Influence of the Protein Binding Site on the Absorption Properties of the Monomeric Bacteriochlorophyll in *Rhodobacter sphaeroides* LH2 Complex†

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ABSTRACT: Resonance Raman spectroscopy was performed on peripheral light-harvesting proteins from *Rhodobacter sphaeroides* in which the residue βArg_{-10} has been modified by site-selected mutagenesis. We show that this residue is indeed involved (as proposed by X-ray crystallographic studies on the LH2 complex from *Rhodopseudomonas acidophila*), in an H-bond with the acetyl carbonyl of the 800 nm-absorbing BChl in these proteins (B800), and that the presence of such an H-bond induces a ca. 10 nm red shift of the lowest energy transition (Q_y) of this molecule. Moreover, other parameters involved in the fine tuning of the absorption of the B800 molecules may be determined from our experiments, and we propose that the local electromagnetic properties of the B800 binding site may induce an additional 10 nm red shift of this transition. These results constitute the first experimental evidence for the parameters able to modify *in vivo* the absorption of "monomeric" BChl molecules, i.e. BChl not involved in strong excitonic interactions, and will be of great help for understanding the absorption properties of such pigments in other light-harvesting systems.

In purple photosynthetic bacteria, light energy is gathered by specialized proteins, namely light-harvesting (LH)[1] pigment−protein complexes. The spectral properties of these proteins 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 (*1*). Usually in purple bacteria this is performed by two different types of light-harvesting proteins, the so-called core (or LH1) complex, which is tightly connected to the reaction center, and the peripheral (or LH2) complex, which transfers light energy to the reaction center *via* the core complexes. In these organisms, LH proteins are made of an assembly of multimers of a minimal unit containing two short transmembrane polypeptides, α and *β*. In LH2 proteins, each of these polypeptides binds a BChl *a* molecule through a histidine residue located in their transmembrane domain, and a third BChl *a* is located near the surface of the intracytoplasmic membrane, principally by the α polypeptide (*2*). The latter molecule absorbs at *ca.* 800 nm and most often exhibits spectroscopic properties typical from a monomeric BChl, i.e. a molecule not involved in strong excitonic interactions (*3*).

Understanding the parameters governing the absorption of the light-harvesting complexes, and thus their function, requires that the relative roles of BChl−protein and BChl−BChl interactions in tuning the absorption of the different BChls be determined. The first evidence that protein−BChl interactions could play a role in modifying the position of the electronic transition of the LH-bound BChl came from the comparison of the sequences of the α polypeptides from 850 and 820 nm absorbing LH2. In 850 nm absorbing LH2, it was noticed that the primary sequence of the α polypeptide at the positions 43 and 44 (that is, at +13 and +14 with respect to the BChl850 ligand His_0) was generally Tyr−Trp or Tyr−Tyr and that the protein sequence was changed in 820 nm absorbing proteins to Phe−Leu (*4*). Replacement of either αTyr_{+13} or αTyr_{+14} with a phenylalanine and a leucine, respectively, in the LH2 from *Rhodobacter sphaeroides* produced the first experimental evidence about the role of these amino acids in tuning the absorption of these complexes. Indeed, each of these mutations induced a *ca.* 10 nm blue shift in the 850 nm absorption transition, and double mutants were shown to exhibit absorption properties close to those observed in LH2 complexes naturally absorbing at 820 nm, such as those of *Rhodopseudomonas acidophila* (*5*). Resonance Raman studies of these mutants showed that each of these mutations induced the breakage of an H-bond between the protein and the C_2 acetyl of one or other of the two BChls responsible for the 850 nm absorption (*6*). It was thus proposed that (i) each of these tyrosines was in the proteic binding site of the 850 nm absorbing BChl *a* molecules and (ii) that the breakage of these tyrosine−BChl *a* H-bonds could be the direct cause of the absorption changes observed in the engineered mutants. The tyrosine−BChl interactions has since been confirmed by the 3-D structure of these proteins derived from X-ray crystallography (*2*), and the direct role in these H-bonds in tuning the absorption of LH2 complexes has since been documented in a number of cases (*7*, *8*). More recently,

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[1] Abbreviations: 3-D, three-dimensional; B(Chl), bacterio(chlorophyll); cm^{-1}, wavenumbers; FT, Fourier transform; LH1, core light-harvesting protein of purple bacteria; LH2, peripheral light-harvesting protein of purple bacteria; *Rb.*, *Rhodobacter*; *Rsp.*, *Rhodospirillum*; *Rps.*, *Rhodopseudomonas*; RR, resonance Raman; WT, wild-type.

