Unexpected Similarities of the B800–850 Light-Harvesting Complex from *Rhodospirillum molischianum* to the B870 Light-Harvesting Complexes from Other Purple Photosynthetic Bacteria†

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ABSTRACT: The B800–850 light-harvesting complex (also called LH2) was isolated from photosynthetic membranes of *Rhodospirillum molischianum* DSM 119 using molecular sieve and ion-exchange chromatography. Its two bacteriochlorophyll *a*-binding polypeptides (α-subunit and β-subunit) were purified with a reverse-phase HPLC system. The complete amino acid sequences of both subunits have been determined. The α- and β-subunits consist of 56 and 45 amino acids, respectively, corresponding to molecular weights of 5939 and 5133. In contrast to the B800–850 complexes from other photosynthetic bacteria, the native B800–850 complex from *Rs. molischianum* is most likely an octamer of monomers with a stoichiometry of three bacteriochlorophyll *a* and 1.5 lycopenes per α,β-subunit. Resonance Raman spectra provide evidence for a 5-coordinated Mg^{2+} in the BChl, and a carotenoid mainly in the all-trans configuration. A comparison between resonance Raman data from different photosynthetic bacteria indicates that the BChl *a*-binding site of the B800–850 complex from *Rs. molischianum* is more similar to the B870 complexes (also called LH1) than to the B800–850 complexes of other photosynthetic bacteria. Sequence similarities especially between the β-subunits of the B800–850 complex of *Rs. molischianum* and the B870 and B800–850 complexes of other photosynthetic bacteria agree with this result and provide information on the mode of pigment binding in bacterial antenna complexes.

The photosynthetic membranes of purple bacteria (Rhodospirillaceae) generally contain two types of protein–pigment complexes: the reaction centers, in which the primary charge separation takes place, and the light-harvesting complexes, the role of which is mainly to gather the light energy and to transfer this energy to the reaction centers. In most purple bacteria, the photosynthetic membranes contain two types of light-harvesting complexes. The B870 complexes are tightly bound to the reaction centers, while the B800–850 complexes constitute the bulk of peripheral antenna: they are assembled independently (Shiozawa et al., 1980; Dierstein et al., 1981) and transfer the energy to the reaction centers via the B870 complexes. Because of the differences in the energy of the Q_y transitions of these complexes, the light energy is funneled efficiently to the reaction centers. Both B870 and B800–850 types of antenna complexes contain two polypeptides, namely, α- and β-subunits, in a multimeric association. The B800–850 complexes from some bacteria contain an additional non-pigment-binding polypeptide (Feick et al., 1979) which may stabilize these complexes (Tichy et al., 1991). α- and β-polypeptides from many different bacterial strains have been sequenced. They contain strictly conserved histidines, thought to bind the two bacteriochlorophylls (BChl)[1] responsible for the 880-nm (or 850-nm) absorption of the light-harvesting complexes. However, the parameters responsible for the tuning of the absorption of these bacteriochlorophylls are still unknown. This is partly due to the fact that, although several B800–850 complexes have been crystallized (Cogdell et al., 1985; Papiz et al., 1989; Welte et al., 1985; Michel, 1991) no X-ray structure has been published. In this paper we present the isolation and characterization of the B800–850 complex from *Rhodospirillum* (*Rs*) *molischianum* strain DSM 119, a facultative photoheterotrophic bacterium, including the chemically determined amino acid sequences of the α- and β-subunits. This complex shows absorption maxima at 850 and 800 nm characteristic for B800–850 complexes. However, its sequence possesses similarities to sequences of B870 complexes. Moreover, resonance Raman spectra of these complexes indicate that the binding site for the bacteriochlorophylls absorbing at 850 nm is similar if not identical to those for the bacteriochlorophylls in B870 complexes. This unique property of the B800–850 complexes from *Rs. molischianum* may provide insights on the protein–pigment interactions which influence absorption properties of B870 and B800–850 complexes. Furthermore, this information may help in designing site-selected mutations that would convert a B800–850 complex to a B870 complex or vice versa.

MATERIALS AND METHODS

Growth of Bacteria. Rs. molischianum strain DSM 119 was cultivated phototrophically in 10-L flasks using the medium described by Pfennig et al. (1971) at a light intensity of 3000 lux (Osram, 120 W) and at a temperature of 30 °C. Cells were harvested by centrifugation using continous flow (Padberg, Cepa Laborzentrifuge LE).

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[1] Abbreviations: BChl *a*, bacteriochlorophyll *a*; RP-HPLC, reverse-phase high-performance liquid chromatography; LDAO, *N*,*N*-dimethyldodecylamine *N*-oxide; LH, light-harvesting complex; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LiDS, lithium dodecyl sulfate; TFA, trifluoroacetic acid; M_w, molecular weight.

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Purification of the B800–850 Complex. Cells were suspended in 20 mM Tris-HCl, pH 8, and 0.5% EDTA and sonified 45 min at 0 °C (Branson Sonifier 250, 70% output, resonator with 12.6-mm tip). Unbroken cells and large fragments were removed by centrifugation (20 min, 24000*g*). The photosynthetic membranes were then sedimented by centrifugation (3 h, 30000*g*) and resuspended in 20 mM Tris/0.5% EDTA by sonication. After removal of debris (20 min, 24000*g*), the supernatant was layered onto a continuous sucrose density gradient [25%–50% (w/w) sucrose] and centrifuged for 15 h at 30000*g*. The photosynthetic membranes formed one pigmented band, which was collected and washed twice by resuspension and sedimentation (1 h, 100000*g*).

