Current Research in Photosynthesis

Volume II

Proceedings of the VIIIth International Conference on Photosynthesis
Stockholm, Sweden, August 6–11, 1989

edited by

M. BALTSCHEFFSKY
Department of Biochemistry,
University of Stockholm,
Stockholm, Sweden

KLUWER ACADEMIC PUBLISHERS
DORDRECHT / BOSTON / LONDON

GENERAL CONTENTS

Volume I

1. Reaction Centers From Purple Bacteria 1
2. Photosystem II 209
3. O_2 Evolution 675

Volume II

4. Prokaryotic Antennae Systems 1
5. Eukaryotic Antennae Systems 209
6. Photoinhibition 349
7. Photosystem I 523
8. Structure, Function and Dynamics of the Thylakoid Membrane 715

Volume III

9. H^+ ATPases 1
10. Cytochrome B/C_1 and B/F Complexes 221
11. Rubisco 323
12. Structure, Function and Regulation of Photosynthetic Genes 423
13. Biosynthesis and Assembly of the Photosynthetic Apparatus 653
14. Chloroplast Differentiation 827

Volume IV

15. Respiration and Photosynthesis 1
16. Regulation of Chloroplast Metabolism 111
17. Adaptation Mechanisms 291
18. CO_2 Concentrating Mechanisms 433
19. Stress and Photosynthesis 549
20. Photosynthesis in Nature 821

CONTENTS TO VOLUME II

General Contents V

Contents Volume II VII

Preface XXI

Acknowledgements XXIII

Organizing Committees XXV

Opening Speech XXVII

4. Prokaryotic Antennae Systems

Analysis of Phycobilisome and Photosystem I Complexes of Cyanobacteria 1
D.A. Bryant, E. Rhiel, R. de Lorimer, J. Zhou, V.L. Stirewalt, G.E. Gasparich, J.M. Dubbs, W.Snyder

Photophysics and Photobiology of the Carotenoid Singlet State 11
T. Gillbro, P.-O. Andersson, R.J. Cogdell

Efficiency and Kinetics of Energy Transfer in Chlorosome Antennas from Green Photosynthetic Bacteria 17
R.E. Blankenship, J. Wang, T.P. Causgrove, D.C. Brune

Antenna Systems of Green Bacteria and Heliobacteria 25
J. Amesz

Effect of Phospholipase A_2 on the Responsiveness of the Electrochromic Carotenoids of *Rhodobacter sphaeroides* Chromatophores 33
L.M. Olivera, R.A. Niederman

Bacteriochlorophyll *C* Monomers, Dimers, and Higher Aggregates in Dichloromethane and Carbon Tetrachloride 37
J.M. Olson, J.P. Pedersen, T.P. Causgrove, D.C. Brune, R.E. Blankenship

Control of Bacteriochlorophyll a and c in Chloroflexus Aurantiacus 41
J. Oelze

Phycocyanin with Modified Chromophores 45
R, Fischer, J. Gottstein, S. Siebzehnrübl, H. Scheer

Carotenoids from an Aerobic Photosynthetic Bacterium, *Erythrobacter longus* Och 101 49
S. Takaichi, K. Shimada, J.-I. Ishidsu

HPLC Analysis of the Pigments of Purple Photosynthetic Bacteria 53
A.E. Connor, G. Britton

Role of B800-850 Light-Harvesting Pigment-Protein Complex in the Morphogenesis of *Rhodobacter sphaeroides* Membranes 57
J.N. Sturgis, R.A. Niederman

The Peripheral Antenna Polypeptides of *Rp.palustris*: Synthesis of Multiple Forms and Structural Variability as a Consequence of Light Intensity 61
R.A. Brunisholz, M.B. Evans, R.J. Cogdell, G. Frank, H. Zuber

Comparison of the Structural Subunit, B820, of Core Light-Harvesting Complexes of Photosynthetic Bacteria 65
B.A. Heller, P.S. Parkes-Loach, M.C. Chang, P.A. Loach

Probing the Bacteriochlorophyll Binding Site Requirements of the Core Light-Harvesting Complex of Photosynthetic Bacteria Using BCHL Analogs 69
P.A. Loach, T. Michalski, P.S. Parkes-Loach

Reconstitution of the Core Light-Harvesting Complex of Photosynthetic Bacteria with Selected Polypeptides 73
P.S. Parkes-Loach, B.A. Heller, M.C. Chang, W.J. Bass, J.A. Ghanatry, P.A. Loach

Phosphorylation of the Chromatophore Membranes of Rhodospirillum Rubrum G9 and Isolation of a B875 Protein Kinase 77
R. Ghosh, P. Tschopp, S. Eicher, R. Bachofen

Phosphoproteins in the Purple Photosynthetic Bacterium, *Rhodospirillum rubrum* 81
A. Cox, J.F. Allen

Interaction of Hydrophilic Succinimidyl Ethers with Polypeptides of Pigment-Protein Complexes in Assembly A890 from Sulfur Photosynthetic Bacterium Chromatium Minutissimum 85
A.A. Moskalenko, O.A. Toropygina, Yu.E. Erokhin

Studies on the Rod-Substructure of the Phycobilisome from the Cyanobacterium *Mastigocladus laminosus* 89
M. Glauser, W. Sidler, G. Frank, H. Zuber

Isolation and Characterization of the Allophycocyanin Complexes of the Cyanobacterium *Mastigocladus laminosus* 93
A. Esteban, W. Sidler, G. Frank, P. Füglistaller, R. Rümbeli, H. Zuber

Studies on the Phycobiliprotein Complexes from the Red Algae *Porphyridium cruentum* 97
W. Sidler, F. Suter, R. Israels, H. Zuber

Role of the "Anchor" Polypeptide in the Architecture of the Phycobilisome Core: A Molecular Approach 101
V. Capuano, N. Tandeau de Marsac, J. Houmard

The Supramolecular Structure of the Light-Harvesting System of Cyanobacteria and Red Algae 105
W. Lange, G.-H. Schatz, C. Wilhelm, E. Mörschel

Quantitation of Reaction Centers by HPLC Analysis of Minor but Key Chlorophyll-Type Pigments 109
T. Watanabe, M. Kobayashi

A Method for Studying Pigment Organization in Photosynthetic Complexes 113
H. van Amerongen, M. van Gurp, F. van Mourik, B. van Haeringen, R. van Grondelle

