# Influence of Carotenoid Molecules on the Structure of the Bacteriochlorophyll Binding Site in Peripheral Light-Harvesting Proteins from *Rhodobacter sphaeroides*[†,‡]

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ABSTRACT: In the LH2 proteins from *Rhodobacter* (*Rb.*) *sphaeroides*, the hydrogen bonds between the bacteriochlorophyll (Bchl) molecules and their proteic binding sites exhibit a strong variance with respect to carotenoid content and type. In the absence of the carotenoid molecule, such as in the LH2 from *Rb. sphaeroides* R26.1, the void in the protein structure induces a significant reorganization of the binding site of both Bchl molecules responsible for the 850 nm absorption, which is not observed when the 800 nm absorbing Bchl is selectively removed from these complexes. FT Raman spectra of LH2 complexes from *Rb. sphaeroides* show that the strength of the hydrogen bond between the 850 nm absorbing Bchl bound to the α polypeptide and the tyrosine $\alpha_{45}$ depends precisely on the chemical nature of the bound carotenoid. These results suggest that the variable extremity of the carotenoid is embedded in these LH2 complexes, lying close to the interacting Bchl molecules. In the LH2 from *Rhodopseudomonas acidophila*, the equivalent part of the rhodopin glucoside, which bears the glucose group, lies close to the amino terminal of the antenna polypeptide. This contrast suggests that the structure of the carotenoid binding site in LH2 complexes strongly depends on the bacterial species and/or on the chemical nature of the bound carotenoid.

In most species of photosynthetic purple bacteria, the light-harvesting system is made up of two types of light-harvesting (LH[1]) antenna proteins, namely, LH1 and LH2. In these complexes, the bacteriochlorophyll (Bchl) and carotenoid (Crt) pigments are noncovalently attached to the individual membrane-spanning apoproteins, α and *β*. The determination of the crystal structure of the LH2 complex from *Rhodopseudomonas acidophila* 10050 to a resolution of 2.5 Å has revealed the arrangement of the pigments within that protein (*1*). It has a nonameric structure. Each monomeric unit contains one α/*β* apoprotein, one carotenoid (rhodopin glucoside), and three Bchl molecules. Two of these molecules absorb prominently at about 850 nm and are known as the Bchl-B850 molecules. The third Bchl molecule lies toward the cytoplasmic side of the membrane and is located between the transmembrane helices of neighboring *β* apoproteins. This pigment, denoted Bchl-B800, absorbs at approximately 800 nm and often exhibits spectroscopic properties typical of monomeric Bchls not involved in strong excitonic interactions (*2*). The LH2 protein from the related species *Rhodobacter sphaeroides* has been the subject of most of the investigations into the molecular interactions assumed by LH proteins because it lends itself readily to site-directed mutagenesis (*3*). Cryoelectron electron microscopy of the LH2 complex obtained from *Rb. sphaeroides* has enabled a projection map to be calculated (*4*). Like *Rps. acidophila*, the projection map of the LH2 from *Rb. sphaeroides* shows a ring of nine α/*β* heterodimers.

Carotenoid molecules, in LH complexes, perform two main photochemical functions, light-harvesting (through their singlet states) and photoprotection, by quenching the Bchl triplet states (through their triplet states). Less understood is their structural role, i.e., how these molecules contribute to the overall structural integrity of the tertiary structures. The X-ray structure of LH2 from *Rps. acidophila* illustrates that there are many carotenoid−polypeptide and carotenoid−bacteriochlorophyll interactions (*1*, *5*, *6*). Shown in Figure 1 is a schematic representation of the arrangement of the carotenoid in the LH2 monomeric unit. The glucosyl group of the rhodopin glucoside molecule is located in a hydrophilic pocket on the cytosolic side of the transmembrane-spanning protein. The polyene chain (shown in black) then runs perpendicular to the macrocycle of the Bchl-B800, progresses to the neighboring α/*β* dimer, and then passes over the macrocycle of the α-Bchl-B850. As the carotenoid snakes

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[‡] PDB code for light-harvesting protein LH2: 1KZU.

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[1] Abbreviations: Bchl, bacteriochlorophyll *a*; $cm^{-1}$, wavenumber; H bond, hydrogen bond; hwhm, half-width at half-maximum; FT-R, Fourier transform Raman spectroscopy; LH, light-harvesting; *Rps.*, *Rhodopseudomonas*; *Rb.*, *Rhodobacter*; B800 and B500, binding sites for Bchl absorbing at 800 and 850 nm, respectively.

