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Biochemical and spectroscopic characterization of the reaction center-LH1 complex and the carotenoid-containing B820 subunit of *Chromatium purpuratum*

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Abstract

Two complexes, the reaction center light-harvesting complex 1 (RC-LH1) and the B820 subunit of the LH1, have been isolated and characterized from the purple-sulfur photosynthetic bacterium *Chromatium purpuratum*. The RC-LH1 consists of the B870 antenna and a P-870 RC with an associated tetraheme cytochrome. This complex can be further fractionated to yield the B820 subunit of the LH1. The *C. purpuratum* B820 subunit is the first isolated from a purple-sulfur bacterium. It is also the first that retains its carotenoid absorption properties. CD spectra in the Q_y region of bacteriochlorophyll *a* in both the RC-LH1 and the B820 subunit are bathochromically shifted as compared to other such complexes. Comparison of the sequence of the LH1β polypeptide to other LH1βs reveals the presence of additional aromatic amino acids in the vicinity of both of the conserved histidines in the *C. purpuratum* β polypeptide. The CD spectra of these *C. purpuratum* pigment-protein complexes can be interpreted in terms of exciton interaction between bacteriochlorophylls in the B820 subunit of the LH1 and in the B870, with additional spectral characteristics arising from interactions of the pigments with their protein environment.

Key words: Photosynthesis; B820 subunit; Light-harvesting complex; Reaction center; Bacteriochlorophyll; Exciton interaction

1. Introduction

The photochemical reaction center (RC) of purple bacteria structurally and functionally resembles that of eukaryotic Photosystem II [1]. The RC of a purple photosynthetic bacterium, *Rhodopseudomonas viridis*, was the first integral membrane pigment-protein complex for which a three-dimensional X-ray crystal structure was determined [2]. Subsequently, the crystal structure of the RC of another purple bacterium, *Rhodobacter sphaeroides*, was reported [3,4]. In *Rb. sphaeroides* the RC is composed of three protein subunits H, M and L. In some purple bacteria there is a fourth subunit, a tetraheme cytochrome, that reduces the photooxidized primary donor. It is not an integral membrane protein but rather, as the *Rps. viridis* structure revealed, is attached to the L and M subunits by a single lipid moiety [5]. It protrudes into the periplasm. Several cofactors are associated with the RC: four bacteriochlorophyll *a* molecules (BChl *a*), two bacteriopheophytin *a* molecules, two molecules of ubiquinone, a carotenoid molecule and a single non-heme iron atom. Two of the BChl *a* molecules are related to one another by an approximate two-fold axis of symmetry and form an exciton coupled dimer; they are known as the special pair or primary electron donor.

Surrounding the RC is at least one and often a second light harvesting complex (LH) that capture light energy and funnel it to the RC. The LH most closely apposed to the RC, the LH1 or B870 (named for its approximate NIR absorption maximum), is encoded by the operon that encodes the L and M subunits of the RC [6–9]. This light-harvesting complex occurs in a

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fixed stoichiometry to the RC [10]. The LH1 is a multimeric complex composed of heterodimers of two small (< 10 kDa) polypeptides, α and β. Sequence analyses of these polypeptides indicate the presence of positionally conserved histidines that are thought to ligate BChl. These are located in the predicted single alpha-helical transmembrane span in each of these polypeptides (reviewed in [11]). In purple-nonsulfur photosynthetic bacteria there are 2 BChl and 1–2 carotenoid molecules per α/β pair in the LH1 [12,13]. In some organisms the LH1 can also be isolated alone as either the large multimeric form that is associated with the RC (the B870) or as a smaller unit, the B820 subunit that is thought to be the basic building block of the B870 [14–19]. Typically, these units have NIR maxima near 820 nm. The polypeptide number of this basic unit is as yet uncertain; estimates range from a single $\alpha\beta$ heterodimer to an $(\alpha\beta)_3$ [14,17,20].

A central question in the study of photosynthesis concerns how the different spectral forms of the chlorophyll- and bacteriochlorophyll-protein complexes arise. For example, free BChl *a* in solution has an absorbance maximum of approximately 770 nm. Association of the pigment with protein in the photosynthetic complexes induces a spectral shift into the NIR. This shift may arise through exciton interactions between pigments [21,22], through interactions between pigments and charged [23] or aromatic amino acids [24], or from some combination of these interactions.

Purple photosynthetic bacteria can be divided into two groups based on their ability to use reduced sulfur compounds as an electron donor in photosynthesis. The photosynthetic complexes from purple-nonsulfur organisms have been well characterized biochemically and at the level of the gene (*Rb. sphaeroides*, *Rhodobacter capsulatus*, *Rps. viridis*) [6–9]. In contrast, little is known about the photosynthetic apparatus of the purple-sulfur organisms (members of the Chromatiaceae and the Ectothiorhodospireaceae). These organisms are thought to be more ancient than their purple-nonsulfur relatives [25].

Chromatium purpuratum is a marine purple-sulfur bacterium first described in 1980 [26]. It is a BChl *a*-containing organism that also contains the uncommon keto-carotenoid, okenone. Deriphat-PAGE resolves the solubilized photosynthetic apparatus of this organism into three distinct pigment-protein complexes: the RC-LH1, the peripheral antenna or LH2 and a carotenoid-protein complex [27]. Further fractionation of the RC-LH1 complex yields the B820 subunit of the *C. purpuratum* B870. Here we describe the *C. purpuratum* RC-LH1 complex and the isolation and characterization of the first B820 subunit from a purple-sulfur bacterium. This is the first B820 subunit isolated from any purple photosynthetic bacteria which retains its carotenoid absorbance properties.