Spectral Shift of Purple Bacterial Carotenoids Related to Solvent and Protein Polarizability 117
P.O. Andersson, T. Gillbro, L. Ferguson, R.J. Cogdell

Polarized Absorption Spectra of the B800-850 Light-Harvesting and the RC-B875 Reaction Center Complexes from Purple Bacteria 121
K. Steck, T. Wacker, G. Drews, N. Gad'on, R. Cogdell, W. Welte, W. Mäntele

Linear Dichroism and Orientation of Chromophores in *Nostoc* sp. Phycobilisomes and their Subunits 125
L.J. Juszczak, B.A. Zilinskas, N.E. Geacintov, J. Breton

Role of Aggregation State in Directed Energy Transfer within the B875 Light-Harvesting Complex of *Rhodobacter sphaeroides* 129
W.H.J. Westerhuis, R. Theiler, R.A. Niederman

Spectroscopic Characterization of a Subunit Form of Light-Harvesting Complex I from *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* 133
R.W. Visschers, M.C. Chang, F. van Mourik, P.A. Loach, R. van Grondelle

A Spectroscopic Characterization of the Low-Light B800-850 Light-Harvesting Complex of *Rhodopseudomonas palustris* 137
F. van Mourik, A.M. Hawthornthwaite, C.A. Vonk, R.J. Cogdell, R. van Grondelle

Pigment Organization in Bchl-*a*-Free and Bchl-*a*-Containing Chlorosomes from *Chloroflexus aurantiacus*, Studied by Absorption Dichroism 141
F. van Mourik, K. Gribenow, B. van Haeringen, A.R. Holzwart, R. van Grondelle

Properties of a Solubilized and Purified Antenna-Reaction Center Complex from Heliobacteria 145
E.J. van de Meent, F.A.M. Kleinherenbrink, J. Amesz

Effects of Conformation and Environment on Bacteriochlorophyll Optical Spectra: Correlation of Calculated Spectra with Structural Results 149
E. Gudowska-Nowak, M.D. Newton, J. Fajer

Temperature Dependence of Energy Transfer from the Long Wavelength Antenna BChl-896 to the Reaction Center in *Rhodospirillum Rubrum, Rhodobacter Sphaeroides* (w.t. and M21 Mutant) from 77 to 177 K, Studied by Picesecond Absorption Spectroscopy 153
K.J. Visscher, H. Bergström, V. Sundström, C.N. Hunter, R. van Grondelle

Excitation Transport and Quenching in Photosynthetic Bacteria at Normal and Cryogenic Temperatures 157
A. Freiberg, V.I. Godik, T. Pullerits, K. Timpmann

Singlet-Singlet Annihilation in the Antenna of *Rhodospirillum rubrum* at Low Temperatures 161
G. Deinum, T.J. Aartsma, R. van Grondelle, J. Amesz

Time-Resolved Measurements of Fluorescence from the Photosynthetic Membranes of *Rhodobacter capsulatus* and *Rhodospirillum rubrum* 165
N. Woodbury, E. Bittersmann

Picosecond Fluorescence Studies of *Rhodopseudomonas viridis* 169
E. Bittersmann, R.E. Blankenship, N. Woodbury

Energy Transfer Dynamics in Three Light Harvesting Mutants of *Rhodobacter* Sphaeroids: A Picosecond Spectroscopy Study 173
H. Bergström, N. Hunter, R. van Grondelle, V. Sundström

Fluorescence Lifetime Measurements of Energy Transfer in Chlorosomes and Living Cells of *Chloroflexus aurantiacus* OK 70-fl 177
M.G. Müller, K. Griebenow, A.R. Holzwarth

Energy Transfer Kinetics in Different Chlorosome Preparations from *Chloroflexus autrantiacus* 181
M. Miller, T. Gillbro, R.P. Cox

Excitation Transfer and Charge Separation in Heliobacterium Chlorum at 15 K 185
P.J.M. van Kan, T.J. Aartsma, J. Amesz

Triplets in the Antenna and Reaction Centre of Heliobacterium Chlorum at Low Temperature 189
F.A.M. Kleinherenbrink, J. Amesz

Excitation Energy Flow in the Aerobic Photosynthetic Bacterium, *Erythrobacter* sp. OCh 114: A Fast Energy Transfer from B806 to B870 193
K. Shimada, N. Tamai, I. Yamazaki, M. Mimuro

Excitation Energy Transfer Among Phycobilisomes from the Phycoerythrin Containing Strain *Anabaena variabilis* ARM310 197
P.S. Maruthi Sai, S. Mahajan

Separation of PS I and PS II from the Prochlorophyte (Oxychlorobacterium) *P. Hollandica* and the Role of CHL *b* 201
H.C.P. Matthijs, H. Reith, G.WM. van der Staay, L.R. Mur

High Resolved Low Temperature Spectroscopy of Photosynthetic Systems 205
J. Hala, J. Dian, O. Prasil, M. Vacha, K. Vacek

5. Eukaryotic Antennae Systems

The Role of Light Harvesting Complex II and of the Minor Chlorophyll a/b Proteins in the Organization of the Photosystem II Antenna System 209
R. Bassi, P. Dainese

Structure of the Light-Harvesting Chlorophyll a/b Protein Complex by High-Resolution Electron Crystallography 217
W. Kühlbrandt

The Functional Organization of the Antenna Systems in Higher Plants and Green Algae as Studied by Time-Resolved Fluorescence Techniques 223
A.R. Holzwarth

Selective Cleavage of the LHCP Precursor May Determine the Relative Abundance of the Two Mature Forms (-26 and 25 kD) Found In Vivo 231
G.K. Lamppa, S.E. Clark, M.S. Abad

The Extraction and Assay of Refractory Chlorophylls and a Simple Method to Correct Data from Arnon's Equations 237
R.J. Porra

Formation of Chlorophyll a-Protein Complexes Absorbing at 685 NM in the Presence of Tetrahydrofuran 241
K. Uehara, Y. Hioki, M. Mimuro

Photooxidation and Pheophytin Formation of Chlorophyll in the Light-Harvesting CHL-a/b-Protein Complex Exposed to Fatty Acids: Protective Role of the Intact Apoproteins 245
D. Siefermann-Harms

