Biochemical Characterization of the Dissociated Forms from the Core Antenna Proteins from Purple Bacteria†

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ABSTRACT: The core light-harvesting protein from *Rhodospirillum rubrum* is of particular interest for studying membrane polypeptide association, as it can be reversibly dissociated in the presence of *n*-octyl-*β*-D-glucopyranoside (*β*OG) into smaller subunit forms, which exhibit dramatically blue-shifted absorption properties (Miller et al. (1987) *Biochemistry 26,* 5055−5062). During this dissociation/reassociation process, two main spectroscopic forms are observed, absorbing at 820 (B820) and 777 (B777) nm, respectively. By using polyacrylamide gel electrophoresis in the presence of *β*OG, these forms were characterized from a biochemical point of view. B777 consist of a mixture of α or *β* polypeptide chains, retaining their bound bacteriochlorophyll (BChl) molecules. The absorption properties of the BChl molecules bound to the monomeric polypeptides do not depend on the chemical nature of the polypeptides they are bound to. B820 is more complex and consist of equilibrium between α*β*-containing oligomers and *β* only containing dimers, all exhibiting very similar electronic absorption properties. Resonance Raman spectroscopy indicates that the binding site provided by the *β*-only B820 to the BChl molecules is very similar to that provided by the α*β* B820. This, together with the observation that the α polypeptide alone is unable to form B820, suggests that the local organization of the BChl molecules tightly depends on BChl-protein interactions. On the other hand, our results suggest that the affinity of the *β*-BChl complexes for itself and for the α-BChl ones are of the same order of magnitude, the formation of heterodimeric complexes being mainly driven by the inability of α-BChl complexes to self-associate.

In purple photosynthetic bacteria, the light-harvesting (LH)[1] pigment−protein complexes collect the solar photons and ensure an efficient funneling of the excitation energy toward the photochemical reaction centers (RCs). There are two types of antenna proteins in these organisms, namely, the LH1 and LH2. The LH1, or "core" antenna complexes, are present in all bacterial species and are closely associated with the RC. The LH2 or "peripheral" antenna complexes are synthesized by many bacterial strains, and they transfer the excitation to the RC via the LH1. Both these complexes have the same basic structural arrangement. They are multimers of a minimal unit containing two small, transmembrane, polypeptides α and *β*, to which are bound the chromophores, bacteriochlorophyll (BChl), and carotenoid (*1*). The atomic structures of the LH2 from *Rhodopseudomonas acidophila* (*2*) and *Rhodospirillum* (*Rsp*) *molischianum* (*3*) have been derived from X-ray crystallographic studies. In these proteins, the α*β* subunits associate in an annular structure, in which the internal ring is constituted by the α polypeptides, while the *β* polypeptides form the external ring.

To date, no atomic resolution structure for a core antenna protein has been obtained. Projection structures obtained from two-dimensional crystals of the core antenna from *Rsp. rubrum* indicated that this protein forms larger rings than the peripheral antenna, containing 16 heterodimeric subunits and large enough to contain a reaction center (*4*). Two-dimensional crystals of LH1−RC complexes from *Rsp rubrum* and *Rhodobacter sphaeroides* (*5*, *6*) have confirmed that these polypeptides surround the RC, though the general existence of such 16-membered, closed rings in intact membranes is still an open question (*7*). In the membrane phase, both the α and *β* polypeptides of LH1 bind a BChl molecule which is located between the two protein rings. These BChl molecules interact with each other in the multimer, and they exhibit a lower energy singlet absorption transition at ca. 875 nm, i.e., dramatically downshifted relative to that of isolated BChl *a* in most solvents (ca. 770 nm). The position of this transition is tightly controlled by the degree of association of the membrane polypeptides (see, e.g., ref *8*).

Besides its role in the photosynthetic process, the LH1 protein from carotenoidless strain of *Rsp. rubrum* G_9+ was used to study membrane polypeptide association. This protein can be reversibly dissociated by the detergent *n*-octyl-*β*-D-glucopyranoside (*β*OG) into smaller subunit forms (*9*−*13*). This dissociation is accompanied by a shift of the lower-energy absorption transition from 873 to 820 nm (*13*, *14*). Additional treatment with *β*OG results in a further shift of this electronic transition to 777 nm (*14*), associated with a

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[1] Abbreviations: BChl, bacteriochlorophyll; LH, light-harvesting complex; RC, reaction center, *β*OG, *n*-Octyl-*β*-D-glucopyranoside; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel; N-PAGE, native-polyacrylamide gel electrophoresis; B873, core light-harvesting complex; B820 and B777, core light harvesting complex subunits absorbing at 820 and 777 nm, respectively; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; *Rsp*, *Rhodospirillum.*

