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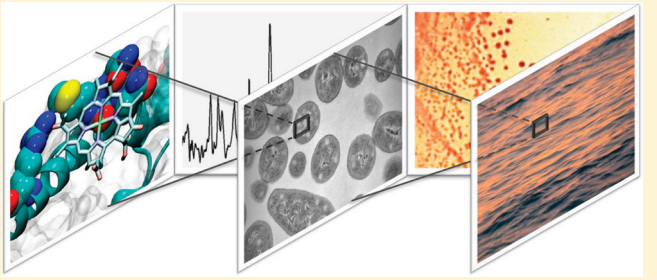
Molecular Origins and Consequences of High-800 LH2 in *Roseobacter denitrificans*

Katia Duquesne, Cecile Blanchard, and James N. Sturgis*

LISM, CNRS - Aix-Marseille University, Marseilles, France

ABSTRACT: *Roseobacter denitrificans* is a marine bacterium capable of using a wide variety of different metabolic schemes and in particular is an anoxygenic aerobic photosynthetic bacterium. In the work reported here we use a deletion mutant that we have constructed to investigate the structural origin of the unusual High-800 light-harvesting complex absorption in this bacterium. We suggest that the structure is essentially unaltered when compared to the usual nonameric complexes but that a change in the environment of the $C_{13:1}$ carbonyl group is responsible for the change in spectrum. We tentatively relate this change to the presence of a serine residue in the α-polypeptide. Surprisingly, the low spectral overlap between the peripheral and core light-harvesting systems appears not to compromise energy collection efficiency too severely. We suggest that this may be at the expense of maintaining a low antenna size.



In the purple photosynthetic bacteria, incident light energy is collected by an extensive system of light-harvesting pigment protein complexes that transfer the absorbed light energy to the photochemical reaction center where it is transduced into chemical potential energy. The properties of the different pigment protein complexes are precisely tuned in order to ensure the efficiency of this process. In particular, the organization of the pigments within the proteins and the nature of their binding sites are used to modulate the absorption and fluorescence properties of the light-harvesting apparatus and the organization of the proteins within the membrane are controlled to ensure optimal functional coupling of the different proteins.[1,2]

The structures of several different light-harvesting complexes have been resolved by X-ray crystallography. These complexes can be divided into two groups: core light-harvesting complexes, or LH1, that surround the reaction centers and peripheral light-harvesting complexes (LH2) which transfer absorbed light energy to the core complexes. The architecture of core light-harvesting complexes varies somewhat between species. They are always formed by an oligomer of LH1 subunits surrounding a reaction center. These oligomers can be formed of apparently closed rings of 16 monomers as in the species *Rhodospirillum* (*Rsp.*) *rubrum*[3] and *Rsp. photometricum*[4] or open rings containing a defect in the ring as observed in *Rhodopseudomonas* (*Rps.*) *palustris*.[5] Furthermore, in some species, notably *Rhodobacter* (*Rb.*) *sphaeroides* the core complexes can be dimeric, containing two reaction centers and an "S-shaped" oligomer of LH1 subunits.[6] In all these structures the LH1 subunits are composed of an α- and a β-polypeptide 2 bacteriochlorophyll *a* (BChl) molecules and a carotenoid molecule. A typical LH2 complex is composed of a ring of 8[7] or 9[8] or more rarely another number of subunits,[9,10] each composed of an α- and a β-polypeptide, 3 bacteriochlorophyll *a* (BChl) molecules, and 1 or 2 carotenoid molecules. The Bchl molecules can be divided into two groups: monomeric BChl that typically absorb near 800 nm and oligomeric BChl molecules that are responsible for the main absorption band usually near 850 nm.

Our knowledge of the organization of the different proteins within the membrane has been greatly advanced recently by atomic force microscopy (AFM) which has allowed a vision of the organization of the native membrane in several different species.[4,11−14] This vision has led to the development of ideas on the importance of this organization for the function of the system at both the level of energy transfer[1,15] and quinol/quinone exchange.[16−18]

In previous work it has been found that H-bonding to the C_3 carbonyl group is important for the modulation of the absorption spectrum. In LH2 complexes with an absorbance maximum at 850 nm the C_3 carbonyl groups have relatively strong H bonds, while in complexes with an absorbance maximum near 820 nm these H bonds are absent.[19] Furthermore, the mutation of the amino acid residues involved in these hydrogen bonds results in a progressive shift of the absorption maximum to 820 nm. This seems to be a general phenomenon as mutations that modify the H bonding to the C_3 acetyl group in LH1 antenna complexes have a similar effect.[20,21] The synthesis of these results indicates a quasi-linear relationship between the change in the absorption maximum and the frequency shift of the C_3 carbonyl modes.[22] This body of work has shown how the position of the main near-IR transition in absorption spectra of LH2 complexes can be

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modulated between 820 and 850 nm. These changes depending on the pair of residues found in the α polypeptide at positions His_{+13} and His_{+14} (residues 13 or 14 after the histidine that ligates the oligomeric Bchl).