Properties of the Minor Chlorophyll a/b Proteins CP29, CP26 and CP24 from *Zea mays* Photosystem II Membranes 249
P. Dainese, M.L. Di Paolo, M. Silvestri, R. Bassi

The 20 kDA Apo-Polypeptide of the Chlorophyll a/b Protein Complex CP24 – Characterization and Complete Primary Amino Acid Sequence 253
M. Spangfort, U.K. Larsson, U. Ljungberg, M. Ryberg, B. Andersson, D. Bartling, N. Wedel, R.G. Herrmann

Phosphorylation of the LHC II-Apoproteins of *Chlamydomonas reinhardii* 257
M. Sigrist, A. Boschetti

The Major Light-Harvesting Chlorophyll a/b Protein (LHC IIb): The Smallest Subunit is a Novel Cab Gene Product 261
D.T. Morishige, J.P. Thornber

Characterization of Isolation Inner and Outer Spinach LHCII 265
B. Andersson, M. Spangfort

Resolution of up to Eighteen Chlorophyll-Protein Complexes from Vascular Plant Thylakoids Using a New Green Gel System 269
K.D. Allen, T.G. Falbel, S.L. Shaw, A. Bennett, L.A. Staehelin

Heterogeneities of the Long-Term Acclimation of Light-Harvesting in Photosystem II 273
P. Mäenpää, B. Andersson

Restoration of an Oligomeric Form of the Light-Harvesting Antenna CP II and of a Fluorescence State II-State I Transition by $_{\Delta}3$-*Trans*-Hexadecenoic Acid-Containing Phosphatidylglycerol, in a Mutant of *Chlamydomonas* 277
J. Garnier, B. Wu, J. Maroc, D. Guyon, A. Tremolieres

Circular Dichroism of Pigment-Protein Complexes of Higher Plants 281
C.L. Chong, C.-H. Xu, Y.-L. Dai, F.-H. Zhou, K.-B. Wang, G.Z. Zhang

Isolation of the LHC I Complex of Barley Containing Multiple Pigment-Proteins 285
S. Anandan, J.P. Thornber

Femtosecond Dynamics of Carotenoid to Chlorophyll Energy Transfer in Thylakoid Membrane Preparation from *Phaeodactylum tricornutum* and *Nannochloropsis* sp. 289
J.K. Trautman, A.P. Shreve, T.G. Owens, A.C. Albrecht

Carotenoid to Chlorophyll *a* Singlet Energy Transfer: Direct Evidence for Involvement of the Carotenoid 'A' State in an Algal Light-Harvesting System 293
A.P. Shreve, J.K. Trautman, T.G. Owens, A.C. Albrecht

Picosecond Chlorophyll Fluorescence from High Plants 297
S.W. McCauley, E. Bittersmann, M. Mueller, A.R. Holzwarth

Interaction Between LHC-11 Antenna and PS 2 Core in Thylakoid Vesicles 301
L.O. Pålsson, T. Gillbro, P. Svensson, P.Å. Albertsson

Competition Between Trapping and Annihilation in PS II 305
W. Leibl, J. Breton, J. Deprez, H.-W. Trissl

Excitation Energy Flow in the Marine Dinoflagellate *Protogonyalux tamarensis* 309
M. Mimuro, N. Tamai, T. Ishimaru, I. Yamazaki

Chlorophyll Spectral Heterogeneity and Energy Transfer to PS II Reaction Centres 313
F.M. Garlaschi, G. Zucchelli, R.C. Jennings

Does LHCP II Have a Long Wavelength Chlorophyll a Spectral Forms? 317
G. Zucchelli, R.C. Jennings, F.M. Garlaschi

A Spectroscopic Study of a Photosystem I Antenna Complex 321
I. Mukerji, K. Sauer

Polarized Pump-Probe Spectroscopy of Photosystem I Antenna Excitation Transport 325
T.P. Causgrove, S. Yang, W.S. Struve

Competition Between Trapping and Annihilation in Photosystem I 329
H.-W. Trissl, J. Breton, J. Deprez, A. Dobek, G. Paillotin, W. Leibl

Cord-Like Phycobilisomes of *Rhodosorus marinus* Geitler: Fine Structure and its Functional Implications 333
T. Dibbayawan, M. Vesk, A.W.D. Larkum

Spectroscopic and Orientational Properties of Chlorophylls a and b in Lipid Membranes 337
M. van Gurp, G. van Ginkel, Y.K. Levine

Reconstitution of Light Harvesting Complexes: A Single Apoprotein Binds CHLa, CHLb, and Xanthophylls 341
K. Cammarata, F.G. Plumley, G.W. Schmidt

Spectral Resolution of the Absorption and Emission Transitions of Chlorophylls 345
M. van Gurp, G. van Ginkel, Y.K. Levine

6. Photoinhibition

On the Mechanisms for the Photoinhibition of the Electron Transfer and the Light Induced Degradation of the D1 Protein in Photosystem II 349
S. Styring, C. Jegerschöld, I. Virgin, A. Ehrenberg, B. Andersson

Zeaxanthin-Associated Energy Dissipation and the Susceptibility of Various Organisms to Light Stress 357
B. Demmig-Adams

Effect of Temperature and PFD on the Susceptibility of Leaves to Photoinhibition and Recovery 365
D.H. Greer, W.A. Laing

Effects of Photoinhibition on the Turnover of P680 in Oxygen Evolving and Tris-Treated PS II-Membrane Fragments from Spinach 373
B. Geiken, H.-J. Eckert, J. Bernarding, Napiwotzki, G. Renger

The Mechanism of Photoinhibition of Spinach Thylakoids 377
A. Wild, M. Richter, W. Rühle

UV-Photoinhibition: Studies *in vitro* and in Intact Plants 381
C.T. Yerkes, D.M. Kramer, J.M. Fenton, A.R. Crofts

The Effect of UV-B Radiation on Photosynthesis and Photosystem II of Phytoplanktonic and Benthic Algae and Seagrasses 385
A.W.D. Larkum, W.F. Wood

Ambiguous Role of Oxygen in the Photoinactivation of PS II Particles 389
J. Masojídek, L. Nedbal, J. Komenda, O. Prášil, I. Šetlík

