Purification and Characterization of the Peripheral Antenna of the Purple-Sulfur Bacterium *Chromatium purpuratum*: Evidence of an Unusual Pigment–Protein Composition†

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ABSTRACT: The purification and characterization of the peripheral antenna and the preliminary characterization of a carotenoid–protein complex from the purple-sulfur bacterium *Chromatium purpuratum* are described. The peripheral antenna of *C. purpuratum* is unusual among purple bacteria in that it can be resolved by SDS-PAGE into six subunits, the largest number observed thus far for a spectrally pure antenna complex. N-terminal sequence analyses of these subunits suggest that they may have an additional bacteriochlorophyll-binding site located outside the transmembrane domain. The results of pigment–protein quantification are also consistent with additional pigment-binding sites in the *C. purpuratum* LH2. Furthermore, CD measurements and sequence analysis suggest the presence of considerable β-type in addition to α-helical secondary structure. Thus, the secondary and quaternary structures of this complex differ significantly from light-harvesting complexes of other purple photosynthetic bacteria. A carotenoid–protein complex is also described; it is an apparent association of three proteins and carotenoid and is closely associated with the peripheral antenna. The purple-sulfur bacteria are evolutionarily older than the relatively better characterized purple-nonsulfur organisms. The phenotypic features described here of the *C. purpuratum* photosynthetic apparatus are related to those of other purple bacteria and green-sulfur bacteria and may reflect the evolutionary position of this organism.

Photosynthesis in green and purple bacteria is a less complex version of that found in eukaryotes; it is accomplished by a single photosystem. The green-sulfur bacterial photosystem closely resembles eukaryotic photosystem I, whereas in purple bacteria it is more analogous to photosystem II (Nitschke & Rutherford, 1991). The purple bacterial photosynthetic apparatus is composed of a photochemical reaction center (RC)[1] surrounded by at least one, and usually two, light-harvesting complexes (LHs) that capture light energy and funnel it to the RC to drive transmembrane-charge separation, beginning the so-called light reactions of photosynthesis. The RC and LHs are complexes of integral membrane proteins, associated pigments, and cofactors and are located in chromatophores, specialized regions of the bacterial plasma membrane.

The RC is composed of three subunits, H, M, and L, and in some organisms, there is a fourth subunit, a tetraheme cytochrome. Surrounding the RC in all purple photosynthetic bacteria is the core antenna or LH1. This complex is composed of small subunits (<10 kDa) termed α and β. These subunits of the LH1 are encoded by the same operon that encodes the L and M subunits of the RC (Youvan et al., 1984; reviewed in Kaplan, 1988; Donohue et al., 1988; Wiessner et al., 1990) and are present in a fixed stoichiometry to the RC (Aagard & Sistrom, 1972). Surrounding the RC–LH1 (the core) in many organisms is a second light-harvesting complex, the LH2 or peripheral antenna. The LH2 is also made up of α and β subunits that have homology to but are distinct from the α and β of the LH1. The LH2 α and β are encoded by a separate operon, and the amount of this complex synthesized by the organism is dependent upon light intensity and other environmental factors (summarized in Kaplan, 1988).

The purple photosynthetic bacteria are distibuted across three of the five 16s-rRNA-derived subdivisions of Gram-negative bacteria (Woese, 1987). They can be divided into two groups depending on their ability to use reduced sulfur compounds as an electron donor in photosynthesis. The purple-nonsulfur organisms have been well characterized biochemically; it is from these organisms that we have the aforementioned knowledge of the organization of the genes that encode the RC and LH apoproteins. In addition, many of the antenna complexes from purple-nonsulfur organisms have been characterized by various spectroscopic methods and by protein sequencing (reviewed in Zuber & Brunisholz, 1991). The results of these studies indicate that the basic structural unit of the antenna is built up of heterodimers of α and β; each of these polypeptides spans the chromatophore membrane once in the form of an α-helix. In contrast, little is known about the photosynthetic apparatus of the purple-sulfur organisms (members of Chromatiaceae and Ectothiorhodospireaceae). These organisms are thought to be more ancient than their purple-nonsulfur relatives (Stackebrandt et al., 1984). There is no information available on their genetic organization, and the pigment–protein complexes of only a

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[1] Abbreviations: A_{830}, absorbance at 830 nm; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; CD, circular dichroism; DM, *n*-dodecyl β-D-maltoside; LH1,2, light-harvesting complex 1, 2; NIR, near infrared; OG, *n*-octyl β-D-glucoside; RC, reaction center; SEC, size-exclusion chromatography.

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few of these organisms have been characterized by spectroscopic methods and by amino acid sequence determination (Zuber & Brunisholz, 1991).