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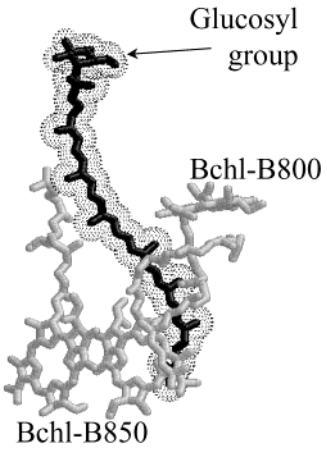


FIGURE 1: Schematic representation of the arrangement of the carotenoid and the proximity of the bacteriochlorophyll molecules in the X-ray crystal structure of LH2 (PDB code 1KZU). The carotenoid is shown in black with its van der Waals surface represented as a dotted surface.

its way through the protein, it establishes direct contacts with two $\alpha/\beta$ pairings. The stabilizing role of carotenoid molecules for LH2 complexes has been suggested, more than three decades ago, by the fact that one of the most prominent phenotypes of bacteria that have lost the ability to synthesize these molecules is unable to synthesize maturated LH2 complexes; for a review, see ref *3*. Similarly, in the R26 blue mutant from *Rb. sphaeroides* (see ref *7*), the LH2 is not assembled. In 1981 a carotenoidless strain of *Rb. sphaeroides* derived from R26, R26.1 was found to contain a LH2 protein (*8*). However, these complexes are quite different from those assembled in the *Rb. sphaeroides* wild type because they have lost the ability to bind a monomeric Bchl that absorbs at about 800 nm. On the other hand, depending on the strain considered and on the growth conditions, the LH2 from *Rb. sphaeroides* may contain different types of carotenoid molecules, such as spheroidene, spheroidenone, neurosporene, etc. The carotenoid binding sites in LH2 are thus able to accommodate the differences in volume and polarity of these different molecules.

In this work, we have used absorption spectroscopy and Fourier transform preresonance Raman (FT-R) spectroscopy to study the influence of the presence and of the chemical nature of carotenoid molecules on the structure and on the electronic transitions of LH2 from *Rb. sphaeroides*. We find that the interactions between the apoprotein and the Bchl-B850 in LH2 depend both on the presence and on the chemical nature of the carotenoid present in the complexes. These results are discussed in light of the absorption properties of the different LH2 complexes studied.

## MATERIALS AND METHODS

*Protein Isolation.* Liquid cultures of *Rb. sphaeroides* (strains 2.4.1, G1C, Ga, and R26.1) were grown in the light in Böse medium (*9*) to optimize their LH2 production. In the case of *Rb. sphaeroides* strain 2.4.1, the cells were grown under (i) strict anaerobic conditions that result in the formation of spheroidene-containing LH2 proteins and (ii) semiaerobic conditions resulting in spheroidenone-containing LH2 proteins (*10*). To obtain complexes containing spheroidenone or spheroidene only, cells were harvested after five culture transfers. In strain GIC, the carotenoid content in LH2 is 98% neurosporene and 2% lycopene while strain Ga possesses a cocktail of three carotenoids: 48% is neurosporene, 37% is chloroxanthin, and 14% is 3,4-dihydrospheroidene (*11*). The amino acid sequences of the LH2 polypeptides in strains Ga and G1C are identical to those of the wild type, as shown recently by matrix assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry (Cogdell and Mitchell, unpublished results). Finally, LH2 complexes were isolated from whole cells as previously described (*12*).

*Pigment-Removal Procedure.* The pigment-removal protocol from *Rb. sphaeroides* LH2 proteins has been extensively described elsewhere (*13*). In short, all of the Bchl-B800 molecules were released from their binding sites by incubating a LH2 sample in 20 mM sodium acetate containing Triton BG-10 (TBG10) at pH 4.75 at 30 °C for 1 h. B850 complexes lacking Bchl-B800 (also known as B800-depleted LH2) were then purified by ion-exchange chromatography using DE52 (Whatman). The detergent TBG10 was exchanged for a buffer containing 0.1% LM and 0.1% BOG. Following the removal of free Bchl, which were eluted by addition of 150 mM NaCl, the B850 protein was eluted with 300 mM NaCl. Finally, the B850 proteins were further purified by gel filtration (Toyopearl TSK-55s, TosoHaas, Montgomeryville, PA) in a salt-free buffer. In the case of the B850-only protein from strain 2.4.1, complete removal of the carotenoid (as evidenced by absorption spectroscopy) was also achieved by extensive incubation at room temperature in the presence of detergent under oxidizing conditions (*14*). This carotenoidless protein was purified as described above and is referred to as the B858 protein (*15*).

