

The Enzymes of Biological Membranes

Volume 3 Membrane Transport

Edited by
Anthony Martonosi
Department of Biochemistry
School of Medicine
Saint Louis University

PLENUM PRESS · NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

The Enzymes of biological membranes.

Includes bibliographies and index.

CONTENTS: v. 1. Physical and chemical techniques. —v. 2. Biosynthesis of cell components. —v. 3. Membrane transport. —v. 4. Electron transport systems and receptors.

1. Membranes (Biology) 2. Enzymes. I. Martonosi, Anthony, 1928-

[DNLM: 1. Biological transport. 2. Cell membrane—Enzymology. QU135 E627]

QH601.E58

574.8'75

75-34410

ISBN 0-306-35083-5 (Vol. 3)

© 1976 Plenum Press, New York

A Division of Plenum Publishing Corporation

227 West 17th Street, New York, N.Y. 10011

United Kingdom edition published by Plenum Press, London

A Division of Plenum Publishing Company, Ltd.

Davis House (4th Floor), 8 Scrubs Lane, Harlesden, London, NW10 6SE, England

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

ADOLPH ABRAMS, Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado.

R. W. ALBERS, Laboratory of Neurochemistry, National Institutes of Health, Bethesda, Maryland.

RANDALL S. ALBERTE, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California

ARNOLD F. BRODIE, Department of Biochemistry, University of Southern California School of Medicine, Los Angeles, California

ERNESTO CARAFOLI, Department of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

MARTIN CROMPTON, Department of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

PATRICIO J. GARRAHAN, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

RICHARD W. HENDLER, Laboratory of Cell Biology, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland

THOMAS R. HINDS, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington

PAUL C. HOLLAND, Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario, Canada

ADAM KEPES, Centre National de la Recherche Scientifique, Institut de Biologie Moléculaire, Laboratoire des Biomembranes, Université Paris VII, Paris, France

MARTIN KLINGENBERG, Institut für Physiologische Chemie und Physikalische Biochemie, Universität München, Munich, Germany

DAVID W. KROGMANN, Department of Biochemistry, Purdue University, West Lafayette, Indiana

WERNER KUNDIG, Department of Biology and The McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland

DAVID H. MACLENNAN, Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario, Canada

ALTON MEISTER, Department of Biochemistry, Cornell University Medical College, New York, New York

RAJENDRA PRASAD, Department of Biochemistry, University of Southern California School of Medicine, Los Angeles, California

ALCIDES F. REGA, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

LEONARD L. ROSS, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pennsylvania

GIORGIO SEMENZA, Laboratorium für Biochemie der ETH Zurich, Zurich, Switzerland

SURESH S. TATE, Department of Biochemistry, Cornell University Medical College, New York, New York

J. PHILIP THORNER, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California

FRANK F. VINCENZI, Department of Pharmaceutical Sciences, School of Pharmacy and Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington

Preface

Much of the information currently available on the transport systems of bacterial and animal cell membranes and their mode of coupling to metabolic supply of energy can be found in this volume. Consideration of the participating enzymes dictated the choice of topics: Several transport systems where little information is available on the enzymology of the process are not included, while separate chapters deal with γ -glutamyl transpeptidase and intestinal disaccharidases which meet many of the requirements of transport enzymes. The volume also includes two chapters on photosynthetic membranes as a general introduction to the topic. Other aspects of biological transport and photosynthesis will be developed in detail in a forthcoming volume now in preparation.

These chapters reveal the excitement and rapid advance of the field, the daily reports of new concepts, new techniques, and new experimental findings which instantly interact to generate further progress. Our aim was to provide a starting point for those who are just beginning, and an opportunity for others to stop, take stock, and start in a new direction.

My warmest thanks to all who contributed to this volume.

