

# Methods in ENZYMOLOGY

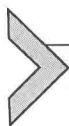
Volume 554

Hydrogen Sulfide in Redox Biology Part A

*Edited by*

Enrique Cadenas and Lester Packer





VOLUME FIVE HUNDRED AND FIFTY FOUR

# METHODS IN ENZYMOLLOGY

## Hydrogen Sulfide in Redox Biology Part A

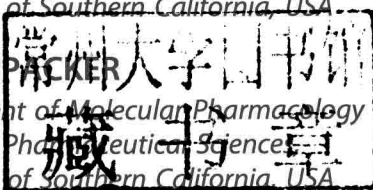
Edited by

**ENRIQUE CADENAS**

*Pharmacology & Pharmaceutical Sciences,  
School of Pharmacy,  
University of Southern California, USA*

**LESTER P. PACKER**

*Department of Molecular Pharmacology and Toxicology,  
School of Pharmaceutical Sciences,  
University of Southern California, USA*



AMSTERDAM • BOSTON • HEIDELBERG • LONDON  
NEW YORK • OXFORD • PARIS • SAN DIEGO  
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier  
225 Wyman Street, Waltham, MA 02451, USA  
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA  
125 London Wall, London, EC2Y 5AS, UK  
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

First edition 2015

Copyright © 2015 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: [www.elsevier.com/permissions](http://www.elsevier.com/permissions).

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

### Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-801512-4

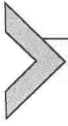
ISSN: 0076-6879

For information on all Academic Press publications  
visit our website at [store.elsevier.com](http://store.elsevier.com)



Working together  
to grow libraries in  
developing countries

[www.elsevier.com](http://www.elsevier.com) • [www.bookaid.org](http://www.bookaid.org)



VOLUME FIVE HUNDRED AND FIFTY FOUR

# METHODS IN ENZYMOLGY

Hydrogen Sulfide in Redox Biology  
Part A

# METHODS IN ENZYMOLOGY

*Editors-in-Chief*

**JOHN N. ABELSON and MELVIN I. SIMON**

*Division of Biology*

*California Institute of Technology*

*Pasadena, California*

**ANNA MARIE PYLE**

*Departments of Molecular, Cellular and Developmental*

*Biology and Department of Chemistry Investigator*

*Howard Hughes Medical Institute*

*Yale University*

*Founding Editors*

**SIDNEY P. COLOWICK and NATHAN O. KAPLAN**

# CONTRIBUTORS

**Abbas Abou-Hamdan**

Inserm U1016; CNRS UM8104, and Université Paris Descartes UMR-S1016, Institut Cochin, Paris, France

**Mireille Andriamihaja**

INRA-CRNH-IdF-AgroParisTech, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France

**Ruma Banerjee**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Jin-Song Bian**

Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

**François Blachier**

INRA-CRNH-IdF-AgroParisTech, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France

**Frédéric Bouillaud**

Inserm U1016; CNRS UM8104, and Université Paris Descartes UMR-S1016, Institut Cochin, Paris, France

**Christopher J. Chang**

Department of Chemistry; Department of Molecular and Cell Biology, University of California, Berkeley, California, and Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

**Taurai Chiku**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Brian W. Dymock**

Department of Pharmacy, National University of Singapore, Singapore

**Hala Guedouari-Bounihi**

Inserm U1016; CNRS UM8104, and Université Paris Descartes UMR-S1016, Institut Cochin, Paris, France

**Takaaki Ito**

Department of Pathology and Experimental Medicine, Graduate School of Medical Science, Kumamoto University, Kumamoto, Japan

**Michael R. Jackson**

Department of Biochemistry and Molecular Biology, College of Medicine, Drexel University, Philadelphia, Pennsylvania, USA

**Marilyn Schuman Jorns**

Department of Biochemistry and Molecular Biology, College of Medicine, Drexel University, Philadelphia, Pennsylvania, USA