*Spectroscopy.* Room-temperature (293 K) electronic absorption spectra were collected using a Cary E5 Varian double-beam scanning spectrophotometer. Fourier transform Raman (FT Raman) spectroscopy in preresonance conditions with the Bchl $Q_y$ transition was performed at 293 K temperature using the 1064 nm excitation from a continuous Nd:YAG laser and a Fourier transform infrared (FTIR) spectrophotometer (Bruker IFS66) equipped with a Raman module (Bruker FRA106), as previously described (*16*). The presented FT-R spectra were the result of 9000 to 15 000 coadded interferograms. No evolution of the Raman signals was observed during data acquisition. Absorption spectra were taken before and after Raman measurements to verify sample integrity. The underlying background fluorescence was removed by fitting polynomial functions to the background signal using Grams Spectral Database (Galactic Industries, Salem, NH).

## RESULTS

To investigate the structural role of the carotenoids in LH2, complexes containing different types of carotenoid molecules (see Figure 2) were isolated from different strains of *Rb. sphaeroides*. LH2 proteins containing spheroidene, spheroidenone, and neurosporene were isolated from strains 2.4.1 anaerobically, from 2.4.1 semiaerobically grown, and from G1C, respectively. LH2 containing a cocktail of three carotenoids (neurosporene, chloroxanthin, and 3,4-dihydrospheroidene) were isolated from *Rb. sphaeroides* strain Ga (Figure 2). As shown in Figure 3, the position of the

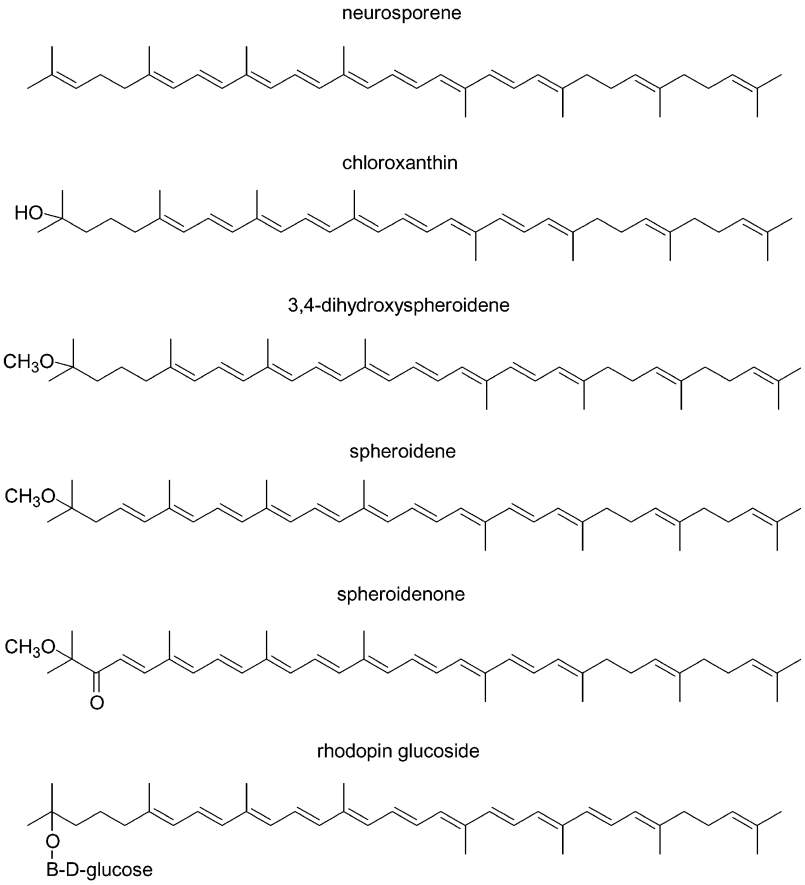


FIGURE 2: Skeletal representation of the different carotenoids found in LH2 proteins from *Rb. sphaeroides* and *Rps. acidophila*.

