A STUDY OF ENZYMES
VOLUME II

A Study of Enzymes

Volume II Mechanism of Enzyme Action

Editor
Stephen A. Kuby
Professor of Biochemistry
Research Professor of Medicine
Laboratory for Study of Hereditary and Metabolic Disorders
Departments of Biochemistry and Medicine
University of Utah
Salt Lake City, Utah

CRC Press
Boca Raton Ann Arbor Boston

Library of Congress Cataloging-in-Publication Data

Mechanism of enzyme action.

(A Sudy of enzymes; v. 2)
Includes bibliographical references and index.
1. Enzymes. I. Kuby, Stephen Allen, 1925—. II. Series.
QP601.S677 vol. 2 574.19'25s 90-2277
ISBN 0-8493-6988-6

90-2277
CIP

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., Boca Raton, Florida 33431

Library of Congress Number 90-2277
International Standard Book Number 0-8493-6988-6

Printed in the United States 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

Lorsqu'il n'est pas en notre pouvoir de discerner les plus vraies opinions, nous devons suivre les plus probables. (When it is not in our power to determine what is true, we ought to act according to what is most probable.)

René Descartes (1596—1650)

Dedicated to all students of enzymology, both past and present, whose fascination with enzyme mechanisms made this treatise possible.

PREFACE

Although there have been several excellent theses on Enzyme Mechanisms, the time seems unusually ripe for a definitive and selected series of reviews on the subject.

In the past several years, there has been a remarkable resurgence of activity and expansion of knowledge in the area of enzyme mechanisms, both in theory and in data acquisition. Many enzyme proteins, whose primary sequence seemed almost beyond the realm of existing technology have been now had their covalent structures deduced from their complementary DNA's. This then permitted a host of chemical derivatization and modification studies designed to shed light on structure-function relationships. With the recent advent of new techniques, especially in the X-ray deduced macromolecular crystal structures and in the NMR deduced solution structures, and coupled with some specific chemically synthesized and in some cases site-directed mutagenesis experiments (where critical, or noncritical, residues may be substituted in the enzyme molecule, or in peptide fragments of the enzyme molecule), an explosion in enzyme mechanistic studies seems almost imminent.

Structure-function relationships in the future will undoubtedly be assisted by computer modeling, where computer techniques making use of the X-ray crystal and NMR solution structure data, will permit an extension of ideas beyond those of conventional methods.

Together with the application of new or updated spectroscopic approaches, e.g., in nuclear magnetic resonance, in fluorescence, in infra-red Fourier analyses, one will be able to study in greater detail the solution structure of the enzyme-substrate complex(s). A hint of these tremendous advances to be made may be found, e.g., a number of the studies reported here (e.g., on superoxide dismutases, Chapter 21 and on adenylate kinase, Chapter 17).

Molecular dynamics of enzyme proteins are now under vigorous investigation. Interactions of the enzyme molecule with its substrate(s) or modifiers or with specific inhibitors may induce changes in shape and in characteristics. Conformational (or fluctuational) changes on a micro scale lead to ideas concerning the dynamics of such systems (cf. Chapter 1).

Metals in enzyme catalysis have long been the serious study a number of investigators, and ideas concerning complexation in biological systems are only now beginning to be codified (see Chapter 2).

Sound Bioorganic and physical chemical approaches to the study of enzymatic mechanisms are still the preferred method of approach in certain problems of enzyme mechanisms (as illustrated e.g., in Chapters 3 and 4, and in Chapters 19 and 20).

The time is ripe for a detailed review of recent studies on allosteric enzymes (e.g., Chapter 5 on glycogen phosphorylase) and on multienzyme complexes (Chapters 4 and 9).

A remarkable headway has been made in the kinetic studies of certain dehydrogenases, whose X-ray structures are now well known (e.g., dihydrofolate reductase, Chapter 8). Very elegant modification and derivatization experiments, together with the application of a fluorometric approach, is shedding much light on the mechanism of glutamate dehydrogenase (e.g., Chapter 7).

Medical advances are to be anticipated as a result of in vivo and in vitro mechanistic studies (e.g., Chapters 6, 22, 23, and 24).