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from rather simple calculations, it was further proposed that these H-bonds should directly influence the absorption of the BChl monomers, which would in turn result in a shift of the absorption resulting from interactions of the excitonically coupled BChl *a* molecules (*9*). Finally, it has been shown that the two residues αTrp_{+13} and βTrp_{+9} in the LH1 complex play an analogous role as H-bond donors to the C_2 acetyl of LH1 and that mutagenesis of each of these residues to Phe results in the loss of H-bonds and produces attendant blue shifts in the Q_y absorbance maximum (*10, 11*).

However, there has not been any formal experimental proof up to now that modifying the H-bond interaction pattern of protein-bound monomeric BChl *a* could actually influence its absorption properties. By contrast, in most cases where the absorption of such monomers has been considered, i.e. in the bacterial reaction centers and in the Fenna−Matthews−Olson protein, the *ca.* 800 nm position of the lowest electronic transition of these monomers has mainly been interpreted both in terms of weak excitonic interactions with the other pigments bound to these proteins, and in terms of BChl conformation (see e.g. ref 12). Although there are noticeable exceptions, such as *Rhodospirillum (Rs.) molischianum,* the LH2 complexes from purple bacteria have a conserved arginine residue at position β_{-10} which has been shown to closely interact with the 2-acetyl carbonyl of the 800 nm absorbing BChl (*2, 13*). This residue was mutated to Glu, and it was shown that this alteration produced a blue shift in the B800 absorbance maximum while leaving the B850 BChls unaffected (*14, 15*). More recently, this residue was mutated to a series of other amino acids, and a blue shift of this 800 nm transition was observed (*16*). In this work, we show that this absorption shift correlates with the presence of an H-bond involving this C_2 acetyl group, this constitutes the first experimental evidence that the presence of such H-bonds modulates the absorption of monomeric BChl in photosynthetic complexes. Moreover, and following on from the work by Fowler *et al.* (*16*), we show that the absorption of BChl *a* monomers is also sensitive to the properties of its proteic environment, such as the local dielectric constant and the refraction index, and we estimate to which extent these parameters may play a role in additionally tuning the absorption of a BChl monomer.

EXPERIMENTAL PROCEDURES

A series of βArg_{-10} mutants was constructed, based on the protocol described by Fowler *et al.* (*5, 16*). To verify that the site-directed alterations had been successfully introduced, plasmid-borne DNA was recovered from the various mutant *Rb. sphaeroides* strains and re-sequenced.

Semi-aerobically, dark-grown cells from each *Rb. sphaeroides* strain containing a mutant LH2 were harvested, and their membranes prepared essentially using the protocol described by Cogdell *et al.* (*17*). Membranes were then further isolated from linear sucrose gradients (0.2−1.2 M sucrose, 20 mM Tris·HCl, pH 8.0) after centrifugation.

Absorption spectra were recorded using 1 cm path length quartz cells, and a Guided Wave model 260 spectrophotometer (Guided Wave Inc., 1590 Golden Foothill Parkway, El Dorado Hills, CA 95630). 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

