# Methods in Enzymology Volume 102

# Hormone Action

Part G

Calmodulin and Calcium-Binding Proteins

EDITED BY

Anthony R. Means

Bert W. O'Mattey

## Methods in Enzymology

## Volume 102

# Hormone Action

Part G

Calmodulin and Calcium-Binding Proteins

#### EDITED BY

Anthony R. Means

DEPARTMENT OF CELL BIOLOGY

BAYLOR COLLEGE OF MEDICINE

HOUSTON, TEXAS O 11 OVI 25 MEDICAL DAME

ACTOR OF MEDICINE AND ACTOR OF THE COLOR OF THE COL

Bert W. O'Malley

DEPARTMENT OF CELL BIOLOGY, rigodom testing in the runne. I
BAYLOR COLLEGE OF MEDICINE
HOUSTON, TEXAS

1983



#### ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

PRINCE OF AN THE UNITED STATES OF AMERICA

New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

COPYRIGHT © 1983, BY ACADEMIC PRESS, 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 PHOTOCOPY, RECORDING, OR ANY
INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT
PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by ACADEMIC PRESS, INC. (LONDON) LTD. 24/28 Oval Road, London NW1 7DX

#### Library of Congress Cataloging in Publication Data

O'Malley, Bert W. Hormone action.

(Methods in enzymology; v. 36-In pts. C. and D Hardman's name is first; pt. G. edited by Anthony R. Means and Bert O'Malley. Includes bibliographical references and indexes. Contents: pt. A. Steroid hormones. -- pt. B. Peptide hormones. -- [etc.] -- pt. G. Calmodulin and calciumbinding proteins. 1. Enzymes. 2. Hormones. 3. Cyclic nucleotides. 4. Cell nucleus. 5. Hormones. 6. Nucleotides, Cyclic. 1. Hardman, Joel G., joint author. II. Series: Methods in enzymology; v. 36-40, [etc.] QP601.M49 vol. 36, etc. 574.19'25

#### PRINTED IN THE UNITED STATES OF AMERICA

Paris Sua Liego San Francisco São Paulo 1 5 4 4 8 7 8 6 9 8 8 88 88

#### Contributors to Volume 102

Article numbers are in parentheses following the names of contributors.

Affiliations listed are current.

- James Michael Anderson (2), United States Department of Agriculture, Agricultural Research Service, and Departments of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27650
- MICHAEL D. BATES (18), Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710
- James G. Chafouleas (9), Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030
- HARRY CHARBONNEAU (3), Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710
- WAI YIU CHEUNG (4, 23), Departments of Biochemistry, St. Jude Children's Research Hospital, The University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38101
- PHILIP COHEN (21, 22), Department of Biochemistry, Medical Sciences Institute, The University of Dundee, Dundee DDI 4HN, Scotland
- P. MICHAEL CONN (18), Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710
- WILLIAM J. COOK (13), Department of Pathology, University of Alabama in Birmingham, Birmingham, Alabama 35294
- MILTON J. CORMIER (3, 18), Department of Biochemistry, University of Georgia, Athens, Georgia 30602
- JOHN R. DEDMAN (1), Department of Physiology and Cell Biology and Department of Internal Medicine (Endocrinology), University of Texas Health Science Center at Houston, Houston, Texas 77025
- JACQUES G. DEMAILLE (25), Centre de Recherches de Biochimie Macromoléculaire du CNRS, and Unité INSERM 249, Biochimie des Regulations des Systemes Contractiles, BP 5015, 34033 Montpellier, France