Photoinactivation of Photosynthetic Electron Transport under Anaerobic and Aerobic Conditions in Isolated Thylakoids of Spinach 393
R. Chaturvedi, M. Singh, P.V. Sane

Photoinhibition of Photosynthesis and Turnover of the 32 KD Chloroplast Protein in Various Atmospheres 397
H. Gong, S. Nilsen

Photoinhibition in Cyanobacteria: Sensitivity to Calcium or Sodium 401
J.J. Brand, J. Zhao, G. Wünschmann, J.H. Golbeck

Studies of the Mechanism for Photoinhibition of the Electron Transport Through Photosystem II 405
C. Jegerschöld, S. Styring

Mechanism of the Light Dependent Turnover of the D1 Protein 409
N. Adir, S. Shochat, Y. Inoue, I. Ohad

The Role of Photosystem II D1 Apoprotein Metabolism in the Physiology of Photoinhibition 415
C.A. Shipton, J.B. Marder, J. Barber

PS2 Proteins Involved in Photoinhibition 419
M.T. Giardi, R. Barbato, P. Dainese, F. Rigoni, G.M. Giacometti

Consequences of Light Induced D1-Protein Degradation on Thylakoid Membrane Organization 423
I. Virgin, T. Hundal, S. Styring, B. Andersson

Mutations in D1 Protein Responsible for a Faster Photoinhibition Process 427
D. Kirilovsky, G. Ajlani, M. Picaud, A.-L. Etienne

Susceptibility of Photosynthesis to Photoinhibition of *psbA* Gene-Inactivated Strains of *Anacystis nidulans* 431
Z. Krupa, G. Öquist, P. Gustafsson, S.S. Golden

Activation of a Reserve Pool of Photosystem II in *Chlamydomonas reinhardtii* Counteracts Photoinhibition 435
P.J. Neale, A. Melis

Effects of Light and Temperature on PSII Heterogeneity 439
E.-M. Aro, E. Tyystjärvi, A. Nurmi

Photoinhibition in the C_4 Grass *Zea mays*. 443
B.S. Ripley, A.M. Amory, N.W. Pammenter, C.F. Cresswell

Photoinhibition of Photosynthesis and Its Recovery in Low and High Light Acclimatized Blue-Green Alga (Cyanobacterium) *Spirulina platensis* 447
R. Shyam, P.V. Sane

Photoinhibition in Thylakoids and Intact Chloroplasts of *Codium fragile* (Suringar) Hariot 451
A.H. Cobb, R.M. Hopkins, M.L. Williams, R.V. Sealey

Adaptations of *Codium fragile* (Suringar) Hariot Fronds to Photosynthesis at Varying Flux Density 455
R.V. Sealey, M.L. Williams, A.H. Cobb

Temperature Dependency of Photoinhibition in Pumpkin 459
E. Tyystjärvi, E.-M. Aro

Photoinhibition of Photosynthesis in a Winter Wheat Crop 463
Q.J. Groom, S.P. Long, N.R. Baker

Spring and Winter Wheats Exhibit Differential Susceptibility to Low Temperature Inhibition of Photosynthesis 467
V.M. Hurry, N.P.A. Huner

Effects of Cold Acclimation on the Susceptibility of Photosynthesis to Photoinhibition 471
G. Öquist, N.P.A. Huner

Intraspecific Variation in Susceptibility to Photoinhibition During Chilling of *Cyperus longus* L Populations from Europe 475
A.E. Gravett, S.P. Long

Photoinhibition in Spinach Leaves at Low Temperature in the Field 479
S. Somersalo, G.H. Krause

Increase in Activities of Scavengers for Active Oxygen in Spinach Related to Cold Acclimation in Excess Light 483
S. Schöner, C. Foyer, M. Lelandais, G.H. Krause

The Effect of Photoinhibition, Salt Stress and Their Interaction on Photosynthesis in Sorghum 487
P.K. Sharma, D.O. Hall

Turnover of the Xanthophyll Cycle During Photoinhibition and Recovery 491
C.H. Foyer, M. Dujardyn, Y. Lemoine

Zeaxanthin Formation in q_E-Inhibited Chloroplasts 495
A.M. Gilmore, H.Y. Yamamoto

Zeaxanthin Formation in Etiolated Leaves 499
L. Montañes, L. Heras, J. Val, E. Monge

Chlorophyll a Fluorescence (77K) and Zeaxanthin Formation in Leaf Disks (*Nicotiana tabacum*) and Isolated Thylakoids (*Lactuca Sativa*) 503
E. Pfündel, R.J. Strasser

Photoinhibition of Photosynthetic Bacteria 507
R.E. Cleland, D. Rees, D.A. Walker, P. Horton

Photoinhibition by High Light in a Shade Plant Role of O_2 Concentration 511
M. Andre, M. Ducamp, T. Betsche

Photoinhibition of Photosystem Two is Increased by Freezing *in vivo* or Inhibition of Water Oxidation *in vitro* 515
W.Q. Wang, D.J. Chapman, J. Barber

Oxygen Quenching of Triplet States in Isolated Photosystem 2 Reaction Centres: A Mechanism for Photodamage 519
L.B. Giorgi, B. Crystall, P.J. Booth, J.R. Durrant, J. Barber, D.R. Klug, G. Porter

7. Photosystem I

Chloroplast Encoded Photosystem I Polypeptides of Barley 523
B.L. Møller, H.V. Scheller, J.S. Okkels, B. Koch, B. Andersen, H.L. Nielsen, I. Olsen, B.A. Halkier, P.B. Høj

Resolution and Reconstruction of the Photosystem I Reaction Center Complex in Green Plants and Cyanobacteria 531
J.H. Golbeck

Electron Transfer Reactions of Photosystem I Involving the Secondary Acceptor A_1 539
P. Sétif, H. Bottin, K. Brettel

On Isolated Complexes of Reaction Center I and X-Ray Characterization of Single Crystals 547
H.T. Witt, M. Rögner, U. Mühlenhoff, I. Witt, W. Hinrichs, W. Saenger, Ch. Betzel, Z. Dauter

Photosystem I Reaction Center of Mastigocladus Laminosus: Structural and Functional Aspects 555
G. Shoham, D. Michaeli, R. Nechushtai

Molecular Weight Determination of an Active and Monomeric PSI Complex 563
M.E. Schafheutle, E. Šetlíková, P.A. Timmins, I. Šetlík, W. Welte