2. Materials and methods

Cells. C. purpuratum cells were grown in modified Pfennig's medium as previously described [28].

Membranes. Photosynthetic membranes (chromatophores) were prepared by French pressure cell breakage of the bacteria and differential centrifugation as previously described [28].

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 $5000 \times g$ to pellet debris.

Ammonium sulfate precipitation. Solubilized membranes were diluted to an A_{830} of 50 with 1 M MES, pH 6.0. Ammonium sulfate was added slowly to 30% saturation. The solution was mixed gently for 30 min at 4°C and the centrifuged at $7600 \times g$. The pellet was discarded and the concentration of ammonium sulfate in the supernatant increased to 45% saturation. After mixing and centrifugation, the pellet was gently resuspended in a small volume of HPLC mobile phase (see below).

Gel electrophoresis. Non-denaturing electrophoresis was carried out as described previously [27,29] on the solubilized membranes. Denaturing polyacrylamide gel electrophoresis was performed on ice by the method of Schagger and von Jagow [30]. For optimal separation of the RC-LH1 subunits, the concentration of acrylamide in the separating gel was increased to 18%. 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 [31]. Molecular mass markers were obtained from BRL (Gibco BRL, Grand Island, New York). Gels were stained with Coomassie Brilliant blue. Alternatively, gels were silver stained [32]. For detection of proteins containing heme groups, the method of Thomas et al. [33] was used.

Electroelution of the Deriphat-PAGE separated photosynthetic complexes. Resolved pigment-protein complexes were excised from the Deriphat-PAGE and electroeluted in an Isco Model 1750 Sample Concentrator (Isco, Lincoln, NE). Electroelution was carried out on ice, in the dark, at a constant current of 6 mA. Deriphat-PAGE reservoir buffer or Deriphat-PAGE reservoir buffer with 0.03% DM substituted for Deriphat was used as the electroelution buffer. Aliquots of the electroeluted material were collected frequently to minimize denaturation.

HPLC-SEC. In preparation for HPLC-SEC, the electroeluted RC-LH1 was concentrated in Centricon 10 concentrators (Amicon, Beverly, MA). The ammonium sulfate-precipitated RC-LH1 was applied directly

to the column. 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 or 0.8% OG (Sigma Chemical, St. Louis, MO) and 0.02% sodium azide. The flow rate was 0.4 ml/min and the eluate was monitored at 280 nm. 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 was increased to 4 ml/min. Peaks were collected individually. Both columns were calibrated with premixed gel filtration standards between 670 and 1350 Da (BioRad, Richmond, CA).

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 [34] onto Immobilon (Millipore Co., Bedford, MA). The blots were briefly stained with Coomassie blue as described [34] and the bands of interest were 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 published antenna sequences [11,35] were performed using FASTA and BESTFIT of the GCG Sequence Analysis Software package (Genetics Computer Group Inc., Madison, WI).

Spectroscopy. Absorbance spectra were recorded on a Shimadzu UV 160 spectrophotometer (Cole Scientific, Moorpark, CA). Chemically induced difference spectra were obtained by the addition of ferricyanide, sodium ascorbate, and sodium dithionite (Sigma Chemical Co., St. Louis, MO). Circular dichroism measurements were carried out on a Jasco J-600 spectropolarimeter (Jasco Inc., Easton, MD.) using a 0.2 cm path length cell. Fluorescence measurements were carried out on an Aminco SPF-500. The B818 sample had an A_{818} of 0.12 cm^{-1}; the RC-LH1 had an A_{870} of 0.100 cm^{-1}, while the whole cells and chromatophores had an A_{830} of > 1.0 cm^{-1}.

Determination of pigment ratios. Pigments were extracted from the complexes using 5 vols. of an acetone/methanol (7:2) mixture followed by centrifugation at 12000 $\times g$ to remove protein. BChl:carotenoid ratios in extracts of the RC-LH1 and the B820 subunit were estimated using an ϵ_{772} of 76 $mM^{-1}cm^{-1}$ for BChl [36] and ϵ_{480} of 134 $mM^{-1}cm^{-1}$ for okenone [28].

3. Results

The RC-LH1 complex

Optimal solubilization of the photosynthetic membranes, assessed by the Deriphat-PAGE pattern [27], was achieved with a mixture of OG and DM. The RC-LH1 complex, prepared either by electroelution or ammonium sulfate precipitation has a size of 550 kDa as determined by analytical SEC in DM (Fig. 1) and 470 kDa in OG. A room-temperature chemical difference spectrum of the ammonium sulfate-prepared RC-LH1 complex (ferricyanide oxidized-ascorbate reduced; Fig. 2a) indicates that the absorption maxima of the special pair is at 875 nm. The absorbance changes at 789 and 808 nm are due to a spectral shift of the accessory BChls. The room-temperature absorbance spectrum of the complex is shown in Fig. 2b. The NIR absorption bands at 760 and 800 nm reflect the presence of the bacteriopheophytin and the accessory BChl *a* of the RC, respectively. The large absorbance at 870 nm is due mainly to the presence of the LH1 (B870) with a small contribution from the special pair, P870. An NIR maximum near 870 nm for the *C. purpuratum* RC-LH1 is the highest energy BChl *a* Q_y transition observed for an LH1 in a purple-sulfur bacterium thus far [37]. In the visible portion of the spectrum, the 590 nm band is due to the Q_x transition of BChl *a* and the region between 500–560 nm contains contributions from carotenoid, the Q_x of bacteriopheophytin, and the tetraheme cytochrome. The 370 nm peak is attributed to the Q_B bands of BChl *a* and of bacteriopheophytin. The ratio of BChl:carotenoid in the B820 complex was estimated to be 1:1, similar to that reported for *Rb. sphaeroides* [12] and in the RC-LH1 it is 4:1. However, the extinction coefficient of okenone is not well established [28] and the BChl content in the RC has fallen short of the expected value in other studies [12], so these estimates must be regarded with caution.

