Transaminases

Edited by
Philipp Christen

David E. Metzler

Transaminases

Edited by

Philipp Christen Universität Zürich

David E. Metzler Iowa State University

A WILEY-INTERSCIENCE PUBLICATION

John Wiley & Sons

New York • Chichester • Brisbane • Toronto • Singapore

Copyright © 1985 by John Wiley & Sons, Inc.

All rights reserved. Published simultaneously in Canada.

Reproduction or translation of any part of this work beyond that permitted by Section 107 or 108 of the 1976 United States Copyright Act without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to the Permissions Department, John Wiley & Sons, Inc.

Library of Congress Cataloging in Publication Data:

(Main entry under title)

Transaminases.

(Biochemistry; v. 2)

"A Wiley-Interscience publication."

Includes bibliographical references and index.

1. Aminotransferases. I. Christen, Philipp,

1937-. II. Metzler, David E. III. Series: Biochemistry (John Wiley & Sons); v. 2.

84-3712

QP606.A43T73 1984 574.19'25 ISBN 0-471-08501-4

Printed in the United States of America

Transaminases

Biochemistry

A Series of Monographs

Editor: ALTON MEISTER. Cornell University Medical College, New York

Volume 1 Woon Ki Paik and Sangduk Kim PROTEIN METHYLATION

Volume 2 Philipp Christen and David E. Metzler TRANSAMINASES

Contributors

- ARTHUR ARNONE, Department of Biochemistry, University of Iowa, Iowa City, IA 52242
- SVIETLANA N. BORISOVA, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR
- VSEVOLOD V. BORISOV, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR
- FRANCESCO BOSSA, Centro di Biologia Molecolare, Istituto di Chimica Biologica, Università di Roma, I-00185 Rome, Italy
- ALEXANDER E. BRAUNSTEIN, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- PATRICK D. BRILEY, Department of Biochemistry, University of Iowa, Iowa City, IA 52242
- PHILIPP CHRISTEN, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- JORGE E. CHURCHICH, Department of Biochemistry, University of Tennessee, Knoxville, TN 37916
- ARTHUR J. L. COOPER, Departments of Neurology and Biochemistry, Cornell University Medical College, New York, NY 10021
- DEVENDRA R. DESHMUKH, Department of Biological Chemistry, University of Michigan, Medical School, Ann Arbor, MI 48109
- SHAWN DOONAN, Department of Biochemistry, University College, Cork, Ireland
- Gregor Eichele, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland
- NOBUYOSHI ESAKI, Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, UJI, Kyoto-FU 611, Japan
- LEONARD A. FAHIEN, Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706

vi Contributors

Paolo Fasella, Istituto di Chimica Biologica, Università di Roma, I-00185 Rome, Italy

- MARGARET L. FONDA, Department of Biochemistry, University of Louisville, Health Sciences Center, Louisville, KY 40232
- GEOFFREY C. FORD, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland
- LESLEY J. FOWLER, Department of Pharmacology, School of Pharmacy, Brunswick Square, London WC1N 1AX, England
- HEINZ GEHRING, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- URSULA GRAF-HAUSNER, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- DARYL K. GRANNER, Department of Physiology, Vanderbilt University, School of Medicine, Nashville, TN 37232
- JAMES L. HARGROVE, Department of Anatomy, Emory University School of Medicine, Atlanta, GA 30322
- EMIL G. HARUTYUNYAN, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR
- ELIZABETH HUBERT, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298
- C. CRAIG HYDE, Department of Biochemistry, University of Iowa, Iowa City, IA 52242
- AKIRA ICHIHARA, Institute for Enzyme Research, School of Medicine, University of Tokushima, Tokushima 770, Japan
- ROBERT J. IRELAND, Department of Biology, Allison University, Sackville, New Brunswick EOA 3CO, Canada
- ANA IRIARTE, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298
- JOHAN N. JANSONIUS, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland
 - ROLF JAUSSI, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- W. TERRY JENKINS, Chemistry Department, Indiana University, Bloomington, IN 47401
- ROBERT A. JOHN, Department of Biochemistry, University College, P.O. Box 78, Cardiff CFI 1XL, Wales, U.K.
- KENNETH W. JOY, Department of Biology, Carleton University, Ottawa, K1S 5B6, Canada
- GALINA S. KACHALOVA, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR

Contributors

ROLAND G. KALLEN, Department of Biochemistry and Biophysics, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104