- TOYOSHI ENDO (24), Department of Pharmacology, Mie University School of Medicine, Edobashi, Tsu 514, Japan
- JOHN R. GLENNEY, JR, (19), The Salk Institute, Laboratory of Molecular Biology and Virology, San Diego, California 92138
- ROGER GRAND (21), Department of Biochemistry, University of Birmingham, Birmingham, England
- JEFFREY F. HARPER (11), Division of Endocrinology, Department of Internal Medicine and Department of Pharmacology, University of Texas Medical School at Houston, Houston, Texas 77025
- RUSSELL C. Hart (3, 18), Department of Biochemistry, University of Georgia, Athens, Georgia 30602
- RITA HICE (3), Department of Biochemistry, University of Georgia, Athens, Georgia 30602
- HIROYOSHI HIDAKA (17, 24), Department of Pharmacology, Mie University School of Medicine, Edobashi, Tsu 514, Japan
- THOMAS R. HINDS (5), Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington 98195
- Shozo Ida (12), Section of Contractile Proteins, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45221
- MARCIA A. KAETZEL (1), Department of Internal Medicine (Endocrinology), University of Texas Health Science Center at Houston, Houston, Texas 77025
- Norio Kajikawa (26), The Kyoto Research Laboratory, Kaken Pharmaceutical Co., Ltd., Kyoto 607, Japan
- KANEFUSA KATO (24), Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kamiya, Kasugai, Aichi 480-03, Japan

- AKIRA KISHIMOTO (26), Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan
- C. B. KLEE (22), Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
- RACHEL E. KLEVIT (8), Department of Microbiology/Immunology, Duke University Medical Center, Durham, North Carolina 27710
- M. H. KRINKS (22), Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
- LISETTE LAGACÉ (9), Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030
- CHRISTIAN J. LE PEUCH (25), Centre de Recherches de Biochimie Macromoléculaire du CNRS, and Unité INSERM U-249, BP 5015, 34033 Montpellier, France
- Danielle A.-M. Le Peuch (25), Centre de Recherches de Biochimie Macromoléculaire du CNRS, and Unité INSERM 249, BP 5015, 34033 Montpellier, France
- A. S. Manalan (22), Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
- DAVID R. MANNING (7), Department of Pharmacology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235
- ANTHONY R. MEANS (9), Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030
- BARBARA E. MILLER (27), Department of Biochemistry, University of California, Riverside, California 92521
- TIMOTHY J. MURTAUGH (15), Department of Pediatrics, University of Wisconsin Medical School, Madison, Wisconsin 53706
- YASUTOMI NISHIZUKA (26), Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan

- ANTHONY W. NORMAN (27), Department of Biochemistry, University of California, Riverside, California 92521
- Bradley B. Ölwin (14), Department of Pharmacology, University of Washington, Seattle, Washington 98195
- COLIN PICTON (21), Searle Research and Development, P.O. Box 53, Lane End Road, High Wycombe, Bucks, HP12 4HL, England
- JAMES D. POTTER (12), Section of Contractile Proteins, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45221
- MARY E. RISER (9), Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030
- GERALD M. ROSEN (18), Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710
- PAUL M. Rowe (15), Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706
- JOHN S. SACK (13), Institute of Dental Research, University of Alabama in Birmingham, Birmingham, Alabama 35294
- RAJENDRA K. SHARMA (20), Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4, Canada
- SHIRISH SHENOLIKAR (21), Department of Pharmacology, University of Texas Health Science Center at Houston, Houston, Texas 77025
- MAKOTO SHIOTA (26), Department of Orthopedics, Hyogo College of Medicine, Nishinomiya 663, Japan
- FRANK L., SIEGEL (15), Departments of Pediatrics and Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706
- PAUL J., SILVER (6), Experimental Therapeutics, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, Pennsylvania 19101

- ALTON L. STEINER (11), Division of Endocrinology, Department of Internal Medicine, University of Texas Medical School at Houston, Houston, Texas 77025
- A. A. STEWART (22), Department of Biological Chemistry, University of California School of Medicine, Davis, California 95616
- Daniel R. Storm (14), Department of Pharmacology, University of Washington, Seattle, Washington 98195
- PRISCILLA STRANG-BROWN (12), Section of Contractile Proteins, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45221
- JAMES T. STULL (6, 7), Department of Pharmacology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235
- E. Ann Tallant (4, 23), Departments of Biochemistry, St. Jude Children's Research Hospital, The University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38101
- Toshio Tanaka (17), Department of Pharmacology, Mie University School of Medicine, Edobashi, Tsu 514, Japan
- WILLIAM A. TAYLOR (20), Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba T3E OW3, Canada
- THOMAS C. VANAMAN (28), Department of Microbiology/Immunology, Duke University Medical Center, Durham, North Carolina 27710