A few LH2 complexes have an unusual High-800 absorption with little or no absorption above 800 nm. This is the case for LH2 complexes from *Rps. palustris* grown at very low light intensity[23] and *Roseobacter* (*Rsb.*) *denitrificans* (see below). Little is known about the structural modifications that give rise to this unusual absorption spectrum. In the case of *Rps. palustris* there is some structural information in the form of a low-resolution X-ray structure of an unusual octameric complex.[23] Further spectral studies been used to propose an origin for the High-800 absorption in polypeptide heterogeneity.[24]

Rsb. denitrificans is a marine aerobic anoxygenic photosynthetic bacterium capable of using cyclic photosynthetic electron transfer in aerobic conditions. The group as a whole are the only known organisms capable of photosynthesis requiring oxygen but not producing oxygen.[25] However, *Rsb. denitrificans* is not only capable of photoheterotrophic growth in the presence of oxygen and light but also anaerobically in the darkness using nitrate or trimethylamine *N*-oxide as an electron acceptor.[25] The genome of the bacterium has been completely sequenced,[26] and it has been found to contain a single *puc* operon encoding the LH2 polypeptides. Recently, we have developed the tools necessary for the genetic manipulation of this species allowing the construction of several mutants.[27]

In the work reported here we have used this ideal model system to investigate the structure of the High-800 LH2 from *Rsb. denitrificans*, the molecular origins of this unusual spectrum, and the functional consequences of such a spectrum.

EXPERIMENTAL SECTION

Sample Preparation. Both the wild-type and Δ*puf-BALMC* strains of *Rsb. denitrificans*[27] were grown aerobically in Marine Broth 2216 (Difco) medium at 28 °C in under filled toxin flasks with agitation at 200 rpm in the dark. Cells were harvested in late log phase.

To prepare photosynthetic membranes, chromatophore cells were resuspended in 10 mM Tris pH 7.5, 8.5% sucrose, 0.6 mg/mL lysozyme, 400 mg/mL DNA'ase, 20 mg/mL RNA'ase, complete antiprotease cocktail (Roche), and 5 mM EDTA and were broken with one passage through a French pressure cell at 900 kPa. The resulting lysate is layered directly onto 10−70% sucrose density gradients and centrifuged 90 min in a SW27 swinging bucket rotor at 25 000 rpm. The colored band in the middle of the gradient is collected, diluted with Tris 20 mM pH 7.5, and pelleted by centrifugation for 90 min at 40 000 rpm in a Ti45 rotor.

For the preparation of LH2 complexes purified membranes were solubilized with dodecyl maltoside (5% final concentration in the same buffer) for 2 h. Unsolubilized material was removed by a centrifugation of 30 min at 60 000 rpm, and the supernatant was loaded directly onto a Resource Q column, equilibrated with Tris 20 mM pH 7.5, 20 mM NaCl, and 0.05% dodecyl maltoside. Light-harvesting complexes are eluted with a linear gradient from 20 mM NaCl to 1.0 M NaCl. The fractions with the highest ratio of absorbance at 800 to 280 nm are pooled and concentrated using centrifugal concentrators (30K cutoff). The concentrated fraction is loaded onto a superose 6 gel-filtration column and eluted with Tris 20 mM pH 7.5, 20 mM NaCl, and 0.05% dodecyl maltoside. The fractions with the highest ratio of absorbance at 800 to 280 nm are again pooled and concentrated using centrifugal concentrators (30K cutoff).

For analytical gel filtration the same column, buffer and elution conditions were used, and the column was calibrated with thyroglobulin, ferritin, aldolase, LH2 from *Phsp. molischianum*[7] and *Rsp. photometricum*,[42] and BSA standard.

Spectroscopy. Fluorescence spectra were recorded on a Fluorolog 3 (Horiba JobinYvon) fluorimeter. Fluorescence emission spectra were recorded with a 90° geometry and 590 nm excitation into the Bchl Q_x transition. The spectra were corrected for the detector sensitivity using a calibrated Si photodetector.

