Functions of Conserved Tryptophan Residues of the Core Light-Harvesting Complex of *Rhodobacter sphaeroides*†

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Received October 8, 1996; Revised Manuscript Received December 23, 1996⊗

ABSTRACT: We have examined mutants in the core light-harvesting complex of *Rhodobacter sphaeroides* in which the tryptophan residues located at positions α_{+11}, β_{+6}, and β_{+9} have been mutated to each of the three other aromatic amino acids, namely tyrosine, phenylalanine, and histidine. We confirm that the α_{+11} residue and show that the β_{+9} residue each form a hydrogen bond to a C_2-acetyl group of a BChl molecule. Mutation of either of these residues to a phenylalanine results in a breakage of the normal hydrogen bond, whereas a histidine in either of these positions is able to form a hydrogen bond to the BChl. Comparison of the absorption spectra with the hydrogen bonding of the C_2-acetyl groups for the various mutants demonstrates a role for this molecular interaction in the tuning of the absorption properties of the complex. We further demonstrate that there is a consistent linear relationship between the downshift in the C_2-acetyl stretching mode and the red shift in the absorption maximum, in both core and peripheral antenna complexes. This linear relationship allows us to estimate the contribution of H bonding to the red shifts of these complexes. Though the residue β_{+6} is found not to be directly involved in interactions with the pigment molecules, mutation of this residue is shown in some cases to result in both a destabilization of the complex and a decrease in the binding site homogeneity. Finally, a consideration of the amount of antenna complex present in the various mutants shows an important role for the reaction center and/or the *pufX* gene product in the assembly or stabilization of this membrane protein.

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 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 "core" antenna (or LH1). In many bacteria, an additional light-harvesting system exists, the "peripheral" antenna (or LH2), which transfers excitation energy to the reaction center (RC) via the core antenna. All these antenna complexes have the same basic arrangement, being constructed from multimers of a heterodimeric subunit containing two polypeptides, α and β, to which are bound the bacteriochlorophyll (BChl) *a* and carotenoid pigments. In LH1, each polypeptide binds a single BChl molecule, and these interact with each other and the protein environment to give a lower-energy singlet absorption transition at ca. 880 nm. In LH2 complexes, three BChl *a* molecules are bound per $\alpha\beta$ polypeptide pair.

Recently, our comprehension of purple bacterial light-harvesting complexes has been greatly advanced by the publication of a number of three-dimensional crystal structures and two-dimensional projections. The LH2 complex of *Rhodopseudomonas* (*Rps.*) *acidophila* strain 10050 has been solved to atomic resolution (McDermott *et al.*,1995) and has been shown to consist of a ring of nine heterodimeric subunits. Within this annular structure, the α polypeptides form an internal protein ring while the β polypeptides form an external ring, and the B850 BChl molecules are situated between these two protein rings. A similar nonameric ring structure has been demonstrated for another LH2 complex, that of *Rhodovulum sulfidophilum* (Savage *et al.*, 1996). However, in the crystal structure determined for the LH2 of *Rhodospirillum* (*Rsp.*) *molischianum* (Koepke *et al.*, 1996), the heterodimeric subunits are arranged in an eight-membered ring.

To date, no atomic resolution structure for a core antenna complex has been determined; however, low-resolution projection structures have been determined from two-dimensional crystals of the LH1 from *Rhodospirillum rubrum* (Ghosh *et al.*,1993; Karrasch *et al.*, 1994). From these structures, it has been determined that the LH1 forms a larger ring than the LH2, containing 16 heterodimeric subunits. This ring is large enough to contain a reaction center which has been proposed to be located within the LH1 ring (Karasch *et al.*, 1994); recent investigations of two-dimensional crystals of core complexes, LH1 associated with RC, from *Rsp. rubrum* and *Rps. acidophila* confirm this location (R. Ghosh *et al.*, submitted for publication; A. Gall *et al.*, submitted for publication). Though there is no three-dimensional structure of an LH1 complex, some idea of the

† This work was supported in part by funding from the BBSRC, the Wellcome Foundation, and the Human Frontier Science Program. J.N.S. was supported in part by grants from the EEC and the Association Claude Bernard.

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⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1997.

[1] Abbreviations: *Rb.*, *Rhodobacter*; *Rsp.*, *Rhodospirillum*; *Rps.*, *Rhodopseudomonas*; LH, light-harvesting; RC, reaction center; BChl, bacteriochlorophyll.