Chromatium purpuratum is a marine purple-sulfur bacterium first described in 1980 (Imhoff and Truper, 1980). It is a bacteriochlorophyll-*a*-containing organism that also contains the uncommon keto–carotenoid, okenone. Deriphat-PAGE (Peter & Thornber, 1991; Ferguson et al., 1991) resolves the solubilized photosynthetic apparatus of this organism into three distinct pigment–protein complexes: the RC–LH1 (or core), the peripheral antenna or LH2, and a carotenoid–protein complex. Here, we report a method for large-scale purification of the peripheral antenna and a closely associated carotenoid–protein complex. The LH2 complex is shown to be composed of multiple polypeptides, rather than the expected two. N-terminal amino acid sequence analysis of these polypeptides and spectral data on the complex indicate that the organization of the peripheral antenna from *C. purpuratum* differs substantially from the model proposed for the purple-nonsulfur peripheral antenna with different secondary structural features and additional potential bacteriochlorophyll-binding sites.

MATERIALS AND METHODS

Cells. *C. purpuratum* cells were grown in modified Pfennig's medium as previously described by Cogdell et al. (1990).

Membranes. Photosynthetic membranes (chromatophores) were prepared by French pressure cell breakage of the bacterial cells and differential centrifugation as previously described (Cogdell et al., 1990).

Solubilization of Membranes. Chromatophores were diluted to an A_{830} of 140 cm^{-1} and solubilized with 1.5% *n*-dodecyl maltoside (DM) and 1.5% *n*-octyl glucoside (OG) (both detergents from Sigma Chemical Co., St. Louis, MO) for 2 h at room temperature. The mixture was centrifuged for 2 min at 5000*g* to pellet debris.

Ammonium Sulfate Precipitation. The solubilized membranes were diluted to an absorption, at 830 nm, of 50 with 1 M MES, pH 6.0. Ammonium sulfate was added slowly to 75% saturation. The solution was mixed gently for 30 min at 4 °C and then centrifuged at 7600*g*. The pellet was discarded and the supernatant, containing the LH2, was concentrated and dialyzed.

Concentration and Dialysis. Vacuum dialysis and concentration were carried out in a ProDiCon apparatus (Biomolecular Dynamics, Beaverton, OR) using a 60-kDa cutoff membrane against 25 mM Tris, pH 8.0, 100 mM glycine, 0.02% sodium azide, and 0.03% DM.

FPLC–Size-Exclusion Chromatography (SEC). A Superose 12 10/30 and a Superose 6 10/30 column (Pharmacia, Uppsala, Sweden) were connected in series and equilibrated with 50 mM Tris, pH 8.0, 100 mM glycine, 0.02% sodium azide, and 0.03% DM (FPLC SEC buffer). The flow rate was 0.3 mL/min, and the eluate was monitored at 280 nm. Fractions were collected every 2 min. The LH2 peak eluted at 78 min and was pooled and concentrated as above. Following the LH2 peak, at 92 min, the carotenoid–protein complex eluted; it was pooled and concentrated as above except with the use of a 30-kDa cutoff membrane.

HPLC–SEC. For analytical SEC, a Toso Haas TSK 3000SW column (Novex Co., San Diego, CA) was equilibrated with 100 mM sodium sulfate, 25 mM sodium phosphate, pH 7.0, 0.03% DM, and 0.02% sodium azide. The flow rate was 0.4 mL/min, and the eluate was monitored at 280 nm. The column was calibrated with premixed gel-filtration standards (670–1.35 kDa) from BioRad (Richmond, CA). For preparative isolation of the complexes, a Toso Haas TSK 3000 column (Novex Co.) was used with the same mobile phase as for analytical SEC and the flow rate increased to 4 mL/min. Peaks were collected individually.

Denaturing and Nondenaturing Gel Electrophoresis. Nondenaturing electrophoresis was carried out as described previously, using the method of Peter and Thornber (1991). Denaturing polyacrylamide gel electrophoresis was performed on ice using the following modifications of the method of Schagger and von Jagow (1987). For resolution of the LH2 polypeptide subunits, the acrylamide concentrations were increased to 14% in the spacer gel and to 20% in the separating gel. Samples of the pigment–protein complexes were prepared for electrophoresis by incubation for 10 min at room temperature with the SDS sample buffer described by Laemmli (1970). Prestained molecular weight markers were obtained from BRL (Gibco BRL, Grand Island, NY). The lane of prestained markers was excised before silver staining (Morrissey, 1981).

N-Terminal Amino Acid Sequencing. After resolution of proteins by SDS-PAGE, 0.5-mm-thick gels were transferred for 30 min at 250 mA in CAPS transfer buffer (LeGendre and Matsudaira, 1988) onto Immobilon (Millipore Co., Bedford, MA) in preparation for N-terminal sequencing. The blots were briefly stained with Coomassie blue as described (LeGendre and Matsudaira, 1988) and the bands of interest excised. N-terminal amino acid sequencing was carried out at the UCLA microsequencing facility.

