# Structure and Properties of the Bacteriochlorophyll Binding Site in Peripheral Light-Harvesting Complexes of Purple Bacteria

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*Received July 22, 1994; Revised Manuscript Received October 26, 1994*®

ABSTRACT: In this paper, we have examined, using FT resonance Raman spectroscopy, the bacteriochlorophyll (BChl) binding sites in the peripheral light-harvesting complexes extracted from a number of purple bacterial strains. A comparison of interactions of the BChl molecules with their binding sites in these LH2 complexes, together with the primary sequences of the α and β polypeptides, allows three amino acids to be proposed to be involved in the hydrogen bonding of the 9-keto carbonyl of one of the 850-nm-absorbing pair of BChl molecules. Specifically, we show that one keto carbonyl group, which is strongly hydrogen bonded in *Rhodobacter sphaeroides* LH2, is involved in much weaker interactions in the LH2 complexes from all the other species studied (i.e., *Rhodobacter capsulatus*, *Rubrivivax gelatinosus*, *Rhodopseudomonas palustris*, *Rhodopseudomonas acidophila*, and *Rhodopseudomonas cryptolactis*). This is correlated with the presence of three polar amino acids in the primary sequence of the α polypeptide of *Rb. sphaeroides* which are absent in the sequences from all the other bacteria and probably close to a chromophore. These three residues are a serine at position −4, a threonine at position +6, and another serine at position +17 (numbering relative to the conserved histidine, considered as position 0), in the α polypeptide of *Rb. sphaeroides*. Furthermore, the study of the interactions in natural B800−820 complexes shows that the two 2-acetyl groups of the 820-nm-absorbing BChl molecules are free from hydrogen-bonding interactions. In the light of previous site-selected mutagenesis studies, the lack of such hydrogen bonds seems to be a general phenomenon, associated with the 820-nm absorption of LH2 complexes, and suggests that hydrogen-bonding interactions have a precise molecular role in finely tuning the functional properties of these complexes.

In purple photosynthetic bacteria, light energy is gathered by an extensive system of light-harvesting (LH)[1] pigment−protein complexes, the spectral properties of which are precisely tuned so as to ensure the efficient funneling of excitation energy toward the photochemical reaction centers (RC) (van Grondelle *et al.,* 1994), where the transduction into chemical potential energy takes place. In all purple bacteria the reaction center is surrounded by a so called "core" antenna (also known as LH1). In many bacteria an additional light-harvesting system exists, the "peripheral" antenna (or LH2); this antenna system transfers excitation energy to the RC via the core antenna. In some species, such as *Rhodopseudomonas (Rps.) acidophila*, *Rps. cryptolactis*, and *Rps. palustris*, a second type of "peripheral" antenna may be induced by cold or light stress (Hawthornthwaite & Cogdell, 1991).

All of these antenna complexes have the same basic arrangement: they are constructed from multimers of a minimal unit containing two polypeptides α and β to which are bound the pigments bacteriochlorophyll (BChl) and carotenoids (Zuber & Brunisholz, 1991). In core antennae, each polypeptide binds a single BChl *a* molecule, and these interact with each other and the protein environment so to exhibit a lower energy singlet absorption transition at ca. 880 nm. In peripheral antenna complexes the two polypeptides, α and β, bind three BChl *a* molecules per αβ polypeptide pair. One of the BChl *a* molecules is responsible for an absorption transition near 800 nm, while the other two are responsible for the absorption transition near either 850 or 820 nm.

Although X-ray crystallography has met remarkable success in solving the molecular structure of the RCs from two different purple bacteria to atomic resolution (Deisenhofer *et al.,* 1985; Allen *et al.,* 1987; El-Kabbani *et al.,* 1991), the three-dimensional structure of the various LH complexes remains unknown. Biochemical studies have allowed the topology of the polypeptides relative to the membrane to be determined (Wiemken *et al.,* 1983), and information on the position and orientation of the pigments in these complexes has been obtained by a number of spectroscopic methods (Kramer *et al.,* 1984). Moreover, the primary sequences of a wide variety of α and β polypeptides are now available (Zuber & Brunisholz, 1991). Precise structural information on these pigment−protein complexes is scarce, and much of that available has been obtained by resonance Raman spectroscopy. This technique allows the interactions of the conjugated substituents of the BChl *a* macrocycle within the binding sites to be described (Robert & Lutz, 1988). Recently, an extension of this technique, FT preresonance Raman spectroscopy, has been shown to be particularly

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® Abstract published in *Advance ACS Abstracts,* December 1, 1994.
[1] Abbreviations: BChl, bacteriochlorophyll; FT, Fourier Transform; LH, light-harvesting; *Rb.*, *Rhodobacter*; RC: reaction center; *Rps.*, *Rhodopseudomonas*; RR, resonance Raman; *Rv.*, *Rubrivivax.*