SDS-PAGE of the DM-electroeluted RC-LH1 complex (Fig. 3) indicates that it retains all six expected subunits although the staining of the cytochrome is diffuse. The proteins of 45, 39, 22 and 19 kDa are

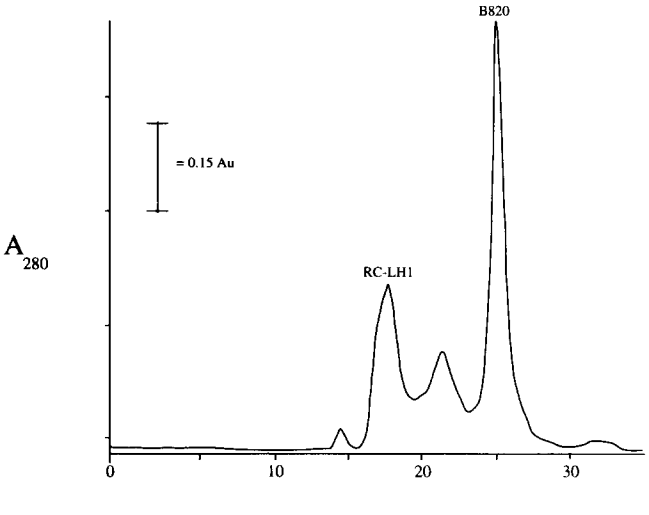

Fig. 1. HPLC elution profile for RC-LH1 electroeluted in DM.

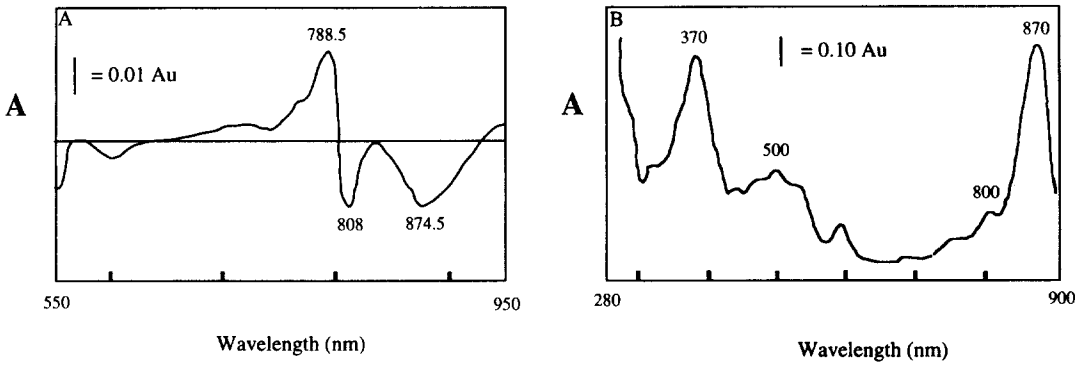

Fig. 2. (A) Room-temperature chemically-induced (ferricyanide-oxidized-ascorbate reduced) difference spectrum of the *C. purpuratum* core. (B) Room-temperature absorbance spectrum of the RC-LH1 complex.

tetraheme cytochrome, H, M, and L, respectively. As in *Rhodocyclus gelatinosus* [38] and *Rhodospirillum salexigens* [39], the Coomassie blue staining of the RC subunits varies in intensity. The 45 kDa subunit stains for heme (Fig. 4) and the multiple absorption bands of its chemically-induced high- and low-potential difference spectra (Fig. 5) are characteristic of a tetraheme cytochrome. There are additional aggregated forms of the RC subunits; this has been noted before in studies of the RC-LH1 of *Rc. gelatinosus* [40]. The smallest subunit (5 kDa) in Fig. 3 has been N-terminally sequenced and is homologous to LH1 β subunits from other purple bacteria (Table 1). The 8 kDa band does not stain well with Coomassie blue (Fig. 3) but can be visualized with silver staining (cp. the α subunit of *Rhodospirillum rubrum* [41]). Attempts to sequence this protein have failed: it is N-terminally blocked, as are other LH1α subunits [11]. These properties and its slower migration relative to the LH1β make it very likely that this is a typical LH1α subunit.

