In Vitro Reconstitution of the Core and Peripheral Light-Harvesting Complexes of *Rhodospirillum molischianum* from Separately Isolated Components†

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ABSTRACT: In most purple bacteria, the core light-harvesting complex (LH1) differs from the peripheral light-harvesting complex (LH2) in spectral properties and amino acid sequences. In *Rhodospirillum* (*Rs.*) *molischianum*, however, the LH2 closely resembles the LH1 of many species in amino acid sequence identity and in some spectral properties (e.g., circular dichroism and resonance Raman). Despite these similarities to LH1, the LH2 of *Rs. molischianum* displays an absorption spectrum similar to the LH2 complexes of other bacteria. Moreover, its crystal structure is very similar to the LH2 of *Rhodopseudomonas* (*Rps.*) *acidophila*. To better understand the basis of the biochemical and spectral differences between LH1 and LH2, we isolated the α and β polypeptides of the LH2 complexes from an LH2-only strain of *Rhodobacter* (*Rb.*) *sphaeroides* as well as the α and β polypeptides from both the LH1 and LH2 complexes from *Rs. molischianum*. We then examined their behavior in reconstitution assays with bacteriochlorophyll (Bchl). The *Rb. sphaeroides* LH2 α and β polypeptides were inactive in reconstitution assays, whether alone, paired with each other, or paired in hybrid assays with the complementary LH1 polypeptides of *Rs. rubrum, Rb. sphaeroides, Rb. capsulatus,* or *Rps. viridis*. The LH1 β polypeptide of *Rs. molischianum* behaved similarly to the LH1 β polypeptides of *Rs. rubrum, Rb. sphaeroides, Rb. capsulatus,* and *Rps. viridis*, forming a subunit-type complex with or without an α polypeptide, and forming an LH1 complex when combined with a native LH1 α polypeptide. Interestingly, the LH2 β polypeptide of *Rs. molischianum*, in the absence of other polypeptides, also formed a subunit-type complex as well as a further red-shifted complex whose spectrum resembled the 850 nm absorbance band of LH2. In the presence of the LH1 α polypeptide of *Rs. rubrum* or *Rs. molischianum*, it formed an LH1-type complex, but in the presence of the LH2 α polypeptide of *Rs. molischianum* it formed an LH2 complex. This is the first reported reconstitution of an LH2 complex using only isolated LH2 polypeptides and Bchl. It is also the first example of an LH2 β polypeptide that can form an LH1 subunit-type complex and an LH1-type complex when paired with an LH1 α polypeptide.

Two major types of light-harvesting complexes are found in purple non-sulfur photosynthetic bacteria. Some species, such as *Rhodospirillum* (*Rs.*) *rubrum*, have only the first kind, the core light-harvesting complex (LH1),[1] which is in close proximity to the photochemical reaction center and has a fixed stoichiometric ratio to it (*1*). Most other species, however, also have an accessory form of light-harvesting complex (LH2), which is more distant from the reaction center, and whose ratio to the reaction center varies depending on environmental conditions such as light intensity, oxygen partial pressure, and temperature (*1*).

Biochemical and spectroscopic studies have shown that LH1 and LH2 are composed of associated heterodimers consisting of bacteriochlorophyll (Bchl) and carotenoid pigments, and an α and β polypeptide, each of which are 5−7 kDa, α-helical, membrane-spanning polypeptides. The amino acid sequences of the light-harvesting polypeptides share many features in common including a hydrophobic membrane-spanning domain flanked by relatively polar N-terminal and C-terminal domains (Figure 1). All LH1 complexes containing bacteriochlorophyll *a* have a single

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[1] Abbreviations: Bchl, bacteriochlorophyll *a*; LH1, core light-harvesting complex (also referred to as B875 after the near-infrared absorption maximum); LH2, a peripheral light-harvesting complex (also referred to as B800−850 after the near-infrared absorption maxima); B800−820, a peripheral light-harvesting complex expressed at low light intensities and temperatures having absorption maxima near 800 and 820 nm; subunit complex (also referred to as B820), subunit form of LH1 or LH2 either isolated from membranes or prepared by reconstitution using native α and β polypeptides and Bchl; LH1-type complex, in vitro reconstituted complex that displays absorption and CD spectra very similar to those of native LH1, but which contains a non-native α and β polypeptide combination and Bchl; subunit-type complex, in vitro reconstituted complex exhibiting absorption and CD spectra very similar to those of the native subunit complex but containing a non-native α and β polypeptide combination or only a native β polypeptide and Bchl; CD, circular dichroism; LDAO, *N,N*-dimethyldodecylamine-*N*-oxide; OG, *n*-octyl-β-D-glucopyranoside; HFA, hexafluoroacetone trihydrate.

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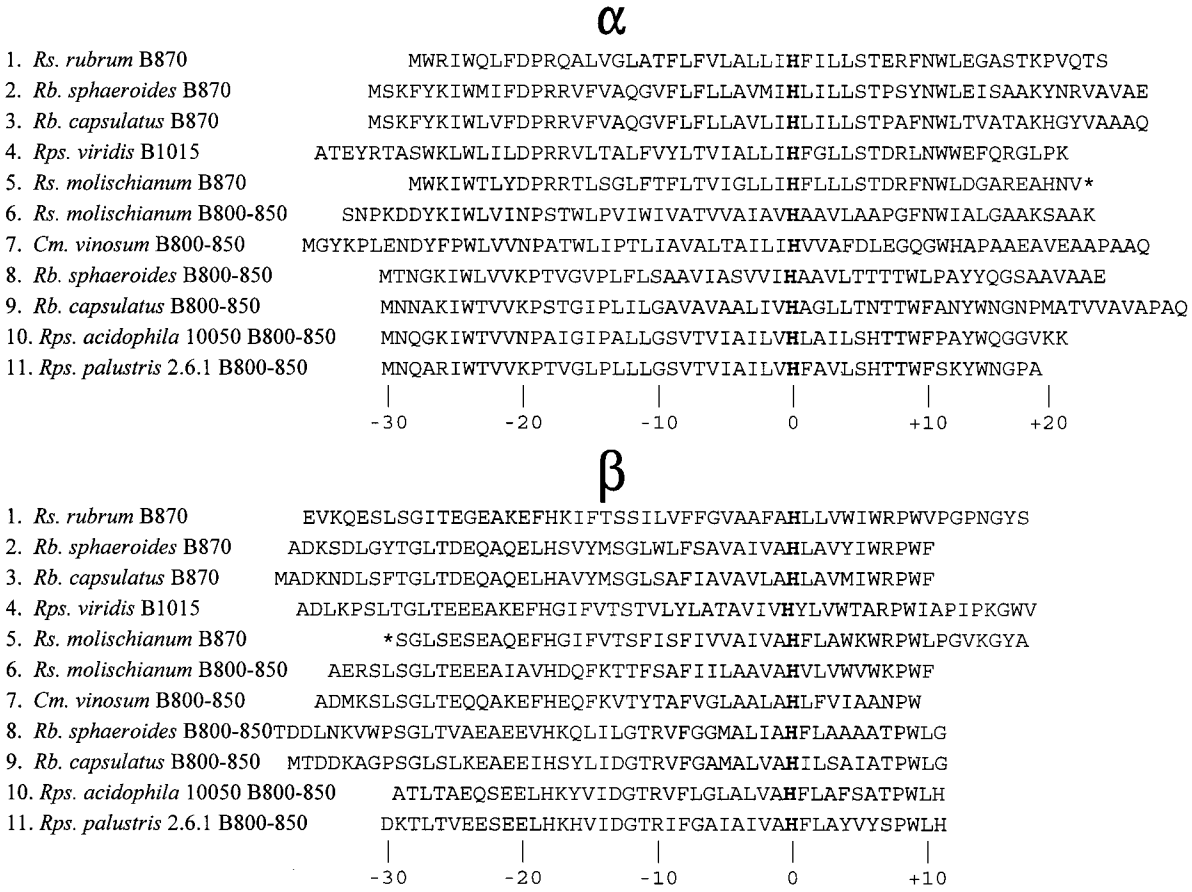

FIGURE 1: Amino acid sequences of the light-harvesting polypeptides of some photosynthetic bacteria. Sources: 1, 2, 4, 7, 8, 10, 11 (*35*); 3, 9 (*40*, *41*); 5 (*3*); 6 (*7*). For ease of comparison, the sequences have been aligned relative to the Bchl-liganding His residue, labeled position 0. Asterisk: The first approximately 7 amino acids of the LH1 β and the last 2 amino acids of the LH1 α polypeptides of *Rs. molischianum* could not be definitely identified by sequencing. See (*3*).

