Biochemical and Spectroscopic Characterization of the B800−850 Light-Harvesting Complex from *Rhodobacter sulphidophilus* and Its B800−830 Spectral Form†

James N. Sturgis,*,‡ Gesine Hagemann,§ Monier H. Tadros,§ and Bruno Robert‡

SBPM/DBCM CEA and URA 1290 CNRS, Centre d'etudes de Saclay, 91191 Gif sur Yvette, France, and Institut für Mikrobiologie, Albert-Ludwigs-Universität, Freiburg, Germany

Received April 5, 1995; Revised Manuscript Received June 2, 1995®

ABSTRACT: We demonstrate that the B800−830 spectral form of the B800−850 peripheral light-harvesting complex of *Rhodobacter sulphidophilus*, which is formed at low ionic strengths in the presence of the zwitterionic detergent LDAO, results from a local modification of the bacteriochlorophyll binding site and not the dissociation of the complex. This perturbation does not result in significant changes to the interactions between the pigments as studied by circular dichroism or fluorescence spectroscopy; however, modifications in the pigment binding sites are inferred from changes in the preresonance Raman spectrum. Specifically, an alteration of the hydrogen bonding of the 2-acetyl group of at least one of the bacteriochlorophyll groups that make up the 850 nm absorbing pair is observed. This implies an alteration in the conformation of the C-terminal domain of the α-polypeptide, in which are located the two tyrosyl residues that are believed to act as H-bond donors to these groups, induced by the protein-bound detergent in the absence of bound cations. We suggest that the ability of this complex to form an 800−830 complex is linked to the presence of an aspartyl residue immediately upstream of the tyrosyl residues. This study therefore provides a further illustration of the importance of hydrogen bonds to the 2-acetyl group of the bacteriochlorophyll in the determination of its spectral properties; furthermore, we provide a description of a conformational change that is able to modulate chromophore binding in these complexes.

In purple photosynthetic bacteria, light energy is gathered by an extensive system of light-harvesting (LH) 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, where the transduction into chemical potential energy takes place. In all purple photosynthetic 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 reaction center via the "core" antenna.

All 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 bacteriochlorophyll *a* (BChl) and carotenoid pigments. In core antennae, each polypeptide binds a single BChl 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 molecules is responsible for an absorption transition near 800 nm, while the other two form a dimer and are together responsible for the absorption transition near 850 nm. In both cases the lowest energy transition is considerably red shifted relative to that for free BChl where this transition is at about 770 nm. A number of these αβ polypeptide pairs are apparently arranged in a cyclic structure to form a native antenna complex (Ghosh *et al.*, 1993; Karrasch *et al.* 1995).

Recently, after more than 10 years of intense effort, the three-dimensional structure of an antenna complex has been solved by X-ray crystallography (McDermott *et al.*, 1995). This development promises to result in rapid advances in our understanding of the molecular mechanisms involved in precisely tuning the various functional properties of these complexes. In particular our understanding of the origin of the considerable red shift in the pigments mentioned above appears within grasp.

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, i.e., in determining their function. Currently there exist a number of examples of considerable blue shifts of the absorption maximum brought about by modifications to the antenna as the result of site-directed mutagenesis (Fowler *et al.*, 1992), detergent treatment (Miller *et al.* 1987), and salt treatment (Jirsakova & Reiss-Husson 1993). The spectral shift observed in the peripheral antenna complex of *Rhodobacter (Rb.) sulphidophilus* when exposed to the zwitterionic detergent LDAO in low ionic strength buffers (Doi *et al.*, 1991; Mäntele *et al.* 1991) has not to date been very extensively studied, and here we investigate its molecular origin. This reversible shift in the absorption maximum, in common with the previously described systems, is accompanied by a hypochromicity and broadening of the near-infrared absorption peak.

Two general mechanisms have been invoked to date in order to explain the large blue shifts in the BChl *a* absorption

† This work was supported by grants from Deutsche Forschungsgemeinschaft. J. S. was supported by the EEC (Human Capital and Mobility Programme).

‡ Centre d'etudes de Saclay.

§ Albert-Ludwigs-Universität.

[1] Abbreviations: BChl, bacteriochlorophyll *a*; CD, circular dichroism; LH, light harvesting; *Rb.*, *Rhodobacter.*

® Abstract published in *Advance ACS Abstracts,* August 1, 1995.

