Characterization of the Different Peripheral Light-Harvesting Complexes from High- and Low-Light Grown Cells from *Rhodopseudomonas palustris*†

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ABSTRACT: In this paper we demonstrate that the spectroscopically different peripheral light-harvesting complexes from *Rhodopseudomonas palustris*, strain 2.6.1, isolated from high- and low-light grown cells have widely differing bacteriochlorophyll *a* (BChl *a*) resonance Raman spectra in the high-frequency carbonyl region (1550−1750 cm^{-1}). Complexes synthesized in low-light grown cells exhibit Raman spectra characteristic of B800−850 and B800−820 complexes, depending on the excitation conditions. The in vivo strategy for low-light adaptation in this bacterium is thus somewhat different from that generally encountered in the Rhodospirillaceae. In these bacteria, as typified by *Rps. acidophila* and *Rps. cryptolactis*, low-light conditions induce the synthesis of B800−820 only complexes in which the hydrogen bonds between the acetyl carbonyl and the B850 binding pocket are broken, inducing changes in the absorption properties of the monomeric bacteriochlorophylls. In the case of *Rps. palustris*, additional spectral effects occur due to the coupling of the electronic levels of the differently interacting dimers. The extensive use of differential α/β-polypeptide expression [Tadros et al. (1993) *Eur. J. Biochem. 217*, 867−875] thus allows *Rps. palustris* to alter its BChl *a* binding site environments causing the observed spread of BChl *a* Q_y transitions, ranging from 801 to 856 nm.

The *Rhodospirillaceae*, or non-sulfur photosynthetic purple bacteria, have a photosynthetic apparatus that consists of the photochemical reaction center (RC) and light-harvesting (LH) complexes that are localized in intracytoplasmic membranes. Typically the Rhodospirillaceae have two types of light-harvesting antennae called LH1 (or core) and LH2 (or peripheral). The RC is always closely associated with the LH1 antennae and generally considered to be of a fixed RC:LH1 stoichiometry (*1*). Located peripheral to the LH1 antennae lie the LH2 pigment−protein complexes. Photons are captured by bound pigments in the LH antennae, and the resulting excitation energy transfer to the RC, and subsequent electron transfer within this protein, produces a chemical potential gradient across the membrane.

Both the LH1 and LH2 complexes are made up of oligomers of two low-molecular-weight transmembrane apoproteins (α and β) with noncovalently attached bacteriochlorophyll *a* (BChl *a*) and carotenoid molecules (*2*). The recent X-ray crystal structures of the B800−850 (LH2) complexes from *Rhodopseudomonas acidophila* (*3*) and *Rhodospirillum molischianum* (*4*) indicate that each LH2 pigment−protein complex is made up of a multiple of eight *(Rs. molischianum)* or nine (*Rps. acidophila*) α/β-heterodimers, each binding three BChl *a*'s (*3*) and either one (*5*) or two (*6*) carotenoid molecules.

The level of LH2 expression and spectral range covered by bound BChl *a* are variable and can depend greatly on environmental conditions such as oxygen tension and the quality and intensity of available light (for a review, see ref *7*). Some species, such as *Rps. acidophila* and *Rps. cryptolactis*, are capable of changing their LH2 antennae turnover from the B800−850 LH2 to a more blue-shifted B800−820 complex. One exception to this rule is the low-light-adapted intracytoplasmic membrane from *Rps. palustris*, strain 2.6.1. In this bacterium the B800−850 is not replaced by a 820-nm-absorbing LH2 complex but rather by a low-850-nm-absorbing B800−850 protein (*8*−*11*). This ability in some members of the Rhodospirillaceae to grow at extreme low-light intensities by adapting their pigment−protein complexes is linked to the presence of multiple LH2 α/β-polypeptides. The environmental regime of the culture causes the different genes within the *puc* operons that encode the α- and β-polypeptides to be differentially expressed (*12, 13*).

Recently, the physicochemical mechanisms underlying the tuning of the absorption of the different LH2 complexes synthesized by *Rps. acidophila* and *Rps. cryptolactis* have been extensively addressed (*14*). It was shown that the molecular origin of the absorption differences between the B800−850 and B800−820 complexes is due to the breakage of H-bonds between the residues +13 and +14 (taking the Mg-coordinating His residue as a point of reference) on the α-polypeptide. In this study we have used a combination of spectroscopic methods to investigate the unusual absorption properties of the low-850-nm-absorbing LH2 complex observed in *Rps. palustris*.

