METAL IONS IN LIFE SCIENCES

edited by

Astrid Sigel,[1] Helmut Sigel,[1] and Roland K. O. Sigel[2]

[1] *Department of Chemistry*
Inorganic Chemistry
University of Basel
Spitalstrasse 51
CH-4056 Basel, Switzerland

[2] *Institute of Inorganic Chemistry*
University of Zürich
Winterthurerstrasse 190
CH-8057 Zürich, Switzerland

VOLUME 3

The Ubiquitous Roles of Cytochrome P450 Proteins

BICENTENNIAL 1807 WILEY 2007 BICENTENNIAL

John Wiley & Sons, Ltd

Metal Ions in Life Sciences

Volume 3

The Ubiquitous Roles of Cytochrome P450 Proteins

METAL IONS IN LIFE SCIENCES

edited by

Astrid Sigel,[1] Helmut Sigel,[1] and Roland K. O. Sigel[2]

[1] *Department of Chemistry*
Inorganic Chemistry
University of Basel
Spitalstrasse 51
CH-4056 Basel, Switzerland

[2] *Institute of Inorganic Chemistry*
University of Zürich
Winterthurerstrasse 190
CH-8057 Zürich, Switzerland

VOLUME 3

The Ubiquitous Roles of Cytochrome P450 Proteins

John Wiley & Sons, Ltd

Copyright © 2007 John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England

Telephone (+44) 1243 779777

Email (for orders and customer service enquiries): cs-books@wiley.co.uk
Visit our Home Page on www.wiley.com

Other Wiley Editorial Offices

John Wiley & Sons Inc., 111 River Street, Hoboken, NJ 07030, USA

Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 42 McDougall Street, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

John Wiley & Sons Ltd, 6045 Freemont Blvd, Mississauga, Ontario L5R 4J3, Canada

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Anniversary Logo Design: Richard J. Pacifico

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 978-0-470-01672-5

Typeset in 10/12pt Times by Integra Software Services Pvt. Ltd, Pondicherry, India
Printed and bound in Spain by Grafos S.A., Barcelona
This book is printed on acid-free paper responsibly manufactured from sustainable forestry in which at least two trees are planted for each one used for paper production.

The figure on the dustcover is part of Figure 2 of Chapter 6 by Andrew K. Udit, Stephen M. Contakes, and Harry B. Gray.

Historical Development and Perspectives of the Series

Metal Ions in Life Sciences

It is an old wisdom that metals are indispensable for life. Indeed, several of them, like sodium, potassium, and calcium, are easily discovered in living matter. However, the role of metals and their impact on life remained largely hidden until inorganic chemistry and coordination chemistry experienced a pronounced revival in the 1950s. The experimental and theoretical tools created in this period and their application to biochemical problems led to the development of the field or discipline now known as *Bioinorganic Chemistry, Inorganic Biochemistry*, or more recently also often addressed as *Biological Inorganic Chemistry*.

By 1970 *Bioinorganic Chemistry* was established and further promoted by the book series *Metal Ions in Biological Systems* founded in 1973 (edited by H.S., who was soon joined by A.S.) and published by Marcel Dekker, Inc., New York, for more than 30 years. After this company ceased to be a family endeavor and its acquisition by another company, we decided, after having edited 44 volumes of the *MIBS* series (the last two together with R.K.O.S.) to launch a new and broader minded series to cover today's needs in the *Life Sciences*. Therefore, the Sigels' new series is entitled

Metal Ions in Life Sciences

and we are happy to join forces in this new endeavor with a most experienced Publisher in the *Sciences*, John Wiley & Sons, Ltd, Chichester, UK.

