7524 *Biochemistry* **2010,** *49,* 7524–7531
DOI: 10.1021/bi101036t

BIOCHEMISTRY
including biophysical chemistry & molecular biology
Article

Light-Harvesting Antenna System from the Phototrophic Bacterium *Roseiflexus castenholzii*†

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Received June 29, 2010; Revised Manuscript Received July 30, 2010

ABSTRACT: Photosynthetic organisms have evolved diverse light-harvesting complexes to harness light of various qualities and intensities. Photosynthetic bacteria can have (bacterio)chlorophyll Q_y antenna absorption bands ranging from ∼650 to ∼1100 nm. This broad range of wavelengths has allowed many organisms to thrive in unique light environments. *Roseiflexus castenholzii* is a niche-adapted, filamentous anoxygenic phototroph (FAP) that lacks chlorosomes, the dominant antenna found in most green bacteria, and here we describe the purification of a full complement of photosynthetic complexes: the light-harvesting (LH) antenna, reaction center (RC), and core complex (RC-LH). By high-performance liquid chromatography separation of bacteriochlorophyll and bacteriopheophytin pigments extracted from the core complex and the RC, the number of subunits that comprise the antenna was determined to be 15 ± 1. Resonance Raman spectroscopy of the carbonyl stretching region displayed modes indicating that 3C-acetyl groups of BChl *a* are all involved in molecular interactions probably similar to those found in LH1 complexes from purple photosynthetic bacteria. Finally, two-dimensional projections of negatively stained core complexes and the LH antenna revealed a closed, slightly elliptical LH ring with an average diameter of 130 ± 10 Å surrounding a single RC that lacks an H-subunit but is associated with a tetraheme *c*-type cytochrome.

Photosynthetic organisms derive chemical energy by utilizing light excitation to facilitate charge separation across the membrane. Light harvesting is achieved in multichromophoric, pigment−protein antenna complexes and serves to increase the absorption cross section of the cell. Light energy absorbed by the antenna is rapidly and efficiently transferred to the RC[1] where a transmembrane potential is generated (*1*). For example, all purple bacteria possess a so-called "core" complex that is comprised of the LH1 antenna and the RC. LH1 is membrane-bound and composed of an inner ring of α-subunits and outer ring of *β*-subunits that encompass the RC. Each α- and *β*-subunit binds one bacteriochlorophyll (BChl) *a* (or BChl *b* in some species) so that the pigments are arranged perpendicular to the plane of the membrane, in a ring between the protein subunits (*2*). Additionally, some species of purple bacteria employ an accessory antenna called LH2 that functions to absorb light and transfer energy to LH1. The structure of LH2 from two different species is known at atomic resolution (*3*, *4*).

The photosynthetic machinery of filamentous anoxygenic phototrophs (FAPs) (previously called green non-sulfur bacteria) has elements that overlap with other groups of phototrophs. The LH antenna is usually exemplified by the chlorosome, which consists of many thousands of bacteriochlorophyll (BChl) *c* and *d* molecules surrounded by a lipid monolayer, forming an assembly that contains relatively little protein (*5*). However, not all FAPs possess chlorosomes (*6*). Chlorosomes are also found in green sulfur bacteria (*7*) and in the recently described genus *Chloracidobacterium* (*8*).

Light excitation absorbed by the chlorosome is funneled through the BChl *a*-containing baseplate complex (*9*), delivered to an integral membrane antenna, and finally transferred to the RC. The membrane antenna has been termed B808−866 in the chlorosome-containing species *Chloroflexus aurantiacus*, and a similar complex termed B800−880 is found in *Roseiflexus castenholzii*, which lacks chlorosomes. These complexes are named according to their respective Q_y absorbance maxima and have also been biochemically and spectroscopically described (*10*, *11*). While extramembraneous chlorosomes are found in other photosynthetic groups, the integral membrane components of the FAP photosystem (the membrane-bound LH antenna and RC) are most closely related to complexes found in purple bacteria. The membrane LH complex from FAPs is comprised of α- and *β*-subunits that are homologous in sequence to portions of both the analogous polypeptides of LH1 and LH2 from purple bacteria (*12*, *13*). Moreover, while the functioning of the complex is like that of LH1 in that it is intimately associated with the RC, spectroscopically, it resembles LH2 in having two absorption bands in the near-infrared (NIR) region (*10*, *11*). For example, most LH2 forms in purple bacteria exhibit an absorption band around 800 nm that arises from a set of principally monomeric BChls (*14*). A similar band is observed in FAPs.

†This material is based upon work supported as part of the Photosynthetic Antenna Research Center (PARC), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Grant DE-SC 0001035. This work was also supported by U.S. Department of Energy Grant DE-FG02-07ER15846 to R.E.B. and a grant from the Biotechnology and Biological Sciences Research Council to C.N.H.

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[1]Abbreviations: RC, reaction center; LH, light-harvesting; FAPs, filamentous anoxygenic phototrophs; LH1, light-harvesting 1 complex; LH2, light-harvesting 2 complex; BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; NIR, near-infrared; HPLC, high-performance liquid chromatography.