**Omer Kabil**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Christopher G. Kevil**

Department of Pathology, Louisiana State University Health Sciences Center–Shreveport, Shreveport, Louisiana, USA

**Gopi K. Kolluru**

Department of Pathology, Louisiana State University Health Sciences Center–Shreveport, Shreveport, Louisiana, USA

**Véronique Lenoir**

Inserm U1016; CNRS UM8104, and Université Paris Descartes UMR-S1016, Institut Cochin, Paris, France

**Zhong-Guang Li**

School of Life Sciences; Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education; Key Laboratory of Biomass Energy and Environmental Biotechnology, Yunnan Normal University, Kunming, Yunnan Province, PR China

**Marouane Libiad**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Vivian S. Lin**

Department of Chemistry, University of California, Berkeley, California, USA

**Alexander R. Lippert**

Department of Chemistry, and Center for Drug Discovery, Design, and Delivery (CD4), Southern Methodist University, Dallas, Texas, USA

**Scott L. Melideo**

Department of Biochemistry and Molecular Biology, College of Medicine, Drexel University, Philadelphia, Pennsylvania, USA

**Philip K. Moore**

Neurobiology Program, Life Science Institute and Department of Pharmacology, National University of Singapore, Singapore

**Nicole Motl**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Noriyuki Nagahara**

Isotope Research Center, Nippon Medical School, Tokyo, Japan

**Masatoshi Nagano**

Department of Pharmacology, Nippon Medical School, Tokyo, Japan

**Péter Nagy**

Department of Molecular Immunology and Toxicology, National Institute of Oncology, Budapest, Hungary

**Chung-Min Park**

Department of Chemistry, Washington State University, Pullman, Washington, USA

**Bo Peng**

Department of Chemistry, Washington State University, Pullman, Washington, USA

**Michael D. Pluth**

Department of Chemistry and Biochemistry, Institute of Molecular Biology, Materials Science Institute, University of Oregon, Eugene, Oregon, USA

**Peter Rose**

University of Lincoln, Lincoln, Lincolnshire, United Kingdom

**Xinggui Shen**

Department of Pathology, Louisiana State University Health Sciences Center–Shreveport, Shreveport, Louisiana, USA

**T. Spencer Bailey**

Department of Chemistry and Biochemistry, Institute of Molecular Biology, Materials Science Institute, University of Oregon, Eugene, Oregon, USA

**Hidenori Suzuki**

Department of Pharmacology, Nippon Medical School, Tokyo, Japan

**Victor Vitvitsky**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Ming Xian**

Department of Chemistry, Washington State University, Pullman, Washington, USA

**Xue Xue**

Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

**Pramod K. Yadav**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

**Shuai Yuan**

Department of Pathology, Louisiana State University Health Sciences Center–Shreveport, Shreveport, Louisiana, USA

## PREFACE

Hydrogen sulfide is viewed as the third gasotransmitter, gaseous signaling molecule, together with nitric oxide and carbon monoxide. The cellular sources of hydrogen sulfide involve enzymes of the trans-sulfuration pathway CBS (cystathionine  $\beta$ -synthase), CSE (cystathionine  $\gamma$ -lyase), and 3MST (3-mercaptopyruvate sulfur-transferase). Storages of hydrogen sulfide occur in mitochondria (iron-sulfur clusters of enzymes) and cytosol (bound sulfane sulfur). Of course, the release of hydrogen sulfide from these storages is tightly regulated by several pathophysiological processes.

In addition to the myriad of effects arising from hydrogen sulfide as a signaling molecule, it also protects against oxidative stress and glutamate toxicity, inhibits the release of insulin, preserves mitochondrial function, and is a modulator of inflammatory responses. These pleiotropic effects of hydrogen sulfide have been the subject of numerous investigations in the last years and are largely accounted for by its role as a gaseous signaling molecule. Hydrogen sulfide may act alone or in conjunction with other gasotransmitters and, in doing so, it regulates a number of physiological processes and is involved in some stages of the pathogenesis of several diseases.

