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Isolation and Characterization of a Subunit Form of the Light-Harvesting Complex of *Rhodospirillum rubrum*†

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Received December 3, 1986; Revised Manuscript Received April 7, 1987

ABSTRACT: A new method is described for the isolation of subunits of the light-harvesting complex from *Rhodospirillum rubrum* (wild type and the G-9 mutant) in yields that approach 100%. The procedure involved treating membrane vesicles with ethylenediaminetetraacetic acid–Triton X-100 to remove components other than the light-harvesting complex and reaction center. In the preparation from wild-type cells, a benzene extraction was then employed to remove carotenoid and ubiquinone. The next step involved a careful addition of the detergent *n*-octyl β-D-glucopyranoside, which resulted in a quantitative shift of the long-wavelength absorbance maximum from 873 to 820 nm. This latter complex was then separated from reaction centers by gel filtration on Sephadex G-100. The pigment–protein complex, now absorbing at 820 nm, contained two polypeptides of about 6-kilodalton molecular mass (referred to as α and β) in a 1:1 ratio and two molecules of bacteriochlorophyll (BChl) for each αβ pair. This complex is much smaller in size than the original complex absorbing at 873 nm but probably is an associated form such as $\alpha_2\beta_2$·4BChl or $\alpha_3\beta_3$·6BChl. The 820-nm form could be completely shifted back to a form once again having a longer wavelength λ_{max} near 873 nm by decreasing the octyl glucoside concentration. Thus, the complex absorbing at 820 nm appears to be a subunit form of the original 873-nm complex.

In recent years, considerable progress has been made in isolating light-harvesting (LH)[1] complexes from photosynthetic organisms (Thornber, 1986; Drews, 1985; Zuber, 1985, 1986; Cogdell, 1986; Gantt, 1986; Anderson & Barrett, 1986; Michel-Beyerle, 1985). Many of these preparations were sufficiently pure that the polypeptide components of the complexes have been well characterized including the determination of their amino acid sequences (Brunisholz et al., 1981, 1984, 1985; Gogel et al., 1983; Tadros et al., 1983, 1985; Theiler et al., 1984a,b, 1985; Wechsler et al., 1985). In addition, the genes coding for the protein of such complexes have been isolated, cloned, and sequenced (Youvan et al., 1984; Coruzzi, et al., 1983; Fish et al., 1985). However, because of the inherent instability of chlorophyll and bacterio-

† This research was supported by research grants from the U.S. Public Health Service (GM 11741) and the National Science Foundation (PCM-7816669).

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[1] Abbreviations: BChl, bacteriochlorophyll; B881, the core light-harvesting complex of wild-type *R. rubrum* whose long-wavelength absorbance maximum is at 881 nm; B873, the core light-harvesting complex of the G-9 mutant (carotenoidless) of *R. rubrum* or the wild-type light-harvesting complex after benzene extraction (also now absorbing at 873 nm); B820, the subunit form of B873 now absorbing at 820 nm after treatment with octyl glucoside; "B873", the reassociated form of B820 whose absorbance band is near 873 nm; B873-α, the polypeptide of B873 soluble in 1:1 $CHCl_3$–CH_3OH and running larger (slower) than the β-polypeptide on SDS–PAGE; B873-β, the other polypeptide of B873 which runs smaller (faster) on SDS–PAGE and does not dissolve in 1:1 $CHCl_3$–CH_3OH; LH, light harvesting; OG, *n*-octyl β-D-glucopyranoside; RC, reaction center; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; kDa, kilodalton(s); EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; PTC, phenylthiocarbamyl; OPA, *o*-phthalaldehyde; cmc, critical micelle concentration; CD, circular dichroism; LDAO, lauryldimethylamine *N*-oxide; BPh, bacteriopheophytin.

0006-2960/87/0426-5055$01.50/0

chlorophyll (BChl) in the aqueous detergent solution required for isolation of the LH complexes, degradative losses of these pigments during preparation have been a problem. As a result, reported BChl to protein ratios have varied considerably (Sauer & Austin, 1978; Cogdell & Thornber, 1979; Picorel et al., 1983; Drews, 1985).