Bchl Q_y absorption band with a maximal absorbance usually between 870 and 900 nm depending on the species (*2*). On the other hand, the LH2 complexes have two Q_y absorption bands, one absorbing near 850 nm, the other near 800 nm [some species, such as *Rhodopseudomonas* (*Rps.*) *acidophila* and *Rs. molischianum*, have additional forms of LH2, synthesized in low-light growth conditions, with absorbance bands at 800 and 820−830 nm (*2*, *3*)]. The crystal structures of LH2 from *Rps. acidophila* (*4*) and *Rs. molischianum* (*5*) show that the 850 nm absorption band arises from an excitonically linked ring of Bchl molecules (B850) liganded to His residues. The second absorption band arises from another, nonexcitonically linked, ring of Bchl molecules (B800) bound more toward the N-terminus (*4*, *5*).

The LH2 complex of *Rs. molischianum* is unique, since some of the spectral properties of its B850 Bchl are more like the B875 Bchl of LH1 than the B850 Bchl of other species' LH2. For example, the circular dichroism (CD) spectrum of the B850 band of *Rhodobacter* (*Rb.*) *sphaeroides* is conservative, while that of *Rs. molischianum* B850 is not, but rather like the CD spectrum of *Rb. sphaeroides* B875 (*3*). Moreover, resonance Raman spectroscopy shows that the $C13^1$ keto carbonyl group [see (*6*) for Bchl numbering system] of the 850 nm Bchl of *Rs. molischianum* is involved in a hydrogen bond similar to the B875 Bchl of the LH1 complex of *Rb. sphaeroides* (*7*). The LH2 crystal structure of *Rs. molischianum* does not show a hydrogen bond donor within the range of this group, however (*5*).

Sequence analyses of the LH2 and LH1 polypeptides of various species have shown that the LH2 polypeptides of *Rs. molischianum* resemble LH1 polypeptides more than do other species' LH2 polypeptides (*8*). In particular, most LH2 β polypeptides contain amino acids that are significantly different than those of LH1 β polypeptides. For example, the *Rb. sphaeroides* LH2 β polypeptide, like most LH2 β polypeptides, has an Arg at position −10 compared with a Trp in the corresponding position in the LH1 β polypeptide. In contrast, the LH2 β polypeptide of *Rs. molischianum* has a Ser in that position, which is similar to residues found in other LH1 β polypeptides. Moreover, whereas most known LH2 β polypeptides have an uncharged residue at the +7 position, the LH2 β polypeptide of *Rs. molischianum* and LH1 β polypeptides have a cationic amino acid (Arg or Lys) at this position.

A powerful tool for evaluating the stabilizing forces in LH1 has been an in vitro reconstitution assay (*9*). The α and β polypeptides of LH1 and Bchl can be isolated, purified, and recombined in detergent solution to form complexes closely resembling LH1 found in vivo. In addition, a subunit complex of LH1 has been isolated from many bacteria (*10*−*12*). This subunit complex can be reconstituted from isolated α and β polypeptides and Bchl in a manner similar to LH1. Interestingly, a subunit-type complex can be reconstituted with Bchl and the LH1 β polypeptides of several bacterial species, without any α polypeptides being present (*6*). Many structural features required for the formation of the subunit

complex have been identified through use of this reconstitution method (*13*−*17*). However, attempts to isolate a subunit complex of LH2 or to reconstitute an LH2 or LH2 subunit complex from separately isolated polypeptides and Bchl have been unsuccessful. In an effort to better understand the interactions determining the distinctive properties of LH1 and LH2, in this paper we report the isolation of the LH2 polypeptides from an LH2-only mutant of *Rb. sphaeroides* and the LH1 and LH2 α and *β* polypeptides from *Rs. molischianum*. The LH2 polypeptides of *Rb. sphaeroides* were inactive in the reconstitution assay, whether alone or in combination with each other or the complementary LH1 polypeptides of *Rb. sphaeroides*, *Rb. capsulatus*, *Rs. rubrum*, or *Rps. viridis*. In contrast, the isolated LH2 α and *β* polypeptides of *Rs. molischianum* formed subunit-, LH1-, and LH2-type complexes in vitro.

MATERIALS AND METHODS

Growth of Bacteria and Membrane Isolation. Rb. sphaeroides LH2 α and *β* polypeptides were obtained from the LH2-only mutant PUHA1 (*18*). The cells were grown in one of two ways. First, PUHA1 grew anaerobically in the dark in Sistrom's minimal medium (*19*) with 60 mM dimethyl sulfoxide and 25 *μ*g/mL kanamycin (*20*). These cells were yellowish green in color and did not grow to high density. Second, PUHA1 grew in Sistrom's minimal medium, with 25 *μ*g/mL kanamycin but without the dimethyl sulfoxide, in 2 L Fernbach flasks which were filled with 1.5 L of media and shaken at 90 rpm at room temperature in the dark. These cells were red in color and grew to a significantly higher density. The near-infrared spectra of the chromatophores and HPLC retention times of the polypeptides isolated from cells grown each way were identical (data not shown).

We obtained *Rs. molischianum* cells from the American Type Culture Collection (catalog number 14031, obtained from NCIB 9957; identical to DSM 120 of the DSMZ collection) and grew them anaerobically in tungsten light in 5.5 L bottles filled with Modified Hutner's medium (*21*) that had been pretreated for 15 min with 0.015% (w/v) sodium sulfide in deionized water. For the standard growth conditions, high light intensity was employed (30−80 mW/cm^2) at 28−30 °C. Thus, we chose conditions that are unfavorable for B800−820 expression and strongly favor LH2 (B800−850) expression (Figure 2). The cells grew very quickly to a high absorbance (A_{847} of 1.5−2.0) within 1 or 2 days. For comparison purposes, two other growth conditions were used to encourage formation of B800−820: 8−12 mW/cm^2 and 0.8−1.2 mW/cm^2 at 20−22 °C.

PUHA1 and *Rs. molischianum* cells were harvested by low-speed centrifugation and washed once with 50 mM potassium phosphate buffer, pH 7.5. Chromatophores were prepared by sonication and differential centrifugation as previously described (*15*). PUHA1 cells grown semiaerobically provided about 200 mg of lyophilized chromatophores per liter of whole cells. One liter of *Rs. molischianum* cells yielded approximately 300 mg of lyophilized chromatophores. Absorption spectra of *Rs. molischianum* chromatophores prepared from cells grown under the various conditions are shown in Figure 2. For isolation of the LH1 and LH2 polypeptides, cells grown at the higher light intensities and temperature were used. Absorption spectra were recorded

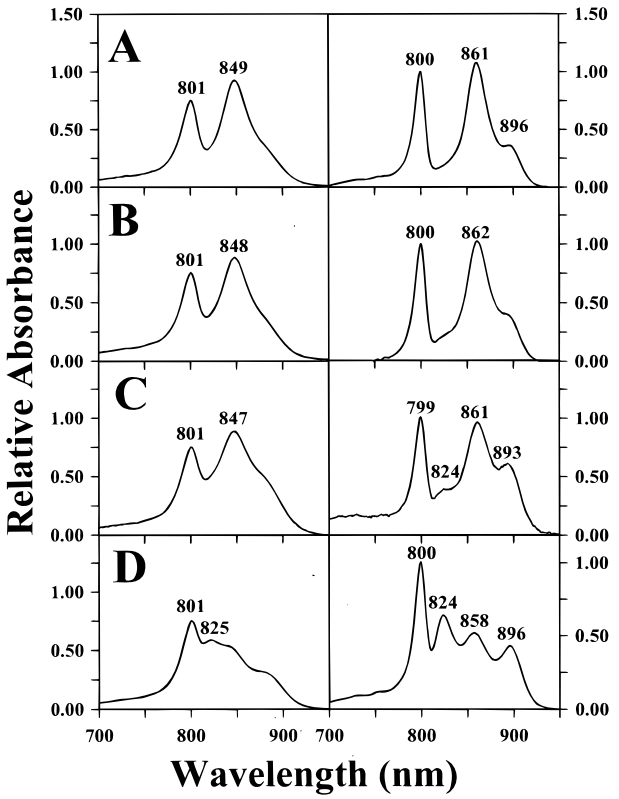

FIGURE 2: Absorption spectra at room temperature (left side) and 77 K (right side) of chromatophores prepared from cells grown at 28−30 °C with irradiation of 80 mW/cm^2 (A) or 30−60 mW/cm^2 (B) or at 20−22 °C with irradiation of 8−12 mW/cm^2 (C) or 0.8−1.2 mW/cm^2 (D). The 77 K data were arbitrarily normalized to an absorbance of 1.0 at 800 nm. The room temperature data were arbitrarily normalized to an absorbance of 0.75 at 800 nm which approximates the ratio of the absorbances at 800 nm for the same sample at room and low temperature.

on a Shimadzu UV160 spectrophotometer interfaced to a Goldstar microcomputer. Low-temperature spectra were recorded using a 1 mm path length cell immersed in liquid nitrogen in a dewar with quartz windows which was placed in the sample compartment. Samples for low-temperature measurements were suspended in 17.5 mM potassium phosphate buffer, pH 7.5, containing 65% glycerol.

