Photosynthetic Light-Harvesting Systems

Organization and Function

Proceedings of an International Workshop
October 12–16, 1987
Freising, Fed. Rep. of Germany

Editors
Hugo Scheer · Siegfried Schneider

W DE G

Walter de Gruyter · Berlin · New York 1988

CONTENTS

List of Participants XIII

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 Mastigocladus laminosus and Cross-linking Experiments
by R. Rümbeli and H. Zuber 61

C-Phycocyanin from Mastigocladus laminosus: Chromophore Assignment in Higher Aggregates by Cystein Modification
by R. Fischer, S. Siebzehnrübl and H. Scheer 71

Photochromic Properties of C-Phycocyanin
by G. Schmidt, S. Siebzehnrübl, R. Fischer and H. Scheer 77

Concerning the Relationship of Light Harvesting Biliproteins to Phycochromes in Cyanobacteria
by W. Kufer 89

Subunit Structure and Reassembly of the Light-Harvesting Complex from Rhodospirillum rubrum G9+
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 Photosynthetic 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-Heteroxanthin/Diadinoxanthin Light-Harvesting Complex from Pleurochloris meiringensis (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 Synchechococcus Sp. PCC 7002, Pseudanabaena Sp. PCC 7409, and Cyanophora paradoxa: 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 Rhodobacter sphaeroides
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

Assigment 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. Juszcak, 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 Mastigocladus laminosus
by S. Schneider, F. Baumann, W. Steiner, R. Fischer, S. Siebzehnrübl 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 Rps. acidophila 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 Rhodopseudomonas acidophila: 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 Photosynthetic 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. Duranton 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 Synechococcus 6301 Strain AN112
by P.Maxson, K. Sauer and A.N. Glazer 439

Picosecond Energy Transfer Kinetics in Allophycocyanin Aggregates from Mastigocladus laminosus
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-Phycocyanin 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 Phormidium luridum: 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

COHERENT ANTI-STOKES RAMAN SPECTROSCOPY OF PHYCOBILISOMES, PHYCOCYANIN AND ALLOPHYCOCYANIN FROM MASTIGOCLADUS LAMINOSUS

S. Schneider, F. Baumann, W. Steiner
Institut für Physikalische und Theoretische Chemie
der TU-München, Lichtenbergstr. 4, D-8046 Garching

R. Fischer, S. Siebzehnrübl, H. Scheer
Botanisches Institut der Ludwig-Maximilians-Universität
Menzingerstr. 67, D-8000 München 19

Introduction

In order to provide a large geometrical and spectral cross-section for the absorption of light, red and blue-green algae produce the so-called phycobilisomes (PBS), which contain the chromoproteins phycoerythrin (or phycoerythrocyanin), phycocyanin (PC) and allophycocyanin (APC). Although PC and APC contain the same chromophore, phycocyanobilin, the spectral properties of these biliproteins (tetrapyrrol chromophore bound to apoprotein) are quite different. Their excitation energies are modified according to their special function in the light harvesting system (see e.g. 1,2) by chromophore-protein interactions (14).
Resonance-enhanced Coherent Anti-Stokes Raman Spectroscopy (CARS) has proved to be a very suitable technique to produce vibrational spectra of highly fluorescing chromophores as e.g. light harvesting pigments (3,4,5). In this contribution CARS-spectra of room-temperature solutions of phycobilisomes and of phycocyanin and allophycocyanin trimers from *Mastigocladus laminosus* are presented and the implications to chromophore structure are discussed.

Photosynthetic Light-Harvesting Systems

Experimental

Resonance-enhanced CARS spectra are obtained by focussing two laser beams with different wavelengths λ_{pump} (fixed) and λ_{stokes} (variable) in the sample, where by a non-linear effect the CARS beam is generated, which holds the equality
$\tilde{\nu}_{CARS} = \tilde{\nu}_{pump} + (\tilde{\nu}_{pump} - \tilde{\nu}_{stokes})$
$\tilde{\nu}_{CARS} > \tilde{\nu}_{pump} > \tilde{\nu}_{stokes}$, with $\tilde{\nu} = 1/\lambda$ [cm^{-1}]
A plot of the CARS intensity as function of $\tilde{\nu}_{pump} - \tilde{\nu}_{stokes}$ reveals a vibrational spectrum of the chromophore. As the CARS frequency is higher than either pump and Stokes frequency, no flourescence problems as in spontaneous Raman spectroscopy can arise. (For more experimental details see ref. 3).