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effective for describing the hydrogen-bonding state of the conjugated 2-acetyl and 9-keto carbonyl groups of BChl *a* (Mattioli *et al.*, 1991). In this method downshifts in the frequencies of the carbonyl stretching modes are analyzed in terms of hydrogen-bonding and ultimately the estimation of hydrogen-bond strengths using the method of Zadorozhnyi and Ischenko (1965). While a number of distortions in the bacteriochlorin macrocycle might be expected to affect the carbonyl vibrational frequencies, apart from hydrogen bonding, these are predicted either to cause an upshift relative to the normal free pigment rather than the routinely observed downshfts or to be nonselective (Lutz *et al.*, 1981). The combination of FT preresonance Raman spectroscopy and site-selected mutagenesis has recently allowed the demonstration that certain amino acids form part of the binding sites of LH1 (Olsen *et al.*, 1994) and LH2 (Fowler *et al.*, 1994) complexes and to permit a role for these amino acids in pigment ligation to be suggested.

A problem of considerable interest is to understand the precise role of the protein, and the molecular mechanisms involved, in tuning the electronic properties of the chromophore in antenna complexes. It has been known for some time that certain residues conserved in the primary sequence of the α polypeptides of B800−850 type LH2 complexes are not conserved in this polypeptide derived from the B800−820 type peripheral antennae. Relative to the conserved histidyl residue, believed to bind the central Mg atom of one of the BChl *a* molecules of the 850 (or 820), nm absorbing pair, these residues are located at positions +13 and +14. Fowler *et al.*, (1992) mutated these residues, both tyrosines in *Rhodobacter (Rb.) sphaeroides* LH2, to phenylalanine and leucine, respectively, two residues naturally found in the 820-nm-absorbing complexes of *Rps. acidophila*. It was observed that each individual mutation induced a ca. 10-nm blue-shift of the absorption transition of the BChl pair, with the absorption maximum of the double mutant was at 826 nm. FT Raman studies performed on these altered complexes have shown that each substitution resulted in the breakage of an hydrogen bond in which the 2-acetyl of one BChl *a* of the 850-nm-absorbing pair was involved (Fowler *et al.*, 1994). However, mutation of the Tyr +14 resulted in more than one modification of the BChl binding site in *Rb. sphaeroides* LH2, and the 826-nm absorption of the doubly mutated LH2 is much broader than that of natural LH2. Although these experiments clearly demonstrated the role of the +13 and +14 residues in tuning the absorption of these complexes, the molecular mechanisms described in the mutants still need to be compared to the situation in natural LH2 complexes, by studying the structure of the binding sites of 850-nm- and 820-nm-absorbing LH2 complexes.

In 1985, it was shown that the structures of the BChl binding sites in LH2 complexes were dependant on the bacterial strain studied (Robert & Lutz, 1985). Variations between 820-nm- and 850-nm-absorbing LH2 complexes should therefore be carefully compared to the natural interspecific variability of the LH2 binding sites, in order to evaluate the relevance of structural changes to the change in absorption maximum. At present, the FT Raman spectra of only two LH2 complexes have been reported, those of *Rb. sphaeroides* (Mattioli *et al.*, 1993) and of the unusual complex from *Rhodospirillum molischianum* (Germeroth *et al.*, 1993) which resembles in a number of respects a core antenna. In this paper, we have studied the binding sites of the BChls in peripheral complexes extracted from various of purple bacterial strains, using FT Raman spectroscopy. The comparison of BChl interactions in the LH2 complexes, together with the primary sequences of the α and β polypeptides they are constructed from, allows three amino acids to be proposed as candidates to be part of the binding site of the 850-nm-absorbing pair of BChl molecules, in contact with the 9-keto carbonyl of a BChl. The study of the interactions in natural B800−820 complexes allows the evaluation of the molecular relevance of previous site-selected mutagenesis studies and of the role of hydrogen-bonding interactions in finely tuning the function of these complexes.

## MATERIAL AND METHODS

The peripheral antennae of *Rb. capsulatus* (2.3.1), *Rb. sphaeroides* (2.4.1), and *Rps. palustris* (2.6.1) were purified by selective extraction from chromatophore membranes with 0.4−0.7% LDAO (Fluka) essentially as described by Cogdell *et al.*, (1983) followed, where necessary to obtain spectrally pure complexes, by DEAE chromatography. Purification of 820- and 850-nm-absorbing LH2 complexes from *Rps. acidophila* (Ac 7750) was performed as previously described Cogdell *et al.*, (1983), and purification from *Rps. cryptolactis* (ATCC 49414) was performed according to the method described in Halloren *et al.*, (1994). Purification of *Rubrivivax (Rv.) gelatinosus* LH2 was performed as described by Jirsakova *et al.*, (manuscript in preparation).