These volumes of *Methods in Enzymology* were designed as a compendium for hydrogen sulfide detection methods, the pharmacological activity of hydrogen sulfide donors, the redox biochemistry of hydrogen sulfide and its metabolism in mammalian tissues, the mechanisms inherent in hydrogen sulfide cell signaling and transcriptional pathways, and cell signaling in specific systems, such as cardiovascular and nervous system as well as its function in inflammatory responses. Three chapters are also devoted to hydrogen sulfide in plants and a newcomer, molecular hydrogen, its function as a novel antioxidant.

In bringing these volumes of *Methods in Enzymology* to fruition, credit must be given to the experts in various aspects of hydrogen sulfide research, whose thorough and innovative work is the basis of these *Methods in Enzymology* volumes. We hope that these volumes will be of help to both new and established investigators in the field.

ENRIQUE CADENAS  
LESTER PACKER

# CONTENTS

<i>Contributors</i>	<i>xi</i>
<i>Preface</i>	<i>xv</i>

## Section I

### Hydrogen Sulfide Detection Methods

<b>1. Mechanistic Chemical Perspective of Hydrogen Sulfide Signaling</b>	<b>3</b>
Péter Nagy	
1. Introduction	5
2. Bioavailability of Sulfide—The Signal	5
3. Inorganic Polysulfides	9
4. Sulfide Signaling Via Protein Sulfhydration	13
5. Sulfide Signaling via Sulfide–Hemeprotein Interactions	18
6. Conclusions	23
Acknowledgments	24
References	24
<b>2. Measurement of H<sub>2</sub>S <i>In Vivo</i> and <i>In Vitro</i> by the Monobromobimane Method</b>	<b>31</b>
Xingguo Shen, Gopi K. Kolluru, Shuai Yuan, and Christopher G. Kevil	
1. Introduction	32
2. Experimental Methods	35
3. Summary	43
Acknowledgment	43
References	43
<b>3. Hydrogen Sulfide Detection Using Nucleophilic Substitution–Cyclization-Based Fluorescent Probes</b>	<b>47</b>
Bo Peng and Ming Xian	
1. Introduction	48
2. Design and Synthesis of the Probes	49
3. Chemistry and Properties of the Probes	50
4. Applications of the Probes in H <sub>2</sub> S Imaging in Cell-Based Experiments	55
5. Conclusions	60
Acknowledgments	61
References	61

<b>4. Azide-Based Fluorescent Probes: Imaging Hydrogen Sulfide in Living Systems</b>	<b>63</b>
Vivian S. Lin, Alexander R. Lippert, and Christopher J. Chang	
1. Introduction	64
2. Fluorescent Azide-Based H <sub>2</sub> S Probes	66
3. <i>In Vitro</i> Characterization of Probes	69
4. Detection of H <sub>2</sub> S in Live Cells Using Fluorescent Probes	72
5. Conclusions	77
Acknowledgments	78
References	78
<b>5. Chemiluminescent Detection of Enzymatically Produced H<sub>2</sub>S</b>	<b>81</b>
T. Spencer Bailey and Michael D. Pluth	
1. Introduction	82
2. Chemiluminescent Probes for the Determination of Sulfide	85
3. Examples of Routine Probe Usage	89
4. Detection of Enzymatically Produced H <sub>2</sub> S	92
5. Conclusions	95
Acknowledgments	96
References	96
<b>6. Quantification of Hydrogen Sulfide Concentration Using Methylene Blue and 5,5'-Dithiobis(2-Nitrobenzoic Acid) Methods in Plants</b>	<b>101</b>
Zhong-Guang Li	
1. Theory	102
2. Equipment	103
3. Materials	104
4. Protocol 1	105
5. Step 1: Quantification of H <sub>2</sub> S Concentration Using MB Method	106
6. Protocol 2	107
7. Step 1: Quantification of H <sub>2</sub> S concentration using 5,5'-dithiobis (2-nitrobenzoicacid) method	108
Acknowledgment	110
References	110
<b>7. H<sub>2</sub>S Analysis in Biological Samples Using Gas Chromatography with Sulfur Chemiluminescence Detection</b>	<b>111</b>
Victor Vitvitsky and Ruma Banerjee	
1. Introduction	112
2. Principle of the GC-Coupled Sulfur Chemiluminescence Method	112