CD spectra were recorded on a Jasco J-815 in 1 mm cuvettes with a sample adjusted to give an absorbance of about 0.5 at the near-IR maximum, in order to maximize the signal-to-noise ratio in this region. Spectra for six scans were averaged, measured with a 5 nm optical resolution and a 2 s time constant.

Raman spectra were recorded with a Labram HR800 (Horiba JobinYvon) using a 1064 nm excitation of Nd:Yag laser. The output laser power was set at 500 mW, and the power arriving on the sample is estimated to be about 170 mW. The sample was deposited on a glass microscope slide and observed microscopically before and after spectrum acquisition; no sample damage was observable. The laser beam was focused on the sample using an objective 100×. The accumulation time was 120 s. A baseline correction was made to eliminate residual fluorescence. Fitting and analysis of spectra were done using the Grams software (Galactic).

RESULTS AND DISCUSSION

The absorption spectrum of *Rsb. denitrificans* cells is unusual and shows only two peaks in the near-IR region centered at 870 and 803 nm, as shown in Figure 1A. This spectrum is that f1 expected of a photosynthetic bacterium with a core complex and a High-800 type peripheral light harvesting complex. As mentioned in the Introduction, the genome of *Rsb. denitrificans* has been completely sequenced,[26] and the species has a single *puc* operon. This makes it an ideal organism in which to study this type of complex in order to understand the molecular origin of this type of spectrum and the functional consequences. As there is only one *puc* operon unlike the other species known to produce such a complex, *Rps. palustris* where sample preparation and the interpretation of the results are hampered by the presence of multiple differentially regulated *puc* operons, with the associated possibility of forming mixed complexes.[15,24]

In order to allow us to study the spectral properties more easily, we have constructed a deletion mutant lacking the *puc* operon,[27] as expected the absorption spectrum of cells from this strain show a single absorption band in the near-IR region (Figure 1A, red line). In the measurements performed here we have used membranes or complexes isolated from this strain to avoid any possible contamination by core complexes.

The absorption spectrum of LH2 complexes isolated from the deletion strain (Figure 1B, red line) shows a single Q_y peak in the near-IR with a maximum at 804.5 nm and a full width at half-maximum of 28 nm. There is no clear resolution of the monomeric and oligomeric absorption bands, and the Q_y peak is rather narrower than in most other complexes. The BChl Q_x maximum is at 587 nm, as in other LH2 complexes; however,

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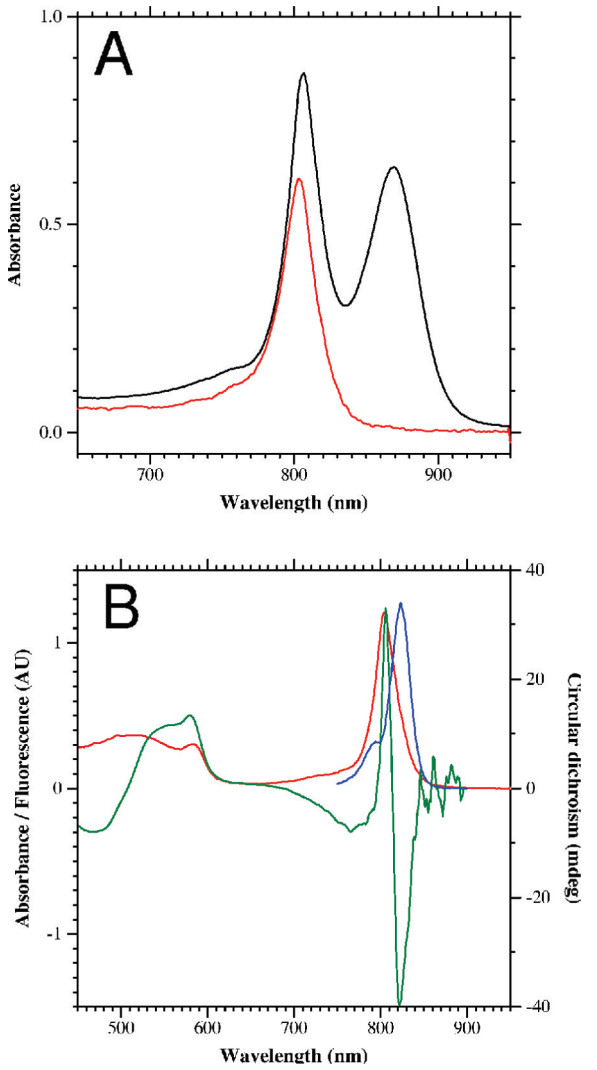