Sequence Analysis. Amino acid sequence comparisons between the *C. purpuratum* LH1 subunit and the published antenna sequences were performed using FASTA and BESTFIT of the GCG Sequence Analysis Software package (Genetics Computer Group, Inc., Madison, WI).

Spectroscopy. Absorbance spectra were taken on a Shimadzu UV 160 spectrophotometer (Cole Scientific, Moorpark, CA). Circular dichroism measurements were carried out on a Jasco J-600 spectropolarimeter (Jasco Inc., Easton, MD). The optical path length was 1 cm between 250 and 1000 nm and 1 mm below 250 nm.

Pigment Analysis and Protein Quantification. Pigments were extracted with an ice-cold (–40 °C) acetone:methanol mixture, 7:2 (v:v). A single extraction with the acetone:methanol mixture was sufficient for complete pigment removal. The protein was collected by centrifugation and quantified using the BCA Protein assay kit (Pierce Rockford, IL) with bovine serum albumin as a standard. A similar quantity of protein was recovered by extraction with five volumes of ice-cold acetone. Bacteriochlorophyll-*a* and carotenoid in the acetone:methanol supernatant were quantitated spectrophotometrically using an extinction coefficient at 772 nm of 76 mM^{-1} cm^{-1} for bacteriochlorophyll-*a* (Clayton, 1963) and an extinction coefficient at 480 nm of 134 mM^{-1} cm^{-1} for okenone (Britton, 1985).

RESULTS AND DISCUSSION

The Peripheral Antenna (LH2). Optimal solubilization of the photosynthetic membranes, assessed by Deriphat-PAGE pattern, was achieved with a mixture of OG and DM. The LH2 of *C. purpuratum* is prepared by adding ammonium sulfate to the diluted solubilized mixture to 75% saturation. After centrifugation, semipure LH2 remains in solution (approximately 60% yield). Its principal contaminants are proteins associated with the carotenoid–protein complex (see

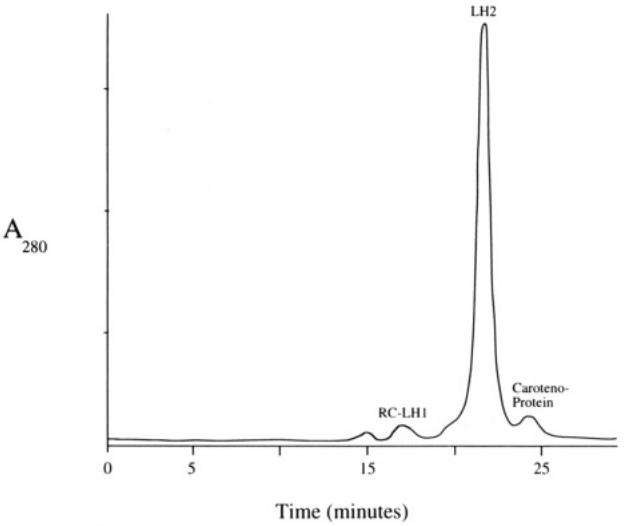

FIGURE 1: Elution profile of the HPLC-SEC purification showing the relative order of elution of the core, the LH2, and the carotenoid–protein complex from *C. purpuratum*.

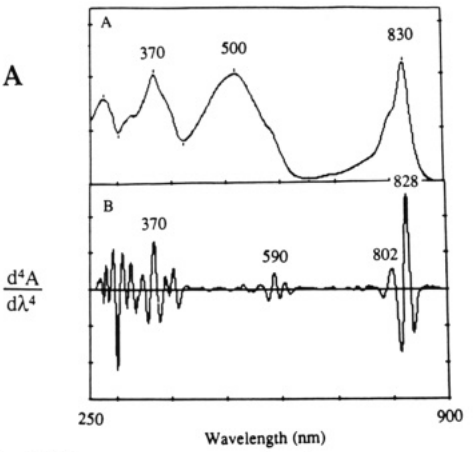

FIGURE 2: (A) Room-temperature absorbance spectrum of the *C. purpuratum* LH2. (B) Fourth-derivative analysis of the LH2 spectrum.

below). After concentration and dialysis, the LH2 is subjected to FPLC- and HPLC–SEC (Figure 1) in which the core- and carotenoid–protein-containing fractions are separated from the LH2. The yield of purified LH2 is typically near 44% of the total LH2 in the crude membrane fraction.