0006-2960/95/0434-10519$09.00/0

maximum of antenna complexes mentioned above. First, changes in the aggregation state of the antenna; these have now been demonstrated in a number of antenna complexes, namely, the LH1 complexes of *Rhodospirillum rubrum*, *Rb. sphaeroides*, *Rb. capsulatus*, *Rhodopseudomonas viridis*, and *Rhodopseudomonas marina* (Loach *et al.*, 1985; Miller *et al.*, 1987; Chang *et al.*, 1990b; Heller & Loach, 1990; Meckenstock *et al.*, 1992), in response to detergent treatment. This treatment results in the dissociation of the carotenoid. Changes in aggregation state are also responsible for the spectral shift of the LH1 complex of *Rubrivivax gelatinosus* in response to ammonium sulfate treatment (Jirsakova & Reiss-Husson, 1993); in this case the carotenoid, though dissociated by treatment, can be reincorporated during reconstitution (Jirsakova & Reiss-Husson, 1994). In all of these examples a large blue shift in the absorption maximum, typically more than 50 nm, accompanies a dissociation in to a spectrally dimeric subunit form. This dissociation also results in some alterations to the pigment binding sites in those examples that have been investigated (Chang *et al.*, 1990a; Visschers *et al.*, 1993; Sturgis & Robert, 1994; Jirsakova *et al.*, 1994).

Secondly, alterations of the residues in the BChl binding pocket by site-specific mutagenesis have been shown to cause blue shifts of up to about 20 nm (Fowler *et al.*, 1992). These mutations have been shown to result in, and the blue shift in the absorption has been ascribed to, the breaking of specific H-bonds to the 2-acetyl carbonyl group of the BChl molecule (Fowler *et al.*, 1994). More recently it has been shown that these changes mimic both the absorption and H-bonding differences observed between the naturally occurring B800−850 and B800−820 peripheral antenna complexes (Sturgis *et al.*, 1995). In these cases the modulation of the absorption is the direct result of changes in the hydrogen bonding of the BChl molecule, and thus due to local effects. We should mention that other mutations, more distant from the binding pocket than those mentioned above, have also been shown to cause large blue shifts, (Fowler *et al.*, 1993), although in these the mechanism is unclear; H-bonds to 2-acetyl groups may again be involved (Sockalingum *et al.* unpublished).

In this paper we attempt to answer various questions about the B800−830 form of the peripheral antenna complex from *Rhodobacter sulphidophilus* and provide a spectral characterization and a comparison with the B800−850 form. Of particular interest is the molecular origin of the 20-nm absorption shift observed in the presence of LDAO at low ionic strengths (Doi *et al.*, 1991). Specifically we determine whether this spectral change can be ascribed to a change in the aggregation state or to a local alteration of the binding site. A similar detergent- and salt-dependent spectral shift has been observed in the B800−850 complex of *Ectothiorhodospira* sp. (Ortiz de Zarate & Picorel, 1994), although in this case the spectral shift is less extensive, only 10 nm, and is not accompanied by the hypochromicity mentioned above. Previous investigations of the interconversion of the B800−850 and B800−830 forms of the LH2 isolated from *Rb. sulphidophilus* have demonstrated that this interconversion is strongly dependant on pH and the nature of the counterions present, the B800−850 form being stabilized by alkaline pH (the pK involved is near 8.5) and by the presence of cations, especially by certain polyvalent cations (M. Beck, M. Doi, M. Skatchkov, N. Gad'on, G. Drews, and W. Mäntele, manuscript in preparation).

MATERIALS AND METHODS

Culture of Bacteria and Preparation of Light-Harvesting Complexes. Rhodobacter sulphidophilus (strain W4) was grown anaerobically in the light as described previously (Doi *et al.*, 1991). The B800−850 light-harvesting complex was purified by sucrose density centrifugation and DEAE chromatography as previously described (Doi *et al.*, 1991); purified complexes were stored frozen until used.