St. Louis, Missouri
January, 1976

ANTHONY N. MARTONOSI

Contents

PART A MEMBRANE TRANSPORT IN MICROORGANISMS

1 Bacterial Membrane Transport Proteins 3

ADAM KEPES

- I. Introduction 3
- II. Transport Proteins as Membrane-Bound Proteins 4
- III. Time and Place of Deposition of Membrane-Bound Transport Proteins in the Membrane 8
- IV. Lateral Mobility of Membrane Proteins in *Escherichia coli* 13
- V. Transmembrane Mobility of Transport Proteins 14
- VI. Substrate Binding Sites, Energization, and Conformational Change 18
- VII. The Coupling of Metabolic Energy to Active Transport 22
- VIII. Mechanics and Energization of Desmoprotein-Dependent Transport Systems 25
- IX. Conclusions 25
- References 26

2 The Bacterial Phosphoenolpyruvate Phosphotransferase System 31

WERNER KUNDIG

- I. Introduction 31
- II. The Phosphotransferase System in Enterobacteriaceae 33
 - A. The Formation of Phospho-HPr 34
 - B. Enzyme II Complexes 35
 - C. Genetics of the Phosphotransferase System 45
 - D. The Physiological Functions of the Phosphotransferase System 47
- III. The Phosphotransferase System in *Staphylococcus aureus* 49
- IV. The Distribution of the Phosphotransferase System in Other Organisms 50
- References 52

3 Structure and Function of Membrane-Bound ATPase in Bacteria 57

ADOLPH ABRAMS

- I. Introduction 57
- II. Molecular Properties 58
 - A. Solubilization and Purification 58 • B. Molecular Weight and Sub-
units 60 • C. Amino Acid Composition 62 • D. Nucleotide Binding 63
- III. Kinetic Properties 65
- IV. Reassembly 66
- V. Inhibitory Action of Dicyclohexylcarbodiimide (DCCD) 67
- VI. Physiological Functions 69
 - A. Function in *E. coli* 69 • B. Function in *S. faecalis* 70
- References 71

4 Respiration and Energy Transduction in *Escherichia coli* 75

RICHARD W. HENDLER

- I. Introduction 75
- II. The Electron Transport Chain of *E. coli* 76
- III. Solubilization and Fractionation of the Electron-Transport
Chain 81
- IV. The Multiplicity of Cytochromes and Their Possible Involvement
in Energy Transduction 83
- V. Oxidative Phosphorylation in *E. coli* 86
- VI. On the Direct Use of Energy from Respiration-Linked D-Lactate
Oxidation for Active Transport 93
- VII. On the Role of Mg^{2+} , Ca^{2+} Adenosine Triphosphatase in Energy
Transduction 100
- References 103

5 Membrane-Bound Enzymes from *Mycobacterium phlei*; Malate Vitamin K Reductase 111

RAJENDRA PRASAD AND ARNOLD F. BRODIE

- I. Introduction 111
- II. Membrane Orientation 112
- III. Nature of Respiratory Chain 114
- IV. Malate Vitamin K Reductase 116
 - A. Assay of Malate Vitamin K Reductase Activity 116 • B. Localization of
Malate Vitamin K Reductase 117 • C. Separation of NAD⁺-Linked
Dehydrogenase from Malate Vitamin K Reductase 118 • D. Purification

of Malate Vitamin K Reductase	118	•	E. Absorption Spectrum and Amino Acid Composition	119
	•	F. Stability of Malate Vitamin K Reductase	119	
G. Phospholipid Requirement	119	•	H. Nature of Phospholipid Binding to Malate Vitamin K Reductase	124
	•	I. FAD Requirement of Malate Vitamin K Reductase	125	
J. Quinone Specificity of Malate Vitamin K Reductase	126	•	K. Nonheme Iron: A Component of Malate Vitamin K Reductase	127
	•	L. Transmembrane Electron Transfer	129	
V. Membrane-Bound Latent ATPase Coupling Factor	130			
A. Localization of Latent ATPase	131	•	B. Solubilization and Purification of Latent ATPase Activity	132
	•	C. Properties of Latent ATPase	132	
D. Role of Latent ATPase in Oxidative Phosphorylation and Active Transport	134	•	E. Lipid Requirement for Latent ATPase Activity	135
VI. Nature of Cytochromes from <i>M. phlei</i>	135			
VII. Conclusion	135			
References	136			

PART B PHOTOSYNTHETIC APPARATUS

6 The Organization of Photosynthetic Enzymes on the Chloroplast Membrane 143

DAVID W. KROGMANN

I. Introduction	143
II. Organization of the Catalysts in a Functional Sequence	144
III. Functional and Structural Subunits of the Chloroplast Membrane	148
IV. Individual Catalysts and Their Interactions with the Membrane and Each Other	149
A. NADP:Ferredoxin Oxidoreductase	149
B. Ferredoxin	151
C. X and P ₇₀₀	152
D. Plastocyanin	153
E. Cytochrome <i>f</i>	155
F. Plastoquinone and Cytochrome <i>b</i> ₅₅₉	155
G. Photosystem II and Oxygen Evolution	156
H. Coupling Factor	156
References	158

