Advances in Enzymology

Edited by

DANIEL L. PURICH

ADVANCES IN ENZYMOLOGY

AND RELATED AREAS OF MOLECULAR BIOLOGY

F. F. Nord, Founding Editor

AMINO ACID METABOLISM, Part A

Volume 72

Edited by DANIEL L. PURICH
University of Florida College of Medicine
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PUBLISHER'S FOREWORD

Many of the most notable achievements in the molecular life sciences were initiated by enzymologists. The assertion of Hopkins that cell life is "an ordered sequence of events governed by specific catalysts" has become a truism in the analytical investigation of all life processes. The field of enzymology has grown by leaps and bounds over the past half-century, and with explosive growth of molecular and cell biology, new enzymes and enzymatic activities are still routinely found. Terms such as "ribozyme," "abzyme" (or catalytic antibodies), "molecular motors" (e.g., dynein and kinesin), and "polyprotein proteinases" have entered the biochemical literature in just the past decade or so.

Professor F. F. Nord began *Advances in Enzymology* in 1941 after he had been appointed Professor of Biochemistry at Fordham University. *Advances* is the successor to *Ergebnisse der Enzymforschung*, a periodic review series started in 1930, while he was still a chemistry professor in Berlin. His idea was to identify areas of enzymology that had undergone significant recent growth. He then sought out expert opinions of others to find an appropriate author to communicate the nature of those strides and to illustrate how these findings could be of broader interest.

This tradition of excellence was maintained by Professor Alton Meister who, with the pubication of Volume 35, succeeded Dr. Nord as the series editor. A man of enormous intellect and awareness of the discipline, Dr. Meister produced 37 volumes of *Advances in Enzymology*. He was proud of the chapters in this series, not so much as a testimony to his good judgment (though certainly his instincts and insights about enzymology were always on the mark), but as contributions of intrinsic worth to other practitioners of a field that has sprung up around enzyme catalysis and regulation. His last effort appeared as Volume 71 about a year after his untimely death in 1995.

The series will now be continued by Daniel L. Purich, Professor of Biochemistry and Molecular Biology at the University of Florida College of Medicine.

New York, New York
March 1998

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PREFACE

Advances in Enzymology is now nearing its seventh decade as the leading periodic and authoritative review of the latest scientific achievements in enzymology. This field concerns itself with the multifaceted nature of enzymes—their reaction properties; their kinetic behavior; their catalytic mechanisms; their regulatory interations; their expression from genes; their zymogen and other storage forms; their mutant forms (both naturally occurring and man-made); their associated pathophysiology; and their virtually limitless use in agriculture, nutrition, biomedicine, and biotechnology. As the new editor of this series, I shall endeavor to maintain the standard of excellence so masterfully established by Dr. Nord and so skillfully pursued by the late Alton Meister.

The value of *Advances in Enzymology* ultimately will only endure if practicing enzymologists judge its chapters to add value to their own pursuits. Accordingly, this volume marks the advent of several changes that should appeal to its readers. This and many subsequent volumes will be thematically organized so that readers of one chapter will be more likely to be interested in several or all of the other contributions. This monograph-within-a-monograph approach places added burdens on the contributors and editor for the timely appearance of each volume, but the final product should provide broader and more integrated perspective on any particular topic. Another new feature will be the inclusion of an abstract describing the scope and content of each chapter (the abstracts can be found in the front matter of the volume). While this was previously an unnecessary feature of earlier volumes in this series, abstracts allow users of information retrieval services to identify material of interest. In the future we may also be able to provide such information in advance of the actual publication date, thereby minimizing the lag time between a volume's first appearance and wider public awareness of its contents.

In seeking to present readers with the latest and most accurate information about enzyme action, I invite other enzymologists to contact me about their interests, their criticisms, and above all their ideas for improving the impact of this series.