Gel Filtration. Size determinations were performed on a calibrated gel-filtration column, either a low-pressure column (26 × 850 mm) packed with Toyoperl TSK resin (Merk, Darmstadt, Germany), developed at a flow rate of 1.0 mL/hour, or an HR10/30 FPLC column packed with Superose 6 (Pharmacia, Uppsala, Sweden) developed at a flow rate of 0.3 mL/min. The columns were calibrated with the following molecular weight standards (Pharmacia, Uppsala, Sweden): blue dextran (M_r 2 000 000), thyroglobulin (M_r 669 000), ferritin (M_r 440 000); catalase (M_r 232 000), aldolase (M_r 158 000), albumin (M_r 67 000), ovalbumin (M_r 43 000), chymotrypsinogen (M_r 25 000), and ribonuclease A (M_r 13 700). Standard curves were constructed by plotting log-(M_r) against retention time in the absence of detergent; this precaution was taken to avoid problems due to detergent binding to the standards. For analysis of the B800−830 form the column was equilibrated with 10 mM Tris, pH 7.0, and 0.05% LDAO containing 25 mM $CsCl_2$, while for analysis of the B800−850 form the column was equilibrated with the same buffer containing 50 mM NaCl in place of the $CsCl_2$. Proteins eluting from the column were detected by their absorption at 280 nm, and the spectral form of eluting antenna complexes was verified by absorption measurements.

Spectroscopic Methods. Absorption spectra were obtained with a Cary 05E UV−vis−IR spectrophotometer (Varian p.l.c., Australia). The machine was adjusted to ensure that the spectral band width remained below 1 nm throughout the region of interest.

Circular dichroism (CD) spectra were take on a Dichrograph III (I.S.A. France) spectrophotometer fitted with a near-IR-sensitive photomultiplier.

Fluorescence spectra were obtained with a SPEX Fluorolog spectrofluorimeter (I.S.A. France), fitted with a calibrated red-sensitive photomultiplier for the sample beam and a photodiode detector in the reference channel. This configuration allowed excitation spectra to be obtained in both the visible and the near-IR corrected for variations in excitation intensity.

FT preresonance Raman spectroscopy was performed as previously described (Mattioli *et al.*, 1991); for these spectra the samples were concentrated to an absorbance of 50−500 OD cm^{-1} in Centricon concentrators (Amicon). The spectral form of the concentrated sample was verified in a short path length cuvette. The spectra shown are the result of averaging 4000−20 000 interferograms. No evolution of the Raman signal was observed during the acquisitions.

RESULTS AND DISCUSSION

Absorption and Circular Dichroism *Spectroscopy.* CD has been extensively used to provide information on the bacterial light-harvesting complexes [e.g., Cogdell and Scheer (1985)], since it is a sensitive probe of the pigment−pigment

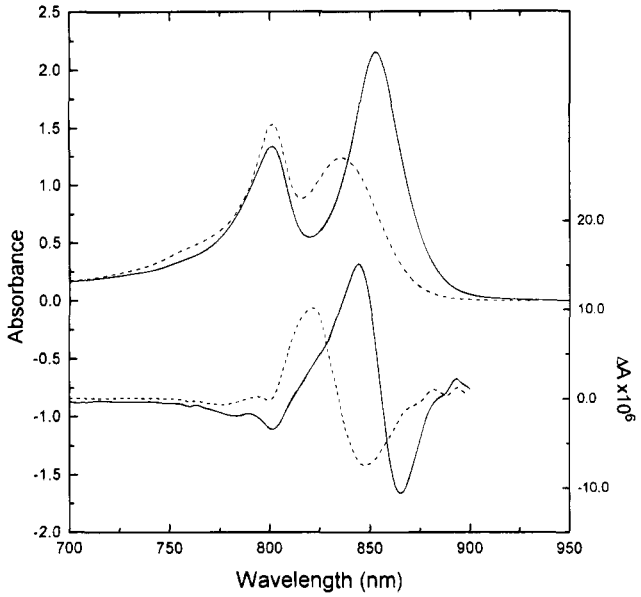

FIGURE 1: Room temperature absorption (above) and circular dichroism (below) spectra of the B800−850 (solid line) and B800−830 (dashed line) forms of the LH2 complex isolated from *Rb. sulphidophilus*. The same sample was used for both spectra, which were obtained in 1 cm path length cuvettes.

interactions in this system and thus of the precise geometrical arrangement of the various BChl molecules. Figure 1 shows the absorption and CD spectra of the B800−850 and B800−830 forms of the LH2 complex purified from *Rb. sulphidophilus*. The native LH2, in common with those isolated from other bacteria, shows a strong near conservative CD signal from the dimeric 850-nm absorbing pair of BChl molecules, with a trough at ~865 nm and a peak at ~844 nm. This strong CD signal is due to pigment−pigment interactions between the BChl molecules that form the oligomeric structure responsible for the 850-nm absorption. There is also a small negative signal from the 800 nm absorbing BChl; it is unclear whether this is due to pigment−protein interactions or to interactions between this chromophore and the other pigments in the complex.

