

Methods in Enzymology

Volume X

Oxidation and Phosphorylation

EDITED BY

Ronald W. Estabrook

DEPARTMENT OF BIOPHYSICS AND PHYSICAL BIOCHEMISTRY
JOHNSON RESEARCH FOUNDATION
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
PHILADELPHIA, PENNSYLVANIA

and

Maynard E. Pullman

DEPARTMENT OF BIOCHEMISTRY
THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC.
NEW YORK, NEW YORK

Bibliothek des
Max-Planck-Institutes
für physikalische Chemie
Göttingen, Bunsenstr. 10

1967



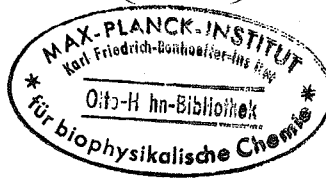
ACADEMIC PRESS New York and London

YMOLOGY

EF
an O. Kaplan

E 4/03 & Vol 10

(X 84)



COPYRIGHT © 1967, BY ACADEMIC PRESS INC.

ALL RIGHTS RESERVED.

NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM,
BY PHOTOSTAT, MICROFILM, OR ANY OTHER MEANS, WITHOUT
WRITTEN PERMISSION FROM THE PUBLISHERS.

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

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 54-9110

PRINTED IN THE UNITED STATES OF AMERICA

L. Lehninger, E. Racker, and
criticism in the organization
also wish to thank Mr. Philip
nyllis Pullman for the excel-
lently, we wish to acknowledge
of the staff of Academic

RONALD W. ESTABROOK
MAYNARD E. PULLMAN

Table of Contents

CONTRIBUTORS TO VOLUME X	v
PREFACE	xi
PREVIOUS VOLUMES	xxi
VOLUMES IN PREPARATION	xxii

Section I. General Techniques for the Measurement of Oxidative and Phosphorylation Reactions

<i>Previously Published Articles from Methods in Enzymology Related to Section I</i>	2
1. Enzyme Profiles in Mitochondria	MARTIN KLINGENBERG 3
2. Criteria of Homogeneity and Purity of Mitochondria	CHRISTIAN DE DUVE 7
3. Manometric Methods and Phosphate Determination	E. C. SLATER 19
4. The Measurement of Oxidative Phosphorylation in the NADH-Cytochrome <i>b</i> Segment of the Mitochondrial Respiratory Chain	GOTTFRIED SCHATZ 30
5. Use of Artificial Electron Acceptors for Abbreviated Phosphorylating Electron Transport: Flavin-Cytochrome <i>c</i>	CHUAN-PU LEE, GIAN LUIGI SOTTOCASA, AND LARS ERNSTER 33
6. Assay of Oxidative Phosphorylation at the Cytochrome Oxidase Region (Site III)	D. R. SANADI AND E. E. JACOBS 38
7. Mitochondrial Respiratory Control and the Polarographic Measurement of ADP:O Ratios	RONALD W. ESTABROOK 41
8. Application of Inhibitors and Uncouplers for a Study of Oxidative Phosphorylation	E. C. SLATER 48
9. Measurement of ATPase, ¹⁴ C-ADP-ATP, and ³² P _i -ATP Exchange Reactions	MAYNARD E. PULLMAN 57
10. The Application of ¹⁸ O Methods to Oxidative Phosphorylation	P. D. BOYER AND DONNA M. BRYAN 60

