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Structures of antenna complexes of several *Rhodospirillales* from their resonance Raman spectra

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Several antenna complexes of *Rhodospirillales* were studied, within the intracytoplasmic membrane or in their isolated states by comparing resonance Raman spectra of their bacteriochlorophyll (BChl) molecules. It has been found that in B880-type complexes the states of BChl are extremely similar, in terms of their local environments. By contrast, at least two families of structures must be distinguished among the B850–800-type complexes, namely B850–800 S (from *Chromatiaceae*) and B850–800 NS (from *Rhodospirillaceae*). It appears that the more the energy of the lower singlet level of antenna BChl is decreased from its value in the isolated state, the smaller is the observed variability in its proteic host sites in the set of complexes observed. On the other hand, resonance Raman spectroscopy permits to conclude that the ground-state interactions assumed by the dihydrophorbin ring of BChl *a* within these complexes most probably are protein–BChl and not BChl–BChl or lipids–BChl interactions. Histidine is a likely candidate as the Mg ligand for the 880 and 850 nm absorbing molecules, but not for the 800 nm absorbing ones.

Introduction

Until recently, structural knowledge about light-harvesting complexes of *Rhodospirillales* (purple bacteria) has been rather scarce, the available experimental information being limited to their characterization by absorption spectroscopy [1,2], to the measurements of the orientations of their bound pigments by linear dichroism [3,4] and to circular dichroism data giving evidence for excitonic interactions among BChl molecules [5,6]. During the last three years, knowledge on the structure of these complexes reached a decisive point: owing to the improvement of purification methods, much better biochemical characterizations became possible, involving:

(i) precise determinations of the relative stoichiometries of the different bound pigments, and the definition of a 'minimal size unit' [7–9],

(ii) a determination of the aminoacid compositions of the polypeptides within this minimal size unit [10,11],

(iii) the first attempts of sequencing these different polypeptides, a subject of intensive current efforts [12–14].

Yet little is known about the detailed structures of these complexes, in particular about those of their 'active sites', i.e., of the regions which bind the BChl molecules and are responsible for their electronic absorption properties. Resonance Raman spectroscopy is currently the only method able to bring this type of information on pigment-protein complexes when X-ray crystallographic data are not available. Indeed, observation of the

Abbreviations: BChl, bacteriochlorophyll *a*; LDAO, lauryldimethylamine *N*-oxide; LiDS, lithium dodecyl sulphate.

vibration modes of Bchl within light-harvesting pigment-protein complexes (whether the last are still part of the intracytoplasmic membrane or isolated) allows the study of several of the interactions between these pigments and their micro-environment, i.e., indirectly of the structures of their host sites within the complexes.

On the other hand, the water-soluble complex of *Prosthecochloris aestuarii* has been crystallized. However, because of taxonomy (*P. aestuarii* is a *Chlorobiale*) and because this complex is not integral to the membrane, the information obtained about its structure from X-ray crystallography [15] cannot be straightforwardly transposed to the membrane-located light-harvesting pigment-protein complexes of *Rhodospirillales*. Resonance Raman spectroscopy permitted a preliminary comparison of the environmental interactions of pigments in complexes of both origins [16]. In this article, we report on a more detailed study of several antenna complexes of *Rhodospirillales* using resonance Raman spectroscopy. From these results, it is possible to estimate the interspecific variability of the host-sites of the pigments within antenna complexes, as classified [1] according to their electronic absorption properties. This method already permitted the two distinct antenna complexes of the revertant R26.1 strain of *Rps. sphaeroides* to be characterized on the basis of the structures of their active sites [17]. Additional information is also presented about intermolecular binding of the magnesium atoms of BChl *a* molecules in light-harvesting complexes, obtained from comparisons of their resonance Raman spectra with those of in vitro models. Preliminary accounts of this work have been given in Refs. 18 and 19.

Material and Methods

Bacteria

We studied eight strains of *Rhodospirillales*: six strains of *Rhodospirillaceae* (purple nonsulfur bacteria): *Rps. sphaeroides*, strains 2.4.1 and R26, *Rps. capsulata, Rps. palustris, Rsp. rubrum*, strains S1 and G9, and two strains of *Chromatiaceae* (purple sulfur bacteria): *Chromatium vinosum* and *Thiocapsa roseopersicina*. The *Rhodospirillaceae* were grown anaerobically in the light in a modified Hutner medium; they were harvested by centrifugation, generally during the exponential phase of growth. Harvesting the cells during this phase allowed us to get intracytoplasmic membranes containing relatively high proportions of B880 pigment-protein complexes (B880 : B850–800 = 1). This facilitated the purification of the B880-type complexes. The *Chromatiaceae* were bought from C.A.M.R. Microbial Products Ltd, and stored frozen until used for the experiments.

Purification of chromatophores

Cells were washed in 10 mM Tris-HCl (pH 8.0) (buffer I) for *Rhodospirillaceae*, or in 25 mM Tris-HCl (pH 8.0) (buffer II) for *Chromatiaceae*, and then disrupted using a French pressure cell. The broken cells were centrifuged first at $6000 \times g$ for 6 min, then at $10000 \times g$ for 15 min. The supernatant contained chromatophores. They were collected by ultracentrifugation at $100000 \times g$ for 45 min. The pellet was resuspended in the initial buffer, and the concentration was adjusted to an $A = 50$ at the highest far-red absorption peak of BChl for the following operations.

Preparation of the antenna complexes

B880 and B850–800 light-harvesting complexes were prepared by ultracentrifugation on sucrose gradients: chromatophores were treated with 1.5% LDAO (*Rhodospirillaceae*) or 1% deoxycholate (*Chromatiaceae*) for 15 min and then centrifuged on a linear sucrose gradient 0.2/0.6 M prepared with buffer I (*Rhodospirillaceae*) or II (*Chromatiaceae*). After 12–15 h of ultracentrifugation at 40000 rpm in a Beckman SW 41 swinging bucket rotor, two strongly pigmented bands appeared in the gradient: the upper band contained B850–800-type complexes, and the lower band B880-type complexes. These bands were collected and dialysed overnight against buffer I or II supplemented with 0.1% detergent. When all these operations were conducted at low temperature (5°C) in the dark, this method allowed a high quality purification of the B850–800 complexes, without any detectable change in the absorbance ratio of the 800–850 nm bands, and with shifts of both of these bands less than 2 nm. B880 complexes from

Rhodospirillaceae were also obtained without any detectable change in their absorption spectra (Fig. 1). This method, however, did not appear as selective when applied to *Chromatiaceae*: the lower band on the sucrose gradient generally contained a mixture of complexes in which the B880/B850–800 ratio was only about 1 or 1.5 : 1. A good separation of B850–800 and of B820–800 complexes occurred in only one experiment and could not be repeated; in this case, fractions collected at the top of the upper band contained B850–800 complexes.

