

Volume 2

Biomedical Applications of Immobilized Enzymes and Proteins



EDITED BY
THOMAS MING SWI CHANG

Biomedical Applications of Immobilized Enzymes and Proteins

Volume 2

EDITED BY

THOMAS MING SWI CHANG, M.D., PH.D., F.R.C.P.(C)

*Professor of Physiology, Professor of Medicine
Associate, Medical Research Council of Canada
Director, Artificial Organs Research Unit
McGill University
Montreal, Quebec, Canada*

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Biomedical applications of immobilized enzymes and proteins.

Includes bibliographical references and index. 1. Immobilized enzymes. 2. Immobilized proteins. I. Chang, Thomas Ming Swi. [DNLM: 1. Enzymes. 2. Proteins. QU135 B617]

QP601.B484 615'.35
ISBN 0-306-34312-6 (v. 2) 76-56231

© 1977 Plenum Press, New York
A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted,
in any form or by any means, electronic, mechanical, photocopying, microfilming,
recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

Contributors

ANN BARTLETT

Nuffield Institute of Comparative Medicine
The Zoological Society of London
London, England

ILYA V. BEREZIN

Laboratory of Bioorganic Chemistry
Lomonosov State University
Moscow, USSR

D. E. BIDWELL

Nuffield Institute of Comparative Medicine
The Zoological Society of London
London, England

R. C. BOGUSLASKI

Ames Research Laboratory and
Library Resources and Services
Miles Laboratories, Inc.
Elkhart, Indiana

JOHN CAMPBELL

Artificial Organs Research Unit
Department of Physiology, McGill
University
Montreal, Canada

HANS E. CARLSSON

Division of Applied Microbiology
National Defence Research Institute
Sundbyberg, Sweden

THOMAS MING SWI CHANG

Department of Physiology
McGill University
Montreal, Canada

STANLEY E. CHARM

New England Enzyme Center
Tufts University School of Medicine
Boston, Massachusetts

CHARLES L. COONEY

Department of Nutrition and Food
Science
Massachusetts Institute of Technology
Cambridge, Massachusetts

EVA ENGVALL

Department of Immunology
Wenner-Gren Institute
University of Stockholm, Stockholm,
Sweden

SCOTT P. FULTON

Department of Interdisciplinary
Science
Massachusetts Institute of Technology
Cambridge, Massachusetts

VICTOR S. GOLDMACHER

Laboratory of Bioorganic Chemistry
Lomonosov State University
Moscow, USSR

LOUIS H. GOODSON

Midwest Research Institute
Kansas City, Missouri

GEORGE G. GUILBAULT

Department of Chemistry
University of New Orleans
New Orleans, Louisiana

WILLIAM E. HORNBY
 Biochemistry Department
 University of St. Andrews
 St. Andrews, Scotland

M. R. IYENGAR
 Department of Animal Biology
 School of Veterinary Medicine
 University of Pennsylvania
 Philadelphia, Pennsylvania

WILLIAM B. JACOBS
 Midwest Research Institute
 Kansas City, Missouri

ISAO KARUBE
 Research Laboratory of Resources
 Utilization
 Tokyo Institute of Technology
 Tokyo, Japan

P. KELLY
 Department of Animal Biology
 School of Veterinary Medicine
 University of Pennsylvania
 Philadelphia, Pennsylvania

ALEXANDER M. KLIBANOV
 Laboratory of Bioorganic Chemistry
 Lomonosov State University
 Moscow, USSR

ROBERT J. LAFFIN
 Department of Microbiology
 Albany Medical College
 Albany, New York

HOWARD M. LENHOFF
 Departments of Developmental and
 Cell Biology
 University of California
 Irvine, California

ALF A. LINDBERG
 Department of Bacteriology
 National Bacteriological Laboratory
 Stockholm, Sweden

T. MACIAG
 Department of Animal Biology
 School of Veterinary Medicine
 University of Pennsylvania
 Philadelphia, Pennsylvania