S0006-2960(96)02524-X CCC: $14.00

α polypeptide MSKFYKIWMIFDPRRVFVAQGVFLFLLAVMIHFILLSTPSYNWLEISAAKYNRVAVAE

β polypeptide ADKSDLGYTGLTDEQAQELHSVYMSGLWPFSAVAIVAHLAVTIWRPWF

FIGURE 1: Amino acid sequences of the LH1 polypeptides from *Rb. sphaeroides*, aligned on the conserved histidine residues that act as a ligands to the BChl molecules. The residues mutated in this study are shown in bold, and residues conserved in the majority of LH1 complexes sequenced are underlined.

molecular arrangement of the BChl binding site should be possible from the recently published crystal structure of the *Rsp. molischianum* LH2 (Koepke *et al.*, 1996), since this LH2 has both sequence and BChl binding sites that are in a number of ways very similar to those usually associated with LH1 (Germeroth *et al.*, 1993).

Resonance Raman spectroscopy provides a powerful tool for examining the interactions of BChl molecules with their environments. In particular, this method yields information on the intensity of the H bonds between the BChls and their host protein. Indeed, protein−BChl H bonds may be currently studied only by vibrational spectroscopy, and in particular Raman spectroscopy. The role of these H bonds in tuning the physicochemical properties of the BChl molecules (such as electronic absorption and redox potential) may thus be precisely determined; this has been recently achieved by Mattioli *et al.* (1994) in bacterial reaction centers and in antenna proteins by Fowler *et al.* (1994), and Sturgis *et al.* (1995a,b). Recently, resonance Raman spectroscopy has been sucessfully used in conjunction with site-directed mutagenesis to determine some of the amino acids involved in the BChl binding sites of LH2 (Fowler *et al.*, 1994) and LH1 (Olsen *et al.*, 1994). For LH2, the conclusions drawn by Raman spectrosocopy have been entirely confirmed by the three-dimensional crystal structure of the *Rps. acidophila* complex. In the absence of a high-resolution structure for LH1, it may thus be hoped that Raman studies on mutant complexes will provide further insights in the BChl binding sites in these proteins.

In this paper, we have used resonance Raman spectroscopy for studying the molecular details of the BChl binding sites in LH1 proteins. The LH1 of *Rhodobacter sphaeroides*, and a large number of other LH1 complexes (Robert & Lutz, 1985; Sturgis & Robert, 1994; Jirsakova *et al.*, 1994; J. N. Sturgis, unpublished; A. Gall, unpublished), have very similar Raman spectra, which has been interpreted to indicate that the BChl molecules associated with the two polypeptide chains (α and β) are in very similar environments, and furthermore that the C_2-acetyl and C_9-keto groups are both involved in hydrogen-bonding interactions. We have previously shown that (Olsen *et al.*, 1994) the tryptophan found at position α_{+11} [in this paper, we use the conserved histidine residue as the reference point for the numbering residues, after Loach *et al.* (1994)] in the LH1 of *Rb. sphaeroides* was a part of the BChl binding pocket, where it formed a hydrogen bond to one BChl C_2-acetyl group within the basic heterodimeric subunit. We extend these previous studies by examining the effects of placing a histidine residue in this position and of altering two other tryptophan residues, which are conserved in the sequences of many LH1 antenna polypeptides [see Zuber and Cogdell (1995) for review], to each of the three other possible aromatic amino acids (phenylalanine, tyrosine, and histidine). In Figure 1, we show the sequences of the two polypeptides for the LH1 of *Rb. sphaeroides*, which we have used for the work reported here, with those residues that are conserved in the majority of other similar sequences underlined. The sequences shown are aligned on a conserved histidine residue, which acts as the ligand for the central magnesium atom of the BChl molecule. The three residues examined are thus the tryptophan at position α_{+11} in the α polypeptide and the tryptophans at positions β_{+6} and β_{+9} in the β polypeptide. Some of the mutations to these residues have an effect on the maximum absorption wavelength of the LH1 proteins. The correlation between this effect and the strength of the protein−BChl H bond, as determined by resonance Raman, together with that already published for LH2 antenna protein leads to a better understanding of how these H bonds modulate the absorption of antenna proteins of purple bacteria.

MATERIALS AND METHODS

Mutagenesis of the LH1 genes was conducted either as described by Kunkel (Kunkel, 1985; Kunkel *et al.*, 1987) or using the pALTER system (Promega) with oligonucleotides designed to change the residues βTrp$_{+6}$ and βTrp$_{+9}$ to Tyr, Phe, and His and αTrp$_{+11}$ to His by introducing the preferred codons for these residues.

