

Photosynthetic Light-Harvesting Systems Organization and Function

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Hugo Scheer · Siegfried Schneider



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CONTENTS

List of Participants	XIII
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SECTION I. ORGANIZATION: BIOCHEMICAL METHODS

Introduction: The Biochemistry of Light-Harvesting Complexes by R.J. Cogdell	1
Phycobilisome-Thylakoid Interaction: The Nature of High Molecular Weight Polypeptides by E. Gantt C.A. Lipschultz and F.X. Cunningham Jr.	11
On the Structure of Photosystem II-Phycobilisome Complexes of Cyanobacteria by E. Mörschel and G.-H. Schatz	21
Structure of Cryptophyte Photosynthetic Membranes by W. Wehrmeyer	35
Structural and Phylogenetic Relationships of Phycoerythrins from Cyanobacteria, Red Algae and Cryptophyceae by W. Sidler and H. Zuber	49
Isolation and Characterization of the Components of the Phycobilisome from <u>Mastigocladus laminosus</u> and Cross- linking Experiments by R. Rübeli and H. Zuber	61
C-Phycocyanin from <u>Mastigocladus laminosus</u> : Chromophore Assignment in Higher Aggregates by Cystein Modification by R. Fischer, S. Siebzehrübl and H. Scheer	71
Photochromic Properties of C-Phycocyanin by G. Schmidt, S. Siebzehrübl, R. Fischer and H. Scheer	77
Concerning the Relationship of Light Harvesting Bili- proteins to Phycochromes in Cyanobacteria by W. Kufer	89
Subunit Structure and Reassembly of the Light-Harvesting Complex from <u>Rhodospirillum rubrum G9+</u> by R. Ghosh, Th. Rosatzin and R. Bachofen	93
Primary Structure Analyses of Bacterial Antenna Polypeptides - Correlation of Aromatic Amino Acids with Spectral Properties - Structural Similarities with Reaction Center Polypeptides by R.A. Brunisholz and H. Zuber	103

The Structure of the "Core" of the Purple Bacterial Photo-synthetic Unit by D.J. Dawkins, L.A. Ferguson and R.J. Cogdell	115
A Comparison of the Bacteriochlorophyll C--Binding Proteins of Chlorobium and Chloroflexus by P.D. Gerola, P. Højrup and J.M. Olson	129
Interactions between Bacteriochlorophyll c Molecules in Oligomers and in Chlorosomes of Green Photosynthetic Bacteria by D.C. Brune, G.H. King and R.E. Blankenship	141
Light-Harvesting Complexes of Chlorophyll c-Containing Algae by A.W.D. Larkum and R.G. Hiller	153
Isolation and Characterization of a Chlorophyll a/c-Hetero-xanthin/Diadinoxanthin Light-Harvesting Complex from <i>Pleurochloris meiringensis</i> (Xanthophyceae) by C. Wilhelm, C. Büchel and B. Rousseau	167
The Antenna Components of Photosystem II with Emphasis on the Major Pigment-Protein, LHC IIb by G.F. Peter and P. Thornber	175

SECTION II: ORGANIZATION: MOLECULAR GENETICS AND CRYSTALLOGRAPHY

Molecular Biology of Antennas by G. Drews	187
High-Resolution Crystal Structure of C-Phycocyanin and Polarized Optical Spectra of Single Crystals by T. Schirmer, W. Bode and R. Huber	195
Crystallization and Spectroscopic Investigation of Purple Bacterial B800-850 and RC-B875 Complexes by W. Welte, T. Wacker and A. Becker	201
Structure of the Light-Harvesting Chlorophyll a/b-Protein Complex from Chloroplast Membranes by W. Kühlbrandt	211
Phycobilisomes of <i>Synchococcus</i> Sp. PCC 7002, <i>Pseudanabaena</i> Sp. PCC 7409, and <i>Cyanophora paradoxa</i> : An Analysis by Molecular Genetics by D.A. Bryant	217
Organization and Assembly of Bacterial Antenna Complexes by G. Drews	233