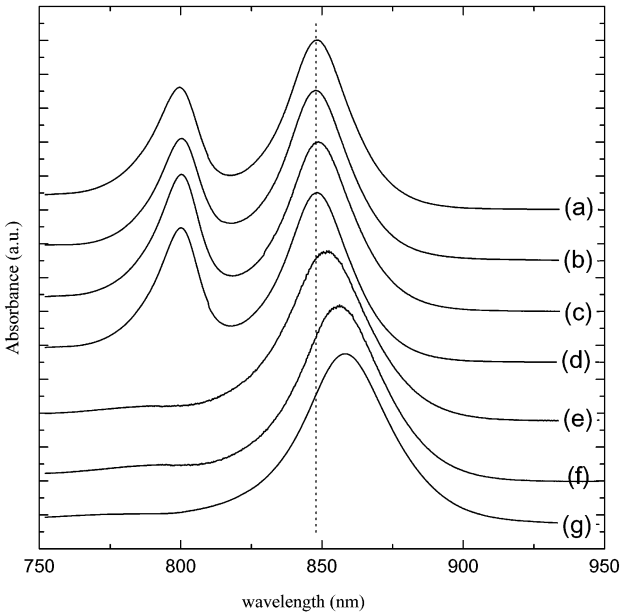


FIGURE 3: Room-temperature absorption spectra of the LH2 from *Rb. sphaeroides*: (a) strain 2.4.1 containing spheroidene; (b) strain 2.4.1 containing spheroidenone; (c) strain G1C containing neurosporene; (d) strain Ga (e) B850 complex from strain 2.4.1; (f) carotenoidless strain R26.1; (g) B858 complex from strain 2.4.1.

electronic transitions in the near-infrared of these different LH2 complexes are nearly identical (see also Table 1).

Fourier transform (FT) Raman spectra of the different *Rb. sphaeroides* LH2 proteins containing the different carotenoid compositions were recorded. This spectroscopy yields precise information on both the conformation of and intermolecular interactions assumed by the bacteriochlorin bound to proteins (*17*−*20*). The frequencies of six bacteriochlorin Raman modes, denoted $R_1$ to $R_6$, were shown to be sensitive to the Bchl macrocycle conformation (*17*), and they were used in particular to establish the conformations of Bchl molecules in LH proteins (*19*). In carotenoid-containing LH2 complexes, most of the bands arising from these modes are masked by carotenoid contributions except for those at 1605 ($R_1$), 1444 ($R_3$), and 1171($R_4$) $cm^{-1}$. The positions of these bands show a virtual invariance to carotenoid content (Table 2). Therefore, we may conclude that the chemical nature of the carotenoid molecules does not influence the conformation of the Bchl molecules in LH2.

Figure 4 displays the higher frequency region of the FT-R spectra of isolated LH2 proteins from different *Rb. sphaeroides* strains. In this region there are contributions arising from the methine bridge stretching mode at 1605 $cm^{-1}$ (*18*) and a cluster of five bands between 1620 and 1710 $cm^{-1}$ that are attributed to the stretching modes of the acetyl and ketocarbonyl groups. In the LH2 from *Rb. sphaeroides*, the position of each carbonyl vibrator has been established. The bands arising from the acetyl and ketocarbonyl stretching modes of the B800 molecules are located at 1628 and 1699 $cm^{-1}$ (*18*, *21*, *22*). The acetylcarbonyl groups of the B850 molecules contribute at 1628 and 1633 $cm^{-1}$ (Bchl bound to the $\alpha$ and $\beta$ polypeptides, respectively) while the keto ones are found at 1651 and 1679 $cm^{-1}$ (Bchl bound to the $\beta$ and $\alpha$ polypeptides, respectively) (*21*, *23*, *24*). The frequency of each band is sensitive to the geometry and strength of the bonding interactions that the carbonyls are involved in

Table 1: Position of the Room-Temperature Absorption Maxima for the $Q_y$ Bchl Transitions for a Range of Different LH2 Proteins from *Rb. Sphaeroides* with Different Carotenoid Content

| | strain (protein) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2.4.1 (LH2) | 2.4.1 (LH2) | Ga (LH2) | G1C (LH2) | 2.4.1 (B851) | R26.1 (B855) | 2.4.1 (B858) |
| carotenoid | spheroidene | spheroidenone | mixture | neurosporene | spheroidene | | |
| Bchl-B800 peak position (nm) | 800.1 | 800.0 | 800.1 | 800.3 | | | |
| Δ peak position ($cm^{-1}$) | | −1.5 | 0.0 | 3.1 | | | |
| Bchl-B850 peak position (nm) | 848.4 | 848.4 | 848.2 | 848.3 | 851.3 | 854.9 | 858.5 |
| Δ peak position ($cm^{-1}$) | | 0.0 | 2.8 | 1.4 | −40.1 | −89.6 | −138.7 |