3. Protocol for GC-Coupled Sulfur Chemiluminescence Detection of H <sub>2</sub> S	114
4. Analysis of Biological Samples	116
5. Additional Technical Details	122
Acknowledgment	122
References	122

## Section II

# Hydrogen Sulfide Donors and Their Pharmacological Activity

## 8. Use of Phosphorodithioate-Based Compounds as Hydrogen Sulfide Donors 127

Chung-Min Park and Ming Xian

1. Introduction	128
2. Synthesis of Phosphorodithioate-Based Donors	129
3. Measurements of H <sub>2</sub> S Generation from the Donors Using Fluorescence Methods	133
4. H <sub>2</sub> S Release from the Donors in Cultured Cells	135
5. Donor's Activity Against H <sub>2</sub> O <sub>2</sub> -Induced Cell Damage	138
6. Summary	140
Acknowledgments	140
References	140

## 9. GYY4137, a Novel Water-Soluble, H<sub>2</sub>S-Releasing Molecule 143

Peter Rose, Brian W. Dymock, and Philip K. Moore

1. Introduction	144
2. Why Slow Releasing H <sub>2</sub> S Donors?	146
3. The Development and Characterization of GYY4137	147
4. Facile Synthesis and Chemical Characterization of GYY4137	148
5. Biological Effects of GYY4137: An Overview and Potential Role in Disease?	149
6. The Effect of GYY4137 in Nonmammalian Systems	159
7. Conclusion	161
References	162

## 10. Neuroprotective Effects of Hydrogen Sulfide in Parkinson's Disease Animal Models: Methods and Protocols 169

Xue Xue and Jin-Song Bian

1. Introduction	170
2. PD Animal Models	170
3. H <sub>2</sub> S and Its Releasing Compound Treatment	176
4. Behavior Tests	177

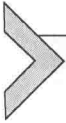
5. Immunohistochemical Assay	178
6. Brain H <sub>2</sub> S Activity Tests	180
7. Prospects of H <sub>2</sub> S Therapy on PD and Conclusions	182
Acknowledgment	184
References	184

## Section III

### Hydrogen Sulfide Metabolism in Mammalian Tissues

<b>11. Assay Methods for H<sub>2</sub>S Biogenesis and Catabolism Enzymes</b>	<b>189</b>
Ruma Banerjee, Taurai Chiku, Omer Kabil, Marouane Libiad, Nicole Motl, and Pramod K. Yadav	
1. Introduction	189
2. Assays for H <sub>2</sub> S Biogenesis	191
3. Assays for Enzymes Involved in H <sub>2</sub> S Catabolism	195
Acknowledgments	199
References	199
<b>12. Oxidation of H<sub>2</sub>S in Mammalian Cells and Mitochondria</b>	<b>201</b>
Abbas Abou-Hamdan, Hala Guedouari-Bounihi, Véronique Lenoir, Mireille Andriamihaja, François Blachier, and Frédéric Bouillaud	
1. Introduction	202
2. Sulfide in the Context of Mitochondrial Bioenergetics	203
3. Practical Issues	208
4. Sulfide Oxidation Experiments	214
5. Treatment, Expression, and Interpretation of Results	222
6. Originality and Interest with Regard to Bioenergetics	225
Acknowledgments	227
References	227
<b>13. Redox Regulation of Mammalian 3-Mercaptopyruvate Sulfurtransferase</b>	<b>229</b>
Noriyuki Nagahara, Masatoshi Nagano, Takaaki Ito, and Hidenori Suzuki	
1. Introduction	230
2. Redox Regulation of Cysteine Metabolism and MST	235
3. Regulation of MST Activity via Redox-Sensing Molecular Switches	235
4. MST Knockout Mouse	245
5. Other Investigation	251
References	252