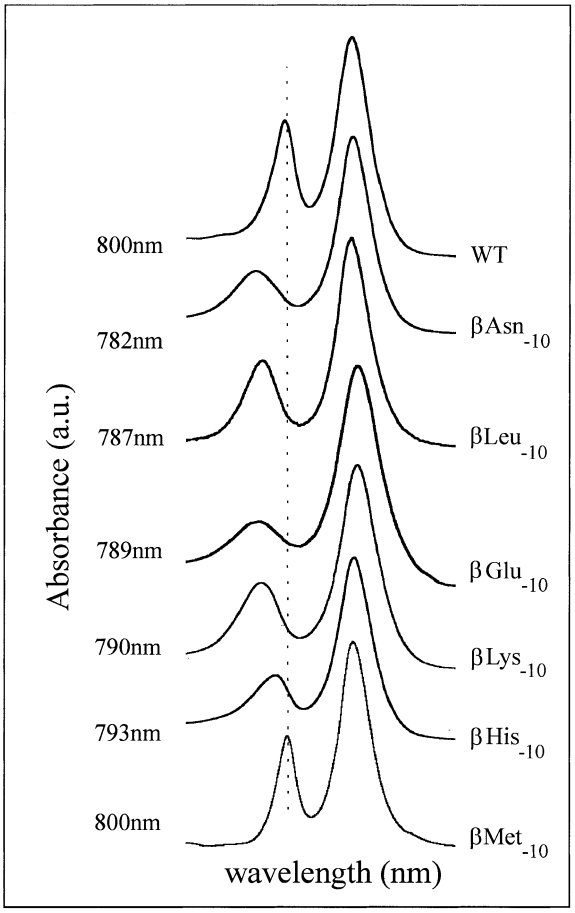

FIGURE 1: Near-infrared 77 K absorbance spectra of membranes of LH2 complexes containing site-directed mutations at the βArg_{-10} residue (700−900 nm). The absorbance maximum for each "B800" is given above each spectrum.

continuous Nd:YAG laser, as described by Mattioli *et al.* (*18*). All spectra were recorded at room temperature with back-scattering geometry from membrane pellets held in standard aluminum cups. The spectra shown were the result of 3000−30 000 co-added interferograms, and no evolution of the Raman signals was observed during data acquisition. Ultraviolet (363.8 nm excitation, provided by a Coherent Innova 100 Ar laser) resonance Raman spectra were recorded with a Jobin Yvon U1000 spectrometer equipped with a UV-coated CCD camera (Jobin Yvon Spectraview 2D). For these experiments, spectra were recorded with a 90° geometry from samples maintained at 77 K in a flow cryostat cooled with liquid nitrogen (TBT, Vitry sur Seine), as described in Ruban *et al.* (*19*).

RESULTS

The near-infrared region of the absorption spectra of the different β_{-10} mutants are displayed in Figure 1. As already described (*16*), in most of these mutants (namely, β_{-10}-His, Lys, Glu, and Leu) the lower energy transition from the monomeric BChl *a* molecule is blue shifted by *ca.* 10 nm (7−13 nm). When an asparagine residue is inserted at position β_{-10} this Q_y absorption transition is further shifted toward the blue (782 nm). Inserting a methionine at this position leads to mutated LH2 complexes exhibiting absorption properties very similar to those of WT LH2 complexes (*16*).

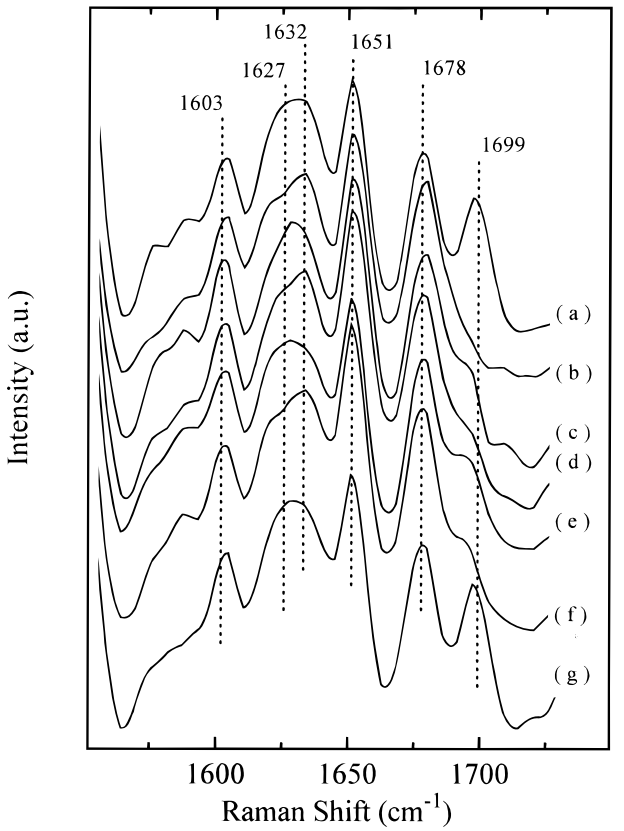

FIGURE 2: FT-Raman spectra of LH2 proteins; 1064 nm excitation, room temperature (1550−1750 cm^{-1}): (a) WT; (b) βAsn_{-10}; (c) βLeu_{-10}; (d) βGlu_{-10}; (e) βLys_{-10}; (f) βHis_{-10}; and (g) βMet_{-10}.