Gainesville, Florida
March 1998

DANIEL L. PURICH

ABSTRACTS

Advances in the Enzymology of Glutamine Synthesis

DANIEL L. PURICH

Meister's proposal of a γ-glutamyl-P intermediate in the glutamine synthetase reaction set the scene for understanding how the stepwise activation of the carboxyl group greatly increased its susceptibility toward nucleophilic attack and amide bond synthesis. Topics covered in this review include: the discovery of the enzymatic synthesis of glutamine; the role of glutamine synthetase in defining the thermodynamics of ATPases; early isotopic tracer studies of the synthetase reaction; the proposed intermediacy of γ-glutamyl-phosphate; the mechanism of methionine sulfoximine inhibition; stereochemical mapping of the enzyme's active site; detection of enzyme reaction cycle intermediates; borohydride trapping of γ-glutamyl-P; positional isotope exchanges catalyzed by glutamine synthetase; regulation of bacterial enzyme; and a brief account of how knowledge of the atomic structure of bacterial glutamine synthetase has clarified ligand binding interactions. Concluding remarks also address how the so-called "Protein Ligase Problem" may be solved by extending the catalytic versatility of carboxyl-group activating enzymes.

Hepatic Glutamine Transport and Metabolism

DIETER HÄUSSINGER

Although the liver was long known to play a major role in the uptake, synthesis, and disposition of glutamine, metabolite balance studies across the whole liver yielded apparently contradictory findings suggesting that little or no net turnover of glutamine occurred in this organ. Efforts to understand the unique regulatory properties of hepatic glutaminase culminated in the conceptual reformulation of the pathway for glutamine synthesis and turnover, especially as regards the role of sub-acinar distribution of glutamine synthetase and glutaminase. This chapter describes these processes as well as

the role of glutamine in hepatocellular hydration, a process that is the consequence of cumulative, osmotically active uptake of glutamine into cells. This topic is also examined in terms of the effects of cell swelling on the selective stimulation or inhibition of other far-ranging cellular processes. The pathophysiology of the intercellular glutamine cycle in cirrhosis is also considered.

Enzymes Utilizing Glutamine as an Amide Donor

HOWARD ZALKIN AND JANET L. SMITH

Amide nitrogen from glutamine is a major source of nitrogen atoms incorporated biosynthetically into other amino acids, purine and pyrimidine bases, amino-sugars, and coenzymes. A family comprised of at least sixteen amidotransferases are known to catalyze amide nitrogen transfer from glutamine to their acceptor substrates. Recent fine structural advances, largely as a result of X-ray crystallography, now provide structure-based mechanisms that help to explain fundamental aspects of the catalytic and regulatory interactions of several of these aminotransferases. This chapter provides an overview of this recent progress made on the characterization of amidotransferase structure and mechanism.

Mechanistic Issues in Asparagine Synthetase Catalysis

NIGEL G. J. RICHARDS AND SHELDON M. SCHUSTER

The enzymatic synthesis of asparagine is an ATP-dependent process that utilizes the nitrogen atom derived from either glutamine or ammonia. Despite a long history of kinetic and mechanistic investigation, there is no universally accepted catalytic mechanism for this seemingly straightforward carboxyl group activating enzyme, especially as regards those steps immediately preceding amide bond formation. This chapter considers four issues dealing with the mechanism: (a) the structural organization of the active site(s) partaking in glutamine utilization and aspartate activation; (b) the relationship of asparagine synthetase to other amidotransferases; (c) the way in which ATP is used to activate the β-carboxyl group; and (d) the detailed mechanism by which nitrogen is transferred.

Mechanisms of Cysteine *S*-Conjugate β-Lyases

ARTHUR J. L. COOPER

Mercapturic acids are conjugates of *S*-(*N*-acetyl)-*L*-cysteine formed during the detoxification of xenobiotics and during the metabolism of such endogenous agents as estrogens and leukotrienes. Many mercaturates are formed from the corresponding glutathione *S*-conjugates. This chapter focuses on (a) the discovery of the cysteine *S*-conjugate β-lyases; (b) the involvement of pyridoxal-5-phosphate; (c) the influence of the electron-withdrawing properties of the group attached to the sulfur atom; and (d) the potential of cysteine *S*-conjugates as pro-drugs.