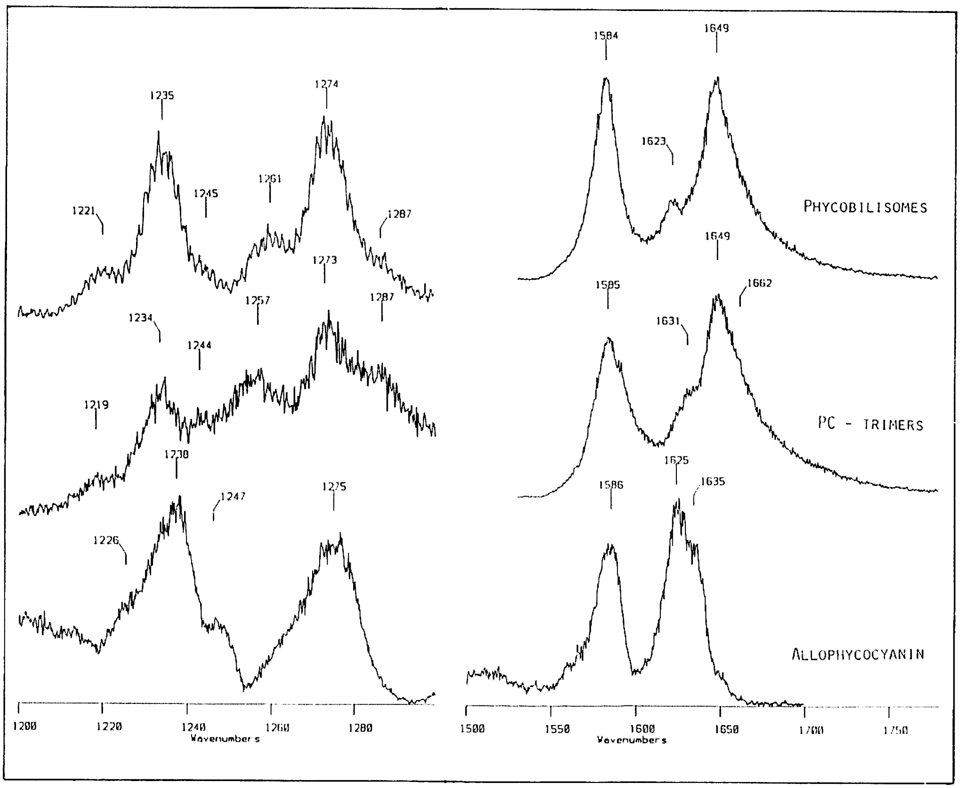
Preparation

PBS were isolated according to the method published by Nies and Wehrmeyer (6), except that the pH of the phosphate buffer was 6 and the purification on the sucrose gradient was done twice. APC was prepared very similar to a procedure described by Füglistaller et. al. (7).
The aggregation state of PC and APC was determined by sedimentation runs according to Martin and Ames (8). Both biliproteins were found to be trimeric. Linker peptides were not present as proved by absorption spectra, sedimentation coefficients and SDS-gel electrophoresis.

Results

As both the fingerprint (1100 - 1300 cm^{-1}) and the double bond stretching region (1500 - 1750 cm^{-1}) have proved to be most suitable for the investigation of chromophore geometry by CARS (3,4,5,9) or Resonance Raman Spectroscopy (10,11), both regions are considered in the following.

The phycobilisome spectra (PBS) are dominated by the contribution of the constituent phycocyanin trimers (PC) and are therefore rather similar to the spectra of the latter. Most of the vibrational frequencies are reproduced within experimental accuracy, though with different intensities (especially between 1200 and 1300 cm^{-1})(fig 1). At 1623 cm^{-1} an additional weak peak is found in PBS, which is due to allophycocyanin (APC). A second interesting feature is that for the pronounced bands a smaller width is found in PBS than in PC. It possibly indicates a lower structural inhomogeneity in PBS due to higher aggregation and the presence of linker peptides.