The B800–850 complexes [≈2000 (OD_{800} × mL)] were solubilized in 20 mM Tris-HCl (pH 8)/10% LDAO, by incubation at room temperature for 1 h. Unsolubilized material was removed by centrifugation (40 min, 185000*g*), and the supernatant was applied to a molecular sieve column (Fractogel TSK HW 55; Merck). Two pigmented peaks consisting of the B800–850 complex and a core complex containing the B870 complex and the reaction center, respectively, were then collected. The B800–850 complexes were further purified by anion-exchange chromatography (Mono Q 10/10, Pharmacia). Pigmented material was eluted at two positions by a continuous salt gradient [300 mM NaCl, 20 mM Tris-HCl, pH 8.5, and 0.1% LDAO (w/v)]. Only a minor amount of free carotenoids (<3%) was observed during the purification procedure.

Purification of α- and β-Subunits. The B800–850 complexes were dialyzed overnight against deionized water, centrifuged (5 min, 14000*g*), and solubilized in acetonitrile/water/TFA (1:1:0.001 v/v). After a short centrifugation (10 min, 14000*g*), the supernatant was applied to a RP-HPLC column (C_{18}, Vydac) and eluted with a continuous gradient with acetonitrile/water/TFA (1:1:0.001 v/v) as starting solvent, and with 2-propanol/0.1% TFA for final elution solvent.

Solvent Extraction of Pigments. Ten samples of B800–850 complex containing different amounts of protein (0.3–3 mg) were extracted several times with acetone/methanol (7:2 v/v) until the corresponding pellets were colorless. Both carotenoid (lycopene) and BChl *a* concentrations were calculated from the acetone/methanol extracts using the millimolar absorption coefficients ϵ_{471} = 173 cm^{-1} (Liaanen-Jensen et al., 1971) and ϵ_{772} = 76 cm^{-1} (Clayton et al., 1966), respectively. In the case of lycopene, the absorption coefficient was calculated for pure acetone. The extraction procedure was repeated with another set of 10 samples from a different purification.

Protein Chemistry. The purified B800–850 α- and β-polypeptides were cleaved chemically and enzymatically.

(*A*) *Cleavage by Chymotrypsin.* One nanomole of α-chain was dissolved in 0.2 mL of 0.1 M NH_4HCO_3, pH 8..0, and digested with chymotrypsin (protein:enzyme ratio = 20:1, w/w) overnight at 37 °C. The reaction was stopped by addition of 0.1 mL of 70% formic acid, and the material was subjected to reverse-phase HPLC.

(*B*) *Cleavage by o-Iodosobenzoic Acid.* One nanomole of α-chain was incubated in 0.2 mL of 70% formic acid with *o*-iodosobenzoic acid (protein:reagent ratio = 1:2, w/w) overnight at room temperature. Then the sample was diluted 3-fold with 70% formic acid and applied to a reverse-phase HPLC.

(*C*) *Cleavage by Pepsin.* One nanomole of β-chain was digested with pepsin (protein:enzyme ratio = 7:1, w/w) in 40% formic acid at 37 °C for 30 min. The cleavage mixture was diluted 2-fold and subjected to reverse-phase HPLC (see section A).

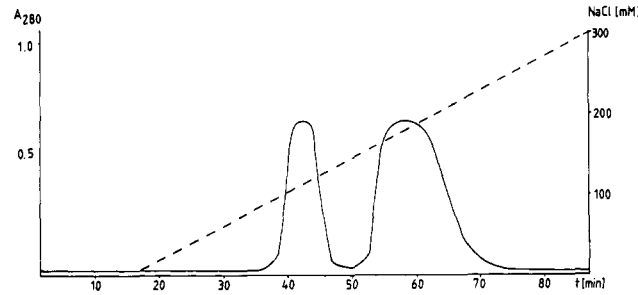

FIGURE 1: Elution profile of the B800–850 complex, separated by anion-exchange chromatography (Mono-Q column, Pharmacia). Elution was performed with a continuous NaCl gradient from a starting buffer of 20 mM Tris-HCl, pH 8.5, and 0.1% LDAO to 20 mM Tris-HCl, pH 8.5, 0.1% LDAO, and 300 mM NaCl at a flow rate of 2 mL/min.

(*D*) *Digestion with Carboxypeptidase.* Five hundred picomoles of β-subunit was digested in 0.05 M sodium citrate, pH 6.38, with 0.4 g of carboxypeptidase Y (sequencing grade, Boehringer Mannheim) for 1 h and overnight, respectively.

The resulting mixtures were injected onto a LiChrospher-100 C_{18} column (Merck, Darmstadt) using a 0.1% trifluoroacetic acid/acetonitrile solvent system at a flow rate of 1 mL/min. A gradient from 0 to 80% acetonitrile/0.1% trifluoroacetic acid in 40 min was applied. The eluted polypeptide fragments were detected at 206 nm.

(*E*) *Amino Acid Sequence Analysis.* Amino acid sequence analysis was performed using a 473A or a 477A gas-phase sequencer (Applied Biosystems, Foster City, CA) according to the instructions of the manufacturer. In addition, intact chains coupled on a DITC support were degraded by solid-phase sequencing using a Milligen sequencer.

Absorption spectra were taken with a Perkin Elmer Lambda 15 spectrophotometer with an optical bandwidth of 2 nm. Resonance Raman spectra were recorded with a Jobin-Yvon HG2S spectrometer at 30 K, using the 363.8-nm excitation of an Argon laser (Coherent Innova 100). FT Raman spectra were recorded at room temperature with an FRA 106 Raman module coupled to an IFS 66 spectrometer (Bruker), and with 1064-nm excitation from a NdYAG laser. Both Raman instuments have been described elsewhere (Robert & Lutz, 1986; Mattioli et al., 1991).