Our laboratory has developed methodology for isolation of the single LH complex of *Rhodospirillum rubrum* in pure form without significant loss of BChl. In this preparation, the native complex (B881 in the wild type or B873 in the G-9 mutant) is apparently converted to a subunit form absorbing at 820 nm (therefore called B820). This is the first example of such a stable subunit preparation. In this paper, we describe the details of our preparative method and the characteristic properties of the subunit form. A preliminary report has appeared in which an in vivo structure was suggested on the basis of properties of the B820 form (Loach et al., 1985).

Materials and Methods

Materials. *n*-Octyl β-D-glucopyranoside (OG) was obtained from Calbiochem, Sephadex G-100 and G-200 were from Sigma Chemical Co., Triton X-100 was from Sigma, and SDS–PAGE chemicals were from Bio-Rad. HPLC solvents were all high-purity HPLC grade.

Growth and Harvesting of the Bacteria. *R. rubrum* wild-type strain S.1 was grown anaerobically in modified Hutner's media (Cohen-Bazire et al., 1957) under low-intensity fluorescent lights as previously described (Loach et al., 1963). *R. rubrum* G-9, the carotenoidless mutant, was grown anaerobically in modified Hutner's media with yeast extract added and illuminated with tungsten light. The cells were harvested during logarithmic growth (usually in 3–5 days), centrifuged, washed once in 50 mM phosphate buffer, pH 7.5, repelleted, and stored at −20 °C as a pellet until use.

Chromatophore Preparation. Chromatophores were isolated from whole cells as previously described (Loach et al., 1963). Briefly, the whole cells were resuspended in 50 mM potassium phosphate buffer, pH 7.5, sonicated for 4–6 min, and centrifuged to separate the chromatophores from the cell debris and unbroken whole cells. The chromatophores were then washed twice in phosphate buffer and twice in deionized water.

EDTA–Triton X-100 Wash. The pellet of washed chromatophores from wild-type cells was homogenized in deionized water, mixed 1:1 with 6% Triton X-100 and 20 mM EDTA in 100 mM Tris, pH 7.3, and rehomogenized (Hall et al., 1973). This solution was diluted in half with deionized water and homogenized. Chromatophores from the G-9 mutant were more sensitive to this procedure so the initial amount of Triton X-100 was reduced to 2% in this case. The chromatophores were centrifuged at 144000*g* for 100 min, followed by four deionized water washes using centrifugation steps at 144000*g* for 90, 80, 70, and 60 min to recover the material. After the washes, the chromatophores were lyophilized and stored at −20 °C. The chromatophores were used over a period varying from 2 weeks to 10 months after the EDTA–Triton treatment.

Benzene Extraction of the EDTA–Triton-Washed Chromatophores. Morrison et al. (1977) showed that benzene extraction of chromatophores from wild-type cells removes free and weakly bound ubiquinone (UQ_B and the UQ_{pool}) and carotenoid (except the reaction center carotenoid) from the membrane. The EDTA–Triton-washed chromatophores were benzene-extracted because it had been found previously that carotenoid removal seemed to weaken the association of the light-harvesting complexes with other membrane protein complexes. The procedure employed here was essentially the same as that used by Morrison et al. (1977) except the lyophilized, EDTA–Triton-washed chromatophores were used instead of untreated chromatophores. They were resuspended at a concentration of 1 mg/mL in benzene, stirred for 20 min at room temperature, and then collected by centrifugation in a table-top centrifuge for 25 min. The pellet was extracted 3 more times with benzene at ratios of 1, 2, and 4 mg/mL. After removal of the supernatant, residual benzene was evaporated with a stream of nitrogen. The resulting pellet was thoroughly dried under high vacuum. The entire procedure was carried out in the dark because the BChl becomes sensitive to degradation in the light during the treatment. The G-9 mutant, lacking carotenoid, was not treated with benzene. The benzene-extracted chromatophores were usually used within a few days of preparation.