MATERIALS AND METHODS

Rps. palustris, strain 2.6.1, was grown photoheterotrophically in Böse medium (*15*) at 28 ± 2 °C in glass bottles located between banks of incandescent lamps at two extreme

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light intensities. The high-light (HL) intensity was in excess of 10 W m^{-2}, while the low-light (LL) intensity was approximately 0.1 W m^{-2}. Cultures were regularly transferred to ensure a constant low culture optical density. This minimized any self-shading caused by the cells themselves, which would precipitate a 'low-light' regime. This ensured that the final inoculums contained only high- or low-light-adapted intracytoplasmic membranes. Cells were harvested and membranes prepared essentially by the method described by ref *10*.

The LH2 complexes were prepared by diluting the membranes to an OD of 25 cm^{-1} at the 800-nm peak with 20 mM Tris·HCl, pH 8.0, and then solubilized for 30 min at room temperature with 0.75% LDAO (Fluka). Following dilution of the detergent, a low-speed centrifugation step ensured removal of any unsolubilized debris (10000*g*, 8 min, 4 °C). On the basis of the protocol in ref *9*, the solubilized photosynthetic membranes were isolated using a linear (0.2−1.2 M) sucrose gradient centrifugation step. The pigmented band was collected, and the LH2 proteins were purified by anion-exchange chromatography (10−400 mM NaCl, 0.075% LDAO, 20 mM Tris·Cl, pH 8.0, Fractogel DEAE-650s, TosoHaas) followed by separation by size exclusion (0.075% LDAO, 50 mM NaCl, 10 mM Tris·Cl, pH 8.0, Toyopearl TSK-55s, TosoHaas). The purified LH2 antennae were then concentrated using Centricon-30s (Amicon) and stored at −20 °C until required. Polyacrylamide gel electrophoresis was used to evaluate polypeptide composition (*16*).

Absorption spectra were collected at 10 K in a SMC-TBT flow cryostat (Air Liquide, Sassenage, France) cooled with liquid helium or at room temperature using a Varian Cary E5 double-beam scanning spectrophotometer. Low-temperature (77 K) fluorescence emission spectra were obtained with a SPEX Fluoromax spectrofluorimeter (ISA, Longjumeau, France) equipped with a red-sensitive photomultiplier R406 (Hamamatsu). Room-temperature circular dichroism spectra were measured on a Dichrograph CD6 spectrophotometer (ISA, Longjumeau, France) equipped with a UV−vis (R-376 Hamamatsu photomultiplier) or near-infrared (IR) detector. Room-temperature Fourier transform preresonance Raman (FT-R) spectra were recorded as described by ref *17*. Soret-excited resonance Raman spectra were recorded with a Jobin Yvon U1000 spectrometer equipped with a back-thinned CCD camera (Jobin Yvon Spectra ONE). Spectra were recorded with a 90° geometry from samples maintained at 10 K in a SMC-TBT flow cryostat (Air Liquide, Sassenage, France) cooled with liquid helium as described in ref *18*. The 363.8-nm excitation was provided by a Coherent Innova 100 Ar laser, with an incident intensity of less than 1 mW. Absorption spectra were taken before and after Raman measurements to verify sample integrity.

RESULTS

The polypeptide composition for the low- and high-850-nm-absorbing LH2 complexes from *Rps. palustris*, strain 2.6.1, was determined using a polyacrylamide gel electrophoresis protocol able to separate low-molecular-weight polypeptides in the 5−10 kDa range (*16*). As previously reported (*10*), each protein sample consists of small molecular weight polypeptides in the 5−8 kDa range (data not shown) and reveals that both the samples are broadly similar although the relative ratios of the bands are somewhat different.