 Published on Web 08/02/2010

However, linear dichroism measurements clearly indicate that the pigments that comprise this band in FAPs are oriented in a manner very different from those of purple bacteria such as *Rhodopseudomonas acidophila*, and at a large angle with respect to the membrane plane (*10*, *15*).

While several kinetic studies have been performed on the integral membrane antenna from *Cfl. aurantiacus* (*16*−*18*), structural data have been elusive. Complications arise in *Cfl. aurantiacus* because the dominant chlorosome antenna can be difficult to separate from the membrane and only a few purification procedures for the B808−866 complex have been described (*11*, *16*). Here we present a full description of the purification of the RC−LH (core complex), LH-only, and RC complexes from *Rfl. castenholzii*, an organism that does not contain chlorosomes (*19*). This provides a unique opportunity to prepare a full complement of LH and RC complexes from a representative member of the FAPs. The genome sequence of *Rfl. castenholzii* has been determined and is available as GenBank entry NC 009767.

In this work, we show that the LH antenna from *Rfl. castenholzii* contains 15 ± 1 $\alpha\beta$-protein subunits and has resonance Raman features akin to purple bacterial LH1, indicating that the environment around the B880 pigments are likely similar. Lastly, detergent-solubilized antenna complexes were negatively stained and analyzed by electron microscopy. This revealed a slightly elliptically shaped closed ring surrounding the RC. The size of the complex is consistent with the number of subunits determined.

MATERIALS AND METHODS

Purification of the RC. *Rfl. castenholzii* strain HLO8^T was grown anaerobically and photosynthetically at 50 °C on 02YE medium (*19*) under continuous illumination from four 60 W incandescent light bulbs in a 15 L fermentor for 7 days. Cells were harvested by centrifugation. These conditions repeatedly yield approximately 3 g of cells/L of medium. Cells were resuspended in 20 mM Tris buffer (pH 8.0) (buffer A), mixed thoroughly, and disrupted using a sonifier. The lysate was centrifuged at 16000*g* for 20 min, and then the supernatant liquid was centrifuged at 225000*g* for 2 h to pellet membranes. Whole membranes (OD_{880} = 20−30 cm^{-1}) were mixed with lauryldimethylamine *n*-oxide (LDAO) dropwise from a concentrated stock (30%) to a final concentration of 0.45% (w/v) and stirred at 0 °C for 90 min. The mixture was diluted to a final LDAO concentration of 0.1% before being subjected to centrifugation at 200000*g* for 1 h. This resulted in a supernatant liquid that was enriched in RCs, a soft pellet that contained primarily the RC−LH complex and some RCs, and a pellet that was still somewhat colored. The supernatant liquid was removed, taking care to avoid the soft pellet. This mixture was filtered through a 0.22 μm filter and immediately loaded onto a Q-Sepharose HP ion-exchange column (GE Healthcare) pre-equilibrated with 0.1% LDAO in buffer A. After being loaded, the column was washed extensively with 0.1% LDAO in buffer A followed by 2 column volumes of 1% LDAO in buffer A. After re-equilibration of the column with 0.1% LDAO in buffer A, the RC fraction was eluted by a linear gradient of NaCl in 0.1% LDAO in buffer A. The RCs eluted with 175−200 mM NaCl. The best RC fractions as judged by the absorption spectrum were pooled, concentrated, and applied to a Superdex 200 column (GE Healthcare) equilibrated with 0.02% *n*-dodecyl β-D-maltoside (β-DDM) and 100 mM NaCl in buffer A and eluted with a flow rate of 0.5 mL/min. If further purification was needed, the RCs were diluted to reduce the NaCl concentration, loaded onto a Mono-Q column (Bio-Rad Laboratories), and eluted with a linear gradient of NaCl in 0.02% β-DDM in buffer A. The best RC fractions had an 814 nm to 280 nm ratio of 2.2.

Purification of LH-only and RC−LH Complexes. Whole membranes (OD_{880} = 20−30 cm^{-1}) were mixed to a final LDAO concentration of 1% and stirred for 60 min at 0 °C. The mixture was diluted two times with buffer A and centrifuged at 200000*g* for 1 h. This resulted in a dark red to brown supernatant liquid and a pellet that was largely devoid of color. The supernatant liquid was diluted two times further in buffer A, filtered through a 0.22 μm filter, and loaded onto a Q-Sepharose HP column pre-equilibrated with 0.1% LDAO in buffer A. The bound material was washed with 50 mM NaCl in 0.1% LDAO in buffer A for several hours to remove weakly bound proteins and free BChl *a* and carotenoids. The NaCl concentration was increased to 100 mM, and washing was continued. The NaCl concentration was increased to 180 mM, and this released some RCs, and the LH-only antenna, which was collected for further purification. Finally, the NaCl concentration was increased to 250 mM, and the RC−LH complex was eluted from the column.

The LH-only and RC−LH complexes were both further purified separately by gel filtration and a final ion-exchange step that was performed as described for the purification of the RC described above. The final 880 nm to 280 nm absorption ratio for the RC−LH complex was 1.5, and the final 884 nm to 280 nm ratio was 1.7 for the LH1 complex. SDS−PAGE was performed according to the methods of Schägger and von Jagow (*20*).