The circular dichroism spectrum of the ammonium sulfate-prepared RC-LH1 in HPLC mobile phase (diluted with water to an $A_{870} = 0.118\ cm^{-1}$) is shown in Fig. 6a. It has a broad positive peak at 877 nm, and a nonconservative double CD that is positive at 810 and

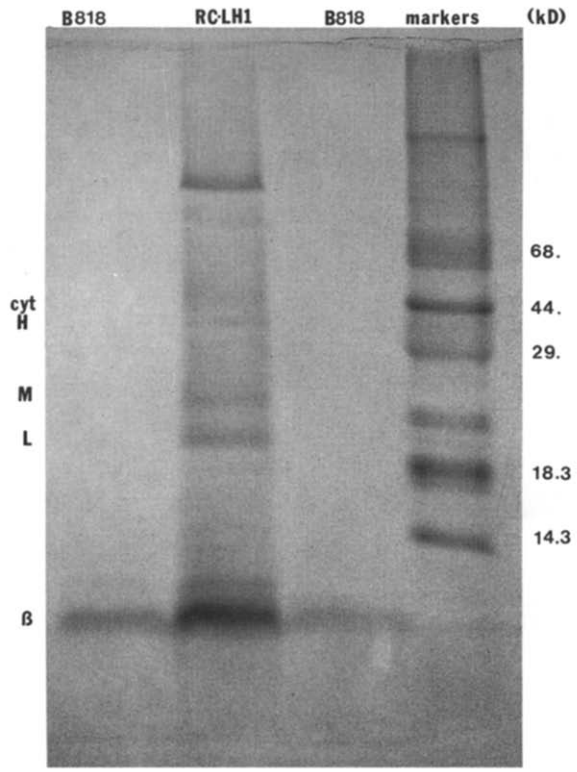

Fig. 3. Coomassie-stained SDS-PAGE of the RC-LH1 complex and the B818 subunit.

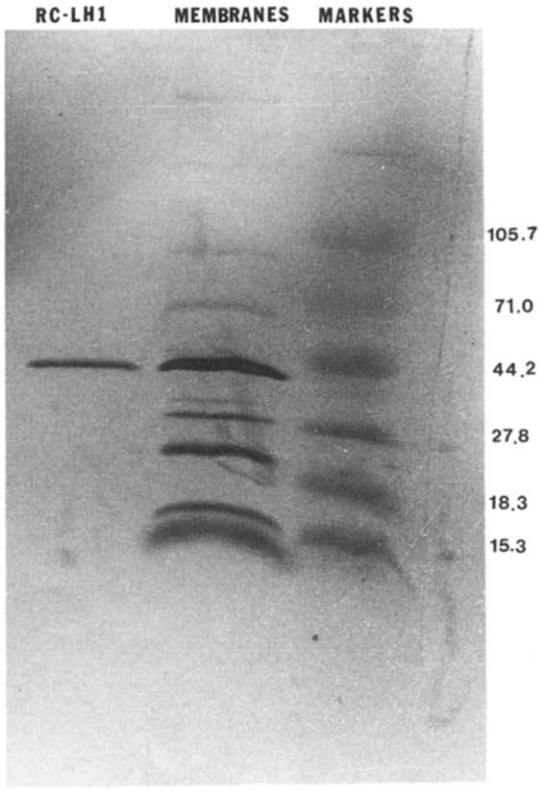

Fig. 4. SDS-PAGE of the *C. purpuratum* chromatophores and RC-LH1 complex stained for heme.

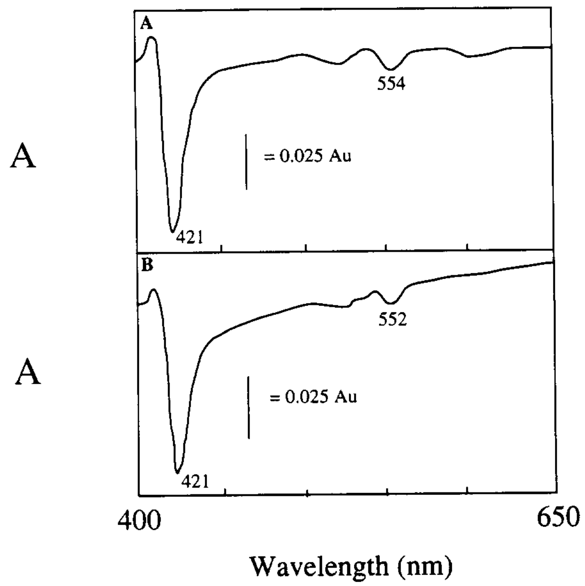

Fig. 5. Chemically-induced (A) high-potential difference spectrum and (B) low-potential difference spectrum of the *C. purpuratum* core.

negative at 829 nm, with the crossover point at 824 nm. The 829 nm lobe has a small shoulder at 822 nm. There is a positive CD band centered at 593 nm corresponding to the Q_x band of BChl *a*, while the Q_B region has double CD, positive at 366 nm and negative at 396 nm. The broad peak centered at approximately 500 nm is due to the carotenoid okenone [28].