Section II. Preparations, Properties, and Conditions for
Assay of Mitochondria

<i>Previously Published Articles from Methods in Enzymology Related to Section II</i>		74
11. The Preparation of Heart Mitochondria from Laboratory Animals	D. D. TYLER AND JEANINE GONZE	75
12. The Large-Scale Preparation and Properties of Heart Mitochondria from Slaughterhouse Material	PAUL V. BLAIR	78
13. Preparation, Properties, and Conditions for Assay of Mitochondria: Slaughterhouse Material, Small-Scale	ARCHIE L. SMITH	81
14. Skeletal Muscle Mitochondria	LARS ERNSTER AND KERSTIN NORDENBRAND	86
15. Isolation of Liver or Kidney Mitochondria	DIANE JOHNSON AND HENRY LARDY	94
16. Preparation and Properties of Brain Mitochondria	R. E. BASFORD	96
17. The Preparation of Thyroid and Thymus Mitochondria	D. D. TYLER AND JEANINE GONZE	101
18. Guinea Pig Mammary Gland Mitochondria	WALTER L. NELSON AND RONALD A. BUTOW	103
19. Preparation and Assay of Phosphorylating Mitochondria from Ascites Tumor Cells	RAY WU AND LEONARD A. SAUER	105
20. Neoplastic Tissue Mitochondria	THOMAS M. DEVLIN	110
21. A Technique for the Isolation of Mitochondria from Bovine Lymphocytes	JOHN TERRANCE DAVIS	114
22. Insect Mitochondria	SIMON G. VAN DEN BERGH	117
23. Avocado and Apple Mitochondria	J. T. WISKICH	122
24. A General Method for the Preparation of Plant Mitochondria	WALTER D. BONNER, JR.	126
25. Mitochondria from Spinach Leaves	I. ZELITCH	133
26. Yeast Mitochondria and Submitochondrial Particles	JAMES R. MATTOON AND WALTER X. BALCAVAGE	135
27. Preparation and Properties of <i>Neurospora</i> Mitochondria	J. W. GREENAWALT, D. O. HALL, AND O. C. WALLIS	142
28. Microbial Phosphorylating Preparations: <i>Alcaligenes faecalis</i>	GIFFORD B. PINCHOT	147
29. Microbial Phosphorylating Preparations: <i>Azotobacter</i>	H. G. PANDIT-HOVENKAMP	152
30. Microbial Phosphorylating Preparations: <i>Mycobacterium</i>	ARNOLD F. BRODIE	157
31. Microbial Phosphorylating Preparations: <i>Micrococcus lysodeikticus</i>	SHINJI ISHIKAWA	169

and Conditions for dria	74
D. D. TYLER AND JEANINE GONZE	75
PAUL V. BLAIR	78
ARCHIE L. SMITH	81
LARS ERNSTER AND KERSTIN NORDENBRAND	86
DIANE JOHNSON AND HENRY LARDY	94
R. E. BASFORD	96
D. D. TYLER AND JEANINE GONZE	101
WALTER L. NELSON AND RONALD A. BUTOW	103
RAY WU AND LEONARD A. SAUER	105
THOMAS M. DEVLIN	110
JOHN TERRANCE DAVIS	114
SIMON G. VAN DEN BERGH	117
J. T. WISKICH	122
WALTER D. BONNER, JR.	126
E. ZELITCH	133
JAMES R. MATTOON AND WALTER X. BALCAVAGE	135
J. W. GREENAWALT, D. O. HALL, AND D. C. WALLIS	142
JEFFORD B. PINCHOT	147
H. G. PANDIT-HOVENKAMP	152
ARNOLD F. BRODIE	157
SHINJI ISHIKAWA	169

Section III. Preparations, Properties, and Conditions for Assay of Submitochondrial Particles

<i>Previously Published Articles from Methods in Enzymology Related to Section III</i>	178
32. Preparation and Properties of Digitonin Particles from Beef Heart	W. B. ELLIOTT AND D. W. HAAS 179
33. Preparation and Assay of Phosphorylating Submitochondrial Particles: Particles from Rat Liver Prepared by Drastic Sonication	CHARLES T. GREGG 181
34. Preparation, Properties, and Conditions for Assay of Phosphorylating Electron Transport Particles (ETPH) and Its Variations	ROBERT E. BEYER 186
35. A-Particles and P-Particles	JUNE M. FESSENDEN AND EFRAIM RACKER 194
36. Stable Phosphorylating Submitochondrial Particles from Bakers' Yeast	GOTTFRIED SCHATZ 197
37. The Keilin-Hartree Heart Muscle Preparation	TSOO E. KING 202
38. Preparation and Properties of Repeating Units of Mitochondrial Electron Transfer	PAUL V. BLAIR 208
39. Preparation and Properties of Succinic-Cytochrome <i>c</i> Reductase (Complex II-III)	HOWARD D. TISDALE 213
40. Preparations of Succinate-Cytochrome <i>c</i> Reductase and the Cytochrome <i>b-c₁</i> Particle, and Reconstitution of Succinate-Cytochrome <i>c</i> Reductase	TSOO E. KING 216
41. The Preparation and Properties of DPNH-Cytochrome <i>c</i> Reductase (Complex I-III of the Respiratory Chain)	Y. HATEFI AND J. S. RIESKE 225
42. Preparation and Properties of Succinate Dehydrogenase-Coenzyme Q Reductase (Complex II)	D. ZIEGLER AND J. S. RIESKE 231
43. Preparation and Properties of DPNH-Coenzyme Q Reductase (Complex I of the Respiratory Chain)	Y. HATEFI AND J. S. RIESKE 235
44. Preparation and Properties of Reduced Coenzyme Q-Cytochrome <i>c</i> Reductase (Complex III of the Respiratory Chain)	J. S. RIESKE 239
45. Cytochrome Oxidase from Beef Heart Mitochondria	DAVID C. WHEARTON AND ALEXANDER TZAGOLOFF 245
46. Electron Transport Particle of Yeast	BRUCE MACKLER 251
47. Preparation and Properties of the Heart Mitochondrial Electron Transporting Particles (Inner Membrane)	KRYSZYNA C. KOPACZYK 253