The Use of Mutants to Investigate the Organization of the Photosynthetic Apparatus of <u>Rhodobacter sphaeroides</u> by C.N. Hunter and R. van Grondelle	247
Mechanisms of Plastid and Nuclear Gene Expression During Thylakoid Membrane Biogenesis in Higher Plants by P. Westhoff, H. Grüne, H. Schrubar, A. Oswald, M. Streubel, U. Ljungberg and R.G. Herrmann	261
SECTION III: ORGANIZATION: SPECIAL SPECTROSCOPY TECHNIQUES AND MODELS	
Assignment of Spectral Forms in the Photosynthetic Antennas to Chemically Defined Chromophores by A. Scherz	277
Linear Dichroism and Orientation of Pigments in Phycobilisomes and their Subunits by L. Juszczak, N.E. Geacintov, B.A. Zilinskas and J. Breton	281
Low Temperature Spectroscopy of Cyanobacterial Antenna Pigments by W. Köhler, J. Friedrich, R. Fischer and H. Scheer	293
Chromophore Conformations in Phycocyanin and Allophycocyanin as Studied by Resonance Raman Spectroscopy by B. Szalontai, V. Csizmadia, Z. Gombos, K. Csatorday and M. Lutz	307
Coherent Anti-Stokes Raman Spectroscopy of Phycobilisomes, Phycocyanin and Allophycocyanin from <u>Mastigocladus</u> <u>laminosus</u> by S. Schneider, F. Baumann, W. Steiner, R. Fischer, S. Siebzehrübl and H. Scheer	317
Optical Absorption and Circular Dichroism of Bacteriochlorophyll Oligomers in Triton X-100 and in the Light-Harvesting-Complex B850; A Comparative Study by V. Rozenbach-Belkin, P. Braun, P. Kovatch and A.Scherz	323
Absorption Detected Magnetic Resonance in Zero Magnetic Field on Antenna Complexes from <u>Rps. acidophila</u> 7050 - The Temperature Dependence of the Carotenoid Triplet State Properties by J. Ullrich, J.U. v. Schütz and H.C. Wolf	339
Effect of Lithium Dodecyl Sulfate on B 800-850 Antenna Complexes from <u>Rhodospseudomonas acidophila</u> : A Resonance Raman Study by B. Robert and H. Frank	349

Bacteriochlorophyll a/b in Antenna Complexes of Purple Bacteria by B. Robert, A. Vermeglio, R. Steiner, H. Scheer and M. Lutz	355
Bacteriochlorophyll c Aggregates in Carbon Tetrachloride as Models for Chlorophyll Organization in Green Photo- synthetic Bacteria by J.M. Olson and J.P. Pedersen	365
Orientation of the Pigments in the Reaction Center and the Core Antenna of Photosystem II by J. Breton, J. Durantou and K. Satoh	375
Non-Linear Absorption Spectroscopy of Antenna Chlorophyll a in Higher Plants by D. Leupold, H. Stiel and P. Hoffmann	387

SECTION IV: FUNCTION: ELECTRONIC EXCITATION AND ENERGY TRANSFER

Excitation Energy Transfer in Photosynthesis by R. van Grondelle and V. Sundström	403
Fluorescence Spectroscopy of Allophycocyanin Complexes from <u>Synechococcus 6301 Strain AN112</u> by P. Maxson, K. Sauer and A.N. Glazer	439
Picosecond Energy Transfer Kinetics in Allophycocyanin Aggregates from <u>Mastigocladus laminosus</u> by E. Bittersmann, W. Reuter, W. Wehrmeyer and A.R. Holzwarth	451
Picosecond Time-Resolved Energy Transfer Kinetics within C-Phycocyanin and Allophycocyanin Aggregates by T. Gillbro, A. Sandström, V. Sundström, R. Fischer and H. Scheer	457
Energy Transfer in "Native" and Chemically Modified C-Phyco- cyanin Trimers and the Constituent Subunits by S. Schneider, P. Geiselhart, F. Baumann, S. Siebzehnrübl, R. Fischer and H. Scheer	469
Effect of Protein Environment and Excitonic Coupling on the Excited-State Properties of the Bilinchromophores in C-Phycocyanin by S. Schneider, Ch. Scharnagl, M. Dürring, T. Schirmer and W. Bode	483
Excitation Energy Migration in C-Phycocyanin Aggregates Isolated from <u>Phormidium luridum</u> : Predictions from the Förster's Inductive Resonance Theory by J. Grabowski and G.S. Björn	491