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further dissociation of the protein. This stepwise formation of the 777 nm-absorbing form (B777) is reversible, and upon removal of the detergent, the polypeptides reassociate to form the B820, and then intact LH1 (B873) complexes (*14*). This possibility of reversibly dissociating LH1 has led to a number of studies, which addressed the parameters underlying the association reaction of its transmembrane polypeptides (see, e.g., ref *15*−*17*). It has been shown by FTIR spectroscopy that neither of these dissociation steps affects the secondary structure of the polypeptides (*18*). It is now clear that, in most cases, B777 consists of isolated, α-helical, α, and β polypeptides still retaining their bound BChl molecule. This was first shown by spectroscopic measurements, on quite highly concentrated samples (*18*). This conclusion was recently confirmed, at much lower concentration, by the determination of the reaction order of the association reaction of B777 into B820 (*19*, *20*). In an early study, it was suggested that B777 is a mixture of monomeric polypeptides and free BChl molecules (*14*). The binding of BChl molecules to the antenna polypeptides could depend on experimental conditions, although it is not yet clear which parameter, in that particular study, led to a different structure of the B777 as compared to (*18*−*20*).

The exact nature of B820 is still unclear. Spectroscopic studies have established that its absorption at 820 nm arises from a BChl dimer (*21*, *22*). However, measurements of hydrodynamic size suggested that B820 behaves as a tetramer (*10*, *13*). The reaction order of the B820 formation from the association of B777 was measured, and it appeared that either two or four B777's were necessary to build up a B820 (*18*, *19*). This situation was recently clarified, as it was shown that this reaction order, and thus the quaternary structure of B820, is dependent on the βOG concentration (*20*). However, the association state of the α and β polypeptides in the B820 form has not yet been precisely described, though this question is of primary importance for understanding the factors which drive the polypeptide association. In this paper, we have addressed this question.

MATERIALS AND METHODS

Enzymes and Chemicals. n-Octyl β-D-glucopyranoside was from Biomol (Germany), and trypsin sequencing grade was from ROCHE (Switzerland). All other chemicals were from Sigma chemical (USA) or Merck-Biochemicals (Germany).

Rsp. rubrum B820 was purified from the carotenoidless strain G_9+ (*23*). The purification procedure was described earlier in (*18*). B820 subunits are first solubilized by treating chromatophores with 2% βOG and loaded on ion exchange chromatography column DEAE sepharose. Then, pure LH1−RC complexes were titrated with 2% βOG and loaded onto a FPLC anion exchange column Resource Q. The quality of the fractions collected were controlled by SDS−PAGE, followed by Coomassie staining. The whole purification procedure was performed in dim light. Protein concentrations were determined by three methods: (i) by measuring the absorption at 280 nm, using absorption coefficients ϵ_{280}1 mg/mL = 3 for α and 2.8 for β subunit, as calculated from the amino acid composition, (ii) by measuring the 777 nm absorption after complex dissociation and/or pigment extraction in organic solvent, and (iii) by using a Bradford assay (Bio-Rad) (*24*), with bovine serum albumin as a standard. Differences not larger than a factor of 2 on the concentration were observed using these different methods.

Spectroscopic Measurements. Room-temperature absorption spectra were recorded on a Cary 5 Spectrophotometer (Varian plc, Sidney). To measure the absorption properties of the different bands after nondenaturing electrophoresis, the gels were mounted on a Microcontrol micro-translator, with the bands parallel to the longer axis of the measuring light. The size of the measuring beam at the focal point was reduced so it corresponds to the width a lane in the gel. Absorption spectra were measured between 700 and 900 nm every millimeter in the gels.

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 degree geometry from samples maintained at 77 K in a flow cryostat cooled with liquid nitrogen (TBT, Vitry sur Seine), as described in (*25*).

Native-PAGE Electrophoresis. For native-PAGE electrophoresis, B820 and/or B777 were concentrated and loading buffer (125 mM Tris pH 8.0 containing 20% glycerol and 2% βOG, final concentration) was added. The electrophoresis was carried out at room temperature as described in ref *26*, except that SDS was replaced by βOG 2%. The acrylamide concentration was 6% for B820 and 10% for B777 (ratio acrylamide/bis 3.5%). The migration was controlled by following the blue-green color of the sample. At the end of migration, the proteins in native-PAGE were detected either by Coomassie staining or by scanning the gel every mm and measuring the absorbance between 700 and 900 nm. For each spectrum, the fraction of B777 and B820 was estimated by fitting the spectra with a linear combination of pure B777 and B820 spectra using the GRAMS 32 software (Galactic, Salem, New Hampshire). The corrected contribution at 777 nm was obtained by this fitting procedure.

Elution from Native-PAGE and Second Dimension Migration. B820 and B777 protein species, separated in native-PAGE and detected by absorption spectroscopy, were eluted passively from native-PAGE overnight at 20 °C in Tris pH8 20 mM buffer containing 100 mM NaCl and 0.2% βOG in dim light. The yield of such passive elution is typically 80%. For characterizing the polypeptide content of the different bands, the eluted proteins were concentrated and subjected to a second dimension migration. A tricine-SDS-PAGE was used to improve the resolution and separate the small LH1 polypeptides. Discontinuous tricine-SDS 16,5% acrylamide gel was carried out as described in (*27*), without "spacer" gel.