Figure 1. Optical spectra of *Rsb. denitrificans* (A) cells and (B) isolated LH2 complexes. (A) absorption spectra of wild-type (black) and Δ*puf* (red) cells of *Rsb. denitrificans*. (B) Absorption (red), fluorescence emission (blue), and circular dichroism (green) spectra of LH2 complexes isolated from the Δ*puf* strain.

the broad carotenoid band with a maximum at 520 nm is rather low compared to other LH2 complexes, suggesting that there may only be a single carotenoid per monomer in these complexes.

The fluorescence emission spectrum (Figure 1B, blue line) shows a typical emission spectrum with a shoulder at 791 nm and the main peak at 823 nm. This corresponds to a Stokes shift of about 19 nm at 300 K, well in line with that observed for B800−820 and B800−850 complexes. This gives a preliminary indication that the excitonic structure could be similar to that of other LH2 complexes, where a relatively large Stokes shift is associated with thermal relaxation of the single exciton manifold.

The observed spectra are in complete agreement with those published recently by another group.[28] The narrow absorption and fluorescence bands compared to those observed for other species are intriguing. In previously studied complexes the width of the absorption and fluorescence bands has two origins: the splitting of the single exciton manifold due to coupling between the pigments and the binding site heterogeneity.[29] The binding site heterogeneity influences the bandwidth through two different mechanisms: first by adding a heterogeneous broadening term and second by increasing the absorption by normally forbidden exciton components. In these complexes it is possible that reduced heterogeneity is responsible for the relatively narrow absorption and fluorescence spectra.

The measured fluorescence emission spectrum has rather poor overlap with the core complex spectrum, suggesting that perhaps excitation trapping by core complexes might be compromised. In order to assess this we measured, in membranes, the effect of solubilization with 1% dodecyl maltoside on the LH2 fluorescence yield. This measurement suggests that trapping is still about 91% efficient in *Rsb. denitrificans*, compared to >98% efficient in the other strains examined.

The circular dichroism spectrum (Figure 1B, green line) is also typical of an LH2 complex with a strong conservative signal associated with the main near-IR transition and a relatively strong negative feature extending to toward the visible originating from the monomeric B800 bacteriochlorophylls.[30] In common with the LH2 of other species, the Q_x band gives a positive CD signal. Intriguingly, the carotenoid region shows a relatively strong positive−negative CD signal.

Overall, these spectra suggest that the High-800 LH2 complex of *Rsb. denitrificans* resembles in most respects previously studied LH2 complexes. Apart from the wavelength of the near-IR absorption maximum, the only unusual feature observed is the multicomponent carotenoid CD signal.

Protein Structure. In order to better understand the possible structure of the High-800 LH2 complex of *Rsb. denitrificans*, we have examined the structure of the protein. For this we have considered both the quaternary structure and the organization of the pigment binding sites. In a first approach we have examined the structure of LH2 complexes in *Rsb. denitrificans* chromatophores by AFM (Figure 2A). The complexes show the typical ring structures associated with the light-harvesting complex, though in vesicles it is hard to obtain excellent resolution. In the complexes we have imaged in which the subunits are visible 9 subunits with a C9 symmetry can be discerned. The size of the complexes observed in both AFM images of native membranes and electron microscope images of close-packed complexes in reconstituted membranes of 80 Å is close to that previously observed for nonameric complexes.[9] These observations would seem to suggest that the quaternary structure is as in the majority of LH2 complexes examined to date, namely a nonameric ring. These microscopic images are consistent with size measurements by gel exclusion chromatography (Figure 2C) which also give an elution profile consistent with nonameric complexes rather than octameric complexes.