Table 2: Raman Frequencies of the Conformation Bands ($R_{4,3,1}$) and High-Frequency Carbonyl Modes of Bacteriochlophyll *a* in LH2[a]

| | frequency ($cm^{-1}$) for protein (carotenoid) | | | | | | |
|---|---|---|---|---|---|---|---|
| mode | 241 (spheroidene) | 241 (spheroidenone) | Ga (mixture) | G1C (neurosporene) | B850 | R26.1 | B858 |
| $R_4$ δCmH(α,γ,δ), δCbH(II,I$_\zeta$) | 1171 | 1171 | 1173 | 1173 | 1174 | 1173 | 1173 |
| $R_3$ νCaN(II,IV),νCaCb(I,II) | 1444 | 1444 | 1443 | 1443 | 1444 | 1445 | 1445 |
| $R_1$ νCaCm(α,β,γ,δ) | 1605 | 1605 | 1604 | 1605 | 1605 | 1606 | 1605 |
| Bchl-850 $C_3$ν(acetyl) | 1627 | 1627 | (1633) | (1633) | 1623 | (1633) | (1632) |
| Bchl-800 $C_{13}$ν(acetyl) | 1628 | 1628 | 1628 | 1628 | | | |
| Bchl-850 $C_3$ν(acetyl) | 1634 | 1634 | (1633) | (1633) | 1632 | (1633) | (1632) |
| Bchl-850 $C_3$ν(acetyl) | 1651 | 1651 | 1651 | 1652 | 1650 | 1653 | 1654 |
| Bchl-850 $C_3$ν(keto) | 1679 | 1679 | 1678 | 1678 | 1678 | 1673 | 1675 |
| Bchl-800 $C_{13}$ν(keto) | 1699 | 1699 | 1699 | 1699 | | | |

[a] The values marked within parentheses belong to carbonyl modes that could not be separated by deconvolution analysis and thus represent an average value of both Bchl-B850 acetyl vibrational modes.

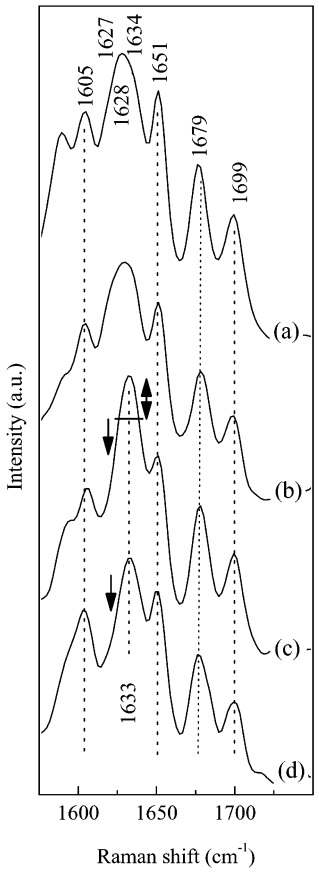


FIGURE 4: Room-temperature FT-R spectra of LH2 from *Rb. sphaeroides*: (a) strain 2.4.1 containing spheroidene; (b) strain 2.4.1 containing spheroidenone; (c) strain G1C containing neurosporene; (d) strain Ga with a mixed carotenoid population.

and may thus be used to probe the structure and intactness of the Bchl binding sites. FT-R spectra of spheroidene- and spheroidenone-containing LH2 proteins are identical (spectra a and b of Figure 4, respectively). The FT-R spectra of the LH2 from strains G1C (Figure 4c) and Ga (Figure 4d) are globally similar to those of strain 2.4.1. However, there is a distinct rearrangement of Raman bands in the 1620−1630 $cm^{-1}$ region in both these spectra. In the case of strain G1C, a loss of intensity at ca. 1627 $cm^{-1}$ is observed, associated with an increase at ca. 1633 $cm^{-1}$ (see single-headed arrows). A similar loss of intensity is observed at 1627 $cm^{-1}$ in strain Ga. However, this loss is not fully compensated by an increase of intensity in the region where the acetylcarbonyl mode usually contributes, i.e., 1620−1660 $cm^{-1}$ (Figure 4d). Only a slight increase of the intensity of the 1651 $cm^{-1}$ band is observed, together with a more pronounced gain intensity of the 1605 $cm^{-1}$ band.

In LH2 from the blue R26.1 mutant, both the carotenoid and the B800 Bchl molecules are absent. In accordance with previous work, this complex has a somewhat red-shifted absorption peaking at 855 nm (Table 1, Figure 3f). In its FT-R spectrum, neither the absence of the carotenoid nor that of the Bchl-B800 molecule induces a change in the conformation of the Bchl-B850 molecules, as indicated by the absence of a shift of the modes sensitive to the macrocycle conformation (Table 2). In the higher frequency region of this spectrum, the contributions of the Bchl-B800 are missing, resulting in a loss of the band at 1699 $cm^{-1}$ and a decrease of intensity around 1628 $cm^{-1}$ (Figure 5c). Three bands only are visible in the carbonyl region: at 1633, 1653, and 1673 $cm^{-1}$. As in spectra from the LH2 complexes purified from strain G1C, the 1633 $cm^{-1}$ band is narrow and intense, suggesting that both acetylcarbonyls of the Bchl-B850 molecules contribute at this position. The shifts experienced by the ketocarbonyl contributions suggest a significant reorganization of the Bchl-B850 molecules in their binding sites.