RESULTS

Isolation of the Native B800–800 Complex. The elution profile (Figure 1) of the B800–850 complex from the anion-exchange column shows two peaks. The material from both peaks shows identical absorption spectra characteristic of B800–850 complexes (Figure 2) and the same electrophoretic mobilities (see Figure 5). The ratio of the two peaks varies with different purifications. The B800–850 complexes of the fractions do not differ in molecular weight as deduced from ultracentrifugation (Kleinekofort et al., 1992) and amino acid sequences of the α- and β-polypeptides as determined by reverse-phase HPLC analysis and amino acid sequencing.

Preliminary results (data not shown) indicate that the two protein fractions contain different amounts of phospholipids.

Purification and Sequencing of B800–850 Polypeptides. The RP-HPLC chromatography step separated two polypeptides (peaks 1 and 2, Figure 3). The stoichiometry of the polypeptides is roughly 1:1 as deduced from the peak

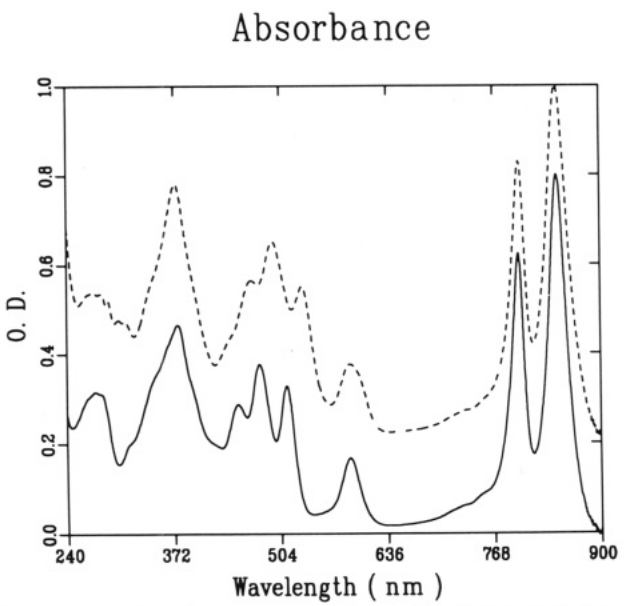

FIGURE 2: Absorption spectra of the purified B800–850 light-harvesting complexes of *Rs. molischianum* DSM 119 (dashed line; for details, see Materials and Methods) and *Rb. sphaeroides* 2.41 (solid line).

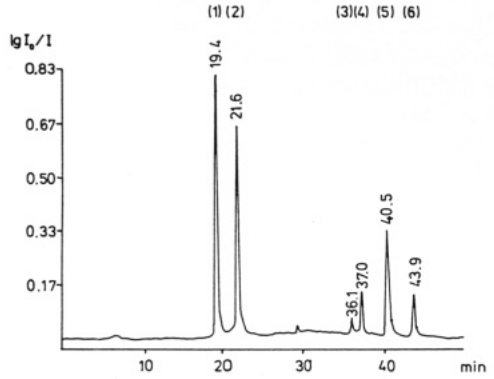

FIGURE 3: Elution profile of the isolated B800–850 complex, separated by reverse-phase HPLC. Dialyzed B800–850 complex (0.5 mg) was solved in the starting solvent (acetonitrile/water/TFA, 1:1:0.001 v/v) and then applied to an RP column (C_{18}, Vydac). Elution was performed with a continuous gradient from starting solvent to 2-propanol/0.1% TFA at a flow rate of 0.5 mL/min. The β- and α-subunits present in peaks 1 and 2 correspond to lanes 2 and 3, respectively, in Figure 4. Pigments elute in peaks 3–6.

integration at the absorption of 280 nm considering the different extinction coefficients of the α- and β-subunits determined at 280 nm. Both polypeptides are highly purified, as shown by SDS–PAGE analysis (Figure 4). Peaks 3–6 visible in Figure 3 are due to pigments (data not shown). The amino acid sequences of these polypeptides were determined by automated Edman degradation. Unique molecular masses were obtained by electrospray mass spectra and indicated that complete sequence information was obtained. The genes coding for the α- and β-subunits have also been cloned and sequenced. The base sequences were in complete agreement with the chemically determined amino acid sequences (data not shown). The molecular weights are 5939 and 5126 as calculated from the amino acid sequences of the α- and β-subunits, respectively.

The amino acid sequence of the α-subunit was determined by gas-phase sequencing [as indicated by (#) in Figure 6] and by solid-phase sequencing (s) of the intact chain and sequence analysis of peptides derived from cleavage with chymotrypsin (c) or o-iodosobenzoic acid (j).

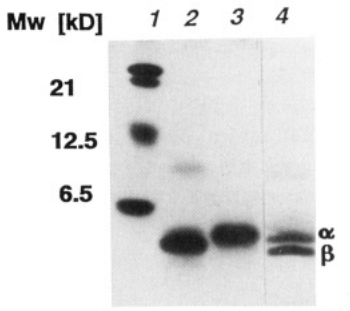

FIGURE 4: SDS–PAGE of the B800–850 α- and β-polypeptides from *Rs. molischianum*. Conditions: 12–22% gradient, in the presence of 4 M urea, stained with Coomassie brilliant blue. Electrophoresis was performed at 80 mA and 0 °C overnight according to Laemmli (1970). Lane 1, standard proteins [trypsin inhibitor (bovine lung), 6.5 kDa; cytochrome *c*, 12.5 kDa; trypsin inhibitor (soybean), 21 kDa]; lane 2, B800–850 β-polypeptide, ≈5 mg of protein applied; lane 3, B800–850 α-polypeptide, ≈5 μg of protein applied; lane 4, B800–850 complex incubated for 3 min at 100 °C, ≈5 μg of protein applied. The band in lane 2 with an apparent molecular mass of approximately 10 kDa represents a dimer of the highly hydrophobic β-subunits.