- HEINZ KIRSTEN, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- VALENTINA M. KOCHKINA, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- TIMO KORPELA, Department of Biochemistry, University of Turku, SF-20500
 Turku, Finland
- TING-KAI LI, Department of Biochemistry, Indiana University School of Medicine, VA Hospital, Indianapolis, IN 46202
- LAWRENCE LUMENG, Department of Biochemistry, Indiana University School of Medicine, VA Hospital, Indianapolis, IN 46202
- VLADIMIR N. MALASHKEVICH, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- JAMES M. MANNING, Department of Biochemistry, Rockefeller University, 1230 York Avenue, New York, NY 10021
- ARTHUR E. MARTELL, Department of Chemistry, Texas A & M University, College Station, TX 77843
- MARINO MARTINEZ-CARRION, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298
- MERLE MASON, Department of Biological Chemistry, University of Michigan, Medical School, Ann Arbor, MI 48109
- YOSHIKAZU MATSUSHIMA, Kyoritsu College of Pharmacy, Tokyo 105, Japan
- JOSEPH R. MATTINGLY, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298
- ALTON MEISTER, Biochemistry Department, Cornell University Medical College, 1300 York Avenue, New York, NY 10021
- CAROL M. METZLER, Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011
- DAVID E. METZLER, Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011
- EDITH WILSON MILES, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, MD 20205
- YOSHIMASA MORINO, Department of Biochemistry, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan
- YURII V. MOROZOV, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- Daniel Picot, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland

viii Contributors

ISOBEL M. RALSTON, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

- PAUL H. ROGERS, Department of Biochemistry, University of Iowa, Iowa City, IA 52242
- COSTANTINO SALERNO, Istituto di Chimica Biologica, Università di Roma, I-00185 Rome, Italy
- FELIX A. SAVIN, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- ELLEN SCHMIDT, Zentrum für Innere Medizin, Medizinische Hochschule Hannover, D-3000 Hannover 61, Federal Republic of Germany
- FRIEDRICH WERNER SCHMIDT, Zentrum für Innere Medizin, Medizinische Hochschule Hannover, D-3000 Hannover 61, Federal Republic of Germany
- IVAN K. SMITH, Department of Botany, Ohio University, Athens, OH 45701
- ESMOND E. SNELL, Departments of Microbiology and Chemistry, University of Texas, Austin, TX 78712
- KENJI SODA, Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, UJI, Kyoto-FU 611, Japan
- Peter Sonderegger, Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
- THOMAS S. SOPER, Rockefeller University, 1230 York Avenue, New York, NY 10021
- NIKITA I. SOSFENOV, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR
- SUMIO TANASE, Department of Biochemistry, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan
- CHRISTINA THALLER, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland
- YURII M. TORCHINSKY, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow 117984, USSR
- HIROSHI UENO, Department of Biochemistry, Rockefeller University, 1230 York Avenue, New York, NY 10021
- BORIS K. VAINSHTEIN, Institute of Crystallography, Academy of Sciences of the USSR, Leninsky Prospect 59, Moscow 117333, USSR
- MICHAEL G. VINCENT, Abteilung Strukturbiologie, Biozentrum der Universität Basel, CH-4056 Basel, Switzerland
- SANTO W. ZITO, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

Preface

This book is the first comprehensive treatise on transaminases. It is written almost half a century after the discovery of transamination as a metabolic reaction and the detection of vitamin B₆, the precursor of the cofactor pyridoxal-5'-phosphate. The broad scope and the depth of insight achieved through investigation of the structural, mechanistic, and functional aspects of transaminases are reflected in this review of present knowledge. The text covers not only the chemical properties and the biological and medical significance of the various transaminases but also the chemical, spectroscopic, and metabolic aspects of the coenzymes pyridoxal phosphate and pyridoxamine phosphate. Transaminases have been the most extensively studied pyridoxal phosphate-dependent enzymes, and the aspartate aminotransferases are the only pyridoxal phosphate-dependent enzymes acting on amino acids whose spatial structures are known in atomic detail. For these reasons we hope that the book will be a source of reference not only in the field of transaminases but of vitamin B₆-dependent enzymes in general. In the coming decades, structural and mechanistic comparisons of the various groups of pyridoxal phosphate-dependent enzymes will doubtless continue to be a fascinating endeavor. Such comparisons may allow the tracing of the evolution not only of the protein structures but also of the mechanisms of catalysis used by these enzymes with their diverse reaction and substrate specificities.

The title of the book is *Transaminases*, the older well-established name for enzymes catalyzing transamination reactions. Within the text, however, we have more often used the name "aminotransferase," which is preferred by the Nomenclature Committee of the International Union of Biochemistry.

We would like to thank Alton Meister, Alexander E. Braunstein, Esmond E. Snell, and W. Terry Jenkins for their advice offered at various stages in the

X Preface

development of this book. We are also grateful to all the authors for their contributions and their cooperation.