Mutants were screened for by sequencing using the dideoxy termination method using the ABI Dyedeoxy system (ABI). Mutant RF M13 DNA and pALTERBX was digested with *Bam*HI and *Xba*I to release the LH1 genes as a 480 bp fragment which was cloned into the expression vectors pRKEK1 or pRKEH1 (Jones *et al.*, 1992). These plasmids were then used to transform *Escherichia coli* S17-1 cells (Simon *et al.*, 1983) for conjugative transfer of the genes into the LH2$^-$ LH1$^-$ RC$^-$ double-deletion strain DD13 (Jones *et al.*, 1992). Colonies harboring the mutant genes were grown chemoheterotrophically in the dark at 34 °C on M22+ agar plates in the presence of neomycin and tetracycline at 20 and 5 μg mL^{-1}, respectively. Screening for expression of the mutant genes was conducted directly on the colonies using a Guided Wave model 260 spectrophotometer (Guided Wave Inc.) with an agar plate holder. A selection of clones expressing the mutant light-harvesting genes were grown in liquid culture, on 10 mL of M22+ containing 0.1% casamino acids, in the dark at 180 rpm in a shaking incubator (antibiotics, temperature as before). Membranes were prepared as described by Olsen *et al.* (1994) and stored at −20 °C until use.

Room-temperature absorption spectra of the membrane samples were run on samples that had been diluted such that the maximum absorption of the LH1 complex was in the range 0.2−0.8 OD unit except in the cases of very poor levels of mutant LH1, under which circumstances the maximum absorption of any part of the spectrum was in the range specified above. The spectra were measured in quartz cells with a 1 cm path length using the Guided Wave model 260 spectrophotometer.

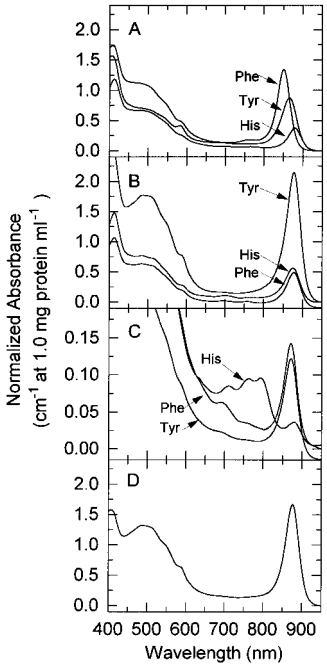

FIGURE 2: Room-temperature absorption spectra of wild type and mutant LH1 in membranes: (A) mutants at position α_{+11}, (B) mutants at position β_{+6}, (C) mutants at position β_{+9}, and (D) the wild type. In each group of spectra (A−C), the traces are labeled with the amino acid that replaces the tryptophan. In panels B and C, the lower traces have been displaced for clarity. The spectra shown have been corrected to be normalized to 1 mg/mL protein.

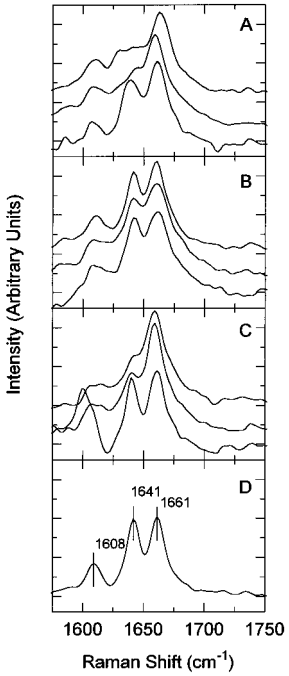

FIGURE 3: Carbonyl stretching region of FT preresonance Raman spectra of wild type and mutant LH1 in membranes: (A) mutants at position α_{+11}, (B) mutants at position β_{+6}, (C) mutants at position β_{+9}, and (D) the wild type. In each group of spectra (A−C), the upper trace is that of the mutant containing a tyrosine residue, the central trace that containing a phenylalanine residue, and the lower trace that containing a histidine residue. As mentioned in the text, the spectrum of the mutant containing histidine at position β_{+9} was obtained from membranes containing the reaction center in addition to LH1.

Protein concentrations in membrane preparations were measured using the Biorad DC Protein Assay (Lowry *et al*., 1951; Peterson, 1979).