F. C. MACINTOSH
 Department of Physiology
 McGill University
 Montreal, Canada

KAREL MARTINEK
 Laboratory of Bioorganic Chemistry
 Lomonosov State University
 Moscow, USSR

BO MATTIASSEN
 Biochemistry II, Chemical Center
 University of Lund
 Lund, Sweden

B. MOCHAN
 Department of Animal Biology
 School of Veterinary Medicine
 University of Pennsylvania
 Philadelphia, Pennsylvania

MARK J. POZNANSKY
 Department of Physiology
 University of Alberta
 Edmonton, Alberta, Canada

E. K. PYE
 Department of Biochemistry and
 Biophysics
 School of Medicine
 University of Pennsylvania
 Philadelphia, Pennsylvania

GENNADY P. SAMOKHIN
 Laboratory of Bioorganic Chemistry
 Lomonosov State University
 Moscow, USSR

IKUO SATOH
 Research Laboratory of Resources
 Utilization
 Tokyo Institute of Technology
 Tokyo, Japan

STANLEY Y. SHIMIZU
Departments of Developmental and
Cell Biology
University of California
Irvine, California

NATHAN L. SMITH III
Department of Developmental and
Cell Biology and Department of
Molecular Biology and Bio-
chemistry
University of California
Irvine, California

R. S. SMITH
Ames Research Laboratory and
Library Resources and Services
Miles Laboratories, Inc.
Elkhart, Indiana

P. V. SUNDARAM
Max-Planck-Institut für
experimentelle Medizin
Abteilung Chemie
Göttingen, West Germany

SHUICHI SUZUKI
Research Laboratory of Resources
Utilization
Tokyo Institute of Technology
Tokyo, Japan

STEVEN R. TANNENBAUM
Department of Nutrition and Food
Science
Massachusetts Institute of Technology
Cambridge, Massachusetts

STUART J. UPDIKE, M.D.
Department of Medicine
University of Wisconsin Medical
School
Madison, Wisconsin

ALISTER VOLLER
Department of Clinical Tropical
Medicine
London School of Hygiene and
Tropical Medicine, and
Nuffield Institute of Comparative
Medicine
The Zoological Society of London
London, England

JAMES C. WEAVER
Research Laboratory of Electronics
and Department of Physics
Massachusetts Institute of Technology
Cambridge, Massachusetts

BING L. WONG
New England Enzyme Center
Tufts University School of Medicine
Boston, Massachusetts

Acknowledgments

This book was made possible by all the contributors, who, despite their busy schedules, spent a great deal of time preparing first-class manuscripts and fulfilling the deadline requirements. Mr. Thomas Lanigan, Senior Editor of Plenum, has been most helpful in the initial planning and throughout the preparation up to the last stages of the page proofs. Mr. Eric P. Otto from Plenum has been most patient and efficient in looking after the printing of the manuscript.

The seven chapters from this Unit include the cumulative result of research over the last twenty years. Throughout this time, the participation, collaboration, and help in other ways by a large number of people is gratefully acknowledged, especially: Dr. P. Barre, Mr. D. Cameron, Dr. J. Campbell, Dr. A. Chawla, Dr. E. Chirito, Dr. S. Chung, Dr. J. Coffey, Dr. C. Cole, Dr. J. Cousineau, Prof. J. Dirks, Mrs. P. Douglas, Dr. H. Duff, Dr. A. Gonda, Dr. M. Habib, Mrs. M. Hewish, Mr. K. Holeczek, Miss L. Johnson, Mrs. N. Kunterian, Mrs. T. Lee-Burns, Mr. B. Lessor, Dr. M. Levy, Mr. C. Lister, Dr. K. S. Lo, Prof. F. C. MacIntosh, Mrs. N. Malave, Prof. S. G. Mason, Dr. M. McGoldrick, Miss M. Migchelsen, Mr. P. Nasielski, Dr. M. Poznansky, Dr. B. Reiter, Mrs. E. Resurreccion, Dr. A. Rosenthal, Dr. J. Seely, Dr. E. Siu Chong, Miss A. Stark, Miss J. Toms, Dr. P. Tung, Miss C. Vaitekunas, Mrs. A. Versaza, Dr. B. Watson, and Miss W. Yensen. The typing of the chapters from this Unit and the secretarial help related to this book by Miss Joanne Toms is gratefully acknowledged. The work in this Unit has been encouraged and enhanced throughout the years by many people, especially, Professor D. Bates, Professor J. Beck, Professor A. V. S. Bergen, Professor A. Burton, Professor R. F. P. Cronin, Professor O. Denstedt, Professor W. Kolff, Professor F. C. MacIntosh, Professor S. G. Mason, Professor M. McGregor, Professor R. J. Rossiter, and others. The support of the Medical Research Council of Canada under the Presidency of Professor G. Malcolm Brown is gratefully acknowledged.