PHILIPP CHRISTEN
DAVID E. METZLER

Zurich, Switzerland Ames, Iowa September 1984

Contents

Abbreviations, xxiii

Chapter 1. Introduction and Historical Survey

1

- A. Transamination and Transaminases, 2
 Alexander E. Braunstein
 - 1. Discovery and Preliminary Characterization of Transamination Reactions and Transaminases, 2
 - 2. Biological Significance of Transamination Reactions, 8
 - Studies of the Structure, Properties, and Catalytic Mechanism of Aspartate Aminotransferases, 13
- Pyridoxal Phosphate in Nonenzymic and Enzymic Reactions, 19
 Esmond E. Snell
 - From Vitamin B₆ to Pyridoxal Phosphate-Dependent Enzymes, 19
 - Early Consideration of Mechanism: Nonenzymic and Enzymic Reactions, 22
 - 3. From Model to Enzymic Reactions, 28

References, Chapter 1, 30

Chapter 2. Chemical and Spectroscopic Properties of Pyridoxal and Pyridoxamine Phosphates

37

Roland G. Kallen, Timo Korpela, Arthur E. Martell, Y. Matsushima, Carol M. Metzler, David E. Metzler,

xii Contents

Yurii V. Morozov, Isobel M. Ralston, Felix A. Savin, Yurii M. Torchinsky, and Hiroshi Ueno

A. The Free Coenzymes, 38

- 1. Chemical Properties, 38
- 2. Acid-Base Chemistry, 41
- 3. Spectra, 45
- 4. Electronic Structures and Photochemistry, 53

B. Schiff Bases, 56

- 1. Formation Constants, 57
- Acid-Base Chemistry and Tautomerism, 64
- 3: Spectra of Schiff Bases, 69
- 4. 4 Metal Complexes, 76
- 5. X-Ray Crystallographic Studies, 81
- 6. Addition and Other Reactions of the C=N Bond, 81
- Transimination and Reactions of Bifunctional Amines, 82
- 8. Electronic Structures and Photochemistry, 87
- 9. PLP as a Protein-Labeling Reagent, 88

C. Quinonoid or Carbanionic Intermediates, 88

- 1. Model Reactions and Absorption Spectra, 89
- Formation and Reactions of Quinonoid Intermediates, 93

D. Spectroscopic Properties of Transaminases, 95

- 1. Electronic Absorption Spectra, 95
- 2. Circular Dichroism, 99
- 3. Resonance Raman Spectra, 102

References, Chapter 2, 103

Chapter 3. Spatial and Covalent Structures of Aminotransferases

109

A. Spatial Structure of Mitochondrial Aspartate
 Aminotransferase, 110
 Johan N. Jansonius, Gregor Eichele, Geoffrey C. Ford,
 Daniel Picot, Christina Thaller, and Michael G. Vincent

- 1. Experimental Procedures, 111
- Three-Dimensional Structure of the Pyridoxal Phosphate Form of Mitochondrial Aspartate Aminotransferase, 114
- 3. The Active Site of Mitochondrial Aspartate Aminotransferase, 129
- 4. Other Forms of the Enzyme, 132
- 5. Conclusion, 137
- B. Crystallographic Studies of Pig and Chicken Cytosolic Aspartate Aminotransferases, 138
 - Pig Cytosolic Aspartate Aminotransferase: The Structures of the Internal Aldimine, External Aldimine, and Ketimine and of the β Subform, 138
 Arthur Arnone, Paul H. Rogers, C. Craig Hyde, Patrick D. Briley, Carol M. Metzler, and David E. Metzler
 - X-Ray Studies of Chicken Cytosolic Aspartate
 Aminotransferase, 155
 Vsevolod V. Borisov, Svietlana N. Borisova, Galina S.
 Kachalova, Nikita I. Sosfenov, and Boris K. Vainshtein
 - 3. Three-Dimensional Structure of the Complex of Chicken Cytosolic Aspartate Aminotransferase with 2-Oxoglutarate, 164

 Emil G. Harutyunyan, Vladimir N. Malashkevich, Valentina M. Kochkina, and Yurii M. Torchinsky
- C. Comparison of Covalent Structures of the Isoenzymes of Aspartate Aminotransferase, 173 Philipp Christen, Ursula Graf-Hausner, Francesco Bossa, and Shawn Doonan
 - 1. Composition of Aspartate Aminotransferase, 173
 - Comparison of Amino Acid Sequences of Aspartate Aminotransferases: Evolutionary Aspects, 178
 - Amino Acid Residues Invariant in Both the Cytosolic and Mitochondrial Isoenzyme. Comparison with Other PLP-Dependent Enzymes, 179
 - Amino Acid Residues Invariant Specifically in the Mitochondrial or Specifically in the Cytosolic Isoenzyme, 181
- D. Quaternary Structure of Aminotransferases, 185 Jorge E. Churchich
 - 1. Aspartate Aminotransferase, Quaternary Structure, 185

xiv Contents

	. Flexibility of Aspartate Aminotransferase, 188
	. Motility of the Cofactor, 190
	. Ornithine Aminotransferase, Quaternary Structure, 191
E.	nteraction of Aminotransferases with Other Metabolically
	inked Enzymes, 192
	Costantino Salerno, Paolo Fasella, and Leonard A. Fahien