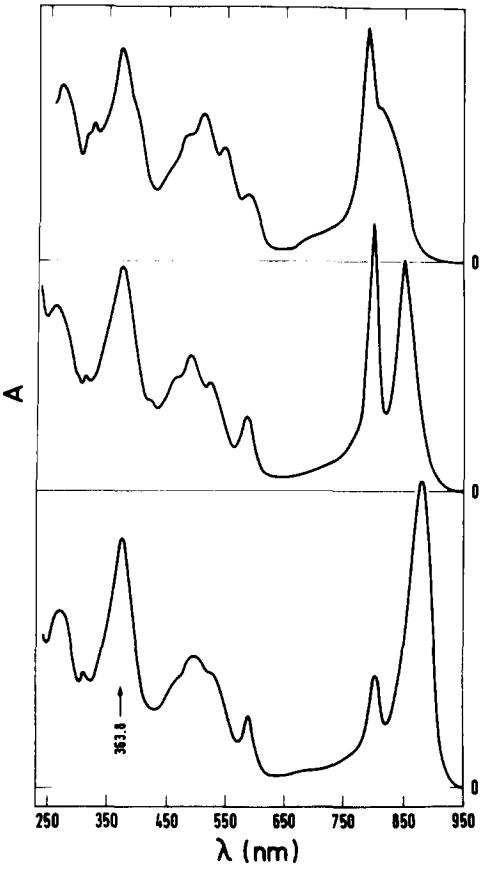

Fig. 1. Room temperature electronic absorption of three types of complexes. (1) B880 complexes: enriched fraction obtained by ultracentrifugation on sucrose gradient of chromatophores from *Rps. palustris*. Samples were suspended in 10 mM Tris-HCl (pH 8.0/0.1% LDAO. (2) B850–800 complexes obtained by ultracentrifugation on sucrose gradient of chromatophores from *Rps. palustris*. Samples were suspended in 10 mM Tris-HCl (pH 8.0)/0.1% LDAO. (3) B820–800 complexes obtained by ultracentrifugation on sucrose gradient of chromatographores from *Thiocapsa roseopersicina*. Samples were suspended in 25 mM Tris-HCl (pH 8.0)/0.1% DOC.

The present preparation constitutes a rapid, often high-quality purification method for most types of complexes. It also offers the advantages of simplicity and of transposability from one species to another, except for *Chromatiaceae*, and appears to induce minimal denaturation of the complexes. B820–800 complexes that we purified from *Thiocapsa roseopersicina* were present in the cells and were not produced during the purification procedure. Some samples of B850–800 were also prepared following the method of Clayton and Clayton [20], by treatment with LDAO or deoxycholate.

Preparation of B850 (800-depleted) complexes

B850–800 complexes selectively depleted in the 800 nm absorbing BChl were prepared from *Rps. palustris*, using a method derived from that of Clayton and Clayton [21], by treatment with LiDS. Depletion of the B850–800 from *Rps. palustris* requires a much higher concentration of LiDS than for *Rps. sphaeroides* (1.5% vs. 0.1%) and still the depletion in the 800 nm absorbing BChl occurs much more slowly (more than one day incubation).

Spectroscopy

Resonance Raman spectra were obtained at 30 K, using a 363.8 nm excitation wavelength from an argon laser (Spectra-Physics, model 171). This wavelength fell close to the top of the Soret band of BChl and insured fixed resonance conditions in any of the complexes studied, which all absorb maximally at 364 nm in this region (Fig. 1). Radiant power reaching the samples was less than 1 mW. Grazing incidence of the excitation laser beam was used in order to prevent reabsorption of the Raman photons by the sample. The Raman spectrometer was a Jobin Yvon HG2S-UV, equipped with d.c. detection. Typical spectral resolution was 8 cm^{-1} at 1000 cm^{-1}. The signal-to-noise ratios were improved by summation of individual spectra in a multichannel analyser (Tracor-Northern 1710). Absorption spectra were recorded at room temperature using a Cary 17 spectrometer.

TABLE I

BACTERIAL STRAINS FROM WHICH LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEXES WERE PURIFIED AND WERE STUDIED USING RESONANCE RAMAN SPECTROSCOPY

B880	B850–800	B830–800	B850
Rps. sphaeroides 2.4.1 [a]	*Rps. sphaeroides* 2.4.1 [a,b]	*Thiocapsa roseopersicina* [c]	*Rps. sphaeroides* 2.4.1 [d]
Rps. sphaeroides R26 [c]	*Rps. palustris* [a,b]	*Chromatium vinosum* [a,c]	*Rps. palustris* [d]
Rps. palustris [a]	*Rps. capsulata* 2.3.1 [b]		
Rsp. rubrum S1 [a,e]	*Thiocapsa roseopersicina* [a]		
Rsp. rubrum G9 [a]			
Chromatium vinosum [a]			
Thiocapsa roseopersicina [a]			

[a] Purified by ultracentrifugation on sucrose gradient.
[b,c] Extracted by the method of Clayton and Clayton [20] with LDAO [b] or deoxycholate [c].
[d] Extracted using LiDS treatment.
[e] Studied in situ in intracytoplasmic membranes.

Results and Discussion

Resonance Raman spectra of BChl in light-harvesting complexes

Resonance Raman spectra of BChl excited at the peak of the Soret transition consist of about 30 bands located between 50 and 1750 cm^{-1}. Most of these bands correspond to complex, in-plane vibrational modes of the dihydrophorbin ring of the molecule. Table II presents the main internal coordinates involved in some of these modes, as proposed by Lutz [22] on the basis of isotopic substitutions and of comparisons with assignments performed for other chlorophylls. In the higher frequencies region (1620–1720 cm^{-1}) occur bands essentially involving the stretching of those carbonyl groups which are conjugated with the dihydrophorbin ring, namely 9-keto and 2-acetyl functions. In the lower frequencies region, around 300 cm^{-1}, some bands arise from modes significantly involving the bonds between the pyrrolic nitrogens and the central Mg atom [23].

In vitro experiments have shown that variations in the local environment may result in important changes of the resonance Raman spectra of BChl *a*. In particular, most of the complex modes of the dihydrophorbin ring of the molecule can shift, by up to 15 cm^{-1}, depending on the number and on the nature of the ligands on the Mg atom, on the dielectric constant of the environment, and on the aggregation state of BChl [22,24]. Also the frequencies, intensities and widths of the bands arising from the stretching vibrations of the carbonyl groups in position 2 and 9 can vary according to

TABLE II

WAVENUMBERS AND ASSIGNMENTS FOR NON-CARBONYL BANDS OBSERVED IN RESONANCE RAMAN SPECTRA OF BChl BOUND TO THE B880 COMPLEX FROM *RPS. PALUSTRIS* AT 30 K, EXCITED AT 363.8 NM

Uncertainties on the wavenumbers of strong bands, ± 2 cm^{-1}; weak bands and shoulders, ± 4 cm^{-1}. s, strong; m, medium; w, weak; v, very; sh, shoulder; c, complex. Assignments: from Ref. 22 and Lutz, M., Reiss Husson, F. and Kléo, J., unpublished results. ν, stretching; δ, in-plane bending.