Peptide Mapping Identification. Digestion. Protein bands were cut from the gel and digested by trypsin as described in (*28*). Digestion products were resuspended in 10 μl formic acid 1%, desalted using Zip Tips C18 from Millipore and eluted with 75% acetonitrile. This method does not permit to identify membrane fragment of the protein, but only soluble fragments.

MALDI-TOF Analysis. Mass spectra were recorded in positive reflectron mode with a Voyager STR -DE (Perseptive Biosystems, Inc., Framingham, MA) MALDI-TOF mass spectrometer equipped with a delayed extraction device.

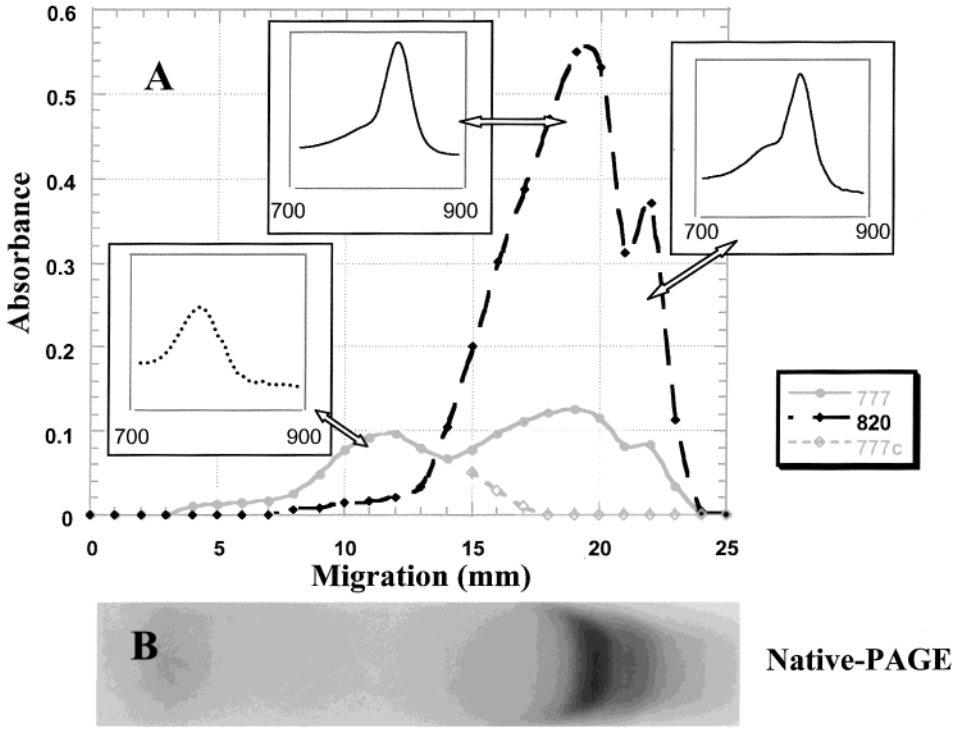

FIGURE 1: Separation of the B820 and 777 nm absorbing forms using Native-PAGE in the presence of 2% βOG. The sample concentration expressed as a monomer was 10 mM. The protein species were detected either by measuring the absorbance between 700 and 900 nm every millimeter (part A) or by Coomassie staining (part B). Insets: spectra associated with the different bands of the gel are presented. In part A, diamonds indicate the absorption at 777 nm, once the contribution at 777 of the 820 form was corrected.