Comparison of the sequence of the *Rsb. denitrificans* LH2 polypeptides with those from *Rps. acidophila* (Figure 2D) shows they are very similar, 40% and 61% identity for the α- and β-polypeptides, respectively, though the α-polypeptide has a 50 amino acid C-terminal extension. This high degree of similarity suggests that the two proteins will have similar structures. Furthermore, as shown in the figure, the similarities include importantly the majority of the residues involved in the interface between the α-polypeptides and the residues important for the BChl binding. Most notably all the determinants for the B800 binding site (Figure 2B) are present,

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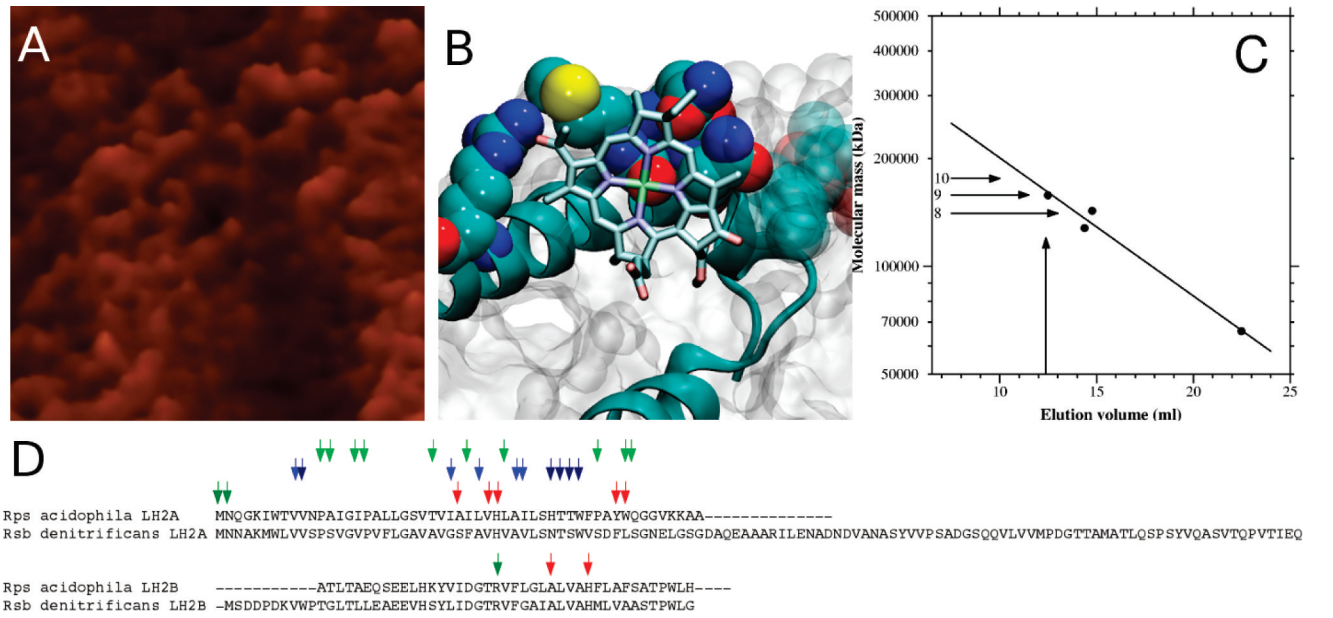

Figure 2. Structure of *Rsb. denitrificans* LH2 complexes compared to *Rps. acidophila* LH2. (A) An AFM image of *Rsb. denitrificans* chromatophores showing LH2 complexes; the size and symmetry of these complexes indicate a nonameric ring. (B) Image of the B800 bacteriochlorophyll binding site in *Rps. acidophila* LH2 showing the interactions of the C3 carbonyl with the arginine residue, the liganding of the Mg by the N terminal formyl group, and the proximity of the $C_{13:1}$ group and the carotenoid (transparent colored atoms). (C) Size determination by gel filtration. The elution of *Rsb. denitrificans* LH2 complexes from a Superose6 gel filtration column is compared with various standard proteins (points); see Experimental Section for details. Arrows mark the expected positions of 8-mer, 9-mer, and 10-mer complexes given the standard curve. (D) Sequence comparison of *Rsb. denitrificans* and *Rps. acidophila* LH2 polypeptides; the arrows mark residues interacting with the monomeric (dark green) and oligomeric (red) BChls and the two interfaces of the α polypeptide (green and blue) that interact to form the cyclic oligomer.

suggesting that the absorption at 800 nm can have contributions from monomeric and oligomeric BChls.

The observed CD spectrum as illustrated above (Figure 1B) is in most respects similar to that observed for other LH2 complexes. Importantly in connection with the protein structure, this is unlike the CD spectra observed for the predominantly eight-membered rings of *Phaeospirillum molischianum* LH2 complexes or low light *Rps. palustris* complexes.[30−32] This lends further support to the idea of a nonameric ring structure like that of *Rsb denitrificans* and furthermore that the relationships between the different oligomeric BChl molecules are similar.