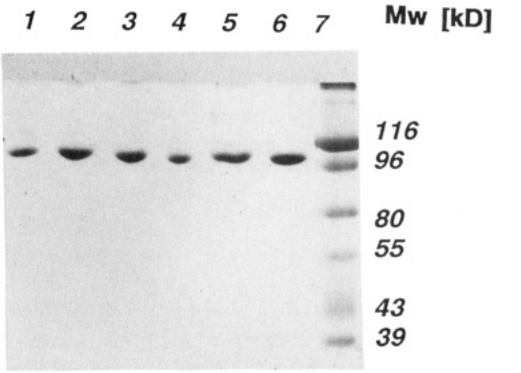

FIGURE 5: SDS–PAGE of the native B800–850 light-harvesting complexes from *Rs. molischianum*. Conditions: 12–22% gradient, stained with Coomassie brilliant blue. Lanes 1–6, the two peak fractions of anion-exchange chromatography (see Materials and Methods) from three different preparations; lane 7, prestained protein standards (triosephosphate isomerase, 39 kDa; lactate dehydrogenase, 43 kDa; ovalbumin, 55 kDa; pyruvate kinase, 80 kDa; fructose-6-phosphate kinase, 96 kDa; β-galactosidase, 116 kDa). All bands are red, and lanes 1–6 show a native spectrum (Figure 2) recorded directly in the gel before Coomassie staining.

The amino acid sequence of the β-subunit was obtained by sequencing the intact chain [gas-phase sequencer (#), solid-phase sequencer (s), and by sequence analysis of a peptide obtained after digestion with pepsin (+)]. The C-terminal amino acid was obtained by carboxypeptidase Y digestion of the intact β-subunit (X).

In the first step, no authentic PTH-alanine could be obtained, but a derivative eluting between dehydroalanine and proline in the PTH separation on the ABI sequencer was observed with the expected UV intensity. However, the DNA sequence indicated an alanine residue in this position and confirmed the presence of a phenylalanine at position 45, which is not part of an overlapping sequence.

Properties of the Native B800–850 Complex. (A) Pigment Composition. The Bchl *a* to carotenoid ratios calculated from two different solvent extractions of purified B800–850 complexes were (2.03 ± 0.04):1 and (2.18 ± 0.02):1, respectively. These results agree best with a Bchl *a* to carotenoid stoichiometry of 2:1. FAB mass spectroscopy with a Kratos MS 902 spectrometer indicated that lycopene (M_w 536) is the major carotenoid present in the complexes. Rhodopsin (M_w 554) is detected as a minor component (<5%). The same

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1        10        20        30        40        50    56
SNPKDDYKIWLVINPSTWLPVIWIVATVVAIAVHAAVLAAPGSNWIALGAAKSAAK
##########################
sssssssssssssssss          sssssssssssssssssss
                                                ccccccccccc
                          jjjjjjjjjjjjjjjjjjj

1   5   10   15   20   25   30   35   40   45
AERSLSGLTEEEAIAVHDOFKTTFSAFIILAAVAHVLVWVWKPWF
*###################################
*sssssssssssssssssssssssssss
                                    ++++++++++++
                                                x
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FIGURE 6: Sequences of the α- and β-subunits from *Rs. molischianum* determined by automated Edman degradation. The symbols mark sequences partly obtained from different polypeptide fragments after chemical and enzymatic cleavage of the subunits. Gas-phase sequencing of the intact subunit (#), solid-phase sequencing of the intact subunit (s), and sequences obtained from peptides after cleavage with chymotrypsin (c), *o*-iodosobenzoic acid (j), pepsin (+), and carboxypeptidase Y (X).

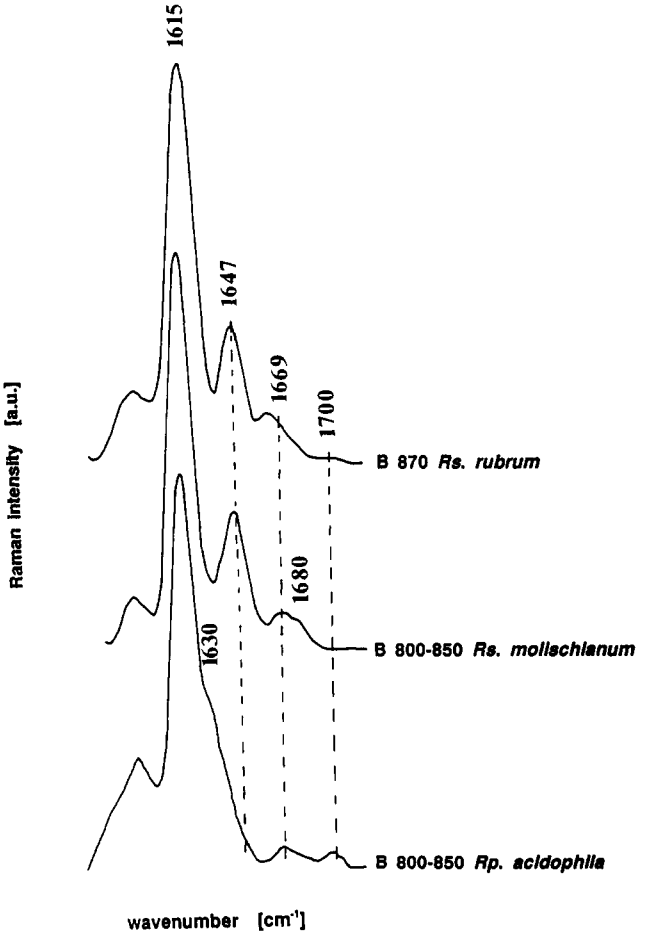

FIGURE 7: Resonance Raman spectra (carbonyl stretch regions) recorded at 30 K, excitation wavelength 363.8 nm, from the B870 complex of *Rs. rubrum* and the B800–850 complexes of *Rs. molischianum* and *Rps. acidophila*. Spectral resolution: 8 cm^{-1} at 1000 cm^{-1}.

experiments indicated a molecular weight of 911 for BChl *a*, demonstrating that BChl *a* possesses a phytyl side chain in *Rs. molischianum* as observed in *Rhodobacter* (*Rb.*) *sphaeroides* (Brockmann et al., 1973).