48. Microbial Electron Transport Particle	F. L. CRANE	259
49. DPNH Oxidase of Heart Muscle	BRUCE MACKLER	261
50. Acetone-Treated Particles (CoQ Deficient)	ROBERT L. LESTER	264
51. Resolved Mitochondrial Translocating Particles	JOHN T. PENNISTON	266

Section IV. Purified Respiratory Chain Oxidation-Reduction Components

<i>Previously Published Articles from Methods in Enzymology Related to Section IV</i>		274
52. Preparations and Properties of Soluble NADH Dehydrogenases from Cardiac Muscle	TSOO E. KING AND ROBERT L. HOWARD	275
53. DPNH Dehydrogenase of Yeast	BRUCE MACKLER	294
54. NADH-CoQ Reductase—Assay and Purification	D. R. SANADI, RICHARD L. PHARO, AND LOUIS A. SORDAHL	297
55. Electron Transfer Flavoprotein	JOHN R. CRONIN, WILHELM R. FRISELL, AND COSMO G. MACKENZIE	302
56. DT Diaphorase	LARS ERNSTER	309
57. Beef Heart TPNH-DPN Pyridine Nucleotide Transhydrogenases	NATHAN O. KAPLAN	317
58. Preparation of Succinate Dehydrogenase and Reconstitution of Succinate Oxidase	TSOO E. KING	322
59. Cytochrome Oxidase: Beef Heart	TAKASHI YONETANI	332
60. Cytochrome <i>c</i> Peroxidase (Bakers' Yeast)	TAKASHI YONETANI	336
61. Cytochrome <i>c</i> from Vertebrate and Invertebrate Sources	E. MARGOLIASH AND O. F. WALASEK	339
62. Isolation and Properties of Cytochrome <i>c</i> ₁	J. S. RIESKE AND H. D. TISDALE	349
63. Purification and Properties of Cytochrome <i>b</i>	J. S. RIESKE AND H. D. TISDALE	353
64. Preparation and Properties of a Respiratory Chain Iron-Protein	J. S. RIESKE	357
65. Isolation of Adrenal Cortex Nonheme Iron Protein	T. OMURA, E. SANDERS, D. Y. COOPER, AND R. W. ESTABROOK	362
66. The Preparation of Bacterial Cytochrome <i>b</i> ₁	LOWELL P. HAGER AND SAMIR S. DEEB	367
67. The Preparation and Properties of Cytochrome <i>b</i> ₅₂₂ from <i>Escherichia coli</i>	LOWELL P. HAGER AND EIJI ITAGAKI	373

F. L. CRANE	259
BRUCE MACKLER	261
ROBERT L. LESTER	264
JOHN T. PENNISTON	266
n Oxidation-Reduction	
.....	274
T. S. KING AND ROBERT L. HOWARD	275
BRUCE MACKLER	294
D. R. SANADI, RICHARD L. PHARO, AND LOUIS A. SORDAHL	297
JOHN R. CRONIN, WILHELM R. FRISSELL, AND COSMO G. MACKENZIE	302
LARS ERNSTER	309
NATHAN O. KAPLAN	317
T. S. KING	322
TAKASHI YONETANI	332
TAKASHI YONETANI	336
E. MARGOLIASH AND O. F. WALASEK	339
J. S. RIESKE AND H. D. TISDALE	349
J. S. RIESKE AND H. D. TISDALE	353
J. S. RIESKE	357
T. OMURA, E. SANDERS, D. Y. COOPER, AND R. W. ESTABROOK	362
LOWELL P. HAGER AND SAMIR S. DEEB	367
LOWELL P. HAGER AND EJI ITAGAKI	373