A Comparison of the PS1 Polypeptide Organization of Soybean and the Cyanobacterium, *Anacystisnidulans* 567
R.L. Henry, M. Li, J.A. Guikema

N.-Terminal Sequence of 5 KDA Polypeptide Associated with Photosystem I Core Complex from Spinach 571
S. Hoshina, N. Kunishima, K. Wada, S. Itoh

Organization of PSI Subunits in Thylakoid Membranes 575
A. Zilber, R.M. Wynn, A. Webber, R. Malkin

Topography of Photosystem I in Cyanobacteria 579
J. Hladík, L. Pospíšilová, D. Sofrová

Extrinsic Membrane Proteins of Photosystem I 583
S. Tjus, B. Andersson

A Photosystem -1 Reaction Center Complex Constituted Only by Two Subunits 587
T. Hiyama, N. Yanai, Y. Takano, H. Ogiso, K. Suzuki, K. Terakado

Isolation and Characterization of cDNA Clones Encoding Five Subunits of Photosystem I from the Green Alga *Chlamydomonas reinhardtii* 591
L.-G. Franzén, G. Frank, H. Zuber

Variations of the Differential Extinction Coefficient of P-700 and Re-Estimation of Stoichiometry of Constituents in Photosystem I Reaction Center Complexes from *Synechococcus elongatus* 595
K. Sonoike, S. Katoh

Characterization of P700 by FTIR Difference Spectroscopy 599
E. Nabedryk, M. Leonhard, W. Mäntele, J. Breton

Multiple Functions of β-Carotene in Photosystem I 607
I. Damm, D. Steinmetz, L.H. Grimme

Energy Transfer Kinetics in Photosystem I Particles Isolated from *Synechococcus sp.* and from Higher Plants 611
A.R. Holzwarth, W. Haehnel, R. Ratajczak, E. Bittersmann, G.H. Schatz

Picosecond Absorption Measurements of Photosystem 1 from the Cyanobacterium, *Chlorogloea fritschii* 615
E.H. Evans, R. Sparrow, R.G. Brown, R. Chittock, W. Toner, M.C.W. Evans

The Transient Photosystem I EPR Spectra Correlated Spin Pair Concept and Structural Conclusions 619
D. Stehlik, C.H. Bock, A.J. van der Est

Fast Electron Transfer Kinetics in Photosystem I from Transient EPR-Spectroscopy at Room Temperature 623
C.H. Bock, K. Brettel, A.J. van der Est, I. Sieckmann, D. Stehlik

Electron Transfer in Photosystem I from Synechococcus sp. under Reducing Conditions 627
K. Brettel

Dark Reduction of the Photosystem-I Electron Acceptor A1 631
H. Bottin, P. Sétif

Nanosecond Spectroscopy of Early Electron Transfers in Photosystem 1. Iron-Sulfur Cluster F_X is Reduced within 5 Nanoseconds 635
J.T. Warden

Functional Role of Phylloquinone on the Acceptor Side of Photosystem 1 639
J. Biggins, N.A. Tanguay, H.A. Frank

Properties of P700-Enriched Particles Isolated from the Thermophilic Cyanobacterium *Synechococcus elongatus* 643
I. Ikegami, S. Itoh, S. Katoh

Function of Substituted Quinones as the Electron Acceptor A-1 (Phylloquinone) in Photosystem I Reaction Center 647
M. Iwaki, S. Itoh

Competitive Bindings of Herbicide and Quinone to Photosystem I Phylloquinone (A-1) Binding Site 651
S. Itoh, M. Iwaki

The Sites of Photoinhibition Around Photosystem I in Chloroplasts 655
K. Inoue, T. Fujii, E.-I. Yokoyama, N. Kusumoto, H. Sakurai

Structure and Function of Ferredoxin-NADP Reductase Complex 659
M. Shin

Reactivation of Dark-Inactivated *Anabaena* FNR 663
M.F. Fillat, C. Gomez-Moreno

Absorbance Transients of Ferredoxin:NADP$^+$ Reductase in Isolated Thylakoid Membranes 667
G. Garab, Y. Hong, S.J. Coughlan, H.C.P. Matthijs, G. Hind

Nearest Neighbour Analysis of the Photosystem I Subunits in Barley and Their Binding of Ferredoxin 671
B. Andersen, B. Koch, H.V. Scheller, J.S. Okkels, B.L. Møller

Identification of the Plastocyanin Binding Subunit of Photosystem I 675
M. Hippler, R. Ratajczak, W. Haehnel

Photosystem I in Barley: Subunit PSI-F is not Essential for the Interaction with Plastocyanin 679
H.V. Scheller, B. Andersen, S. Okkels, I. Svendsen, B.L. Møller

Simulation of the Flash Induced $P_{700}+$ Reduction Kinetics Using a Modified Simplex Method Scope and Limitation of the Approach 683
E. vander Donckt, R. Vandeloise, S. Mauro

Low Potential Cytochrome C_{550} Function in Cyanobacteria and Algae 687
D.W. Krogmann, S. Smith

Photoelectrochemical Monitoring of Photosystem I Electron Transport with Oxygen as Acceptor 691
D.C. Goetze, R. Carpentier

PS I Particles Assembled on Crista Membranes can Drive Electron Transport and Phosphorylation 695
S.-J. Li, Y.-N. Xu, J. Wan, W.-L. Ying, Y.-S. Guo

Characteristics of Photosystem Activity of Cyanobacteria Immobilized in Polymer Foams 699
C. Garbisu, H. Wen, D.O. Hall, J.L. Serra

Labeling by a Fluorescent Maleimide of Peptides of Chromatophores from a Greensulfur Bacterium, *Chlorobium limicola* 707
S.-I. Shirasawa, H. Sakurai

Heliobacterium chlorum, An EPR Investigation of the Electron Acceptors 711
M.R. Fischer, A.J. Hoff

8. Structure, Function and Dynamics of the Thylakoid Membrane

The Fluid-Mosaic Nature of the Thylakoid Membrane 715
J. Barber

The Structure of Photosystem I and II 725
D.J. Simpson

Cycling of the Photosystem II Reaction Center Core Between Grana and Stroma Lamellae 733
M.L. Ghirardi, F.E. Callahan, S.K. Sopory, T.D. Elich, M. Edelman, A.K. Mattoo

