ADVANCES IN INORGANIC BIOCHEMISTRY 6

Editors:

GUNTHER L. EICHHORN

LUIGI G. MARZILLI

ADVANCES IN INORGANIC BIOCHEMISTRY 6

Editors:

GUNTHER L. EICHHORN
NIH Gerontology Research Center, Baltimore, Maryland, U.S.A.

LUIGI G. MARZILLI
Department of Chemistry, Emory University, Atlanta, Georgia, U.S.A.

with the assistance of Patricia A. Marzilli

ELSEVIER
NEW YORK • AMSTERDAM • OXFORD

Published by:

Elsevier Science Publishing Company, Inc.
52 Vanderbilt Avenue, New York, New York 10017

Sole distributors outside of the United States and Canada:

Elsevier Science Publishers B.V.
P.O. Box 211, 1000 AE Amsterdam The Netherlands

ISSN: 0190-0218
ISBN: 0-444-00825-X

Manufactured in the United States of America

PREFACE

This volume is like Volumes 1 and 4 of this series in that it is not dedicated to any one subtopic in inorganic biochemistry. We have put together a variety of topics of current interest. The first chapter deals with one well-thought-out view of carboxypeptidases, which is one of the most thoroughly studied enzymes, and yet its mechanism of action is still controversial. Chapter 2 is concerned mainly with other zinc proteins, and their study by cobalt substitution, and Chapter 3 summarizes the recent NMR studies on metallothionein ligand clusters. Chapter 4 is about a very interesting iron enzyme, and Chapter 5 deals with advances in Mössbauer spectroscopy with iron proteins. Chapters 6, 7 and 8, and 9 deal with copper, nickel, and vanadium biochemistry, respectively.

Chapters 1, 2, 5, and 6 contain recent advances in topics previously covered in Inorganic Biochemistry, while the other chapters are concerned with developments in topics that have reached major importance more recently.

Gunther L. Eichhorn
Luigi G. Marzilli
October 1984

CONTRIBUTORS

Numbers in brackets indicate the page on which the author's contribution begins.

ROBERT K. ANDREWS, Department of Biochemistry, University of Queensland, St. Lucia, Queensland, Australia 4067 [245]

IAN M. ARMITAGE, The Departments of Molecular Biophysics and Biochemistry, and Diagnostic Imaging, Yale University, New Haven, Connecticut 06510 [113]

IVANO BERTINI, Department of Chemistry, University of Florence, Florence, Italy [71]

ROBERT L. BLAKELEY, Department of Biochemistry, University of Queensland, St. Lucia, Queensland, Australia 4067 [245]

DONALD W. BOYD, Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254 [311]

MAX COSTA, Department of Pharmacology, University of Texas, Medical School at Houston, P. O. Box 20708, Houston, Texas 77025 [285]

DAVID C. DALGARNO, The Departments of Molecular Biophysics and Biochemistry, and Diagnostic Imaging, Yale University, New Haven, Connecticut 06510 [113]

J. DANIEL HECK, Lorillard Research Center, P. O. Box 21688, 420 English St., Greensboro, North Carolina 27420 [285]

BOI HANH HUYNH, Physics Department, Emory University, Atlanta, Georgia 30322 [165]

SA-OUK KANG, Department of Biophysics and Theoretical Biology, The University of Chicago, Cummings Life Science Center, 920 E. 58th Street, Chicago, Illinois 60637 [1]

THOMAS A. KENT, Gray Freshwater Biological Institute, University of Minnesota, Navarre, Minnesota 55392 [163]

KENNETH KUSTIN, Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254 [311]

CLAUDIO LUCHINAT, Department of Chemistry, University of Florence, Florence, Italy [71]

MARVIN W. MAKINEN, Department of Biophysics and Theoretical Biology, The University of Chicago, Cummings Life Science Center, 920 E. 58th Street, Chicago, Illinois 60637 [1]

BRUNO MONDOVÌ, Institute of Applied Biochemistry, University of Rome and CNR Center of Molecular Biology, Rome, Italy [225]

PIERLUIGI RICCIO, Institute of Applied Biochemistry, University of Rome and CNR Center of Molecular Biology, Rome, Italy [225]

GERRIT A. VELDINK, Department of Bio-Organic Chemistry, State University of Utrecht, Croesestraat 79, NL-3522 AD UTRECHT, The Netherlands [139]