The development of *Biological Inorganic Chemistry* during the past 40 years was and still is driven by several factors; among these are (i) the attempts to reveal the interplay between metal ions and peptides, nucleotides, hormones or vitamins, etc.; (ii) the efforts regarding the understanding of accumulation, transport, metabolism and toxicity of metal ions; (iii) the development and application of metal-based drugs; (iv) biomimetic syntheses with the aim to understand biological processes as well as to create efficient catalysts; (v) the determination of high-resolution structures of proteins, nucleic acids, and other biomolecules; (vi) the utilization of powerful spectroscopic tools allowing studies of structures and dynamics; and (vii), more recently, the widespread use of macromolecular engineering to create new biologically relevant structures at will. All this and more is and will be reflected in the volumes of the series *Metal Ions in Life Sciences*.

The importance of metal ions to the vital functions of living organisms, hence, to their health and well-being, is nowadays well accepted. However, in spite of all the progress made, we are still only at the brink of understanding these processes. Therefore, the series *Metal Ions in Life Sciences* will endeavor to link coordination chemistry and biochemistry in their widest sense. Despite the evident expectation that a great deal of future outstanding discoveries will be made in the interdisciplinary areas of science, there are still 'language' barriers between the historically separate spheres of chemistry, biology, medicine, and physics. Thus, it is one of the aims of this series to catalyze mutual 'understanding'.

It is our hope that *Metal Ions in Life Sciences* proves a stimulus for new activities in the fascinating 'field' of *Biological Inorganic Chemistry*. If so, it will well serve its purpose and be a rewarding result for the efforts spent by the authors.

Astrid Sigel, Helmut Sigel
Department of Chemistry
Inorganic Chemistry
University of Basel
CH-4056 Basel
Switzerland

Roland K. O. Sigel
Institute of Inorganic Chemistry
University of Zürich
CH-8057 Zürich
Switzerland

October 2005

Preface to Volume 3

The Ubiquitous Roles of Cytochrome P450 Proteins

Cytochrome P450 monooxygenases (P450s) own a dual functionality and are often referred to as 'mixed-function oxidases' because they possess both 'oxygenase' and 'oxidase' reactivities, meaning that they incorporate an oxygen atom from atmospheric dioxygen into a substrate molecule oxidizing it. The electron transfer functionalities of P450s also earned them the label of 'cytochrome'. The signature of all P450s is the heme Soret band at about 450 nm in the absorption spectrum of the Fe(II)-CO complex; this spectroscopic feature is diagnostic of a cysteinate residue bound *trans* to the carbon monoxide ligand.

This volume encompasses the breadth of research efforts focused on P450s from structure to function, including an appreciation of the diversity and complexity of the biotransformations they catalyze. The introductory Chapter 1, setting the scene, explores the many different levels at which these P450 enzymes (and their respective genes) have diverged in the process of evolution to yield the plethora of enzymes that are now termed 'P450 monooxygenases'. This broad and unprecedented reactivity of P450 enzymes which contain a heme iron center (most commonly iron(III)protoporphyrin IX) with a deprotonated cysteine side chain providing the fifth ligand to iron, has challenged the 'biomimetic community'. Indeed, much has been accomplished over the past five decades, e.g., regarding the understanding of the unusual activation of paramagnetic dioxygen by reductive oxygen cleavage, as is evident from Chapter 2 which provides an in-depth overview of structural and functional mimics of P450s.

The structures of P450 proteins and their molecular phylogeny are detailed in Chapter 3, together with the P450 nomenclature and classification. It is made clear in this account that the P450 protein fold is unique and highly conserved independent of the organism; astonishingly, the same fold is also used by some enzymes that catalyze non-P450 redox transformations. It is worthwhile to note that some P450s function at extremes of pH and heat, as was recently discovered with archaeans. The diversity of P450s is also reflected in aquatic species as outlined in Chapter 4 where expecially P450 activities in invertebrates are in the focus.

Chapter 5 examines the ability of electrochemical techniques to unravel fundamental aspects of the electron transfer process of P450 enzymes. This process is central to P450 catalysis and thus, in Chapter 6 extensive studies are summarized which have shown that interprotein electron transfer is facilitated by proper positioning, e.g., of the flavin mononucleotide- and heme-containing domains. Clearly, generation of a truly catalytic system that utilizes non-native redox cofactors in place of the reductase proteins is a 'holy grail' of P450 research.