Preparation of LH1 from Rs. molischianum. Rs. molischianum LH1 was isolated by the method described in (*22*). Briefly, a solution of chromatophores in deionized water (A_{847} = 50) was mixed with 2 volumes of 1% *N*,*N*-dimethyldodecylamine-*N*-oxide (LDAO, diluted from a 30% aqueous solution purchased from Fluka) containing 10 mM Tris-HCl (pH 8.0). This solution was subjected to a step gradient of 0.3, 0.6, and 1.2 M sucrose, each containing 0.2% LDAO (diluted from the same source as above) and 10 mM Tris-HCl (pH 8.0), and centrifuged at 200000g for 10−14 h at 4 °C; 300 *μ*L fractions were collected from the bottom of the gradient using a Buchler peristaltic pump at 0.6 mL/min. These fractions were diluted with 700 *μ*L of Tris−0.2% LDAO, and their absorbances were measured at 885, 800, and 483 nm (primarily reflecting LH1, LH2, and free carotenoid, respectively) in a 2 mm path length quartz cuvette. The fractions in the peak absorbing maximally at 800 and 885 nm were separately pooled and dialyzed in 12 000−14 000 molecular weight cutoff tubing at room temperature in the dark against deionized water over the

course of 3 days, lyophilized to dryness, and stored in the dark at −20 °C. To calculate yields, we defined the OD units present in a solution to be the absorbance of a sample at a particular wavelength multiplied by the volume of the solution. To determine the number of OD units present in the starting material, the LH2 and LH1 absorbances in the starting chromatophores were deconvoluted using the method described in the appendix. The yield of LH1 was 30−40%, while the LH2 yield was significantly higher, 70−80%.

Characterization of Isolated LH1 and LH2. LH1 and LH2 fractions prepared from sucrose density gradient centrifugation as indicated above were extracted with benzene and methanol as described below. The pellets were solubilized in hexafluoroacetone trihydrate (HFA) and chromatographed by HPLC on a Waters 501 solvent delivery system interfaced to an NEC Powermate SX Plus microcomputer using a reverse-phase C-18 column and solvent gradient (G1) described in (*15*). The absorption spectrum (not shown) of the LH1 fraction occurring at the interface of the 0.6 and 1.2 M sucrose layers was highly comparable to that of LH1 of *Rps. palustris* (*22*). This spectrum and the HPLC chromatogram of this LH1 preparation (Figure 3B) indicated that this fraction was free from LH2 contamination. The concentrations of reaction centers in the LH1 and LH2 fractions were determined by measuring their electron paramagnetic resonance signals under continuous saturating light (*23*). These measurements indicated reaction centers were present to a similar extent in both LH2 and LH1 fractions.

Isolation of Polypeptides. PUHA1 and *Rs. molischianum* polypeptides were extracted from lyophilized chromatophores with 1:1 (v/v) chloroform/methanol containing 0.1 M ammonium acetate. The polypeptides were then chromatographed at 4 °C on a Sephadex LH60 column equilibrated in the same solvent (*24*). The broad peak following the void volume was collected and concentrated to less than 10 mL by removing the chloroform and most of the methanol by rotary evaporation. This fraction was dialyzed against deionized water in 3500 molecular weight cutoff tubing and lyophilized to dryness.

The PUHA1 polypeptides were purified by HPLC using a reverse-phase C-18 column and solvent gradient (G1) described in (*15*). HPLC retention times of these *Rb. sphaeroides* LH2 α and β polypeptides can be found in (*20*). To purify the *Rs. molischianum* polypeptides to homogeneity, the LH60 fraction was extracted with approximately 3 × 1 mL of methanol per milligram of protein to remove residual chlorophyllous pigments. Alternately, the LH1 and LH2 fractions isolated by sucrose density gradient centrifugation were extracted with approximately 3 × 1 mL of benzene to remove carotenoids and then 3 × 1 mL of methanol per milligram of complex to remove Bchl, with the pellet dried under a stream of argon between the benzene and methanol extractions. The polypeptides were then dissolved in HFA and further purified by HPLC using a reverse-phase C-18 column and solvent gradient (G1) described previously (*15*).

Previously described methods were used to isolate LH1 polypeptides from *Rs. rubrum* (*9*), *Rb. sphaeroides* (*14*), *Rb. capsulatus* (*12*), and *Rps. viridis* (*25*).

Identification of Rs. molischianum Polypeptides. Each major HPLC polypeptide peak (Figure 3A,B) was collected and its identity determined by N-terminal amino acid sequencing. N-terminal sequencing was performed either by

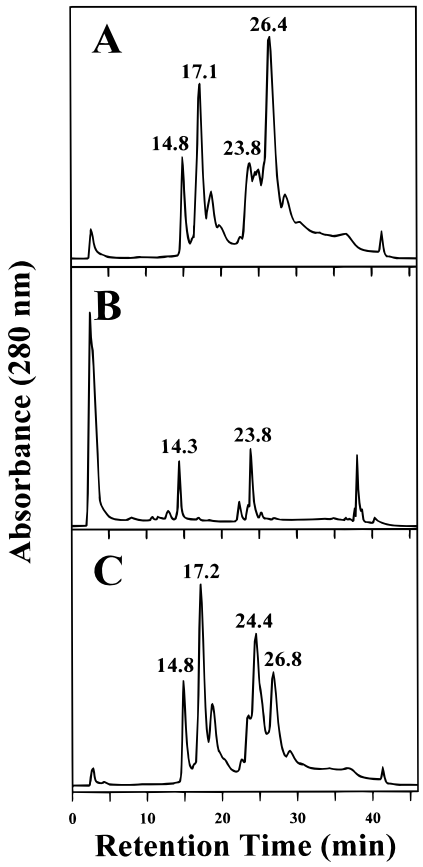

FIGURE 3: HPLC chromatograms of *Rs. molischianum* LH1- and LH2-containing preparations. (A) *Rs. molischianum* polypeptides extracted from chromatophores of cells grown at 28−30 °C with high light intensity whose absorption spectra are shown in Figure 2A. After extraction by 1:1 chloroform/methanol containing 0.1 M ammonium acetate, they were subjected to LH60 chromatography. The dialyzed and lyophilized material was dissolved in HFA for HPLC. (B) LH1 isolated by detergent solubilization of chromatophores and subsequent sucrose gradient centrifugation. The dialyzed and lyophilized material was extracted with benzene and methanol, and the pellet was dissolved in HFA for HPLC. The peak eluting at 14.3 min was identified as the LH1 β polypeptide, and the peak eluting at 23.8 min was identified as the LH1 α polypeptide. (C) *Rs. molischianum* polypeptides extracted from chromatophores of cells grown at 20−22 °C with very low light intensity whose absorption spectra are shown in Figure 2D. After extraction by 1:1 chloroform/methanol containing 0.1 M ammonium acetate, the extracts were subjected to LH60 chromatography. The dialyzed and lyophilized material was dissolved in HFA for HPLC.

the Macromolecular Structure Facility at Michigan State University or by the University of Illinois at Champaign−Urbana, IL. At Michigan State, LH1 and LH2 polypeptides were subjected to automated Edman degradation on an Applied Biosystems (Foster City, CA) Model 494 sequencer. The expected N-terminal five amino acids of LH1 β have been tentatively assigned as SGLSE (*3*); the sequence determined from the 14.3 min peak shown in Figure 3B was SGLSE, which confirms this assignment. Similarly, the expected sequence for the LH2 α polypeptide was SNPKDD (*7*); the sequence determined from the 26.4 min eluting peak shown in Figure 3A was identical.