Raman spectra, in preresonance with the Q_y transition, were recorded at 4 cm^{-1} resolution using a Bruker IFS 66 infrared spectrophotometer coupled to a Bruker FRA 106 Raman module equipped with a continuous Nd:YAG laser, essentially as described by Mattioli *et al.* (1993). All spectra were recorded at room temperature with back scattering geometry either from membrane pellets held in standard aluminum cups or from solutions in sapphire microcuvettes. The spectra shown were the result of 3000−30000 co-added interferograms; no evolution of the Raman signals was observed during data acquisition.

RESULTS

In Figure 2A, we show the room-temperature near-IR absorption spectra of membrane-associated mutant LH1 in which the tryptophan at α_{+11} has been altered; for reference, the spectrum of membranes containing the wild type antenna is shown in Figure 2D. As previously reported, the mutation of this tryptophan to a phenylalanine or a tyrosine results in a blue shift of the absorption peak by 24 and 12 nm, respectively. In contrast, the introduction of a histidine residue at this position results in a red shift in the Q_y absorption maximum of 3 nm, from 877 to 880 nm.

We show in Figure 3A the carbonyl stretching region of the preresonance Raman spectra obtained from these LH1 complexes with modifications at the α_{+11} position, and in Figure 3D the wild type antenna complex. In this region, in the wild type spectrum, the coordination sensitive methine bridge stretching mode can be observed at 1608 cm^{-1} and the peaks corresponding to the two carbonyl group stretching modes at 1641 and 1661 cm^{-1}. These last two peaks, at 1641 and 1661 cm^{-1}, have been assigned to the stretching modes of the hydrogen-bonded C_2-acetyl and C_9-keto groups of the BChl *a* molecules, respectively (Robert, 1985). In each case, the narrow peaks imply that the two different pigment molecules, associated with the α and β polypeptide, have remarkably similar hydrogen bonding environments. As has been previously reported (Olsen *et al.*, 1994), the replacement of the tryptophan residue at α_{+11} with a phenylalanine residue results in the breakage of a hydrogen bond to a C_2-acetyl group while its replacement with a tyrosine residue causes a strengthening of the hydrogen bond between the BChl *a* C_2-acetyl group and the protein. These changes to the BChl *a* environment are reflected in the Raman spectra by an upshift of part of the 1641 cm^{-1} peak to near 1656 cm^{-1}, where it merges with the 1661 cm^{-1} portion giving a resultant peak at 1658 cm^{-1}, in the phenylalanine-containing mutant and a downshift of part of the same peak to 1630 cm^{-1} in the tyrosine-containing mutant. As in the other mutants, the replacement of the tryptophan at α_{+11} with a histidine causes a change to the 1641 cm^{-1} peak, specifically a downshift of the peak to 1638 cm^{-1} associated with a broadening. We interpret this spectral change as the result of the C_2-acetyl peak splitting to give two bands, one centered at 1635 cm^{-1} and the other unperturbed at 1641 cm^{-1}, and believe that the component at 1635 cm^{-1} is due to a strengthening of the same hydrogen bond as that which is affected by the replacement of the α_{+11} residue with tyrosine or phenylalanine.

In the three cases we have studied, mutation of the residue at α_{+11} results in a reduction in the amount of LH1 complex

accumulated in the membranes, which is at least partially overcome when the mutated genes are coexpressed with the rest of the *puf* operon, which encodes the reaction center L and M subunits and the pufX protein.

Alterations to the β_{+6} residue result in only very minor changes in the absorption spectra, as shown in Figure 2B. From this figure, it is clear that the modification of the tryptophan residue normally present to tyrosine, phenylalanine, or histidine had very little effect on the position of the Q_y absorption maximum. However, each of the mutants studied, with the exception of the tyrosine β_{+6} mutant, had reduced levels of antenna complex. Again, this reduction could be at least partially overcome by coexpression with the reaction center.

Raman spectra of these mutants show only slight changes relative to the wild type spectrum (Figure 3B). These changes appear to be mainly associated with a broadening of the peak at 1661 cm^{-1} attributed to the C_9-keto stretching mode. This broadening, which is accompanied by an increase in the integrated intensity relative to that of the wild type, is particularly evident in the LH1 in which a histidine or phenylalanine is present at position β_{+6}. It seems probable that the spectral alterations we observe are due to a change in the environment of the C_9-keto groups, and more specifically a loss of order around the C_9-keto group. These spectral changes are not evident in the spectra of these LH1 obtained in the presence of reaction centers (not shown), probably indicating a restoration of order to the BChl binding site.