Frequency	Assignment	Frequency	Assignment
1615 vs	νC_aC_m(νC_bC_b, δC_mH)	950 w	
1587 m		900 w	
1535 s	νC_bC_b, νC_aC_b	850 vwc	
	pyrrole ν_{15}	795 w	δ C N C
1476 m	νC C	767 w	
1452 m	ν C C	736 w	δC N C, ...
1430 m	ν C C, ...	700 w	
1400 w		689 wsh	
1378 w	pyrrole ν_5	627 vwsh	δ C C C
1360 msh	ν C N, ...	590 vw	
1348 mc	δ C_m H ?	570 vwsh	
1291 s	ν C N, ...	446 vw	δC=O?
1263 m		380 w	
1219 w		360 vw	Mg N_4
1165 mc	ν C N, ...	295 w	Mg N_4
1119 m	ν C N, (δ CNC)	274 w	
1068 vs	ν C N, ...		
972 wsh			

the nature of the intermolecular bonding that these groups may assume and also according to their orientation relative to the plane of the dihydrophorbin ring [24]. When the 2-acetyl and the 9-keto carbonyls are free from bonding, their stretching modes occur at 1660 and 1700 cm^{-1}, respectively. Intermolecular bonding may decrease the frequencies of these bands down to 1620 and 1640 cm^{-1}, respectively [24].

The variability of the resonance Raman spectra of BChl in various antenna complexes from various *Rhodospirillales* is smaller than that observed for various in vitro systems. Actually, only very few significant frequency shifts were observed for the modes of the dihydrophorbin ring among all the complexes studied, and the maximum amplitude of these shifts is 6 cm^{-1} only. Table II displays most of the frequencies observed in resonance Raman spectra of B880 complexes of *Rps. palustris*. The only shifts observed for other complexes concern the 570 cm^{-1} band, the position of which may vary between 570 and 576 cm^{-1}, and the carbonyl stretching bands. On the other hand, significant relative intensity variations occur between the spectra of different types of complexes. Most of the observed bands exhibit these variations. The subsequent comparisons between these spectra are based on certain of the larger of these variations, which are located in the following spectral regions: 350–380 cm^{-1} (a region); 570–590 cm^{-1} (b region); the complex cluster of bands between 700 and 800 cm^{-1} (c region); the 1068 band (e region) and 1620–1700 cm^{-1} (C=O stretching modes; f region). In addition, the intensities of the bands of frequencies lower than 300 cm^{-1} and the double band at 900–950 cm^{-1} (d region) also vary according to the type of complexes. These variations were more difficult to quantify, because of the weakness of the bands, and because of the presence of carotenoid bands in the d region. Indeed, resonance Raman spectra of antenna complexes excited in the Soret band of BChl *a* contain contributions from the carotenoids. These contributions can vary considerably, relative to those of BChl, according to the type of the complexes and to the chemical nature of the carotenoids, for unclear reasons. The presence of carotenoid bands around 950 cm^{-1} and between 1100 and 1500 cm^{-1} thus impairs relative intensity comparisons for BChl between complexes of different origins. In the case of B880 complexes from *Chromatiaceae*, the carotenoid contributions were so strong that they prevented any reliable intensity measurement of BChl bands under 1600 cm^{-1}.

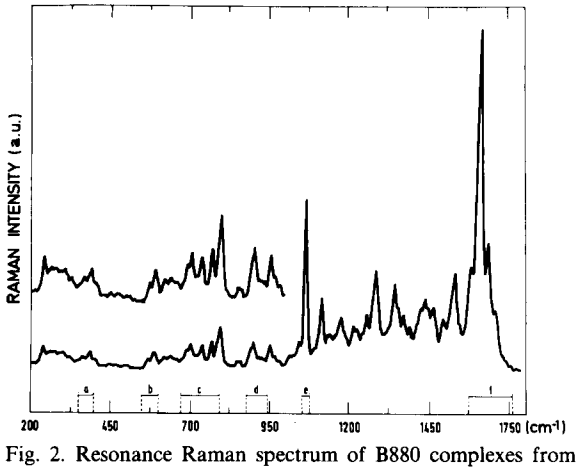

Fig. 2. Resonance Raman spectrum of B880 complexes from *Rsp. rubrum* strain G9 at 30 K, excitation wavelength 363.8 nm, upper trace: expanded twice. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

Bands of the f region arise from stretching modes of the C=O groups in positions 2 and 7 of BChl. Their relative intensity variations can reliably be interpreted in terms of differences in populations of vibrators involved in a given intermolecular interaction and in terms of differences in conjugation of the C=O groups with the dihydrophorbin rings, depending on their conformation. Relative intensity changes amid skeletal bands in regions a–e are more difficult to interpret, inasmuch as they may have various origins: they indeed may depend on very short distance parameters (e.g., the nature of the ligands on the conjugated carbonyls and on the Mg atom, and the geometry of the central MgN_4(pyrrole)-ligands grouping) but also on longer distance parameters of the local environment (e.g., nature and charges of amino-acids located near the ligand of the central Mg of the molecule).

Comparison of resonance Raman spectra of BChl a in the different types of antenna complexes

B880-type complexes

All of the B880 complexes from *Rhodospirillales*

studied have extremely similar resonance Raman spectra regardless of the bacterial species or genus. Those spectra are quite characteristic of this type of complexes and can be characterized as follows (Figs. 3 and 4):

(i) The a region is definitely dominated by the 380 cm^{-1} band ($I_{360}/I_{380} = 0.5$).

(ii) In the b region, the 590 cm^{-1} band is clearly stronger than the the 570 cm^{-1} band ($I_{590}/I_{570} = 2$).

(iii) In the c region, the intensity of the 767 cm^{-1} band is close to that of the 700 cm^{-1} band ($I_{767}/I_{700} = 1$), and the 795 cm^{-1} band is twice as strong as any other band of the cluster.

(iv) In the d region, when the carotenoid bands do not contribute, the I_{950}/I_{900} ratio is about 0.6.

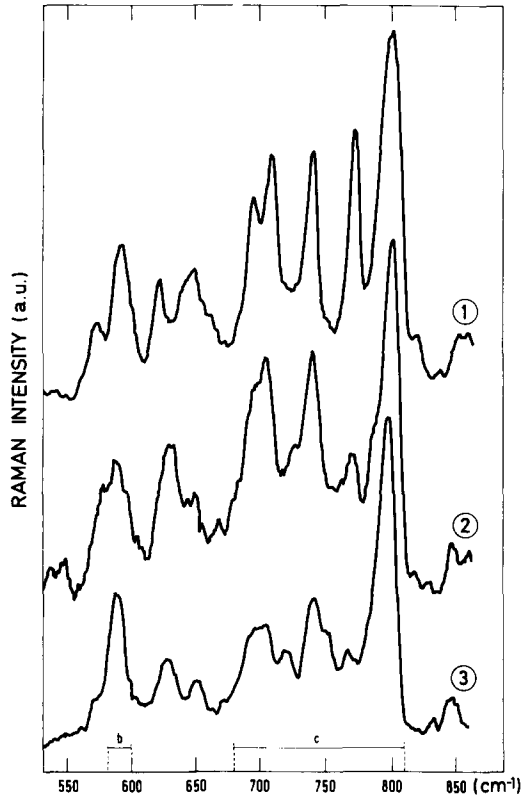

Fig 3. Resonance Raman spectra (b and c regions, see text) obtained at 30 K, excitation wavelength 363.8 nm, from (1) B880 complexes from *Rsp. rubrum* strain S1, (2) B850-800 complexes from *Rps. sphaeroides* strain 2.4.1; (3) B850-800 complexes from *Thiocapsa roseopersicina*. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