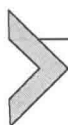
<b>14. Role of Human Sulfide:Quinone Oxidoreductase in H<sub>2</sub>S Metabolism</b>	<b>255</b>
Michael R. Jackson, Scott L. Melideo, and Marilyn Schuman Jorns	
1. Introduction	256
2. Expression of Human SQOR in <i>E. coli</i>	257
3. Purification of Recombinant Human SQOR	257
4. Catalytic Assays	259
5. Spectral Properties of Recombinant Human SQOR	260
6. Survey of Potential Sulfane Sulfur Acceptors for Human SQOR	261
7. Spectral Course of SQOR Catalytic Assays with Sulfite, Cyanide, or Sulfide as Sulfane Sulfur Acceptor	263
8. Steady-State Kinetic Parameters for H <sub>2</sub> S Oxidation by SQOR with Sulfite, Cyanide, or Sulfide as Sulfane Sulfur Acceptor	265
9. Role of Human SQOR in H <sub>2</sub> S Metabolism	267
Acknowledgment	268
References	269
<b>15. H<sub>2</sub>S Regulation of Nitric Oxide Metabolism</b>	<b>271</b>
Gopi K. Kolluru, Shuai Yuan, Xinggui Shen, and Christopher G. Kevil	
1. Introduction	272
2. Techniques Determining Enzymatic Activity and Expression of NOS	275
3. Detection of NO and Its Metabolites	278
4. Novel Adducts from H <sub>2</sub> S–NO Interactions	287
5. Conclusion	292
Acknowledgments	293
References	293
<i>Author Index</i>	<b>299</b>
<i>Subject Index</i>	<b>317</b>



## SECTION I

# Hydrogen Sulfide Detection Methods





# Mechanistic Chemical Perspective of Hydrogen Sulfide Signaling

Péter Nagy<sup>1</sup>

Department of Molecular Immunology and Toxicology, National Institute of Oncology, Budapest, Hungary

<sup>1</sup>Corresponding author; e-mail address: peter.nagy@oncol.hu

## Contents

1. Introduction	5
2. Bioavailability of Sulfide—The Signal	5
2.1 Endogenous sulfide production	6
2.2 Sulfide catabolism	8
2.3 Endogenous sulfide buffers	9
3. Inorganic Polysulfides	9
3.1 Biological relevance	9
3.2 Speciation and redox capacity of polysulfides	10
3.3 Polysulfide formation by sulfide oxidation	11
3.4 Stability of polysulfides	12
4. Sulfide Signaling Via Protein Sulfhydration	13
4.1 Mechanisms of persulfide formation	14
5. Sulfide Signaling via Sulfide–Hemeprotein Interactions	18
5.1 Sulfide mediates heme protein functions	19
5.2 Heme proteins generate sulfide oxidation products	21
5.3 Antioxidant properties of sulfide via reduction of metal centers with higher oxidation states	22
6. Conclusions	23
Acknowledgments	24
References	24

## Abstract

Hydrogen sulfide is now a well-appreciated master regulator in a diverse array of physiological processes. However, as a consequence of the rapid growth of the area, sulfide biology suffers from an increasing number of controversial observations and interpretations. A better understanding of the underlying molecular pathways of sulfide's actions is key to reconcile controversial issues, which calls for rigorous chemical/biochemical investigations.

Protein sulfhydration and coordination/redox chemical interactions of sulfide with heme proteins are the two most extensively studied pathways in sulfide biochemistry. These pathways are important mediators of protein functions, generate bioactive