7 Chlorophyll-Proteins: Membrane-Bound Photoreceptor Complexes in Plants 163

J. PHILIP THORNER AND RANDALL S. ALBERTE

I. Introduction	163
II. Demonstration of Existence of Multiple Chlorophyll-Proteins in Higher Plants	164
III. The P ₇₀₀ Chlorophyll <i>a</i> -Protein	167
A. Isolation	167
B. Characteristics	168
C. Function	170
IV. Light-Harvesting Chlorophyll <i>a/b</i> -Protein	171
A. Isolation	171
B. Characteristics	172
C. Function	173

V. Content of Chlorophyll-Proteins in Photosynthetic Membranes	174
VI. Biosynthesis of the Chlorophyll-Protein Complexes	177
VII. Chloroplast Membrane Polypeptides	178
A. Characteristics	179
B. Function	180
C. Biosynthesis	182
VIII. Summary and Concluding Remarks	183
References	186

PART C SOLUTE TRANSPORT IN MAMMALIAN CELLS

8 Binding Proteins and Membrane Transport 193

ERNESTO CARAFOLI AND MARTIN CROMPTON

I. Introduction	193
II. Nonmammalian Cells	195
A. Periplasmic Proteins	195
B. The Phosphotransferase System	199
C. The Lactose Permease	200
D. The Dicarboxylate-Transporting System	201
III. Mammalian Cells	202
A. The Ca ATPase of Erythrocytes	202
B. The Ca-Binding Protein from Intestinal Mucosa	203
C. The Sucrase-Isomaltase Complex	205
IV. Mitochondria	205
A. The Ca-Binding Proteins	205
B. Anions	207
V. Conclusions	211
References	212

9 The Calcium Transport ATPase of Sarcoplasmic Reticulum 221

DAVID H. MACLENNAN AND PAUL C. HOLLAND

I. Structure of the Sarcotubular System	221
II. Function of the Sarcotubular System	222
III. Isolation of the Sarcoplasmic Reticulum	223
IV. Protein Composition	225
V. Purification of the Membrane-Bound ATPase Enzyme	226
A. Lipid Composition of ATPase	228
B. Proteolipid	230
C. Reconstitution of Calcium Transport	230
VI. Ultrastructure of Isolated Sarcoplasmic Reticulum Vesicles and of ATPase	232
A. Tryptic Fragmentation of ATPase	233
B. Ionophoric Activity in the ATPase	235
C. Control of Function through Phosphorylation	236
D. Membrane-Binding Sites for Calcium	236
VII. Biosynthesis of Sarcoplasmic Reticulum	239

VIII. Reaction Mechanism	240
A. The Phosphorylated Intermediate	241
B. Formation of the Phosphorylated Intermediate (EP)	242
C. Decomposition of EP	245
D. Substrate Specificity	246
E. Inhibitors of Ca^{2+} -Dependent ATPase Activity and Ca^{2+} Transport	246
IX. Model for ATP-Driven Ca^{2+} Transport	247
X. Conformational Probes	249
A. Spin Labels	249
B. Chromophoric Probes	249
C. Hydrogen Exchange	250
D. Circular Dichroism	250
XI. Summary	251
References	251

10 Plasma Membrane Calcium Transport and Membrane-Bound Enzymes 261

FRANK F. VINCENZI AND THOMAS R. HINDS

I. Introduction	261
A. Red Blood Cell Membrane Preparations	262
B. Red Blood Cell Membrane-Bound ATPases	263
II. Plasma Membrane Calcium Transport	265
A. Calcium Transport in Red Blood Cells	265
B. Calcium Transport in Other Systems	269
C. Cellular Significance of Plasma Membrane Calcium Transport	270
D. Active Ca^{2+} Transport and Na^{+} - Ca^{2+} Exchange	272
III. Calcium Transport and Disease	273
IV. Summary	274
References	276

11 The (Sodium plus Potassium)-Transport ATPase 283

R. W. ALBERS

I. Physiological Background	283
II. Characteristics of Ouabain-Sensitive Na^{+} and K^{+} Fluxes	284
III. General Properties of the ATPase	285
IV. Molecular Events	286
V. The Ionophoric Process	288
VI. Reversal of the $(\text{Na}^{+} + \text{K}^{+})$ -ATPase Reaction	290
VII. Arguments Against a Sequential Transport Model	292
VIII. Regulation of Na^{+} and K^{+} Active Transport	293
IX. Hormonal Control	294
X. Regulation at the Cellular Level	295
XI. Enzyme Preparations	295
XII. Properties of Purified $(\text{Na}^{+} + \text{K}^{+})$ ATPases	296
XIII. Conclusion	296
References	297