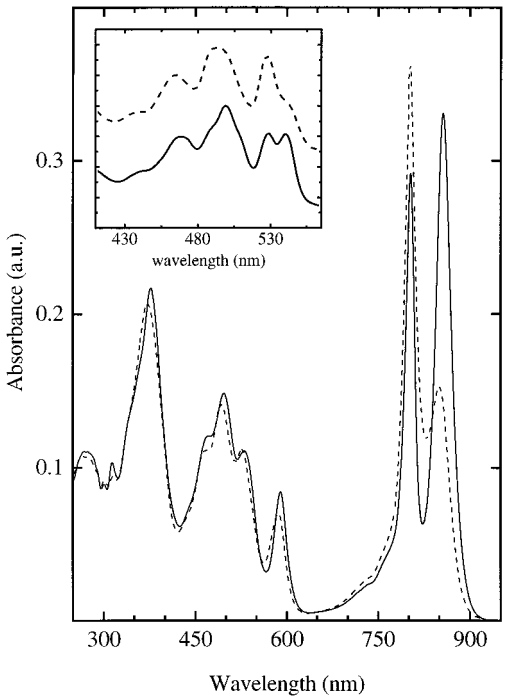

FIGURE 1: Room-temperature absorption spectra of LH2 proteins isolated from high-light grown cells (solid line) and low-light grown cells (broken line). Insert: Low-temperature absorption spectra of the carotenoid electronic transitions from the HL LH2 (solid line) and the LL LH2 (broken line), $T = 10$ K. The spectra are offset for enhanced clarity.

Figure 1 shows room-temperature absorption spectra for both the high-850 nm-absorbing (HL) and low-850 nm-absorbing (LL) LH2 complexes. In the HL LH2 complex the Soret, Q_x, and both Q_y BChl *a* transitions are located at 375, 589, 802, and 856 nm, respectively. The corresponding maxima for the LL LH2 complex are located at 370, 585, 802, and 850 nm.

In *Rps. palustris* LH2 there is a heterologous carotenoid population that changes depending on the light regime, therefore, under HL and LL conditions these complexes have different carotenoid absorption characteristics (*10*). The absorption peaks at 468, 496, and 530 nm are attributed to the carotenoids in the HL LH2 complex. In the LL form of LH2 the absorption peaks are at 466, 491, and 526 nm. At 10 K, as we move from HL- to LL-adapted membranes, the carotenoid absorption transitions at 468, 499, and 528 nm are blue-shifted to 464, 497, and 526 nm, respectively (Figure 1, insert). Of the two absorption maxima that do not alter their wavelength position upon changing light regime, we nevertheless see changes. As the light level is reduced we see a reduction of the 539-nm band with an associated increase at 489 nm.

The strength of the H-bond network formed between the BChl molecules and the polypeptides has been shown to tune the absorption of the B800 (*18*) and B850 molecules (*21, 22*). The modulation caused by the perturbation of the H-bond network within the B850 pairs should result in the presence of a series of absorption transitions between about 820 and 850 nm, the precise position depending on how the different α-polypeptides are positioned within the ring. In agreement with previous works (*10, 11, 19*) low-temperature

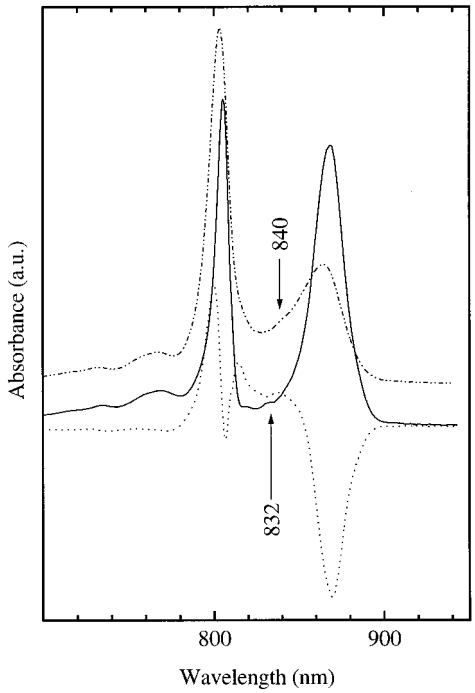

FIGURE 2: Low-temperature absorption spectra of the Q_y electronic transitions from the HL LH2 (solid line) and the LL LH2 (broken line) and their difference spectrum (dotted line), $T = 10$ K.