Absorption spectra were recorded with either a Varian Cary 2300 or a Varian Cary 5E double-beam spectrophotometer. Room temperature FT Raman spectra in preresonance with the BChl $Q_y$ transition were measured using 1064-nm excitation from a continuous Nd-Yag laser and a Fourier transform infrared (FTIR) spectrophotometer (Bruker IFS66) equipped with Raman module (Bruker FRA106) as described by Mattioli *et al.*, (1991). No time-dependent spectral changes were observed during the various Raman experiments reported here, indicating the absence of significant photodamage. The spectra shown are the results of 2000−10000 coadded interferograms. Samples for FT Raman spectroscopy, were concentrated to an OD of 500−1500 with Centricon microconcentrators (Amicon) and introduced into the spectrophotometer as previously described (Mattioli *et al.*, 1991).

## RESULTS AND DISCUSSION

*Comparison of the BChl Binding Site in the LH2 Complexes of Various Purple Bacteria.* Until now the FT preresonance Raman spectrum of only one typical LH2, that of *Rb. sphaeroides*, has been reported (Mattioli *et al.*, 1993; Fowler *et al.*, 1994). As the binding site of this type of complex varies depending on the bacterial strain from which it originates (Robert & Lutz, 1985), and since FT-Raman spectroscopy yields a much more precise picture of the interactions assumed by the carbonyl groups of BChl pigments than the previously used Soret-excited, resonance Raman spectroscopy (Mattioli *et al.*, 1991), this study was expected to yield FT-Raman spectra clearly differing from each other and a better understanding of the molecular origin of this variation. Figure 1 displays the FT-Raman spectra of LH2 complexes extracted from four different species: *Rb.*

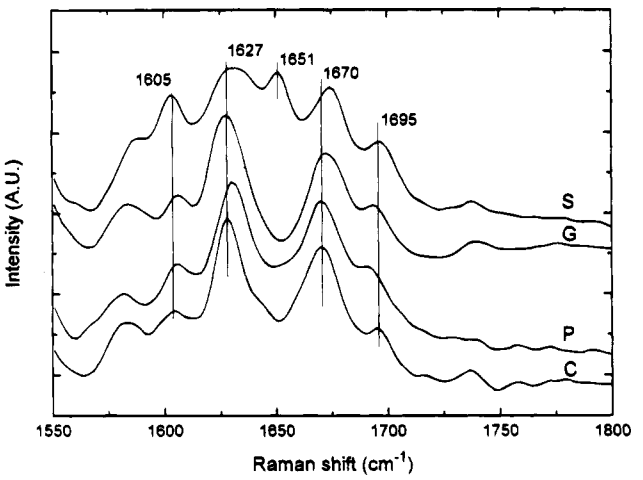


FIGURE 1: FT resonance Raman spectra in the carbonyl stretching region of the B800−850 complexes isolated from (S) *Rb. sphaeroides*, (G) *Rv. gelatinosus*, (P) *Rps. palustris*, grown under high light conditions, (C) *Rb. capsulatus*. spectra of concentrated samples ($A_{850}$ ca. 1000) were obtained as described under Materials and Methods and normalized on the basis of the intense 730-$cm^{-1}$ BChl skeletal mode.

*capsulatus* (C), *Rb. sphaeroides* (S), *Rps. palustris* (P), and *Rv. gelatinosus* (G) in the high frequency region (1550−1750 $cm^{-1}$). In these spectra a number of bands arising from the stretching modes of those carbonyl groups conjugated with the BChl macrocycle are clearly visible in the 1620−1710-$cm^{-1}$ region. More precisely, the stretching modes of the 2-acetyl carbonyl groups of BChl molecules give spectral contributions around 1660 $cm^{-1}$ when free from interaction, and these bands may shift down to ca. 1620 $cm^{-1}$ upon hydrogen-bond formation (Robert & Lutz, 1988; Mattioli *et al.*, 1993). The stretching modes of the 9-keto carbonyl groups of BChl molecules are observed *in vitro* at 1685 $cm^{-1}$ when these groups are free from interactions and may be observed to shift to lower energies upon hydrogen-bond formation, to 1650−1660 $cm^{-1}$ (Lutz, 1984; Robert & Lutz, 1988). In protein-bound BChl molecules frequencies up to 1701 $cm^{-1}$ have been reported for these 9-keto carbonyl stretching modes, both in purple bacterial reaction centers (Mattioli *et al.*, 1991, 1994) and in LH2 complexes (Mattioli *et al.*, 1993; Fowler *et al.*, 1994).