In Figure 2C, we show the room-temperature near-IR absorption spectra of the LH1 obtained from membranes containing mutant antennae in which the tryptophan at β_{+9} has been altered. As with the LH1 complexes in which the tryptophan at α_{+11} is altered, which we have discussed above, mutation of the tryptophan at β_{+9} results in significant shifts to the Q_y transition of the complex. Mutation of the normal tryptophan residue to a phenylalanine or a tyrosine results in a blue shift of the absorption peak by 6 and 5 nm, respectively. As found for the histidine α_{+11} mutant, the introduction of a histidine residue at this position results in a small red shift in the Q_y absorption maximum, in this instance of 2 nm, from 877 to 879 nm.

As previously, mutation of the residue at β_{+9} results in a reduction in the amount of LH1 complex accumulated in the membranes, and again, this reduction in the amount of antenna is at least partially overcome when the mutated genes are coexpressed with the rest of the *puf* operon. The reduction in the amount of complex accumulated in the membranes was particularly severe in the case of the mutant with a histidine residue in this position, as can be seen from the absorption spectrum. In this case, in addition to the absorption due to the assembled complex, there is considerable absorption due to other pigments at shorter wavelengths, between 650 and 800 nm; this absorption is believed to be due to BChl biosynthetic intermediates and breakdown products.

In Figure 3C, we show the preresonance Raman spectra of the series of mutants at the β_{+9} position. Due to the very low levels of LH1 accumulation in the mutant in which histidine replaces tryptophan when expressed without the reaction center, we show here spectra obtained from membranes that also included reaction centers. In this series of spectra, clear alterations are visible, reminiscent of those seen in Figure 3A where the α_{+11} residue was altered. In the spectrum from membranes in which the LH1 contains a phenylalanine residue at position β_{+9}, there is a decrease in the intensity of the 1641 cm^{-1} band and a concomitant increase in the intensity of the 1661 cm^{-1} peak. This difference is interpreted as being due to the splitting of the 1641 cm^{-1} band, as before, and the upshift of a part of this band to near 1660 cm^{-1}, a frequency expected for a C_2-acetyl group stretching mode when this group is not involved in a hydrogen bond. Therefore, it seems clear that in the wild type the tryptophan at β_{+9} is involved in a hydrogen bond with the C_2-acetyl group of one of the BChl *a* molecules within the heterodimeric minimal unit and that this hydrogen bond is broken when the tryptophan residue is replaced by a phenylalanine residue. In addition to the displacement of the C_2-acetyl stretching mode, a splitting of the band near 1608 cm^{-1} attributed to a methine bridge stretching mode is apparent, giving rise to two components with one upshifted to about 1615 cm^{-1}.

The Raman spectrum of membranes with antenna complexes containing a tyrosine residue at position β_{+9} is very similar to that described for those containing a phenylalanine residue. This is unlike the situation observed with the mutation of the α polypeptide where a tyrosine residue resulted in the downshift of a part of the peak at 1641 cm^{-1} as it was able to replace the tryptophan in forming the hydrogen bond. There is again a displacement of a part of the peak originally at 1641 cm^{-1}, though in this case to near 1638 cm^{-1}, and a splitting of the peak at 1608 cm^{-1}.

When the residue at position β_{+9} is a histidine, there is very little difference from the FT-Raman spectra of the wild type except for the possible appearance of a shoulder near 1638 cm^{-1} on the band at 1641 cm^{-1}. This may again represent a splitting of the 1641 cm^{-1} band with the downshift of one part of it to about 1638 cm^{-1}, though such an attribution is at the limit of the resolution and signal to noise ratio of this spectrum. This would therefore suggest that, as with the α_{+11} position, a histidine at position β_{+9} is able to replace the normal tryptophan and form a normal strength, or possibly slightly stronger, hydrogen bond. The differences apparent in the lower frequencies (near 1600 cm^{-1}) shown are due to the presence of reaction centers.

DISCUSSION

Tryptophans at α_{+11} and β_{+9} Each Bind a C_2-Acetyl Carbonyl of the 880 nm-Absorbing BChl Pair. Our results provide evidence of the nature of the hydrogen bond donors to each of the BChl C_2-acetyl groups in the LH1 heterodimeric subunit. One BChl is hydrogen bonded by the tryptophan at position α_{+11} in the α polypeptide, in agreement with a previous paper (Olsen *et al.*, 1994), the other by the tryptophan at position β_{+9}, on the β polypeptide. Substitution of either of these residues with a histidine residue results in the assembly of a complex in which the BChl Q_y absorption maximum is slightly red shifted and one hydrogen bond to a C_2-acetyl group is most probably strengthened. Replacement of either tryptophan by a phenylalanine results in a complex with a blue-shifted absorption maximum and only one of the normal two C_2-acetyl hydrogen bonds present. In the case of substitution with tyrosine, the effects appear to be different depending on whether the site can accommodate a hydrogen bond from a η-hydroxyl in place of the normal ϵ-amino group or not. Thus, the geometric constraints for hydrogen bond formation seem to be more severe for the