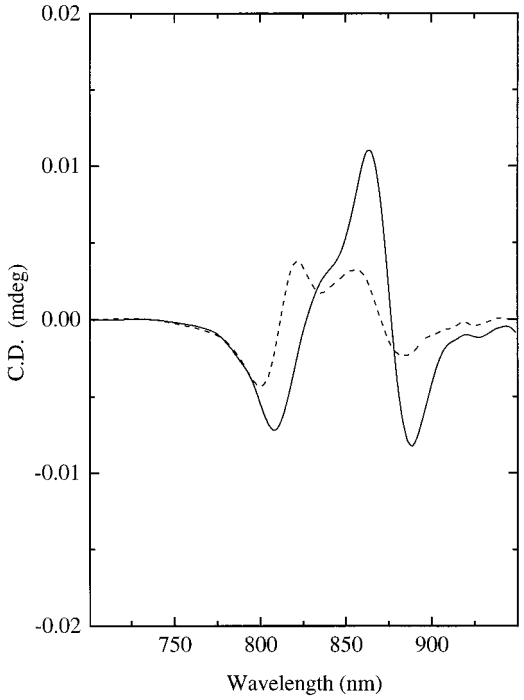

FIGURE 3: Near-IR circular dichroism spectra of the HL (solid line) and LL (broken line) LH2 proteins.

near-IR absorption spectra of same proteins (Figure 2) reveal complex electronic transitions in both samples. The Q_x and B800 Q_y absorption maxima shift by 1−5 nm. In both LH2 complexes the B850 Q_y band red-shifts by approximately 12 nm. Apart from the major peaks at 805 and 868 nm, the HL LH2 complex has a very small absorption peak at 832 nm (Figure 2, solid line). In the LL LH2 (Figure 2, broken line) in addition to the Q_y maxima at 803 and 863 nm, we observe another peak at 840 nm. Recently the identification of the upper exciton component of the B850 bacterichlorophylls of the LH2 antennae has been ascribed to the absorption maxima located at approximately 780 nm at 77 K (*26*). Therefore, in our 10 K absorption spectra the similar maxima at ∼780 nm is possibly due to this spectral component.

Figure 3 shows the normalized low-temperature fluorescence emission spectra upon excitation of the Q_x transition of BChl *a*. The fwhm for the spectra from both HL and LL samples are very similar. The fwhm of the LL LH2 is 3 nm narrower. Compared to the fluoresence peak of the LL LH2, the HL sample is 5 nm red-shifted (900 nm compared to 895 nm). This corresponds to the same 5-nm red shift seen in the low-temperature absorption spectra (Figure 2).

The near-IR circular dichroism (CD) spectra (Figure 4) are in broad agreement with those previously reported (*19*) for high- and low-light grown cells. The LL LH2 complex exhibits at least one additional peak between 820 and 840 nm and probably corresponds to different B800 structures in the two samples (*19*). As expected from the low-temperature absorption spectra (Figure 2, insert) small shifts are observed in the carotenoid UV−vis region CD signal (data not shown).

We measured room-temperature Raman spectra of both LH2 complexes under preresonance conditions with the BChl *a* Q_y electronic transitions, using the 1064-nm line of a Nd:

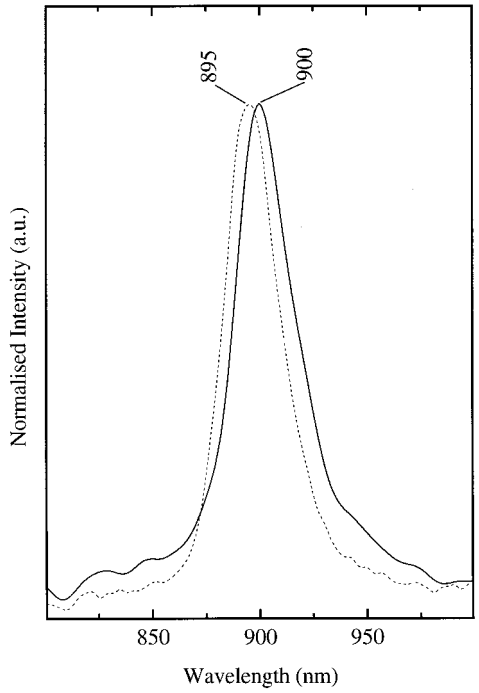

FIGURE 4: Low-temperature fluorescence emission spectra of the HL (solid line) and LL (broken line) LH2 proteins from *Rps. palustris*. The antennae were excited in the BChl *a* Q_x band, $T =$ 77 K.