The LH2 of *C. purpuratum* has a strong NIR absorption peak at 830 nm with a shoulder at 802 nm (Figure 2A); it is the predominant component of the absorbance spectrum of *C. purpuratum* chromatophores with lesser contributions to the whole membrane spectrum from the core and the carotenoid–protein. This unusual peripheral antenna absorbance has only been previously reported for *Chromatium okenii* (Thornber et al., 1978). Other organisms have LH2 maxima typically centered at 850 nm with a definite peak at 800 nm. Other spectrally shifted peripheral antenna absorbances do occur as alternative forms of the *Rhodopseudomonas acidophila* and *Chromatium vinosum* LH2, with maxima at 820 nm (B820) (Zuber & Brunisholz, 1991). Fourth-derivative analysis indicates that there are no other NIR absorbance forms in the *C. purpuratum* LH2 except the 802-nm shoulder (Figure 2B). We and others (R. J. Cogdell, personal communication) have been unable to induce alternative spectral forms of the LH2 of *C. purpuratum* by modifying growth conditions for this organism. The bacteriochlorophyll-*a* to carotenoid ratio in this LH2 preparation

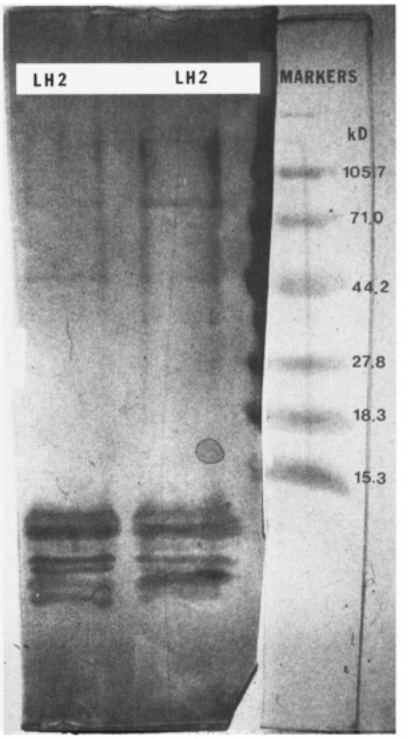

FIGURE 3: Silver-stained SDS-PAGE of the *C. purpuratum* LH2 complex (left two lanes). Prestained molecular weight markers and their sizes are given at right.

is 1.21, essentially identical to that reported for an alternative LH2 preparation from this organism (Cogdell et al., 1990). It was then concluded that the ratio is more probably 1.0 (Cogdell et al., 1993) because of the uncertainty of the extinction coefficient of okenone.

The size of the LH2 complex as determined by analytical HPLC–SEC is 180 kDa, similar to that reported for *Rhodobacter capsulatus* (Shiozawa et al., 1982). Initial attempts to sequence α and β polypeptides that had been isolated by standard SDS-PAGE indicated that there were more than two polypeptides present (R. J. Cogdell, personal communication). A modified SDS-PAGE system enabled resolution of the spectrally pure complex into six polypeptides (Figure 3) of sizes between 4 and 8 kDa. This silver-stained gel illustrates the purity of this preparation. The N-terminal sequence of each polypeptide has been obtained (Table 1). The amount loaded into the amino acid sequencer varied between 180 and 800 pmol for each of the different polypeptides, and the yield of the initial residues varied between 25 and 200 pmol. One of these sequences (α2) was independently duplicated from a different sample to verify the reproducibility of the amino acid assignments. Each of the resulting sequences resembles either an LH2 α- or β-type polypeptide. It is unlikely that any of these polypeptides are a contaminant from another organism because of *C. purpuratum*'s relatively high-salt growth requirement (5%); it is easy to maintain an axenic culture. Furthermore, we argue that none of these polypeptides are derived from LH1 because we have also obtained nearly the full-length sequence of the LH1 β subunit from our core (RC–LH1) preparation and it is highly homologous to LH1 βs from other purple bacteria and does not resemble the LH2 β polypeptides reported here. The LH1 α polypeptide is N-terminally blocked (Kerfeld et al., in press), as is true for LH1 αs in general (Zuber & Brunisholz, 1991), and, therefore, cannot be one of the six polypeptides we have sequenced.

Table 1: Comparison of the *C. purpuratum* Peripheral Antenna (LH2) Sequences with Some Published α and β Sequences[a]

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                        β-----**  *   !       +
C. purpuratum B830α1    MKVPVMMADENAKLNNPEDDRKKFFV....
                                  || :|||||:| ::|
C. vinosum B850α2                 SNVAKPKNPEDDWKIWLVVN....
                                  || ::||||:|
C. purpuratum B830α2    MQVPVMLADKNAKLNHPEDDRKR....
                              ***  *   !

                                !                +
C. vinosum B850α2               SNVAKPKNPEDDWKIWLVVNPATWLIP....
                                      :  |  ||
C. purpuratum B830α3                  RESKANLVQS....
                                      ::|  ||
Rb. sphaeroides B850α                 MTNGKIWLVVKP....