Titration of Chromatophores with OG. The most difficult step in the preparation is that of appropriately titrating the benzene-extracted, EDTA–Triton-washed chromatophores with OG. The goal was to add only enough OG to shift the entire 873-nm absorbance band to 820 nm. Too much OG can cause some BChl to dissociate from the protein (indicated by increased absorbance at 777 nm and by subsequent separation on gel filtration chromatography). Unfortunately, the amount of OG required varies with the chromatophore preparation, the age of the sample, and the concentration of the sample. The amount of OG usually required for a sample with an $A_{820} = 0.7$ cm^{-1} was 0.80% (0.80 g/100 mL final volume). A typical titration curve is shown in Figure 1. As may be seen from the data of Figure 1, the first additions of OG have the effect of increasing the absorbance at 873 nm. Because the EDTA–Triton-washed and lyophilized chromatophores are difficult to completely resuspend in water without adding detergent, it is assumed that the lower absorbance observed before adding small amounts of OG is due to aggregation. The majority of the shift in the λ_{max} due to the addition of OG occurred within minutes, but as the OG concentration increased to above 0.6%, there was a small amount of change still occurring after several hours. For column chromatography, a sample was used with an $A_{873} = 6$–10 cm^{-1} (actually measured with a 1-mm path-length cuvette) and usually required between 0.9% and 1.2% OG. Addition of OG was stopped when the A_{777}/A_{820} ratio started increasing, even if there was still some absorbance at 873 nm. Inappropriate addition of OG by only a few hundredths of a percent could cause quite a noticeable increase in the 777-nm absorbing material (excess OG) or incomplete conversion (too little OG) of B873, leaving a long-wavelength shoulder or small peak. Once the majority (85–95%) of the absorbance had shifted to 820 nm, the sample was applied to a Sephadex G-100 column. Because of the high sensitivity of B820 to light, the entire procedure was performed in the dark. Material prepared from wild-type cells and the G-9 mutant behaved identically in this titration with OG.

Separation of B820 and Reaction Centers by Gel Filtration. Prior to titration of the sample for application to the column, a small amount of sample was solubilized in buffer at one-tenth the concentration applied to the column and titrated with OG until the λ_{max} shifted to 820 nm. This was done because it was found that the sample became diluted by 5–10-fold on the column, thus requiring less OG to keep it in the 820-nm form. Enought OG had to be present, though, or the λ_{max} would shift back to longer wavelengths. The range of OG used varied between 0.75% and 0.95%. The Sephadex G-100 was then equilibrated in 50 mM phosphate buffer, pH 7.5, containing 5 mM $MgSO_4$ and the predetermined percent OG by