- PAMELA L. VINCENT (15), Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706
- FRANK F. VINCENZI (5), Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington 98195
- PATRICIA L. WALKER (12), Section of Contractile Proteins, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45221
- ROBERT W. WALLACE (4, 23), Department of Pharmacology, The University of Alabama in Birmingham, Birmingham, Alabama 35294
- JERRY H. WANG (20), Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4, Canada
- KLAUS WEBER (19), Department of Biochemistry, Max-Planck Institute for Biophysical Chemistry, D-3400 Goettingen, Federal Republic of Germany
- BENJAMIN WEISS (16), Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129
- MICHAEL J. WELSH (10), Department of Anatomy and Cell Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109
- LYNDA S. WRIGHT (15), Department of Pediatrics, University of Wisconsin Medical School, Madison, Wisconsin 53706

# Preface

It is now well accepted that calmodulin represents the major intracellular calcium receptor in all nonmuscle and smooth muscle eukaryotic cells. Although this protein was first reported in the late 1960s, the impact it would have upon the multidisciplinary fields involving calcium transport, metabolism, and action was not realized until 1976. Calmodulin research is one of the most rapidly advancing areas of investigation in cell regulation since the early years of cyclic nucleotides. This rapidly expanding field, comprised of investigators from a broad range of disciplines, has generated an impressive array of methodological advances. These advances led to the addition of this volume to the Hormone Action series of volumes that specifically deal with steroid and peptide hormones.

The volume includes methods for assaying calmodulin, purifying the protein, and determining many of its multiple and unique physicochemical properties. Chapters are included that describe approaches to the utilization of calmodulin to purify calmodulin-dependent enzymes and to the detection of previously unknown calmodulin-binding proteins. The hydrophobic properties of this protein have led to the discovery of various pharmacological agents that bind it and can be utilized not only to purify calmodulin but also to probe potential physiological roles. Finally, methods are presented for the production and purification of polyclonal and monoclonal antibodies. These antibodies have been used to develop radioimmunoassays, localize calmodulin in cells at both light and electron microscopic levels, study the turnover of the protein in cells and tissues, and to isolate the calmodulin mRNA and gene.

It should be emphasized that in this exponentially growing field methodological advances have been made since this volume went to press. Calmodulin mediates many of its effects through the activation of various protein kinases. Some of the apparent omissions dealing with these enzymes are covered in Volume 99 of Methods in Enzymology.

We would like to express our appreciation to the contributors for their willingness to participate in this venture and for their excellent research that made this volume possible. Thanks are also extended to the competent and courteous staff of Academic Press for their untiring efforts and advice.

ANTHONY R. MEANS BERT W. O'MALLEY

## METHODS IN ENZYMOLOGY

#### EDITED BY

# Sidney P. Colowick and Nathan O. Kaplan

protein kinases. Some of the apparent antisame dealing with these en

SCHOOL OF MEDICINE NASHVILLE, TENNESSEE

VANDERBILT UNIVERSITY DEPARTMENT OF CHEMISTRY UNIVERSITY OF CALIFORNIA AT SAN DIEGO LA JOLLA, CALIFORNIA research is one of the most rapidly silving at the stress of the seligation in cel