It is clear from the CD spectrum that the blue-shifted B800−830 form of the complex largely maintains the interactions present in the native B800−850 form; notably, the CD signal remains conservative and moves with the absorption peak, giving a minimum at 848 nm and a maximum at 821 nm. The slight increase in the peak to trough separation from 21 nm in the native form to 27 nm in the B800−830 form is probably linked to the general spectral broadening, and is not due to any increase in the excitonic splitting. Since both the size and shape of the CD spectrum are extremely sensitive to alterations in the relationship between the chromophores, it seems unlikely that the formation of B800−830 results in any very large changes in the relationship between the BChl molecules responsible for the 850-nm absorption. It appears rather that the spectral shift is due either to small changes in the relationship between the pigments or changes in the intrinsic transition energies of the pigment molecules. Nevertheless, from the absorption and CD spectra of the B800−830 form it is clear that the peak at 834 nm is considerably broader than that of the B800−850 form at 854 nm. At least some of this effect can be ascribed to an increase in the heterogeneity of the B830 binding site. It is interesting to

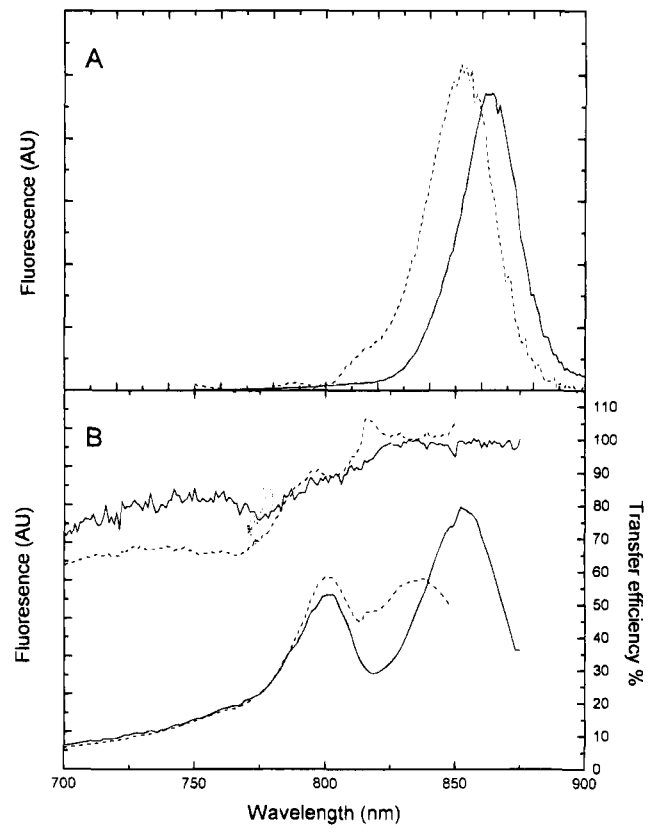

FIGURE 2: Room temperature fluorescence emission (A) and fluorescence excitation (B) spectra of the B800−850 (solid line) and B800−830 (dashed line) forms of the LH2 complex isolated from *Rb. sulphidophilus*. The excitation spectra also show the calculated energy transfer efficiency curves (left-hand scale). Sample concentrations were adjusted to give a maximum absorption of about 0.25 in order to avoid problems of fluorescence reabsorption. Emission spectra were measured with excitation at 600 nm, while excitation spectra were measured by monitoring emission at 860 and 890 nm for the B800−830 and B800−850 forms, respectively.

note that this broadened absorption band and reduced peak extinction coefficient appear to be widespread in complexes absorbing near 830 nm, being observed both in natural B800−820 complexes and in the various blue-shifted complexes mentioned above.

Also visible in the absorption spectrum is a small absorption increase near 770 nm upon conversion of B800−850 to the B800−830 form of the LH2 complex. This increase appears to reflect a destabilization of the complex in this spectral form and results in an increased susceptibility to denaturation and the release of free BChl and its oxidation products. In the absence of such denaturation the conversion between the two forms appears to be completely reversible.