The RC−LH1 complexes from *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* were purified as previously described (*21*, *22*). These samples and the RC−LH complex from *Rfl. castenholzii* were analyzed using a gel filtration column (BioSep-SEC-S 3000, Phenomenex) using 0.03% β-DDM in 10 mM HEPES buffer (pH 7.5) to compare their respective retention times.

Pigment Extraction and Subunit Determination by HPLC. The number of subunits in the LH antenna was determined using a modification of the method described by Qian et al. (*23*). Fifty microliters of the purified RC−LH (OD_{880} = 4 cm^{-1}) or RC (OD_{814} = 1 cm^{-1}) complex was suspended in 500 μL of an ice-cold 8:2 (v/v) methanol/acetone mixture and incubated at −20 °C in the dark for 30 min. The mixture was then centrifuged at 14000*g* in a microfuge. The supernatant was drawn off and dried under a stream of argon gas in the dark before being resuspended in 100 μL of an 8:2 (v/v) methanol/acetone mixture and immediately injected into the HPLC instrument (Agilent 1100 system). Centrifugation and drying of the pigments took less than 20 min to complete. Additionally, the single extraction described above was enough to extract essentially all of the pigments from the protein as determined by resuspension of the pellet and measurement of the OD. Reverse-phase HPLC was performed with a Zorbax Eclipse XDB-C18 column, an isocratic flow rate of 1 mL/min, and an 8:2 (v/v) methanol/acetone solvent system. The eluent was monitored from 200 to 850 nm using an array detector. Under the conditions described above, the eluted BChl *a* and BPhe *a* were separated by several minutes and the area under each elution peak was integrated.

Resonance Raman Spectroscopy. Resonance Raman (RR) measurements were performed on the LH-only sample contained in tightly sealed 1 mm inside diameter capillary tubes that were mounted directly onto the cold tip of a closed-cycle liquid He refrigeration system (ADP Cryogenics DE-202). The excitation wavelength was 364 nm. The data acquisition times were ~2 h

(40 × 180 s scans). Cosmic ray spikes were removed prior to addition of the data sets. The laser power was ~10 mW, and the spectral resolution was ~2 cm^{-1}. Further details of the experimental setup can be found elsewhere (*24*).

Electron Microscopy (EM) and Particle Analysis. For negative stain EM, materials were diluted to a protein concentration of ~0.1 mg/mL. Five microliters of a protein solution of RC−LH and LH-only complexes was applied separately to glow-discharged, carbon-coated copper grids (Agar Scientific), blotted, stained with 0.75% (w/v) uranyl formate, blotted, and dried in air. Electron micrographs were recorded on a Philips CM100 electron microscope fitted with a 1K × 1K Gatan Multiscan 794 CCD camera at a magnification of 52000×. Single-particle analysis was performed using IMAGIC-5 (Image Science Software GmbH) (*25*, *26*); 6193 and 15474 single particles of LH-only and RC−LH complexes, respectively, were boxed from electron micrographs using a box size of 64 pixels × 64 pixels (~250 Å × 250 Å). Particles were subjected to iterative normalization and band-pass filtering before alignment as described in the references cited above.

RESULTS

Purification of RC, LH-only, and RC−LH Complexes. The RC, LH-only, and RC−LH complexes were separated and purified from *Rfl. castenholzii*. The respective absorption spectra are shown in Figure 1. The absorbance features of the RC−LH complex have been described in detail elsewhere (*10*). The LH-only complex lacks absorption shoulders at 410 and 760 nm that are ascribed to the Soret band of oxidized cytochrome *c* and the Q_y band of the RC-associated BPhe *a*, respectively. The Q_y band of B880 redshifts 4 nm to 884 nm when the RC is removed. Moreover, there is a slight shift in the carotenoid region, suggesting a possible difference in the carotenoid environment or composition for this sample. Lastly, the RC has an NIR absorption spectrum very similar to that of the RC of *Cfl. aurantiacus* (*27*, *28*) with prominent absorption in the NIR region at 760, 814, and 864 nm. However, the visible portion of the spectrum clearly identifies an oxidized *c*-type cytochrome that copurifies with the RC as reported previously (*29*).

The purity as well as the polypeptide composition of the RC, LH-only, and RC−LH complexes can be assessed by SDS−PAGE analysis (Figure 2). These bands have been identified previously (*29*) as the RC C-subunit (37000 kDa), L-subunit (26000 kDa), M-subunit (24000 kDa), and antenna α-subunit (7000 kDa) and *β*-subunit (5000 kDa).