Like other LH1 α polypeptides [footnote to Figure 4 in (*2*)], the N-terminus of the polypeptide comprising the 23.8 min HPLC peak in Figure 3B was blocked, presumably by a formylmethionine group. The blocking group was removed by a protocol previously reported (*26*). The expected

sequence for the LH1 α polypeptide was MWKIWTLYDP (*3*); the sequence determined for the 23.8 min peak shown in Figure 3B was M_KI_TLYmEP, where mE represents a methylated Glu residue. The low yields of detected amino acids in cycles 2 and 5 are consistent with Trp's vulnerability to oxidation in Edman degradation. The sequence then agrees with the expected sequence of LH1 α at all but one position: mGlu replaces Asp_9, a conservative change. The strain of *Rs. molischianum* sequenced in (*3*) was the DSM-119 strain, while the strain we obtained from the American Type Culture Collection was catalog number 14031 (*27*), which is identical to DSM-120. Assuming there are a small number of point mutations arising from the different lineages of the strains, we concluded that the 23.8 min peak was the LH1 α polypeptide.

The expected N-terminal 10 amino acids of the LH2 *β* polypeptide were AERSLSGLTE (*7*). Sequencing of the 17.1 min HPLC peak shown in Figure 3A (when isolated from chromatophores by organic solvent extraction and LH60 chromatography) yielded two sequences: XEKSLSGLTE (80%) and ELSLSGLTEE (20%). The major component differs from the published *Rs. molischianum* LH2 *β* sequence at only two positions: an unknown amino acid analogue, X, replaces Ala at the N-terminus, and a conservative change is seen from Arg to Lys at position 3. Again, we conclude that this peak represents the LH2 *β* polypeptide, with a small number of point mutations arising from differences in the strains.

Interestingly, if the unknown amino acid analogue is removed from the N-terminus of the major component referred to above, the result is identical to the minor component at all but the second position, where there is a Leu instead of a Lys. Similarly, when the 17−18 min HPLC peak was isolated from the LH2 fraction obtained by detergent solubilization of chromatophores and sucrose gradient centrifugation and then sequenced, a family of three sequences was obtained that was in 80% agreement with the published sequence but differed only in the number of amino acids at the N-terminus. These results may indicate the presence of an amino peptidase activity which modified some of the LH2 *β* polypeptides during their isolation from chromatophores.

Reconstitution Assay. Bchl*a* with a geranyl-geraniol esterifying alcohol was isolated from the G9 carotenoidless mutant of *Rs. rubrum* as previously described (*15*). This form of Bchl was chosen rather than Bchl*a* with a phytol esterifying alcohol, the form of Bchl found in *Rs. molischianum* LH2 (*5*), because it is more easily isolated, and previous studies with *Rb. sphaeroides* and *Rs. rubrum* (*28*) have shown that the nature of the esterifying alcohol has no effect on formation of subunit and LH1 complexes.

A crude carotenoid preparation was obtained by extracting *Rs. molischianum* carotenoids from chromatophores with petroleum ether, boiling point 30−60 °C, as described in (*14*). Pure preparations of lycopene and rhodopin, the main carotenoid components of *Rs. molischianum* LH2 (*7*), were obtained by HPLC of benzene extractions of chromatophores. The column and chromatographic methods used are described in (*14*). Lycopene was also purchased from Sigma.

Reconstitutions of the LH1 and LH2 complexes were performed as described (*15*), using *n*-octyl-*β*-D-glucopyranoside (OG). In some experiments, carotenoid was also added as described in (*14*). All absorption spectra were recorded on a Shimadzu UV160 spectrophotometer interfaced to a Goldstar microcomputer. Opal glass filters were placed in both the sample and reference beams to reduce the effects of light scattering. CD spectra were measured on a Jasco J500C spectropolarimeter interfaced to a Leading Edge microcomputer using 0.1, 1, or 2 cm quartz cuvettes. The absorbance of the CD samples was between 0.1 and 0.8 at the long-wavelength absorption band. Association constants for the formation of the subunit complex were determined as previously described (*29*) after deconvoluting the spectra using the method described in the appendix.

RESULTS

Reconstitutions with Native LH2 Polypeptides of Rb. sphaeroides. When the *Rb. sphaeroides* LH2 α and *β* polypeptides or the LH2 *β* polypeptide alone was tested in the reconstitution assay, the Bchl Q_y absorption band did not red shift but remained at 777 nm (data not shown). When the LH2 *β* polypeptide of *Rb. sphaeroides* was combined with the LH1 α polypeptide from *Rb. sphaeroides*, *Rb. capsulatus*, *Rs. rubrum*, or *Rps. viridis*, the results were the same (no red shifted Q_y absorption bands were observed; data not shown). If the LH2 α polypeptide was combined with the LH1 *β* polypeptide from any of those four species, results similar to those obtained with only LH1 *β* polypeptides were observed [(*13*); data not shown]. Association constants calculated for these assays were comparable to samples with LH1 *β* polypeptide alone. Thus, the LH2 α polypeptide of *Rb. sphaeroides* did not seem to interact with the LH1 *β* polypeptides in the reconstitution assay.

Isolation and Identification of LH1 and LH2 Polypeptides from Rs. molischianum. Because the strain of *Rs. molischianum* available to us (DSM 120) can produce the B800−820 complex as well as the LH2 complex (*3*), we sought to grow cultures under conditions in which little, if any, B800−820 complex was produced. For *Rps. acidophila*, it has been shown that expression of LH2 is greatly favored by high light intensity and higher temperatures (*30*). Accordingly, we investigated the LH2 and B800−820 contents in *Rs. molischianum* grown at a series of light intensities and at temperatures of either 28−30 °C or 20−22 °C (Figure 2). The results from the 77 K spectra (Figure 2, right side) show that no, or very little (approximately 3−5% of the LH2 level), B800−820 is expressed at the higher light intensity and temperatures routinely used in these studies. Even at a 10-fold lower light intensity and lower temperature (Figure 2C), B800−820 is expressed to only about 10% of the LH2 level. If a 1−100-fold lower light intensity is employed at the lower temperature, a higher content of B800−820 is obtained (Figure 2D). For all preparations of LH1 and LH2 polypeptides reported here, *Rs. molischianum* cells were grown at higher light intensities and temperature.

When the light-harvesting polypeptides from *Rs. molischianum* were extracted, separated by size-exclusion chromatography on LH60, and analyzed by HPLC, many peaks were apparent (Figure 3A). To help determine which peaks corresponded to which light-harvesting polypeptides, we isolated the LH1 complex by detergent solubilization and sucrose density gradient centrifugation using a discontinuous

gradient of 0.3, 0.6, and 1.2 M sucrose. The heavier greenish brown LH1 complex migrated to the interface of the 0.6 and 1.2 M sucrose layers, the reddish LH2 complex migrated to the 0.3 and 0.6 M interface, and displaced carotenoids remained at the top of the gradient. Polypeptides present in these fractions were evaluated by HPLC. The 14.3 and 23.8 min peaks in Figure 3B were presumed to be LH1 polypeptides, and by implication the 17.1 and 26.4 min peaks in Figure 3A were presumed to be LH2 polypeptides. These assignments were confirmed by N-terminal sequencing (see Materials and Methods).

The locations of the B800−820 α and β polypeptides on HPLC were surmised by comparing the HPLC profiles in Figure 3A,C. According to the absorbance spectrum of the chromatophores (Figure 2D) used for the HPLC profile shown in Figure 3C, the populations of LH1, LH2, and B800−820 were similar, with perhaps twice as much B800−820 as LH2. In Figure 3C, the HPLC peak at 26.8 min, assigned to the LH2 α polypeptide, is much reduced relative to the corresponding peak at 26.4 min in Figure 3A. Concomitantly, a prominent peak at 24.4 min appears in Figure 3C, suggesting that this peak is the B800−820 α polypeptide. One would also expect the peak at 17.2 min in Figure 3C (assigned to the LH2 β polypeptide in Figure 3A) to be diminished similarly to the peak at 26.8 min. It is highly likely, however, that this is also the location of the B800−820 β polypeptide, whose amino acid sequence was reported to be identical to that of the LH2 β polypeptide except for three very conservative changes (*31*) (see Discussion). No attempt was made to further purify or characterize the B800−820 polypeptides.