Lateral Diffusion of Plastocyanin and Plastoquinol in Thylakoid Membranes 739
W. Haehnel, R. Mitchell, R. Ratajczak, A. Spillmann, H. Robenek

Redox Control and Sequence Specificity of a Thylakoid Protein Kinase 747
H.P. Michel, W.E. Buvinger, J. Bennett

Molecular Order and Dynamics in Membranes Containing α-Tocopherol, (Trans 16:3)-Phosphatidyl-Glycerol and Other Thylakoid Lipids 755
G. van Ginkel, L.J. Korstanje, R. Moormans, G. Pruijsen, M. van Zandvoort, A.A. van 't Veld, Y.K. Levine

Effects of Bicarbonate on Thylakoid Phosphorylation and on Photoinhibition 759
C. Sundby, U.K. Larsson, T. Henrysson

Inorganic Pyrophosphate (PPi) Dependent LHCII and PSII Protein Phosphorylation 763
A. Pramanik, S. Bingsmark, H. Baltscheffsky, M. Baltscheffsky, B. Andersson

Investigation of Thylakoid Protein Kinase Substrate Specificity Using Synthetic Peptides 767
I.R. White, P.A. Millner, J.B.C. Findlay

Regulation of Photosynthetis: α- to β-Conversion of Photosystem *II* and Thylakoid Protein Phosphorylation 771
M. Timmerhaus, E. Weis

On the Phosphorylation and Dephosphorylation of LHC-II and of the Protein Kinase of Spinach Thylakoids 775
G. Forti, C. Resta, A. Sangalli

Specific Loss of LCHII Phosphorylation in a *Chlamydomonas* Mutant Lacking the Cytochrome b$_6$/f Complex 779
A. Gal, L.J. Mets, I. Ohad

LHCII Kinase Activity Associated with Isolated Cytochrome b$_6$/f Complex 783
A. Gal, T.S. Mor, G. Hauska, R. Hermann, I. Ohad

Characterisation of a Guanine Nucleotide Binding Protein Associated with the Thylakoid Membrane 787
P.A. Millner, K.-D. Hinsch, J. Clarkson

Dynamic Rearrangements in Different Photosynthetic Membranes Induced by Phosphoproteins 791
S.M. Kochubey, O.I. Volovik

The Participation of LHCP II in Adaptive Reorganization of Photosynthetic Membranes 795
O.I. Volovik, T.T. Zhuravel, S.M. Kochubey

Phosphorylation of LHCII at Low Temperatures 799
S. Bingsmark, U.K. Larsson, B. Andersson

The Granal Margins of Plant Thylakoid Membranes: An Important Nonappressed Domain 803
J.M. Anderson, D.J. Goodchild, W.W. Thomson

The Specific Surface of Thylakoid Membranes as Determined by Absorption Spectroscopy of Single Blebs 809
B. Stolz, D. Walz

Surface Charge Densities, Lipid Compositions and Fluidities of Thylakoid Membranes Showing Different Degrees of Stacking 813
C. Dahlin, I.M. Møller, H. Ryberg, A.S. Sandelius

The Role of Electrical Potential in Light-Induced Swelling and Shrinkage of Thylakoid Membranes 817
H.G. Ding, Y.K. Shen

Experimental Investigation of Energy Transfer Processes in the Model Photosynthetic Systems I 821
P. Pančoška, M. Urbanová, L. Bednárová, K. Vacek

Theoretical Investigation of Energy Transfer Processes in the Model Photosynthetic Systems II 825
L. Skála, V. Kapsa, K. Vacek

Cytochrome F as Indicator for the Interaction of the Two Photosystems in the State 1 and State 2 827
W. Rühle, A. Weinzettel, P. Baur, A. Wild

Heterogeneity of the Functional Antenna Size of Photosystem I from Spinach Thylakoids 831
E. Andreasson, P. Svensson, P.-Å. Albertsson

Heterogeneity of PSIIα 835
P.-Å. Albertsson, S.-G. Yu, U.K. Larsson

Properties of a Non-Detergent PSII Membrane Preparation 839
P. Svensson, S.-G. Yu, U.K. Larsson, E. Andreasson, P.-Å. Albertsson

Photosynthetic Characterization of Thylakoid Membrane Preparations Isolated from Spinach Grow Under Different Light Conditions 843
S.-G. Yu, E. Andreasson, P.-Å. Albertsson

Effects of Acridine Derivatives on Energy Transduction in Thylakoid Membranes of Anabaena sp. 847
H.A. Simoes, C.B.C. Silva, R.M. Chaloub

The Influence of the Temperature in the Solubilization by SDS of the Chlorophyll-Protein Complexes of *Anabaena variabilis* 851
M.A. Rodrigues, C.P. Santos

Reconstitution of the Light-Harvesting CHL *a*/*b* Protein Complex of the CHL *b*-Less Mutant cbn1-48 of *Chlamydomonas reinhardtii* with a Pigment Extract Derived from Wildtype 855
D. Steinmetz, I. Damm, L.H. Grimme

Turnover of LHC-1 and LHC-11 Apoproteins in CHL *B*-Deficient Mutants of Rice and Barley 859
T. Terao, S. Katoh

Light Availability Influences *psbA* Expression and the Ratio of Two Forms of D1 in Cyanobacterial Thylakoids 863
M.R. Schaefer, S.A. Bustos, S.S. Golden

Characterisation of a Temperature-Sensitive Mutant of Barley 867
J. Knoetzel, D. Simpson

Biosynthesis and Distribution of the Ferredoxin-NADP Oxidoreductase Binding Protein 871
R.H. Vallejos, R.L. Chan, F.C. Soncini

The ATP-Dependent Post Translational Modification of Ferredoxin: NADP$^+$ Oxidoreductase 875
M. Hodges, M. Miginiac-Maslow, P. Le Marechal, R. Remy

Organization of the Plastoquinone Pool in Chloroplasts: Evidence for Clusters of Different Sizes 879
P. Joliot, J. Lavergne, D. Béal

Flash-Induced Absorbance Changes in Thylakoid Membranes in the 490-550 mm Wavelength Region. The Gramicidin-Insensitive Component 883
W.J. Vredenberg, W. Versluis, J.J.J. Ooms

