyme systems in homogeneous solution. Some new methods are provided for solving the differential rate equations describing consecutive enzyme reactions, so as to allow parameter evaluation (for noninteracting as well as for interacting enzyme systems), optimization of coupled assays, *inter alia*. Chapter 8 includes some theoretical and experimental studies on the behavior of immobilized systems, wherein we see how standard reaction–diffusion equations must be altered for heterogeneous states, and how some interesting biological phenomena in such states can be modeled. As an indication of new directions in which "cellular biochemistry" may be going, Chapter 9 presents a somewhat speculative integrative view of the kind of functional coherence that may be operative in organized states *in vivo*. This view is based on a melding of the following features: (i) the reactivity of the very protein fabric of enzymes, (ii) the nature of the material substratum in which enzymes are organized *in vivo*, and (iii) the ambience of long-range "energy continua" (e.g., electrical fields, mobile protonic states) in cytological substructures.

The accumulation of knowledge on the behavior of enzymes in organized states is bound to yield fruits for basic and applied biochemistry alike. It is hoped that this

volume will help foster continued dialogue and cooperation between these two sides of biochemistry. To this end, I gratefully acknowledge the editors of the "Biotechnology and Applied Biochemistry Series" for their receptiveness to the subject matter herein. In particular, I owe a large debt of gratitude to Dr. Lemuel B. Wingard, Jr., for his interest and encouragement and, also, for his assistance on numerous questions regarding organization of the book, style, etc. Also, I appreciate the assistance and patience of the Academic Press editorial staff in the construction of the text. Of course, my heartiest thanks go to the contributing authors, for their time and effort invested in producing such a fine collection of review chapters. It is, after all, *their* book.

G. RICKEY WELCH

# Contents

# 1

# Organization of Proteins within the Mitochondrion

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## I. BIOLOGICAL ORGANIZATION

In no biological entity, be it a subcellular organelle, a bacterial cell, or a eukaryote, does it appear that structure depends only upon random juxtaposition of its components. Organized arrays of biological components permit effective use of the elements by the reduction in the numbers of molecules needed to achieve the same thermodynamic chemical potentials and separation of elements allows reactions with seemingly opposite objectives to be achieved within the same biospace.

The structural complexity of cells—even of the simplest microorganisms—has been made clearly visible by electron microscopy. In spite of the apparent need for, usefulness of, and physical existence of compartments in



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cells, a major problem has existed in terms of the difficulty of obtaining experimental evidence showing the metabolic functioning of compartments *in vivo*. Further, what about the existence and function of unseen compartments, the microenvironments? Do they exist and, if so, what is their role?

There are many easily recognized forms of biological compartmentation. (1) In the biosphere each life form is a separate compartment and the interaction between them is referrred to as the study of ecology. (2) In each multicellular species the carrying out of special functions by differentiated cells is another example of compartmentation. (3) Within the individual cells compartmentation is achieved with organelles (nuclei, mitochondria, chloroplasts, lysosomes, peroxisomes, vacuoles) separated from each other by enclosing membranes. Even prokaryotic cells, at one time thought to be devoid of organelles, have minimally the separate regions of cytosol and periplasmic space. (4) In the absence of membrane-limited organelle compartmentation, functional separation exists by means of isolatable, stable complexes of enzymes and by skeletal networks of proteins found in various subcellular compartments. (5) Finally, it seems probable that compartmentation exists even in the absence of membranes and stable enzyme complexes. In the latter case the compartment may be the microenvironment in a region of weakly interacting proteins or the microenvironment near a surface due to unstirred water layers or due to a proposed water structure in the cell. Indeed certain catalytic systems have been shown to become an oscillating structure, which is indicative that inhomogeneties can be formed in initially homogeneous solutions.

Below the level of stable multienzyme complexes direct evidence for microenvironmental effects in cells is unavailable. If separate microenvironments exist within the same physical compartment of a cell, all the present experimental attempts to evaluate which metabolites are regulatory in the various pathways are far from the mark.

Many aspects of metabolic regulation can be attributed to the kinetic properties of enzymes and the amounts of the enzymes that are present in cells. The metabolic advantages of microenvironments and compartmentalization are manifold but the data to support their role in regulation or even their existence are at present little more than suggestive. A number of reviews have appeared that discuss various aspects of the subject of compartmentation (Srere and Mosbach, 1974; Srere and Estabrook, 1978; Welch, 1977; Mosbach and Mattiasson, 1978; Ottaway and Mowbray, 1977; Masters, 1977; Nover *et al.*, 1980).

This paper will present and examine the available evidence for the existence of quinary structures (interactions between proteins) (McConkey, 1982) of metabolically related enzymes within the matrix and membranes of mitochondria.

## II. THE MITOCHONDRION

### A. Introduction

A mitochondrion has four separate metabolic compartments: the outer membrane, the intermembrane space, the inner membrane, and the matrix space (Racker, 1970; Tandler and Hoppel, 1972, Lloyd, 1974; Munn, 1974; Fleischer *et al.*, 1978; Tzagoloff, 1982) (Fig. 1).