                                                       +
C. vinosum B820β1                             AELSGLTDQQAKEF....
                                              |:||| ||
C. purpuratum B830β3                        DESANLSGSTDTLQA....
                                            ||| :
C. purpuratum B830β1                  AKIPGLXGDESIHTIRMPET....

                                              +
C. purpuratum B830β1                  AKIPGL.GDESIHTIRMPET....
                                      | :||   |:||
Rb. capsulatus B850β                MTDDKAGPSGLKEAEEIHSYLID....
                                          | | |
C. purpuratum B830β2                AFLSALTDAQAKEFHE....
                                    | ||:||| ||||||
C. vinosum B820β1                   AELSGLTDQQAKEFHE....
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[a] Sequences named in bold identify data obtained for this paper. X = unidentifiable residue. ! = the position of H/N*. * = conserved antenna primary structure features. β = predicted β-type secondary structure. The underlined region is identical to the pigment-binding motif of *P. aestuarii*. + = the proposed beginning of the transmembrane region.

Six subunits is the largest number of subunits reported for any purified LH complex described thus far. The number of subunits does not vary in cells grown under different environmental conditions (data not shown), and the complex comprises a single spectral form, the B800-830 (Figure 2). Thus, the moderately halophilic *C. purpuratum* is similar to the thermophilic *Chromatium tepidum* in that it apparently does not modify the absorbance of its peripheral antenna in response to environmental conditions. This is in contract to the mesophilic *C. vinosum* (Zuber & Brunisholz, 1991). Perhaps the ability to modify the spectral features of these polypeptides to compensate for changing light intensities is an evolutionary adaptation that began as a multiplicity of subunits that initially contributed to a single spectral form.

Many LH α and β polypeptides from purple-nonsulfur bacteria have been sequenced. Their proposed structural features are conserved (Figure 4): a single transmembrane α-helical domain with polar N- and C-termini protruding into the cytoplasm and periplasm, respectively (Tadros et al., 1987). Within the proposed transmembrane span of these polypeptides are conserved histidine residues that are thought to ligate bacteriochlorophyll. On the basis of these conserved primary structural features, we are able to assign three of the *C. purpuratum* LH2 sequences as α-types and three as β-types. Sequence homology between α and β polypeptides is approximately 6% (Zuber & Brunisholz, 1991), facilitating the distinction between these two types of polypeptides. Table 1 shows the N-terminal sequences of the six *C. purpuratum* LH2 subunits and their best alignments to some published antenna sequences.

LH2 α Subunits. Alignments of the *C. pupuratum* LH2 α1 and α2 sequences with those of other LH α polypeptides suggest that the N-terminal region we have sequenced extends just into the proposed transmembrane span (Table 1). These

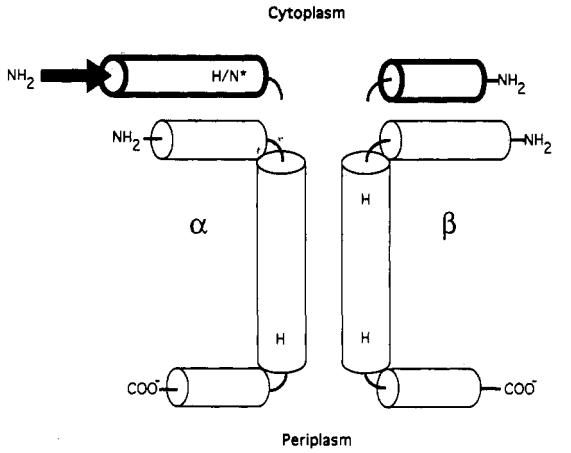

FIGURE 4: Schematic diagram of the α and β polypeptides in the membrane (adapted from the model of Zuber & Brunisholz, 1991). The canonical bacteriochlorophyll-binding sites are indicated with H. The sequenced regions of the LH2 α and β polypeptides from *C. purpuratum* are shown in bold above the model. The N-terminal extension with β-strand character is drawn as an arrow. H/N* points to a possible additional bacteriochlorophyll-binding site.

two α subunits of *C. purpuratum* LH2 are distinctive for having the longest N-terminal sequence preceding the membrane span reported thus far for LH polypeptides (Zuber & Brunisholz, 1991). Also, it is unusual that the N-terminal portion of the α1 and α2 subunits is longer than those of the β subunits. The primary structure of *C. purpuratum* LH2 α1 and α2 subunits most closely resembles that of the *C. vinosum* B850 α1 (Table 1) subunit rather than that of the more spectrally similar *C. vinosum* B820 α (not shown). The presence of aromatic amino acids in antenna polypeptides is thought to be partly responsible for conferring the spectral shift that occurs when bacteriochlorophylls are conjugated to their apoproteins (Brunisholz & Zuber, 1988; Fowler et al., 1992). In the *C. purpuratum* LH2 α1 subunit, there is a phenylalanine at position 24; in the analogous position of the B850 α subunits of *C. vinosum*, there is a tryptophan or a tyrosine. *C. vinosum*'s other spectral form of the LH2 complex, the B820, lacks an aromatic amino acid in this position in the α polypeptide.