Fluorescence Spectroscopy. Fluorescence spectroscopy can provide a sensitive probe of the functioning of native and modified light-harvesting complexes. Figure 2A shows the room temperature fluorescence emission spectra of the B800−830 and B800−850 forms of the LH2 complex excited at 600 nm. The B800−850 form shows a single emission peak at 863 nm, corresponding to a Stokes shift of about 10 nm; this is typical of what is observed for LH2 complexes isolated from other bacteria. In the B800−830 form two emission bands are clearly visible, one at 853 nm and the other near 812 nm. The increased Stokes shift, now about 20 nm, observed for the dimeric BChls can probably

Table 1: Size Determinations of the B800−830 and B800−850 Spectral Forms of the Peripheral Antenna Isolated from *Rhodobacter sulphidophilus*, Estimated by *Gel Filtration*[a]

spectral form	buffer	estimated size (M_r)	estimated size (Å)	eluted fraction λ_{Max} (nm)
B800−830	10mM Tris, pH 7.5, 0.05% LDAO, and 25 mM $CsCl_2$	195 000	50	835
B800−850	10mM Tris, pH 7.5, 0.05% LDAO, and 50 mM Na Cl	225 000	52	854

[a] Estimations were performed as described in Materials and Methods. The values shown are the average of three measurements on two columns.

be explained by the increased spectral heterogeneity mentioned above, though such effects would be expected to be more pronounced at low temperature. The increased emission from the 800 nm absorbing chromophore, here observed near 812 nm, can be explained as a result of thermal equilibration rather than being due to partially denatured complexes. This is reinforced by the observation that in samples with increased absorption at 770 nm an additional emission band near 795 nm is observed. At 300 K and 830 nm the Boltzmann constant multiplied by the temperature, k_BT, is equivalent to a spectral shift of about 15 nm. Population of the B800 excited state in the B800−830 form is expected to be considerable, about 5% of the excitations being located on the monomeric BChl; this percentage compares well with the degree of B800 emission.

Figure 2B shows the fluorescence excitation spectra and energy-transfer efficiencies calculated from the ratio of the fluorescence excitation and the (100% − % transmission) spectra, assuming a 100% efficiency at the red edge of the reddest absorption transition. These spectra show that the modified complexes remain fully capable of transferring energy between the various BChl molecules, and particularly that the transfer efficiency between the B800 BChl and the other BChls remains near unity. This is consistent with the thermal origin for the emission at 812 nm proposed above, since emission from functionally disconnected B800 BChl molecules would seriously compromise the transfer efficiency. The drop in the energy-transfer efficiency seen on the blue side of the absorption bands may be due to the accumulation of free pigment absorbing at about 770 nm in the absorption spectrum.

Size Determination by Gel Filtration. The sizes of the complexes as determined by gel filtration are shown in Table 1. This shows that the complex does not change its apparent relative molecular weight of about 200 000 when its near-IR absorption maximum is shifted from 850 to 830 nm. A relative molecular weight of 225 000, measured by gel filtration, corresponds to a Stokes radius of about 50 Å. The constancy of the hydrodynamic size observed here is in stark contrast to what has been observed for the B820 complexes derived from LH1 type antennae which appear much smaller than the native complexes when analyzed by this method. For example, the B820 subunit of *Rhodospirillum rubrum* has an apparent molecular weight as determined by gel filtration of about 40 000 compared to the ~200 000 obtained for the native B875 complex (Miller *et al.*, 1987). It should be emphasized that the position of the absorption maximum in the fractions eluting from the column was determined in order to ensure that there was no alteration in this property during the chromatography; see Table 1. From this result it can be safely concluded that there is no dissociation of the LH2 complex into smaller subunits accompanying the spectral shift, and in this respect the shift observed here, though dependant on detergent effects, differs from the detergent-induced spectral shifts observed in LH1 complexes that are caused by dissociation.