YAG laser as the excitation line. In these conditions of resonance, the main contributions observed arise from the red-most-absorbing pigments (*18*). The high-frequency regions (1550−1750 cm^{-1}) of these spectra are shown in Figure 5. The spectra are offset for enhanced clarity. In the 1620−1700-cm^{-1} region the Raman bands arise from the keto and acetyl carbonyl stretching modes of BChl *a*. The exact positions of the carbonyl stretching modes depend on the strength of the intermolecular interactions between the

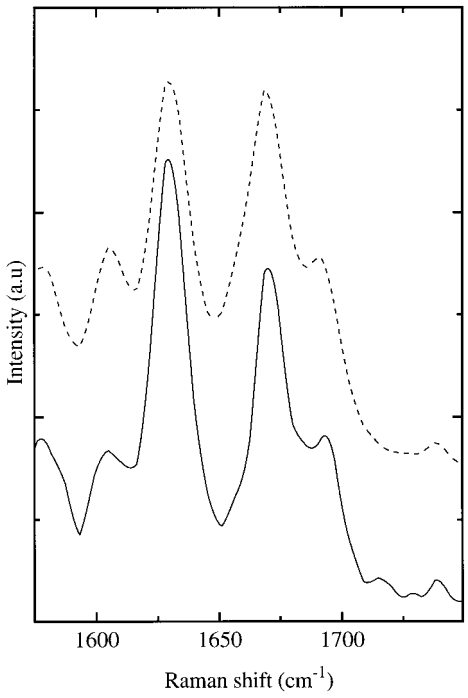

FIGURE 5: Room-temperature Fourier transform preresonance Raman spectra of the 1550−1750-cm^{-1} region of the HL (solid line) and LL (broken line) LH2 complexes from *Rps. palustris*, strain 2.6.1.

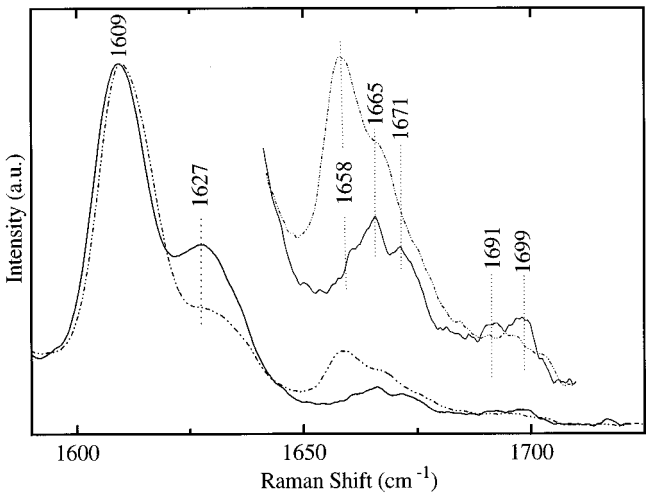

FIGURE 6: Low-temperature UV resonance Raman spectra of the 1550−1750-cm^{-1} region of the HL (solid line) and LL (broken line) LH2 complexes. Excitation wavelength of 363.8 nm, $T = 10$ K.

different pigments and their immediate environment. Only minor differences are observed between the two LH2 complexes and concern mainly the relative intensity of the 1631/1660-cm^{-1} bands. In the LL complex (Figure 5, broken line) the 1668-cm^{-1} band has a slightly broader fwhm (20 cm^{-1}) than in the HL LH2 (16 cm^{-1}).