residue at the β_{+9} position, and as a result, tyrosine is unable to form a hydrogen bond. In both the β_{+9} and α_{+11} positions, introduction of a benzene ring appears to be difficult and to result in a certain amount of structural rearrangement. In the β_{+9} position, this rearrangement results in a splitting of the band at 1608 cm^{-1}, while at the α_{+11} position, as described previously (Olsen *et al.*, 1994), it probably causes a rearrangement of the BChls within the site (R. Frese, J. D. Olsen, C. N. Hunter, and R. van Grondelle, unpublished results). The frequency shift of the 1608 cm^{-1} methine bridge stretching mode probably reflects a small movement of the central magnesium out of the bacteriochlorin plane and a consequent change in the core size (A. Näveke *et al.*, submitted for publication). Such a structural alteration, which is presumably induced by an increase in the distance between one BChl and the polypeptide, might also be expected to alter the position of the Q_x transition, though the position of the Q_y transition seems relatively insensitive to such deformations, at least as judged by the sensitivity of these transitions to coordination-induced core size changes (Evans & Katz, 1975).

The residue at position β_{+9} has previously attracted attention as it is altered in a number of suppressor mutants of the *pufX* deletion strain of *Rb. sphaeroides* in which it was converted into an arginine residue (Barz & Oesterhelt, 1994). Though the mutant with arginine at position β_{+9} did not apparently accumulate intact antenna complexes, a related mutant with a glycine residue at this position accumulated blue-shifted complexes, results which appear entirely consistent with our own. The presence of a blue-shifted complex in this mutant is consistent with the role of the tyrosine residue in BChl binding and the effect of H bonds on the BChl spectrum discussed below.

As mentioned above, the recent publication of a three-dimensional structure for the LH2 of *Rps. molischianum* (Koepke *et al.*, 1996) allows us to compare our results concerning the BChl *a* binding site of the *Rb. sphaeroides* LH1 with the structure of the binding site in the *Rps. molischianum* LH2 which is expected to be very similar (Germeroth *et al.*, 1993). For the purposes of this comparison, it should perhaps be remarked that within the crystals the C-terminal region of the polypeptides, in particular the α polypeptide, are involved in a certain number of crystallographic interactions, and a number of differences are to be expected between LH1 of *Rb. sphaeroides* and the LH2 of *Rsp. molischianum*, due in particular to the differing sizes of the two complexes. From the published structure, it is clear that the tryptophan residues at positions α_{+11} and β_{+9} are well placed to form hydrogen bonds with the C_2-acetyl groups of the BChls, and indeed, this possibility is invoked. The H-bonding pattern in this region of the LH1 complex could involve bonds from the tryptophan at α_{+11} to the BChl liganded to the α polypeptide and from the tryptophan at β_{+9} to the Bchl liganded by the β polypeptide. The tryptophan at β_{+6} is located at the base of the antenna ring in a position to interact either with the membrane or with the neighboring subunits of the oligomer. This residue is located at the beginning of a bend in the C-terminal region of the β polypeptide that allows the tryptophan at position β_{+9} to approach the BChl C_2-acetyl group. It thus appears that our present study confirms that of Germeroth *et al.* (1993), which concluded that the Bchl a binding sites in LH2 from *Rsp. molischianum* are closely related to those of LH1 complexes.