Reconstitutions with Rs. molischianum LH Polypeptides. When the LH2 β polypeptide was combined with Bchl in the reconstitution assay, a subunit-type complex was formed which absorbed at 822 nm (Figure 4A). The association constant was 2.7×10^{16} M^{-3} at 0.90% OG, comparable to LH1 β polypeptides previously characterized (*13*). The CD spectrum of this complex (Figure 4B) was also characteristic of previously described LH1 subunit complexes' CD spectra (*10*−*12*). In the Q_y band region of Bchl, there is a nonconservative trough and peak at 818 and 779 nm, respectively, with the trough having a larger magnitude than the peak. In the Q_x band region of Bchl, there is a small CD band, with a trough at a longer wavelength and a peak at a slightly shorter wavelength. Finally, in the Soret band region, there are a nonconservative trough and peak at 379 and 347 nm, respectively, with the trough again having the greater magnitude. After chilling the sample overnight, the Q_y absorption maximum shifted to about 848 nm. The CD spectrum of this complex was nearly conservative, with a zero crossing at 847 nm, the maximum of the absorption spectrum (Figure 4C). There was some variation in the time required for formation of this red shifted complex, with some samples requiring only a few hours chilling and others requiring as long as 3−5 days chilling at 4 °C.

When the LH2 β polypeptide, Bchl, and the LH2 α polypeptide of *Rs. molischianum* were present in a reconstitution assay, a subunit complex absorbing at 817 nm was formed (Figure 5A) with an association constant of 5.3×10^{17} M^{-3} at 0.90% OG. This association constant is about 20-fold greater than that for the system containing only the

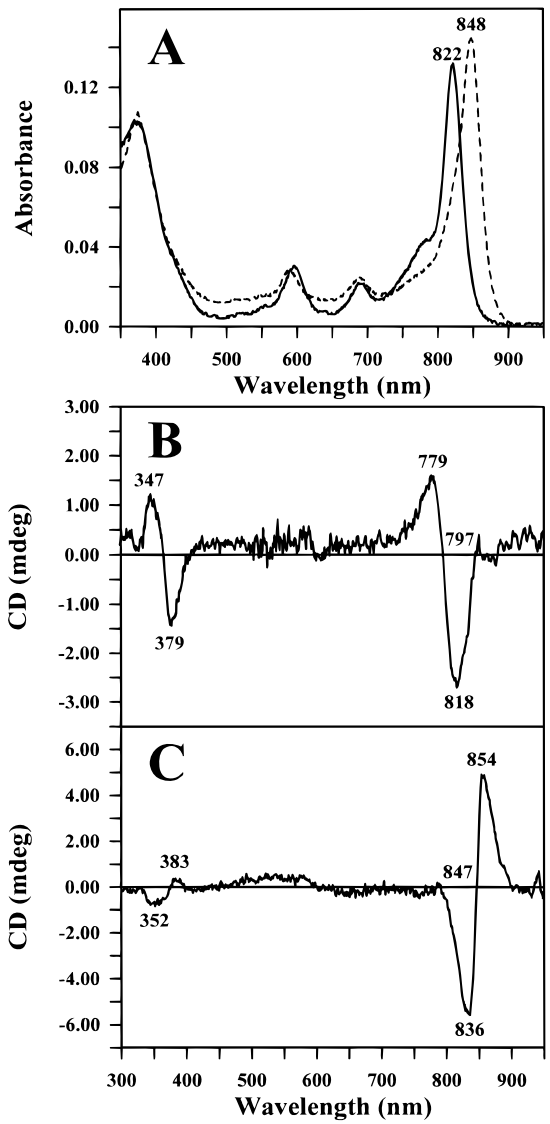

FIGURE 4: Reconstitution assay performed with the *Rs. molischianum* LH2 β polypeptide and Bchl. (A) Absorption spectrum under subunit-forming conditions of 0.75% OG (solid line) and under LH1-forming conditions of 0.66% OG after incubating at 4 °C overnight (dashed line). Concentrations: $\beta = 6.5$ μM, Bchl = 1.8 μM at 0.75% OG. Spectra were recorded in 1 cm cuvettes. (B) CD spectrum of the subunit-type complex at 0.9% OG and room temperature. Concentrations: $\beta = 3.6$ μM, Bchl = 2.1 μM. (C) CD spectrum of the red-shifted complex. The sample was the same as in (B), only at 0.66% OG, chilled overnight at 4 °C, and measured at approximately 13 °C. CD parameters (500−950 nm): time constant = 4 s, slit width = 240 μm, sensitivity = 2 mdeg/cm, step resolution = 1 nm; (300−650 nm): slit width was variable because the spectral bandwidth was set to 2 nm. The spectra in (B) and (C) were recorded in a 2 cm cuvette. In (B), two scans were averaged and an averaged base line was subtracted. In (C), the recorded spectrum appeared to be a mixture of the subunit-type complex and the red-shifted complex. After subtracting an averaged base line, 86.68% of the spectrum in (B) was subtracted to yield the spectrum shown in (C).

β polypeptide, indicating that the α polypeptide helped to stabilize this complex. The CD spectrum of the subunit complex (Figure 5B) was similar to that of LH1 subunit complexes (*10*−*12*) except for small changes in the Soret region. Upon chilling the sample to achieve LH1-forming conditions, the peak of maximal absorbance shifted to 847 nm, and the spectrum also developed a peak at 799 nm similar to LH2. The CD spectrum of this LH2 complex

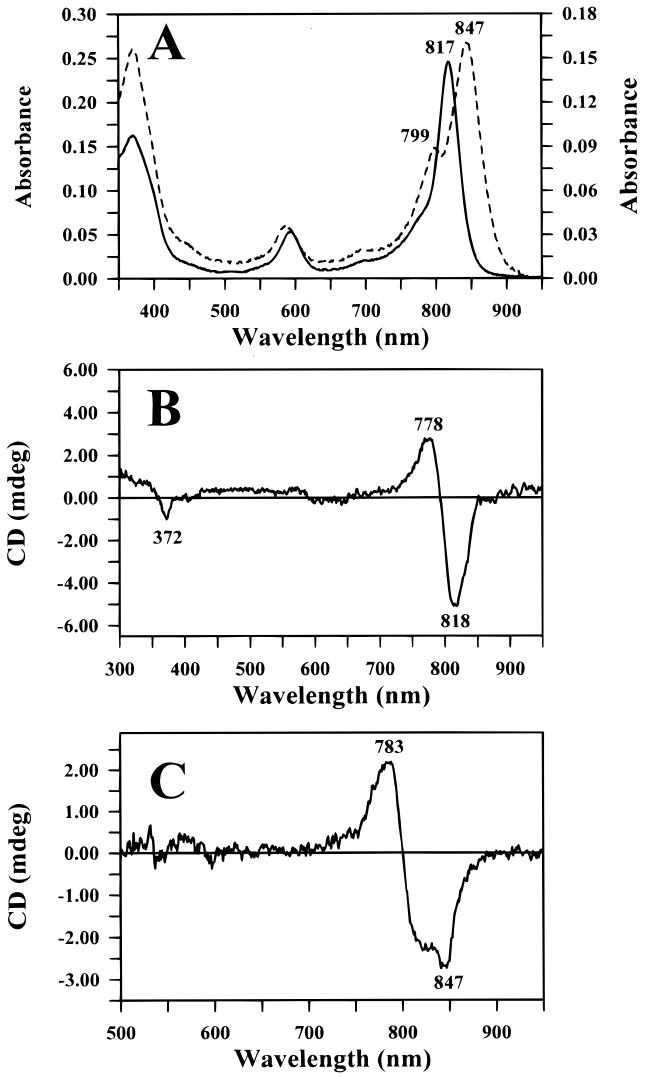

FIGURE 5: Reconstitution assay performed with the *Rs. molischianum* LH2 α and *β* polypeptides and Bchl. (A) Absorption spectrum under subunit-forming conditions of 0.75% OG (solid line, left absorbance scale) and under LH2-forming conditions of 0.66% OG after incubation at 4 °C overnight (dashed line, right absorbance scale). Concentrations: α = 3.9 *μ*M, *β* = 6.5 *μ*M, Bchl = 1.6 *μ*M at 0.75% OG. (B) CD spectrum of the LH2 α and *β* polypeptide reconstitution under subunit-forming conditions of 0.9% OG at room temperature. Concentrations: α = 5.3 *μ*M, *β* = 3.6 *μ*M, Bchl = 3.0 *μ*M. (C) CD spectrum of the LH2 α and *β* reconstitution under LH2-forming conditions of 0.66% OG after chilling overnight at 4 °C and measured at approximately 13 °C. Concentrations: Same as in (A). The spectra in (B) and (C) were recorded in a 2 cm cuvette. CD parameters were the same as in Figure 5. Note the differences in the scales of the abscissae in (B) and (C).