- 1. Aspartate Aminotransferase-Cystathionase Complex, 192
- 2. Aspartate Aminotransferase and Malate Dehydrogenase, 196
- Aspartate Aminotransferase-Glutamate Dehydrogenase Complex, 200
- 4. Conclusions, 206

References, Chapter 3, 208

Chapter 4. Interactions of Aminotransferases with Coenzymes, Substrates, and Inhibitors

- A. Kinetics, Equilibria, and Affinity for Coenzymes and Substrates, 216
 W. Terry Jenkins and Margaret L. Fonda
 - 1. Equilibria, 216
 - 2. Kinetics, 226
 - 3. Conclusion, 234
- B. Studies with Coenzyme Analogues, 235

 David E. Metzler and Margaret L. Fonda
 - 1. Synthetic Methods, 235
 - 2. Apoenzymes and the Binding of Analogues, 236
 - 3. Modifications at Position 1, 237
 - 4. Modifications at Positions 2 and 6, 238
 - 5. Modifications a Position 3, 241
 - Modifications at Position 4, 244
 - 7. Modifications at Position 5, 245
 - 8. Reactive Coenzyme Analogues, 249
 - 9. Labeling of Proteins, 250
- C. Quasisubstrates and Irreversible Inhibitors of Aspartate Aminotransferase, 251 Yoshimasa Morino and Sumio Tanase

- 1. Ouasisubstrates, 251
- 2. Site-Specific Inhibitors, 254
- Enzyme-Activated Inhibitors of Pyridoxal-Phosphate Enzymes, 266

Thomas S. Soper and James M. Manning

- Inhibitors Activated by Rearrangement during Processing, 268
- Inhibitors Activated by Elimination during Processing, 273
- 3. Inhibitors that React with the Coenzyme, 279
- 4. Conclusions, 281
- E. Kinetic Properties of Microcrystalline Aspartate Aminotransferase, 285 Philipp Christen and Heinz Kirsten
 - Experimental Procedure for Determining the Activity of Crystalline Aspartate Aminotransferase, 287
 - Criteria for Exclusion of Rate Limitation by Diffusion, 287
 - Catalytic Parameters and Functional Nonequivalence of Subunits of the Crystalline Aspartate Aminotransferase Dimer, 288

References, Chapter 4, 291

Chapter 5. Mechanism of Aminotransferase Action

- A. Stereochemistry of Transamination, 308

 Marino Martinez-Carrion, Elizabeth Hubert, Ana Iriarte,
 Joseph R. Mattingly, and Santo W. Zito
 - Historical Perspective, 308
 - 2. The Mechanism of Transamination, 309
 - 3. Coenzyme Chirality and Stereospecificity, 311
 - 4. The Coenzyme Substrate Complex, 312
 - 5. Dicarboxylic Acid and Inhibitor Binding, 313
 - 6. Transamination of D- and L-Amino Acids, 314
 - Transamination as "Job Fatigue" Expression of Pyridoxal-Dependent Enzymes, 314
 - Pyridoxylamino Acids—Stereochemical Considerations, 315

- Summary, 316
- B. Chemical Modification Studies, 317
 Heinz Gehring
 - 1. Chemical Modification of Active-Site Residues, 317
 - Detection of Conformational Changes in Aspartate Aminotransferase by Chemical Modification of Nonactive-Site Residues, 321
 - 3. Proteolytic Modification, 323
- C. Hypothetical Mechanism of Action of Aspartate Aminotransferases, 326 Arthur Arnone, Philipp Christen, Johan N. Jansonius, and David E. Metzler
 - 1. Experimental Studies Relevant to the Mechanism, 326
 - 2. The Proposals of Ivanov and Karpeisky, 328
 - 3. Current Proposals, 331
 - 4. Further Discussion of Various Mechanistic Questions, 342
 - Spectroscopy of Aspartate Aminotransferase Crystals Using Polarized Light, 349

References, Chapter 5, 357

Chapter 6. Special Aspects of Various Transaminases

- A. Introductory Remarks, 365 W. Terry Jenkins
 - 1. Problems of Nomenclature, 365
 - 2. Range of Specificity, 371
 - 3. Physiological Implications, 373
 - 4. Compendium on Aspartate Aminotransferases, 373

 Heinz Gehring
- B. Plant Transaminases, 376
 Robert J. Ireland and Kenneth W. Joy
 - 1. Functions, 376
 - 2. Properties, 379
 - 3. Subcellular Localization, 383
 - 4. Isoenzymes, 384