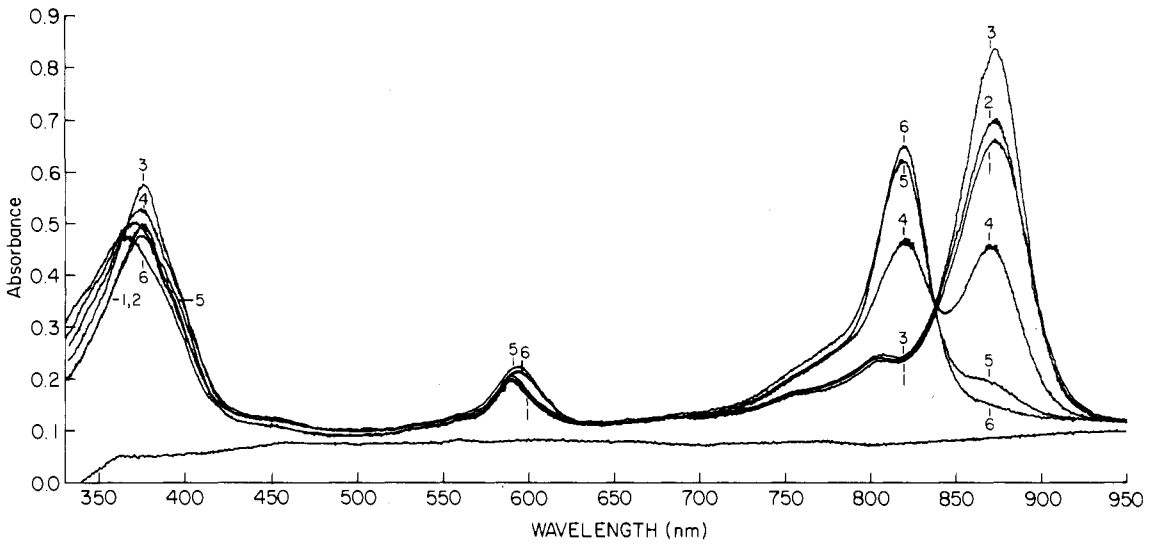

FIGURE 1: Absorbance spectra of EDTA–Triton X-100 treated G-9 chromatophores (curve 1) upon addition of increasing amounts of octyl glucoside at room temperature in the dark (0.05 M phosphate buffer, pH 7.5, containing 5 mM $MgSO_4$). (Curve 2) 10 min after adding 0.25% octyl glucoside; (curve 3) 10 min after 0.5%; (curve 4) 10 min after 0.75%; (curve 5) 4 h after 0.75%; (curve 6) 24 h after 0.75%. The bottom trace is the base line taken with 0.75% octyl glucoside in buffer.

running several void volumes of buffer through the column (2.5 × 100 cm); 7–10 mL of the sample was applied to the column which was run at room temperature in the dark with a pressure head of 70 cm. The progress of B820 was monitored by measuring its absorbance at 820 nm with an LKB Ultrospec 4050 attached to an LKB recorder, Model 2065.

Two gel filtration columns were used for molecular weight determinations. One of these was the column described above for isolation of B820. Standards, applied at a concentration of 2 mg/mL, were cytochrome *c*, ovalbumin, and bovine serum albumin, and the void volume was determined with blue dextran. In the second molecular weight determination, a Sephadex G-200 column (2.5 × 21.5 cm) was equilibrated with 50 mM potassium phosphate buffer, pH 7.5, with 10 mM $MgSO_4$ and 0.9% OG. Blue dextran, cytochrome *c*,γ-globulin, and bovine serum albumin were applied separately to the column (2.75 mL of a 3 mg/mL solution was applied) and their elution volumes determined. A B820 sample was run in the same manner. To determine the size of a "B873" complex (the reaggregated 860–870-nm absorbing form of B820), a B820 sample was applied to a Sephadex G-25 column and eluted with buffer free of OG in order to remove OG from the sample. The sample (now absorbing at about 870 nm) was concentrated by using poly(ethylene glycol) and redissolved in phosphate buffer containing 10 mM $MgSO_4$ and 0.1% OG. After the G-200 column was equilibrated with buffer containing 0.1% OG, the "B873" sample was applied, and its elution volume at its peak was determined.

Absorbance Spectra. Absorbance spectra from 350 to 950 nm were taken on a Cary 14 spectrophotometer with a scattered transmission attachment. UV spectra were recorded by using a Cary 219 spectrophotometer.

Determination of Reaction Center Activity. The activity of reaction centers was determined by light minus dark absorbance change measurements at 602 nm using the kinetic spectrometer described by Loach and Loyd (1966) and modified as in Woodle et al. (1984).