To further establish the organization of the BChl *a* environment, we measured Raman spectra at 10 K in resonance conditions with the Soret electronic transition (363.8 nm). Under these conditions, all the BChl *a* molecules of the LH2 complexes are expected to contribute more or less equally (*18*). Figure 6 displays the high-frequency spectra (1590−1725 cm^{-1}) for both LH2 complexes. The spectra are dominated by a band at 1609 cm^{-1} attributed to the methine bridge stretching modes of BChl *a* molecules having their central Mg 5-coordinated (*20*). The contributions from the carbonyl stretching modes (1620−1710 cm^{-1}) are weak and partially hidden by this band. The insert in Figure 6 shows an enlarged view of most of the carbonyl region (1640−1710 cm^{-1}). Both LH2 preparations have carbonyl modes at 1627, 1658, 1665, and 1671 cm^{-1}. It is evident that in the LL LH2 (Figure 6, broken line) there is a strong reduction of the 1627-cm^{-1} component which is accompanied by an increased intensity at 1658 cm^{-1}. The mode at ca. 1700 cm^{-1} is present with components between 1691 and 1699 cm^{-1}. This mode has been attributed to free-from-interaction C_9-keto carbonyl groups of the B800 (*20*). The splitting of this band into two distinct modes occurs in the HL complex suggesting the presence of at least two BChl *a* free-from-interaction C_9-keto pools. In contrast, in the LL LH2 no such separation between different carbonyl pools is detectable, and this results in a broad single band peaking at ca. 1696 cm^{-1}.

DISCUSSION

As multiple Q_y transitions are observed in the absorption spectra of the LH2 complexes from *Rps. palustris* at low temperature, these proteins are more complex than the LH2 antennae from *Rps. acidophila*, *Rps. molischianum*, and *Rb. sphaeroides*. The mechanisms underlying the absorption changes in LH2 from bacteria able to synthesize more than one pair of α/β-polypeptides have been extensively addressed (*2, 7*). The direct evidence that protein−BChl *a* interactions are responsible for these changes arose from the work of Fowler et al. (*21*). These authors showed that replacement of either the αTyr_{+13} or αTyr_{+14} induced a ca. 10-nm blue shift in the 850-nm Q_y absorption transition. They also demonstrated that double mutants at these two positions exhibit absorption properties (826 nm) close to LH2 complexes naturally absorbing at ca. 820 nm. Raman studies of the mutants have shown that each of these mutations was associated with the breakage of an H-bond between the polypeptide and the acetyl of one, or other, of the two BChls responsible for the 850-nm absorption (*22*). The removal of the H-bond to the acetyl carbonyl groups induced the frequency upshift expected for free-from-interaction acetyl carbonyls (i.e., from ca. 1630 to 1660 cm^{-1}).

Upon excitation at 363.8 nm major differences are observed in the carbonyl stretching frequency region between the LL and HL LH2 complexes from *Rps. palustris*. Compared to the HL complex, the LL complex has a reduced intensity at 1627 cm^{-1} attributed to the acetyl stretching modes. This is accompanied by dramatic increases in the 1660-cm^{-1} region where free-from-interaction acetyl groups are expected to contribute. It can be concluded that protein−BChl H-bonds which are present in HL LH2 are broken in the LL form. This same phenomenon is observed when comparing the B800−850 and B800−820 complxes from *Rps. acidophila* leading to the previous hypotheses (*23*) that the LL LH2 from *Rps. palustris* and the LH2 complex from *Rps. acidophila* had similar structures. This implied that the mechanisms underlying the conversion of B800−850 into B800−820 (*Rps. acidophila*) or into a low-850 nm-absorbing B800−850 complex (*Rps. palustris*) were the same. However, in the case of the B800−820 from *Rps. acidophila*, the differences in the Raman spectra between the B800−

850 and B800−820 complexes may be observed independently of excitation conditions, as expected from a homogeneous BChl *a* population. In contrast when exciting at 1064 nm *Rps. palustris* exhibits few differences between the FT-Raman spectra of HL and LL LH2 complexes. Furthermore, the spectra obtained in these conditions are very similar to the FT-Raman spectra of B800−850 complexes from *Rubrivivax gelatinosus* and *Rb. capsulatus* (*14*). As in most B800−850 LH2 complexes, this is a direct evidence that the intermolecular interactions assumed by the 850-nm-absorbing BChl *a* are the same in both spectral forms of LH2 from *Rps. palustris*. From this we may conclude that in both the HL and LL complexes both acetyls of the red-most-absorbing BChls are H-bonded. In *Rps. palustris*, it seems that the BChl *a*'s contributing to the Soret-excited resonance Raman spectra of the LL complexes assume different intermolecular interactions than those contributing to the 1064-nm-excited ones.