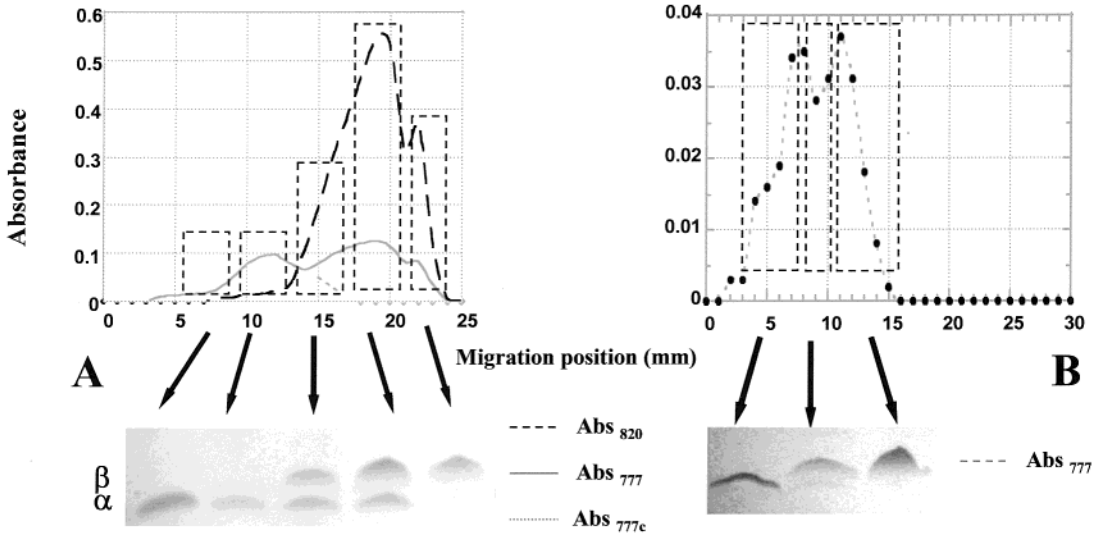

FIGURE 2: Identification of the polypeptide composition of the different protein species observed in native-PAGE (see methods) of B820 (part A) and B777 (part B). In part A and B, a concentrated sample (10 mM) and a diluted sample (1.5 mM) were loaded onto the native gels, respectively. Bottom: second dimension denaturing electrophoresis. Each lane in the denaturing gel corresponds to a dotted box in the native gel.

Samples were analyzed with the dried droplet method using a saturated solution of Di Hydroxy Benzoic Acid as a matrix. Internal calibration was performed using peptide from the auto-digestion of trypsin.

RESULTS

Native Polyacrylamide Gel Electrophoresis of B777 and B820 Subunits. Native-polyacrylamide gel electrophoresis (N-PAGE) in the presence of 2% βOG was used to analyze the B777 and B820 form. B777 and B820 sample were loaded on such a gel, at identical βOG concentration. The conversion of B820 into B777 before loading the samples onto the gels was ensured by a proper dilution of the sample at fixed detergent concentration (*18*). Gels were analyzed both by Coomassie staining and by measuring the absorption between 700 and 900 nm every millimeter in each lane (before staining).

When B820 samples are loaded on N-PAGE, Coomassie staining reveals the presence of three bands in the gel, the two major bands visible on Figure 1A and one minor band. The latter is not visible on Figure 1A, as at the protein concentration necessary for revealing it by Coomassie stain-