The B820 subunit of C. purpuratum

Electroelution of the RC-LH1 complex band from Deriphat PAGE was initially carried out in Deriphat reservoir buffer. This material, when applied to the SEC, yielded a small amount of intact RC-LH1 as well as a B820 subunit form of the *C. purpuratum* LH1 (Fig. 1). The room-temperature absorption spectrum of the latter complex is shown in Fig. 7a. The NIR maximum is centered at 822 nm. The carotenoid absorbance is retained and the BChl *a* Q_B band is pronounced, this

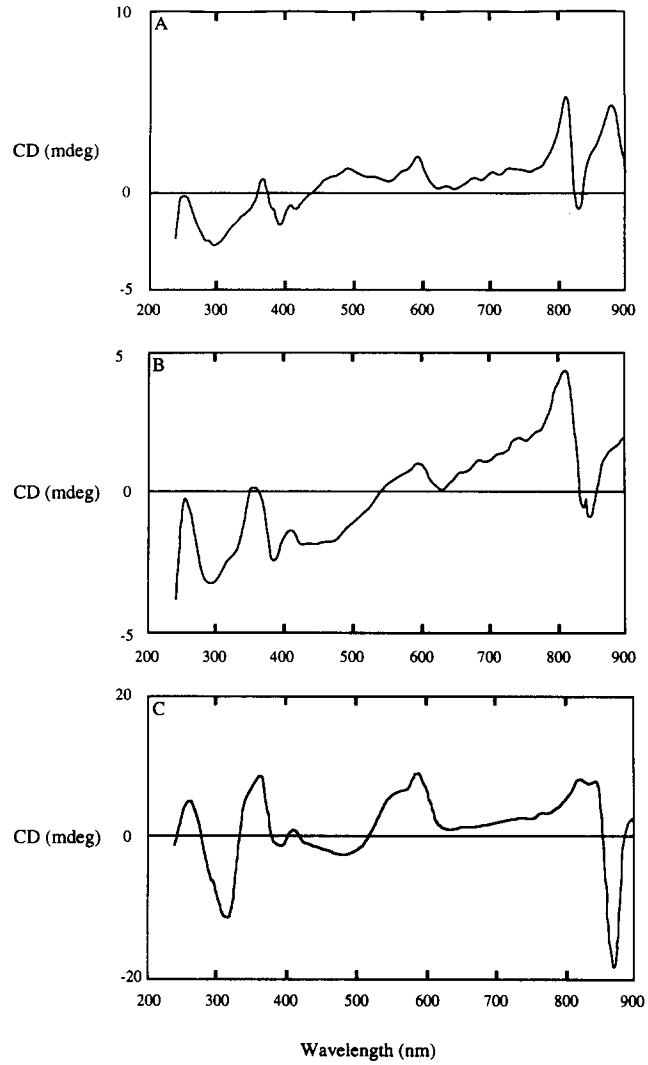

Fig. 6. Circular dichroism spectra of (A) the RC-LH1 complex; (B) the B818 form of the B820 subunit and (C) chromatophores of *C. purpuratum*.

latter feature is observed in other B820 subunits [14,15,42]. This B822 form, however, was labile and decayed irreversibly into free pigment and protein