Since the early days of Michaelis' pioneering experiments, studies of flavo-protein catalysis have been at the forefront of investigations into the mechanisms of redox reaction (Chapters 10, 11, and 12); and ideas concerning semi-quinones and free radicals originated in these studies. Free-radical oxygen (e.g., superoxide anion) has also absorbed the attention of those interested in the dismutation H_2O_2 (e.g., Chapter 21), and the oxygen utilizing reactions, e.g., the monooxygenases and dioxygenases (Chapter 13), will continue to receive the attention they deserve. Now, however, new approaches have permitted deeper insight into the role that the very short-lived intermediates (e.g., with oxygen) play in their mechanisms.

The mechanism of action of the ATP-utilizing enzymes and carriers will continue to occupy much attention especially because of their intrinsic biological importance (Chapters 14, 15, 17, and 18). Their modes of action and those of the phosphotransferases (Chapters 17 and 18) and the nucleotidyl transferases (Chapter 16) are fascinating and absorbing topics for the mechanistic bent of mind.

It, therefore, was the original goal to follow Volume I, which deals with a sound description on enzyme kinetics and ligand binding with Volume II, to deal in detail, with carefully selected topics in enzyme mechanisms, as written by those investigators with a special interest in the particular enzyme mechanism(s) they discuss here.

We hope that we have not been unsuccessful in both of these goals.

THE EDITOR

Stephen A. Kuby, Ph.D., is a Professor of Biochemistry and Research Professor of Medicine at the University of Utah School of Medicine, Salt Lake City, Utah. He is also the head of the Biochemical Division of the Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah Research Park, Salt Lake City, Utah 84108. He graduated *Summa cum Laude* from New York University in 1948 with an A.B. in Chemistry. He obtained his M.S. in Biochemistry in 1951, and his Ph.D. in Biochemistry in 1953 at the University of Wisconsin under Professor H. A. Lardy. As a recipient of the U.S. Public Health Service Fellowship, he spent his post-doctorate period with Professor B. Chance at the University of Pennsylvania (Johnson Foundation for Medical Physics) and with Professor H. Theorell at the Karolinska Institute (Medical Nobel Institute, Stockholm, Sweden). Following a period at the Enzyme Institute (University of Wisconsin) as an Assistant Professor, in 1963 he joined the faculty at the University of Utah, and has held his present positions since 1969. His research interests have dealt with many aspects of enzyme and protein chemistry and certain aspects of medicinal chemistry and inherited disorders (including the muscular dystrophies). Mechanistic enzymology is his current interest, and "state of the art" approaches are being applied to study of enzyme action, e.g., of the kinases.

He is a member of the American Society of Biochemistry and Molecular Biology, the American Chemical Society Division of Biochemistry (Chemistry), Sigma Xi, Phi Beta Kappa, American Association for the Advancement of Science, and the New York Academy of Sciences. He has been a member of the Subcommittee on Enzymes, Committee on Biological Chemistry, Division of Chemistry and Chemical Technology, National Academy of Sciences and National Research Council; and also a member of the Physiological Chemistry Study Section, Division of Research Grants, National Institutes of Health, Publich Health Service, Department of Health, Education and Welfare. His long list of publications reflect these research interests.

CONTRIBUTORS

Gilbert Arthur
Assistant Professor
Department of Biochemistry and Molecular Biology
University of Manitoba
Winnipeg, Manitoba, Canada

Patrick C. Choy
Professor
Department of Biochemistry and Molecular Biology
University of Manitoba
Winnipeg, Manitoba, Canada

Roberta F. Colman
Professor
Department of Chemistry and Biochemistry
University of Delaware
Newark, Delaware

Cindy L. Fisher
Postdoctoral Fellow
Department of Molecular Biology
Research Institute of Scripps Clinic
La Jolla, California

David C. Fry
Department of Biological Chemistry
Johns Hopkins School of Medicine
Baltimore, Maryland
Research Scientist
Department of Physical Chemistry
Hoffmann LaRoche
Nutley, New Jersey

Elizabeth D. Getzoff
Assistant Member
Department of Molecular Biology
Research Institute of Scripps Clinic
La Jolla, California

Robert W. Gracy
Professor and Chairman
Department of Biochemistry
University of North Texas/Texas College of Osteopathic Medicine
Fort Worth, Texas