Number of BChl a Molecules and Polypeptide Subunits in the LH Antenna. In the absence of high-resolution structural data, the number of antenna subunits can be determined indirectly by comparing the amount or ratio of BChl to bacteriopheophytin (BPhe) in the core complex and the isolated RC. Such approaches have given estimates of the number of subunits in the detergent-solubilized antenna from *Rhodobium marinum* (*23*, *30*) and a variety of other purple bacteria containing LH1 as the only antenna complex (*31*). These methods utilize pigment extraction followed by separation and quantification by HPLC and rely on knowing the ratio of BChl to BPhe in the RC and the number of BChls in each antenna subunit. These values are 2:1 in the purple bacterial RC and two BChl molecules for each LH1 antenna subunit, respectively (*32*, *33*), and most likely 1:1 and three BChl molecules in the RC and in each subunit of the LH antenna in FAPs, respectively (*15*, *27*). A representative chromatogram for

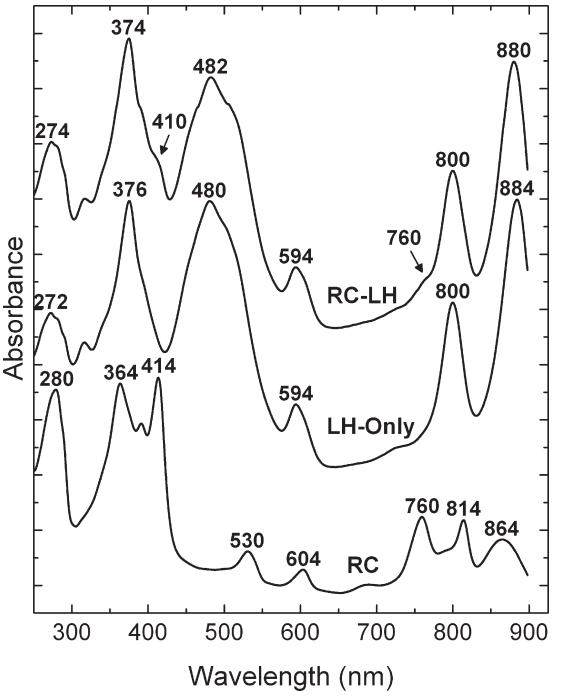

FIGURE 1: Absorption spectra of the isolated RC−LH, LH-only, and RC complexes from *Rfl. castenholzii* with wavelength maxima indicated in nanometers. The spectra have been vertically displaced for the sake of clarity.

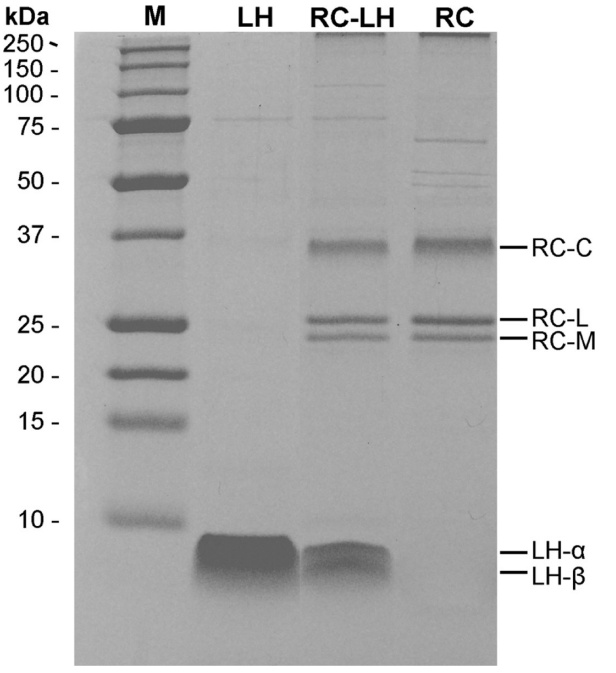

FIGURE 2: Coomassie blue-stained SDS−PAGE of the RC−LH, LH-only, and RC complexes from *Rfl. castenholzii* along with molecular mass markers (M). The apparent masses determined from the gel are 37000 kDa for the RC C-subunit, 26000 kDa for the L-subunit, 24000 kDa for the M-subunit, 7000 kDa for the LH α-subunit, and 5000 kDa for the LH *β*-subunit. The protein assignments are based on those of Yamada et al. (*29*).

the isolated RC and RC−LH complexes recorded at 750 nm is presented in Figure 3. The peak ascribed to BChl *a* matched the elution time of BChl *a* esterified with phytol, isolated from *Rba. sphaeroides* (results not shown), verifying that phytol is the esterifying alcohol in the antenna and the RC in *Rfl. castenholzii*.

There are almost certainly also three BChl *a* and three BPhe *a* molecules associated with the RC L- and M-subunits in *Rfl. castenholzii*, because the NIR absorption spectrum of the RC is nearly identical to that of *Cfl. aurantiacus*. The ratio of BChl to BPhe (X) from pigments extracted from the isolated RC can then be represented as

$$X = \frac{A^{RC}_{BChl}}{A^{RC}_{BPhe}} = \frac{3\varepsilon_{BChl}}{3\varepsilon_{BPhe}} = \frac{\varepsilon_{BChl}}{\varepsilon_{BPhe}} \quad (1)$$

where A_{BChl} and A_{BPhe} are the integrated absorbances from the HPLC chromatogram (at 750 nm) for BChl and BPhe, respectively, and ε_{BChl} and ε_{BPhe} are extinction coefficients for BChl and BPhe at the detection wavelength of 750 nm, respectively.