Section V. Isolation and Determination of Other Mitochondrial Constituents

<i>Previously Published Articles from Methods in Enzymology Related to Section V</i>	380
68. Isolation and Determination of Ubiquinone	E. R. REDFEARN 381
69. Isolation, Characterization, and Determination of Polar Lipids of Mitochondria	GEORGE ROUSER AND SIDNEY FLEISCHER 385
70. Removal and Binding of Polar Lipids in Mitochondria and Other Membrane Systems	SIDNEY FLEISCHER AND BECCA FLEISCHER 406
71. Preparation of Mitochondrial Structural Protein	DAVID W. ALLMANN, ALBERT LAUWERS, AND GIORGIO LENA Z 433
72a. Isolation of a Mitochondrial Membrane Fraction Containing the Citric Acid Cycle and Ancillary Enzymes	DAVID W. ALLMANN AND ELISABETH BACHMANN 438
72b. Isolation and Purification of the Outer Membrane and Inner Membrane of Liver Mitochondria	D. F. PARSONS AND G. R. WILLIAMS 443
72c. Separation and Some Enzymatic Properties of the Inner and Outer Membranes of Rat Liver Mitochondria	GIAN LUIGI SOTTOCASA, BO KUYLENSTIERNA, LARS ERNSTER, AND ANDERS BERGSTRAND 448
73. Determination of Nonheme Iron, Total Iron, and Copper	PHILIP E. BRUMBY AND VINCENT MASSEY 463
74. The Fluorometric Determination of Mitochondrial Adenine and Pyridine Nucleotides	RONALD W. ESTABROOK, JOHN R. WILLIAMSON, RENE FRENKEL, AND PABITRA K. MAITRA 474
75. Assay of Nucleotides and Other Phosphate-Containing Compounds by Ultramicro Scale Ion-Exchange Chromatography	HANS W. HELDT AND MARTIN KLINGENBERG 482
76. The Quantitative Determination of Mitochondrial Hemoproteins	J. S. RIESKE 488
77. Quantitative Determination of Mitochondrial Flavins	N. APPAJI RAO, S. P. FELTON, AND F. M. HUENNEKENS 494
78. Extraction and Estimation of Cytochrome <i>c</i> from Mitochondria and Submitochondrial Particles	GIORGIO LENA Z AND DAVID H. MACLENNAN 499
79. Preparation and Properties of a Factor Conferring Oligomycin Sensitivity (F_0) and of Oligomycin-Sensitive ATPase ($CF_0 \cdot F_1$)	YASUO KAGAWA 505
80. Preparation and Properties of a Naturally Occurring Inhibitor of Mitochondrial ATPase	GLADYS C. MONROY AND MAYNARD E. PULLMAN 510

Section VI. Isolation and Assay of Coupling Factors for Oxidative Phosphorylation

<i>Previously Published Articles from Methods in Enzymology Related to Section VI</i>		518
81. The Isolation, Properties, and Assay of ATP Synthetase II	ROBERT E. BEYER	519
82. Preparation and Properties of Mitochondrial ATPase (Coupling Factor 1)	HARVEY S. PENEFSKY	522
83. Preparation of ³ H-Acetyl-ATPase (Coupling Factor 1)	YASUO KAGAWA	526
84a. Coupling Factor 2 (F ₂)	JUNE M. FESSENDEN, M. ANNE DANNENBERG, AND EFFRAIM RACKER	528
84b. Coupling Factor 3 (F ₃)	JUNE FESSENDEN AND EFFRAIM RACKER	530
85. Coupling Factor 4 (F ₄)	T. E. CONOVER AND H. ZALKIN	532
86. Extraction and Purification of an ATP-ADP Exchange Enzyme from Beef Liver Mito- chondria	CHARLES L. WADKINS AND ROBERT P. GLAZE	537
87. Energy-Coupling in Nonphosphorylating Submitochondrial Particles	CHUAN-FU LEE AND LARS ERNSTER	543

Section VII. Microsomal Electron Transport

88. Microsomal DPNH-Cytochrome <i>c</i> Reduc- tase	BRUCE MACKLER	551
89. Cytochrome <i>b_s</i>	PHILIPP STRITTMATTER	553
90. Isolation of Cytochromes P-450 and P-420	TSUNEO OMURA AND RYO SATO	556
91. NADH-Cytochrome <i>b_s</i> Reductase	PHILIPP STRITTMATTER	561
92. The Preparation and Properties of Micro- somal TPNH-Cytochrome <i>c</i> Reductase from Pig Liver	BETTIE SUE SILER MASTERS, CHARLES H. WILLIAMS, JR., AND HENRY KAMIN	565
92a. Microsomal Lipid Peroxidation	LARS ERNSTER AND KERSTIN NORDENBRAND	574

Section VIII. Special Assays and Techniques

<i>Previously Published Articles from Methods in Enzymology Related to Section VIII</i>		582
93. Diffuse Reflectance Spectrophotometry	GRAHAM PALMER	583
94. Electron Paramagnetic Resonance	GRAHAM PALMER	594