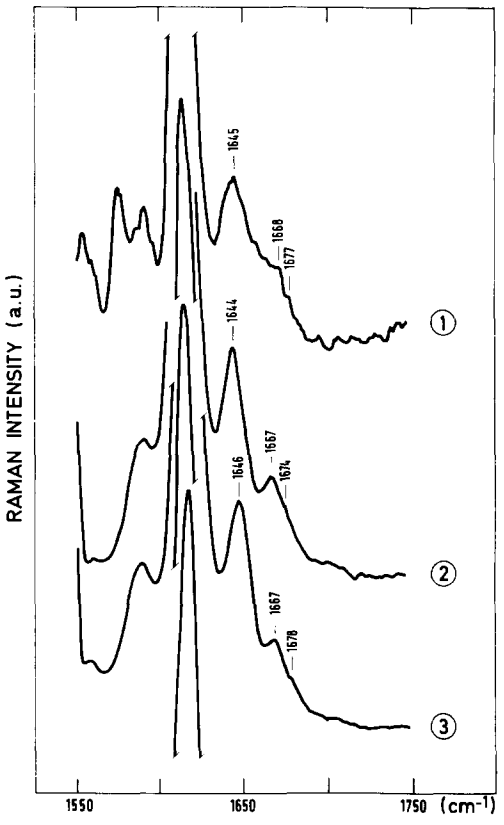

Fig. 4. Resonance Raman spectra (carbonyl stretching region) obtained at 30 K, excitation wavelength, 363.8 from B880 complexes of (1) *Chromatium vinosum*, (2) *Rsp. rubrum* G9, (3) *Rps. palustris*. Spectral resolution; 8 cm^{-1} at 1000 cm^{-1}.

(v) The 1068 cm^{-1} band is strong ($I_{1068}/I_{1615} = 0.5$)

(vi) The f region, which alone could be studied for the whole set of *Chromatiaceae*, contains a strong band close to 1645 cm^{-1} (Fig. 4), a broad, weaker band around 1667 cm^{-1} and a very weak band near 1676 cm^{-1}. The structure of this region is consistent with the stoichiometry suggested for B880 complexes from biochemical data [25,26], i.e., with the presence in the minimal size unit of two unequivalent BChl a: indeed the 1667 and 1676 cm^{-1} bands probably arise from two 9C=O vibrators, involved in different intermolecular interactions, and the 1645 cm^{-1} band may well account for two C=O vibrators, bound to identical, or very similar, sites. The absence of any 1700 cm^{-1} band most probably implies that no 9C=O groups are free.

The reproducibility of resonance Raman spectra of B880 complexes from one species to another and more particularly in the f region, indicates that in all B880 complexes the host sites of BChl are extremely similar, most probably providing the same binding groups to both the central Mg atoms and the conjugated carbonyl groups of the molecules, and insuring very similar environment to their dihydrophorbin rings.

B850–800 type complexes

Considering their resonance Raman spectra, B850–800-types complexes definitely constitute a heterogenous population among *Rhodospirillales*. Indeed, the spectra of B850–800 complexes extracted from *Chromatiaceae* clearly differ from those of B850–800 complexes extracted from *Rhodospirillaceae* in any of a–f spectral regions (Figs. 3 and 5). All of these spectra also differ from those of B880 complexes.

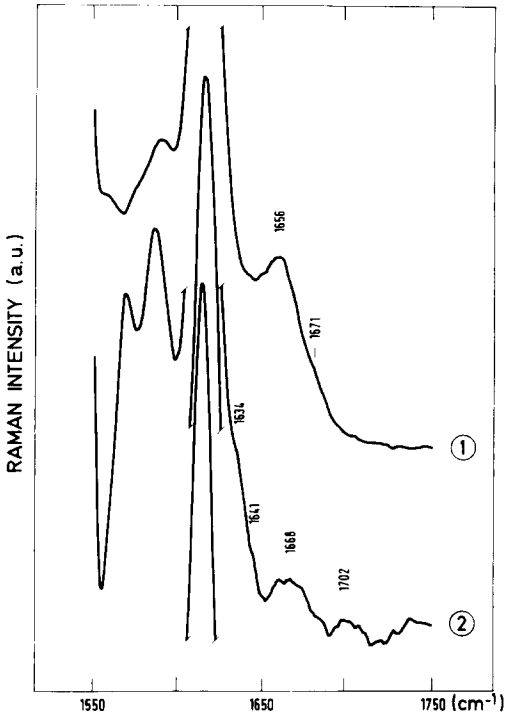

Fig. 5. Resonance Raman spectra (carbonyl stretching region) obtained at 30 K, excitation wavelength 363.8 nm, from B850–800 complexes of (1) *Thiocapsa roseopersicina* and (2) *Rsp. sphaeroides* 2.4.1. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

B850–800 and B820–800 complexes of Chromatiaceae. There is good evidence that these two forms of complexes are not independent: for example, it is now well known that many of their biochemical characteristics are identical [26] and that it is possible, by using detergents such as LDAO to reversibly transform the B850–800 form into the B820–800 form [27]. Resonance Raman spectroscopy fully confirms this structural analogy: for example, no difference in any band was observed between resonance Raman spectra of a B850–800 enriched fraction and those of a pure B820–800 fraction of *Thiocapsa roseopersicina* (not shown). The absence of any observable difference in the 1620–1700 cm^{-1} spectral region indicates that the carbonyl groups of the BChl molecules interact with the same ligands, in the same relative topology; the similarity observed in the a–e regions provides evidence that the microenvironment of the dihydrophorbin ring of BChl is the same in both B820–800 and B850–800 forms. It thus appears that the differences observed in the absorption spectra are probably due to a change of the tertiary structure of the protein, which could modify the excitonic interactions between pigments by changing distances and/or relative orientations between these pigments without otherwise modifying their local environments.

Resonance Raman spectra of B850–800 and B820–800 complexes from *Chromatium vinosum* and *Thiocapsa roseopersicina* can be characterized as follows.

(i) The 360 cm^{-1} band dominates the a region.

(ii) In the b region the 570 cm^{-1} band is very weak ($I_{590}/I_{570} = 4$) only as a shoulder of the 590 cm^{-1} band (Fig. 2).

(iii) In the c region, the 767 cm^{-1} band is very weak ($I_{767}/I_{700} = 0.4$) whereas the 795 cm^{-1} band is clearly stronger than in resonance Raman spectra of B880 complexes ($I_{795}/I_{700} = 3$).

(iv) In the d region, the 950 cm^{-1} band is stronger than in any other types of complexes ($I_{950}/I_{900} = 0.75$).

(v) The e band is weaker than in B880 complexes ($I_{1068}/I_{1615} = 0.4$).

(vi) In region f, the cluster of the C=O stretching bands has a very characteristic profile, consisting of a single complex broad band around 1655 cm^{-1}, with a shoulder at 1680 cm^{-1}. This band is prob-

ably composed of more than two convoluted components: indeed, shoulders around 1667, 1670 and 1680 cm^{-1} may represent stretching vibration bands of the ketone C=O groups (Fig. 5). The absence of any band near 1700 cm^{-1} indicates that all the ketone groups are involved in intermolecular interactions. The precise frequencies of the stretching bands of the acetyl groups are difficult to assess because of the convolution of many components in this region.