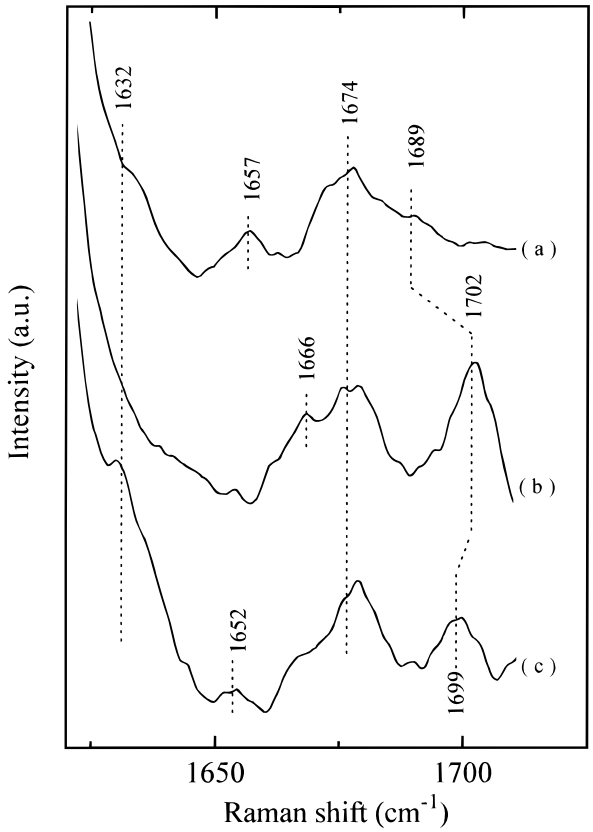

FIGURE 3: Resonance Raman of LH2 proteins; 363.8 nm excitation; 77 K (1620−1715 cm^{-1}): (a) β_{-10}-Asn; (b) β_{-10}-His; and (c) WT.

Figure 2 displays room temperature FT-Raman spectra in the higher frequency region of the different β_{-10} mutants. In these spectra, the bands contributing between 1620 and 1710 cm^{-1} arise from the carbonyl stretching modes of the C_2 acetyl and C_9 keto carbonyl groups of the different BChl molecules bound to the LH2 complexes. In the WT spectrum (Figure 2a), the BChl molecules responsible for the 850 nm absorption of these complexes contribute at 1627, 1632, 1651, and 1678 cm^{-1} (*6*, *8*), while the carbonyl stretching modes of the 800 nm absorbing BChl molecule are observed at *ca*. 1626 and 1699 cm^{-1} (*6*, *20*). In the FT-Raman spectra of all the β_{-10} mutants exhibiting absorption properties different from those of the WT (i.e. all the mutants but βMet_{-10}) the 1699 cm^{-1} band is much weaker than in the WT. Moreover, upon deconvolution of the spectra, the 1699 cm^{-1} band seems slightly shifted toward the lower frequencies in the β_{-10}-Glu, Leu, and Lys, and in the βAsn_{-10} mutant (Figure 2b) it appears only as a shoulder of the 1678 cm^{-1} band. In these mutants, the cluster of bands arising from the stretching modes of those C_2 acetyl groups involved in BChl−protein H-bonds (in the 1625−1640 cm^{-1} region) seems slightly distorted relative to that observed in the WT spectra, although these changes are often at the limit of the uncertainty of our experiments. In addition, the 1651 cm^{-1} band is slightly more intense in the spectra of these mutants than in the WT. Therefore it may be concluded that these mutations induce very small changes in the spectral region corresponding to the C_2 acetyl stretching frequencies, and that their effect mainly affects the C_9 keto carbonyl group of the 800 nm absorbing BChl. By contrast, FT-Raman spectra of the βMet_{-10} mutant (Figure 2g) is indistinguishable from that of the WT LH2 protein (Figure 2a) in this region.

