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

Walter de Gruyter · Berlin · New York 1988

CONTENTS

SECTION II: ORGANIZATION: MOLECULAR GENETICS AND CRYSTALLOGRAPHY

SECTION III: ORGANIZATION: SPECIAL SPECTROSCOPY TECHNIQUES AND MODELS

SECTION IV: FUNCTION: ELECTRONIC EXCITATION AND ENERGY TRANSFER

V. CONCLUDING REMARKS

BChl a/b IN ANTENNA COMPLEXES OF PURPLE BACTERIA

Bruno ROBERT*, André VERMEGLIO**, Robert STEINER+, Hugo SCHEER+ and Marc LUTZ*

* : Service de Biophysique, Département de Biologie, C.E.N. Saclay, 91191 Gif sur Yvette CEDEX FRANCE

** : A.R.B.S., CEN Cadarache, B.P.1, St Paul Lez Durance,

+ : Botanisches Institut der Universitat Munchen, D 8000 Munchen 19, GFR

Introduction

During the last five years, decisive progresses has been made in the knowledge of the structures of the protein-pigment complexes involved in the first steps of bacterial photosynthesis. However, this progress has largely concerned reaction centers (1), and has been slower for light harvesting complexes. As far as Rhodospirillales (purple bacteria) are concerned, the two subunits of many B 870 and B 850-800-type antenna complexes have now been sequenced (2). However, up until now, none of the several crystallization attempts yet allowed a highly diffracting crystals to be obtained. Resonance Raman spectroscopy is thus currently the only method able to provide information about the structure of these complexes. This technique indeed permits selective observation of the 'active site' of the antenna, i.e., the regions of the proteins which bind the BChl molecules and are responsible for their electronic absorption properties : studying the Raman-active vibration modes of BChl within the native light harvesting pigment-protein complexes indeed permits to characterize several of the interactions occurring between these pigments and their micro-environment, and thus gives some indications of the structures of BChl host sites within the protein. In this article, we will briefly summarize conclusions recently drawn from

Photosynthetic Light-Harvesting Systems
© 1988 Walter de Gruyter & Co., Berlin · New York

resonance spectroscopy of BChl a and b-containing complexes of Rhodospirillales.

Resonance Raman Spectroscopy of Antenna complexes from Rhodospirillales

Excitation of both BChl a and BChl b near the tops of their Soret transitions provides resonance Raman spectra which contain much structural information (3,4,5). The 1620-1720 cm^{-1} regions of the Raman spectra obtained in these conditions of resonance contain bands arising from the stretching modes of the 2-acetyl and 9-keto carbonyl groups, which are conjugated with the dihydrophorbin cycles of the molecules. The frequencies of these modes are sensitive to existence and strength of intermolecular bonds in which these chemical groups may be engaged (6) : typically, 2-acetyl groups of BChl a and b vibrates at 1665 cm^{-1} and 1670 cm^{-1} respectively when free from intermolecular bonding. These frequencies may shift down to ca 1630 when the groups assumes hydrogen bonding (6). The 9-keto groups of both BCHl a and b vibrates near to 1700 cm^{-1} when free from intermolecular interactions, and their stretching frequency may shift down to 1660 cm^{-1} when they are hydrogen bonded (6). Moreover, in the same spectral region, the frequency of a band located near to 1600 cm^{-1} , and which mainly arises from methine bridge stretching, is sensitive to the number of axial ligands that bind the central Mg of the molecule for both BChl a and b (6). This band indeed occurs at 1600 cm^{-1} when two axial ligands are bound to the Mg atom, and around 1615 cm^{-1} when it is five-coordinated. Thus, spectral analysis of the 1550-1750 cm^{-1} region brings information about the interaction states of the central Mg and of both the conjugated C=O of BChl a or b. These groups are known to be predominantly involved in intermolecular interactions of chlorophylls both in vivo and in vitro (6).