Rhodospirillaceae. Spectra of native B850-800 complexes. Resonance Raman spectra of B850–800-type complexes of *Rhodospirillaceae* present significant variations according to the bacterial species. In the f region particularly, very conspicuous spectral differences can be noted (Fig. 6). Nevertheless, these complexes have common spectral characteristics in the a–e regions, which distinguish them from B880 complexes and from B850–800 complexes of *Chromatiaceae*. These characteristics are the following,

a region: the bands located around 360 and 380 cm^{-1} have the same intensities ($I_{380}/I_{360} = 1$).

b region: the 570 and 590 cm^{-1} bands have the same intensities in spectra of complexes from *Rps. sphaeroides* and *Rps. capsulata*. For *Rps. palustris*, these bands are not resolved. This may be accounted for by a 5 cm^{-1} upshift of the 570 cm^{-1} band, which is weaker than for all the other species ($I_{590}/I_{570} = 1.3$).

c region: the 767 cm^{-1} band is weak for all three species ($I_{767}/I_{700} = 0.55$). The intensity of the 795 cm^{-1} band is close to that of the B880 complexes ($I_{795}/I_{700} = 2$).

d region; no accurate measurements of relative intensity ratios of the BChl *a* bands were possible in this region due to interference with carotenoid bands for all three species studied. However, it is possible to conclude that the I_{950}/I_{900} ratio is always weaker than for any other type of complexes (around 0.45).

e region: the 1068 cm^{-1} band is weaker than for any other types of complexes ($I_{1068}/I_{1615} = 0.25$).

f region; in the stretching region of the carbonyl groups, three bands located around 1632, 1642 and 1667 cm^{-1}, are common to the three species studied. A very weak band may also exist around 1680 cm^{-1}. However, complexes from *Rps. sphaeroides* and *Rps. capsulata* yielded resonance Raman bands at 1700 cm^{-1} accounting for interaction-free ketone carbonyl groups [16], whereas the B850–800 complexes from *Rps. palustris* did not yield any visible band at this frequency. This spectral difference indicates a major difference in the interaction states of the keto carbonyl of at least one of the three unequivalent BChl molecules present in the B850–800 complexes from *Rps. palustris* and from *Rps. sphaeroides* and *Rps. capsulata*.

Spectra of B850–(800-depleted) complexes from *Rps. sphaeroides* and from *Rps. palustris*. Resonance Raman spectra of B850–800 complexes selectively depleted of their 800 nm absorbing BChl reinforce the conclusion that the microenvironment of the BChl within this type of complexes depends upon the species of *Rhodospirillaceae* considered. Indeed, the f region of the resonance Raman spectra of B850–(800-depleted) complexes from *Rps. sphaeroides* exhibits weak bands at 1634 and 1641 cm^{-1} and a broad band at

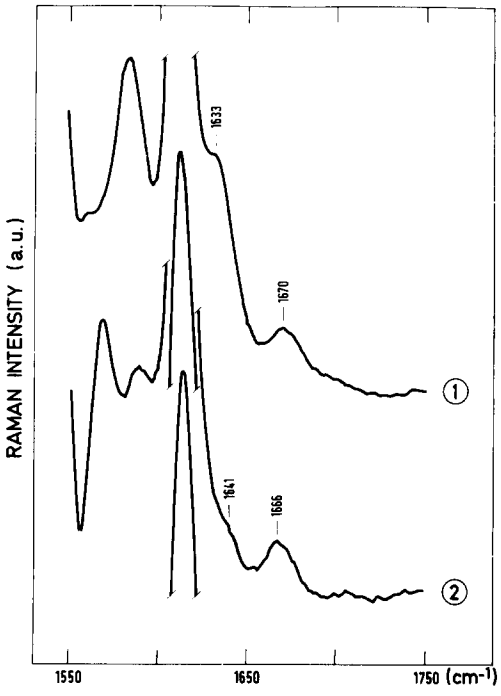

Fig. 6. Resonance Raman spectra (carbonyl stretching region) obtained at 30 K, excitation wavelength 363.8 nm, from B850 (800-depleted) complexes of (1) *Rps. palustris* and (2) *Rps. sphaeroides* 2.4.1. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

1667 cm^{-1}, whereas for *Rps. palustris* this region only consists of an intense band at 1633 cm^{-1} and of a broad band at 1667 cm^{-1} (Fig. 6). The variability in the local environments of the pigments within the B850–800 complexes thus concerns a least one of the two BChl a responsible for the absorption at 850 nm. Indeed, for *Rps. sphaeroides*, the bands at 1634 and 1641 cm^{-1} indicate that the acetyl carbonyls of the two 850 nm absorbing BChl are engaged in unequivalent intermolecular bonding, whereas for *Rps. palustris* the two groups vibrate at the same frequency, and hence may well be engaged in identical intermolecular bonding. However, it may be noted that for the three species of *Rhodospirillaceae* both BChl molecules responsible for the absorption at 850 nm have both their conjugated carbonyls engaged in intermolecular bonding.

Resonance Raman spectrum of the 800 nm absorbing BChl

It is possible to obtain the contribution of the 800 nm absorbing BChl to the spectrum of the whole complex, by subtracting the resonance Raman spectra of the B850–(800-depleted) complexes from the spectra of the B850–800 complexes. For this, it was necessary to attribute to the spectra relative weightings corresponding to the numbers of BChl molecules they contain. This operation gave essentially the same results, whether the normalization between the spectra was done on the carotenoid bands or on the strongest BChl bands at 1615 cm^{-1}. This confirmed the $3:x$ and $2:x$ relative stoichiometries of BChl *a* and carotenoids in these two complexes. For *Rps. palustris* the recording and addition of 8–15 successive spectra of each complex insured signal-to-noise ratios high enough to yield a difference spectrum of the 800 nm absorbing BChl *a* of good quality throughout the 100–1700 cm^{-1} region. For *Rps. sphaeroides*, the observation was limited to the f region of the spectrum of this BChl. We purposely limited our interest to the frequencies of the Raman bands in these difference spectra and did not consider their relative intensities, the reliability of which might have been impaired by the normalization procedure.

Only a few frequency differences appear between resonance Raman spectra of the 800 nm absorbing BChl and of the other two of B850–800 complexes. In the regions corresponding to the vibrations of the dihydrophorbin ring of the BChl only one band can possibly be shifted: it is the 800 cm^{-1} band, which dominates the c region. This band is located at 792 cm^{-1} for the 800 nm absorbing BChl and at 795 cm^{-1} in all the other cases. It probably arises from a bending mode of CNC bonds of the pyrroles. In the f region, resonance Raman spectra of the 800 nm absorbing BChl are very different according to the bacterial species considered (Fig. 7). For *Rps. sphaeroides*, this region exhibits a band at 1632 cm^{-1} accounting for the stretching of an intermolecularly bound acetyl carbonyl, and a band at 1700 cm^{-1} accounting for the stretching of a ketone carbonyl free from bonding. For *Rps. palustris*, the strongest bands in this region are located at 1645 and 1663 cm^{-1}. In this latter case, both conjugated carbonyls of the 800 nm absorbing BChl thus are involved in intermolecular interactions. Quite different host-sites, hence, should accommodate the B800 molecules in these two species.