If we assume that the RC−LH complex is composed of one RC and n antenna subunits and that in each antenna subunit there are three BChl *a* molecules, then the ratio of BChl to BPhe for the RC−LH complex (Y) from the chromatogram may be represented as RC-LH

$$Y = \frac{A^{RC\text{-}LH}_{BChl}}{A^{RC\text{-}LH}_{BPhe}} = \frac{(3n+3)\varepsilon_{BChl}}{3\varepsilon_{BPhe}} = (n+1)\frac{\varepsilon_{BChl}}{\varepsilon_{BPhe}} \quad (2)$$

Combining eqs 1 and 2 and solving for the number of subunits n gives $n = Y/X - 1$. Six separate extractions of the RC−LH complex (Y) yielded values of 15.5, 15.9, 16.6, 16.2, 16.6, and 16.2. Three extractions of the isolated RC (X) yielded 1.02, 0.99, and 1.04. Using the average values of X and Y above and solving for the number of antenna subunits yields a value of 14.9 ± 0.9 subunits. If three BChl molecules are bound in each subunit, then there are 45 ± 3 BChl *a* molecules esterified with phytol that comprise the antenna complex.

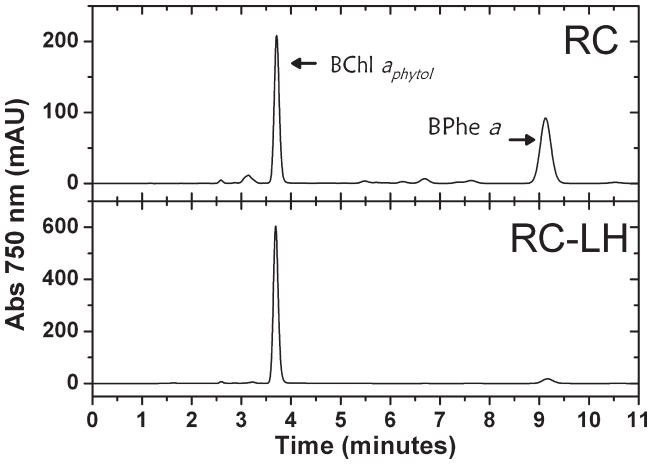

FIGURE 3: Representative HPLC elution profiles, monitored at 750 nm, for pigment extracted from the purified RC (top) and RC−LH (bottom) complex.

The gel filtration elution times of the purified RC−LH complex from *Rfl. castenholzii* and RC−LH1 complexes from two representative purple bacteria were compared and were found to be similar for each of the monomeric complexes (Table 1). Thus, gel filtration analysis is consistent with approximately 15 LH1 $\alpha\beta$ subunits, and with the number of BChls quantified by extraction from the RC−LH complex.

Hydrogen Bonding in the Antenna. One of the most interesting features of the LH antenna from FAPs is that although it resembles the peripheral antenna LH2 from purple bacteria in having 800 nm-absorbing BChls, it is more akin to an LH1 complex in being associated with a RC. An explanation for these findings is not apparent from a superficial examination of the primary sequence of the α- and β-subunits. However, resonance Raman (RR) is a suitable spectroscopic method for investigating the interactions of BChl ring substituents with the protein environment by reporting the stretching modes of C3-acetyl and $C13^1$-keto groups of BChl molecules bound to a protein scaffold (*34*). The Raman stretching frequencies of these groups are shifted by tens of inverse centimeters if they are involved in intermolecular interactions as compared to their unbound states, giving insight into the local environment around the antenna pigments.

RR spectra were recorded for the LH-only complex at 30 K with an excitation wavelength of 364 nm (Figure 4). The 1600−1720 cm^{-1} region is sensitive to conjugated carbonyls and in the context of BChl *a* reports on C3-acetyl and $C13^1$-keto groups. Considering the carbonyl stretching region, the dominant stretching frequency observed was a symmetric band at 1613 cm^{-1} and is consistent with the methine bridge stretching frequency for a pentacoordinated BChl (*35*). An additional band is clearly resolved at 1641 cm^{-1}, and two minor bands of approximately the same amplitude are observed at 1666 and 1672 cm^{-1}. The distinct but asymmetric band centered at 1520 cm^{-1} most likely represents the C═C stretch of the antenna carotenoid molecules.

A survey of seven LH1 complexes investigated by RR all produce a band close to 1645 cm^{-1} that is thought to originate exclusively from bound C3-acetyl groups (*36*). Interestingly, no reported LH2 complex has a RR stretching band in this region with the exception of *Phaeospirillum molischianum* which is known to be homologous in sequence to LH1 (*37*). From the same report by Robert and Lutz, all LH1 complexes had a weak mode around 1667 cm^{-1} and a yet smaller mode near 1676 cm^{-1}. These bands were provisionally assigned to 13^1-keto carbonyls involved in inequivalent hydrogen bonding (*36*) but can also occur from unbound C3-acetyls. Moreover, many LH2 complexes also possess a mode around 1660−1670 cm^{-1} that is

Table 1: Comparison of Gel Filtration Elution Times for RC−LH1 Complexes from Representative Purple Bacteria and the RC−LH Complex from *Rfl. castenholzii*[a]

	Rba. sphaeroides dimeric RC−LH1	*Rba. sphaeroides* monomeric RC−LH1	*Rsp. rubrum* monomeric RC−LH1	*Rfl. castenholzii* RC−LH
composition	2(L-,M-,H-subunits), 28 $\alpha\beta$-subunits, 2 PufX	L-,M-,H-subunits, 16 $\alpha\beta$-subunits	L-,M-,H-subunits, 16 $\alpha\beta$-subunits	L-,M-,C-subunits, 15 ± 1 $\alpha\beta$-subunits
approximate mass (kDa)	623	333	331	332 ± 14
elution time (min)	14.8	16.1	15.9	15.9

[a]The approximate masses were determined by adding the peptide masses for each subunit and the mass of the number of total pigments that are known to bind to each complex. For *Rfl. castenholzii*, gene product masses were used.