JOHANNES F. G. VLIEGENTHART, Department of Bio-Organic Chemistry, State University of Utrecht, Croesestraat 79, NL-3522 AD UTRECHT, The Netherlands [139]

GREGG B. WELLS, Department of Biophysics and Theoretical Biology, The University of Chicago, Cummings Life Science Center, 920 E. 58th Street, Chicago, Illinois 60637 [1]

BURT ZERNER, Department of Biochemistry, University of Queensland, St. Lucia, Queensland, Australia 4067 [245]

CONTENTS OF PREVIOUS VOLUMES

VOLUME 1. ADVANCES IN INORGANIC BIOCHEMISTRY

VOLUME 2. METHODS FOR DETERMINING METAL ION ENVIRONMENTS IN PROTEINS: STRUCTURE AND FUNCTION OF METALLOPROTEINS

VOLUME 4. ADVANCES IN INORGANIC BIOCHEMISTRY

1. BIOINORGANIC CHEMISTRY OF NITROGENASE
   Mark J. Nelson, Paul A. Lindahl, and William H. Orme-Johnson
2. PEROXIDASES
   H. B. Dunford
3. BLEOMYCIN
   James C. Dabrowiak
4. CARBONIC ANHYDRASE
   Sven Lindskog
5. THE ENZYMATIC AND NON-ENZYMATIC DECARBOXYLATION OF OXALACETATE
   Daniel L. Leussing
6. LANTHANIDE ION PROBES OF BIOMOLECULAR STRUCTURE
   William DeW. Horrocks, Jr.
7. THE STRUCTURE AND MECHANISM OF THE SARCOPLASMIC RETICULUM Ca^{2+}-ATPase: A BIO-INORGANIC PERSPECTIVE
   Eileen M. Stephens and Charles M. Grisham
8. NMR AND EPR INVESTIGATIONS OF *BI*-METALLOENZYMES
   J. J. Villafranca and F. M. Rauschel

VOLUME 5. IRON BINDING PROTEINS WITHOUT COFACTORS OR SULFUR CLUSTERS

1. FERRITIN: STRUCTURE, FUNCTION, AND REGULATION
   Elizabeth C. Theil
2. THE SPATIAL STRUCTURE OF HORSE SPLEEN APOFERRITIN
   D. W. Rice, G. C. Ford, J. L. White, J. M. A. Smith and P. M. Harrison
3. THE CHEMISTRY OF HEMERYTHRIN
   R. G. Wilkins and P. C. Harrington
4. RIBONUCLEOTIDE REDUCTASE
   Britt-Marie Sjöberg and Astrid Gräslund
5. UTEROFERRIN AND THE PURPLE ACID PHOSPHATASES
   Bradley C. Antanaitis and Philip Aisen
6. SIDEROPHORES
   J. B. Neilands
7. THE CATECHOL DIOXYGENASES
   Lawrence Que, Jr.
8. TRANSFERRIN: A PERSPECTIVE
   N. Dennis Chasteen
9. PHOSVITIN
   George Taborsky

CONTENTS

1

STRUCTURE AND MECHANISM OF CARBOXYPEPTIDASE A

MARVIN W. MAKINEN, GREGG B. WELLS, and SA-OUK KANG

Department of Biophysics and Theoretical Biology
The University of Chicago
Cummings Life Science Center
920 E. 58th Street
Chicago, Illinois 60637

I. INTRODUCTION

In a variety of Zn(II)-containing metalloenzymes, the catalytically essential metal ion in the active site is tetra-coordinate. Three of the ligands to the metal ion are provided by amino acid side chains in distorted tetrahedral geometry with a water molecule as the fourth ligand. Chief examples of such enzymes are carboxypeptidase A (1-3), thermolysin (4,5), carbonic anhydrase (6,7), and liver alcohol dehydrogenase (8,9). The coordination environment of the catalytically essential Zn(II) in these enzymes has been defined through X-ray diffraction studies, and for each of these enzymes the structures of a variety of inhibitor complexes have been determined by difference Fourier methods. A particularly noteworthy structural feature observed in most of the inhibitor complexes of these metalloenzymes is that the metal-bound water molecule is displaced by the inhibitor. For this reason, it is generally assumed that substrates also displace the metal-bound water molecule in the course of the enzyme-catalyzed reaction. However, the results of a variety of studies for some time have suggested that this assumption may not be valid. In this review we shall pay particular attention to those aspects of structural, spectroscopic, and chemical studies of carboxypeptidase A that indicate that the zinc-bound water molecule is not displaced by substrates in catalytically competent reaction intermediates but rather that it has an important role in substrate hydrolysis. We also emphasize those aspects of spectroscopic and X-ray diffraction studies that define substrate configuration and the coordination environment of the metal ion, in efforts to identify stereochemical interactions between the substrate and active site residues that are catalytically significant.