The extended N-terminal regions of the *C. purpuratum* LH2 α1 and α2 polypeptides have a surprisingly high degree of homology with LH β subunits. Nevertheless, they have more α characteristics than β.

Indications of Additional or Alternate Pigment-Binding Sites and β-Strand Structure in LH2. In the N-termini of two of the *C. purpuratum* LH2 α subunits, there is a histidine or asparagine at position 16, H/N* (Table 1; Figure 4). In the aligned position of the homologous B850 α subunit of *C. vinosum*, there is an asparagine (N*). Histidine is the canonical ligand for bacteriocholophyll found in the membrane-spanning region of light-harvesting polypeptides. There are two positionally conserved histidine residues in the β polypeptides and one conserved histidine in the α polypeptides (Zuber & Brunisholz, 1991) (Figure 4). These histidines and/or also asparagine, when they appear in these conserved positions, are thought to ligate bacteriochlorophyll. Conserved primary structure features have been noted in the vicinity of these bacteriochlorophyll-binding residues. Several of these are also found in the extramembranous environment of the *C. purpuratum* H/N*. For example, at the His/Asn −4 position, there is an alanine or, occasionally, a glycine. These small amino acids have been suggested to be necessary in this position to provide an interaction point for pigment in the

presumed α-helical structure of the polypeptide (Zuber & Brunisholz, 1991). An alanine is also found in the –4 position to H/N* of the *C. purpuratum* α polypeptides (Table 1). There are several other conserved features in the vicinity of the conserved N-terminal histidine of β subunits that appear near H/N* in the *C. purpuratum* α polypeptides: at the His-7 position, there is an acidic residue, and there is an alanine (analogous small residue) at the His-8 position in the *C. purpuratum* LH2 α1 and 2. There is an additional leucine in the His-9 position of *C. purpuratum* α2—this is another typical feature near the N-terminal conserved β histidine. The His* of the *C. vinosum* B850 α1 shares only the –4 conserved feature, but its N-terminal region is too short to have any of the other conserved features.

The results of pigment–protein quantification concur with the hypothesis that the *C. purpuratum* LH2 may contain more than three bacteriochlorophyll molecules per α, β pair. Assuming an average molecular weight of 12 kDa for any of the three α, β pairs in the *C. purpuratum* LH2, the molar ratio of bacteriochlorophyll to protein was found to be 4.5 ± 0.5 in three separate pigment/protein quantifications.

Additional histidines in light-harvesting α polypeptides besides those found in the conserved position occur in *Rb. capsulatus*, in *Ectothiorhodospira halophila* and *Ectothiorhodospira halochloris* (Wagner-Huber et al., 1992), in *Rhodopseudomonas palustris* (Brunisholz et al., 1990), and in *Rps. acidophila* (Zuber & Brunisholz, 1991). In *Rb. capsulatus*, this histidine is located in the C-terminal extramembranous region. The others are found within the proposed membrane span of the Ectothiorhodospireae antenna polypeptides or at the cusp of the transmembrane and extramembranous regions in *Rps. acidophila* and *Rps. palustris* (Zuber & Brunisholz, 1991). In contrast, the large number of charged residues following H/N* and N* in the extended N-terminal regions of the *C. purpuratum* LH2 α subunits makes it unlikely that this sequence is embedded in the membrane—a presumed requirement for coordination of the nonpolar pigment molecules to the conserved LH α and β histidines. Thus, if bacteriochlorophyll is ligated to H/N* of the *C. purpuratum* α polypeptides, the protein would have to shield the cofactors from the polar environment; for example, the N-terminal region may form amphipathic secondary structure that could fold over and protect the pigment. Secondary structure prediction (Chou & Fasman, 1978) for the *C. purpuratum* LH2 α1 and α2 sequences suggests that, unlike N-termini in purple-nonsulfur organisms, their N-terminal regions have β-strand character up to the H/N*-9 position followed by an α-helical region that contains H/N* and the conserved primary structure features found in the vicinity of pigment-coordinating histidines (see Table 1). The presence of alanine at position H/N*-9 further implicates this position as the site of a change in secondary structure; alanine is commonly found at α/β connections (Richardson & Richardson, 1989). CD measurements of the *C. purpuratum* LH2 in the peptide region indicate a mixture of α- and β-type secondary structure (Figure 5) (Greenfield & Fasman, 1969; Brahms & Brahms, 1980; Johnson, 1988). This is similar to the UV–CD spectrum for *C. vinosum* LH2 polypeptides, which indicates a predominance of β-sheet structure rather than the α-helical structure that was observed for the LH complexes from purple-nonsulfur organisms (Cogdell & Scheer, 1985). CD and Raman spectroscopy data (summarized in Zuber & Brunisholz, 1991) indicate that the pigment organization and environment in the *C. vinosum* peripheral antenna are different from those of purple-nonsulfur organisms. Perhaps this is