12 Potassium-Activated Phosphatase 303

ALCIDES F. REGA AND PATRICIO J. GARRAHAN

- I. Introduction 303
 - A. K-Activated Phosphatase and $(\text{Na}^+ + \text{K}^+)$ ATPase 303
- II. Estimation of Phosphatase Activity 305
- III. Substrate Requirements 305
- IV. Effects of Cations 307
 - A. Magnesium 307 · B. Potassium 307 · C. Sodium 308
- V. Effects of Inhibitors 309
- VI. Effects of ATP 310
- References 312

13 Membrane-Bound γ -Glutamyl Transpeptidase 315

ALTON MEISTER, SURESH S. TATE, AND LEONARD L. ROSS

- I. Introduction 315
- II. Background 316
- III. Histochemical Studies 320
- IV. Studies on Purified γ -Glutamyl Transpeptidase 327
 - A. Methods of Purification 327 · B. Some Chemical and Physical Properties of the "Light" and "Heavy" Forms of the Enzyme 329
 - C. Specificity 330 · D. Inhibition 333 · E. Ontogeny 335
- V. Physiological Function of γ -Glutamyl Transpeptidase 337
- References 343

14 Small Intestinal Disaccharidases: Their Properties and Role as Sugar Translocators across Natural and Artificial Membranes 349

GIORGIO SEMENZA

- I. Small Intestinal Oligo- and Disaccharidases 349
 - A. Maltases-Glucoamylases 350 · B. Sucrase-Isomaltase Complex 351
 - C. Trehalase 352 · D. β -Glycosidase Complex 353
- II. Some Molecular Properties of the Sucrase-Isomaltase Complex from Rabbit Small Intestine 354
- III. The Hydrolytic Mechanism of Sucrase and Isomaltase 358
 - A. The Kinetic Mechanism 358 · B. The Configuration of C_1 of Glucose in the Products 359 · C. The Participation of a Carboxylate Group 359
 - D. The Bond Split by Sucrase and Isomaltase 362 · E. The Effect of Para Substituents in the Aglycone Moiety: the Hammett-Hansch Equation 362
 - F. The Secondary Deuterium Effect 363 · G. A Minimal Reaction Mechanism 363

- IV. The Role of Brush-Border-Bound Disaccharidases in Intestinal Sugar Transport in Intact Cells 367
- V. Reconstitution of the Sucrase-Dependent Sugar-Transport System into Artificial Membranes 369
- References 376

15 The ADP-ATP Carrier in Mitochondrial Membranes 383

MARTIN KLINGENBERG

- I. Introduction 383
- II. Fundamentals of Defining Mitochondrial ADP-ATP Transport 384
 - A. Metabolic Localization of ADP-ATP Transport 384 · B. The Mitochondrial Adenine Nucleotide Pool 385 · C. The Carrier Concept 387
- III. Kinetics 388
 - A. Specificity 389 · B. Temperature Dependence 390
- IV. Regulation of Carrier Activity 391
 - A. Concentration Dependence of ADP-ATP Transport 392 · B. Energy Control of Reversed and Forward Rates 393 · C. Electrical Charge Movement and Exchange 395
- V. Inhibitors of ADP-ATP Transport 397
- VI. Definition of the Carrier Sites 399
 - A. Binding of ADP and Interaction with ATR 399 · B. Binding of ADP and Interaction with Bongkrekate 402 · C. The Reorientation Mechanism 405 · D. Endogenous ADP, ATP under the Influence of BKA 406
- VII. Conformational Changes of the Membrane on Binding of ADP 407
- VIII. The Binding of [35 S]ATR and [35 S]CAT and the Interaction with Other Ligands 412
- IX. The Sensitivity of ADP-ATP Carrier to Maleimide 419
- X. The ADP-ATP Carrier in Submitochondrial (Sonic) Particles 421
- XI. Carrier Mechanisms 425
 - A. Translocation Step 426 · B. Activation Step 428
- XII. Isolation of the Carrier Protein 430
 - A. [35 S]CAT as a Marker for Carrier Isolation 430 · B. NEM as a Marker of the Carrier Protein 434 · C. Conclusions 435
- References 435

Author Index 439

Subject Index 453

PART A

MEMBRANE TRANSPORT IN MICROORGANISMS

I

Bacterial Membrane Transport Proteins

ADAM KEPES

I. Introduction

Bacterial transport systems are historically associated with the acceptance of the idea that the crossing of the cell membrane by a physiologically significant solute was mediated by the specialized operation of a protein or an array of molecules including specific proteins. This idea was in opposition to the predominant-view of permeability, a membrane property, as the principal factor governing the passage of solutes.