(Figure 5C) closely resembled that of the LH2 complex of *Rs. molischianum*, whether present in chromatophores or isolated in detergent (Figures 6A,B, respectively). Very little LH1 CD can be observed because of the relatively high concentration of LH2 in the membrane and because LH1 CD signals are generally much weaker than LH2 CD signals (*32*). The LH1 isolated in this work also had a very weak CD signal (data not shown). It should be noted that the extent of formation of the band at 800 nm and the CD spectra varied considerably in separate experiments; the B800 band was sometimes only a shoulder on the B850 band, or, at the other extreme, exhibited a distinct band whose absorption was half as intense as the 850 nm band. The data shown in Figure 5

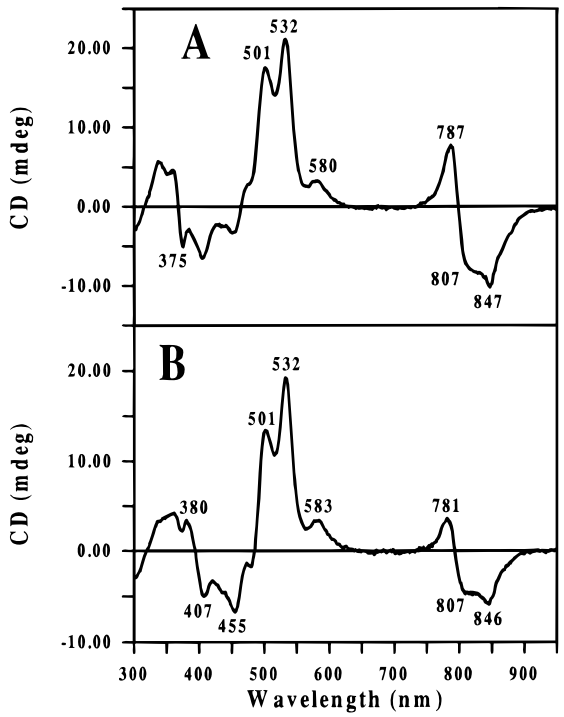

FIGURE 6: CD spectra of *Rs. molischianum* chromatophores (A) and LH2 isolated by detergent solubilization and sucrose gradient centrifugation (B). The chromatophores had a concentration of 0.25 mg/mL in deionized water. The LH2 was taken directly from the sucrose gradient and diluted with 2 volumes of 10 mM Tris (pH 8.0) with 0.2% LDAO. The spectrum in (A) was recorded in a 1 cm cuvette, and the spectrum in (B) in a 0.1 cm cuvette, both at room temperature. CD parameters were the same as in Figure 5.

were for experiments displaying an intermediate size 800 nm band.

Carotenoid was added to these reconstitution assays in the same way that it was successfully reconstituted into LH1 preparations (*14*). Isolated lycopene and rhodopin were found to be without effect on formation of the subunit and LH2 complexes with Bchl and the LH2 α and *β* polypeptides (data not shown). A crude petroleum ether carotenoid extract was also added to reconstitution systems and found to be without effect on the near-infrared absorption spectra. However, in this case, some native-type CD spectra were observed in the carotenoid absorbing region (data not shown).

Upon combining the LH1 *β* polypeptide with Bchl in the reconstitution assay, a subunit-type complex was partially formed (data not shown) which had an absorbance maximum of 818 nm and an association constant at 0.75% OG of 1.5×10^{15} M^{-3}. After chilling overnight, the absorption maximum reverted to 777 nm, the same as that of Bchl free in detergent solution. When the LH1 *β* polypeptide of *Rs. molischianum* was assayed with the LH1 α polypeptide of *Rs. molischianum* (data not shown) or the LH1 α polypeptide of *Rs. rubrum* (Figure 7A), subunit complexes were more readily formed absorbing at 818 nm, with association constants at 0.90% OG of 3.4×10^{16} M^{-3}. After chilling these samples overnight, typical LH1 complexes were formed with an absorption maximum at 878 nm.

When a hybrid reconstitution was conducted with the LH2 *β* polypeptide of *Rs. molischianum*, the LH1 α polypeptide of *Rs. rubrum*, and Bchl (Figure 7B), a subunit-type complex readily formed with an association constant of 9.9×10^{16}

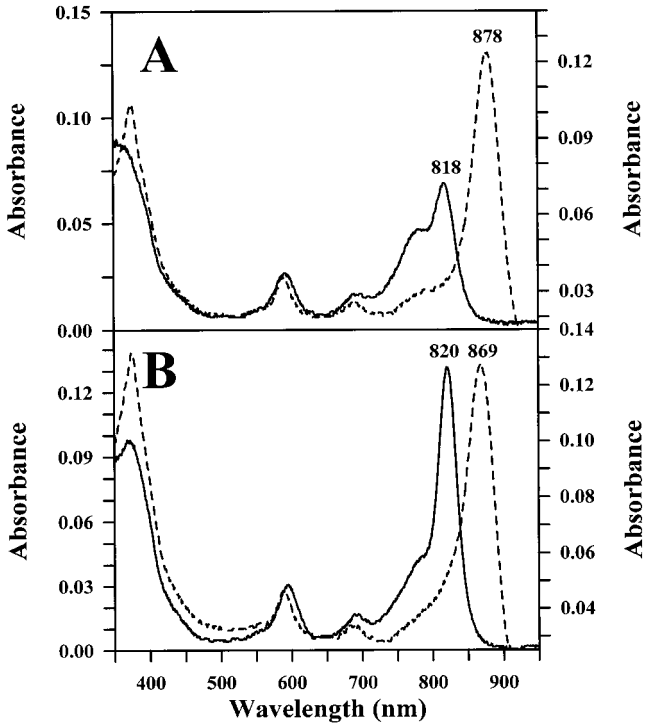

FIGURE 7: Reconstitution assays performed with the *Rs. rubrum* LH1 α polypeptide and the LH1 (A) or LH2 (B) β polypeptide of *Rs. molischianum*. Absorption spectra were recorded under subunit-forming conditions of 0.75% OG at room temperature (solid lines, left absorbance scales), or LH1-forming conditions of 0.66% OG after chilling at 4 °C overnight (dashed lines, right absorbance scales). Concentrations: (A) α = 2.7 μM, β = 1.9 μM, Bchl = 1.3 μM at 0.75% OG. (B) α = 2.7 μM, β = 6.5 μM, Bchl = 1.8 μM at 0.75% OG.

M^{-3} at 0.90% OG and absorbing at 820 nm. Interestingly, upon chilling, an LH1-type complex also formed with an absorption maximum at 869 nm (Figure 7B) instead of 848 nm where the LH2 β-only sample or the LH2 α + β sample absorbed.

It is important to emphasize the high-affinity interactions exhibited by the reconstituted systems. The subunit complexes can be formed from nanomolar to micromolar concentrations of polypeptides and Bchl, and the measured association constants were quite high, indicating highly specific interactions. Multiple experiments conducted in this laboratory have shown that Bchl, by itself, does not form red-shifted species under subunit-forming conditions. The native α polypeptides of these bacteria never form red-shifted complexes with Bchl unless a β polypeptide is also present. Other unrelated proteins do not interfere with these reconstitutions at the low concentrations chosen (*6*).