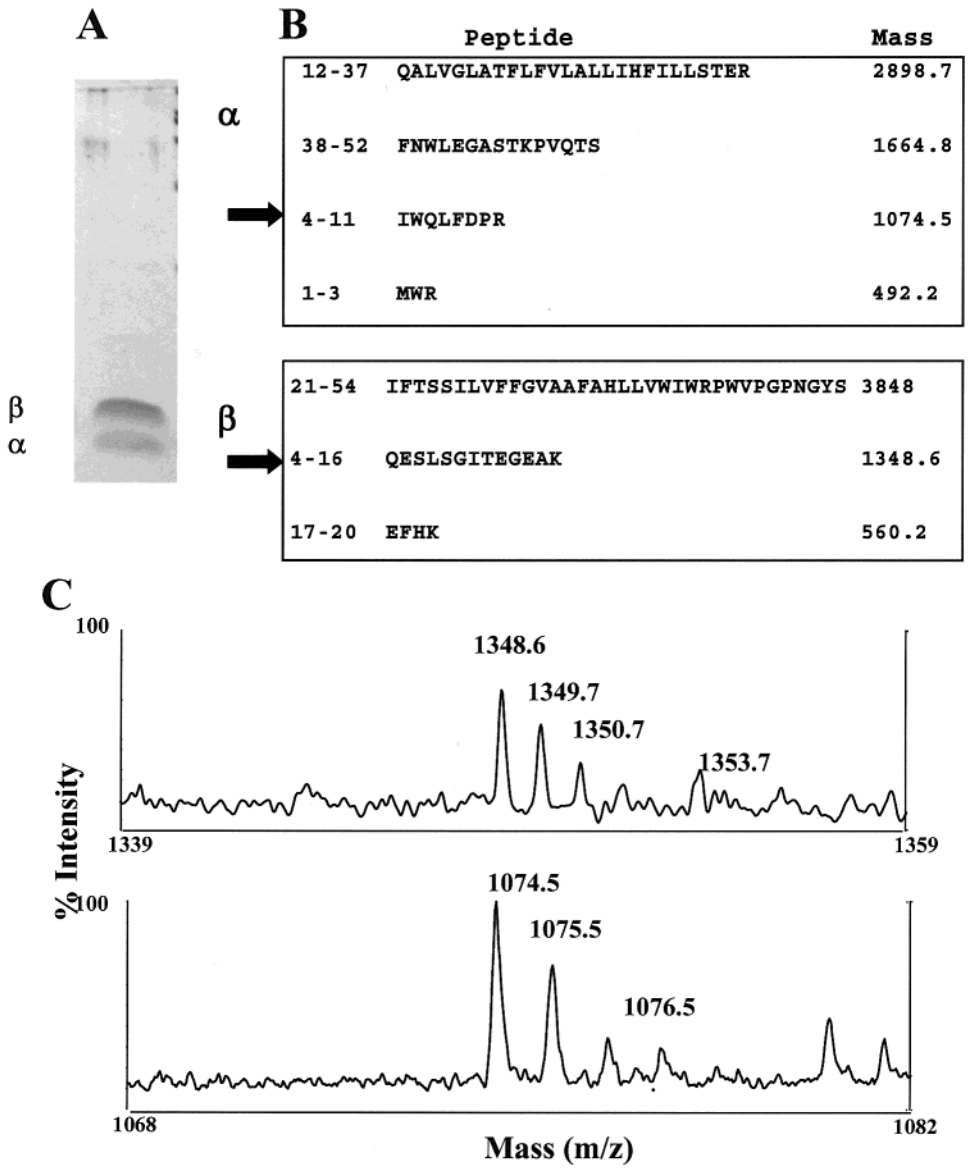

FIGURE 3: Part A: Separation of polypeptides α and *β* using Tris-tricine-SDS-PAGE. Part B: Theoretical sequences of peptides after complete trypsic digestion. Part C: Mass spectra of the peptides after trypsin digestion and elution from the gel. Arrows in part B indicate the peptides which can be used in part C for polypeptide identification.

ing, the two major bands become saturated and are not resolved anymore on the gel. This band is, however, revealed by absorption measurement in Figure 1A. Figure 1 displays the absorbance values obtained at 777 and 820 nm as a function of the migration distance in the gel. The 777 nm absorption alone is mainly observed at the migration position of the minor band centered at 11 nm. At further migration distances, the 777 nm absorption corresponds to the foot of the electronic transition located at 820 nm (see below). Indeed, when the contribution of the latter is subtracted from the absorption spectra, no absorbance at 777 nm remains in the difference spectra (Figure 1). Plotting the absorbance at 820 nm according to the migration distance in the gel results in a quite complicated profile. Two major bands, which exhibit different migration behavior in the gel, possess an absorption maximum at 820 nm. It is worth noting that the first of the 820-nm absorbing band is quite broad, possibly degenerate and containing more than one proteic species which could not be separated by N-PAGE in our experimental conditions. When B777 samples are loaded on the N-PAGE gels, only two bands are observed. Both these bands exhibit absorption transitions at 777 nm, of equal intensity (Figure 2).

The polypeptide composition of the proteic species observed by the N-PAGE experiments was analyzed by electrophoresis. Each of the bands of the N-PAGE gels was isolated, passively eluted from the gel, and dialyzed against water. After concentration, proteins were heat-denatured in the presence of SDS 4% and loaded on a Tris-tricine-SDS-PAGE. Figure 3 displays the polypeptide composition of each band from the native-PAGE, absorbing either at 777 or 820 nm.

Identification of α *and β Polypeptides on Tris-Tricine-SDS−PAGE Gels.* The different bands observed in Tris-tricine-PAGE experiments were assessed to the α or *β* polypeptides by mass spectrometry. The molecular masses of α and *β*, as calculated from their amino acid compositions, are 6076 and 6077 Da, respectively. These polypeptides were submitted to tryptic digestion in the gel. Such digestion is expected to produce fragments of masses different enough

to allow α and *β* polypeptide identification (Figure 3B). The mass spectra are dominated by trypsin autodigestion fragments. However, for each band a set of masses allows an unequivocal identification of the polypeptides (Figure 3C). As could be expected, these fragments correspond to the N-terminus soluble part of the polypeptides (see methods). These experiments show that the upper and lower band, in the Tris-tricine-SDS-PAGE gels, correspond to the *β* and α polypeptides, respectively.

Characterization of the B777 and B820 Solution. The different protein species evidenced in N-PAGE gels can now be unambiguously identified. In Figure 2B, the bands migrating between 3 and 15 mm correspond to B777 forms, containing pure α and pure *β* polypeptides, respectively. The positions of the Q_y absorption transitions of these two fractions in the gels are identical at room temperature (data not shown). It can thus be concluded that the binding of BChl molecules to the α and/or *β* polypeptides results in a similar shift of their Q_y absorption transition. Moreover, as these polypeptides are produced by the dissociation of LH1 proteins, they are expected to be in equimolar concentration. As the absorbance values detected for each of these bands are roughly equal, it may be concluded that the extinction coefficient for BChl molecules bound to α and/or *β* polypeptides are similar. In Figure 2A, the species absorbing at 777 nm, which migrates at 11 mm, corresponds to B777 containing only the α polypeptide. The following, broad band, absorbing at 820 nm, which extends between 15 and 20 mm, corresponds to protein species which contain both α and *β* polypeptides. If this form corresponds to a mixture of species, which are not resolved in our present gels, all containing equimolar ratios of α and *β* polypeptides. It was recently shown that B820 may, depending on detergent concentration, adopt different quaternary structures (*20*). This band could correspond to a mixture of these different α*β* oligomeric forms, although alternative explanations cannot be excluded. However, post-transcriptional modifications of the α and/or *β* polypeptides, which have been shown to occur (*29*), cannot explain the complexity of this band. Indeed in Figure 2B, both the α and *β* polypeptides containing B777 fractions migrate as a single band. If these polypeptides were exhibiting structural inhomogeneity, such as different degrees of oxidation, this would necessarily result in the presence of additional bands in Figure 2B. Finally, the narrow band, centered at 22 mm, also absorbing at 820 nm, contains *β* polypeptides only. It must thus correspond to *β*-only oligomers, likely β_2 dimers (see below).