II. THE THREE-DIMENSIONAL STRUCTURE OF CARBOXYPEPTIDASE A

A. Active Site Structure and Metal Ion Coordination Geometry

Carboxypeptidase A (CPA)* is synthesized in the mammalian pancreas as the proteolytically inactive zymogen procarboxypeptidase A. Upon secretion into the intestinal tract, the zymogen is converted into catalytically active forms by the action of trypsin. Under conditions of controlled tryptic hydrolysis in the laboratory, at least four chromatographically separable species are observed, differing in the length of the peptide segment that is cleaved (10,11). Of these various forms, termed α, β, γ and δ, the α- and γ-forms of CPA have been the most widely used in chemical and structural studies. In contrast to the α-, β-, and δ-forms, apocarboxypeptidase A_γ cannot be fully activated with Zn^{2+} (12). The molecular structure of only the α-form has been defined through X-ray crystallographic studies. Our review is consequently based primarily on the structural, chemical, and spectroscopic studies of the α-form and of its metal-substituted derivatives that have provided insight into the molecular basis of CPA action.

Carboxypeptidase A specifically catalyses the hydrolysis of esters and peptides in which the terminal residue has a free COOH-group and a branched aliphatic side chain or aromatic group in an L-configuration (13,14). The catalytic action of CPA is a multi-point, cooperative process that involves three separate regions of the enzyme. These are schematically illustrated in Figure 1. They are: (i) the hydrophobic pocket which binds the aromatic side chain together with Arg-145, forming a salt-link to the terminal carboxylate group; (ii) the bond cleavage site which consists of the side chain of Glu-270 and the active site Zn(II) to which the carbonyl oxygen of the scissile amide or ester bond is ligated; and (iii) amino acid side chains known as the sites of secondary substrate recognition that serve to assist in the binding of extended oligopeptide substrates and that distort the substrate in the active site for bond cleavage. The correlated results of chemical and X-ray diffraction studies

---

*Abbreviations: MMBP, (-)-2-benzyl-3-p-methoxybenzoylpropionate; Bz-, Benzoyl-; CBZ-, carbobenzoxy-; ClCPL, 3-O-(trans-p-chlorocinnamoyl)-L-β-phenyllactate; CML, O-(trans-cinnamoyl)-L-mandelate; CPA, carboxypeptidase A (in this review, CPA designates the α-form in general); ZnCPA, native Zn(II)-containing carboxypeptidase A; correspondingly, CoCPA, NiCPA, etc., Co(II)-, Ni(II)-, etc. substituted carboxypeptidase A; CPL, O-(trans-cinnamoyl)-L-β-phenyllactate; Dns-, 5-(N,N-dimethylamino)-naphthalene-1-sulfonyl-; EPR, electron paramagnetic resonance; HBNA, 2-O-(hippuryl)-butanoate; HPLA, O-(hippuryl)-L-β-phenyllactate; hippuryl, N-(benzoyl)-glycyl-; NMR, nuclear magnetic resonance; βPP, L-β-phenylpropionate; TEPOPL, O-3-(2,2,5,5-tetramethylpyrrolinyl-1-oxyl)-propene-2-oyl-L-β-phenyllactate. Peptides are abbreviated as, e.g., Gly-Tyr, glycyl-L-tyrosine; CBZ-Gly-Phe, N-(carbobenzoxy)-gly-L-phenylalanine, etc.

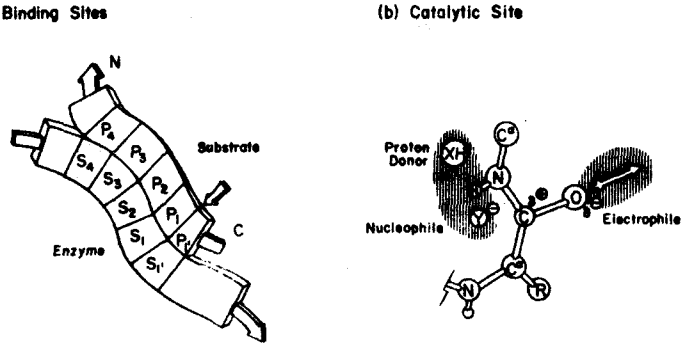

Fig. 1. Schematic illustration of the active site of CPA. In Part (a) the binding interactions of an oligopeptide substrate are illustrated while in Part (b) the molecular and electronic interactions required for bond cleavage in the active site are illustrated. The active site is comprised of five subsites S_1 to S_4 and S'_1 (15,16), and the catalytic site is designated by the arrow. Each subsite accommodates one amino acid residue and the positions P are counted from the site of cleavage.