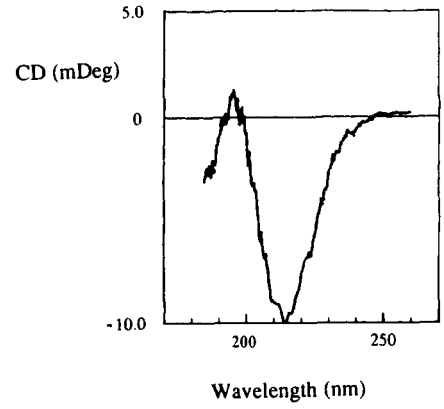

FIGURE 5: Circular dichroism spectrum of the ultraviolet region of the *C. purpuratum* LH2.

due to coordination of a bacteriochlorophyll molecule by *C. vinosum*'s N*. The unusual spectroscopic data may be the result of the additional pigment and the concomitant structural features of the protein necessary to accommodate it. CD-based prediction of secondary structure of pigment–proteins can really be only qualitative; however, Cogdell and Scheer (1985) concluded that the chromophore contribution below 230 nm is minor and that scattering due to the complexes is negligible.

The possibility of a β-sheet structure being involved in shielding the bacteriochlorophyll-*a* molecules in the N-terminal region of *C. purpuratum* α polypeptides recalls the bacteriochlorophyll-*a*-binding protein from another moderately halophilic organism, the green-sulfur organism *Prosthecochloris aestuarii*. The three-dimensional structure of this water-soluble protein shows that the pigments are shielded by amphipathic β-sheets from the polar environment (Tronrud et al., 1986). It is therefore interesting to note that the L-N-H/N*-P motif in the *C. purpuratum* LH2 αs is identical to that of a bacteriochlorophyll-binding segment of the *P. aestuarii* protein; His 282 ligands bacteriochlorophyll no. 4 (Daurat-Larroque et al., 1986). In addition, the putative β-strand element of the *C. purpuratum* LH2 α polypeptides is similar to a segment of the *P. aestuarii* protein (residues 144-148, M-L-V-P-L) that participates in β-sheet formation and functions as a shield for bacteriochlorophyll no. 1 in that protein (Daurat-Larroque et al., 1986; Tronrud et al., 1986).

The LH2 of *C. purpuratum* is unusually stable, able to withstand high concentrations of SDS, salts, and organic solvents. Porin, the only entirely β-sheet-type membrane protein for which the crystal structure is known (Weiss & Shulz, 1992), is also extremely stable (Rosenbusch & Mueller, 1977); extensive hydrogen bonding within and between the subunits stabilizes this homotrimeric complex.

The third subunit assigned to the LH α class lacks the N-terminal extension. It has the most sequence similarity to the B850 α antenna polypeptides of *Rhodobacter sphaeroides* and *C. vinosum* (Table 1).

The LH2 β Subunits. Unlike the α1 and α2 subunits of the LH2 of *C. purpuratum*, the three β subunits are not highly homologous to each other (Table 1). *C. purpuratum* β2 has high sequence similarity to a B820 β subunit of *C. vinosum*. In that organism, the three B820 β subunits are highly homologous to one another.

The LH2 β1 subunit from *C. purpuratum*, surprisingly, has the highest amino acid sequence similarity to a LH2 β polypeptide of a purple-nonsulfur organism, *Rb. capsulatus*, far exceeding the alignment with any of its closer purple-sulfur relatives (Table 1). The N-terminal region of the *C.*

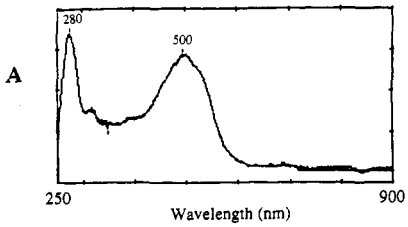

FIGURE 6: Room-temperature absorbance spectrum of the carotenoid–protein complex.

purpuratum β1 is shorter than that of the *Rb. capsulatus* polypeptide, and it is not as acidic. The acidic stretch in the N-terminal sequence of β polypeptides is highly conserved and is thought to be important in the interaction of β polypeptides with αs. The *C. purpuratum* β1 is thus somewhat unusual which may be reflected in the unusual quaternary structure of the LH2 in this organism.