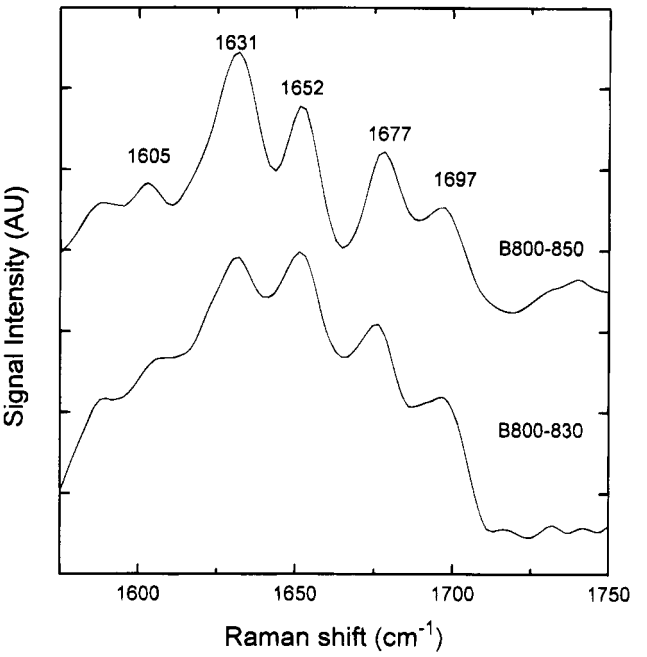

FIGURE 3: Fourier transform preresonance Raman spectra in the carbonyl stretching region of the B800−850 (above) and B800−830 (below) forms of the LH2 complex isolated from *Rb. sulphidophilus*.

Fourier Transform Preresonance Raman Spectroscopy. Figure 3 shows the high-frequency carbonyl stretching region (1575−1750 cm^{-1}) of the Fourier transform preresonance Raman spectra obtained from the B800−850, above, and B800−830, below, forms of the isolated LH2 from *Rb. sulphidophilus*. The spectra that we show here are broadly similar to the previously published Fourier transform preresonance Raman spectra of this complex (Mäntele *et al.*, 1991), except that a significant improvement in the signal to noise ratio permits a more precise interpretation. The bands visible in these spectra have been assigned to bacteriochlorin skeletal modes 1575−1620 cm^{-1}, and the stretching modes of the conjugated carbonyl substituents around the macrocycle, 1620−1750 cm^{-1} (Lutz 1984). The frequency of the bacteriochlorin skeletal vibration near 1605 cm^{-1} is sensitive to the coordination state of the central magnesium atom of the BChl molecule (Cotton & van Duyne, 1981), the position observed here is typical of the five coordinated BChls found in antenna complexes when measured at room temperature in the preresonance with the Q_y transition (Sturgis *et al.*, 1995). The frequency of the carbonyl vibrations is dependent on their chemical nature and their interactions with the environment. The 2-acetyl carbonyl group gives a peak at 1660 cm^{-1} when free from interactions, while the 9-keto group gives a peak near 1695 cm^{-1} when not involved in intramolecular interactions. The frequencies of these vibrators can be downshifted by hydrogen bonding to about 1620 and 1650 cm^{-1}, respectively (Robert & Lutz 1985).

The Raman spectrum of the B800−850 form of the LH2 complex in Figure 3 shows peaks due to carbonyl stretching modes at 1631, 1652, 1677, and 1697 cm^{-1}. These can be assigned, by comparison with our previous assignments for LH2 complexes from other species (Sturgis *et al.* 1995), as follows: the 1631-cm^{-1} band, to all three 2-acetyl group modes, one from the B800 BChl and two from the B850 BChls, all involved in strong hydrogen bonds; the bands at 1652 and 1677 cm^{-1}, to the hydrogen-bonded 9-keto groups of the two B850 BChl molecules; and the band at 1697 cm^{-1}, to the free keto group of the B800 BChl.

It is instructive to compare these spectra to those of B800−850 complexes from other species (Sturgis *et al.*, 1995). From such a comparison it is clear that the environments of the 9-keto groups are very similar to those observed in *Rb. sphaeroides*. In particular, the presence of a peak at 1650 cm^{-1} has previously only been observed in spectra of *Rb. sphaeroides* LH2. The peak due to the 2-acetyl groups is similar to that observed for the other B800−850 complexes studied, though slightly narrower than that observed in *Rb. sphaeroides* and less downshifted than that found for most other complexes (Sturgis *et al.* 1995).

On the basis of site-directed mutagenesis it has been possible to suggest the hydrogen bond donors responsible for binding the 2-acetyl groups of the 850 nm absorbing BChls (Fowler *et al.*, 1994). In *Rb. sulphidophilus* two tyrosine residues, Y_{+13} and Y_{+14} (numbered relative to the conserved histidyl residue) on the α-polypeptide, are found in the appropriate positions in the sequence (Tadros *et al.*, 1995); these are the same as are found in *Rb. sphaeroides* and are unlike the other B800−850 complexes studied. It is therefore interesting to note that the peak due to the 2-acetyl group stretching modes is much narrower in *Rb. sulphidophilus* than the similar peak in spectra of *Rb. sphaeroides* LH2. This difference in the spectra probably results from a reduced separation of the energies of the three carbonyl stretching modes that make up the peak at 1630 cm^{-1} in the spectrum of *Rb. sulphidophilus* LH2.