The electronic absorption spectra at room temperature of the different B820 forms were measured. To avoid mixed contributions of these different species, these absorption spectra were recorded with the measuring beam positioned either on the left side of the lower band or on the right side of the upper band (Figure 4). The spectrum of the species containing both α and *β* is similar to that of B820 in solution (*18*). The spectrum of *β*-only B820 is nearly identical to that of the α*β*; however, the maximum of the electronic transition is slightly shifted toward the blue, by 2 nm. Absorption spectra of α*β*- and *β*-only species were recorded after passive elution from the gel, in the presence of low *β*OG concentration (Figure 5). As expected, elution from the gel of the α*β*-containing B820 species at low *β*OG concentration induces a red shift of the main absorption Q_y transition of this

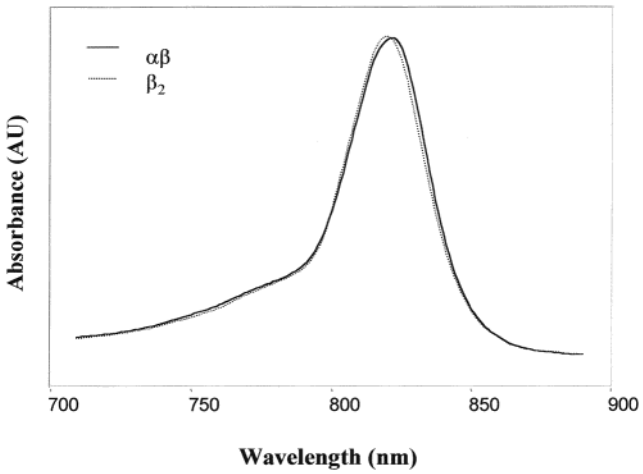

FIGURE 4: Absorption spectra measured directly in native-PAGE of α*β* and *β* only containing B820 species.

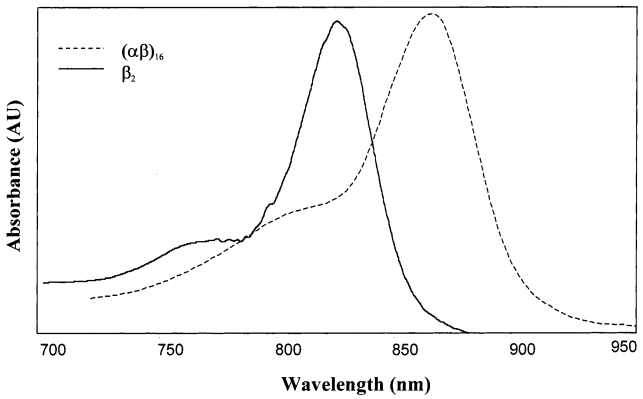

FIGURE 5: Absorption spectra of the same species after elution from the gel, at low octyl glucoside concentration.

complex to 869 nm, indicating its reassociation into larger aggregates. The precise position of this transition is not identical to that of intact LH1 (873 nm) but the observation of such 869 nm-absorbing aggregates has been already reported when quickly diluting the B820 at low detergent concentration (*15*). By contrast, eluting the 820-nm absorbing *β*-only species in the presence of low detergent concentrations does not affect its absorption spectra, suggesting that this form has lost the ability to further associate. As a control, the species absorbing at 777 nm containing pure α monomer was eluted from native-PAGE in similar *β*OG concentrations. Decreasing the detergent concentration does not affect its absorption spectrum, indicating that this monomer is unable to form any homodimeric aggregates (result not shown). These results are summarized on Figure 6.

To gain insight into the BChl binding site at a structural level, the different B820 species were examined by resonance Raman spectroscopy. Resonance Raman provides a convenient method for studying the conformation of, and the intermolecular interactions assumed by, the BChl molecules in their proteic binding sites (*30*). Care must though be taken to ensure that the sample is uncontaminated with longer and/or shorter wavelength absorbing forms (i.e., B820 in the B777 or B873 in the B820 samples) at the concentrations used. This was performed by measuring in the Raman spectrometer, the fluorescence of the samples in the near-infrared (data not shown). Figure 7 shows the resonance

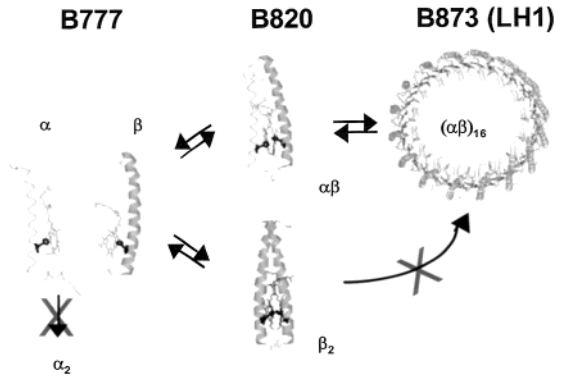

FIGURE 6: Model of the different protein species in equilibrium at each step of LH1 assembly, in vitro. For simplicity, the possibility of $\alpha\beta$-containing species to form higher oligomeric form was not represented in this diagram.