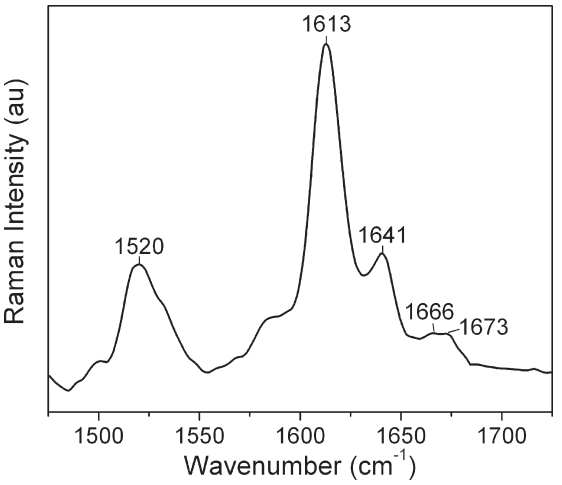

FIGURE 4: Resonance Raman spectrum of the LH-only complex recorded for the high-frequency region recorded at 30 K with an excitation wavelength of 364 nm.

proposed to originate from the B850 pigments. Two bands of nearly equal magnitude were observed in this same region for the *Rfl. castenholzii* LH-only complex (1666 and 1672 cm^{-1}). The additional band intensity on the higher-frequency mode may reflect contributions from the B800 pigments in *Rfl. castenholzii* that are not present in LH1.

Some LH2 complexes display a band at 1700 cm^{-1} (for example, *Rba. sphaeroides*) that is indicative of free $C13^1$-keto carbonyls and is thought to arise from the B800 pigments because RR analysis of LH2s depleted of B800 did not contain this stretching frequency (*36*). The clear absence of a 1700 cm^{-1} mode in the antenna of *Rfl. castenholzii* suggests that the 13^1-keto groups of all the BChl are involved in intermolecular interactions. Overall, the RR spectrum of the LH-only complex from *Rfl. castenholzii* is more similar to that of LH1 than that of LH2 and probably reflects a very similar BChl environment for B880 pigments in these complexes.

Single-Particle Analysis of the Negatively Stained RC−LH Complex. Single RC−LH and LH-only complexes were negatively stained and imaged by electron microscopy (Figure 5A,B). With the RC−LH complex, the majority of particles appeared to sit flat on the carbon support film of the EM grid (Figure 5A, green box). However, some particles appeared to be side views (Figure 5A, red box). Of the top viewed particles, some were observed to be circular and others more elliptical, with an average diameter of 130 ± 10 Å. Many of the side view projections had a clear density extending from only one side of the complex, presumed to be the C-subunit that copurifies with the RC. However, it can be noted that the presence of the presumed C-subunit was not always obvious. The height of the side projections with and without the C-subunit was measured to be 110−115 and 70−75 Å, respectively.

EM images of the LH-only sample revealed some circular and elliptical particles with most lacking density in the center, confirming the lack of the RC (Figure 5B, green box). The dimensions of closed rings observed for the LH-only sample were approximately the same as for the RC−LH complex. There appeared to be many fewer side projections, and many of the rings were not complete (Figure 5B, red box).

Single projections (6193 LH-only and 15474 RC-LH) were boxed (Figure 5C,D) and used to create image classes. Three projections for each type of complex are shown in Figure 6.