Table 1
Comparison of the *C. purpuratum* LH1 β-subunit sequence to the LH1 β from other organisms for which structural subunits have been isolated

```
                                  [ *                        *         ]
Rps. marina (B820)           AEIDRPVSLSGLTEGEAREFHGVFMTSFMVFIAVAIVAHI__LAWM_WRPWIPGPEGYA
                                   |::|||::||: ||| ||:::  ||:||  |||:
C. purpuratum (B818)            ANEKSVTGLTDEEAQWFHGFFMQGMSGFIGVAAFAHL__LAWF(FR_DM)...
                                |: | ||||||:|| :|: :|:|:|:||:||::|||  : |
Rb. capsulatus (B816)        MADKNDLSFTGLTDEQAQELHAVYMSGLSAFIAVAVLAHLAVMIWR_PWF
                                  [ *                        *       ]
Rb. sphaeroides (B825)       ADKSSDLGYTGLTDEQAQELHSVYMSGLWPFSAVAIVAHLAVYI_WRPWF
                                    ||||||:|| :|: :|:|:  | :||  ||| :::
C. purpuratum (B818)            ANEKSVTGLTDEEAQWFHGFFMEGMSGFIGVAAFAHLLAWF(FR_DM)...
R. rubrum (B820)               EVKQESLSGITEGEAKEFHKIFTSSILVFFGVAAFAHLLVWI_WRPWVPGPNGYS
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The NIR wavelength maximum for the structural subunit is given in parentheses. Note: The two positionally conserved histidines are denoted with *. The position of the proposed transmembrane span is bound by [ ]. Amino acids in parentheses are tentative identifications. The %identity/number of amino acids in the overlapping region between *C. purpuratum* β and the others is: *Rps. marina*, 53.8%/39; *Rb. capsulatus*, 53.7%/41; *Rb. sphaeroides*, 48.6%/37; *R. rubrum*, 47.6%/42.

within minutes of its isolation. Substitution of 0.03% DM for Deriphat in the electroelution buffer yielded a form of the B820 subunit after SEC that was stable indefinitely if stored in the dark at 4°C. The NIR absorbance maximum of this complex is at 818 nm (Fig. 7b) and again, the carotenoid absorbance is retained. However, the yield of the B818 form is slightly lower than that of B822, due to more of the RC-LH1 complex remaining intact (as estimated by SEC peak height: the B818:RC-LH1 is 2.1, while the B822:RC-LH1 is 2.3). The Q_y absorption maxima of both forms of the B820 subunit have shoulders at 770 nm, likely due to free BChl *a*. The ratio of this breakdown product to the absorption at 818 or 822 nm is approximately the same. The BChl:carotenoid ratio in the B818 complex (1:2) indicates that this complex appears to have selectively lost BChl. However, uncertainty of the extinction coefficient for okenone again precludes a conclusive assessment of the pigment ratio. The SEC determined size for the B818 form is 70.5 kDa and that of B822 is 59.6 kDa; the difference in these estimates is likely due to the size and quantity of detergent bound to the two complexes. The only two polypeptides in the complex visible by SDS-PAGE are the LH1 α and β (Fig. 3). The DM prepared material (B818) was subsequently used for fluorescence and CD measurements.

There is no detectable long-wavelength fluorescence from the B818 complex so we are unable to determine whether the carotenoids are participating in energy transfer in this complex. However, *C. purpuratum* is unusual in that there is no long-wavelength fluorescence detectable in the RC-LH1 preparation, in chromatophores or in fresh, whole cells. The CD spectrum of B818 subunit ($A_{820} = 0.190$; Fig. 6b) exhibits a non-conservative double CD in the NIR and small peaks in the Q_x and Q_B regions of BChl *a*. The CD of the carotenoid has decayed slightly relative to that observed in chromatophores (Fig. 6c) and is similar to that observed in carotenoid-protein preparations (Kerfeld et al., unpublished data). It is opposite in sign and broader than the carotenoid CD of the RC-LH1 (Fig. 6a). The CD signal in the 250–300 nm region is negative and relatively intense, indicating a rigid orientation of aromatic amino acids within the complex [43].

4. Discussion

The RC-LH1 complex

In the purification procedure presented here the RC-LH1 is isolated in what is likely an intact form. In many purple photosynthetic bacteria the RC-LH1 complex is dissociated into RC and B870 during purification procedures. Exceptions to this include *Rc. gelatinosus* [38,44], *Rhodopseudomonas palustris* [45], *Rb. sphaeroides* [46] and *Chromatium vinosum* [47], organisms for which partial purifications of the RC-LH1 have been devised. The *C. purpuratum* P-870 RC is composed of the M, L, H subunits and also a tetraheme cytochrome; this subunit contains both low- and high-potential hemes. The function of this cytochrome, especially of the low-potential hemes, is unclear, but it presumably reduces oxidized P870. All *Chromatium* species characterized thus far have an associated tetraheme cytochrome, although the strength of the association of this subunit to the RC varies. This subunit is thought to be characteristic of the more primitive photosynthetic bacteria [1]. It is also found in *Rc. gelatinosus*, where it is easily removed during purification without concomitant loss in the rate of RC reduction [48]. The difference spectrum shown in Fig. 5a shows the typical *c*-type cytochrome α, β and γ bands at approx. 550, 520 and 420 nm, respectively. The α peak is slightly broadened, probably due to contributions from two high-potential hemes associated with the RC preparation. The dithionite-minus-ascorbate difference spectrum (Fig. 5b) indicates that there are also low-potential hemes present with the RC. Preliminary redox titrations confirm that there are four *c*-type hemes (Knaff, D., personal communication).

Fig. 4 shows the large number of heme-staining proteins associated with the membrane fraction. Sev-

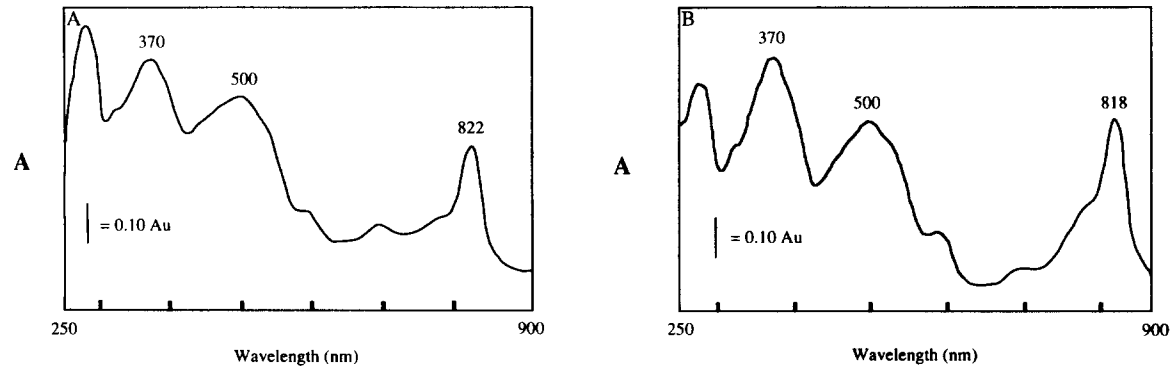

Fig. 7. Room-temperature absorption spectra of the (A) B822 and (B) B818 form of the B820 subunit.