DISCUSSION

Isolated LH2 α and β polypeptides of *Rb. sphaeroides* did not form complexes with red-shifted Q_y bands with Bchl, either alone, with each other, or with complementary LH1 polypeptides from *Rb. sphaeroides*, *Rb. capsulatus*, *Rs. rubrum*, or *Rps. viridis*. From these results, it can be concluded that the significant differences in the amino acid sequences (Figure 1) of the LH2 and LH1 β polypeptides prevent the *Rb. sphaeroides* LH2 β polypeptide from forming subunit, LH1-type, or LH2 complexes in vitro.

In contrast, the LH2 β polypeptide of *Rs. molischianum* formed an LH1 subunit-type complex as well as an LH1-type complex in the presence of an LH1 α polypeptide, or an LH2 complex in the presence of the *Rs. molischianum* LH2 α polypeptide. At higher concentration, it also formed a subunit-type complex and a further red-shifted complex with a Q_y band at 847 nm in the absence of any added α polypeptide. This red-shifted complex resembled native LH2 except for the lack of the 800 nm band. Addition of pure carotenoid, either lycopene or rhodopin, or crude carotenoid obtained by extracting chromatophores with petroleum ether had no effect on the near-infrared absorption spectrum of reconstituted LH2. This result seems consistent with the fact that detergent can occupy the carotenoid binding site in the *Rb. sphaeroides* reaction center (*33*) and may do so in the *Rps. acidophila* and *Rs. molischianum* LH2 crystal structures (*4*, *5*) without loss of functional properties.

Formation of Subunit Complexes. The similarities of the CD and absorption spectra of the subunit complexes, as well as the very large association constants, indicate that these complexes are similar in structure to LH1 subunit complexes previously isolated from chromatophores (*10*−*12*, *34*) or reconstituted in vitro using native LH1 α and β polypeptides and Bchl (*6*). With only minor variations in the absorption maxima and minima of the CD bands, the CD spectra of subunit complexes all exhibit the same features. Such similarities in subunit complexes support the idea of a common evolutionary origin of both LH2 and LH1, as has been suggested by comparison of the amino acid sequences of LH1 and LH2 α and β polypeptides (*35*).

Possible Presence of B800−*820.* Because a B800−820 complex has been reported to be inducible in the same strain of *Rs. molischianum* used in these studies (*3*), it is important to determine whether such a complex is present under our growing conditions. The amino acid sequences of the B800−820 α and β polypeptides for a pseudorevertant of an LH2-(−) mutant of the DSM 119 strain were identical in the first 26 and 13 amino acids of the N-termini to those of the B800−850 α and β polypeptides, respectively (*31*). Therefore, the N-terminal amino acid sequence determination would not distinguish between the α or β polypeptides of the two species. Three observations allow us to conclude that the possible expression of B800−820 is not a significant concern in these studies. First, growth conditions were selected which highly favor LH2 expression. The chromatophores used for isolation of the α and β polypeptides of LH1 and LH2 had absorption spectra at low temperature which displayed a possible B800−820 content of only between 0 and 10% (most preparations were between 3 and 7%) relative to the LH2 content (Figure 2). Second, the major components of LH2 separated by HPLC (Figure 3) are accounted for by B800−850 and B875 α and β polypeptides. Whereas the near-identity in amino acid sequence of the β polypeptides of B800−820 and B800−850 would likely result in comigration during HPLC, the amino acid sequences of the α polypeptides are significantly different and a unique peak was observed which is attributed to the B800−820 α polypeptide (Figure 3C). Finally, comparison of the amino acid sequences of the B800−850 and B800−820 β polypeptides reveals only three changes: Ile_{-21} to Val, Ile_{-6} to Leu, and Val_{+4} to Ile (numbering scheme of amino acids is that of Figure 1). These highly conservative changes make it very

likely that the B800−820 β polypeptide would show the same behavior as the LH2 β polypeptide. When evaluating the identity of the 820 nm component in Figure 2, it is important to recall that the LH2 β polypeptide is able to combine with Bchl with high affinity (with or without an α polypeptide) to form a subunit-type complex. Thus, conditions could exist in these cells where some 820 nm absorbance could arise due to the existence of small populations of the LH2 subunit complex and not due to the B800−820 complex.

The differences between the amino acid sequences of the B800−850 and B800−820 α polypeptides are more significant, however, in that 13 differences were reported, including several that are not of a conservative nature (*31*). These changes include Trp_{+11} to Phe and a three amino acid substitution from ThrValVal at −7 to −5 to LeuLeuThr. The Trp_{+11} to Phe alteration is especially interesting since a similar mutation in LH1 of *Rb. sphaeroides* shifts the absorbance maximum to the blue by up to 20 nm (*36*). Thus, the location of the Q_y band is likely to be a good probe for whether the isolated α polypeptide is from a B800−820 or a B800−850 complex. The fact that the complex reconstituted using the 26.4 min peak from HPLC (Figure 3A) had a Q_y band at 847 nm (Figure 5A) strongly argues that the polypeptide is the B800−850 α polypeptide. Further evidence in support of this assignment comes from comparing the HPLC profiles in Figures 3A and 3C. In the chromatophores from which the material in Figure 3A was isolated, there was no evidence for the presence of a B800−820 complex (Figure 2A), while in the chromatophores from which the material in Figure 3C was isolated, a high content of the B800−820 complex was indicated (Figure 2D). Thus, the prominent 26.4 min peak of Figure 3A is substantially reduced in Figure 3C, while a peak at 24.4 min becomes prominent.

Amino Acid Similarities between LH2 and LH1 β Polypeptides. The LH2 β polypeptide of *Rs. molischianum* shares several amino acids in common with the LH1 β polypeptides of other species, which the LH2 β polypeptides of other species lack (Figure 1). These include Lys_{+7}, which has a cationic side chain like the Arg_{+7} of LH1 β polypeptides; an uncharged Ser residue at the −10 position, unlike the somewhat conserved Arg residue found in the LH2 β polypeptides of many other bacteria; and Trp_{+4} and Trp_{+6}, which are conserved in all LH1 β polypeptides (except for *Rb. sphaeroides*, which has a Tyr at the +4 position). In LH1 β polypeptides, three of these four residues have been shown to be important in stabilizing subunit and LH1 complexes. In *Rb. sphaeroides*, the Tyr_{+4} stabilizes the subunit by 1.6 kcal/mol (*16*), and the Trp_{+6} and Arg_{+7} stabilize the subunit by about 1.4 and 2.0 kcal/mol, respectively (*17*). Moreover, mutation of the Trp_{+6} in the LH1 of *Rb. sphaeroides* has been shown to destabilize the complex, perhaps by altering the Bchl binding site (*36*). A residue shared by all LH1 and LH2 β polypeptides, Trp_{+9}, stabilizes the subunit complex in *Rb. sphaeroides* by 3.7 kcal/mol (*17*), and has been shown by resonance Raman to form a hydrogen bond to the $C3^1$ acetyl carbonyl of Bchl (*36*). Thus, the *Rs. molischianum* LH2 β polypeptide possesses all the same residues that have thus far been identified in stabilizing the LH1 subunit complex. The results reported in this paper independently confirm the importance of these residues. Moreover, the ability of the LH2 β polypeptide of *Rs. molischianum* to form a subunit-type complex in vitro suggests that if the LH2 β polypeptide of *Rb. sphaeroides* were altered at some or all of these five positions to resemble the LH1 β polypeptides, it would gain the ability to form a subunit complex in vitro. Studies into this possibility are in progress.

Role of the α Polypeptide. Interestingly, the *Rs. molischianum* LH2 β polypeptide displayed different activity when paired with LH1 and LH2 α polypeptides. When the LH2 β polypeptide was combined with the LH1 α polypeptide of *Rs. rubrum* or *Rs. molischianum*, an LH1-type complex formed (Figure 7B), whereas when it was combined with the LH2 α polypeptide of *Rs. molischianum*, an LH2 complex formed (Figure 5A). Clearly, the α polypeptide has a key role in determining the oligomerization product. One unique aspect of the LH2 α polypeptide is that it provides the ligand to the B800 Bchl in both LH2 crystal structures (*4*, *5*). Another interesting aspect is that the *Rs. molischianum* LH2 α polypeptide shares two amino acids with the LH1 α polypeptides, Asn_{+10} and Trp_{+11}, that are near the Bchl binding pocket and have hydrogen bonding capability. These two residues are not conserved in LH2 α polypeptides. Several observations suggest an important role for Trp_{+11}. First, as noted above, mutating Trp_{+11} of the LH1 α polypeptide of *Rb. sphaeroides* to Phe causes a 17 nm blue shift to 853 nm of LH1 in vivo (*36*) and of LH1-type complexes reconstituted in vitro (*16*). Moreover, resonance Raman has shown that Trp_{+11} forms a hydrogen bond to a $C3^1$ acetyl carbonyl oxygen of Bchl (*36*), an observation that has been confirmed by the crystal structure of LH2 (*5*). These results all suggest an important role for Trp_{+11} in the Bchl binding site of LH1. Thus, Trp_{+11} may be the determining factor that allows the LH2 α polypeptide of *Rs. molischianum* to interact with β polypeptides and Bchl, in contrast to the LH2 α polypeptide of *Rb. sphaeroides*, which had no activity when combined with LH1 β polypeptides in the reconstitution assay. In the crystal structure of the *Rs. molischianum* LH2 (*5*), Asn_{+10} protrudes away from the B850 Bchl, and is thus not in a position to influence the structure of the Bchl binding site. However, it may form a hydrogen bond to its own main chain carbonyl, since the distance from the amide N to the carbonyl O is 2.82 Å.