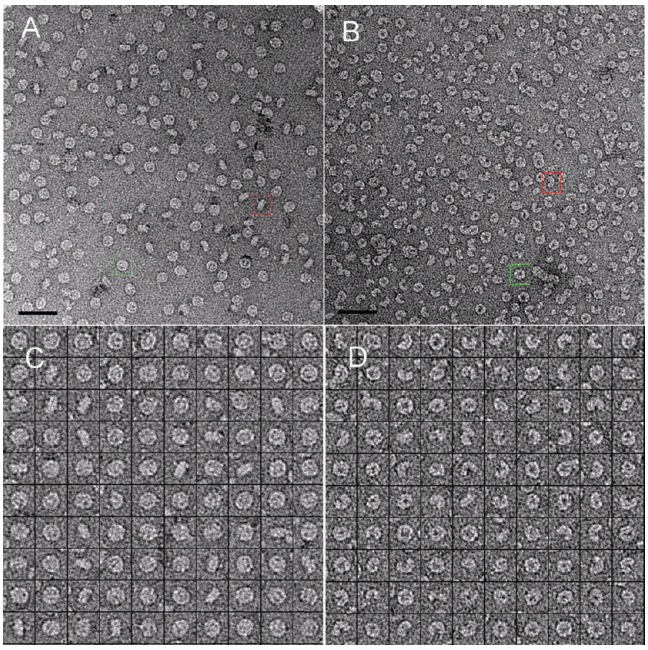

FIGURE 5: (A) Representative electron micrograph at 52000× magnification of the detergent-solubilized RC−LH complex. (B) Same as panel A, only for the LH-only complex. An example of a top or bottom view of the RC−LH complex is shown in a green box, while the presumed side view is shown in a red box in panel A. In panel B, the green box represents a top or bottom view of the LH-only complex while the red box shows an incomplete ring. The scale bar represents 50 nm. (C and D) One hundred selected single particles for the RC−LH and LH-only complex, respectively. Each box is ~250 Å × 250 Å.

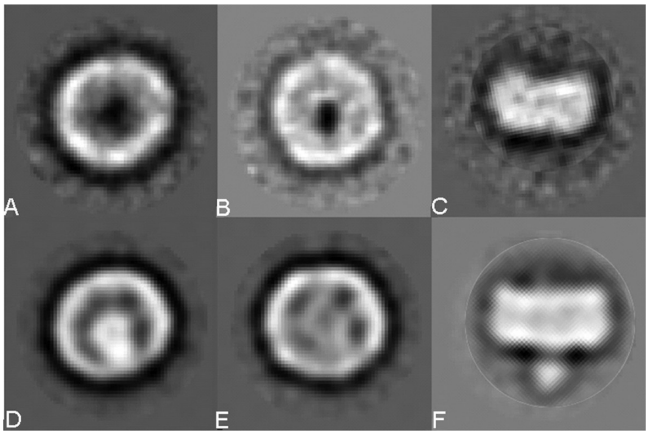

FIGURE 6: Three different views of the LH-only and RC−LH complexes. The individual view was selected from 200 classes. The number of single-molecule particles used for the average is shown in parentheses. Top row: presumed periplasmic (A, $n = 56$), cytoplasmic (B, $n = 47$), and side (C, $n = 68$) projections of the LH-only complex. Bottom row: presumed periplasmic (D, $n = 28$), cytoplasmic (E, $n = 36$), and side (F, $n = 28$) projections of the RC−LH complex. Each box is ~250 Å × 250 Å.

Of these projections, the following views are assumed: (1) a bottom projection looking from the periplasmic side of the complex, (2) a top projection looking from the cytoplasmic side of the complex, and (3) a side view, presumably in the plane of the ring. The top and bottom projections (Figure 6A,B,D,E) reveal that the slightly elliptical structure is a closed ring regardless of whether the RC is present. The protrusion extending perpendicular to the plane of the ring structure in the side view (Figure 3F) is likely to be the C-subunit, because the RC−LH complex from *Rfl. castenholzii*

lacks the H-subunit found in all purple bacteria but maintains a large C-subunit. This subunit is probably responsible for the weak staining that is observed in the center of the complex that extends to the edge of the ring on the periplasmic side (Figure 6D). If this is indeed the C-subunit protrusion, it is interesting that it is observed to orient along the short axis of the elliptical ring.

DISCUSSION

The study of photosynthetic membrane complexes among FAPs has been largely limited to *Cfl. aurantiacus* because of its relative ease of growth and lack of other species that can be cultured in high yield. However, *Cfl. aurantiacus* contains chlorosomes that often dominate the optical properties of samples. Despite this, procedures have been developed to prepare cytoplasmic membranes devoid of chlorosomes (*38*). The discovery and isolation of *Rfl. castenholzii* by Hanada et al. provides an opportunity to study a representative member of the FAPs without chlorosomes (*39*). Here we present a description of the purification of a full complement of photosynthetic complexes from *Rfl. castenholzii*: RC, LH-only, and RC−LH complexes.

Size and Organization of the LH Antenna in Rfl. castenholzii. Pigment extraction followed by HPLC to quantify the ratio of BChl to BPhe pigments allowed the number of subunits in the antenna to be determined. It is assumed that the isolated RC contains three BChl and three BPhe molecules while the antenna is comprised of 3*n* BChl molecules, where *n* is the number of subunits. This method affords the advantage of not needing to determine extinction coefficients to quantify the total amount of pigments in the sample and is advantageous considering that extinction coefficients can depend strongly on the solvent system used. On the basis of six extractions of the RC−LH complex and three from the isolated RC, it was determined that the antenna contains 15 ± 1 subunits. Using a similar HPLC method, it was shown that there were 16 subunits in the detergent-solubilized antenna of *Rh. marinum* (*23*). The 8.5 Å projection structure of the RC−LH1 complex from *Rsp. rubrum* revealed a ring of 16 subunits completely surrounding the RC (*40*), while the crystal structure of the RC−LH1−PufW complex from *Rhodopseudomonas palustris* revealed an elliptical ring of 15 antenna subunits surrounding the RC. We note also that LH1 rings of 15 and 16 $\alpha\beta$-subunits, both circular and elliptical, have been reported on the basis of AFM studies conducted on native photosynthetic membranes (*2*, *41*, *42*). In terms of gel filtration behavior, the complex from *Rfl. castenholzii* resembles the other monomeric cores that contain 16 $\alpha\beta$-subunits and it deviates in only filtration behavior from the *Rba. sphaeroides* core dimer, which is expected given that the dimer, on the basis of an electron density map and BChl extraction, is known to be composed of two RCs, 28 LH1 $\alpha\beta$-subunits, and two PufX molecules (*22*).

