Methods in Enzymology

Volume LII

Biomembranes

Part C: Biological Oxidations Microsomal, Cytochrome P-450, and Other Hemoprotein Systems

EDITED BY

Sidney Fleischer
Lester Packer

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Preface

A great deal of progress has taken place in biological oxidations and bioenergetics since "Oxidation and Phosphorylation" edited by Ronald W. Estabrook and Maynard E. Pullman (Volume X of "Methods in Enzymology") became available in 1967. To update this field five volumes on biomembranes (Volumes LII–LVI, Parts C–G, respectively) have been prepared, three dealing with biological oxidations and two with bioenergetics.

In this volume, Part C of "Biomembranes," subtitled "Biological Oxidations: Microsomal, Cytochrome P-450, and Other Hemoprotein Systems," we aim to bring together the new methodology that has accompanied the development of essentially a new field that has great relevance to molecular pharmacology, endocrinology, chemical carcinogensis, and environmental toxicology.

We single out for special thanks the contributions of Dr. Ronald W. Estabrook of our Advisory Board for his extensive input in organizing this volume and for his wisdom in solving a number of problems that developed. Drs. M. J. Coon and I. C. Gunsalus have also provided valuable counsel in matters concerning this volume.

We are pleased to acknowledge the good counsel of the members of our Advisory Board for these five volumes. Special thanks are also due Drs. E. Carafoli, G. Palmer, H. Penefsky, and A. Scarpa for their helpful comments on our outlines for these volumes. We were very gratified by the enthusiasm and cooperation of the participants in the field of biological oxidations and bioenergetics whose advice, comments, and contributions have enriched and made possible these volumes. The friendly cooperation of the staff of Academic Press is gratefully acknowledged.

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METHODS IN ENZYMOLOGY

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I. Preparation and Assay of Enzymes
II. Preparation and Assay of Enzymes
III. Preparation and Assay of Substrates
IV. Special Techniques for the Enzymologist
V. Preparation and Assay of Enzymes
VI. Preparation and Assay of Enzymes *(Continued)*
Preparation of Assay of Substrates
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Section I

General Overview

[1] Molecular Oxygen in Biological Oxidations—An Overview

By THOMAS KEEVIL and HOWARD S. MASON

In this overview of molecular oxygen in biological oxidations, we have surveyed the properties of the oxidases in an attempt to generalize on the mechanisms of oxygen use by living organisms. To do this, we assembled a complete catalog of 220 oxidases.[1] The oxidases are classfied according to their prosthetic groups, and within prosthetic group classes according to reaction types and biological occurrence. We are then able to discuss some general questions, such as the following: (1) What prosthetic group structures account for the biological reactions of molecular oxygen? (2) How many different kinds of reaction of molecular oxygen are enzyme catalyzed, and are there any common properties among them? (3) Are there any relationships between active site types and reaction types? and (4) Do any generalizations regarding mechanism emerge from this survey?

Good comprehensive reviews of molecular oxygen enzymology have appeared recently.[2-5] Our goal in this short article is to give a brief overview that will provide some framework for the articles in this volume. We will show that, in the broadest sense, the enzymology of molecular oxygen arises from its strong electrophilic nature. The reaction types in which molecular oxygen participates are a consequence of the amount of electronic charge that oxidases and their substrates present to molecular oxygen. The reaction products are those that in model systems are produced by singlet oxygen, superoxide, hydrogen peroxide, atomic oxygen, and free hydroxyl radical.

[1] "Oxidases," here, is a collective term signifying the enzymes that catalyze reactions of molecular oxygen, rather than any specific type of reaction.

[2] P. D. Boyer, ed., "The Enzymes," 3rd ed., Vol. XII. Academic Press, New York, 1975.

[3] O. Hayaishi, ed., "Molecular Mechanisms of Oxygen Activation." Academic Press, New York, 1974.

[4] I. C. Gunsalus, T. C. Pederson, and S. G. Sligar, *Annu. Rev. Biochem.* **44**, 377 (1975).

[5] K. T. Yasunobu, H. F. Mower, and O. Hayaishi, eds., "Iron and Copper Proteins." Plenum, New York, 1976.