eral of these are likely to be carried over from the soluble fraction; however, the heme-staining band at 21 kDa is especially prominent in the membrane fraction and is not observed in the soluble fraction. It is also visible as a straw-colored band in Deriphat-PAGE, migrating just ahead of the carotenoid-protein complex. It is unlikely that this heme-staining band is a degradation product of the RC tetraheme cytochrome because it does not appear in RC-LH1 preparations (Figs. 3 and 4). Instead this presumably *c*-type cytochrome may be analogous to other similarly-sized *c*-type cytochromes found in other *Chromatium* species [49,50] and in cyanobacteria and green-sulfur bacteria [51–54]. The function of these small *c*-type cytochromes is as yet unknown, although it has been suggested for *C. vinosum* that the small membrane-associated cytochrome is involved in photosynthesis because of its high redox potential [50]. Its primary structure suggests that it may be an evolutionary precursor to the tetraheme cytochrome [50]. Another possibility for the identity of the *C. purpuratum* 21 kDa heme-staining protein is a cyt c_y homolog [55].

The LH1 is retained with the RC during purification (Fig. 3). It is the main source of the strong NIR absorption peak at approximately 870 nm which also contains an additional small contribution from P-870 (Fig. 2). There is no evidence of the presence in the RC-LH1 preparation of the *puf* X gene product, a 9 kDa protein, which has been found associated with the RC-LH1 of *Rb. sphaeroides* [46].

The molecular mass of the intact RC-LH1 complex is surprisingly large. The estimated size of the RC-LH1 from *Rc. gelatinosus* (520 kDa) is similar [56]. When analytical SEC is carried out in the presence of OG, the size of the *C. purpuratum* RC-LH1 is estimated to be 470 kDa. The size difference is likely due to the smaller size of the OG micelle. Based on measurements of bound detergent to various integral membrane photosynthetic complexes and to cytochrome *c* oxidase [17,57–59], the micelle size for DM is estimated to be 30–80 kDa, that of OG, 8–25 kDa. Taking into account the mass contribution from bound detergent, the *C. purpuratum* RC-LH1 complex has a molecular mass of approx. 450 kDa. Assuming a bound detergent contribution no greater than that of one micelle [60,61], an equal stoichiometry of all RC subunits and an associated LH1 $(\alpha\beta)_6$ [62,63] or $(\alpha\beta)_{12}$ [11], a single RC-LH1 complex has a calculated size of about 170–230 kDa. The much larger observed molecular mass for the *C. purpuratum* RC-LH1 suggests that the RC may exists as a dimer. Redox kinetic studies have suggested previously that the bacterial RC may occur as a dimer [64]. In addition in the analogous core of the eukaryotic photosystem, PSII, is also thought to exist as a dimer [57,65,66].

The circular dichroism spectrum for the isolated RC-LH1 complex (Fig. 6a) has features in similar relative positions in the NIR region as the RC-LH1 of *C. vinosum*, but each is opposite in sign [67]. The *C. purpuratum* RC-LH1 CD spectrum is similar in overall shape to that of *Rps. palustris* RC-LH1 [68] although in the *C. purpuratum* RC-LH1 the position of the double CD in the NIR is bathochromically shifted, as it is in the B818. It has been noted before that BChls of pigment-protein complexes from different species often have similar absorption spectra without having equivalent CD spectra [22,69]. The 879 nm peak is due to the presence of the antenna. Spectral kinetic studies of B820 units indicate that the 870 nm shift is due to the interaction of at least two B820 s [20]. In addition, the NIR region of CD spectra of reassociated B870 s can vary considerably, even within preparations from the same organism [70].

The B820 subunit

The isolation of pigmented B820 subunits of the B870 was first reported in 1987 [14]. B820 subunits have since been obtained from six species of purple-nonsulfur photosynthetic bacteria [14–19]. They have similar spectral properties and have gained considerable acceptance as the fundamental building block of the B870. The isolation reported here of such a complex from a purple-sulfur bacterium strengthens this assertion. The estimated size and spectral characteristics of the B820 subunit from *C. purpuratum* are similar to those of purple-nonsulfur organisms. The two forms of the B820 subunit from *C. purpuratum* are spectrally quite similar, although the B818 has less carotenoid absorbance relative to BChl's Q_y absorbance (Fig. 7). Presumably the labile nature of the B822 form is due to exposure of the pigments to light and oxygen. DM is a larger surfactant (molecular weight = 511) than Deriphat (molecular weight = 327). The larger size of DM perhaps allows it to more effectively shield the chromophores of the B818 complex. It also should be noted that this complex is only obtained if the RC-LH1 is isolated by Deriphat-PAGE and subjected to electroelution; other RC-LH1 preparations produced initially by ammonium sulfate fractionation of solubilized *C. purpuratum* membranes do not exhibit this complex during SEC (Kerfeld et al., in press).

For the first isolation of B820 subunits, it proved necessary to remove carotenoids or begin with a carotenoidless mutant strain [14–19]. This was ascribed to the essential role of carotenoids in the in vivo association of B820 subunits into B870. However, B820 subunits have been shown to reform native-like B870s in vitro without addition of carotenoids [71]. Recently B820 subunits from *Rsp. rubrum* and from *Rb. sphaeroides* were obtained without prior extraction of carotenoids by use of the detergent n-octyl *rac*-2,3-di-

propylsulfoxide (molecular weight = 250) [72]. However, the resulting complex was irreversibly depleted of carotenoids, supporting the suggestion that in order to dissociate B870 into B820 the loss of carotenoids is essential. For *C. purpuratum* this is not the case. The B820 subunit from *C. purpuratum* is unique in that its carotenoids are retained. They absorb at 500 nm, similar to the carotenoid absorption maximum in the intact RC-LH1 complex (Figs. 2 and 7) but different from the carotenoid absorption in the peripheral antenna of *C. purpuratum* which is centered at 520 nm [77]. Whether the ability to isolate a B820 subunit that retains its carotenoid absorbance is due to the detergent we have used or is a special property of the purple-sulfur LH complex or its unusual carotenoid composition remains to be tested. Unlike B820 subunits isolated thus far from purple-nonsulfur organisms, the *C. pupuratum* B818 cannot be induced to reform the native B870 complex by dilution of the detergent concentration. It is plausible that the presence of carotenoids inhibits this reassociation. The sequence data obtained from the *C. purpuratum* β polypeptide as well as those from other Chromatiaceae indicate a high degree of sequence similarity to those of purple-nonsulfur organisms (Table 1) making a primary structural basis for these differences unlikely.