In the FT-Raman spectrum of *Rb. sphaeroides*, five different carbonyl stretching bands are resolved, and, by combining FT-Raman spectroscopy and site-selected mutagenesis, they were recently all attributed (Fowler *et al.*, 1994). It was concluded that both the 1627- and 1635-$cm^{-1}$ bands arise from the stretching modes of the hydrogen-bonded 2-acetyl groups of those BChls involved in the 850-nm absorption of the LH2 complexes. The stretching mode of the 2-acetyl of the 800-nm-absorbing BChl, which contributes at ca 1628 $cm^{-1}$ (Robert & Lutz, 1985), is much weaker than those due to reduced resonance and so not resolved. The remaining bands at 1651, 1677, and 1701 $cm^{-1}$ have been attributed to stretching modes from the 9-keto carbonyl groups of the different BChl molecules present in the LH2 complex. The 1701-$cm^{-1}$ band comes from the 800-nm-absorbing BChl (Robert & Lutz, 1985), while the 1651 and 1677 $cm^{-1}$ arise from the stretching modes of the keto carbonyl groups from the 850-nm-absorbing BChl molecules, the first strongly hydrogen-bonded.

In Figure 1, it is clear that the FT-Raman spectrum of *Rb. sphaeroides* differs from those of *Rb. capsulatus*, *Rv. gelatinosus*, and *Rps. palustris* in the region shown. Indeed, the latter all exhibit apparently less complex spectra, with three clearly resolved bands in the carbonyl region, one located between 1627 and 1631 $cm^{-1}$, one around 1672 $cm^{-1}$, and the last at ca. 1697 $cm^{-1}$. Both the major contributions, at ca. 1631 and ca. 1672 $cm^{-1}$, are degenerate in these three spectra, and it is likely that the small changes in the shape and position of these bands observed between these species result from minor alterations in the positions and intensities of their components. It is worth mentioning at this point that FT-Raman spectra of *Rps. acidophila* and *Rps. cryptolactis* 850-nm-absorbing LH2 complexes (see Figure 3) are very similar to those of *Rb. capsulatus*, *Rv. gelatinosus*, and *Rps. palustris*, shown here.

Between 1985 and 1988 (Robert *et al.*, 1985; Robert & Lutz, 1985, 1988), LH2 complexes from many purple bacteria were studied by UV-excited resonance Raman spectroscopy. The frequencies reported in these previous studies for the carbonyl stretching modes in *Rb. sphaeroides* LH2 match well with those reported in the present work, as already mentioned by Mattioli *et al.*, (1993).

In the *Rb. capsulatus* LH2 complex most bands in this region were previously reported to be similar to those observed with the *Rb. sphaeroides* LH2 complex (Robert, 1983). However, in FT-Raman experiments with 1064-nm excitation, carbonyl modes are much more active relative to the band arising from the methine bridge stretching modes, than in the previous experiments with excitation in the $B_x$ and $B_y$ transitions. Therefore, in the present investigation it is possible to assess with much greater precision the position and intensity of the bands in the carbonyl stretching region. Nevertheless, reexamination of the original resonance Raman spectra has allowed us to conclude that the small changes seen in the previous resonance Raman study were in fact consistent with the present interpretation.

In the LH2 from *Rps. palustris* (Robert & Lutz, 1985) it was previously concluded that there were no free carbonyl groups on the BChl molecules; however, the present FT Raman study shows a clear band at 1693 $cm^{-1}$ which we would now assign to the B800 BChl by analogy with the other LH2 spectra. A similar apparent discrepancy has already been noted between the reported frequencies observed for the primary electron donor in *Rb. sphaeroides* reaction centers between UV-excited Raman spectra and FT-Raman spectra (Mattioli *et al.*, 1991). Both of these discrepancies seem to arise because stretching modes of the keto carbonyl groups, in particular when free from hydrogen-bonding interactions, are only very weakly active in resonance Raman spectra excited at 363.8 nm.

The FT-Raman spectra of *Rb. capsulatus*, *Rv. gelatinosus*, *Rps. palustris*, *Rps. acidophila*, and *Rps. cryptolactis* (shown in Figures 1 and 3) differ from that of *Rb. sphaeroides* (Figure 1) in three important respects. First, the cluster of bands observed in *Rb. sphaeroides* between 1627 and 1635 $cm^{-1}$ is narrower in the other species. In *Rb. sphaeroides*, this cluster arises mainly from the 2-acetyl carbonyl groups of the two BChl a molecules responsible for the 850 nm absorption the position of these bands being modulated by the presence of Tyr $\alpha_{+13}$ and Tyr $\alpha_{+14}$ (Fowler *et al.*, 1994). Second, there is a band at 1651 $cm^{-1}$ in *Rb. sphaeroides* spectra which is missing in the spectra of complexes from