- I. Preparation and Assay of Enzymes
- II. Preparation and Assay of Enzymes and but an accompany to the second
- III. Preparation and Assay of Substrates design and accomplishing approximation and assay of Substrates design and accomplishing a complishing accomplishing an accomplishing accomplishing an accomplishing accomplishing an accomplishing accomplishing an accomplishing accomplishing accomplishing accomplishing accomplishing a complishing accomplishing accomplishing accomplishing an accomplishing accompli
- IV. Special Techniques for the Enzymologist
- V. Preparation and Assay of Enzymes bodises abbutom amulov ad I
- VI. Preparation and Assay of Enzymes (Continued) Preparation and Assay of Substrates Dallari on a substrated assays of Substrates Special Techniques to pash-n below the fitting of all mounts to not
- VII. Cumulative Subject Index located a worden visuorears to noncored in any abic early such of this measure rule had to the dispersions of framious

## METHODS IN ENZYMOLOGY

#### **EDITORS-IN-CHIEF**

Sidney P. Colowick Nathan O. Kaplan

VOLUME VIII. Complex Carbohydrates

Edited by ELIZABETH F. NEUFELD AND VICTOR GINSBURG

VOLUME IX. Carbohydrate Metabolism

VOLUME X. Oxidation and Phosphorylation

Edited by Ronald W. Estabrook and Maynard E. Pullman

VOLUME XI. Enzyme Structure

Edited by C. H. W. Hirs

VOLUME XII. Nucleic Acids (Parts A and B)

Edited by Lawrence Grossman and Kivie Moldave

VOLUME XIII. Citric Acid Cycle

Edited by J. M. LOWENSTEIN

VOLUME XIV. Lipids

Edited by J. M. LOWENSTEIN

VOLUME XV. Steroids and Terpenoids Edited by RAYMOND B. CLAYTON

VOLUME XVI. Fast Reactions . Edited by KENNETH KUSTIN

VOLUME XVII. Metabolism of Amino Acids and Amines (Parts A and B) Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME XVIII. Vitamins and Coenzymes (Parts A, B, and C)

Edited by Donald B. McCormick and Lemuel D. Wright

Volume XIX. Proteolytic Enzymes and syndalisms of HIXXX standard Edited by Gertrude E. Perlmann and Laszlo Lorand and Market and Mar

VOLUME XX. Nucleic Acids and Protein Synthesis (Part C)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXI. Nucleic Acids (Part D)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXII. Enzyme Purification and Related Techniques

Edited by WILLIAM B. JAKOBY

VOLUME XXIII. Photosynthesis (Part A)

Edited by Anthony San Pietro Bords and Stating formed and Market Stating for the Stating formed and Market Stating formed and Market Stating for the Stating formed and Market Stating for the Stating formed and Market Stating for the Statin

VOLUME XXIV. Photosynthesis and Nitrogen Fixation (Part B)

Edited by Anthony San Pietro

VOLUME XXV. Enzyme Structure (Part B)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVI. Enzyme Structure (Part C)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVII. Enzyme Structure (Part D)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVIII. Complex Carbohydrates (Part B) Edited by VICTOR GINSBURG

VOLUME XXIX. Nucleic Acids and Protein Synthesis (Part E)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXX. Nucleic Acids and Protein Synthesis (Part F)

Edited by Kivie Moldave and Lawrence Grossman

VOLUME XXXI. Biomembranes (Part A)

Edited by Sidney Fleischer and Lester Packer

VOLUME XXXII. Biomembranes (Part B) as animally ALIVX and BV Edited by SIDNEY FLEISCHER AND LESTER PACKER OF ANOTHER PACKER OF ANOTHER PACKER.