The next four chapters center on mechanistic considerations. At first leakage reactions are considered which occur during the P450 catalytic cycle, followed by detailed evaluations of the structural basis for substrate recognition and catalysis, including the architecture of the active site. Roles of the secondary coordination sphere, i.e., at the proximal (thiolate) and the distal side (where activation of dioxygen and substrate binding occurs) are described next, as is the coordination to the heme iron of several small-molecule inhibitors, such as nitrogen monoxide (nitric oxide), carbon monoxide, cyanide, and imidazole.

P450-catalyzed hydroxylations and epoxidations, the biosynthesis of steroid hormones, and the catalyzed carbon–carbon bond cleavage are discussed in Chapters 11–13. The design and engineering of cytochrome P450 systems is detailed in Chapter 14 regarding the oxidation of non-natural substrates. Evidently, potential applications of altered P450s in the environmentally benign synthesis of chemical products and intermediates are expected in this research area.

In the terminating three chapters of *The Ubiquitous Roles of Cytochrome P450 Protein*s first the biotransformation of xenobiotics is comprehensively dealt with, i.e., the 'chemical defense' or response of an organism to foreign chemicals. Thereafter the metabolism of drugs by human P450 systems is described, an area of particular significance for drug development and finally also for daily life in the clinic, as is emphasized by a clinical pharmacist.

Astrid Sigel
Helmut Sigel
Roland K. O. Sigel

Contents

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Stephen G. Bell Department of Chemistry, Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QR, UK, <stephen.bell@chem.ox.ac.uk> (437)

Rita Bernhardt Institut für Biochemie, Universität des Saarlandes, P. O. Box 151150, D-66041 Saarbrücken, Germany, <ritabern@mx.uni-saarland.de> (361)

Alan M. Bond School of Chemistry, Monash University, Clayton, Victoria 3800, Australia, <alan.bond@sci.monash.edu.au> (127)

Peggy L. Carver University of Michigan College of Pharmacy, Clinical Sciences Department, 428 Church St., Ann Arbor, MI 48109-1065, USA, <peg@umich.edu> (591)

Stephen M. Contakes The Beckman Institute, California Institute of Technology, Pasadena, CA 91125, USA, <contakes@caltech.edu> (157)

Max J. Cryle School of Molecular and Microbial Sciences, University of Queensland, Brisbane, QLD 4072, Australia (397)

John H. Dawson Department of Chemistry and Biochemistry, and School of Medicine, University of South Carolina, Columbia, SC 29208, USA, <dawson@sc.edu> (319)

James J. De Voss School of Molecular and Microbial Sciences, University of Queensland, Brisbane, QLD 4072, Australia, <j.devoss@uq.edu.au> (397)

Barry D. Fleming School of Chemistry, Monash University, Clayton, Victoria 3800, Australia, <barry.fleming@sci.monash.edu.au> (127)

Elizabeth M. J. Gillam School of Biomedical Sciences, The University of Queensland, St. Lucia, Brisbane, Qld 4072, Australia, <e.gillam@uq.edu.au> (477)

Hazel M. Girvan Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK (285)

Harry B. Gray The Beckman Institute, California Institute of Technology, Pasadena, CA 91125, USA, <hbgray@caltech.edu> (157)

F. Peter Guengerich Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, 638 Robinson Research Building, 23rd & Pierce Avenues, Nashville, TN 37232-0146, USA, <f.guengerich@vanderbilt.edu> (561)

Nicola Hoskins Department of Chemistry, Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QR, UK (437)

Dominic J. B. Hunter School of Biomedical Sciences, The University of Queensland, St. Lucia, Brisbane, Qld 4072, Australia, <d.hunter1@uq.edu.au> (477)

Shengxi Jin Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA, <jinshengxi@gmail.com> (319)

Christiane Jung Max-Delbrück Center for Molecular Medicine, Research Group Protein Dynamics, D-13125 Berlin, Germany. Present address: KKS Ultraschall AG, Surface Treatment Division, Frauholzring 29, CH-6422 Steinen, Switzerland, <christiane_jung@hotmail.com> (187)