SDS–PAGE. The SDS discontinuous buffer system of Laemmli (1970) was used following the recipes and techniques described by Hames (1981). The chromatophore polypeptides were resolved on 8–20% acrylamide gradient slab gels (1.5 mm × 16 cm × 32 cm). The samples were dissolved in sample buffer containing 0.5% SDS, 1% dithiothreitol, 63 mM Tris-HCl, pH 6.8, 8% glycerol, and 1% bromophenol blue and incubated at room temperature for 30 min to 2 h. Heating the sample, higher concentrations of SDS, and the presence of urea were found to increase protein aggregation. The gel was run until the tracking dye reached the bottom of the gel, usually for 24 h at 10–15 mA. It was then stained for 4 h with Coomassie blue (1 g/L) in 50% CH_3OH–7% acetic acid in water and destained in 20% CH_3OH, 7% acetic acid, and 3% glycerol in water for 2 days.

BChl Determination. Samples for BChl determination were first dialyzed extensively against water, lyophilized, and then extracted several times with CH_3OH. In later determinations, the aqueous samples were extracted directly with CH_3OH without first removing the OG. These extracts were combined, evaporated to dryness using a rotary evaporator, and redissolved in diethyl ether. BChl absorbance at 770 nm was determined, and the amount of BChl was calculated by using the extinction coefficient $\epsilon_{770} = 95\ mM^{-1}\ cm^{-1}$ (Weigl, 1953; Holt & Jacobs, 1954).

Protein Determination. Because the light-harvesting protein is so hydrophobic and insoluble in water, many of the usual ways of determining protein were not suitable. Assays based on a modified Lowry procedure (Lowry et al., 1951; Ohnishi & Barr, 1978) and on the Bradford procedure (Bradford, 1976) could not be used because the protein precipitated when the working reagent was added or the detergent or solvent required to keep the protein in solution interfered. OG also interfered even though protein samples were extensively dialyzed to remove it. The two methods used successfully were UV absorbance at 290 nm (a tryptophan λ_{max}) and a Pierce BCA (bicinchoninic acid) protein assay (Pierce Chemical Co.) which is compatible with nonionic detergents such as OG. The latter assay is also compatible with 1% SDS which was used to dissolve the protein samples and standards. The extinction coefficient at 290 nm used for determination from UV spectra was 20.4 $mM^{-1}\ cm^{-1}$ which was based upon the value of 10.2 $mM^{-1}\ cm^{-1}$ obtained in independent determinations for both B873-α and B873-β in trifluoroethanol, in 1% SDS, and in 1:1 (v/v) $CHCl_3$–CH_3OH with 2% (v/v) HCl. These were the solvents used to dissolve the protein for UV absorbance measurements. A small amount of scattering was taken into account by drawing a line from the spectra between 300 and 350 nm and extending it for a base line at 290 nm. In the

Pierce protein assay, bovine serum albumin (Sigma) and B873-α were used as standards.

Amino Acid Analysis. Protein for amino acid analysis was hydrolyzed under a vacuum with HCl (phenol added as a scavenger) at 110 °C. The amino acid analyses were conducted either by conversion to the phenylthiocarbamyl (PTC) derivatives followed by separation and quantitation on HPLC or by *o*-phthalaldehyde (OPA) precolumn derivatization and separation by reverse-phase HPLC (Pfeifer & Hill, 1983; Cariello et al., 1984). For the latter method, a Perkin-Elmer Series 4 liquid chromatograph with a LC-85B spectrophotometric detector and a Sigma 15 chromatography data station were used.

Separation of Polypeptides by HPLC. The polypeptides of B820 were separated by reverse-phase chromatography using a modification of a procedure by Heukeshoven and Dernick (1985). The protein was dissolved in a small volume (100–200 μL) of 60% formic acid, vortexed briefly, and centrifuged 6 min in an Eppendorf 5414 centrifuge at room temperature. The protein was then injected immediately onto the column, or it was stored up to 1 week at −15 °C.

A Perkin-Elmer HC-ODS C_{18} column (25 cm × 1.6 cm i.d.) was used to separate the polypeptides. A solvent gradient of tetrahydrofuran in 30% formic acid (v/v) was used. The protein peaks were detected at 280 nm and collected separately in test tubes. Because 30% formic acid is damaging to the bonded silica surface of the packing material, exposure of the column to the elution buffers was kept to a minimum. The column was equilibrated in 60:40 MeOH–water (v/v) for storage. The pooled protein collected from the HPLC was vacuum desiccated in the presence of KOH pellets with a dry ice–acetone trap between the desiccator and the pump.