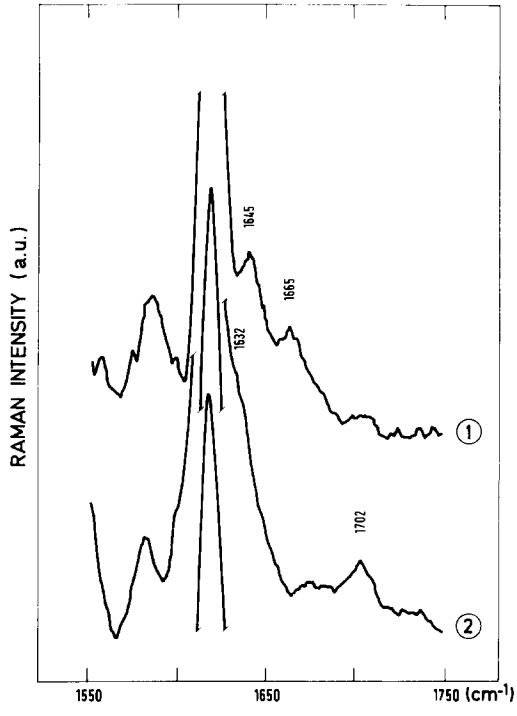

Fig. 7. Spectra (carbonyl stretching region) obtained by subtracting resonance Raman spectra of B850 (800-depleted) complexes to resonance Raman spectra of B850–800 complexes from (1) *Rps. palustris* and (2) *Rps. sphaeroides* 2.4.1.

Hence, in the B850–800 complexes, the variability is greater for those sites accommodating 800 nm absorbing BChl molecules than for those accommodating BChl 850. It thus appears that the more the energy of the lower singlet of antenna BChl is decreased from its value in the isolated state, the smaller is the observed variability in its proteic host-sites, in the set of complexes observed.

Environmental interactions on BChl within antenna complexes

Coordination numbers of the central Mg of BChl

Soret-excited resonance Raman spectra of BChl contain a 290–305 cm^{-1} band most probably arising form an in plane mode involving stretching of the Mg-N(pyrrole) bonds [22]. The frequencies and structure of this band depend on the coordination number of the Mg atom, being higher than 300 cm^{-1} if this atom binds two external ligands, and lower than 300 cm^{-1} if it binds a single external ligand [24]. For all of the complexes investigated, the BChl spectra yield bands at 290–300 cm^{-1}, [28] indicating that these pigments generally have five-coordinated magnesiums. In particular, the B800 molecule of *Rps. palustris* yields a 290 cm^{-1} band, and hence has a five coordinated Mg atom [28]. Shoulders however may occur on the high frequency side of the 295 cm^{-1} band, particularly for the B880-type complexes [28]. Such shoulders were initially taken as indicative of six coordinated BChl molecules [29]. However, the presence of a 300 cm^{-1} shoulder in spectra of the soluble complex from *P. aestuarii* in which all seven BChl molecules are expected to share five-coordinated Mg atoms [15] led us to assign them to an intrinsic complexity of the 295 cm^{-1} band [16].

Cotton and Van Duyne [30] also proposed that the wavenumber of the 1600 cm^{-1} band of BChl which arises from stretching of the methine bridges [22] can be used for diagnosing the coordination number of the magnesium atoms. Five coordination should correspond to a wavenumber 15 cm^{-1} higher than in the case of six coordination. In vitro experiments done in our laboratory on BChl in presence of substituted imidazoles (1-methyl and 2-methyl derivatives) corroborate this proposal. This band was observed at 1602 cm^{-1} in the presence of 1-methylimidazole and at 1615 with a 1605 cm^{-1} component in the presence of 2-methylimidazole. For all of the antenna complexes, this band is located around 1615 cm^{-1}. Moreover, it always has a symmetrical shape, making improbable any contribution from a 1600 cm^{-1} component overlapping with the main band (Figs. 4–7). Hence, it is concluded that, in all of the antenna complexes considered in the present study, the central Mg atoms of all of the BChl molecules are five-coordinated.

Existence of BChl–BChl bonding

The possibility that some of the pairs of BChl molecules present in antenna complexes from *Rps. sphaeroides* might be interconnected by Mg..O = C or Mg..OH_2..O = C bonds [31] has been largely ruled out [16,22–24] by comparisons of the νC=O frequencies of these molecules in the complexes, in simple in vitro systems involving monomers or oligomers, and in the soluble complex from *Prosthecochloris aestuarii*, for which the presence of such bonds has been excluded on the basis of X-ray data [15].

The present study permits this conclusion to be extended to other *Rhodospirillales*, and provides another piece of evidence against a possible bonding between antenna BChl molecules: in vitro studies [22,24] have shown that the formation of oligomers, and particularly of hydrated oligomers from BChl *a* monomers, induces frequency shifts of certain modes of their dihydrophorbin skeletons, independently of shifts due to a change in coordination number of the Mg atom. On the other hand, the 800 nm absorbing molecule of B850–800 complexes is generally considered not to be closely associated with the other two molecules, because of the lack of detectable electronic interactions between them [5]. It hence constitutes a valuable model of a monomer BChl inserted in an environment very similar to those of other antenna BChl, e.g., the B850 pair, for which dimeric states might be possible. Table III shows that actually all of the skeletal resonance Raman bands which are sensitive, in vitro, to oligomer formation occur at the same frequencies in spectra of B800 and of B850 molecules from *Rps. palustris*. As mentioned earlier the latter band frequencies are also those observed for any antenna complex from

TABLE III

WAVENUMBERS (cm^{-1}) OF RESONANCE RAMAN BANDS PARTICULARLY SENSITIVE TO SELFAGGREGATION OF BChl IN VITRO COMPARED TO THOSE OBSERVED IN VIVO ON THE SAME BANDS MEASURED AT 30 K WITH 363.8 nm EXCITATION

Bacteriochlorophyll *a* monomers [a]	590	893	1123	1341	1497	1577
Bacteriochlorophyll *a* oligomers [a]	570	893	1123	1346	1504	1541
Hydrated polymers of bacteriochlorophyll *a* [a]	583	905	1115	1345	(...)e	(...)e
Prosthecochloris aestuarii [b]	587	896	1123	1349	1476	1584
800 nm absorbing BChl of *Rps. palustris* [c]	587	898	1118	1346	1472	1585
Antenna complexes of *Rhodospirillales* [d]	590	899	1118	1348	1473	1586

[a] From ref. 24.
[b] From ref. 16.
[c] Obtained on calculated spectra (see text).
[d] No detectable shift between all complexes studied.
[e] No detectable bands.

Rhodospirillales. This confirms that no BChl–BChl dimer or oligomer (in terms of ground state interactions) may occur in any of the complexes studied. Interactions between antenna BChl molecules thus essentially occur between electronically excited levels, while their groundstate electronic levels are hardly perturbed by the presence of nearby pigments [16].

BChl–lipids interactions

The recent sequencing of antenna polypeptides of *Rhodospirillaceae* allowed Zuber and coworkers [32] to propose a three-dimensional model for the structures of B850–800 and B880-type complexes. According to this model, the polar heads of the BChl molecules should lie at interfaces between the polypeptides and the lipids. This possibility appears questionable, however, on the basis of resonance Raman data.