Together these different lines of evidence—AFM images, chromatography, sequence conservation, and CD spectra—all suggest that the protein structure is similar to that of other nonameric LH2 complexes, in both the degree of oligomerization and the positioning and organization of the pigments in the binding sites.

Binding Site Structure. To probe more closely the structure around the BChl binding sites, we collected (pre)resonance Raman spectra of membranes from the Δ*puf* mutant strain exciting at 1064 nm (Figure 3). The overall spectrum is typical of a light harvesting complex, and both BChl and carotenoid bands can be observed. The BChl Raman bands can be used to obtain precise information on the configuration and environment of the pigments.

Six Raman bands have been identified as being sensitive to ring distortions caused by changing the size of the central metal, the so-called core size of the bacteriochlorin.[33] Of these bands four can be clearly identified in the spectrum shown in Figure 3A and are indicated on the figure. From the positions on these bands the core size can be predicted to be 2.03 Å; this is somewhat smaller than in other LH2 complexes, suggesting the bacteriochlorin rings are slightly less distorted. The degree of distortion has in turn been shown to influence the position of the carbonyl vibrations,[34] and a 2.03 Å core is expected to give modes in the absence of interactions at 1660 and 1692 cm^{-1} for the C_3 and $C_{13:1}$ carbonyl groups, respectively. The reduced degree of distortion might also be expected to slightly increase the fluorescence lifetime.[35]

The vibrational frequencies of the carbonyl bands are particularly sensitive to their environment and can also strongly influence the electronic transition energy of the Q_y transition.[19] In Figure 3B we show the carbonyl region of the Raman spectrum together with the various Gaussian components that we identified and attribute to carbonyl vibrations; the red line shows the fitted spectrum, illustrating the good agreement between the model and the experimental spectrum, in black. In this region we identify four different spectral components arising from the C_3 and $C_{13:1}$ carbonyl groups of the three different BChl molecules in the structure: 2 oligomeric Bchls and 1 monomeric BChl per subunit. The frequencies of the bands and their attributions are assigned in Table 1. In this assignment the monomeric BChl is assigned modes at 1625 and 1692 cm^{-1}, as in other LH2 complexes,[19] and in agreement with the conservation of the residues implicated in the binding site. The oligomeric BChl's are assigned C_3 carbonyl modes at 1660 cm^{-1}, corresponding to carbonyl groups free from interactions; this is as seen in B800−820 type LH2 complexes[19] and completely coherent with the sequences of the *Rsb. denitrificans* Puc polypeptides (Figure 2D). Indeed, in these sequences the residues at the positions His_{+13} and His_{+14} are Phe and Leu, respectively, both unable to form H bonds to the C_3 carbonyl group. The $C_{13:1}$ carbonyl groups of the oligomeric BChl's are assigned frequencies of 1672 and 1692 cm^{-1}. That is

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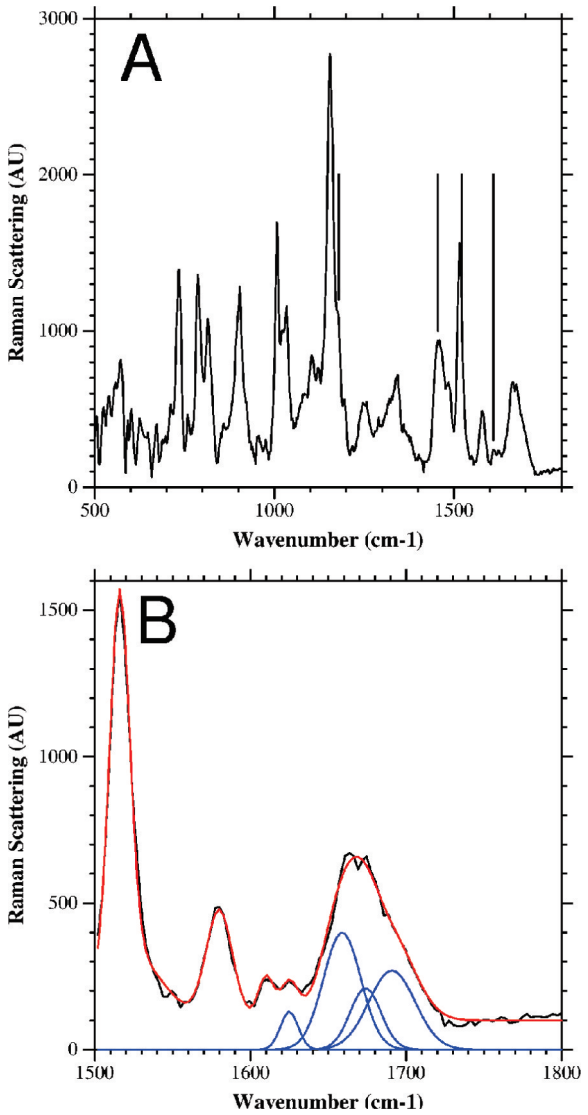