VOLUME XXXIII. Cumulative Subject Index Volumes I-XXX Edited by MARTHA G. DENNIS AND EDWARD A. DENNIS

VOLUME XXXIV. Affinity Techniques (Enzyme Purification: Part B)

Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XXXV. Lipids (Part B) Township of the Edited by John M. Lowenstein Township of the Edited by Townsh

VOLUME XXXVI. Hormone Action (Part A: Steroid Hormones)

Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVII. Hormone Action (Part B: Peptide Hormones)

Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVIII. Hormone Action (Part C: Cyclic Nucleotides)

Edited by Joel G. Hardman and Bert W. O'Malley

VOLUME XL. Hormone Action (Part E: Nuclear Structure and Function) Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XLI. Carbohydrate Metabolism (Part B)

Edited by W. A. WOOD

VOLUME XLII. Carbohydrate Metabolism (Part C) I Y TAGE VA DOLLAR Edited by W. A. WOOD

VOLUME XLIII: Antibiotics ADULIGE AND HER BANK TO LEADER Edited by JOHN H. HASH

VOLUME XLIV. Immobilized Enzymes YBONAK A MAKELIN IN THE Edited by KLAUS MOSBACH

(O 1189) RESIDENCE RECORD TO A CONTRACT OF THE PROPERTY OF T

VOLUME XLV. Proteplytic Enzymes (Part B) August M. Servick of Landa Edited by Laszlo Lorand

VOLUME XLVI. Affinity Labeling was also as a gradio M sivil vd bands. Edited by William B. Jakoby and Meir Wilchek

VOLUME XLVII. Enzyme Structure (Part E) and W. H. O of bound Edited by C. H. W. Hirs and Serge N. Timasheff

此为试读,需要完整PDF请访问: www.ertongbook.com

VOLUME XLVIII. Enzyme Structure (Part F)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLIX. Enzyme Structure (Part G)

Edited by C. H. W. Hirs and Serge N. Timasheff

VOLUME L. Complex Carbohydrates (Part C)

Edited by Victor Ginsburg

VOLUME LI. Purine and Pyrimidine Nucleotide Metabolism

Edited by Patricia A. Hoffee and Mary Ellen Jones

VOLUME LII. Biomembranes (Part C: Biological Oxidations)

Edited by Sidney Fleischer and Lester Packer

VOLUME LIII. Biomembranes (Part D: Biological Oxidations)

Edited by Sidney Fleischer and Lester Packer

VOLUME LIV. Biomembranes (Part E: Biological Oxidations)

Edited by Sidney Fleischer and Lester Packer

VOLUME LV. Biomembranes (Part F: Bioenergetics)

Edited by Sidney Fleischer and Lester Packer

VOLUME LVI. Biomembranes (Part G: Bioenergetics)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVII. Bioluminescence and Chemiluminescence

Edited by Marlene A. DeLuca

VOLUME LIX. Nucleic Acids and Protein Synthesis (Part G)

Edited by Kivie Moldave and Lawrence Grossman

VOLUME 61. Enzyme Structure (Part H)

Edited by C. H. W. HIRS AND SERGE Na TIMASHEFF AND ALL AND ADDITIONAL ADDITIONAL AND ADDITIONAL ADDITI

VOLUME 62. Vitamins and Coenzymes (Part D) Edited by Donald B. McCormick and Lemuel D. Wright

VOLUME 63. Enzyme Kinetics and Mechanism (Part A: Initial Rate and Inhibitor Methods)

Edited by Daniel L. Purich

VOLUME 64. Enzyme Kinetics and Mechanism (Part B: Isotopic Probes and Complex Enzyme Systems) and good and control of the American America

VOLUME 65. Nucleic Acids (Part I) A Hard And Strend AT AND TOWN Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE MOLE VIS WALLEY

VOLUME 66. Vitamins and Coenzymes (Part E) and after the Property of the Vitamins and Coenzymes (Part E) and after the Vitamins and Coenzymes (Part E) and After

VOLUME 67. Vitamins and Coenzymes (Part F) JUNIODE AND LEMUEL D. WRIGHT AND LEMUEL D. WRIGHT

VOLUME 68. Recombinant DNA H Traff sand demonstrated by RAY Wu

VOLUME 69. Photosynthesis and Nitrogen Fixation (Part C)

Edited by ANTHONY SAN PIETRO

VOLUME 70. Immunochemical Techniques (Part A)