Clark J. Gubler
Professor Emeritus of Biochemistry
Department of Chemistry
Brigham Young University
Provo, Utah

Robert Hallewell
Director, Protein Engineering
Chiron Research Laboratories
Chiron Corporation
Emeryville, California

Minoru Hamada
Professor and Chairman
Department of Hygiene
Miyazaki Medical College
Miyazaki-gun, Miyazaki, Japan

D. J. Hupe
Senior Research Fellow
Merck Institute for Therapeutic Research
Rahway, New Jersey

Kazutomo Imahori
President
Mitsubishi-Kasei Institute of Life Sciences
Tokyo, Japan

Yuzuru Ishimura
Professor and Chairman
Department of Biochemistry
School of Medicine
Keio University
Tokyo, Japan

Mark S. Johnson
Research Associate
Department of Biochemistry
Loma Linda University
Loma Linda, California

Nobuhiko Katunuma
Professor and Director
Institute for Enzyme Research
School of Medicine
The University of Tokushima
Tokushima, Japan

Seiichi Kawashima
Chief
Department of Biochemistry
Tokyo Metropolitan Institute of Gerontology
Tokyo, Japan

Martin Klingenberg
Professor
Institute for Physical Biochemistry
University of Munich
Munich, West Germany

Stephen A. Kuby
Professor of Biochemistry
Research Professor of Medicine
Laboratory for Study of Hereditary and Metabolic Disorders and the Departments of Biochemistry and Medicine
University of Utah
Salt Lake City, Utah

Rufus Lumry
Professor
Department of Chemistry
University of Minnesota
Minneapolis, Minnesota

Neil B. Madsen
Professor
Department of Biochemistry
University of Alberta
Edmonton, Alberta, Canada

Vincent Massey
Professor
Department of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

Albert S. Mildvan
Professor
Department of Biological Chemistry and Chemistry
Johns Hopkins School of Medicine
Baltimore, Maryland

Mikund J. Modak
Professor
Department of Biochemistry
UMDNJ/New Jersey Medical School
Newark, New Jersey

John F. Morrison
Professor
Department of Biochemistry
John Curtin School of Medical Research
Australian National University
Canberra A. C. T., Australia

Yoichi Nakamura
Instructor
Central Laboratory of Clinical Investigation
Miyazaki Medical College
Miyazaki-gun, Miyazaki, Japan

Hiromichi Okuda
Professor
Department of Medical Biochemistry
School of Medicine
Ehime University
Onsen-gun, Ehime, Japan

Virendra Nath Pandey
Scientific Officer D
Department of Biochemistry
Bhabha Atomic Research Center
Bombay, India

Victoria A. Roberts
Research Associate
Department of Molecular Biology
Research Institute of Scripps Clinic
La Jolla, California

Lawrence M. Schopfer
Assistant Research Scientist
Department of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

Engin H. Serpersu
Assistant Professor
Department of Biochemistry
University of Tennessee
Knoxville, Tennessee

Michihiro Sumida
Associate Professor
Department of Medicine
Ehime University
Onsen-gun, Ehime, Japan

Koichi Suzuki
Chief
Department of Molecular Biology
The Tokyo Metropolitan Institute of Medical Sciences
Tokyo, Japan

John A. Tainer
Assistant Member
Department of Molecular Biology
Research Institute of Scripps Clinic
La Jolla, California

Hitoshi Takenaka
Instructor
Department of Hygiene
Miyazaki Medical College
Miyazaki-gun, Miyazaki, Japan

Kosaku Uyeda
Professor
Department of Biochemistry
University of Texas Health Science Center
Dallas, Texas

R. J. P. Williams
Professor
Inorganic Chemistry Laboratory
University of Oxford
Oxford, United Kingdom

Kunio Yagi
Director
Institute of Applied Biochemistry
Mitake, Gifu, Japan

Shozo Yamamoto
Professor
Department of Biochemistry
School of Medicine
Tokushima University
Tokushima, Japan

K. Ümit Yüksel
Director, Biopolymer Analysis Laboratory
Department of Biochemistry
University of North Texas
Texas College of Osteopathic Medicine
Fort Worth, Texas

TABLE OF CONTENTS

Volume I

TABLE OF CONTENTS

Volume II