(*B*) *Resonance Raman Spectroscopy*. Resonance Raman spectra of B800–850 complexes were first recorded at low temperature with 364–nm excitation. The high frequency range (1600–1720 cm^{-1}) of such spectra is displayed in Figure 7. In this region, spectra are dominated by an intense 1615-cm^{-1} band, which arises from the methine bridge stretching mode of the BChl *a* molecule, and which is sensitive to the number of axial ligands on the central Mg ion being located at 1600 cm^{-1} when this atom binds two external ligands, and at 1615 cm^{-1} when only one axial ligand interacts with the Mg ion (Cotton et al., 1981). In the 1600–1720-cm^{-1} frequency region, bands are observed which arise from the stretching modes of the conjugated carbonyl groups, namely, the 2-acetyl and 9-keto groups. When these groups are not involved in intermolecular binding, their peak frequencies are observed at 1660 and 1700 cm^{-1}, respectively, and these may shift down 40 wavenumbers upon H-bonding. Figure 7 shows the high-frequency region of resonance Raman spectra of the *Rs. molischianum* B800–850 complex excited in the Soret band, compared to resonance Raman spectra of the *Rs. rubrum* B870 complex (Robert et al., 1985) and of the *Rhodopseudomonas* (*Rps.*) *acidophila* B800–850 complex (Robert et al., 1988). All these spectra exhibit a strong 1615-cm^{-1} band, which indicates that all Mg atoms of all BChl molecules in these complexes are 5-coordinate. In the carbonyl stretching region, resonance Raman spectra of the *Rs. molischianum* B800–850 complex exhibit a strong 1647-cm^{-1} band, which can arise from bound 2-acetyl groups only, a 1669-cm^{-1} band, which may arise both from unbonded 2-acetyl C=O groups and from bound 9-keto ones, and a 1680-cm^{-1} band, which can readily be attributed to bound keto carbonyl groups. Previous resonance Raman studies on the antenna complexes of more than 12 bacterial species have shown that the resonance Raman spectra of all B870 complexes share common Raman patterns and, in particular, that they all possess a strong 2-acetyl carbonyl mode at 1641/1645 cm^{-1}, while this band was always absent in resonance Raman spectra of B800–850 complexes (Robert & Lutz, 1985; Robert et al., 1988). Resonance Raman spectra of the B800–850 complex of *Rs. molischianum* thus look much more like the resonance Raman spectra of B870 complexes than those of peripheral antennae (see Figure 7). Because of the striking similarity between the resonance Raman spectra of the *Rs. molischianum* B800–850 complex and resonance Raman spectra of any B870 complex studied up to now, we attribute the main bands observed in this spectrum to the carbonyl modes of the B850 bacteriochlorophylls. According to Robert and Lutz (1985), the bands at 1647 and 1660 cm^{-1} can be attributed both to 2-acetyl carbonyl groups and to 9-keto carbonyl groups of the 850-nm-absorbing bacteriochlorophylls, respectively. Contributions of the BChl absorbing at 800 nm should also be present in the spectra. Under these conditions of excitation, however, the intrinsic weakness of the bands arising from the carbonyl stretching vibrations does not allow accurate characterization of these bands. Fourier transform (FT) resonance Raman experiments at room temperature had to be conducted in order to record the whole set of carbonyl bands present in the resonance Raman spectra of the B800–850 complex from *Rs. molischianum*.

Figure 8 presents FT-resonance Raman spectra, i.e., resonance Raman spectra excited in the preresonance with the Q_y transitions of the BChl molecules at 1064 nm, of the *Rs. molischianum* B800–850 complex and the *Rb. sphaeroides* B870 complex. Under these conditions of excitation, the intensity of the carbonyl vibrations is much higher compared to excitation in the Soret band, and, moreover, the intensity of the methine bridge stretching mode is weaker and shifted to lower frequencies (Mattioli et al., 1991). *Rs. molischianum* B800–850 complex FT-Raman spectra exhibits two bands at 1643 and 1668 cm^{-1}, and two shoulders at 1632 and 1678 cm^{-1}, while *Rb. sphaeroides* B870 complex spectra exhibit only two bands at 1643 and 1665 cm^{-1}, as previously reported

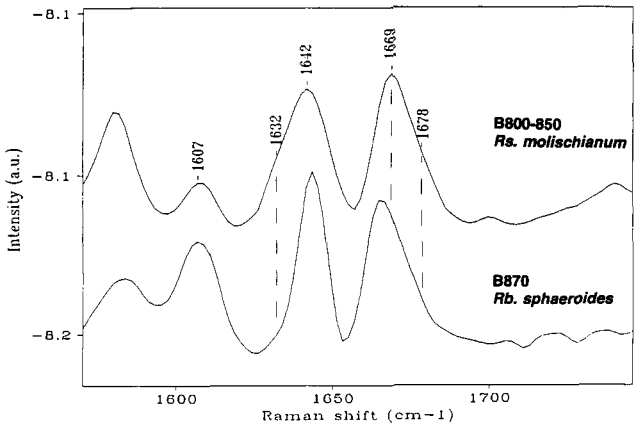

FIGURE 8: Fourier transform resonance Raman spectra, recorded at room temperature, excitation wavelength 1064 nm, from the B870 complex of *Rb. sphaeroides* and the B800–850 complex of *Rs. molischianum*. Spectral resolution: 4 cm^{-1} at 1000 cm^{-1}.