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|---|---|---|---|
| α polypeptides | | | |
| *Rhodobacter sphaeroides* | 2.4.1 | SAAFIASVVIHAAVLTTTTWLPAYYQGSAAVAA | |
| *Rhodobacter capsulatus* | 2.3.1 | GAVAVAALIVHAGLLTNTTWFANYWNGNPMATV | |
| *Rubrivivax gelatinosus* | DSM149 | GAVAVTALILHGGLLAKTDWFGAYWNGGKKAAA | |
| *Rhodopseudomonas acidophila* | Ac 7750 | GSVTVIAILVHLAILSHTTWFPAYWQGGVKKAA | |
| *Rhodopseudomonas palustris* | 2.6.1 | GSVTVIAILVHFAVLSHTTWFSKYWNGKAAAIE | $\alpha_a$ |
| *Rhodopseudomonas palustris* | 2.6.1 | GSVTVIAILVHYAVLSNTTWFPKYWNGATVAAA | $\alpha_b$ |
| *Rhodopseudomonas palustris* | 2.6.1 | GSVAAIAFAVHFAVLENTSWVAAFMNGKSVAAA | $\alpha_c$ |
| *Rhodopseudomonas palustris* | 2.6.1 | GSVAIMVFLAHFAVLTHTTWVAKFMNGKAAAIE | $\alpha_d$ |
| *Rhodopseudomonas palustris* | 2.6.1 | GSVTVIAILVHFAVLSNTTWFSKYWNGKAAAIR | $\alpha_e$ |
| β polypeptides | | | |
| *Rhodobacter sphaeroides* | 2.4.1 | RVFGGMALIAHFLAAAATPWLG | |
| *Rhodobacter capsulatus* | 2.3.1 | RVFGAMALVAHILSAIATPWLG | |
| *Rubrivivax gelatinosus* | DSM149 | RIFGVIAILAHILAYAYTPWLH | |
| *Rhodopseudomonas acidophila* | Ac 7750 | RAFLGIALVAHFLAFSMTPWLH | |
| *Rhodopseudomonas palustris* | 2.6.1 | RIFGAIAIVAHFLAYVYSPWLH | $\beta_a$ |
| *Rhodopseudomonas palustris* | 2.6.1 | RIFGAIAIVAHFLAYVYSPWLH | $\beta_b$ |
| *Rhodopseudomonas palustris* | 2.6.1 | RIFVAIAIVAHFLAYVYSPWLH | $\beta_c$ |
| *Rhodopseudomonas palustris* | 2.6.1 | RIFVAIAIVAHFLAYVYSPWLH | $\beta_d$ |
| *Rhodopseudomonas palustris* | 2.6.1 | RIFGAIAIVAHFLAYVYSPWLH | $\beta_e$ |

FIGURE 2: Aligned sequences of the α and β polypeptides in the region of the conserved histidyl residue believed to ligand the central magnesium atom of the long wavelength absorbing BChl molecules.

all of the other bacteria. Third, the contributions around 1677 $cm^{-1}$ are broader and more intense in the spectra where the 1651-$cm^{-1}$ band is missing. The 1651-$cm^{-1}$ band has been attributed to the keto carbonyl group of one BChl molecule of the 850-nm-absorbing pair, involved in strong intermolecular interactions. As the frequency range expected for the stretching mode of such a chemical group is 1650−1700 $cm^{-1}$ (Robert *et al.,* 1988), the disappearance of the 1651-$cm^{-1}$ band is expected to be correlated with the appearance of a new contribution at higher frequencies. It is thus possible to conclude that one keto carbonyl group, which is strongly intermolecularly bound in *Rb. sphaeroides* LH2 (contributing at 1651 $cm^{-1}$), is involved in weaker interactions in the LH2 complexes from all the other species studied in this work, contributing to the band near 1670 $cm^{-1}$.

As the primary sequences of the α and β polypeptides from LH2 of *Rb. sphaeroides*, *Rb. capsulatus*, *Rv. gelatinosus*, *Rps. palustris*, and *Rps. acidophila* have all been published, it is of interest to look for a sequence pattern which could account for the differences in the intermolecular interactions assumed by the 2-acetyl carbonyls, and one 9-keto carbonyl of the BChls of the 850-nm-absorbing pair in the LH2 of *Rb. sphaeroides*. Figure 2 shows the primary sequences of the polypeptides of these various LH2 complexes in the region of the conserved histidines which are believed to be the Mg ligands of these BChl molecules (Zuber & Brunisholz 1991).

A slight complication in this analysis is the presence of a multigene family for the LH2 complexes of *Rps. palustris* (Tadros *et al.,* 1993). It is, however, noteworthy that the FT-Raman spectrum of *Rps. palustris* LH2 does not seem to be more complex than the other spectra displayed in Figure 1. This is despite the fact that the LH2 complexes, as isolated, might be expected to be composed of several different polypeptides, all exhibiting small differences in their primary sequence, and the purification procedure applied to these light-harvesting complexes would not be expected to separate any subtypes. However, since the complexes studied derive from cells grown at relatively high light intensity, as previously described by Robert *et al.,* (1985), it is probable that the major sequences are those encoded by the $\alpha\beta_a$ and $\alpha\beta_b$ gene clusters (Tadros *et al.,* 1993). This may explain why the FT-Raman spectra of this LH2 preparation does not appear to be any more complex than those from the other strains.