The antenna α- and β-subunits from *Rfl. castenholzii* are homologous in sequence to subunit polypeptides from both LH1 and LH2 complexes (for sequence alignments, see ref *10*). For example, the His residues found on the α- and β-subunit that is known to centrally coordinate the B880 or B850 BChls in LH1 and LH2 complexes are conserved among FAPs. The resonance Raman spectrum for the LH-only complex had features similar to those measured for LH1 rather than most LH2 complexes [with the exception of *Phs. molischianum* (see below)]. The differences in the Raman spectra for LH2 and LH1 are manifested in the hydrogen bonding differences for the B850 and B880 pigments, respectively. From the three-dimensional structure of LH2 from *Rps. acidophila*, the BChl pigments that make up B850 are centrally coordinated by His residues on the α- and β-subunits (*43*). An H-bond is present between the C3-acetyl group on ring A of BChl *a* and αW_{+14}. Another H-bond is formed between the C3-acetyl group of the other BChl and αW_{+13} of an adjacent protomer (*44*, *45*). The result is that each α-subunit forms H-bonds with two BChl C3-acetyl groups, but the two pigments are not in the same antenna subunit.

The situation is different for the H-bonding pattern in B880 from LH1. Both the α- and β-subunits coordinate a BChl with His (*46*). Although there is no high-resolution crystal structure for LH1, resonance Raman and mutagenesis have clearly demonstrated that the C3-acetyl groups of BChl *a* in LH1 are hydrogen bonded to αW_{+11} and βW_{+9} and that these interactions are internal to the same $\alpha\beta$-pair (*47*, *48*). In fact, LH2 from *Phs. molischianum* was shown to have Raman features similar to those of LH1 and peptide sequences that conserve both αW_{+11} and βW_{+9} (*37*). The crystal structure later confirmed the participation of these two residues in H-bonding to the B850 pigments (*4*). While the resonance Raman spectrum cannot be unequivocally assigned for *Rfl. castenholzii*, both αW_{+11} and βW_{+9} are maintained (*10*). It is probably the case that the molecular interactions of the BChls that embody B880 in *Rfl. castenholzii* are similar to those of LH1. The interactions of the BChls in the B800 band are not determined here, and the residue that coordinates these BChls is still unknown. For example, residue R_{-10}, which is conserved in LH2 β-polypeptides and which forms an H-bond with the C3-acetyl carbonyl of the B800 BChls in LH2 complexes (*49*), is absent in *Rfl. castenholzii* (*10*).

The structural significance of the H-bonding differences between LH1 and LH2 has been thought to allow flexibility in LH1, specifically to permit the escape of quinol formed by RC photochemistry (*40*, *50*). The similar H-bonding pattern for the B880 pigments in *Rfl. castenholzii* and its analogous functionality indicate that a comparable antenna organization surrounding the RC may be expected.

Images generated of the RC−LH and LH-only complexes by single-particle analysis revealed both circular and elliptical complexes that appeared to be closed rings slightly larger than the dimensions reported for most purple bacterial reaction center−antenna complexes (~10.5 nm) (*40*, *51*). However, negatively stained, detergent-solubilized complexes will have an increased measured diameter due to bound detergent molecules (*52*, *53*). For example, negatively stained, core complexes from *Phs. molischianum*, solubilized in β-DDM, were measured to have a diameter of 14.1−14.6 nm (*54*). In contrast, measurements of the LH1 ring in its native membrane environment for this same species using AFM gave short and long axis diameters of 8.5 and 9.5 nm, respectively (*55*). However, it can be noted that AFM, by recording only the surface topology, can underestimate the full width of the complex if much of the structure lies beneath the surface of the membrane.

Images of individual LH-only complexes displayed rings of variable diameter and what appeared to be portions of rings. This result might not be unexpected because LH1, in the absence of the RC, was observed to have a variable number of subunits in *Rba. sphaeroides*. This variation was attributed to the relatively weak associations between the LH1 $\alpha\beta BChl_2$ subunits, which are normally stabilized by the enclosed RC complex (*50*, *56*). Although the resolution of the two-dimensional projections was not sufficient to directly count the number of antenna subunits,

the determination by pigment extraction as well as the overall size of the complex revealed by EM indicates that 15 ± 1 subunits is reasonable.

Higher-resolution structural information is required to investigate the finer details of the complex, such as the binding of the B800 pigments and the distance relationships between chromophores. However, we show here that that the membrane-bound LH antenna from *Rfl. castenholzii* is similar in size and shape to monomeric LH1 complexes from purple bacteria. The size is consistent with a ring of antenna subunits that surround a singular RC.

ACKNOWLEDGMENT

We acknowledge Dr. Svetomir Tzokov (University of Sheffield) for electron microscopy support.

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