Energy Transfer Calculations for two C-Phycocyanins Based on Refined X-Ray Crystal Structure Coordinates of Chromophores by K. Sauer and H. Scheer 507

Energy Transfer in Light-Harvesting Antenna of Purple Bacteria Studied by Picosecond Spectroscopy by V. Sundström, H. Bergström, T. Gillbro, R. van Grondelle, W. Westerhuis, R.A. Niederman and R.J. Cogdell 513

Excitation Energy Transfer in the Light-Harvesting Antenna of Photosynthetic Purple Bacteria: The Role of the Long-Wave-Length Absorbing Pigment B896 by R. van Grondelle, H. Bergström, V. Sundström, R.J. van Dorssen, M. Vos and C.N. Hunter 519

The Function of Chlorosomes in Energy Transfer in Green Photosynthetic Bacteria by R.J. van Dorssen, M. Vos and J. Amesz 531

Energy Transfer in Chloroflexus aurantiacus: Effects of Temperature and Anaerobic Conditions by B.P. Wittmershaus, D.C. Brune and R.E. Blankenship 543

Interpretation of Optical Spectra of Bacteriochlorophyll Antenna Complexes by R.M. Pearlstein 555

Time Resolution and Kinetics of "F680" at Low Temperatures in Spinach Chloroplasts by R. Knox and S. Lin 567

Picosecond Studies of Fluorescence and Absorbance Changes in Photosystem II Particles from Synechococcus Sp. by A.R. Holzwarth, G.H. Schatz and H. Brock 579

Analysis of Excitation Energy Transfer in Thylakoid Membranes by the Time-Resolved Fluorescence Spectra by M. Mimuro 589

V. CONCLUDING REMARKS

Future Problems on Antenna Systems and Summary Remarks by E. Gantt 601

Author Index 605

Subject Index 609

HIGH-RESOLUTION CRYSTAL STRUCTURE OF C-PHYCOCYANIN AND POLARIZED
OPTICAL SPECTRA OF SINGLE CRYSTALS

T.Schirmer^{*)}, W.Bode, R.Huber

Max-Planck-Institut für Biochemie, Am Klopferspitz, D-8033
Martinsried, F.R.G.

Several biliprotein crystals are composed of oligomeric aggregates which most probably closely resemble the trimeric and hexameric discs of native phycobilisomes (1-3). Therefore, X-ray analysis of these crystals yields not only information about the monomeric structure, but about the interaction of the monomers and relative geometry of their chromophores as well. Particularly, the structure of C-phycoerythrin (C-PE) hexamers from *Agmenellum quadruplicatum* (2, 4) shows that there is a multitude of paths for intra-hexameric energy transfer. Chromophore $\alpha 84$ and $\beta 84$ of the neighbouring monomer (same trimer) have the most favourable relative position, whereas $\alpha 84 - \alpha 84$ and $\beta 155 - \beta 155$ seem to represent the main paths for inter-trimeric energy transfer. Energy transfer across the interface of hexamers in the phycobilisome rods most probably proceeds from $\beta 84$ to $\beta 84$.

The crystal structure of C-PC from *Mastigocladus laminosus* has recently been refined (4). The high resolution (2.1 Å) of this structure allowed an accurate determination of the geometry of all three chromophores. All chromophores are defined in their entirety and exhibit very similar geometry in their tetrapyrrole part. The structure of a phycocyanobilin chromophore resembles a cleaved porphyrin which has been unfolded by a twist of roughly 180° around the C5-C6 and C14-C15 bonds. Accordingly, the configuration / conformation of

*) present address: Laboratory of Molecular Biology, M.R.C.,
Cambridge CB2 2QH, U.K.



Fig. 1:

a) Stereoscopic view of chromophore $\beta 84$, the side-chain of aspartate $\beta 87$ and the $C\alpha$ -backbone (part of helix E).

b) Same model, rotated by approximately 90 degree around the vertical direction.