In the *Rb. sphaeroides* α polypeptide sequence the residues located at the +13 and +14 positions are both tyrosines which have been implicated in the hydrogen bonding of the 2-acetyl groups of the 850-nm- absorbing BChl molecules (Fowler *et al.,* 1994). In contrast, in *Rb. capsulatus*, *Rv. gelatinosus*, *Rps. acidophila*, and some *Rps. palustris* sequences, the amino acids located at the +13 and +14 positions in the α polypeptide are a tyrosine and a tryptophan (see Figure 2), which are both able to form an hydrogen bond with the 2-acetyl carbonyl groups. However, the strength of this hydrogen bond is likely to vary according to the nature of the amino acid side chain (i.e., Tyr or Trp) as well as the distance and orientation of the hydrogen-bond donor. Although it has not been formally demonstrated that these amino acids interact directly with the 2-acetyl groups of the BChl a molecules of the 850-nm-absorbing pair, this is likely to be the case. The variation in the width of this cluster of bands between *Rb. sphaeroides* and the other bacteria can thus be explained by this sequence difference. Indeed, the strength of the hydrogen bond to these groups is adjusted according to the chemical nature of these amino acids, located at the +13 and +14 positions in the α polypeptide. Among the *Rps. palustris* LH2 α polypeptide

sequences are shown two which appear to contribute little in cells grown at high light, lack the Tyr-Trp motif, and have instead Phe-Met. It will be of interest to investigate the nature of the B850 binding site in complexes with this motif and compare them to those containing the Tyr-Trp and Tyr-Tyr motifs.

To identify possible hydrogen-bond donors to the 9-keto carbonyl group of the *Rb. sphaeroides* complex, it is necessary to compare the primary sequences of the B800−850 type LH2 polypeptides shown. In the primary sequence of the $\beta$ polypeptide of *Rb. sphaeroides* all of the polar amino acids around the histidine are conserved in the primary sequences of the other bacteria. However, in the primary sequence of the $\alpha$ polypeptide of *Rb. sphaeroides* there are four polar amino acids which are replaced in the sequences from all the other bacteria: a serine at position −10, a serine at position −4, a threonine at position +6, and another serine at position +17. There is also the tyrosine doublet at position +13 and +14 discussed above.

The serine at position −10, replaced by glycine in the other sequences shown, is within the presumed transmembrane $\alpha$ helical segment of the $\alpha$ subunit (Zuber & Brunisholz, 1991). The side chain of this residue is expected to be on the same side of the helix as the histidyl residue but considerably above it within the membrane. As the $Q_y$ transitions of the 850-nm-absorbing BChl molecules are approximately parallel to the membrane plane (Morita & Miyazaki, 1971; Breton, 1974; Kramer *et al.*, 1984), this residue cannot interact with the conjugated carbonyls of the BChl ligated to the $\alpha$ subunit histidyl residue and is unlikely to be involved in such binding of the other BChl since there is no large displacement in the position of the ligating histidine within the hydrophobic portion of the sequence. The situation with regard to the serine at position −4 is identical; however, in this case the side chain might be able to interact with the BChl a ligated by the histidine of the $\beta$ polypeptide.

The threonine at position +6 in the sequence of the $\alpha$ polypeptide of *Rb. sphaeroides* is replaced by an asparagine (in *Rb. capsulatus* and some of the $\alpha$ polypeptides synthesized by *Rps. palustris*), a histidine (in *Rps. acidophila* and the remaining $\alpha$ polypeptides synthesized by *Rps. palustris*), or a lysine (in *Rv. gelatinosus*). In the threonine structure, the OH group is attached to the $C_\beta$ carbon and is thus nearer to the main chain of the protein than the potential NH ligands of three other amino acids found at that position. Moreover, asparagine, histidine, and lysine side chains all bear at least two potential hydrogen-bonding chemical groups. It is thus likely that all these residue are involved in a similar hydrogen-bonding network which differs markedly from that found in the *Rb. sphaeroides* LH2 complex.

Finally, the serine at position +17, this is replaced by asparagine in *Rb. capsulatus*, glycine in *Rv. gelatinosus* and *Rps. acidophilla*, and lysine or alanine in *Rps. palustris*. Although this residue seems, at first sight, to be rather far from the conserved histidine for involvement in the binding of the keto carbonyl group of one of the BChl a molecules in the 850-nm-absorbing pair, since the tyrosines at +13 and +14 are involved in the hydrogen bonding of the 2-acetyl carbonyls of these molecules, this residue may well be positioned close to the binding site. Therefore the possible involvement of this residue should not be ignored.