Yi Lu Department of Biochemistry and Department of Chemistry, University of Illinois at Urbana-Champaign, A322 Chemical and Life Science Building, Box 8-6, 600 S. Matthews Avenue, Urbana, IL 61801, USA, <yi-lu@uiuc.edu> (267)

Lisandra L. Martin School of Chemistry, Monash University, Clayton, Victoria 3800, Australia, <lisa.martin@sci.monash.edu.au> (127)

Kirsty J. McLean Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK (285)

Yergalem T. Meharenna Department of Biochemistry and Molecular Biology, Physiology and Biophysics, and Chemistry, University of California, Irvine, CA 92697-3900, USA, <ymeharen@uci.edu> (57)

Andrew W. Munro Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK, <andrew.munro@manchester.ac.uk> (285)

Roshan Perera Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA, <perera@scripps.edu> (319)

Thomas D. Pfister Department of Biochemistry, University of Illinois at Urbana-Champaign, A322 Chemical and Life Science Building, Box 8-6, 600 S. Matthews Avenue, Urbana, IL 61801, USA (267)

Thomas L. Poulos Department of Biochemistry and Molecular Biology, Physiology and Biophysics, and Chemistry, University of California, Irvine, CA 92697-3900, USA, <poulos@uci.edu> (57)

Ilme Schlichting Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany, <ilme.schlichting@mpimf-heidelberg.mpg.de> (235)

Mary A. Schuler Departments of Cell and Developmental Biology, Biochemistry and Plant Biology, 190ERML, 1201 W. Gregory Drive, University of Illinois, Urbana, IL 61801, USA, <maryschu@uiuc.edu> (1)

Stephen G. Sligar Departments of Biochemistry, Chemistry and the College of Medicine, 116 Morrill Hall, MC-119, 505 S. Goodwin Avenue, Urbana, IL 61801, USA, <s-sligar@uiuc.edu> (1)

Mark J. Snyder Department of Clinical Pharmacology, University of California at San Francisco, Kenwood, CA 95452, USA. Contact address: P. O. Box 609, Kenwood, CA 95452, USA, <snyder181@comcast.net> (97)

Masanori Sono Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA, <sono@mail.chem.sc.edu> (319)

Andrew K. Udit Department of Chemistry, Occidental College, Los Angeles, CA 90041, USA, <udit@oxy.edu> (157)

Konstanze von König Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany, <konstanze.von.koenig@mpimf-heidelberg.mpg.de> (235)

Michael R. Waterman Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146, USA, <michael.waterman@vanderbilt.edu> (361)

Christopher J. C. Whitehouse Department of Chemistry, Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QR, UK (437)

Wolf-D. Woggon Department of Chemistry, Organic Chemistry, University of Basel, St. Johannsring 19, CH-4056 Basel, Switzerland, <wolf-d.woggon@unibas.ch> (27)

Luet L. Wong Department of Chemistry, Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QR, UK, <luet.wong@chem.ox.ac.uk> (437)