I am grateful to my wife, Lancy, for her cheerful acceptance of the extra time I needed for editing this book, especially when it came so shortly after the publication of the monograph *Artificial Cells*. She has carried out the very laborious work of preparing and typing the entire index section and reading over the manuscripts of the entire book.

Contents of Volume 1

Chapter 1 • *Introduction* • T. M. S. CHANG

I. CLASSIFICATION AND CHEMISTRIES OF IMMOBILIZED ENZYMES

Chapter 2 • *Covalent Linkage: I. Enzymes Immobilized by Covalent Linkage on Insolubilized Supports* • RICHARD D. FALB

Chapter 3 • *Covalent Linkage: II. Intramolecular Linkages* • ROSA UY AND FINN WOLD

Chapter 4 • *Covalent Linkage: III, Immobilization of Enzymes by Intermolecular Cross-Linking* • OSKAR R. ZABORSKY

Chapter 5 • *Immobilization of Enzymes by Adsorption* • OSKAR R. ZABORSKY

Chapter 6 • *Gel-Entrapment of Enzymes* • ANN-CHRISTIN KOCH-SCHMIDT

Chapter 7 • *Encapsulation of Enzymes, Cell Contents, Cells, Vaccines, Antigens, Antibodies, Cofactors, Hormones, and Proteins* • T. M. S. CHANG

II. EXPERIMENTAL APPLICATIONS IN THERAPY

Chapter 8 • *Rationale and Strategies for the Therapeutic Applications of Immobilized Enzymes* • T. M. S. CHANG

Chapter 9 • *L-Asparaginase as a Model for Enzyme Therapy of Substrate-Dependent Tumors* • E. D. SIU CHONG AND T. M. S. CHANG

Chapter 10 • *A Biomedical View of Enzyme Replacement Strategies in Genetic Disease* • CHARLES R. SCRIVER

Chapter 11 • *Experimental Therapy Using Semipermeable Microcapsules Containing Enzymes and Other Biologically Active Material* • T. M. S. CHANG

Chapter 12 • *Stabilized Urease Microencapsulated* • DAVID L. GARDNER AND DONALD C. EMMERLING