Edited by Helen Van Vunakis and John J. Langone

Volume 71. Lipids (Part C)

Edited by John M. Lowenstein and Teamedonnemal 18 amulov

Volume 72. Lipids (Part D) What all days and an amount of bonds Edited by John M. Lowenstein

Volume 73. Immunochemical Techniques (Part B) on the College Apparatus and the College (Part B) on the Edited by John Langone and Helen Wanning and Edited by Dixhesina and Helen Wanning and Edited by Dixhesina and Helen Wanning and Edited by Dixhesina and Edited by Dixh

VOLUME 74. Immunochemical Techniques (Part C) galeot 4.8 SHAULOV Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS LIW of belief

VOLUME 75. Cumulative Subject Index Volumes XXXI, XXXII, and XXXIV-LX

Edited by Edward A. Dennis and Martha G. Dennis

Volume 76. Hemoglobins

Edited by Eraldo Antonini, Luigi Rossi-Bernardi, and Emilia
Chiancone

VOLUME 77. Detoxication and Drug Metabolisma and value of the Edited by WILLIAM B. JAKOBY

VOLUME 78. Interferons (Part A) A MAN A SAME AND A SAME

VOLUME 79. Interferons (Part B) COCHAYMER and COCHAY BONALD B MCCORMICE AND LEW ATTERPRESENTATION OF BONALD B. MCCORMICE AND LEW ATTERPRESENTATION OF THE PROPERTY OF THE PROP

VOLUME 80. Proteolytic Enzymes (Part C) bas among the start of the sta

VOLUME 81. Biomembranes (Part H: Visual Pigments and Purple Membranes, I)

Edited by LESTER PACKER

VOLUME 82. Structural and Contractile Proteins (Part A: Extracellular Matrix)

Edited by LEON W. CUNNINGHAM AND DIXIE W. FREDERIKSEN

VOLUME 83. Complex Carbohydrates (Part D) Edited by VICTOR GINSBURG

VOLUME 84. Immunochemical Techniques (Part D: Selected Immunoassays)

Edited by John J. Langone and Helen Van Vunakis

VOLUME 85. Structural and Contractile Proteins (Part B: The Contractile Apparatus and the Cytoskeleton) pindoot is important and the Cytoskeleton) pindoot is important and the Cytoskeleton pindoot is important and Edited by Dixie W. Frederiksen and Leon W. Cunningham besided

VOLUME 86. Prostaglandins and Arachidonate Metabolites AT HIMLE Edited by WILLIAM E. M. LANDS AND WILLIAM L. SMITH OF VALUE AND AND WILLIAM L. SMITH OF VALUE AND AND WILLIAM L.

Volume 87. Enzyme Kinetics and Mechanism (Part C: Intermediates, Stereochemistry, and Rate Studies)

Edited by Daniel L. Purich

VOLUME 88. Biomembranes (Part I: Visual Pigments and Purple Membranes, II)

Edited by LESTER PACKER

VOLUME 89. Carbohydrate Metabolism (Part D) Edited by WILLIS A. WOOD

VOLUME 90. Carbohydrate Metabolism (Part E) Edited by Willis A. Wood

VOLUME 91. Enzyme Structure (Part I)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 92. Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 93. Immunochemical Techniques (Part F: Conventional Antibodies, Fc Receptors, and Cytotoxicity)

Edited by John J. Langone and Helen Van Vunakis

Volume 94. Polyamines

Edited by Herbert Tabor and Celia White Tabor

VOLUME 95. Cumulative Subject Index Volumes 61–74, 76–80 (in preparation)

Edited by EDWARD A. DENNIS AND MARTHA G. DENNIS

VOLUME 96. Biomembranes [Part J: Membrane Biogenesis: Assembly and Targeting (General Methods; Eukaryotes)]