Depending on the polypeptide under consideration, in the primary sequences of the α-polypeptides from *Rps. palustris*, YW or FL sequences are present at the +13 and +14 positions where the amino acids interacting with the acetyl carbonyl of the coupled BChl *a* are expected to be located. As the global structures of these LH2 complexes are closely related to that of the LH2 from *Rps. acidophila* (*2, 24*), we must conclude that most of the α-polypeptides constituting the HL complexes have a YW doublet at positions +13 and +14. However, in the LL complexes there must be a greater mixture of YW and FL at these positions. It is then of particular interest to know whether the LL complexes from *Rps. palustris* are constituted of a mixture of homogeneous rings (i.e., each only containing a single type of α-polypeptide) or from rings containing mixtures of different α-polypeptides.

The near-IR absorption peak of every LH2 containing only a $\alpha F_{+13}/\alpha L_{+14}$ sequence is observed at ca. 826 nm (*2, 21, 24*). Since *Rps. palustris* expresses multiple α-polypetides under HL conditions (*10, 11*), the slight absorption maximum at 832 nm is likely due to a small percentage of $\alpha F_{+13}/\alpha L_{+14}$ sequences in the preparation. In agreement with previous works, we were never able to get a typical B800−820 absorption spectrum from any LL LH2 fraction although mutiple α-polypeptides are present. If the LL LH2 complexes from *Rps. palustris* consist of a ring containing a mixture of different α-polypeptides, then in such a ring some B850 pairs would be H-bonded with the $\alpha Y_{+13}/\alpha W_{+14}$ residues while in other BChl pairs no such H-bonding interactions would occur due to the presence of FL sequences. Since these pigment−protein interactions modulate the H-bond environment this would lead to the presence of nonequivalent dimers in the rings. As these dimers further interact with each other, via excitonic interactions and BChl−BChl interactions, a range of different Q_y transitions between 820 and 870 nm would occur. This is exactly what we observe in our proteins at low temperature. Such a heterologous coupling between BChl *a* dimer systems possessing their first singlet excited state at different energy levels may induce the appearance of upper energy components which could at least partly account for the apparent increase in the 800-nm absorption band of the LL LH2 complexes (see Figure 2). Combined with the lack of any previous report of a simplified LH2 absorption spectrum, it would explain why heterologously expressed LH2 complexes containing only one type of α-polypeptide from *Rps. palustris* exhibit completely different properties than those observed in the parent strain (*25*). Conversely, in the HL preparation, where the mixed α-polypeptide population contains fewer $\alpha F_{+13}/\alpha L_{+14}$ residues, results in more defined Q_y electronic transitions, i.e., a classic B800−850 spectrum, albeit with some slight spectral contamination.

Our low-temperature fluorescence spectra appear fully consistent with this hypothesis. However, they also may be explained by a model where rings are homogeneous and very fast excitation transfer between rings takes place to the most red pigment. At very low temperature, in an excitation condition, a contribution of short wavelength fluorescence should be detected. One irresputable approach to identify whether the different Q_y electronic transitions are in the same protein ring would be to follow, at a femtosecond time scale, the spectral evolution of the bleaching induced by a femtosecond flash. Spectroscopic measurements have been carried out to measure the B800 → B850 transfer time in LL *Rps. palustris* (*24*). However, these authors have not yet obtained the spectral evolution of the bleached transition after the femtosecond flash.

We propose that the molecular strategy used by *Rps. palustris* for adapting to adverse light conditions drastically differs from that of the well-characterized *Rps. acidophila*. According to our hypothesis, under low-light conditions, if the α-polypeptides were synthesized form homogeneous pigment−protein rings, they will result in 820-nm-absorbing LH2 complexes. This is what is observed for the LL LH2 from *Rps. acidophila* 7750, where only one type of α-polypeptide is expressed. However, we do not observe any isolated B800−820 complexes in our *Rps. palustris* preparation adapted for low-light conditions. Therefore, as *Rps. palustris* produces multiple α-polypeptide species under low-light conditions, we suggest that this bacterium may produce rings where each ring consists of multiple α-polypeptide species.

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