BChl a-containing Complexes

Core antenna (B 890,870 complexes)

The very close similarity of resonance Raman spectra of core antenna complexes from any species of Rhodospirillales that wa have studied so far indicates that, in all these complexes, the proteic host sites of BChl are extremely similar, most probably providing the same binding groups to the conjugated carbonyls of BChl a molecules (3). For more than ten species and strains (Rhb sphaeroides, capsulatus, Rps palustris, acidophila, Rsp rubrum, Chromatium vinosum Thiocapsa roseopersicina) the RR spectra of the core antenna obtained at Soret resonance are extremely similar to that presented in fig 1a. The strong 1645 cm^{-1} band most probably arises from both of the acetyl carbonyl groups of both of the BChl a molecules known to be present in B 870-type complexes. These groups thus assume similar if not identical intermolecular interactions. The frequency of this band is species-dependent, being up or downshifted by a few wavenumbers from the 1645 cm^{-1} mean value. These variations likely arise from slight variations in the environment or geometry of the intermolecular C=O --- X bond rather than from a variability in the nature of the site X. Two bands are observed in the keto stretching region of RR spectra : interactions assumed by keto groups of both BChl in B 870-type are thus not the same. However both of them are most probably H-bonded, vibrating respectively at 1667 and 1678 cm^{-1}. The 1615 cm^{-1} frequency of the methine bridge stretching mode clearly indicates that, in B 880-type complexes as well as in any BChl-containing antenna complexes studied to date (3,4,5), the central magnesiums of the BChls are five-coordinated.

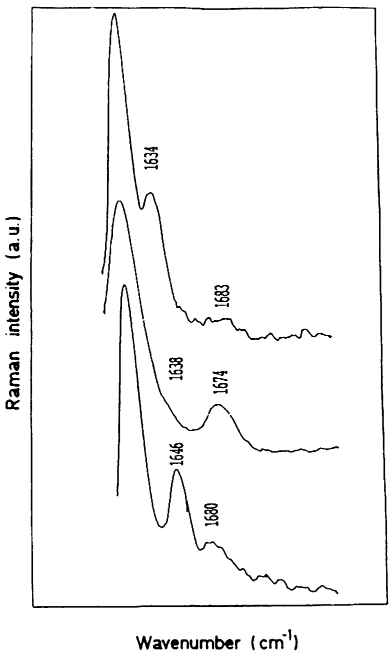
Peripheric antenna (B 850-800 or 820-800-type complexes)

From the BChl host-site structures revealed by RR spectroscopy at least two families must be distinguished among the B 850-800-type complexes : intermolecular interactions assumed by the three BChl in B 850-800 complexes extracted from Rhodospirillaceae indeed differ from those extracted from Chromatiaceae (fig 2) (3). In RR spectra of B 850-800 complexes from Chromatiaceae, the C=O stretching modes consitute as a complex cluster located around 1656 cm^{-1} , with a shoulder at 1671 cm^{-1} , and involving very weakly bound acetyl and H-bonded keto groups. RR spectra of the Rhodospirillaceae complexes, exhibit resolved bands at 1633, 1641, 1666-1670 and occasionally 1700 cm^{-1} .

Moreover, intergeneric variability has been observed among the RR spectra of B 850-800 complexes from Rhodospirillaceae thus revealing intergeneric structural variability (4): in RR spectra of the B 850-800 complexes extracted from bacteria of the Rhodobacter genus (sphaeroides, capsulatus), the keto C=O group of the 800-nm absorbing BChl is free from intermolecular bonding, vibrating at 1700 cm^{-1} whereas it is intermolecularly bound in B 850-800 complexes of bacteria from the Rhodopseudomonas genus (palustris, acidophila, type II complexes). This intergeneric variability is also observed for the acetyl groups of the 800 nm-absorbing and one of one of the 850 nm-absorbing BChls. In this case only changes in the strengths of the intermolecular interactions of these groups are observed. Two types of B 850-800 complexes have been extracted from Rps acidophila (type I and type II (7)). Host sites of BChls within type I complexes are very similar to those of B 850-800 complexes extracted from the Rhodobacter genus (B. ROBERT and R.J. COGDELL, unpublished results).