An interesting question to consider is what determines whether the LH2 β polypeptide of *Rs. molischianum* forms LH1 or LH2 in vivo, since it displays such versatility in its activity in vitro, and presumably both α polypeptides are available during biosynthesis. One possibility is suggested by studies on the LH1 polypeptides of *Rb. capsulatus* showing that the insertion of LH1 α and β polypeptides into the membrane after translation is a concerted event. *Rb. capsulatus* LH1 α and β polypeptides do not accumulate in the membrane if they are not accompanied by their complementary polypeptide (*37*). Moreover, although the *Rb. capsulatus* LH1 α and β polypeptides can be inserted into a membrane separately in a cell-free translation system, they are harder to extract from the membrane if they are incorporated together (*38*). Thus, the insertion of the LH1 α and β polypeptides seems to be a concerted event, as is probably also the case for LH2. Therefore, the role of the LH2 β polypeptide of *Rs. molischianum* is probably determined from the time of its translation, since it is almost

certainly already paired with an LH2 α polypeptide. These studies imply that once LH1 and LH2 are formed the α and β polypeptides do not mix; i.e., LH1 and LH2 do not reversibly dissociate into subunit complexes in the membrane.

Rs. molischianum LH1 β Polypeptide. The LH1 β polypeptide of *Rs. molischianum* formed a subunit-type complex by itself, but this complex was relatively unstable. Moreover, this complex reverted to an absorbance of 777 nm, the absorption maximum of Bchl free in detergent solution, when chilled overnight. This behavior is comparable to the activity of *Rs. rubrum* and *Rps. viridis* LH1 β polypeptides in β-only reconstitution assays (*9*, *25*). The *Rs. molischianum* LH1 β polypeptide formed subunit-type complexes with an association constant approximately 10-fold less than the *Rs. rubrum* β polypeptide, but still about 10-fold more than the *Rps. viridis* β polypeptide. Moreover, the α polypeptide seemed to stabilize the subunit complexes formed by the *Rs. molischianum* LH1 β polypeptide, a fact which is also similar to the *Rs. rubrum* and *Rps. viridis* systems (*9*, *25*).

Conclusions. The LH2 β polypeptide of *Rs. molischianum* seems to be very versatile in its role, since it can form a red-shifted complex by itself, an LH1-type complex when combined with an LH1 α polypeptide, or an LH2 complex when combined with the *Rs. molischianum* LH2 α polypeptide. Moreover, unlike other LH2 β polypeptides, the LH2 β polypeptide of *Rs. molischianum* is capable of forming subunit complexes very similar in structure to those formed by LH1 β polypeptides. We attribute the ability of the LH2 β polypeptide to form these complexes to key amino acids it shares in common with LH1 β polypeptides, especially His_0, Trp_{+4}, Trp_{+6}, Arg_{+7}, Trp_{+9}, and possibly Ser_{-10}. Nevertheless, it exhibits the characteristics of an LH2 β polypeptide in the membrane in vivo and in vitro when combined with an LH2 α polypeptide. Perhaps the *Rs. molischianum* LH2 β polypeptide is an evolutionarily ancient LH β polypeptide, dating from a time soon after the LH2 complex evolved from the LH1 complex (*35*), so that this LH2 β polypeptide is still capable of fulfilling both roles. In this case, the LH2 β polypeptide of *Rs. molischianum* should prove to be a valuable model molecule for structure−function studies into both the core and peripheral light-harvesting complexes of photosynthetic bacteria.

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APPENDIX

Deconvolution of Absorption Spectra. In reconstitution spectra, the absorbance of free Bchl ($\lambda_{max} = 777$ nm) and the subunit complex ($\lambda_{max} = 820$ nm) overlap to some extent. Since the calculation of association constants depends on an accurate measure of A_{777} and A_{820}, this overlap should be taken into account. Moreover, the calculation of the yield of LH1 and LH2 from the detergent solubilization/sucrose gradient centrifugation procedure requires an accurate measure of the amount of starting material in the chromatophores. Since the B875 band of LH1 and the B850 band of LH2 overlap extensively, a deconvolution of these spectra is also desirable. The expressions derived below were used to estimate the concentrations of these species in the work reported here.

Consider a sample containing two components, A and B, with absorbance maxima at λ_a and λ_b, respectively. We can define α to be the ratio of A's absorbance at λ_b to its absorbance at λ_a when pure in solution. Similarly, we can define β to be the ratio of B's absorbance at λ_a to its absorbance at λ_b when pure in solution. Note that because λ_a is greater than λ_b for A, and vice versa for B, α and β will both be less than 1. In a mixture of A and B, A's true absorbance at λ_a will be the apparent absorbance at λ_a minus B's contribution to the absorbance at λ_a, which is equal to the absorbance at λ_b times β. Thus:

$$A_{\lambda a} = A_{\lambda a}' - A_{\lambda b}\beta$$

where $A_{\lambda a}'$ is the measured, uncorrected absorbance at λ_a. But the apparent absorbance at λ_b needs to be adjusted similarly for the absorbance of A at λ_b. Thus:

$$A_{\lambda b} = A_{\lambda b}' - A_{\lambda a}'\alpha$$

where, again, $A_{\lambda b}'$ is the measured, uncorrected absorbance at λ_b. Substituting, we have

$$A_{\lambda a} = A_{\lambda a}' - \beta(A_{\lambda b}' - A_{\lambda a}'\alpha) \quad (1)$$

A similar process yields, for $A_{\lambda b}$

$$A_{\lambda b} = A_{\lambda b}' - \alpha(A_{\lambda a}' - \beta A_{\lambda b}') \quad (2)$$

These equations could be further refined by including higher order corrections, but these corrections were found to be unnecessary for the cases reported here. One could also deconvolute the absorption spectra using an appropriate algorithm (*39*). Such a treatment yielded numbers essentially identical to those obtained using eqs 1 and 2.

Each experimental system will obviously have different α and β values because the exact absorption maxima and shape of the absorption spectra of the components vary. For example, the *Rb. sphaeroides* and *Rs. rubrum* subunit complexes have absorption maxima at 824 and 820 nm, respectively (*10*, *11*).

The absorption spectrum of pure Bchl was determined at several concentrations of OG and found to have similar shapes and absorbance maxima. The absorption maximum and spectrum of each subunit complex were taken from our best reconstitutions where little or no free Bchl or LH1 was present. Thus, the relevant equations for the *Rb. sphaeroides* system are

$$A_{824} = A_{824}' - 0.06(A_{777}' - 0.17A_{824}')$$

$$A_{777} = A_{777}' - 0.17(A_{824}' - 0.06A_{777}')$$

while the equations for the *Rs. rubrum* system are

$$A_{820} = A_{820}' - 0.10(A_{777}' - 0.22A_{820}')$$

$$A_{777} = A_{777}' - 0.22(A_{820}' - 0.10A_{777}')$$

Deconvolution of the LH1 and LH2 absorbance in chromatophores was accomplished similarly. We used the

absorption spectra of LH1 and LH2 isolated by detergent solubilization and sucrose gradient centrifugation to determine the ratios. The resulting equations are

$$A_{878} = A_{878}' - 0.17(A_{844}' - 0.35A_{878}')$$

$$A_{844} = A_{844}' - 0.35(A_{878}' - 0.17A_{844}')$$

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