γ-Glutamyl Transpeptidase: Catalytic Mechanism and Gene Expression

NAOYUKI TANIGUCHI AND YOSHITAKA IKEDA

The γ-glutamyl transpeptidases are key enzymes in the so-called γ-glutamyl cycle involving glutathione synthesis, the recovery of its constituents, and in the transport of amino acids. This membrane-bound ectoenzyme thus serves to regulate glutathione synthesis. This chapter deals with the active site chemistry of γ-glutamyl transpeptidase, including the role of side-chain groups on the light subunit as well as several serine residues in the catalytic process. Also considered are genomic studies indicating (a) the presence of a single gene in mouse and rat; (b) the occurrence of multiple genes in humans; (c) the involvement of multiple promoters for gene expression; and (d) how these multiple promoters may play a role in the tissue-specific expression of γ-glutamyl transpeptidases.

Enzymology of Bacterial Lysine Biosynthesis

GIOVANA SCAPIN AND JOHN S. BLANCHARD

Bacteria have evolved three strategies for the synthesis of lysine from aspartate via formation of the intermediate diaminopimelate (DAP), a metabolite that is also involved in peptidoglycan formation. The objectives of this chapter are descriptions of mechanistic studies on the reactions catalyzed by dihydrodipicolinate synthase,

dihydrodopicolinate reductase, tetrahydrodipicolinate *N*-succinyltransferase, *N*-succinyl-*L,L*-DAP aminotransferase, *N*-succinyl-*L,L*-DAP desuccinylase, *L,L*-DAP epimerase, *L,L*-DAP decarboxylase, and DAP dehydrogenase. These enzymes are discussed in terms of kinetic, isotopic, and X-ray crystallographic data that allow one to infer the nature of interactions of each of these enzymes with its substrate(s), coenzymes, and inhibitors.

Collagen Hydroxylases and the Protein Disulfide Isomerase Subunit of Prolyl 4-Hydroxylases

KARI I. KIVIRIKKO AND TAINA PIHLAJANIEMI

Prolyl 4-hydroxylases catalyze the formation of 4-hydroxyproline in collagens and other proteins with an appropriate collagen-like stretch of amino acid residues. The enzyme requires Fe(II), 2-oxoglutarate, molecular oxygen, and ascorbate. This review concentrates on recent progress toward understanding the detailed mechanism of 4-hydroxylase action, including: (a) occurrence and function of the enzyme in animals; (b) general molecular properties; (c) intracellular sites of hydroxylation; (d) peptide substrates and mechanistic roles of the cosubstrates; (e) insights into the development of antifibrotic drugs; (f) studies of the enzyme's subunits and their catalytic function; and (g) mutations that lead to Ehlers-Danlos Syndrome. An account of the regulation of collagen hydroxylase activities is also provided.

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Amino Acid Metabolism, Part A

Volume 72

Edited by DANIEL L. PURICH

This volume is dedicated to the late Professor Alton Meister, the immediate past Editor of *Advances in Enzymology,* in recognition of his major contribuitons to amino acid metabolism. This is Part A in a subseries, entitled "Amino Acid Metabolism." Topics in Part A should be of immediate interest to those who are broadly concerned with amino acid assimilation and metabolism. Investigators interested in enzyme mechanism and regulation will also find this volume to be especially valuable.

CONTENTS

Reprint of Meister's Classic Paper from Journal of Biological Chemistry, 1960, PC39 (by permission of American Society for Biochemistry and Molecular Biology):

ACTIVATED GLUTAMATE INTERMEDIATE IN THE ENZYMATIC SYNTHESIS OF GLUTAMINE

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