Effect of Phosphonic Acid Ester PAE-6 on Electron Transport in Thylakoid Membranes of Wheat and Broad Bean 887
O. Górnicka, K. Strzałka

State Transitions in Photomorphogenetic Mutants of Tomato 891
W.F. Buurmeijer, J.H.A.M. Wonders, W.J. Vredenberg

Serine Protein Phosphorylation in the Non-Appressed (Stroma) Thylakoid Membranes 895
E. Garcia-Véscovi, H.A. Lucero

The Role of LHCII Phosphorylation and D1 Turnover in the Structure and Function of PSII 899
C. Critchley

A New Green Gel System that Resolves Up to Twenty Chlorophyll-Protein Complexes from *Chlamydomonas reinhardtii* Thylakoids 903
K.D. Allen, L.A. Staehelin

Surface-Enhanced Resonance Raman Scattering (Serrs) Spectroscopy of Bacterial Membranes: The Flavoproteins 907
R. Picorel, T. Lu, R.E. Holt, T.M. Cotton, M. Seibert, E.E. Aula Dei

Complete Suppression of Oxygen Evolution in Open PS2 Centers by Non-Photochemical Fluorescence Quenching? 911
J.F.H. Snel, W. van Ieperen, W.J. Vredenberg

How Does Protein Phosphorylation Control Protein-Protein Interactions in the Photosynthetic Membrane? 915
J.F. Allen

P-700 Photooxidation in State 1 and in State 2 in Cyanobacteria Upon Flash Illumination with Phycobilin and Chlorophyll Absorbed Light 919
N.F. Tsinoremas, J.A.M. Hubbard, M.C.W. Evans, J.F. Allen

Organization of the Thylakoid Membrane with Respect to the Four Photosystems, PSIα, PSIβ, PSIIα and PSIIβ 923
P.-Å. Albertsson, E. Andreasson, A. Persson, P. Svensson

Chlorophyll Fluorescence Quenching and Protein Phosphorylation in Rice Mutants with Different Contents of Chlorophyll B 927
Y. Kobayashi, K., Chyaen, T. Yoshihira, K. Yamamoto, N. Iwata, T. Oku

The Effect of Various Detergents on the Fragmentation of Thylakoid Membranes During Leaf Ontogeny 931
N. Wilhelmová, Z. Sesták

Light Regulation of the Photosystem II and Photosystem I Reaction Centres of Plant Thylakoid Membranes 935
W.S. Chow, J.M. Anderson

Structure of an Aminoglycoosphingolipid from Chlorobium 939
M.T. Jensen, J. Knudsen, J.M. Olson

The Interaction of State Transitions and Chlororespiration in the Xanthophycean Alga Pleurochloris Meiringensis 943
C. Büchel, C. Wilhelm

Index of Names 947

PHYCOCYANIN WITH MODIFIED CHROMOPHORES

FISCHER, R., GOTTSTEIN, J., SIEBZEHNRÜBL, S., SCHEER, H.
Botanisches Institut der Universität, Menzinger Str. 67,
D-8000 MÜNCHEN 19, FRG

1. INTRODUCTION

Phycocyanin plays an important role in collecting light energy and transfering it to the reaction center (1,2). *In vivo*, this chromoprotein is highly aggregated as part of the phycobilisomes (3-5). *In vitro*, in buffers of low ionic strenght, the phycobilisomes dissociate. Under these conditions, phycocyanin of *Mastigocladus laminosus* is aggregated to ring-shaped trimers $(\alpha ß)_3$. The α-subunit carries one (α-84) the ß-subunit two covalently linked cyanobilin-chromophores (ß-84 and ß-155) (6,7). These trimers are generally associated with linker-peptides, which can principally function as structural elements, as "terminators" of antenna rod building, but are also involved in fine tuning of the chromophore spectra for optimum energy transfer (3-6). In order to investigate the role of individual chromophores in energy transfer, we are modifying distinct chromophores within energy transfering chromophore ensembles. During such studies, we have now found that chromophore modifications have also a profound influence on protein-chromophore interactions and chromoprotein aggregation. It is this aspect which is presented here.

2. MATERIAL and METHODS

Phycocyanin was isolated according to ref. 8. Subunits were prepared via isoelectric focusing under denaturing, anaerobic conditions (9).

Chromophore modifications: 1) **Reduction with borohydride** (modified from ref. 10). A solution of PC or isolated subunits (chromophore concentration 7-21μM, 0.9M potassium phosphate, pH 7, 8M urea) was treated with $NaBH_4$ (170 mM). After complete reduction (spectrum, ≈45 min) excess reductant was destroyed by glucose. For reoxidation experiments, the samples (100mM potassium phosphate, pH 7, containing 70% ammonium sulfate to prevent protein degradation) were allowed to stand at room temperature in the dark for up to nine days. This was followed by recombination (if not yet done), denaturation (8M urea in 100mM potassium phosphate, pH7) and renaturation as described below. 2) **Photobleaching** of the α-subunit (8μM protein, 100 mM phosphate, 8M urea, pH 7.5) was

M. Baltscheffsky (ed.), Current Research in Photosynthesis, Vol. II, 45–48.
© 1990 *Kluwer Academic Publishers. Printed in the Netherlands.*

carried out with 350 nm light over four periods of 30 min each (11).
3) **Indirect chromophore modification** was done by titration of trimeric phycocyanin $(\alpha\beta)_3$ with PCMS (para-chloro-mercury-benzenesulfonate) in a 1.1-1.2 fold excess (12).

Recombination: Isolated subunits were modified and then hybridized with the respective "partners" by dialysis of combined samples containing 5 mM mercaptoethanol, against 100mM potassium phosphate, pH 7 , 25°C over night, and then with new buffer at 4°C for 5hrs. In control experiments, PC was modified *in toto*. Linker-peptide preparations were also added in some experiments. The modification and recombination products were tested by UV-vis-absorption, SDS-PAGE, sedimentation behaviour (13) and (in some cases) gel-filtration on sephadex-G-75 (Serva, Heidelberg). The chromoprotein bands (which were distinct by their color) were marked and quantitated by absorption spectroscopy.

concentration of chromophores (μM)	0.025	0.05	0.09	0.12	0.2	0.39
sedimentation						
phycocyanin	93/7	-	-	12/88	8/92	-
phycocyanin + PCMS	92/8	68/32	52/48	45/55	32/68	16/84
gel-filtration						
phycocyanin	-	-	-	2/98	-	-
phycocyanin + PCMS	-	-	-	>4/<96	-	-

Table 1: Influence of the protein-bound mercurial PCMS on the monomer/trimer $(\alpha\beta)_1/(\alpha\beta)_3$ equilibrium of phycocyanin. Comparison of two different methods of determination.