- Chapter 13 • *Liquid-Membrane-Encapsulated Enzymes* • SHELDON W. MAY AND NORMAN N. LI
- Chapter 14 • *Liposomes as Carriers of Enzymes and Proteins in Medicine* • GREGORY GREGORIADIS
- Chapter 15 • *Enzyme-Loaded Erythrocytes* • GARRET IHLER AND ROBERT GLEW
- Chapter 16 • *Enzyme Entrapment in Erythrocytes and Liposomes for the Treatment of Lysosomal Storage Diseases* • R. J. DESNICK, M. B. FIDDLER, S. R. THORPE, AND L. D. STEGER
- Chapter 17 • *Strategy for Enzyme Therapy: Immobilization in Hypoallergenic Gel Versus Entrapment in Red Blood Cell* • STUART UPDIKE
- Chapter 18 • *Immobilized Enzymes for Therapeutic Applications and for Large-Scale Production of Biologically Active Compounds* • ICHIRO CHIBATA, TETSUYA TOSA, AND TAKAO MORI
- Chapter 19 • *Artificial Kidney, Artificial Liver, and Detoxifier Based on Artificial Cells, Immobilized Proteins, and Immobilized Enzymes* • T. M. S. CHANG
- Chapter 20 • *Removal of Bilirubin from Blood by Affinity-Competition Chromatography over Albumin-Agarose Gel* • PAUL D. BERK, PAUL H. PLOTZ, AND BRUCE F. SCHARSCHMIDT
- Chapter 21 • *Membrane-Immobilized Liver Microsome Drug Detoxifier* • WILLIAM COHEN, WILLIAM H. BARICOS, PETER R. KASTL, AND ROBERT P. CHAMBERS
- Chapter 22 • *Some in Vivo and in Vitro Studies of Biologically Active Molecules on Organic Matrixes for Potential Therapeutic Applications* • L. S. HERSH AND H. H. WEETALL
- Chapter 23 • *Therapeutic Perspectives of Enzyme Reactors* • FRED R. BERNATH, LAWRENCE S. OLANOFF, AND WOLF R. VIETH
- Chapter 24 • *Possible Roles of Enzymes in Development of a Fuel Cell Power Source for the Cardiac Pacemaker* • SIDNEY K. WOLFSON, LEMUEL B. WINGARD, JR., CHUNG C. LIU, AND SHANG J. YAO
- Chapter 25 • *The Use of Enzymes for Oxygenator Membranes* • GEORGES B. BROUN AND TRAN MINH CANH

Contents

<i>Contents of Volume 1</i>	xix
-----------------------------------	-----

III. DIAGNOSTICS AND PUBLIC HEALTH

Chapter 26

The Use of Immobilized Enzymes in Automated and Semiautomated Systems of Analysis

JOHN CAMPBELL AND WILLIAM E. HORNBY

1. Enzymatic Analysis	3
2. Automation of Analytical Procedures	4
3. Immobilized Enzymes in Analysis	9
4. Conclusion	23
5. References	24

Chapter 27

Immobilized Enzymes and Proteins in Urinalysis

R. C. BOGUSLASKI AND R. S. SMITH

1. Introduction	27
2. Carbohydrates	28
3. Nonprotein Nitrogenous Compounds	30
4. Amino Acids	32
5. Organic Acids	33
6. Proteins	34
7. Hormones	38
8. Toxicology	43
9. Miscellaneous Substances	45
10. Future Prospects	46
11. References	47

Chapter 28

Immobilized Enzymes for Detection and Monitoring of Organophosphates and Carbamates

LOUIS H. GOODSON AND WILLIAM B. JACOBS

1. Introduction	55
2. Automatic Analysis with an Electrochemical Cell	57

3. The Continuous Aqueous Monitors, CAM-1 and CAM-2	58
4. Monitoring Air for Enzyme Inhibitors	63
5. Conclusions	68
6. References	69

Chapter 29

Simplification and Automation of Radioimmunoassay by Gel Entrapment of Antibody

STUART J. UPDIKE

1. Introduction	71
2. Elimination of Centrifugation Step	71
3. Preparation of Gel Antibody	72
4. Elimination of Pipetting Step	74
5. Elimination of High-Molecular-Weight Interferences	77
6. Elimination of Low-Molecular-Weight Interferences	80
7. Gel Antibody RIA and Determination of High-Molecular-Weight Antigens	81
8. RIA Automation	81
9. Reuse of Solid-Phase Antibody	84
10. Summary	85
11. References	86

Chapter 30

Enzyme-Linked Immunosorbent Assay, ELISA

EVA ENGVALL

1. Introduction	87
2. Reagents for ELISA	88
3. Determination of Antigens	90
4. Determination of Antibodies	93
5. Conclusion	94
6. References	94

Chapter 31

Application of ELISA for the Diagnosis of Bacterial Infections

HANS E. CARLSSON AND ALF A. LINDBERG

1. Introduction	97
2. Detection and Quantitation of Antibodies	98
3. Detection and Quantitation of Antigens	108
4. Conclusions	109
5. References	110