B 830-800 complexes (or 'high 800nm-absorbing complexes') have been purified from bacteria of the Rhodopseudomonas genus grown in low light. These complexes exhibit absorption spectra very similar to those of B 850-800 complexes extracted from Chromatiaceae. However, on the basis of the structures of the BChl host sites, revealed by RR spectroscopy, these complexes appear to be actually related to 'regular' B 850-800 complexes of Rhodospirillaceae, genus Rhodopseudomonas, from which they appear to only differ by the presence of an additional BChl molecule (4).

Fig 1 : RR spectra (363.8nm excitation) of
a)(lower trace) B 880 from Rps acidophila
b)(middle trace)B 1015 from Rps viridis
c)(upper trace) B 910 from Chromatium tepidum



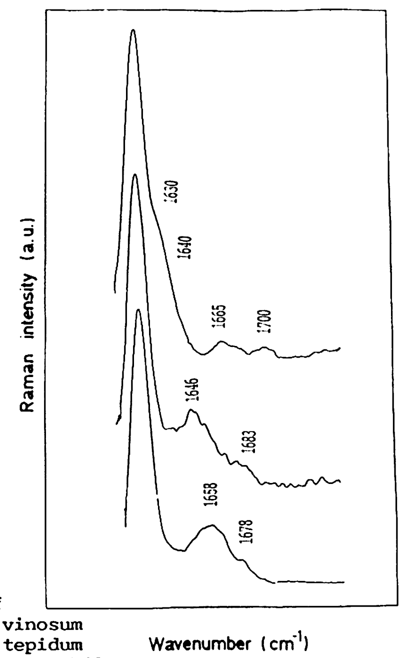


Fig 2 : RR spectra (363.8 nm excitation) of
a)(lower trace) B 850-800 from Chromatium vinosum
b)(middle trace) B 850-800 from Chromatium tepidum
c)(upper trace) B 850-800 type I from Rps acidophila

The thermophilic bacterium Chromatium tepidum

We have studied antenna complexes extracted from the thermophilic bacterium Chromatium tepidum : this bacterium synthesizes both a core antenna (B 920) and a peripheric one (B 855-800) which are thermostable up to 70 C in the intact membrane (8). In both these complexes, the central magnesiums of all of the BChl molecules are five-coordinated, as in the mesophilic strains. On the other hand, in both of these complexes, a ca 13 cm^{-1} downshift of the whole set of frequencies of the acetyl carbonyl vibrators is observed, indicating that these groups are all engaged in stronger intermolecular interactions than in the mesophilic Chromatium vinosum strain. At such wavenumbers, a 13 cm^{-1} downshift of a C=O vibrator approximately corresponds to an increase in H-bond strength of 2 kcal/M. In B 920 complexes, the keto carbonyl stretching frequencies are also downshifted (16 cm^{-1}) relative to the mesophilic strain. Hence, in antenna complexes extracted from this thermophilic strain, proteic host sites appear to provide BChl molecules with noticeably stronger interactions on most of their conjugated carbonyls.
Following suggestion by BABCOCK and CALLAGHAN (9) it would be tempting to relate the 880->920 nm absorption difference to the difference in ligation strength of the carbonyl groups of the BChls in the core complexes of the mesophilic and thermophilic species. Nevertheless, the liganding of these groups (at least of the acetyl ones) appears to have only a limited influence on the electronic absorption of the pigments since the peripheric antennae of both tepidum and vinosum species exhibit the same absorption spectra, whereas the ligation strength of the acetyl carbonyl of their BChl a also differ by hte same amounts.

BChl b-Containing Antenna Complexes

Core antenna of Rps viridis (B 1015)
RR spectra of Bchl b-containing antenna indicate that, as in the case

of BChl a-containing complexes, the central Mg of the pigments within these complexes generally are singly liganded (methine bridge frequency at 1615 cm^{-1}). However, the ligation of BChl b in B 1015 from Rps viridis appears to be different from that of BChl a in B 880-type complexes : indeed, in the carbonyl stretching region of the former complex, a weak 1639 cm^{-1} arises from a H-bonded acetyl C=O, a strong 1670 cm^{-1} most probably arises from a free acetyl group and from a H-bonded keto grouping, and a 1682 cm^{-1} , band arises from a weakly interacting keto carbonyl (fig 1c).