The effects observed in the Raman signature from R26.1 LH2 may arise from the absence of either the carotenoid or the Bchl-B800 molecule, or possibly a combination of the two. We thus prepared two new samples from the LH2

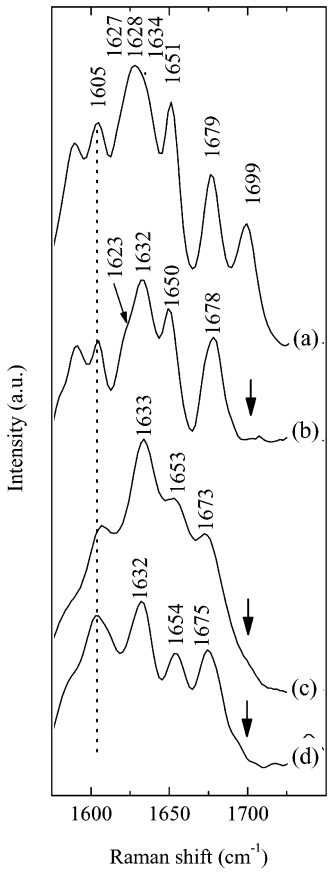


FIGURE 5: Room-temperature FT-R spectra of modified LH2 proteins from *Rb. sphaeroides*: (a) native LH2 from strain 2.41 containing spheroidene; (b) B850 complex from strain 2.4.1 containing spheroidene; (c) LH2 from strain R26.1 possessing neither carotenoid nor Bchl-B800; (d) the carotenoidless B858 complex from strain 2.4.1.

protein from *Rb. sphaeroides* 2.4.1 by selectively removing (i) only the Bchl-B800 and (ii) both the Bchl-800 and the carotenoid molecules. Removing the Bchl-800 from LH2 has no drastic effect on the position of the $Q_y$ transitions at 850 nm while it upshifts by ca. 8 nm upon disruption of both the B800 site and removal of the carotenoid (Table 1, Figure 3g,h). According to the positions of their $Q_y$ transitions, these proteins are referred to as B850 and B858 complexes, respectively (*15*). As expected from the results obtained with R26.1 LH2, the conformation of the Bchl-B850 molecules is not affected by the changes in pigment composition in these complexes (Table 2). In the higher frequency region, the FT-R spectra of the Bchl-B800 depleted LH2 exhibit Raman bands peaking at 1623, 1632, 1650, and 1679 $cm^{-1}$ (Figure 5b, Table 2), which nearly perfectly matches the Bchl-B850 modes in intact complexes. By contrast, the FT-R spectrum of the B858 complex (Bchl-B800 and carotenoid-depleted) displays bands at 1632, 1654 and 1675 $cm^{-1}$ (Figure 5d, Table 2). The main perturbations of the FT-R spectrum in these proteins, and in consequence in the R26.1 LH2 complex, thus results from the absence of carotenoid, and not from the removal of the Bchl-B800 molecule.

## DISCUSSION

*Influence of the Presence of Carotenoid Molecules on the LH2 Structure*. It has been known for a long time that the absorption properties of LH2 from *Rb. sphaeroides* R26.1 are different from those of the wild-type strain. In this work, we show that these differences are accompanied by a significant reorganization of the binding site of the Bchl-B850 molecules. In these complexes, both the carotenoid and the Bchl-B800 molecules are missing. To precisely assess which molecule is at the origin of the observed perturbations, we selectively removed from the LH2 from strain 2.4.1 the Bchl-B800 or the Bchl-B800 together with the bound carotenoid. The removal the Bchl-B800 alone from the complex induces a small upshift of the Bchl-B850 $Q_y$ electronic transition and has little effect on the FT-R spectrum of the protein. The removal of this molecule thus has no long-range influence on the binding site of the Bchl-B850 molecules. In contrast, removing both the Bchl-B800 and the carotenoid molecule induces the Bchl-B850 $Q_y$ electronic transition to upshift to 858 nm, which corresponds to an energy shift of some 140 $cm^{-1}$. The FT-R spectra of these doubly depleted complexes are similarly perturbed compared to those of R26.1 but to a slightly lesser extent. It is thus possible to conclude that the differences between R26.1 and the carotenoid-containing LH2 primarily arise from the absence of the carotenoid molecule and not from the absence of Bchl-B800.