Figure 3. Preresonance Raman spectra of membranes from the Δ*puf Rsb. denitrificans* mutant measured with a 1064 nm Nd:YAG laser. (A) An overview of the spectrum with marked by lines the four Raman modes sensitive to bacteriochlorin distortion. (B) The carbonyl stretching region of the spectrum showing (black) the measured scattering, (red) the fitted spectrum, using a total of eight Gian components, and (blue) the four peaks identified in the carbonyl region.

Table 1. Frequency Assignments for BChl Carbonyl Groups

freq (cm^{-1})	% area	assignment
1625	4	monomeric Bchl C_3 (interacting with arginine)
1660	52	oligomeric Bchl C_3 (two unperturbed)
1674	11	oligomeric BChl $C_{13:1}$ (? liganded by β polypeptide)
1691	33	oligomeric BChl $C_{13:1}$ (? liganded by α polypeptide)
		monomeric BChl $C_{13:1}$ (unperturbed)

one, as in most LH2 complexes, with a downshifted frequency due to the interaction with the histidine ligating the neighboring BChl and the second, unusually, free from such an interaction. This unusual assignment is based on the strength of the band observed at 1692 cm^{-1}, which would suggest that two BChl molecules contribute. This assignment poses several questions. What effect might such a change in the binding site have on the spectrum? What could the structural origin of this modification be?

For the C_3 carbonyl it has been shown that hydrogen bonding reduces the frequency of the carbonyl vibration and reduces the energy of the Q_y transition with a relatively linear dependence; thus, a 20 cm^{-1} reduction in the C_3 carbonyl frequency of 1 BChl is associated with a 200−450 cm^{-1} increase in the Q_y single site transition energy.[22,36] This relation can be understood as the hydrogen bonding favors the excited state electron density. This by interacting favorably with the electron density on the carbonyl oxygen which increases as electron density moves toward the periphery of the molecule, along the Q_y axis, with excitation.

The C_3 carbonyl group configuration can also modify the absorption spectrum by being twisted out of plane.[23,35,37,38] Such a structural modification can cause increases of a similar order of magnitude in the Q_y single site transition energy.[36] On the basis of quantum mechanical calculations,[38] it is expected that such a modification, in addition to increasing the vibrational frequency of the carbonyl group, reduces the intensity of the resonance Raman band.

Both the C_3 $C_{13:1}$ carbonyl group environments are expected to affect the electronic properties similarly. This is because the changes in electron density upon excitation are similar for both groups,[39] as indeed is expected from the molecular symmetry. We can thus expect the proposed modification in the environment of a $C_{13:1}$ carbonyl group, loss of a hydrogen bond, or proton-induced polarization that causes a 17 cm^{-1} upshift of the $C_{13:1}$ carbonyl group mode to lead to an increase in the Q_y transition energy of those BChl molecules concerned by ∼400 cm^{-1}. Such a shift coupled with the shift due to the loss of hydrogen bonding to the C_3 carbonyl groups is sufficient to shift the absorption of an oligomeric BChl from 850 nm to about 807 nm. This prediction is in reasonably good agreement with the observed absorption spectrum. We would thus propose that the High-800 absorption spectrum of the Roseobacter LH2 is due to a coincidence of the monomeric and oligomeric Bchl absorption transitions. This is possible because the particular environment of the oligomeric BChl is able to raise the single site transition energy sufficiently. This is associated with the absence of hydrogen bonding to both C_3 carbonyl groups and one $C_{13:1}$ carbonyl group.