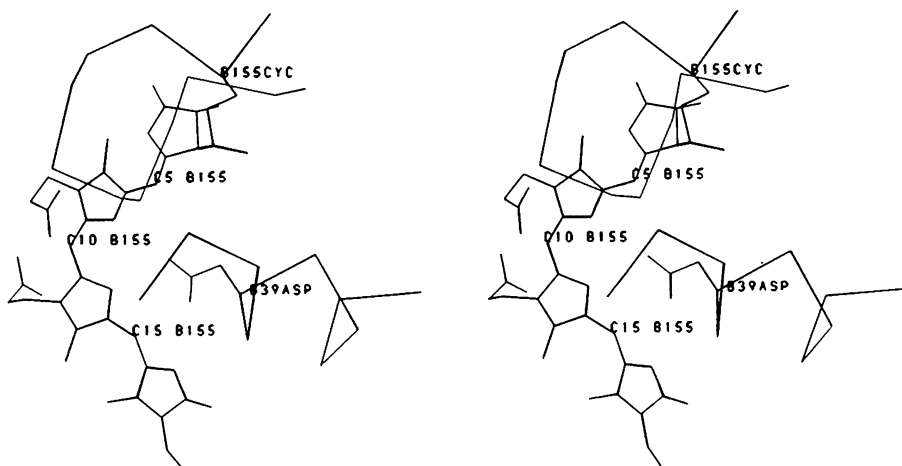


Fig. 2:

a) Stereoscopic view of chromophore $\beta 155$, the side-chain of aspartate $\beta 39$ and the α -backbone (G-H loop and part of helix A).

the chromophores is Z-anti, Z-syn, Z-anti (with the exception of the "configuration" of C14-C15 of chromophore $\beta 155$, which is almost midway between Z and E).

All three chromophores show a common principle of interaction with the protein. They arch around aspartate residues ($\alpha 87$, $\beta 87$, $\beta 39$), the nitrogen atoms of pyrroles B and C being within hydrogen-bonding distance to one of the carboxylate oxygens (Figs. 1 and 2). Most of the propionic side-chains of the chromophores form salt-bridges with arginine and lysine residues. In view of the local symmetry between the α - and β -subunit (1), the similarity of the structures of $\alpha 84$ and $\beta 84$ is not surprising, although it has to be considered that they occupy quite different positions in the trimeric aggregate. $\alpha 84$ is shielded by the neighbouring monomer, whereas $\beta 84$ is located at the inner side of the central channel. It is striking,

however, that also $\beta 155$ exhibits a very similar geometry, despite its distinct position in the subunit at the G-H loop. Moreover, the same chromophore-aspartate interaction is observed, the interacting aspartate $\beta 39$ being provided by helix B (Fig. 2).

The three chromophores of C-PC differ in their spectral properties (see e.g. ref. 5). To assign the different spectra to the individual chromophores, polarized optical spectroscopy on single crystals together with the knowledge of the chromophore positions and orientations provides a powerful method.

Polarized absorption and fluorescence spectra of single C-PC crystals from *Mastigocladus laminosus* have been recorded (6). The crystals show dichroism. The position of the absorption maximum shifts from 612 nm (E parallel to the optical axis c) to 626 nm (E perpendicular to c). The polarized absorption spectra can be simulated well only - using the deconvoluted solution spectra of the individual chromophores (5) and the orientations of the chromophores (4) - if the short and long wavelength component spectra of the β -subunit (β_s and β_f , respectively) are assigned to chromophores $\beta 155$ and $\beta 84$, respectively.

The polarization component of the fluorescence from single crystals is about 2.9 times more intense perpendicular to the three-fold trimer axis than parallel to it. From the known inclination of the chromophores towards the trimer axis intensity ratios of 11.0, 1.6 and 0.25 would be expected, if one of the chromophores $\alpha 84$, $\beta 84$ and $\beta 155$, respectively, would fluoresce solely. Fluorescence originating from both chromophores $\alpha 84$ and $\beta 84$, however, would yield an intensity ratio of 3.0, comparing favourable with the observed ratio. It can be excluded that $\beta 155$ contributes a substantial part to the observed fluorescence. This gives further direct evidence that $\beta 155$ is the β_s -chromophore. It can be deduced, therefore, that the net energy flow in the trimers (and hexamers) is directed

from the peripheral β 155 chromophores to the α 84 - β 84 pair.

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