(Robert & Lutz, 1985). Furthermore, a mode at 1632 cm^{-1} is clearly visible in these spectra, which was previously hidden between the strong 1615- and 1642-cm^{-1} modes, as well as a shoulder at 1681 cm^{-1}. The mode at 1632 cm^{-1} can be readily attributed to a H-bonded 2-acetyl carbonyl group (Lutz & Robert, 1986). Its frequency perfectly matches that reported for the 2-acetyl carbonyl group of the 800-nm-absorbing molecule in *Rb. sphaeroides* B800–850 complexes (Robert & Lutz, 1985). However, the additional shoulder at 1678 cm^{-1} arises from a most likely H-bonded 9-keto group located at lower frequencies than that observed in the *Rb. sphaeroides* B800–850 complex, in which the 9-keto group of the 800-nm-absorbing molecule is free from interactions (Robert & Lutz, 1985).

(*C*) *Molecular Weight and Oligomeric State.* The molecular weight of the complex was determined by analytical ultracentrifugation (Kleinekofort et al., 1992) and SDS–PAGE, from which values of 114 500 ± 8000 and about 100 000 were obtained, respectively, for the molecular weight (Figure 5) of the native pigment–protein complex, which agrees very well with an octamer ($\alpha_8\beta_8$). This octamer appears to be very stable since the optical absorbance spectra of the 100-kDa band of the SDS–polyacrylamide gel still look very much like optical absorbance spectra of the isolated complex (Figure 2). Crystallographic data (Michel, 1991) are in agreement with an octamer of the basic $\alpha\beta$ heterodimer being the native B800–850 complex of *Rs. molischianum*.

DISCUSSION

In the present study, we describe the purification and characterization of the B800–850 complex from *Rs. molischianum*. The purified complex can be crystallized. The crystals obtained are well-ordered and diffract X-rays to 2.4-Å resolution (Michel, 1991).

To our surprise, the B800–850 complex was eluted from the final Mono-Q column in the form of two peaks at different NaCl concentrations. The relative amounts of the B800–850 complexes found in both peaks varied. Despite much effort, we have been unable thus far to unequivocally explain the presence of two peaks in the elution profiles from the final Mono-Q column. Our suspicion, that the existence of two peaks in the elution profile might be due to different α- and/or β-subunits, which are found in the B800–850 complexes from *Rhodopseudomonas* (*Rps.*) *palustris* (Tadros et al., 1989), *Rps. acidophila* (Bissig et al., 1988), and *Chromatium vinosum* (Bissig, 1989), could be ruled out. The α- and β-subunits of both peaks from the Mono-Q column eluted at the same positions from the RP-HPLC, and the amino acid sequences found upon Edman degradation were identical. In addition, identical sedimentation profiles were obtained upon analytical ultracentrifugation (Kleinekofort et al., 1992), and the material from both peaks yielded the same crystal forms (unpublished observations). Preliminary results indicate that B800–850 complexes of the two fractions might differ in phospholipid content.

The determined Bchl *a* to carotenoid ratio is 2:1, which was also reported for the B800–850 complexes of *Rb. capsulatus* (Feick et al., 1978) and *Rb. sphaeroides* (Radcliffe et al., 1984; Evans et al., 1988). Considering the generally accepted model that one α,β-subunit binds three Bchl *a*, this pigment ratio indicates the α:β:carotenoid:Bchl *a* stoichiometry to be 1:1:1.5:3 per α,β heterodimer of the B800–850 complex from *Rs. molischianum*.

Examining the electrophoretic mobility of the native B800–850 complex, we obtain an apparent molecular weight of about 100 000 (Figure 5). In order to determine the molecular weight more accurately, sedimentation analysis of the B800–850 complex was performed (Kleinekofort et al., 1992). A value of 114 500 ± 8000 was obtained. The molecular weights determined by these two different methods are in good agreement and suggest an octameric structure of the native B800–850 complex unit.

The crystallographic data are also in agreement with an octamer of the basic α,β heterodimer: the B800–850 complex from *Rs. molischianum* crystallizes in the uncommon space group $P42_12$ with cell dimensions of 92 × 92 × 209 Å. Most likely, four octamers containing 2-fold and 4-fold symmetry elements are present in the crystallographic unit cell. An octameric association is of variance with the generally accepted model that B800–850 light-harvesting complexes are hexamers [for a review, see Zuber and Brunisholz (1991)]. However, the octameric state of the B800–850 complex from *Rs. molischianum* suggests also an intermediate position between B800–850 and B870 complexes, which forms dodecamers (Stark et al., 1984). Such an intermediate position, which is also indicated by sequence similarities and the resonance Raman data, could give new insights into the evolution of the light-harvesting complexes, since most likely the B870 and B800–850 complexes arose from a single ancestral gene by gene duplications (Youvan et al., 1985).

Resonance Raman spectra of the B800–850 complex from *Rs. molischianum* indicate that the bacteriochlorophylls absorbing at 850 nm are most likely involved in the same protein interactions as the bacteriochlorophylls absorbing at 870 nm in B870 complexes. Although the bands cannot be assigned unequivocally, it is likely that the 1642- and 1668-cm^{-1} modes belong to the bacteriochlorophylls absorbing at 850 nm, each of these bands being degenerate as in the B870-type complexes. This hypothesis is consistent with the results of the sequence analysis. According to this hypothesis, the 2-acetyl carbonyl group of the bacteriochlorophyll absorbing at 800 nm vibrates at a frequency close to that observed in *Rb. sphaeroides*. However, whereas in *Rb. sphaeroides* the 9-keto carbonyl group of this molecule is free from interactions, it is bonded in *Rs. molischianum*. This difference in pigment–protein interactions of the 800-nm-absorbing BChl could explain why this molecule is very insensitive to LiDS treatment, whereas in *Rb. sphaeroides* the same treatment results in loss of the 800-nm absorption. The 9-keto carbonyl group of the 800 BChl has been reported to be H-bonded in, e.g., the B800–850 complex from *Rps. palustris*, and it also is difficult to get

FIGURE 9: Sequence comparison of β- and α-subunits of the B800–850 complex from *Rs. molischianum* with β- and α-subunits of B870 and B800–850 complexes from other photosynthetic bacteria. The arrows define the presumed hydrophobic transmembrane segment of the polypeptides.

rid of the 800-nm absorption in this latter case (Robert & Lutz, 1985).