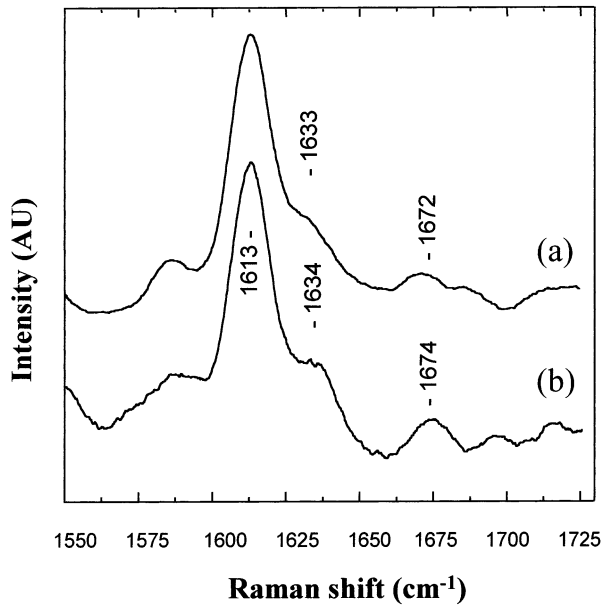

FIGURE 7: Resonance Raman spectra (higher frequency region, 1550−1725 cm^{-1}) of B820 complexes containing (a) both α and β polypeptides and (b) β polypeptide only. Excitation wavelength: 363.8 nm. Temperature: 77 K.

Raman spectra, excited in resonance with the Soret electronic transition of BChl molecules at 363.8 nm, of $\alpha\beta$ and β2 B820 species. The most intense peak in these spectra is the methine stretching mode, located at 1612 cm^{-1}. This position indicates that the central Mg atom of the BChls is 5-coordinated in both samples as previously reported for the B820 subunit (*31*). The modes corresponding to the acetyl and keto carbonyl stretching modes contribute in the 1620−1700 cm^{-1} region. Their frequency tightly depends on the nature of the intermolecular interactions these modes are involved in (*30*), as well as on, to a lesser extent, the dielectric constant of their local environment (*32*). The spectra of both $\alpha\beta$ and β2 B820 are nearly identical in this region, containing a band at 1633−1634 cm^{-1} and a broad, weak feature centered at 1672−1674 cm^{-1}, as previously reported for the B820 subunit (*31*). It may thus be concluded, from these spectra, that the β_2 B820 subunit is able to provide a binding site for the BChl dimer nearly identical to that provided by the $\alpha\beta$ one.

DISCUSSION

Biochemical Characterization of the Dissociated Forms of LH1 from Rsp rubrum. From the experiments conducted in this work, a number of conclusions may be drawn about the biochemical nature of both the B777 and B820 dissociated forms of LH1 from *Rsp rubrum*. The presence of 777 nm-absorption in the N-PAGE gels always coincides with the presence of the α and β polypeptides. In our gels, in the absence of SDS, migration of the macromolecules is driven by their intrinsic charge only. Comigration of 777 nm-absorbing BChl molecules with the polypeptides thus constitutes a direct biochemical evidence that B777 is constituted by α and/or β-bound BChl molecules. The dissociated B777 form thus consists of isolated, monomeric polypeptides still retaining their bound chromophore, in agreement with resonance Raman spectroscopy (*18*). The protein:pigment stoichiometry of B777 was evaluated with Coomassie staining and absorption measurements. Such measurements are consistent with a 1:1 stoichiometry. In this work, we show that the α- and β-bound BChl molecules share identical absorption properties in the B777 form. In particular, N-PAGE experiments show, for the first time, that the α- and β-bound BChl molecules share the same extinction coefficient, i.e., that the latter is not influenced by the chemical nature of the transmembrane polypeptide. These results strongly suggest, in particular, that the conformations of both α-and β-bound BChl molecules are identical in the B777 form. From resonance Raman experiments and taking into account the crystallographic structure of the LH2 protein from *Rps acidophila*, it was previously concluded that β-bound BChl was distorted in LH1 proteins (*33*−*36*). Furthermore, analysis of the circular dichroism spectra of LH from purple bacteria led to the conclusion that, in intact complexes, the absorption properties of the α- and β-bound BChl molecules are unequivalent (*34*−*36*). Our results strongly suggest that both the conformational change of the β-bound BChl, and the unequivalent spectra properties of the LH-bound BChl molecules do not occur upon the binding of these molecules to their host polypeptides, but rather upon the formation of the quaternary structure of the LH proteins.