The extraction procedures used in isolating the antenna complexes result in an extensive replacement of membrane lipids by detergent molecules [33]. The latter, e.g., deoxycholate, may be chemically quite different from the lipids, and their coming into close contact with the polar heads of the BChl molecules should result in two types of detectable effects, namely:

(i) alterations in the intermolecular bonding involving the carbonyls and the magnesium of BChl and

(ii) changes in the nonbonding interactions on the dihydrophorbin rings brought about by changes in distribution of local charges, in Van der Waals interactions or local permittivities. Effects of the first type should in particular be observed in the f regions of resonance Raman spectra. The second type of environmental changes should result in detectable changes in Raman bands arising from modes of the dihydrophorbin skeleton. Indeed, these bands have been shown to be very sensitive to the nature of the solvent, in simple binary solutions of chlorophylls, particularly in the case of BChl [22,24]. Yet, no significant difference could be detected, in any spectral region, between resonance Raman spectra of chromatophores and of B880 complexes from *Rsp. rubrum*, which contains this single type of complex. Also, resonance Raman spectra of B850–800 extracted from *Rps. palustris* using either LDAO or deoxycholate are identical. More particularly referring to intermolecular bonding of conjugated carbonyls, it has been possible to precisely reconstitute the f spectral region for chromatophores of the R26.1 strain of *Rps. sphaeroides* using resonance Raman spectra of B880 and B850 (800-depleted) complexes [17]. In addition, the 800 nm absorbing molecule

of the B850–800 complex from *Rps. sphaeroides*, in the membrane, has a keto carbonyl free from intermolecular bonding (Fig. 7). This carbonyl remains free throughout the extraction procedure of the complex. Hence, although this molecule is easily extractible from the complex, its keto carbonyl is not accessible to the external medium.

A possible objection to the above reasoning is that current preparations of antenna complexes, including ours, are generally aggregated [34]. However, it clearly appears that detergent molecules have easy access to all of the complexes during the extraction. For instance, when adding LDAO to B850–800 complexes from *Thiocapsa roseopersicina*, their transformation into a B820–800 form is rapid (a few seconds) and complete. In these conditions, the absence of modification of resonance Raman spectra after extracting complexes by action with detergent indicates that the dihydrophorbin rings of BChl are not in contact with the lipids. This conclusion derived from resonance Raman data about the location of the dihydrophorbin rings of antenna BChls should not be extended to their phytyl or geranylgeraniol chains, which do not contribute to resonance Raman spectra. It cannot be excluded that these chains are at the protein surface, where they could help protecting the dihydrophorbin rings from the external medium.

The presence of carotenoids in antenna complexes has no detectable influence on the resonance Raman spectra of BChl: indeed, resonance Raman spectra of B880 complexes from *Rsp. rubrum* S1 (wild type) and G9 (carotenoidless) are identical (not shown). Hence, no detectable ground-state interactions occur between carotenoids and the dihydrophorbin ring of BChls. We are thus left with the conclusion that the ground-state interactions assumed by the dihydrophorbin rings of antenna BChls and detected by resonance Raman spectroscopy occur with the peptidic moieties of the complexes.

Magnesium ligands of antenna bacteriochlorophylls

In addition to their sensitivity to the coordination number of its Mg atom, resonance Raman spectra of BChl are sensitive to the nature of the magnesium ligand. Evidence for this sensitivity was obtained by comparison of in vitro resonance Raman spectra of BChl, the Mg atom of which was singly liganded to various molecules. These ligands were: conjugated carbonyls of BChls (in BChl oligomers), water (in hydrated polymers), 2-methylpyridine and 2-methylimidazole. Resonance Raman spectra of these samples differed by several band structures and frequencies in the 250–1620 cm^{-1} region (Table X in Ref. 28). A group of bands in the 670–810 cm^{-1} region appeared particularly sensitive to the nature of the Mg ligands (Fig. 8).

Using this spectral region as well as the whole spectrum as a fingerprint for a given Mg ligand, it was found that resonance Raman spectra of B880 and B850 antenna BChl were closer to that of the 2-methyl-imidazole-BChl complex than to any other (Fig. 8) despite the expected differences in dielectric permittivities of the environments [16].

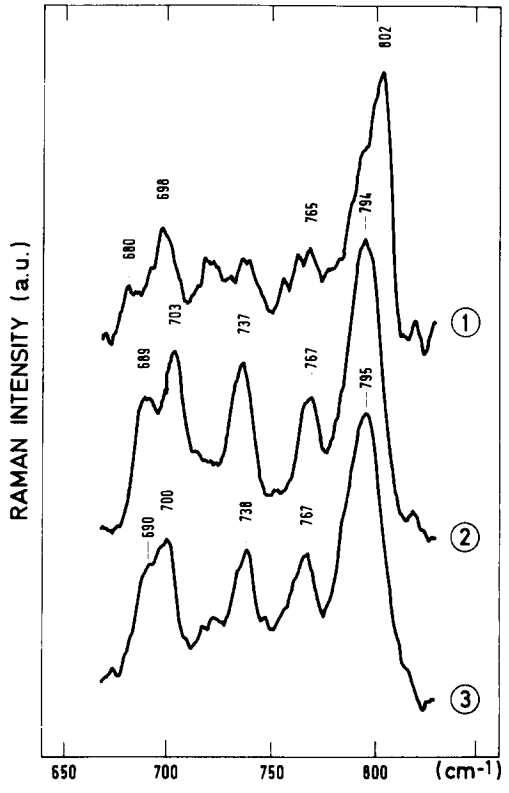

Fig. 8. Resonance Raman spectra (d region) obtained at 30 K, excitation wavelength 363.8 nm of (1) BChl in 2 methylpyridine; (2) B880 complexes from *Rps. palustris* and (3) BChl and 2 methylimidazole codissolved in tetrahydrofuran. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

This gives a good indication that histidine side chains may be the Mg ligands of both the 880 and 850 nm absorbing BChl pairs. Another indication for this possibility may be found in the close similarity of resonance Raman spectra of antenna complexes of *Rhodospirillales* with those of the soluble complex of *P. aestuarii*, for which X-ray crystallography indicated that at least six of the unequivalent BChl molecules should be liganded to His side chains through their Mg atoms [15]. If the present proposal is correct, the occurrence of a single His residue in the sequences of each of the two polypeptides constituting B880 and B850–800 complexes of *Rhodospirillaceae* [32] would imply that each of the two polypeptides constitutes an anchoring site for the Mg atom of one BChl molecule (but not necessarily for its other functional groups). Another possibility is that both BChl molecules of the 880- or 850 nm absorbing pairs could be liganded at the same histidine through an histidinate form (Ref. 35, see also pyrazine adducts in Ref. 30).

On the other hand, it is of interest that the resonance Raman spectrum of the 800 nm absorbing BChl present in the B850–800 complexes from *Rps. palustris* differs from that of the 850 or 880 nm absorbing pairs by the frequency of the 795 cm^{-1} band, observed at 792 cm^{-1} for B800. This may indicate that this molecule has a Mg ligand different from those of the other antenna molecules, and hence which should not be histidine. This possibility again is consistent with the likely presence of one His residue only in each of the two polypeptides constituting the B850–800 type complexes [32].