It thus appears that the interactions between 850-nm-absorbing BChl pair and the amino acid residues in their binding site depend on the bacterial strain considered and can be summarized as follows. The 2-acetyl carbonyl groups of these molecules are always involved in intermolecular hydrogen-bonding interactions, the strength of these interactions varies little (less than about 1 kcal $mol^{-1}$; Zadorozhnyi & Ishchenko, 1965) between species. In contrast, the 9-keto carbonyl group of these BChl molecules shows considerable species specific variation in the strength of their hydrogen-bonding interactions (perhaps as much as 3 kcal $mol^{-1}$). Surprisingly, this variation in hydrogen-bonding strength does not appear to affect the position of the near infrared absorption maximum. Taking into account the primary sequences of the various LH2 complexes, three different residue positions, all on the $\alpha$ polypeptide, are proposed as candidates for interacting with the 9-keto carbonyl group of one of the 850-nm-absorbing pair in *Rb. sphaeroides*. The targeting of these residues by site-selected mutagenesis may help in determining which of these amino acids are involved in the binding site of the LH2 complexes and specifically the role of these residues in the hydrogen-bonding of the 9-keto group. Such a study would also permit the verification of the insensitivity of the absorption spectrum to the hydrogen-bonding environment of the 9-keto group.

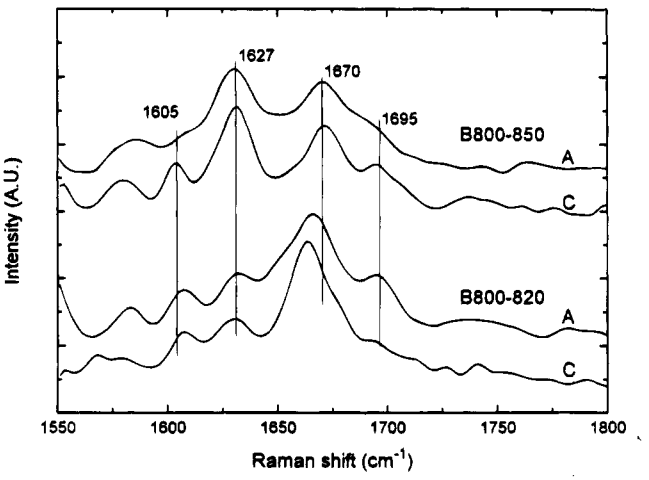


FIGURE 3: FT resonance Raman spectra in the carbonyl stretching region of the B800−850 (above) and B800−820 (below) complexes isolated from (A) *Rps. acidophila* and (C) *Rps. cryptolactis*. Spectra were obtained from concentrated samples as outlined above.

*Comparison of the Binding Sites Of 850- And 820-nm-Absorbing LH2 Complexes.* Figure 3 displays the FT-Raman spectra of 850 nm- and 820-nm-absorbing LH2 complexes from *Rps. acidophila* and *Rps. cryptolactis*, in the higher frequency region (1550−1750 $cm^{-1}$). As already discussed above, spectra of the 850-nm-absorbing LH2 complexes from both these strains are very similar to those of *Rb. capsulatus*, *Rv. gelatinosus*, and *Rps. palustris*, shown in Figure 1, exhibiting three main bands at 1630, 1671, and 1695 $cm^{-1}$. The FT-Raman spectra of the 820-nm-absorbing complexes from the same two bacteria are clearly different from these. The relative intensity of the 1630-$cm^{-1}$ band is drastically reduced (more than 5-fold). The 1671-$cm^{-1}$ band is more intense but downshifted to ca. 1665 $cm^{-1}$ and shows some substructure. In the spectrum of the complex derived from *Rps. acidophila* there is a clear shoulder at lower frequencies, near 1652 $cm^{-1}$, while in that derived from *Rps. cryptolactis* there is one shoulder between 1652 and 1660 $cm^{-1}$ and another at higher frequencies near 1670 $cm^{-1}$. The decrease of the 1630-$cm^{-1}$ band together with the appearance of higher

frequency components, around 1652 and 1660 $cm^{-1}$, clearly indicates that in 820-nm-absorbing LH2 complexes both of the 2-acetyl carbonyl groups are free from interactions, although in slightly different environments. It is worth noting that this type of binding site, with free 2-acetyl groups, has not been observed for the 850-nm-absorbing LH2 complexes.