Coupling Factors for Oxidation		95. The Preparation of Cytochrome-Deficient Mutants of Yeast	FRED SHERMAN	610
.....	518	96. Methods of Determining the Photochemical Action Spectrum	OTTO ROSENTHAL AND DAVID Y. COOPER	616
ROBERT E. BEYER	519	97. Determination of Oxygen Affinity	FREDERICK J. SCHINDLER	629
HARVEY S. PENEFSKY	522	98. Determination of Acid-Labile Sulfide and Sulfhydryl Groups	TSOO E. KING AND ROY O. MORRIS	634
YASUO KAGAWA	526	99. Sensitive Measurements of Changes of Hy- drogen Ion Concentration	B. CHANCE AND M. NISHIMURA	641
JUNE M. FESSENDEN, M. ANNE DANNENBERG, AND EFRAIM RACKER	528	100. ¹⁴ CO ₂ Measurements during Mitochondrial Respiration	S. C. STUART AND G. R. WILLIAMS	650
JUNE FESSENDEN AND EFRAIM RACKER	530	101. New Developments in Electron Microscopy As Applied to Mitochondria	D. F. PARSONS	655
T. E. CONOVER AND H. ZALKIN	532	102. Methods for Studying Cytochrome-Mito- chondrial Structural Protein Interaction	RICHARD S. CRIDDLE	668
CHARLES L. WADKINS AND ROBERT P. GLAZE	537	103. Characterization of Respiratory Chain Com- ponents by Polyacrylamide Gel Electro- phoresis	KUNI TAKAYAMA AND CLINTON D. STONER	676
CHUAN-PU LEE AND LARS ERNSTER	543	104. Means of Terminating Reactions	MARTIN KLINGENBERG AND ERICH PFAFF	680
Electron Transport		105a. Energy-Linked Low Amplitude Mitochon- drial Swelling	LESTER PACKER	685
BRUCE MACKLER	551	105b. Measurement of Mitochondrial Swelling and Shrinking-High Amplitude	F. EDMUND HUNTER, JR. AND E. E. SMITH	689
PHILIPP STRITTMATTER	553	106. Preparation and Use of Antisera to Respira- tory Chain Components	STARKEY D. DAVIS, THOMAS D. MEHL, RALPH J. WEDGWOOD, AND BRUCE MACKLER	696
TSUNEO OMURA AND RYO SATO	556	107. Antibody against F ₁	JUNE M. FESSENDEN AND EFRAIM RACKER	699
PHILIPP STRITTMATTER	561	108. Synthesis of γ - ³² P-ATP	HARVEY S. PENEFSKY	702
BETTIE SUB SILER MASTERS, CHARLES H. WILLIAMS, JR., AND HENRY KAMIN	565	109. Methods for the Elevation of Hepatic Mi- crosomal Mixed Function Oxidase Levels and Cytochrome P-450	HERBERT REMMER, HELMUT GREIM, JOHN B. SCHENKMAN, AND RONALD W. ESTABROOK	703
LARS ERNSTER AND KERSTIN NORDENBRAND	574	110. Thermal Measurements during Oxidative Reactions	M. POE, H. GUTFREUND, AND R. W. ESTABROOK	708
and Techniques		111. Biological Applications of Ion-Specific Glass Electrodes	BERTON C. PRESSMAN	714
.....	582			
GRAHAM PALMER	583			
GRAHAM PALMER	594			

Section IX. Measurement of Energy-Linked and Associated Reactions

112. Energy-Linked Reduction of NAD ⁺ by Succinate	LARS ERNSTER AND CHUAN-PU LEE	729
113. Energy-Linked Pyridine Nucleotide Transhydrogenase	LARS ERNSTER AND CHUAN-PU LEE	738
114. Energy-Linked Transport of Ca ⁺⁺ , Phosphate, and Adenine Nucleotides	ERNESTO CARAFOLI AND ALBERT L. LEHNINGER	745
114a. Fatty Acid Activation and Oxidation by Mitochondria	SIMON G. VAN DEN BERGH	749
115. Measurement of Protein Synthesis in Mitochondria	MELVIN V. SIMPSON, MAURILLE J. FOURNIER, JR., AND DOROTHY M. SKINNER	755
116. Sodium and Potassium-Stimulated ATPase	R. L. POST AND A. K. SEN	762
117. ³² P-Labeling of Mitochondrial Protein and Lipid Fractions	P. D. BOYER AND L. L. BIBBER	768
118. ³² P-Labeling of a (Na ⁺ + K ⁺)-ATPase Intermediate	R. L. POST AND A. K. SEN	773
119. Analysis of Phosphoproteins	K. AHMED AND J. D. JUDAH	777
AUTHOR INDEX		781
SUBJECT INDEX		798