It has previously been reported,[40] in contradiction with our hypothesis, that hydrogen bonding to the $C_{13:1}$ carbonyl group does not modify the BChl absorption spectrum. This in the context of the *Rb. sphaeroides* LH2, which shows a similar resonance Raman spectrum to that of *Rsb. denitrificans* LH2 in the $C_{13:1}$ carbonyl group region. However, in view of the theoretical and experimental support for the sensitivity of the BChl transition energy to carbonyl hydrogen bonding and local environment,[37−39] and the experimental evidence presented here for an unusual environment significantly changing the vibrational frequency, but not the Franck−Condon factor, we believe that the absorption is sensitive to hydrogen bonding to the $C_{13:1}$ carbonyl group but that this can be masked, for example in the study of Braun et al.,[40] by other factors.

The Raman spectrum also allows some conclusions about the configuration of the spheroidenone carotenoid molecule in the complex. The three major carotenoid bands ν_1 at 1516 cm^{-1}, ν_2 at 1155 cm^{-1}, and ν_3 at 1007 cm^{-1} are intense and well resolved, while the ν_4 band is hidden. These Raman bands are

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particularly sensitive to distortions of the condjugated system which lead to shifts and splittings of these modes.[41] We can thus conclude that the carotenoid is in an essentially undistorted planar all-trans configuration as in other LH2 complexes. The CD signal observed above (Figure 1) would thus appear from asymmetric interactions with the environment rather than distortion of the carotenoid molecule

CONCLUSIONS

At the energetic level the results presented here suggest that the loss of an interaction between the $C_{13:1}$ carbonyl group of one BChl and the protein adjusts the site energies of half the oligomeric BChls and that this modification leads directly to the observed absorption and fluorescence properties. We would propose, on the basis of the polypeptide sequence, that the molecular origin of the changed site energy is the presence of a serine residue at the position His_{-4} in the α-polypeptide; this residue is close to the $C_{13:1}$ carbonyl and could well alter the H-bonding network in this region. It is important to point out here that we attribute the lack of a H-bond normally present in the Bchl binding site[19] to the presence of the serine residue. In the case of *Rb. sphaeroides* LH2 where this residue is also present it has been associated with the presence of a H-bond[40] that is much stronger than usual.

It is also possible that the reduced width of the Q_y absorption peak compared to other types of LH2 is associated with the modification to the BChl binding site that is responsible for the change in Raman spectrum, in particular if part of the site heterogeneity is due to fluctuations in H-bond strength. Further structural and spectroscopic studies will be necessary to elucidate these points.

The interpretation we propose predicts that the loss of the remaining interaction of a $C_{13:1}$ carbonyl group would shift the main absorption peak to about 790 nm; such complexes have not been observed, and it is intriguing to consider which of several reasons is the most important: maintaining spectral overlap with the core complex for efficient energy transfer between LH2 and core complexes, avoiding equilibration with the monomeric BChls to maintain good energy transfer with both other LH2 complexes and core complexes, or requiring sufficient fixation sites to hold the BChl's firmly in their correct location and avoid excessive disorder.

We remarked above that the carotenoid spectrum is unusual: both the low extinction compared to many other complexes and the two-lobed CD spectrum. This is intriguing, as one of the roles of the carotenoid molecules is to protect the photosynthetic apparatus against oxidative damage. Since the complex studied comes from an aerobic photosynthetic organism, it might be expected to have a highly evolved photoprotection system. Thus, this observation deserves to be followed up with further spectral and structural measurements to understand the way carotenoids manage to maintain the photosynthetic system operational in this organism.

At a functional level moving the LH2 absorption toward the visible appears to be an adaptation to low light intensities in both *Rps. palustris* and *Rsb. denitrificans*. Indeed, *Rsb. denitrificans* does not normally synthesize its photosynthetic apparatus when illuminated under laboratory conditions but will put in place the apparatus in the dark.[27] However, this shift seems to require balancing two different effects: the reduced efficiency of transfer to the core complex and the increased light flux in the solar spectrum at these wavelengths. Certainly in *Rsb. denitrificans* the amount of LH2 per core complex is much smaller than in many other photosynthetic bacteria, suggesting that energy transfer is becoming critical. The different trade-offs that exist between increasing antenna size while maintaining efficient transfer and changing the absorption maximum to match the incident spectrum while reducing transfer efficiency deserve further investigation.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sturgis@ifr88.cnrs-mrs.fr. Tel: +33 491164127.

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ABBREVIATIONS

AFM, atomic force microscopy; Bchl, bacteriochlorophyll *a*; *Rb.*, *Rhodobacter*; *Rps.*, *Rhodopseudomonas*; *Rsb.*, *Rhoseobacter*; *Rsp.*, *Rhodospirillum*.

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