The recent paper of Sauer *et al.* (1996) provides a different interpretation of a Raman spectrum from the 850 nm-absorbing LH2 of *Rsp. molischianum*, which has previously been shown to be very similar to that of LH1s (Germeroth *et al.*, 1993). These authors interpret their spectrum, which contains two bands in the carbonyl stretching region, at 1641 and 1665 cm^{-1}, as indicating that only one C_2-acetyl group is hydrogen bonded, the other being free from interactions. This interpretation appears to be at odds with the interpretation of the structural data (Koepke *et al.*, 1996) and does not account for any contributions from the C_9-keto groups of the Bchl, which are expected to be present in these spectra. For this reason, Germeroth *et al.* (1993) concluded that each of these bands was degenerate, accounting respectively for the stretching modes of both C_2 -acetyl and both C_9-keto groups. Moreover, for LH1 complexes, it has been demonstrated here and elsewhere (Olsen *et al.*, 1994; J. D. Olsen *et al.*, manuscript in preparation) that each of these bands is indeed degenerate, since in each case one H bond can be selectively removed by mutagenesis. As our data show that the binding sites of LH1 and of LH2 from *Rsp. molischianum* are indeed related, we propose that each of the bands observed by Sauer *et al.* (1996) actually is degenerate and thus accounts for the same modes as those observed in LH1 Raman spectra. Moreover, the disappearance of the 1640 cm^{-1} band in 820 nm-absorbing complexes would be consistent with the breakage of two C_2-acetyl hydrogen bonds upon going from the 850 to the 820 nm form of LH2 in *Rsp. molischianum*, as in other systems investigated (Fowler *et al.*, 1994; Sturgis *et al.*, 1995a).

LH1 Protein Stability in Mutant Complexes, with and without Coexpression of Reaction Centers. In all of the mutants investigated, with the notable exception of the tyrosine β_{+6} mutant, there was an appreciable decline in the amount of LH1 complex accumulated in the membranes. In all cases, this reduction was at least partially reversed by coexpression of the *pufBA* gene pair with the rest of the *puf* operon, which resulted in the accumulation of core complexes rather than LH1. In view of the published structure of LH1, which is a large ring of heterodimer subunits (Ghosh *et al.*, 1993; Karrasch *et al.*, 1995), and of the core complex structure, where the same ring contains a reaction center (R. Ghosh *et al.*, submitted for publication; A. Gall *et al.*, submitted for publication), it is possible to appreciate how the other components of the core complex could aid in the assembly and stabilization, and hence accumulation, of the core complex by acting as templates or scaffolds for the structure. This presumed templating function, and its effect on complex stabilization, is visible in the mutants in which the β_{+6} residue was changed. In these mutants, it may be responsible for the narrowing of the broad 1661 cm^{-1} peak in the Raman spectra, when the LH1 genes are coexpressed with the rest of the *puf* operon. We hypothesise that in these mutants the absence of a tryptophan in this turn region results in a certain flexibility that is transmitted to the BChl and its environment. However, in the presence of the other core components, the flexibility of the LH1 complex is much reduced and this ensures both a greater stability for the LH1 complex and a reduction in the disturbance of the pigment binding site. In *Rb. sphaeroides*, the region of the *puf* operon downstream of the LH1 genes contains three open reading

frames, the reaction center L and M subunit genes and the *pufX* gene of unknown function. It is unclear as yet whether the stabilizing and templating is due to the reaction centers, the *pufX* gene product, or both. However, the recent work of McGlynn *et al.* (1996) suggests that the *pufX* gene product does not act as a stabilizing element.

In this work, we have replaced three different conserved tryptophan residues with each of the three other aromatic amino acids; this allows us some insight into the relative importance of different interactions in the stability of these proteins. It is clear that, for the hydrogen bonding between the protein and the BChl, histidine represents a much more conservative replacement than phenylalanine or tyrosine, in both the α_{+11} and β_{+9} positions. This must clearly be related to the presence in both histidine and tryptophan of an aromatic ϵ-amino group hydrogen bond donor. Surprisingly, tyrosine is not a good substitute for this role; in one case, at the β_{+9} position, it fails to form a hydrogen bond, and in the other, at the α_{+11} position, it does so only after perturbation of the binding site (Olsen *et al.*, 1994). Presumably, this is related both to the much larger distance from the peptide backbone to the hydrogen bond donor, which is a. 6.5 Å as opposed to ca. 4.5 Å in tryptophan, and to the presence of an alcohol donor as opposed to an amino group donor. Conversely, the introduction of a tyrosine at the exterior β_{+6} position appears to be much more conservative than either phenylalanine or histidine, at least as far as allowing accumulation of normal levels of complex within the membrane is concerned. However, the precise role, or roles, of this residue is not yet known; an analysis of the position of the equivalent residue in the structure of *Rsp. molischianum* LH2 (Koepke *et al.* 1996) may shed some light on this.