Chapter 32*Application of Enzyme-Linked Immunosorbent Assays to Parasitic Diseases***ALISTER VOLLE AND EVA ENGVALL**

1. Introduction	111
2. Helminth Diseases	112
3. Protozoal Diseases	114
4. Future Developments	115
5. References	116

Chapter 33*Microplate Enzyme-Linked Immunosorbent Assays in Virology***ALISTER VOLLE, D. E. BIDWELL, AND ANN BARTLETT**

1. Introduction	119
2. Rubella	119
3. Cytomegalovirus	121
4. Measles	122
5. Arboviruses	123
6. Adenoviruses, Coxsackies, and Herpes	124
7. Hepatitis B	124
8. Viruses of Veterinary Importance	125
9. Plant Viruses	127
10. Conclusions	127
11. References	128

Chapter 34*The Immunoadsorption of Hepatitis B Antigen from Blood Plasma***BING L. WONG AND STANLEY E. CHARM**

1. Introduction	131
2. Methodology	133
3. Immunoabsorption of Hepatitis B Antigen	138
4. Purification of Hepatitis B Antigen	143
5. References	145

Chapter 35*Visual Detection of Hepatitis B Surface Antigen and Antibody***ROBERT J. LAFFIN**

1. Review of the Literature	147
2. Choice of a System	150
3. Principles of the Reactions	150
4. Materials	154
5. Methods	155

6. Results	156
7. Discussion	160
8. Future Prospects	161
9. References	162

Chapter 36

Enzyme Electrodes

GEORGE G. GUILBAULT

1. Introduction	163
2. Enzyme Electrodes	164
3. References	175

Chapter 37

Electrochemical Preparation of Enzyme–Collagen Membrane for Enzyme Electrode

SHUICHI SUZUKI, ISAO KARUBE, IKUO SATOH

1. Introduction	177
2. Electrochemical Preparation of Enzyme–Collagen Membrane	177
3. Properties of ADH–Collagen Membrane	179
4. Application of Enzyme–Collagen Membrane	182
5. References	188

Chapter 38

Possible Biomedical Applications of the Thermal Enzyme Probe

JAMES C. WEAVER, CHARLES L. COONEY, STEVEN R. TANNENBAUM, AND
SCOTT P. FULTON

1. Introduction	191
2. Thermal Enzyme Probe	192
3. Related Configurations and Devices	193
4. Simple Description of TEP	195
5. Future Directions for Biomedical Applications	199
6. References	204

Chapter 39

Possible Biomedical Applications of the Volatile Enzyme Product Method

JAMES C. WEAVER

1. Introduction	207
2. Qualitative Description of VEP and Comparison to Gas Chromatography–Mass Spectrometry	208
3. Theoretical Performance	210
4. Measurements of Substrates Using Volatile Products	213

5. Measurements of Nonvolatile Substrates by Use of Volatile Substrates	215
6. Measurements of Enzymes	215
7. Measurements of Inhibitors or Activators	216
8. Coupled Assays	217
9. Possible Clinical Applications	217
10. Biomedical Research Applications	220
11. Measurements on Cells	221
12. Spatial Mapping of Enzyme Distributions	222
13. References	224

IV. PERSPECTIVES

Chapter 40

Biomedical Aspects of Immobilized Enzymes and Proteins: A Physiologist's View of the Prospects

F. C. MACINTOSH

1. Introduction	229
2. Microcapsules as Injectable Prostheses	229
3. Microvascular Templates for the Miniaturization of Artificial Organs	233
4. Further Possibilities	235
5. References	235

Chapter 41

Mechanosensitive and Sound-Sensitive Enzymatic Systems as Chemical Amplifiers of Weak Signals