This lack of hydrogen bonds corresponds exactly to what was observed when Tyr +13 and +14 were selectively mutated to phenylalanyl residues in *Rb. sphaeroides* LH2 (Fowler *et al.*, 1994). By analogy, in the LH2 complexes of *Rps. acidophila* the stretching modes of these two 2-acetyl carbonyl contribute at ca. 1630 $cm^{-1}$ and are likely to interact with the Tyr and Trp residues located at the +13 and +14 position relative to the conserved histidyl residue (see above). In the 820-nm-absorbing LH2 complexes, these residues are replaced by a Phe and a Leu, respectively. Their FT-Raman spectra thus contain two bands arising from acetyl carbonyl groups that are not involved in hydrogen bonds, the positions of which closely correspond to those observed in the *Rb. sphaeroides* mutants.

As with the double mutant from *Rb. sphaeroides* with Phe +13 and Leu +14, the 820-nm-absorbing LH2 complexes exhibit a weak 1630-$cm^{-1}$ band (1628 $cm^{-1}$ in this double mutant) which is assigned to the 2-acetyl carbonyl of the 800-nm-absorbing BChl. From these data, it is possible to conclude that the molecular mechanisms responsible for the appearance of the 820-nm absorption in the mutated LH2 complexes from *Rb. sphaeroides* do indeed correspond to those employed *in vivo* in the LH2 complexes of bacteria that naturally synthesize this type of complex.

Thus it is clear that the site directed mutagenesis work is biologically relevant and further allows us to suggest a possible explanation for the "side effects" observed in the different site-directed mutants, in particular the wide absorption peak and perturbed resonance Raman spectrum. As the BChl a binding site of *Rb. sphaeroides* LH2 is clearly different from that of *Rps. acidophila*, it seems probable that the *Rb. sphaeroides* LH2 is unable to fully accommodate the site-directed molecular changes which were introduced in comparison with the naturally occurring B800−820 complexes. In particular, the band at 1651 $cm^{-1}$ in the FT-Raman spectra of *Rb. sphaeroides* LH2 implies the presence of an hydrogen-bond donor near a keto carbonyl group of the 850-nm-absorbing BChl pair, which is absent, or at least farther away in *Rps. acidophila*. The reorganization of the binding site in *Rb. sphaeroides*, which necessarily follows each mutation to ensure that the structure reaches a new potential energy minimum, will thus involve different side chains from those that form the B820 binding site in *Rps. acidophila*. This may well result in a slightly perturbed binding site for the 820-nm-absorbing pair of BChl a molecules and may be the cause of the decreased stability of the mutated *Rb. sphaeroides* complexes. Once the amino acid responsible for the 1651, $cm^{-1}$ band in the FT-Raman of *Rb. sphaeroides* LH2 has been identified, it will be possible to test this hypothesis.

The breakage of two hydrogen bonds in which the two 2-acetyl carbonyl of the 850-nm-absorbing pair of BChl a seems thus to be a general phenomenon, associated with the 820-nm absorption of LH2 complexes. The absorption of the 850-nm-absorbing BChl a pair is expected, as an exciton coupled dimer, to be sensitive both to changes in interactions within the binding site and to even minor alterations in chromophore chromophore interactions. The breakage of the hydrogen bonds in which the 2-acetyl carbonyl of the BChl a of the 850-nm-absorbing pair could thus result in or be the result of a slight reorganization of the topology of the pair which would be itself the origin of the blue-shift of the absorption transition of the pair. However, as reported by Cogdell and Scheer (1985) the circular dichroism spectra of 820-nm- and 850-nm-absorbing LH2 complexes are similar. Indeed the variations between different B800−850 complexes appear more important than those between B800−850 and B800−820 complexes. Thus, though the intermolecular, excitonic interactions between the BChl pair might differ slightly between these two types of complex, the origin of the absorption difference must probably be looked for elsewhere. Differences in the interactions of the BChl a molecules with their binding sites between these two types of complex certainly could be responsible for their spectral differences in the absence of changes in chromophore−chromophore geometry. In this regard it is noteworthy that previous calculations (Hanson *et al.*, 1988) have shown that blue-shifts of up to 10 nm can be expected as the result of the formation of hydrogen bonds to the 2-acetyl carbonyl group. However, recent work on site-directed mutants of LH1 complexes (Olsen *et al.*, 1994), where blue-shifts accompanied both a strengthening and a weakening of a hydrogen bond to a 2-acetyl group, suggests that such calculations may not be universally valid, particularly in systems with many extended chromophore−chromophore interactions. Nevertheless, the breakage of two of such hydrogen bonds may well be sufficient to explain the observed blue-shift from 850 to 820 nm in LH2 complexes, in the absence of rearrangement of the chromophore−chromophore interactions.

## ACKNOWLEDGMENT

J.S. was supported by a fellowship from the EEC (Human Capital and Mobility programme). R.J.C. acknowledges support from the SERC and the EC and thanks Evelyn Halloran for expert technical assistance.

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BI941669J