The Carotenoid–Protein Complex. A Deriphat-PAGE band that contains carotenoid was tentatively identified as a detergent-complexed pigment (Ferguson et al., 1991) and was subsequently found to contain three proteins of 55, 18, and 15 kDa (data not shown). We are currently obtaining N-terminal sequence data on these proteins. It will be of interest to see if they are related to the *Rb. capsulatus* γ subunit or other photosynthetic carotenoid–proteins of similar size that have been described (Engle et al., 1991; Markwell et al., 1992). These three proteins together with carotenoids comprise a single peak in HPLC–SEC and FPLC–SEC of 70 kDa. The absorbance spectrum of the complex indicates that carotenoid is the only type of pigment present (Figure 6). The peak at 500 nm is blue-shifted as compared to the central carotenoid absorption peak in the LH2 (Figure 2). TLC analysis confirms that the carotenoid is essentially okenone only (Cogdell et al., 1990). Okenone is a keto–carotenoid with an aromatic ring; carotenoids containing aromatic groups are synthesized by strictly anaerobic organisms (Schmidt, 1978) including green-sulfur organisms (Goodwin, 1980). Okenone is not found in the Rhodospirilleae (Goodwin, 1980), and its CD spectrum has not been previously described. The CD spectra of the *C. purpuratum* carotenoid–protein complex, the LH2, and whole chromatophores are compared in Figure 7. Okenone's CD spectrum in the carotenoid–protein complex is similar to that of isolated LH2 (Figure 7B) and of chromatophores (Figure 7C), yet the carotenoid absorbance in the LH2 (520 nm, Figure 2) is blue-shifted in the carotenoid–protein complex (500 nm, Figure 6). Raman spectroscopy indicates that the okenone molecules in the *C. purpuratum* LH2 are actively involved in light harvesting (Cogdell et al., 1993).

CONCLUDING COMMENT

Since few purple-sulfur organisms have been evolutionarily cataloged by 16s rRNA analysis, it is uncertain how ancient the phenotype is (Stackebrandt & Woese, 1981). Of the Rhodospirilleae, which are thought to have evolved from the purple-sulfur organisms, *C. purpuratum* shares many phenotypic features with *Rb. capsulatus*, *Rps. acidophila*, *Rhodopseudomonas viridis*, and *Rhodocyclus gelatinosus*. These organisms fall into three distinct branches of the purple photosynthetic bacteria on the basis of 16s rRNA analyses (Woese, 1987), perhaps indicative of the existence of *C. purpuratum* well before the branching points that ultimately gave rise to these organisms. *C. purpuratum's* phenotypic relation to other Chromatiaceae is also broad. Also, its ability to synthesize the aromatic carotenoid okenone further suggests

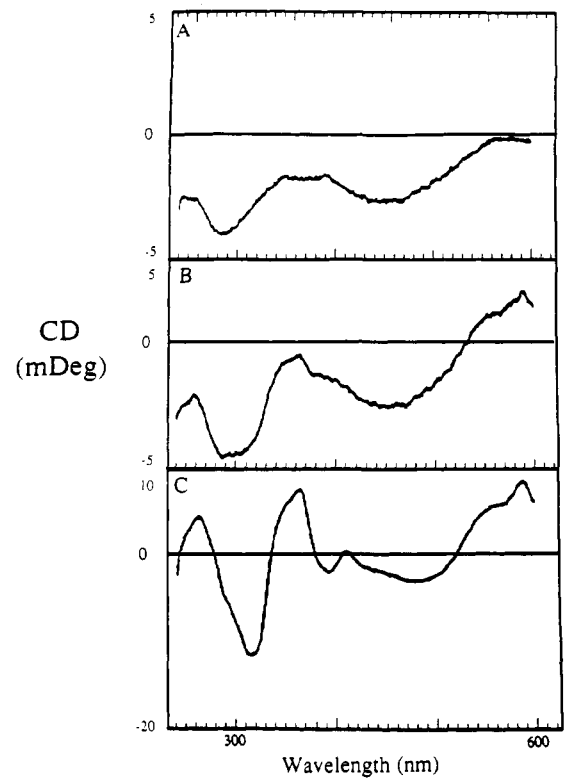

FIGURE 7: Circular dichroism spectra of (A) the carotenoid–protein complex, (B) the isolated LH2, and (C) *C. purpuratum* chromatophores.

that *C. purpuratum* is one of the more ancient organisms in this group; thus, its photosynthetic apparatus may share some structural and functional characteristics with that of green-sulfur organisms. The three-dimensional structure of the *C. purpuratum* LH2 will be especially interesting because of its unusual subunit composition and its secondary structure. In addition, it will reveal how this organization of pigments and proteins confers the unusual spectroscopic properties of this complex. To this end, we have prepared crystals of the photosynthetic pigment–protein complexes of *C. purpuratum* for X-ray diffraction experiments (Kerfeld et al., 1993).

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