To gain more insight into the vibrational contributions of the 800 nm absorbing BChl in the different β_{-10} mutants, their Raman spectra were recorded. In resonance conditions with the Soret electronic transitions of the bound BChl molecules. Figure 3 displays the high-frequency regions of the spectra of the WT, and two mutants exhibiting the extreme limits of altered carbonyl frequencies, namely the βHis_{-10} and βAsn_{-10} mutants (RR spectra of the βLeu_{-10}, βLys_{-10}, and βGlu_{-10} mutants were all very similar to each other, see Table 1). In these conditions of excitation, the Raman spectra are dominated by a strong band arising from the stretching modes of the BChl methine bridges, and the contributions from the carbonyl stretching modes are weak and partially hidden by this intense band. The RR spectrum of WT LH2 (Figure 3c) is similar to those previously reported (*20*), and they contain mainly three bands attributable to the carbonyl stretching modes at *ca*. 1632, 1674, and 1699 cm^{-1}. An additional, very weak contribution is observed at *ca*. 1652 cm^{-1}. It is worth noting that there is fair agreement between these frequencies and those observed in FT-Raman spectra. In the RR spectra of all β_{-10} mutants exhibiting absorption properties different from that of the WT, there is strong attenuation of the 1632 cm^{-1} component. This attenuation is accompanied by the appearance of new components between 1657 cm^{-1} (βAsn_{-10}, Figure 3a) and 1666 cm^{-1} (βHis_{-10}, Figure 3b), all indicating the presence of a C_2 acetyl carbonyl that is free from interaction. The band which arises from the stretching mode of the free-from-interaction keto carbonyl group of the B800 BChl of LH2 (*20*) is present in all these spectra in the 1689−1700 cm^{-1} region. In agreement with the FT-Raman data, the position of this band is dependent on the β_{-10} mutation, and it is shifted toward the low frequencies in the βLeu_{-10} mutant to 1695 cm^{-1},

Table 1: Positions of Q_y and of the Acetyl and Keto Stretching Frequencies of the B800 Molecules in the Different LH2 Complexes

	WT	β_{-10}-Met	β_{-10}-His	β_{-10}-Lys	β_{-10}-Glu	β_{-10}-Leu	β_{-10}-Asn
Q_y position (nm)	800	800	793	790	789	787	782
acetyl frequency (cm^{-1})	1632	1631	1666		~1660		1657
keto frequency (cm^{-1})	1700	1699	1702		~1695		1689

and it is more downshifted in the βAsn$_{-10}$ mutant to 1689 cm^{-1}, appearing only as a shoulder of the 1675 cm^{-1} band (see Table 1 and Figure 2b). RR spectra of the βMet$_{-10}$ LH2 mutant exhibit features very similar to those of the WT complexes.

DISCUSSION

The relative insensitivity of the FT-Raman spectra to the β_{-10} mutation indicates, in agreement with previous works (*6*, *8*) that most of the contributions present in the FT-Raman spectra of LH2 complexes arise from the 850 nm absorbing BChl molecules. As noticed before, only the 1699 cm^{-1} mode originates from the B800 BChls, and more precisely from their free-from-interaction C_9 keto group (*21*). The band arising from the H-bonded C_2 acetyl of the B800 pigments seems to be very weak in these spectra, and this was attributed to the fact that the preresonance conditions ensured by an excitation at 1064 nm preferentially enhances the signal from the most red shifted pigments (*6*). However, it must be noted that there is no clear explanation for the relatively more intense 1699 cm^{-1} band arising from the stretching mode of the B800 C_9 keto group seen in these conditions. The relative insensitivity of the FT-Raman spectra to the alterations of the arginine at β_{-10} indicates that the binding sites of the B850 pigments are probably not perturbed in these LH2 mutants. The slight distortion of the C_2 acetyl stretching band cluster, at *ca.* 1627 cm^{-1}, together with the slight increase observed at *ca.* 1651 cm^{-1}, might suggest that a weak band, arising from the H-bonded C_2 acetyl from the 800 nm-absorbing BChl is upshifting upon mutating the β_{-10} residue. However the spectral variations are much too weak for drawing any conclusions solely on this basis. Furthermore these spectra suggest that the 1699 cm^{-1} band position can be altered by mutations at the LH2 βArg$_{-10}$ residue, but the precise position of this band in the different mutants is difficult to measure, since it becomes a shoulder of the 1678 cm^{-1} band, and band intensity varies depending on the mutation.