Titles of Volumes 1–44 in the *Metal Ions in Biological Systems* Series

edited by the SIGELs
and published by Dekker/Taylor & Francis

Volume 1: **Simple Complexes**
Volume 2: **Mixed-Ligand Complexes**
Volume 3: **High Molecular Complexes**
Volume 4: **Metal Ions as Probes**
Volume 5: **Reactivity of Coordination Compounds**
Volume 6: **Biological Action of Metal Ions**
Volume 7: **Iron in Model and Natural Compounds**
Volume 8: **Nucleotides and Derivatives: Their Ligating Ambivalency**
Volume 9: **Amino Acids and Derivatives as Ambivalent Ligands**
Volume 10: **Carcinogenicity and Metal Ions**
Volume 11: **Metal Complexes as Anticancer Agents**
Volume 12: **Properties of Copper**
Volume 13: **Copper Proteins**
Volume 14: **Inorganic Drugs in Deficiency and Disease**
Volume 15: **Zinc and Its Role in Biology and Nutrition**
Volume 16: **Methods Involving Metal Ions and Complexes in Clinical Chemistry**
Volume 17: **Calcium and Its Role in Biology**
Volume 18: **Circulation of Metals in the Environment**
Volume 19: **Antibiotics and Their Complexes**
Volume 20: **Concepts on Metal Ion Toxicity**
Volume 21: **Applications of Nuclear Magnetic Resonance to Paramagnetic Species**
Volume 22: **ENDOR, EPR, and Electron Spin Echo for Probing Coordination Spheres**
Volume 23: **Nickel and Its Role in Biology**
Volume 24: **Aluminum and Its Role in Biology**
Volume 25: **Interrelations Among Metal Ions, Enzymes, and Gene Expression**
Volume 26: **Compendium on Magnesium and Its Role in Biology, Nutrition, and Physiology**

Contents of Volumes in the *Metal Ions in Life Sciences* Series

edited by the SIGELs
and published by John Wiley & Sons, Ltd, Chichester, UK
<http://www.wiley.com/go/mils>

Volume 2: Nickel and Its Surprising Impact in Nature

Comments and suggestions with regard to contents, topics, and the like for future volumes of the series are welcome.

Met. Ions Life Sci. ***3***, 1–26 (2007)

1

Diversities and Similarities in P450 Systems: An Introduction

Mary A. Schuler[1] ***and Stephen G. Sligar***[2]

[1]Department of Cell and Developmental Biology, University of Illinois,
Urbana, IL 61801, USA
<maryschu@uiuc.edu>

[2]Department of Biochemistry, University of Illinois,
Urbana, IL 61801, USA
<s-sligar@uiuc.edu>

Metal Ions in Life Sciences, Volume 3 Edited by Astrid Sigel, Helmut Sigel and Roland K. O. Sigel

1. OXYGENASES: MEDIATORS OF BIOCHEMICAL DIVERSITY

The introduction of oxygen into biochemical processes has had a profound effect on the evolution of life. An appreciation for this traumatic event was presented in a beautiful recent review on the linkages of gene development that occurred at this juncture [1]. Although the first important utilization of atmospheric dioxygen was perhaps through its use as a terminal electron acceptor in metabolic energy conversion, an equally important leap in complexity and diversity was appreciated when, in the mid-1950s, Osamu Hayaishi and Howard Mason discovered the oxygenases [2,3]. This advance changed the simplistic view of how Nature uses atmospheric dioxygen from that of a simple electron acceptor and pointed to the rich metabolic diversity allowed by the incorporation of atmospheric dioxygen into substrate molecules. The discovery, naming and mechanistic understanding of the first 'oxygenase' enzymes have provided wonderful opportunities and scientific impetus to understand the great diversity of these systems in the synthesis and catabolism of organic molecules.

Before describing their various levels of diversity, one must consider the prime biochemical similarity that categorizes nearly all of them within the class of 'oxidases' that use atmospheric dioxygen as a terminal electron acceptor and, hence, yield an oxidized substrate molecule. In technical terms, the 'oxygenases' that exist within this broader oxidase category are classed as 'mono-' or 'di-', depending on whether one or both atoms of atmospheric dioxygen are incorporated into their respective substrates. In their reaction cycles, the classic stoichiometry of monooxygenases represents a sort of 'half-way' point on the pathway for the full four-electron reduction of dioxygen to generate two molecules of water as is typical of the redox counting of cytochrome 'oxidases'. Positioned midstream in this pathway, monooxygenases require only two electrons and two protons to reductively cleave atmospheric dioxygen, producing only a single water molecule in the process while saving the second atom for the incorporation and formal oxidation of the organic substrate molecule. As a result of their dual functionality, cytochrome P450 monooxygenases (P450s) are often referred to as 'mixed-function oxidases' since they possess both 'oxygenase' and 'oxidase' reactivities. The electron transfer functionalities of P450s also earned them the label of 'cytochrome'.