Native-PAGE experiments evidence that the B820 form is inhomogeneous and that it may be formed by the association of both α and β or β only polypeptides. Our experiments do not allow the unequivocal assessment of the oligomerization state of these different B820 forms. It was recently shown that the latter actually depends on the detergent concentration present in the solution (*20*) and that it is a dimer at low detergent concentration and a tetramer when the concentration of βOG is higher that 1.25%. The β-only B820 migrates as a thin band in the N-PAGE gels. This, together with the fact that it is unable to further associate, strongly suggests that it is actually a $(BChl)_2$-β_2 form. By contrast, no B820 form containing α polypeptide only could be found, neither in the N-PAGE gels, nor after elution of the α polypeptide-containing B777 form from the gels. Our results are thus in perfect agreement with those of Parkes-Loach et al. (*14*). Using polypeptides which had been purified first in the presence of organic solvents, these authors also concluded that the α polypeptide was unable to self-associate and that the B820 form was the highest oligomeric form reachable when promoting the association of the β

polypeptide alone. However, these authors found that the 777 nm-absorbing form was arising from free BChl. Formation of B820, in this case, was thus involving two steps, the binding of the BChl to the polypeptides and the subsequent oligomerization of the BChl-polypeptide complexes. The absence of α-only B820 was thus more difficult to interpret than in the experiments conducted in this work. As in our conditions the B777 corresponds to BChl bound to the α and/or β polypeptide, our results indicate that the lack of α-only B820 is due to the inability of the BChl-α polypeptide complex to self-associate. Moreover, the presence of $(BChl)_2\beta_2$ complex in the B820 samples obtained from dissociating LH1 indicates that this form is in equilibrium with $\alpha\beta$-containing oligomers and is able to compete with these forms. However, no more than 10% of β_2 is present in the B820 solution; thus, the apparent reaction order determined by Arluison et al (*20*) mainly reflects the $\alpha\beta$ formation.

The β polypeptides only B820 form exhibits absorption properties at room temperature which are very similar to those of the $\alpha\beta$ B820, as was already underlined in (*14*). As the absorption of these complexes is strongly dependent on the BChl-BChl distance and relative geometry, the similarity between the absorption of β-only and $\alpha\beta$ complexes indicates that the local organization of BChl molecules in these complexes is nearly identical. This could suggest that the local organization of the BChl molecules are mainly driven by BChl/BChl interactions, and not by the chemical nature of the polypeptides involved, as suggested by (*37*). However, resonance Raman experiments show that the complex formed by the β polypeptides only is able to provide a binding site to the BChl molecules nearly identical to that of $\alpha\beta$ B820. It is thus also possible that the formation of a nearly identical dimer in both $\alpha\beta$ and $\beta\beta$ complexes structure is driven by the ability of the polypeptides to provide a specific binding sites to the BChl molecules of the dimer. The fact that no αα association is observed present evidence for the crucial role of the chemical nature of the polypeptide in the formation of transmembrane polypeptide dimers. As developed below, the fact that α is unable to interact with itself may play an important role in the formation of heterodimeric $\alpha\beta$ subunits.

Analysis of the B820/B777 Equilibrium. The B820 spectral form consists of at least two protein species that exhibit different migration behavior, namely an heterodimer $\alpha\beta$ and an homodimer β_2. These forms are possibly in equilibrium with higher oligomeric forms of $\alpha\beta$. This equilibrium may thus be described, in its simplest form (i.e., neglecting the possible existence of higher oligomeric forms), as follows:

$$2\alpha + 2\beta \rightleftharpoons \begin{matrix} 2\alpha\beta \\ 2\alpha + \beta_2 \end{matrix} \qquad (1)$$

As the α polypeptide is unable to form dimers, even if the ΔG associated with the formation of β_2 is of the same order of magnitude as that associated with the formation of $\alpha\beta$, the equilibrium will be pushed toward the formation of $\alpha\beta$. More precisely, neglecting for simplicity the formation of $(\alpha\beta)_2$ tetramers, if $\Delta G_{\alpha\beta}$ is equal to $\Delta G_{\beta\beta}$ the ratio $[\beta_2]/[\alpha\beta]$ should be equal to $[\beta]/[\alpha]$. In Figure 2A, the concentration of free β polypeptide is barely detectable and should not account for more that 10% of the free α polypeptide. Thus, our results suggest, in contrast with previous studies (*38*), that the stability of the B820 constituted by β_2 dimers should be not dramatically different from that of the $\alpha\beta$ ones. It must be here underlined that introducing higher oligomeric forms of $\alpha\beta$ such as $(\alpha\beta)_2$ in the equilibrium (1) is reinforcing this conclusion. Indeed, as β_2 seems unable to further associate, presence of these forms should push the equilibrium even more toward the formation of $\alpha\beta$ B820.

Perspectives. In this paper, we report a method for analyzing B777 and B820 forms of LH1 by nondenaturing βOG-PAGE. With this method, we could put to evidence the existence of β_2 dimers in the B820 form obtained by treating B873 with the detergent octylglucoside and analyze the relative role of the polypeptides and the BChl molecules in the B820 formation. It is clear that this method represents a promising tool for understanding the dissociation/association process of LH1 polypeptides. We are currently purifying large amounts of pure β_2 B820 from B873 by N-PAGE to analyze the electronic and structural properties of these complexes and test its stability by direct, thermodynamic measurements.

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