In RR spectra recorded at low temperature with a 363.8 nm excitation, the contribution of the stretching mode of the H-bonded C_2 acetyl of the 800 nm-absorbing BChl is expected to be clearly visible, at *ca.* 1632 cm^{-1} (*20*). Most of the β_{-10} mutations induce drastic changes around that position in these spectra, and the disappearance of this band, together with the appearance of a new component in the 1655−1668 cm^{-1} range may be interpreted as the result of breakage of an H-bond between the protein and a C_2 acetyl of a BChl molecule (*21*). As the arrangement of the 850 nm absorbing pigments is probably not affected by these mutations (see above), it can be concluded that the C_2 acetyl carbonyl affected is that of the 800 nm absorbing BChl. These experiments thus constitute direct evidence that, as suggested by crystallographic studies on the LH2 complex from *Rps. acidophila* (*2*), βArg$_{-10}$ forms an H-bond with the C_2 acetyl carbonyl of the monomeric BChl in LH2 complexes from purple bacteria, and in particular *Rb. sphaeroides*. On the other hand, the similarity between the spectra of the βMet$_{-10}$ mutant and the WT LH2 indicates that, in this particular case, this carbonyl group is able to find another H-bond partner. The chemical nature of this partner is rather unclear, since it is obvious that the methionine side chain cannot directly interact with the C_2 acetyl carbonyl of the 800 nm absorbing BChl. As discussed below, it appears from the Raman spectra of the β_{-10} mutants that the replacement of the bulky Arg residue at this position may induce reorganization of the three-dimensional structure of the 800 nm absorbing BChl binding site. It could thus be possible that replacement of the βArg$_{-10}$ by a methionine brings the B800 pigment nearer to an amino acid group or a water molecule that is able to interact with its C_2 acetyl.

We previously stated the βArg$_{-10}$ is replaced by a serine and the βAsp$_{-13}$ is replaced by a threonine in *Rs. molischianum* but this LH2 also has a Q_y transition maximum at 800 nm. Using the X-ray crystal structure of *Rs. molischianum* (*22*) we find that the βThr$_{-13}$ and the reactive carbonyl group of the monomeric BChl are separated by only 1.93Å, thus allowing an H-bond to be formed which in turn maintains the 800 nm absorption maximum. From this work and the crystal structures of LH2 (*2*, *13*, *22*) it is apparent that the overriding factor in maintaining a Q_y maximum at *ca.* 800 nm is the ability for the local environment around the monomeric BChl to H-bond, *via* variations in the permittivity and polarizability of the pigment binding pocket, and is achieved by BChl−βArg$_{-10}$ interactions although other solutions are possible.

From these results, it can thus be inferred that at least part of the blue shift observed in the β_{-10} mutants is induced by the H-bond between the βArg$_{-10}$ residue and the C_2 acetyl carbonyl of the 800 nm absorbing BChl. To assess more precisely the role of this H-bond, the mutants in which the β_{-10} mutation seems to have the least effect on the Raman spectra must be considered. Indeed, in the βAsn$_{-10}$ mutant, the downshift of the C_9 keto carbonyl frequency suggests that the breakage of the H-bond in which the B800 C_2 acetyl group is involved is accompanied by additional effects on the structure of this BChl binding site, as shown by the down shift of the band arising from its keto stretching mode (Figure 2c). For the other mutants, only small variations of the frequency of this mode are observed, and it may thus be concluded that the direct effect of the C_2 acetyl-β_{-10} residue H-bond induces a red shift of the monomer BChl of *ca.* 10 nm (7−13 nm).

The Q_y absorption transition of the monomeric BChl *a* in LH2 complexes is therefore probably situated at *ca.* 790 nm in the absence of the H-bond between the β_{-10} residue and its C_9 keto carbonyl group, when the binding site of this molecule is not otherwise perturbed. Relative to the position at which this band is observed for BChl in ether (771 nm), this represents a *ca.* 330 cm^{-1} red shift, the origin of which remains to be characterized. In the βAsn$_{-10}$ mutant, this transition is observed at 782 nm, and this additional blue shift of the Q_y transition of the monomer BChl is associated

with a downshift of the frequencies of the C_9 keto carbonyl group of this molecule. This stretching mode ($\nu_{C=O}$) is particularly sensitive to the properties of the local environment, and it is well-known that its frequency depends on both the dielectric constant (ϵ) and the refractive index (n_D) of the environment (*23*−*25*), according to the following formula:

$$\nu_{C=O} = A[(\epsilon - 1)/(2\epsilon + 1)][({n_D}^2 - 1)/(2{n_D}^2 + 1)] + B \quad (1)$$

where the *B* parameter, which describes the stretching frequency of the keto carbonyl group at ϵ or n_D equal to 1, strongly depends on the molecule that is being studied (see for example refs 26 and 27).