As mentioned above, the Raman bands derived from the 9-keto group stretching modes are very similar of those observed in *Rb. sphaeroides* LH2, and as such they differ from those seen in LH2 complexes derived from other bacteria. Recently a number of residues were suggested as possibly responsible for this difference between *Rb. sphaeroides* and the other strains studied (Sturgis *et al.* 1995). Specifically three amino acids in the α-polypeptide were suggested as possibly responsible for the band at 1650 cm^{-1} of *Rb. sphaeroides*, namely, S_{-4}, T_{+6}, and S_{+17}. Unfortunately in the *Rb. sulphidophilus* LH2 α polypeptide the residues present in these positions are all identical to those found in *Rb. sphaeroides*, so no refinement of the suggestion can be made on the basis of these results. However, in the light of the recently published structure (McDermott *et al.*, 1995) it seems possible that this feature may be linked to the serine residue S_{-4} present in the α polypeptide of both *Rb. sphaeroides* and *Rb. sulphidophilus*, which is expected to be close to the 9-keto group of the BChl bound to the β polypeptide.

The spectrum of the B800−830 form of the LH2 complex shown in Figure 3 shows a number of differences from that of the B800−850 form, despite the slightly poorer resolution. The differences between the two spectra can be largely ascribed to a loss of intensity from the 2-acetyl-associated band at 1631 cm^{-1} in the B800−850 form which is displaced and broadened to near 1660 cm^{-1} in the spectrum of B800−830 where it partially fills the trough near 1665 cm^{-1}. Such an alteration in the spectrum is diagnostic of the breaking of a hydrogen bond to one of the 2-acetyl groups associated with the B850-absorbing BChl molecules.

From these results it is clear that the spectral shift observed here, though dependant on detergent and ionic effects, is in many ways similar to the differences between native B800−850 and B800−820 type LH2 complexes and to that observed upon mutation of residue Y_{+13} by Fowler *et al.* (1994) in *Rb. sphaeroides* LH2. Specifically the spectral alteration of the B800−850 complex of *Rb. sulphidophilus* is due to a perturbation in the hydrogen-bonding environment of at least one and possibly both of the 2-acetyl groups of the dimeric BChl responsible for the long-wavelength absorption and not to a change in the aggregation state of the polypeptides. This confirms the importance of hydrogen bonds in tuning the specific spectral properties of this type of complex (Sturgis *et al.*, 1995). Structurally such an alteration in the hydrogen bonding would involve an alteration in the conformation of the C-terminus of the α polypeptide which provides the hydrogen bond donors probably in the form of the hydroxyl groups of the Y_{+13} and Y_{+14} residues (Fowler *et al.*, 1994; McDermott *et al.*, 1995).

In view of the important role played by cations in the formation of the B800−830 (M. Beck, M. Doi, M. Skatchkov, N. Gad'on, G. Drews, and W. Mäntele, Manuscript in preparation) it is interesting to note the presence of an aspartyl residue, D_{+12}, immediately before these amino acids in the primary structure of the α polypeptide (Tadros *et al.*, 1995), the residue in this position in the LH2 of *Rb. sphaeroides* is an alanine. This aspartyl residue is not found in the primary structures of the other LH2 α polypeptides published. It will be of interest to see whether the introduction of such a residue into the α polypeptide of *Rb. sphaeroides* will render it susceptible to a similar transformation and whether its replacement with a non-acidic residue in *Rb. sulphidophilus* will prevent the transformation. It will also be interesting to compare this system with that of *Ectothiorhodospira* (Ortiz de Zarate & Picorel, 1994). Beck *et al.* (M. Beck, M. Doi, M. Skatchkov, N. Gad'on, G. Drews, and W. Mäntele, Manuscript in preparation) noted that a pK_a of 8.6 played an important role in the formation of the B800−830 spectral form, it is therefore surprising and noteworthy that there are no basic residues in the C-terminal domains of either the α or the β polypeptide, and so it is unclear as to what, on a molecular level, this pK_a corresponds.