The circular dichroism spectrum of *C. purpuratum* B818 is very similar to CD spectra of other B820 subunits, especially in the Q_B and Q_x regions. The NIR region, although of similar basic shape: nonconservative double CD with positive CD at the high-energy transition (800 nm), negative CD at the low-energy transition (840 nm with a pronounced shoulder at 830 nm), is bathochromically shifted as compared to other previously studied B820 s. In the NIR CD region of B820 s from purple-nonsulfur bacteria, the higher energy transition of the CD couple is positioned between 765–795 nm with the lower transition energy between 815 to 830 nm. Only *Rps. marina* B816 has its negative lobe near the position of that of the *C. purpuratum* B818, at 830 nm [17]. However, its higher energy transition peak is similar to that of other B820 subunits.

The presence of positive CD in the 860–900 nm region is unusual for B820 subunits, although it has recently been observed in the B820 subunit from *R. rubrum* (isolated without prior removal of carotenoids) [72]; in this case it was ascribed to the presence of incompletely dissociated B870. However, fourth derivative analysis of the B818 absorbance spectrum, performed after the CD measurements were taken, indicates the presence of very little B870 (data not shown). Furthermore, we have not yet observed an intact B870 complex from *C. purpuratum*.

The CD in the carotenoid region of B818 is difficult to interpret. The CD of okenone in the caroteno-protein is rather broad and featureless [77]. The carotenoid region of the CD spectrum of chromatophores is similar in sign and is only slightly narrower than that of B818, while the carotenoid region in the RC-LH1 CD spectrum is opposite in sign.

NIR shifts in B820s are likely to be mainly due to intra-dimer excitonic interactions of BChls [73]. However, exciton coupling alone cannot fully account for CD spectra of B820 subunits. In B818 the high and low exciton transitions occur near 800 and 835 nm; additional factors must influence the spectral properties of B820 s. For example since the composition of the B820 form of LH1 is yet uncertain and may be a multimer of $\alpha\beta$ heterodimers, there may be additional interdimer interactions contributing to the CD spectrum of these complexes. In addition, transition state mixing between the exciton couple also may affect the size and position of the components of the NIR CD signal. The crosspoint shift and the nonconservative nature of the double CD in the region 790–825 nm may also be explained by interactions with aromatic amino acids; the BChls are in dissimilar environments dictated by the surrounding amino acids of α and β, making the high- and low-energy components of unequal strength.

Reconstitution studies of B820 subunits from purple-nonsulfur bacteria have led to the suggestion that the β subunit is predominantly responsible for the spectral properties of the LH1 [71]. Moreover, B820 complexes can be reconstituted from β subunits and free BChl alone; these complexes are unable to form B870 unless α subunits are added [71]. We have obtained what is likely to be nearly the full-length sequence of the LH1 β subunit from *C. purpuratum* (Table 1). It shares basic primary structure features with other LH1β polypeptides [11] and is similar in length. This is in contrast to the LH2 subunits from *C. purpuratum* with extended N-termini [77]. Of the organisms from which B820 subunits have been obtained, the *C. purpuratum* β subunit has the highest homology with *Rps. marina* (B820) and *Rb. capsulatus* (B816; see Table 1). It is noteworthy that the *C. purpuratum* β subunit contains additional aromatic amino acids in the vicinity of the conserved histidines. The C-terminal histidine in the *C. purpuratum* β subunit has an aromatic amino acid in the conserved His + 4 site as well as at +5, +6 and −2. Interestingly, additional aromatic residues are also found in the vicinity of the N-terminal histidine that are not found in other β subunits: at His + 2 there is a Phe and at His − 2 there is a tryptophan. An acidic residue is strongly conserved in this latter position at the beginning of the presumed membrane span. However, a tryptophan at this position at the polar-apolar interface is consistent with its distribution in other membrane proteins including the L and M subunit of the RC [74,75] and at the putative C-terminal membrane-periplasm interface of LH1β

subunits (Table 1). The N-terminal histidine is not thought to bind BChl, although the evidence is indirect [11] and it is absolutely conserved.

The photosynthetic apparatus of *C. purpuratum* contains the rare carotenoid okenone which may influence the organism's spectral properties. Carotenoids have also been shown to modify the absorbance of BChls in the LH1 [14,76]. Because we are unable to measure long-wavelength fluorescence from the RC-LH1 or from the B818 of *C. purpuratum* we cannot determine whether the carotenoids are participating in energy transfer in these complexes. Thus, we are unable to state with confidence that the okenone present in B818 is influencing the CD of B818. Another factor that may be influencing the CD spectrum of the *C. purpuratum* complexes is structural variations of BChl *a*: the esterifying tail on the C-17 propionate of Ring IV has been thought to influence spectroscopic properties [71]. The BChl *a* in *C. purpuratum* has not been characterized in this manner. Finally, this is the first report of the use of electrophoretic methods and the detergent DM in the preparation of B820 subunits; choice of detergent has been shown to be responsible for small band shifts in optical spectra; large effects are not generally observed, but rather dissociation into free pigment and protein results [36]. The method described here may be applicable to the isolation of other B820 subunits; it will be of interest to determine whether B820 complexes of purple-nonsulfur bacteria can retain their carotenoid absorbance properties if prepared similarly.

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