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 97. Biomembranes [Part K: Membrane Biogenesis: Assembly and Targeting (Prokaryotes, Mitochondria, and Chloroplasts)]

Edited by Sidney Fleischer and Becca Fleischer

VOLUME 98. Biomembranes [Part L: Membrane Biogenesis (Processing and Recycling)] (in preparation)

Edited by Sidney Fleischer and Becca Fleischer

VOLUME 99. Hormone Action (Part F: Protein Kinases) Edited by Jackie D. Corbin and Joel G. Hardman

VOLUME 100. Recombinant DNA (Part B)

Edited by RAY WU, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

Volume 101. Recombinant DNA (Part C)

Edited by RAY Wu, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

Volume 102. Hormone Action (Part G: Calmodulin and Calcium-Binding Proteins)

Edited by Anthony R. Means and Bert W. O'Malley:

VOLUME 103. Hormone Action (Part H: Neuroendocrine Peptides) (in preparation)

Edited by P. MICHAEL CONN

VOLUME 104. Enzyme Purification and Related Techniques (Part C) (in preparation)

Edited by WILLIAM B. JAKOBY

VOLUME 105. Oxygen Radicals in Biological Systems (in preparation)

Edited by Lester Packer

Vollving 96. Biomer.francy 15st 1. A scharte E. g. nests socially and Engeting (General Methods). Edited of Edited by Stoney Figure and Breck Figuretts.

Edition for East and A. Derkars and Marriera G. Debars

Volume 97. Biomembranes (Part K.: Membrane Biogenesis: Assembly and Targeting (Prokaryotes, Mitochondria, and Chloroplasts)]. Edified by Sunway Puriscurer and Bucca Fill Ischik.

## Table of Contents

Co	NTRIBUTORS TO VOLUME 102		ix
PRI	EFACE		XII
Vo	LUMES IN SERIES	********	XV
1.	Calmodulin Purification and Fluorescent Labeling	JOHN R. DEDMAN AND MARCIA A. KAETZEI	1
2.	Purification of Plant Calmodulin	JAMES MICHAEL ANDERSON	9
3.	Purification of Calmodulin by Ca <sup>2+</sup> -Dependent Affinity Chromatography	HARRY CHARBONNEAU. RITA HICE, RUSSELL C. HART, AND MILTON J. CORMIER	17
4.	Assay of Calmodulin by Ca <sup>2+</sup> -Dependent Phosphodiesterase	ROBERT W. WALLACE. E. ANN TALLANT, AND WAI YIU CHEUNG	39
5.	The Red Blood Cell as a Model for Calmodulin- Dependent Ca <sup>2+</sup> Transport	THOMAS R. HINDS AND FRANK F. VINCENZI	47
6.	Myosin Light Chain Phosphorylation in Smooth Muscle and Nonmuscle Cells as a Probe of Calmodulin Function	PAUL J. SILVER AND JAMES T. STULL	62
7.	Assessment of Ca <sup>2+</sup> -Calmodulin Formation in Intact Vertebrate Skeletal Muscle	DAVID R. MANNING AND JAMES T. STULL	74
8.	Spectroscopic Analyses of Calmodulin and Its Interactions	RACHEL E. KLEVIT	82
9.	Production of Polyclonal and Monoclonal Anti- bodies to Calmodulin and Utilization of These Immunological Probes	MARY E. RISER,	104
10.	Localization of Calmodulin and Calmodulin Acceptor Sites by Fluorescence Methods	MICHAEL J. WELSH	110
11.	Immunofluorescence Localization of Calmodulin in Unfixed Frozen Tissue Sections	JEFFREY F. HARPER AND ALTON L. STEINER	122
10.	beideaten and Dawietin a W. L. Chart	JAMES D. POTTER, PRISCILLA STRANG-BROWN, PATRICIA L. WALKER, AND SHOZO IIDA	135
13.	Preparation of Calmodulin Crystals	WILLIAM J. ÇOOK AND JOHN S. SACK	143