3. RESULTS and DISCUSSION

The bilin chromophores of either the α- or the β-subunit of phycocyanin, or both, were (photo)chemically modified and recombined with the respective missing subunits. The modifications consisted of photobleaching, or reduction of the verdin- to rubin-type chromophore(s). It was not possible to obtain trimeric phycocyanin $(\alpha\beta)_3$ from such modified preparations by the recombination procedures used, irrespective of the modification being done with PC *in toto*, or with isolated subunits which were then recombined with the complementary subunit containing unmodified chromophores. All products are at most dimeric aggregates (Fig. 1). Also, addition of a functionally active linker peptide (22 kDa), which in unmodified samples stabilized trimeric aggregates, did not yield trimers containing rubin type chromophores. Control experiments showed, that this was not due to any irreversible modification on the peptide chain. They were based on the fact that the reduced chromophore(s) were not stable over longer periods of time under aerobic conditions, but rather reoxidized to the native dihydrobilin chromophores. Aggregate analysis of such reoxidation products obtained and with hybrids containing a reduced α-subunit, showed that reoxidation leads to reformation of trimers (Fig. 1).

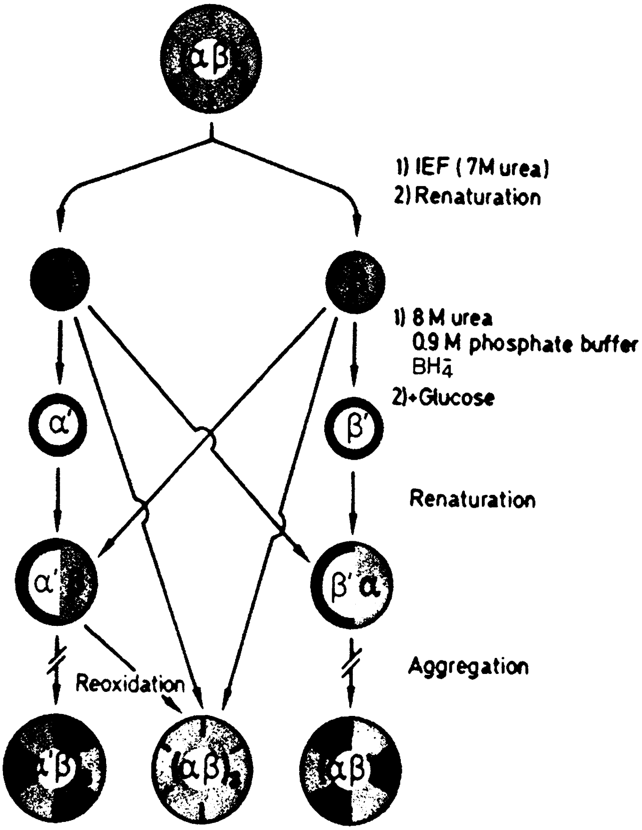

Figure 1: Scheme of the modification procedure at phycocyanin with borohydride. α and β are the subunits, "prime" indicates reduced chromophores; IEF = isoelectric focusing; for more details see text.

While the chemical modifications tested prevent trimer formation completely, modification with PCMS has only a moderately destabilizing effect on aggregation. The reagent binds to the single free cystein of phycocyanin, near the ß-84-chromophore and quite close to the monomer-monomer $(\alpha\beta)_1$-$(\alpha\beta)_1$ contact region (7). In the concentration range where the "natural" pigment contains both monomers $(\alpha\beta)_1$ and trimers $(\alpha\beta)_3$, the monomer portion is increased in PCMS titrated samples. This can be concluded from both the sedimentation runs and the gel filtration experiments (Table 1). However, in all tests it was found that the monomer concentration was higher in the sedimentation than in the gel-filtration experiments, which might be a high pressure effect. The desaggregating effect of modified chromophores is reminescent of the situation in phycoerythrocyanin, where a reversible photochemical reaction of the violobilin-chromophore leads to a reversible modulation of the aggregation (see poster Siebzehnrübl et al.).

Acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft, Bonn (SFB 143).

REFERENCES

1 Schneider, S., Geiselhart, P., Baumann, F., Siebzehnrübl, S., Fischer, R. and H. Scheer (1988) in Photosynthetic Light Harvesting Systems. Organization and Function (Scheer, H. and Schneider, S., eds.), pp. 469-483, deGruyter, Berlin
2 Sauer, K. and Scheer, H. (1988) Biochim. Biophys. Acta 936, 157-170
3 Gantt, E. (1986) in Photosynthesis (Staehelin, L. A. and Arntzen, C. J., eds.), pp. 260-268, Springer, Berlin
4 Glazer, A. N. (1985) Ann. Rev. Biophys. Chem. 14, 47-77
5 Wehrmeyer, W. (1983) in Proteins and nucleic acids in plant systematics (Jense, U. and Fairbrother, D. E., eds.), pp. 144-167, Springer, Berlin
6 Zuber, H. (1986) in Photosynthesis III (Staehelin, L. A. and Arntzen, C. J., eds.), pp. 238-251, Springer, Berlin
7 Schirmer, T., Bode, W. and Huber, R. (1987) J. Mol. Biol. 196, 677-695
8 Füglistaller, P., Rümbeli, R., Suter, F. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chemie 365, 1085-1096
9 Schmidt, G., Siebzehnrübl, S., Fischer, R. and Scheer, H. (1988) in Photosynthetic Light Harvesting Systems. Organization and Function (Scheer, H. and Schneider, S., eds.), pp. 77-89, deGruyter, Berlin
10 Kufer, W. and Scheer, H. (1982) Z. Naturforsch. 37c, 179-192
11 Scheer, H. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), pp. I.1.143-149, Martinus Nijhoff Publishers
12 Siebzehnrübl, S., Fischer, R. and Scheer, H. (1987) Z. Naturforsch. 42c , 258-262
13 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372-1379