From the FT-R spectra, the extent of structural reorganization around the Bchl-B850 molecules can be precisely assessed. The merging of the contributions from both the acetylcarbonyls of the Bchl-B850 molecules indicates that the H bond between the acetyl Bchl bound to the α polypeptide and the Tyr $\alpha_{45}$ residue is weaker in *Rb. sphaeroides* R 26.1. Both the ketocarbonyl group stretching frequencies are perturbed by the absence of carotenoid, though to different extents. In *Rb. sphaeroides* 2.4.1, the ketocarbonyl group of $Bchl_\beta$ vibrates at 1651 $cm^{-1}$, and it is H-bonded to Ser $\alpha_{27}$. This H bond is very slightly weakened by the absence of the carotenoid. The $Bchl_\alpha$ ketocarbonyl stretching frequency is at 1679 $cm^{-1}$, a position that is only 6 $cm^{-1}$ downshifted from that observed in apolar solvents. This indicates that this carbonyl is embedded in a polar environment or involves a very weak H bond with the surrounding protein. This group is only 3.8 Å from the His 31, which serves as ligand for $Bchl_\beta$, in the *Rps. acidophila* crystallographic structure, and the proximity of this polar side chain accounts well for its rather low stretching frequency. In the absence of carotenoid, the downshift of the 1679 $cm^{-1}$ band to 1673 $cm^{-1}$ indicates a shortening of the carbonyl−histidine distance. An estimate of this variation may be provided by the FT-R spectrum of LH2 from *Rps. acidophila*. In these complexes, where there is no equivalent for Ser 27, the ketocarbonyl group of $Bchl_\beta$ is only 3.5 Å from the histidine that serves as a ligand to $BChl_\alpha$, and its stretching frequency occurs at 1670 $cm^{-1}$. A 0.2−0.0.4 Å shortening of the distance between the histidine 31 and the ketocarbonyl group of $Bchl_\beta$ would thus account for the variation in the stretching frequency observed in the absence of carotenoid. It is therefore possible to conclude that, although it does not affect the conformation of the Bchl-B850s, the absence of carotenoid induces a small but global reorganization of the Bchl-B850 molecules. This is likely to be at the origin of the known electronic differences between the LH2 from *Rb. sphaeroides* R26.1 and 2.4.1, such as the changes in the $Q_y$ position, and the extent of its charge-

transfer character, which has been observed by Stark spectroscopy (*25*).

*Influence of the Chemical Nature of the Carotenoid Molecules on the LH2 Structure*. Although the chemical nature of the carotenoid bound to the LH2 complexes from *Rb. sphaeroides* does not affect the position of the $Q_y$ electronic transitions of the bound Bchl molecules, it may have a significant influence on the structure of the B850 binding site. This is not the case when exchanging spheroidene for spheroidenone. In that particular case, no change occurs in the FT-R spectra of LH2 and thus in any reorganization of the binding sites of these molecules. In contrast, the presence of neurosporene induces a weakening of the H bond between Tyr $\alpha_{45}$ and the acetylcarbonyl group of $Bchl_x$. By use of a Badger-type relation (*26*), it may be evaluated that the downshift from 1627 to 1633 $cm^{-1}$ corresponds to the weakening of this H bond by about 0.9 kcal/mol. This corresponds to either a slight change of the distance between, or the relative geometry of, Tyr $\alpha_{45}$ and this Bchl partner molecule. This reorganization is, however, too small to have any sizable influence on the absorption of the LH2 complexes, or it is accompanied by other, Raman silent, small structural reorganizations (such as very small motions of neighboring Bchl molecules relative to each other) that compensate the effect of the tyrosine motion. Results obtained with the LH2 from *Rb. sphaeroides* strain Ga, which incorporates a mixture of very different carotenoids (neurosporene, dihydrospheroidene and chloroxanthin), are similar to those obtained with LH2 from strain G1C. The incorporation of the carotenoid mixture does not affect the absorption properties of the complex; however, a significant change is observed in the structure of the B850 site. FT-R spectra of the LH2 form stain Ga are, however, more complicated to interpret. It mainly consists of a reduction of intensity of the band arising from the stretching mode of the $Bchl_\alpha$-B850 (contributing at 1627 $cm^{-1}$). Such a loss may correspond to a change in the resonance conditions because it is accompanied by changes in intensity affecting bands arising from the methine bridge stretching mode and, to a lesser extent, that arising from the ketocarbonyl stretching mode of the other Bchl-B850. It may also arise from a highly localized molecular reorganization, such as a change in the angle between this acetylcarbonyl and the macromolecular plane of the $Bchl_\alpha$-B850 molecule. Indeed, such reorganization in a Bchl molecule has already been postulated to occur in the B800−820 LH2 from *Rps. acidophila* based on crystallographic studies (*27*) and actually observed in mutated reaction centers from *Rb. sphaeroides* (*28*). In the crystal structure of the RC mutant FM197R, a 20° out-of-plane rotation of the acetyl grouping of $P_m$ occurs and was concomitant with a reduction in intensity of its FT-R stretching mode (*28*). In the case of the LH2 from strain Ga, the extent of this potential reorientation is difficult to evaluate because the loss of intensity of the 1627 $cm^{-1}$ band is difficult to quantify (see Results). If such a rotation occurred, the absence of changes in the position of the $Q_y$ transition of the LH2 complexes would suggest that the rotation of this carbonyl, by itself, does not affect the electronic transition of the Bchl-B850.