B 800-1020 complex from Ectothiorhodospira halochloris

B 800-1020 complex from Ectothiorhodospira halochloris is a BChl b-containing core antenna. Biochemical analysis (10) indicated that it contains five BChl b, two of them being responsible for the 1015 nm absorption peak. RR data largely confirm these hypothesis : at least four unequivalently interacting BChl b are present in these complex, as far as 8 different frequencies may be observed in the C=O stretching region. Moreover, HCl-treatment, which results in shifting the 1015 nm transition down to 960 nm (10), affects the stretching vibrations of no more than two acetyl and two keto carbonyl groups.

Overview

RR spectroscopy shows that, although the sequences of antenna complexes of purple bacteria appear to be highly conservative (2), interactions assumed by BChls in many of these complexes are largely species- (or genus-) dependent. Despite this interspecific variability, antenna complexes of purple bacteria share common structural features at their active sites :

- all BChl molecules have singly liganded Mg. From both sequence data and RR spectra (3), these ligands are most probably imidazole side chains of histidines in most cases. The 800 nm-absorbing BChl of Rhb sphaeroides B 850-800 complexes may constitute an exception in this respect.

- In all of these complexes, BChl appears not to be directly exposed to the lipidic phase or aqueous detergent : indeed, RR spectra are insensitive to the isolation procedures, to the nature of the detergent as well as to the size of the complex multimers. Moreover, when direct comparison are possible (Rsp rubrum) RR spectra of the membrane-bound and extracted complexes are identical.

Now that several sequences are available for (2) antenna complexes of purple bacteria hypotheses can be drawn from RR spectroscopy about the nature of the aminoacids interacting with the pigments For example, B 1015 complex from Rps viridis is the only core antenna for which no 1645 cm^{-1} RR band is observed but for which 1639 cm^{-1} and 1670 bands are observed. This specificity may well be related to the fact that its polypeptides each have specific amino acid changes relative to all of the other polypeptides of core antenna : phenylalanine -8 (taking the conserved His as 0 position) in the alpha polypeptide is replaced in viridis by a tyrosine which could provide a H-bond to the C=O acetyl group so that its frequency could be downshifted to 1639 cm^{-1} ; in the beta one the +6 Trp is also replaced by an Ala. These aminoacids are thus likely candidates as binding sites of acetyl C=O groups. Similarly, when more antenna complex polypeptides are sequenced, it will most probably be possible to locate the BChl molecules in the polypeptide matrix taking into account both orientations of electronic transition moments and RR data.

References

1) MICHEL, H., EPP, O. and DEISENHOFER, J. (1986) EMBO J. 5-10 2445-2451

2) ZUBER, H., SIDLER, W., FUGLISTALLER, P., BRUNISHOLZ, R. and THEILER, R. (1987) Trends in Biochem. Sc., 11, 414-419

3) ROBERT, B. and .LUTZ, M. (1985) Biochim. Biophys. Acta 807, 10-23

4) ROBERT, B. ANDRIANAMBININTSOA, S. and .LUTZ, M. (1985) Biophys J. (Tokyo), 100, 6-11

5) ROBERT, B., STEINER, R., ZHOU, Q., SCHEER, H. and LUTZ, M. (1987) in : Progess in Photosynthesis Research (BIGGINS, J. ed) Martinus Nijhoff, Dordrecht, The Netherlands

6) LUTZ, M. and ROBERT, B. (1987) in Biological applications of Raman spectroscopy (SPIRO, T.G. ed) Vol III Chap 9

7) COGDELL, R.J., DURANT, I., VALENTINE, J., LINDSAY, J.G. and SCHMIDT, K. (1983) Biochim. Biophys. Acta 722, 427-455

8) MADIGAN, M.T. (1984) Science 225, 313-315

9) CALLAGHAN, P.M. and BABCOCK, G.T. (1983) Biochemistry 22, 452-461

10)STEINER, R (1985) Thesis, Ludwig Maximilian University (Munchen)