The concept of the specialized transport protein gained general recognition primarily because of the possibility of varying the amount of transport protein separately from that of surface area of the membrane through specific genetic change and through inductive or repressive regulation of its biosynthesis (Cohen and Monod, 1957). The word "permease" (Rickenberg *et al.*, 1956) stirred up considerable discussion which served to emphasize the transport proteins. It suggested in a condensed form the involvement of a protein with enzyme-like specificity and catalytic (i.e., cyclic) activity. Unfortunately, it failed to stress distinction between permeability and active transport which later was recognized to be a nearly universal feature of all transport systems to which the term has ever been applied (Kepes, 1964). The enzyme-like denomination "-ase" can be justified on the ground that a typically enzymatic event, the breakdown of chemical (or electrochemical) energy, is partly or totally dependent on the transport protein. The phosphoenolpyruvate sugar phosphotransferase system (Kundig *et al.*, 1964) (see also Kundig, this volume) is an illustration of this statement, although since the discovery of its chemical

ADAM KEPES • Centre National de la Recherche Scientifique, Université Paris VII, Institut de Biologie Moléculaire, Laboratoire des Biomembranes, Tour 43-2, place Jussieu, 75221 Paris cedex 05, France.

mechanism it has seldom been included among permeases. Presumably, once similarly well-defined biochemical mechanisms have been established for all transport systems, the term permease will have completely lost its usefulness.

In this chapter both old and recent evidence concerning the membrane location of transport proteins will be reviewed. This view is supported by their limited freedom of movement along the membrane surface. Some facts will be reported which permit speculation about the insertion of membrane protein into membrane during biosynthesis; other facts and speculations will be relevant to the possibility of transmembrane movements.

The likelihood of multiple configurational changes during the transport cycle will be documented by several lines of indirect evidence. The energy coupling to active transport will be discussed briefly on the basis of experiments with inhibitors (Kepes, 1974) and isolated membrane vesicles (Kaback, 1971, 1972). The chemi-osmotic theory and its methodology (Harold, 1972; West, 1970; West and Mitchell, 1972, 1973; Rosen, 1973) and the genetic analysis of the energy pathways in bacterial transport will be briefly reviewed (Haddock and Schairer, 1973; Devor *et al.*, 1974; Simoni and Shallenberger, 1972). For a large class of transport systems the results can be tentatively summarized as the utilization of an energized state of the membrane to fuel active transport. Finally, recent evidence for transport systems utilizing chemical-bond energy independent from the *energized state of the membrane* will be briefly described (Berger, 1973).

II. Transport Proteins as Membrane-Bound Proteins

The plasma membrane is the main diffusion barrier to penetration of hydrophilic solutes from the medium and to the escape of many hydrophilic metabolites from the cell. This basic impermeability is the cause of the inaccessibility of intracellular metabolic enzymes to their substrates dissolved in the medium (Deere *et al.*, 1939). This inaccessibility (termed crypticity) helped establish the generalized requirement for transport systems to carry out the uptake of all exogenously furnished nutrients (Cohen and Monod, 1957).

The logical counterpart of the virtual impermeability of the plasma membrane is the necessary location within its hydrophobic fabric of essential parts of the transport machinery. Some of these must either encompass the thickness of the barrier permanently or be able to shuttle occasionally or periodically from one face to another. Such logic does not unambiguously designate a membrane protein. The best examples of nonprotein carriers are the polyisoprenoid molecules which help the hydrophilic building blocks of murein or lipopolysaccharide to cross the hydrophobic membrane. The logic is somewhat tightened when a transport process is performed in isolated membranes in the absence of cytoplasmic factors, since only structures permanently linked to the membrane can participate in the translocation process from the recognition of the solute to its release on the other side. Such a sequence implies a stereospecific site for the transport substrate and strongly supports the presence of the key protein.