For the purpose of this review I will adopt the accepted notion that the cristal membrane is identical to the inner boundary membrane, even though there does exist some contrary evidence (Brdiczka *et al.*, 1974). The question that I will examine in this article is whether or not an organization of proteins exists in the inner membrane and in the matrix space of the mitochondrion.

Both the inner membrane and matrix compartments are characterized by their high protein concentration. The inner membrane is reported as being 72% protein and 28% lipid (see Capaldi, 1982, for review), while the matrix space has a varying protein concentration, depending on its physiological state, of 36 to 50% (Hackenbrock, 1968). One of the main metabolic activities of these two contiguous compartments is to oxidize reduced carbon of carbohydrates, fats, and amino acids to $CO_2$ and $H_2O$ and efficiently trap the energy as ATP. This is accomplished by three sets of enzymes: one set (the converting enzymes) converts carbohydrates, lipids, and amino acids to tricarboxylic acid cycle intermediates; a second set is the tricarboxylic acid

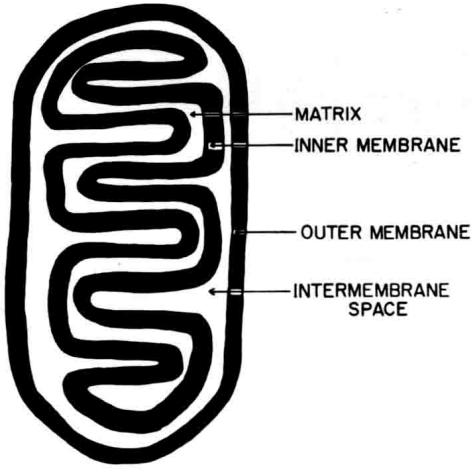


**Fig. 1.** The four mitochondrial compartments.

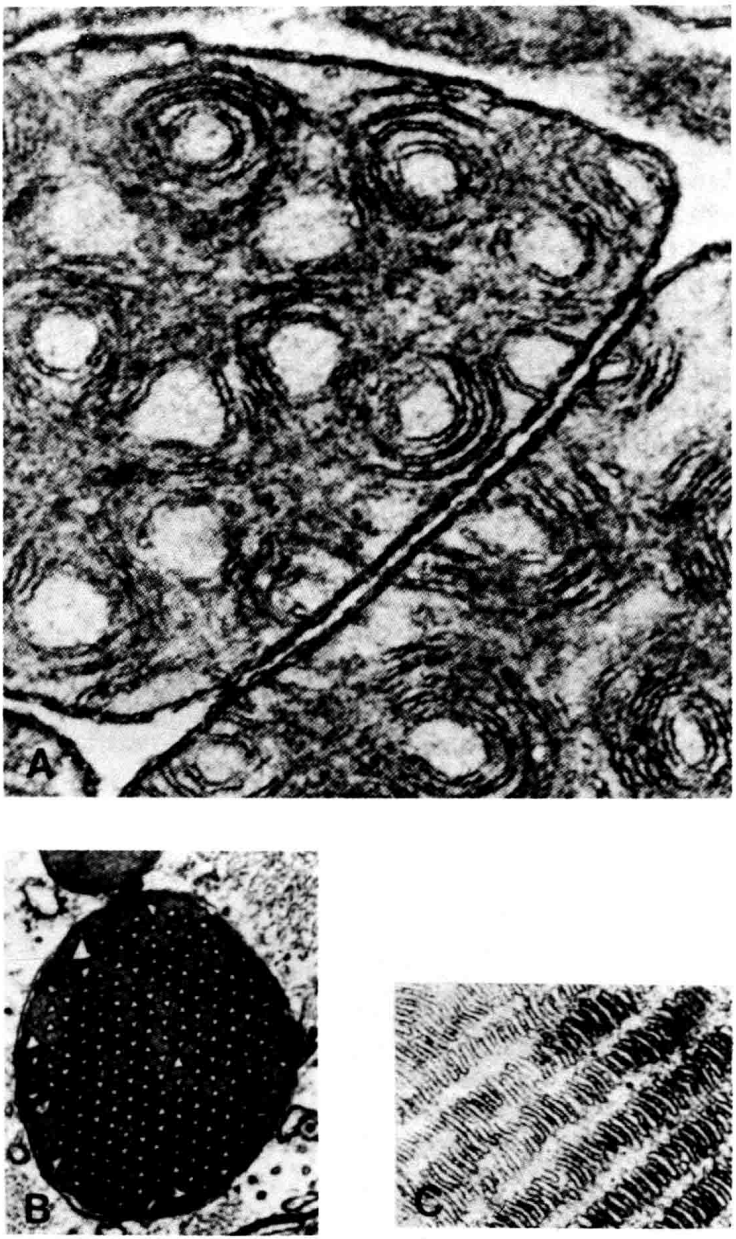


**Fig. 2.** Electron micrographs of some mitochondria showing a variety of cristal forms (A) Part of a mitochondrion from the ellipsoid of the retina of a tree shrew (Samorajski *et al.* 1966) with permission of the Rockefeller Univ. Press. (B) Mitochondrion in an astrocyte of the hamster brain