leading to the identification of these three regions of the active site have been reviewed previously by Lipscomb and co-workers (1,2). Therefore, we shall direct our discussion only to those aspects of high resolution studies (3,17,18) that have further clarified the description of the active site environment of the metal ion and the structural basis of CPA action.

The polypeptide chain of the α-form of CPS with 307 amino acid residues described through X-ray crystallographic studies at 1.54 Å resolution is comprised of eight segments of α-helix ranging from 8-22 amino acid residues in length, eight regions of β-pleated sheet ranging from 3 to 8 residues in length, and 20 reverse β-turns (17). Thirty-seven percent of the amino acid residues are in α-helical conformation, sixteen percent in β-sheet, and forty-seven percent in random coil. Two aspects of the secondary structure may be of importance in the catalytic function of the enzyme: (i) the random coil structure is localized primarily within a region in which conformational changes are observed upon binding of inhibitors, and (ii) a groove near the active site is lined on one side by chains of β-sheet structure. The amino acid side chains in this region probably influence the binding of distal portions of an oligopeptide substrate that extend beyond the neighborhood of the Zn(II) when the COOH-terminus is bound at the P'_1 site since this groove contains many of the resi-

TABLE I

SUMMARY OF ZINC-LIGAND BOND ANGLES AND BOND DISTANCES IN CARBOXYPEPTIDASE A

Zinc-ligand bond angles[a,b]		Zinc-ligand bond distances[c]	
		Ligand	Distance, Å
$N_{\varepsilon 1}(69)$-Zn-$N_{\varepsilon 1}(196)$	99°		
$N_{\varepsilon 1}(69)$-Zn-O (72)	108°	$N_{\varepsilon 1}(69)$	2.10
$N_{\varepsilon 1}(69)$-Zn-O(H_2O)	116°	$N_{\varepsilon 1}(196)$	2.08
$N_{\varepsilon 1}(196)$-Zn-$O_{\varepsilon}(72)$	128°	$O_{\varepsilon 1}(72)$	2.23
$N_{\varepsilon 1}(196)$-Zn-O(H_2O)	99°	$O_{\varepsilon 2}(72)$	2.33
O(H_2O)-Zn-$O_{\varepsilon}(72)$	107°	H_2O	1.96

[a] The bond angles at 1.54 Å resolution involving Glu-72 are determined by combining the two carboxylate oxygens into a hypothetical atom O_{ε}, placed midway between the two oxygen atoms

[b] Ref. 17.

[c] Ref. 3.

dues responsible for the so-called secondary interactions of substrate recognition. Also, six amino acid residues Ser-197 and Tyr-198, Pro-205, Tyr-206, and Arg-272, and Asp-273 form cis peptide bonds and are located near this groove (3).

In the bond cleavage site, the Zn(II) is coordinated to the side chain residues of His-69, His-196, and Glu-72 in a distorted tetrahedral configuration with a water molecule as a nonprotein ligand. Table I provides a listing of valence angles and bond distances calculated from refined X-ray data. Figure 2 illustrates the environment of the active site metal ion. Recent interpretations of the electron density map calculated on the basis of high resolution, refined data have introduced a change in the assignment of the configuration of donor-ligand atoms to the metal ion from that of previous studies. Originally Lipscomb and co-workers (1,2) assigned only one oxygen to the carboxylate group of Glu-72 as a donor-ligand atom on the basis of the electron density map calculated at 2.0 Å resolution by the multiple isomorphous replacement method (19). In more recent studies, this map, after application of the real space refinement method of Diamond (20,21) provided the starting model for refinement by the constrained, least-squares method of Konnert and Hendrickson (22,23), producing an electron density map at 1.75 Å resolution (3). At this higher resolution, the improved map shows that the carboxylate oxygens of Glu-72 are nearly equidistant from the metal ion.

The X-ray data reveal unambiguously that there is only one water molecule within the inner coordination shell of the metal ion. The electron density features and temperature factors of the residues in the active site of the native