H Bonding and Antenna Protein Absorption. It has been proposed for LH2 complexes that a certain proportion of the red shift observed in the BChl *a* Q_y transition, relative to isolated BChl *a*, is due to the hydrogen bonding of the C_2-acetyl group (Fowler *et al.*, 1994; Sturgis *et al.*, 1995a,b; Sauer *et al.*, 1996; J. N. Sturgis and B. Robert, submitted for publication). Our examination in this paper of a number of mutants in which the hydrogen bond environment of the BChl molecules has been altered allows a further estimation of the sensitivity of the LH1 absorption spectrum to the hydrogen bonding of the BChl *a* C_2-acetyl group. In the comparisons below, we compare Q_y absorption shifts, in nanometers, with the average C_2-acetyl stretching mode displacements, in inverse centimeters. It is unclear, in the absence of a precise understanding of the physical origin of this relationship, whether this is indeed the best comparison or whether some other relationship is more appropriate. In Figure 4, we show the correlation between the Q_y absorption maximum and the average stretching mode displacement of the C_2-acetyl vibrators from 1660 cm^{-1}. As can be seen, if we exclude the mutants containing phenylalanine or tyrosine at position α_{+11} which we discuss below, there is a good correlation for the LH1 complexes examined here ($R = 0.99$), and a sensitivity of the absorption spectrum of approximately 0.80 nm cm^{-1}. We also show data for LH2 complexes, both native (Sturgis *et al.*, 1995a) and mutant (Fowler *et al.*, 1992, 1994) complexes which were fitted with a single line ($R = 0.99$), and the sensitivity was determined to be 0.88 nm cm^{-1}. The LH1 mutants containing phenylalanine or tyrosine at position α_{+11} and previously described by Olsen *et al.* (1994)

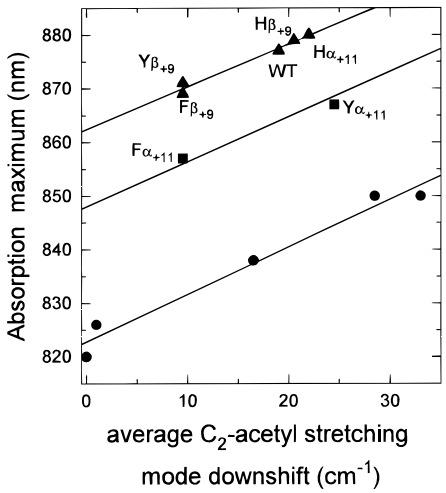

FIGURE 4: Relationship between the Q_y absorption maximum and the downshift of the C_2-acetyl stretching modes. We plot here the position of the room-temperature near-infrared absorption maximum and the total downshift observed for the two C_2-acetyl vibrators from the noninteracting frequency of 1660 cm^{-1}. The triangles relate to LH1 mutants discussed here and are labeled to indicate the mutant; the circles show data for LH2 complexes taken from Sturgis *et al.* (1995a) and Fowler *et al.* (1994). The lines show linear regression fits to the LH2 data and the LH1 data excluding the two points (squares), $Y\alpha_{+11}$ and $F\alpha_{+11}$.

appear to fall on a separate line with a slope similar to the other two. In this regard, it is interesting to note that a rearrangement of the binding site has been inferred in these mutants (Olsen *et al.*, 1994). These results indicate that in a series of homologous complexes there is a linear correlation between the strength of the hydrogen bond to the acetyl group, as reflected by the Raman frequency of the stretching mode of this group, and the position of the long wavelength absorption band; however, the physical origin of this correlation remains uncertain. Nevertheless, the values for the sensitivity, 0.80 and 0.88 nm cm^{-1}, correspond reasonably closely; this observation extends the generality of the direct link between the strength of hydrogen bond interactions with the C_2-acetyl group of the BChl and the absorption spectrum to a different, though undoubtedly similar, system (Sturgis *et al.*, 1995a,b; Fowler *et al.*, 1994). The observation of similar sensitivities in complexes with different structures, and in particular different degrees of pigment coupling, provides additional evidence for the effect being mediated through alterations in the absorption spectrum of the BChl monomer (J. N. Sturgis and B. Robert, in press). The different intercepts of the two lines, 822 nm for the LH2 complexes and 865 nm for the LH1 complexes, imply that there are other important factors governing the position of the Q_y absorption maximum, notably the coupling between pigments.

In conclusion, in this paper, we have identified the two amino acids that are responsible in LH1 complexes for binding the C_2-acetyl groups of the Bchl molecules. We have further demonstrated a role for these residues in tuning the Q_y absorption maxima of these complexes. Mutation of a tryptophan located away from the pigment binding site has been shown to cause a loss of binding site integrity and a destabilization of the complex. Consideration of the LH1 levels of the various mutants shows an important role for the reaction center and/or the *pufX* gene product in stabilizing the LH1 complex in the membrane.

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BI962524A