The B800–850 complex of *Rs. molischianum* exhibits resonance Raman spectra similar to B870 complexes from other photosynthetic bacteria and optical absorbance spectra nearly identical to B800–850 complexes. In order to identify amino acid residues possibly involved in pigment binding in the B800–850 complex from *Rs. molischianum*, we have aligned the sequences of the α- and β-polypeptides and sequences of both types of light-harvesting complexes from other bacteria. The two polypeptides of *Rs. molischianum* possess three distinct domains which are found also in all other light-harvesting polypeptides from purple bacteria, namely, a hydrophobic transmembrane stretch (localized by the arrows in Figure 9) and the hydrophilic C- and N-terminal parts of the polypeptides.

The N-terminus of the α-subunit from *Rs. molischianum* differs in length and charge distribution pattern from the B800–850 α-polypeptides of the other bacteria. Most of the B800–850 α-subunits have the same N-terminal length, whereas the α-polypeptide of *Rs. molischianum* possesses four additional amino acids. Two aspartic acids (positions 5 and 6) enclosed by two lysines (positions 4 and 8) provide a unique charge distribution pattern compared to the subunits of both antenna types. Therefore, the positive charges at the N-terminus of the α-polypeptides do not influence the spectral properties directly. However, they may contribute to electrostatic interactions between the α,β-subunits forming the native oligomers.

Those amino acids which are also found in the B800–850 α-polypeptides and not in the B870 α-polypeptides are of special interest, because they might contribute to the specific absorption characteristics of the B800–850 complexes. Only valine-12 and proline-20 are conserved among all B800–850 α-subunits. Proline-20 might be present for structural reasons, either to enable specific interactions between the two bacteriochlorophylls absorbing at 850 nm or to provide an appropriate conformation for the binding site of the 800-nm-absorbing bacteriochlorophyll. The C-terminus of the B800–850 α-subunit from *Rs. molischianum* does not show many similarities to either type of antenna complex except at positions 44 and 45, where asparagine and tryptophan residues are strictly conserved between all B870 α-subunits but not found in any of the other B800–850 α-subunits. We suggest that these two amino acids are necessary for the formation of the B870-like resonance Raman spectra of the B800–850 complex from *Rs. molischianum*. Especially tryptophan-45 might form a hydrogen bond with a 2-acetyl carbonyl oxygen of a bacteriochlorophyll absorbing at 850 nm (1647-cm^{-1} band). This band is present in B800–850 complex of *Rs. molischianum* and B870 complexes from other photosynthetic bacteria, but it is absent in B800–850 complexes from other photosynthetic bacterial strains (see Figure 7). Similar hydrogen bonds are observed in the photosynthetic reaction center from *Rps. viridis* between both bacteriopheophytins and nearby tryptophan residues (Michel et al., 1986).

The similarities of the C-terminal portions from the B800–850 complex β-subunit of *Rs. molischianum* to B870 β-subunits of other strains are even more pronounced. Tryptophan-41 and a positively charged amino acid at position 42 are highly conserved within the B870 β-polypeptides, whereas the B800–850 β-polypeptides contain an amino acid with a hydroxyl residue at position 42. Both types of amino acids could be involved in H-bonding, but it is energetically unfavorable for the charged amino acid side chains to be buried in the membrane.

Therefore, similar to tryptophan-45 in the B800–850 α-subunit, tryptophan-41 is considered to be a possible hydrogen bond donor to the 2-acetyl carbonyl group of a bacteriochlorophyll absorbing at 850 nm (1647-cm^{-1} band).

The membrane-spanning part of B800–850 β-polypeptides contains a strictly conserved arginine at position 25 (*Rs. molischianum* numbering) which is absent in the β-subunit of the B800–850 complex from *Rs. molischianum*. We therefore can definitively exclude that this residue is responsible for the specific absorption characteristics of B800–850 complexes.

The N-terminus of the B800–850 β-subunit is at least two amino acids shorter compared to the other B800–850 β-subunits, whereas the C-terminus is one amino acid shorter than the C-termini of the other B800–850 polypeptides, which all possess the same length. The first three amino acids of the β-polypeptide, alanine, glutamic acid, and arginine, show a B870-like charge distribution pattern, whereas the other B800–850 β-polypeptides start with two negative charges followed by one positively charged residue. The environment of histidine-17, which may be a ligand to the bacteriochlorophyll absorbing at 800 nm, is different at position 19 compared to the B870 and B800–850 β-polypeptides. Most B800–850 β-polypeptides including the B800–850 β-polypeptide of *Rs. molischianum* possess potential H-bond donors at this position, except the B800–850 β-subunit from *Rhodocyclus* (*Rc.*) *gelatinosus* which possesses a glycine at position 19, and histidine-17 is replaced by glutamine. In contrast, the B870 β-polypeptides contain only hydrophobic amino acids at position 19. Therefore, one might speculate that the glutamine at position 19 in the β-subunit from *Rs. molischianum* interacts with the bacteriochlorophyll absorbing at 800 nm.