Conclusion. A classification of antenna complexes types according to local environments of the BChl molecules

The structural information provided by resonance Raman spectroscopy, which specifically concerns narrow regions around pigments, is of particular relevance when characterizing antenna complexes, because their optical properties essentially depend on local interactions. It thus appears justified to use resonance Raman spectral data as parameters for classifying antenna complexes from *Rhodospirillales* on a structural basis. In 1978, Thornber hypothesized from electronic absorption data that there were only two universal structural forms of antenna complexes in *Rhodospirillales*, B880 and B850–800 [1]. From resonance Raman data, it can be concluded that

(1) the structure of the host sites of BChl is most probably the same in any B880 antenna complexes of *Rhodospirillales*, and

(2) these host site structures vary among B850–800 complexes, depending on the bacterial species and genus. At least two different types of B850–800 complexes must be distinguished on this basis, that we propose to name B850–800 S (from sulfur purple bacteria or *Chromatiaceae*) and B850–800 NS (from nonsulfur purple bacteria or *Rhodospirillaceae*). B820–800 complexes of *Chromatiaceae* clearly belong to the B850–800 S type. On the other hand, in spite of that (limited) variability, the microenvironments of BChl have several characteristics in common, as indicated by constant frequencies of most of the Raman-active vibrational modes of these molecules regardless of the complex type and bacterial species.

Simultaneously with this proposal [18,19], Thornber and co-workers recently proposed another classification of antenna complexes of *Rhodospirillales*, involving two structurally distinct types of B880 and two or three types of B850–800 complexes [26]. It appears from Raman data that the biochemical properties which differentiate the B890 and B875 subclasses defined by these authors, e.g., carotenoid stoichiometry and polypeptide composition [26] do not affect their 'active site' structures. As discussed above for B850–800 and B820–800 complexes of *Chromatiaceae*, such differences should only result in minor changes in the relative positioning of the chromophores. On the opposite, a current Raman investigation of complexes from *Rps. acidophila* indicates that the B850–800 complex from cells grown in low light structurally differs from B850–800 of *Chromatium vinosum*.

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References

1 Thornber, J.P., Trosper, T.L. and Strouse, C.E. (1978) in The Photosynthetic bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 133–160, Plenum Press, New York

2 Morita, S., Hayashi, H., Miyao, M., Nosawa, T. and Hatano, M. (1983) in Photosynthesis (Akoyunoglou, ed.), Vol. 3, pp. 513–522, Balaban International Science Services, Philadelphia, PA

3 Breton, J. and Vermeglio, A. (1982) in Photosynthesis. Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. 1, p. 152–174 Academic Press, New York

4 Bolt, J.D., Sauer, K., Shiosawa, J.A. and Drews, G. (1983) Biochim. Biophys. Acta 635, 535–541

5 Sauer, K. and Austin, L.A. (1978) Biochim. 17, 2011–2018

6 Hayashi, H., Nosawa, T., Hatano, M. and Morita, S. (1982) J. Biochim. 91, 1029–1038

7 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 403–416

8 Shiosawa, J., Cuendet, P., Zuber, H. and Drews, G. (1981) in Photosynthesis (Akuyonoglou, G., ed.), Vol. 3, pp. 427–434, Balaban International Science Services, Philadelphia, PA

9 Kramer, H.J.M., Van Grondelle, R., Hunter, C.N., Westerhuis, W.H.S. and Amesz, J. (1984) Biochim. Biophys. Acta 765, 156–165

10 Shiosawa, J., Cuendet, P. Drews, G. and Zuber, H. (1980) Eur. J. Biochem. 111,455–460

11 Cogdell, R.J., Durant, I., Valentine, J., Lindsay, J.G. and Schmidt, K (1983) Biochim. Biophys. Acta 722, 427–435

12 Brunisholz, R.A., Cuendet, P., Theiler, R. and Zuber, H. (1981) FEBS Lett. 123, 150–154

13 Tadros, M.H., Suter, F., Seydewitz, H.H., Witt, I, Zuber, H. and Drews, G. (1984) Eur. J. Biochem. 138, 209–212

14 Gogel, GE., Parkes, P.S., Loach, p.a., Brunisholz, R.A. and Zuber, H (1983) Biochim. Biophys. Acta 746, 32–39

15 Matthews, B.W., Fenna, R.E., Bolognesi, M.C., Schmid, M.F. and Olson, J.M. (1979) J. Mol. Biol. 181, 259–285

16 Lutz, M., Hoff, H.F. and Brehamet, L. (1982) Biochim. Biophys. Acta, 679, 331–341

17 Robert, R., Vermeglio, A. and Lutz, M. (1984) Biochim. Biophys. Acta 766, 250–262

18 Robert, B., Vermeglio, A. and Lutz, M. (1983) in Advances in Photosynthesis Research (Sybesma C., ed.), Vol. II, pp. 199–202, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, the Netherlands

19 Lutz, M. and Robert, B. (1983) Workshop on Molecular Structure and Function of Light Harvesting Pigment Protein Complexes and Reaction Centers, Zürich, Abstract No. II-11

20 Clayton, R.K. and Clayton, B.J. (1972) Biochim. Biophys. Acta 283, 692–698

21 Clayton, R.K. and Clayton, B.J. (1983) in Photosynthesis (Akoyunoglou, G., ed.), Vol. 3, pp. 461–465, Balaban International Science Service, Philadelphia, PA

22 Lutz, M. (19840 in Advances in Infrared and Raman spectroscopy (Clark, R.J.H. and Hester, R.E., eds.), Vol. 11, pp. 211–360, Wiley Heyden, London

23 Lutz, M., Kléo, J. and Reiss-Husson, F. (1976) Biochem. Biophys. Res. Comm. B.B.R.C. 69, 711–717

24 Lutz, M. (1979) Thèse de Doctorat d'Etat, Université Pierre et Marie Curie, Paris

25 Broglie, R.M., Hunter, C.N., Delepelaire, P., Niederman, R.A., Chua, N.H. and Clayton, R.K. (1980) Proc. Natl. Acad. Sci. USA 77, 87–91

26 Thornber, J.P., Cogdell, R.J., Seftor, R.E.B., Pierson, B.K. and Tobin, E.M. (1983) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 25–32, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands

27 Erokhin, Yu. and Sinegub, O.A., (1970) Mol. Biol. USSR (English translation) 4, 437–440

28 Robert, B. (1983) Thesis, Université Pierre et Marie Curie, Paris

29 Lutz, M. (1983) in Photosynthesis (Akoyunoglu, G., ed.), Vol. 3, pp. 461–476, Balaban International Science Services Philadelphia, PA

30 Cotton, T.M. and Van Duyne R.P. (1981) J. Am. Chem. Soc. 103, 6020–6024

31 Katz, J.J., Norris, J.R. and Shipman, L.L. (1976) Brookhaven Symp. Biol. 28, 16–55

32 Theiler, R. and Zuber, H. (1983) Workshop on Molecular Structure and Function of Light Harvesting Pigment Protein Complexes and Reaction Centers, Zürich, Abstract No. II–8

33 Radcliffe, C.W., Pennoyer, J.D., Broglie, R.M., Niederman, R.A. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 215–219, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands

34 Van Grondelle, R., Hunter, C.N., Bakker J.C.G. and Kramer H.J.M. (1982) Biochim. Biophys. Acta 723, 30–36

35 Landrum, J.T., Hatano, K., Scheidt, W.R. and Reed, C.A. (1980) J. Am. Chem. Soc. 102, 6729–6734