In conclusion, the LH2 of *Rb. sulphidophillus* provides another example of a peripheral antenna complex whose absorption spectrum is to be modulated by the degree of hydrogen bonding to the 2-acetyl carbonyl groups. This contrasts with the core antenna complexes in which the changes in the absorption spectrum characterized to date are due to changes in the aggregation state.

ACKNOWLEDGMENT

The authors would like to thank Françoise Reiss-Husson for assistance with the CD spectroscopy and Jobin-Yvon/SPEX for their help with the fluorescence measurements.

REFERENCES

Chang, M. C., Callahan, P. M., Parkes-Loach, P. S., Cotton, T. M., & Loach, P. (1990a) *Biochemistry 29*, 421−429.

Chang, M. C., Meyer, L., & Loach, P. A. (1990b) *Photochem. Photobiol. 52*, 873−881.

Cogdell, R. J., & Scheer, H. (1985) *Photochem. Photobiol. 42*, 669−689.

Cotton, T. M., & van Duyne, R. P. (1981) *J. Am. Chem. Soc. 102*, 6020−6026.

Doi, M., Shioi, Y., Gad'on, N., Golecki, J. R., & Drews, G. (1991) *Biochim. Biophys. Acta 1058*, 235−241.

Fowler, G. J. S., Visschers, R., Grieff, G. G., van Grondelle, R., & Hunter, C. N. (1992) *Nature 355*, 848−850.

Fowler, G. J. S., Crielaard, W., Visschers, R. W., van Grondelle, R., & Hunter, C. N. (1993) *Photochem. Photobiol. 57*, 2−5.

Fowler, G. J. S., Sockalingum, G. D., Robert, B., & Hunter, C. N. (1994) *Biochem. J. 299*, 695−700.

Ghosh, R., Hoenger, A., Hardmeyer, A., Mihailescu, D., Bachofen, R., Engel, A., & Rosenbusch, J. P. (1993) *J. Mol. Biol. 231*, 501−504.

Heller, B. A., & Loach, P. A. (1990) *Photochem. Photobiol. 51*, 621−627.

Jirsakova, V., & Reiss-Husson, F. (1993) *Biochim. Biophys. Acta 1183*, 301−308.

Jirsakova, V., & Reiss-Husson, F. (1994) *FEBS Lett. 353*, 151−154.

Jirsakova, V., Reiss-Husson, F., Robert, B., & Sturgis, J. (1994) *Liet. Fiz. Zurnalas 34*, 344−347.

Karrasch, S., Bullough, P. A., & Ghosh, R. (1995) *EMBO J. 14*, 631−638.

Loach, P. A., Parkes, P. S., Miller, J. F., Hinchigeri, S., & Callahan, P. M. (1985) in *Molecular Biology of the Photosynthetic Apparatus* (Antzen, C., Bogorad, L., Bonitz, S., & Steinback, K., Eds.) pp 197−209, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Lutz, M. (1984) in *Advances in IR and Raman Spectroscopy* (Clark, R. J. H., & Hester, R. E., Eds.) Vol 11, pp 211−300, John Wiley & Sons Inc., New York.

Mäntele, W., Sawatzki, J., Doi, M., Gad'on, N., & Drews, G. (1991) *Biochim. Biophys. Acta 1057*, 367−372.

Mattioli, T. A., Hoffman, A., Robert, B., Schrader, B., & Lutz, M. (1991) *Biochemistry 30*, 4648−4654.

McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., & Isaacs, N. W. (1995) *Nature 374*, 517−521.

Meckenstock, R. U., Brunisholz, R. A., & Zuber, H. (1992) *FEBS Lett. 311*, 128−134.

Miller, J. F., Hinchigeri, S. B., Parkes-Loach, P. S., Callahan, P. M., Sprinkle, J. R., Riccobono, J. R., & Loach, P. A. (1987) *Biochemistry 26*, 5055−5062.

Ortiz de Zarate, I., & Picorel, R. (1994) *Photosynth. Res. 41*, 339−347.

Sturgis, J. N., & Robert, B. (1994) *J. Mol. Biol. 238*, 445−454.

Sturgis, J. N., Jirsakova, V., Reiss-Husson, F., & Robert, B. (1995) *Biochemistry 34*, 517−523.

Tadros, M. H., Hagemann, G. E., Gad'on, N., Dierstein, R., & Schiltz, E. (1995) *FEBS Lett.* (submitted).

Visschers, R. W., van Grondelle, R., & Robert, B. (1993) *Biochim. Biophys. Acta 1183*, 369−373.

BI950772L