Recently it was reported (Fowler et al., 1992) that single and double mutations of B800–850–Tyr-47 and B800–850–Tyr-48 (see Figure 9) of the α-polypeptide of the B800–850 complex from *Rb. sphaeroides* cause 11- and 24-nm blue shifts of the 850-nm absorption, respectively. These results demonstrated that tyrosine residues could influence the absorption of BChl *a* in vivo; more recent data (Hunter et al., 1992) suggest that the aromatic character of this residue is not the determining factor but that hydrogen bonding is the modulator of BChl absorbance. The C-terminus of the α-polypeptide from *Rs. molischianum* does not contain tyrosine residues at positions 47 and 48 nor any other potential hydrogen bond donors or aromatic amino residues, which are proposed to influence the BChl absorption by interaction of π–π^* orbitals (Brunisholz & Zuber, 1988). Thus, different mechanisms may ensure the absorption at 850 nm in the B800–850 complexes, depending on the complex studied. In conclusion, the optical properties of light-harvesting complexes are controlled not only by amino acids which directly interact with bacteriochlorophylls but also by amino acid residues that influence the spectral and structural properties of the protein.

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REFERENCES

Bissig, I. (1989) Ph.D. Thesis, No. 8945, ETH Zürich.
Bissig, I., Brunisholz, R. A., Suter, F., Cogdell, R. J., & Zuber, H. (1988) *Z. Naturforsch. 43C*, 77–83.
Brockmann, H., Knobloch, G., Schweer, I., & Trowitzsch, W. (1973) *Arch. Mikrobiol. 90*, 161.
Brunisholz, R. A., & Zuber, H. (1988) in *Photosynthetic Light-harvesting Systems. Organisation and Function* (Scheer, H., & Schneider, S., Eds.) pp 103–114, de Gruyter, New York.
Clayton, R. K. (1966) *Photochem. Photobiol. 5*, 669–677.
Cogdell, R. J., & Thornber, J. P. (1980) *FEBS Lett. 122(1)*, 1–8.
Cogdell, R. J., Woolley, K. J., MacKenzie, R. C., Lindsay, J. G., Michel, H., Dobler, J., & Zinth, W. (1985) *Springer Ser. Chem. Phys. 42*, 85–87.
Cotton, T. M., & Van Duyne, R. P. (1981) *J. Am. Chem. Soc. 103*, 6020–6024.
Dierstein, R., Schumacher, A., & Drews, G. (1981) *Arch. Microbiol. 128*, 376–383.
Evans, M. B., Cogdell, R. J., & Britton, J. (1988) *Biochim. Biophys. Acta 935*, 292–298.
Feick, R., & Drews, G. (1978) *Biochim. Biophys. Acta 501*, 499–513.
Feick, R., & Drews, G. (1979) *Z. Naturforsch. 34C*, 196–199.
Fowler, G. J. S., Visschers, R. W., Grief, G. G., van Grondelle, R., & Hunter, C. N. (1992) *Nature 355*, 848–850.
Hunter, C. N., Fowler, G. J. S., Grief, G. G., Olson, J. D., & Jones, M. R. (1992) *Biochem. Soc. Trans.*, 41–43.
Kleinekofort, W., Germeroth, L., van den Broek, J. A., Schubert, D., & Michel, H. (1992) *Biochim. Biophys. Acta 1140*, 102–104.
Laemmli, U. K. (1970) *Nature 227*, 680–685.
Liaaen-Jensen, J., & Jensen, A. (1971) *Methods Enzymol. 23*, 586–602.
Mattioli, T. A., Hoffmann, A., Robert, B., Schrader, B., & Lutz, M. (1991) *Biochemistry 30*, 4648–4654.
Michel, H. (1991) in *Crystallization of membrane proteins* (Michel, H., Ed.) pp 82–83, CRC Press, Boca Raton, FL.
Michel, H., Epp, O., & Deisenhofer, J. (1986) *EMBO J. 5*, 2445–2451.
Papiz, M. Z., Hawthornthwaite, A. M., Cogdell, R. J., Woolley, K. J., Wightman, P. A., Ferguson, L. A., & Lindsay, J. G. (1989) *J. Mol. Biol. 209*, 833–835.
Pfennig, N., & Trüper, H. G. (1971) *Int. J. Syst. Bacteriol. 21*, 19–24.
Radcliffe, C. W., Pennoyer, J. D., Broglie, R. M., & Niederman, R. A. (1984) in *Advances in Photosynthesis Research* (Sybesma, C., Ed.) Vol. II, pp 215–220, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht.
Robert, B., & Lutz, M. (1985) *Biochim. Biophys. Acta 807*, 10–23.
Robert, B., & Lutz, M. (1986) *Biochemistry 25*, 2303–2309.
Robert, B., Vermeglio, A., Steiner, R., Scheer, H., & Lutz, M. (1988) in *Photosynthetic Light-Harvesting Systems*, de Gruyter & Co., Berlin and New York.
Shiozawa, J. A., Cuendet, P. A., Drews, G., & Zuber, H. (1980) *Eur. J. Biochem. 111*, 455–460.
Stark, W., Kühlbrandt, K., Wildhaber, I., Wehrli, E., & Mühlethaler, K. (1984) *EMBO J.* 3, 773.
Tadros, M. H., & Waterkamp, K. (1989) *EMBO J. 8*, 1303–1308.
Tichy, H. V., Albien, K. U., Gad'on, N., & Drews, G. (1991) *EMBO J. 10*, 2949–2955.
Welte, W., Wacker, T., Leis, M., Kreutz, W., Shiozawa, J., Gad'on, N., & Drews, G. (1985) *FEBS Lett. 182*, 260–264.
Youvan, D. C., & Ismail, S. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 58–62.
Zuber, H., & Brunisholz, R. (1991) in *Chlorophylls* (Scheer, H., Ed.) pp 669–672, CRC Press, Boca Raton, FL.