In the *Rps. acidophila* structure, the rhodopin glucoside is inserted in the LH2 structure with the glucoside group on the amino-terminal side of the $\alpha$ and $\beta$ polyeptides. At its other end, the carotenoid molecule runs parallel to the $Bchl_\alpha$-B850, close to the cycle IV and I of this molecule (the minimal distance between these two molecules is 3.62 Å), and its extremity is in the vicinity of the His $\alpha_{31}$, which coordinates the central Mg atom of the $Bchl_\alpha$-B850. This position of the carotenoid is consistent with the FT-R data reported in this work. Upon changes in the carotenoid composition, the most perturbed carbonyl group is the acetyl of the $Bchl_\alpha$-B850, which is the closest of the carotenoid in the *Rps. acidophila* structure. The same phenomenon is observed when the carotenoid molecule is absent; however, in this case, the structural perturbation extends to the surroundings of both ketocarbonyl groups of the Bchl-B850 molecules. The keto of the $Bchl_\beta$-B850 that interacts with a serine residue, which is replaced in the *Rps. acidophila* structure by an alanine (Ala $\alpha_{27}$), is only 4 Å from the carotenoid molecule. The absence of the carotenoid is thus likely to perturb the precise position of this residue. On the other hand, the change in the environment of the ketocarbonyl of the $Bchl_\alpha$-B850, which we attribute to a change in the distance between this carbonyl and the histidine liganding the $Bchl_\beta$-B850, cannot be explained by a localized effect. It is thus more likely that the void due to the absence of the carotenoid, in close vicinity of the $Bchl_\alpha$-B850, induced a slight reorganization of the whole ensemble of the Bchl-B850 molecules, which in turn results in a shortening of the ketocarbonyl−histidine distance.

The carotenoids used in this study, i.e., spheroidene, spheroidenone, neurosporene, chloroxanthin, 3,4-dihydroxyspheroidene, and rhodopin glucoside, possess a common chemical structure on one end, and the chemical differences generally occur on the other end, where the glucose group of rhodopin glucoside (Figure 2) also sits. In the *Rps. acidophila* structure, the rhodopin glucoside is bound with its glucose group on the amino-terminal side of the $\alpha$ and $\beta$ polypeptides. It could thus be expected that in *Rb. sphaeroides* LH2 the variable end of the carotenoid molecules also sits close to the amino-terminal end of the polypeptides, while the other end of the molecule lies close to the Bchl-B850 molecules. However, if true, the differences described in this work in the binding site of the latter molecules are extremely difficult to account for. To explain them, long-range perturbations of the protein structure should be invoked, induced by changes as small as the presence (or the absence) of, for example, a $CH_3O$ group (which is the only chemical difference between spheroidene and neurosporene). The structural perturbations of the B850 site induced by the differences in the chemical nature of the carotenoid molecules are much more likely to be due to local effects. This implies that, in the LH2 from *Rb. sphaeroides*, the variable end of the carotenoid is embedded inside the protein, in the vicinity of the B850 Bchl molecules, and that the identical end of these molecules sits on the amino-terminal side of the $\alpha$ and $\beta$ polypeptides. In *Rb. sphaeroides* and *Rps. acidophila* LH2, the carotenoid molecules are thus inserted inside the LH2 in a completely different way: the variable end in *Rb. sphaeroides* and the conserved end in *Rps. acidophila*. This conclusion indicates that the structure of the binding site of carotenoid molecules in LH2 complexes is highly dependent on the bacterial strain and/or the chemical nature of the considered molecule.

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