ILYA V. BEREZIN, ALEXANDER M. KLIBANOV, GENNADY P. SAMOKHIN, VICTOR S. GOLDMACHER, AND KAREL MARTINEK

1. Introduction	237
2. Regulation by Mechanical Device of the Rates of Enzymatic Processes Occurring in Polymer Gels	239
3. Ultrasound Initiation of Enzymatic Reactions in Thixotropic Gels	240
4. Mechanical Regulation of the Catalytic Activity of Enzymes Covalently Attached to Elastic Supports	241
5. Ultrasound Regulation of the Catalytic Activity of the Enzymes Covalently Attached to Polymer Gels	243
6. Mechanical Regulation of the Interaction Rate of Macromolecular Substances with Enzymes Chemically Bound to Elastic Supports	244
7. Release of Immobilized Enzyme into the Solution on Mechanical Deformation of the Support	246
8. Regulation by Mechanical Action of Reactions of Two Proteins Attached via Long Spacers to Different Surfaces	247

9. Release of Enzymes from Microcapsules and Liposomes under the Action of Ultrasound	248
10. Ultrasound Regulation of Enzymatic Processes Limited by Mass Transfer	249
11. Ultrasound Regulation of Diffusion Rate across the Monolayers	249
12. Ultrasound Initiation of Cooperative Conformational Transitions in Enzymatic Systems	250
13. References	250

Chapter 42

Biochemical Applications and Perspectives of Immobilized Multistep Enzyme Systems

BO MATTIASSEN

1. Introduction	253
2. Theoretical Aspects	253
3. Model Studies on Multistep Enzyme Systems	254
4. Medical and Biochemical Applications of Multistep Enzyme Systems ..	261
5. Perspectives	265
6. References	267

Chapter 43

Sequential and Cyclical Actions of Immobilized Enzymes that Require Pyridine Nucleotides: Their Potential Use in Biochemical Diagnostics and Syntheses

HOWARD M. LENHOFF, STANLEY Y. SHIMIZU, AND NATHAN L. SMITH III

1. Introduction	271
2. Description of Some Sequential Enzyme Systems	272
3. Special Considerations for Using Enzymes Acting in Sequence and Bound to the Same Carrier	276
4. Conclusions and Applications	278
5. References	279

Chapter 44

Immobilized Multienzyme Systems and Coenzyme Requirements: Perspectives in Biomedical Applications

JOHN CAMPBELL AND THOMAS MING SWI CHANG

1. Introduction	281
2. Coenzyme Supply	282
3. Maximization of Coenzyme Use	287
4. Pseudo-Cellular Organization	298
5. Conclusion	299
6. References	299

Chapter 45*Plasminogen Activators for Therapeutic Applications*

T. MACIAG, B. MOCHAN, P. KELLY, E. K. PYE, AND M. R. IYENGAR

1. Introduction	303
2. Rationale for Enzymatic Therapy of Thromboembolic Diseases	304
3. Urokinase, Its Purification and Availability for Clinical Use	304
4. Standardization of Plasminogen Activator Preparations for Therapeutic Evaluations	306
5. Diagnostic Potential of Plasminogen Activators	308
6. Perspectives into Fibrinolysis	309
7. References	313

Chapter 46*Potentials of Enzymes Attached to Nylon Tubes in Analysis*

P. V. SUNDARAM

1. Introduction	317
2. Chemical Structure of the Solid Support	318
3. Activation Methods	319
4. Coupling of Enzymes	325
5. Coupled- and Multienzyme Systems	326
6. Assay Methods	328
7. Coupled-Enzyme Systems	330
8. Enzyme Reactor Tube for Estimation of Urea, Uric Acid, and Citrulline	335
9. Estimation of Amino Acids	338
10. Estimation of Carbohydrates and Sugars	339
11. Estimation of Drugs and Pollutants	339
12. Production of Radioactively Labeled Compounds	339
13. Concluding Remarks	340
14. References	340

Chapter 47*Perspectives of Soluble Cross-Linked Enzyme Polymers for Enzyme Therapy*

MARK J. POZNANSKY

1. Introduction	341
2. <i>In Vitro</i> Experiments	344
3. <i>In Vivo</i> Experiments	349
4. Conclusions	351
5. References	353
<i>Index</i>	355

III. Diagnostics and Public Health