Of course, in a proteic binding site, which is highly anisotropic, application of such a rule constitutes an approximation in which properties of the BChl environment have been reduced to average, isotropic values, but it may still give an indication of the important parameters which should be taken into account. In the different βArg$_{-10}$ mutants exhibiting absorption properties differing from that of the WT, the keto stretching frequency of the B800 is observed between 1689 and 1702 cm^{-1} while the acetyl frequency is located between 1657 and 1666 cm^{-1}. It is worth noting that within the resolution of our CCD detector, the frequencies of the acetyl and keto modes may be correlated to some extent with the B800 Q_y maximum (Table 1) in that the most blue-shifted Q_y absorption maximum (βAsn$_{-10}$, Figure 2b) appears to have the most perturbed carbonyl frequencies. Despite the fact that the 9-keto and 2-acetyl carbonyl groups are situated at opposite sides of the B800 BChl macrocycle, the two groups seem to react to changes in the local environment upon the introduction of mutations at the β_{-10} position, which leads to the suggestion that the physicochemical properties of the environment of the B800 pigment depends on the identity of the amino acid at position β_{-10}. It is difficult to fully ascertain the exact relationship between the different amino acids, Q_y absorption maxima, and Raman bands, as there are not yet enough examples of other mutant series with which to compare our data. Nevertheless, the observed frequency variations for the 9-keto and 2-acetyl carbonyls groups may be linked to the position of the B800 in the LH2 protein, namely, near the interface between the membrane and the cytoplasm, and they may be explained if the precise nature of the β_{-10} residue determines, for example, a partial accessibility of the BChl monomer binding site to water molecules. Therefore it is possible that in most cases, replacement of the bulky arginine by other amino acids induces not only the breakage of one BChl-protein H-bond, but also a partial, structural, reorganization near the B800 binding site. This may explain why, although some of the mutations described in this work are rather conservative (e.g. Arg for Lys), in all of the LH2 mutants except for the Arg for Met mutant, the β_{-10}-BChl H-bond is broken. Although this structural reorganization could be a consequence of protein−B800 H-bond breakage, it is more likely that it is induced by other structural factors, such as how the different amino acids fit at the Arg position, or how the different interactions between amino acids (either electrostatic or van der Waals interactions) are perturbed by these replacements. In the particular case of the methionine, it could be that this particular amino acid allows the conservation of all these interactions, thus maintaining a WT-like BChl binding site for the 800 nm absorbing molecule. In these conditions, as this amino acid is shorter than arginine, a cavity that may be left empty near the B800 could be filled either by a neighboring polar side chain (although none able to play this role is readily observable in the 3-D crystallographic structure) or by a water molecule.

It thus appears that mutations in the β_{-10} locus induce changes in the electrostatic properties of the B800 BChl environment and that the largest changes are found in the most blue shifted mutant, i.e. βAsn$_{-10}$. The dependence of the position of the Q_y transition of the chlorophyllic pigments according to the permittivity and polarizability of the surrounding media has been extensively studied, for both chlorophyll (*24*, *25*) and bacteriochlorophyll (*28*, *29*) molecules, and can account for shifts of this transition as large as 300 cm^{-1}. It is thus possible to account for the absorption shifts in the different β_{-10} mutants, which represent a spread of *ca.* 177 cm^{-1}, by examining the variations of the local permittivity and polarizability of the B800 binding site. We thus propose the red shift induced by the binding of the B800 to its proteic site to be a result from two distinct phenomena: (i) a *ca.* 160 cm^{-1} red shift (from 780 to 790 nm) due to the local electromagnetic properties of the BChl binding site and (ii) an additional 160 cm^{-1} red shift (from 790 to 800 nm) due to the formation of an H-bond between the β_{-10}-arginine and the acetyl carbonyl of this molecule. Over the 470 cm^{-1} total red shift (from 771 to 800 nm) experienced by the Q_y transition of this molecule, *ca.* 320 cm^{-1} may be thus be accounted by these two phenomena. This indicates that any other interaction between this molecule and the protein, including possible conformational strains on the BChl macrocycle, which have been evoked for the tuning of the absorption of these molecules, should not account for a shift greater than at most 150 cm^{-1}. Although this could not be a general case, such an evaluation will be extremely important for reassessing the absorption properties of the BChl monomers in other light-harvesting proteins containing weakly a interacting BChl molecule, such as the Fenna−Matthews−Olson (FMO) protein, in order to see whether such values could explain more accurately the global absorption properties of these complexes.

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