<u>Fig</u>. 1: Resonance-enhanced CARS spectra of phycobilisomes, phycocyanin trimers and allophycocyanin trimers from <u>Mastigocladus laminosus</u>. Pump wavelength 640 nm.

APC spectra are very different from PC spectra (see also ref. 10). The 1257 cm^{-1} and 1287 cm^{-1} bands observed in PC are absent in APC. In the double bond stretching region the dominant peak in PC is found at 1649 cm^{-1} with shoulders at ≈1631 and ≈1662 cm^{-1}. In APC spectra, however, the dominant peak is found at 1625 cm^{-1} with a strong shoulder at ≈1635 cm^{-1} and a weak one at ≈1650 cm^{-1}.

Discussion

According to preliminary normal mode calculations using the coordinates provided by X-ray analysis (12,13), the bands found between 1200 and 1300 cm^{-1} represent extensively mixed C-C stretch / C-H in plane bending modes and can presently not be interpreted in a straight-forward manner. The band around 1585 cm^{-1} is related to C=C stretching vibrations in the ring B/C fragment. That this band exhibits nearly no change in frequency, most likely indicates a conservation of the geometry of this part of the chromophore.
The C=C double bonds of the methine bridges between ring A-B and ring C-D, on the other hand, should give rise to localized modes with frequencies between 1650 and 1700 cm^{-1}. The C=O stretch mixes with the C=C stretch in ring D to produce vibrational frequencies around 1630 cm^{-1}. The actual value of the calculated frequencies is found to be sensitive to structural changes.
From these theoretical results and the comparison of CARS spectra of model compounds (9) with the presented CARS spectra a significant difference in chromophore geometry and/or Π-electron distribution between PC and APC must be postulated. Interpretation of e.g. fluorescence decay data of APC should therefore not be based on structural information gained from PC.

Additional measurements on antenna pigments and a more elaborate normal mode analysis are in preparation.

Acknowledgement

Financial support by Deutsche Forschungsgemeinschaft (SFB 143) and Fonds der Chemie is gratefully acknowledged.

References

(1) Gantt E. 1981. Phycobilisomes . Ann. Rev. Plant Physiol. 32, 327 - 347.

(2) Scheer H. 1982. In: Light Reaction Path of Photosynthesis (F.K. Fong, ed.). Springer, Berlin. pp. 7 - 45.

(3) Schneider S.,F. Baumann, U. Klüter, 1987. Z. Naturforsch. 42c(11/12).

(4) Schneider S., F. Baumann, P. Geiselhart, S. Siebzehnrübl, R. Fischer, H. Scheer, 1987. Proc. of the Sec. Europ. Conf. on the Spectr. of Biol. Mol., J.Wiley, Chichester.

(5) Schneider S., F. Baumann, P. Geiselhart, H. Kayser, H. Scheer, 1987. Photochem. Photobiol., submitted.

(6) Nies M. and W. Wehrmeyer, 1980. Planta 150, 330 - 337.

(7) Füglistaller P., H. Widmer, G. Frank and H. Zuber, 1981. Arch. Microbiol. 129, 268 - 274.

(8) Martin R.G. and B.N. Ames, 1961. J. Biol. Chem. 236, 1372 - 1379

(9) Schneider S., P. Geiselhart, F. Baumann, H. Falk and W. Medinger, 1987. J. Biochem. Biophys., submitted.

(10) Szalontai B., Z. Gombos, V. Csizmadia, 1985. Biochem. Biophys. Res. Com. 130, 358-363.

(11) Szalontai B., Z. Gombos, V. Csizmadia and M. Lutz, 1987. Biochim. Biophys. Acta 893, 296-304.

(12) Schirmer T., W. Bode and R. Huber, 1985.
J. Mol. Biol. 184, 257-277.

(13) Schirmer T., W. Bode and R. Huber, 1987.
J. Mol. Biol., 196, 677-695.

(14) Schneider S., C. Scharnagl, M. Duerring, T. Schirmer
and W. Bode, 1987. Contribution in this volume.