RESULTS

Preparation of B820. The logic behind the LH isolation procedure was to first gently remove all membrane components that were not essential to the functioning photoreceptor complex. Toward this end, chromatophores were first exposed to an EDTA–Triton wash step which had previously been shown to remove most protein components that were not part of the LH or RC without affecting primary photophysical and photochemical events (Hall et al., 1973). It should be noted that a lower Triton X-100 concentration must be used for G-9 chromatophores than for chromatophores from wild-type cells (see Materials and Methods).

After the EDTA–Triton wash, the chromatophores were washed several times with water and then lyophilized to dryness. A benzene extraction step was next employed to remove carotenoid from the LH complex (see later Figure 3). Because this extraction was not required with material from the G-9 mutant in order to obtain good conversion to B820 when OG was added in the next step, it is assumed that carotenoid is important in binding the B820 subunits together to form B881. Without benzene extraction, wild-type chromatophores are much more resistant to interaction with OG, and a quantitative conversion to a stable B820 form was not obtained. It is interesting that upon benzene extraction of wild-type chromatophores, the far-red λ_{max} shifts 8 nm toward the blue to 873 nm. Since the λ_{max} of the in vivo G-9 LH complex is also at 873 nm, a specific interaction for carotenoid is again implied. In an attempt to reverse this shift, the benzene extract of the wild-type material was added back to the residue fraction and the benzene evaporated. Upon resuspension of this residue in buffer, the far-red λ_{max} was found to be at 877 nm, suggesting partial, but not complete, rebinding of the carotenoid to the LH complex. In a recent study by

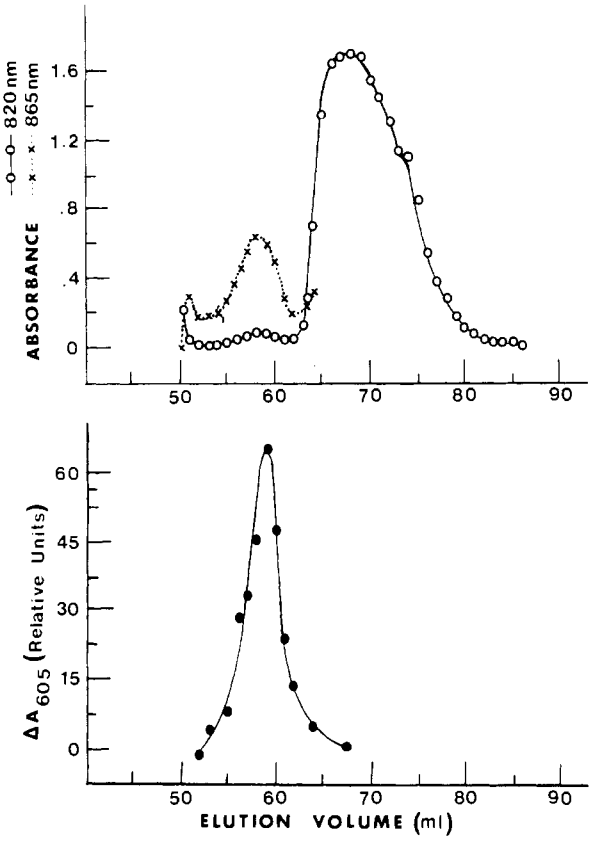

FIGURE 2: Elution profile for the Sephadex G-100 column used in the isolation of B820 from the G-9 mutant of *R. rubrum*. The elution buffer was 0.05 M phosphate buffer, pH 7.5, which contained 5 mM $MgSO_4$ and 0.75% OG. (Top) Absorbance at 820 nm shows location of B820, and that at 865 nm shows location of the reaction center. The absorbance values for the 865-nm measurements were multiplied by 10 to compare on the same scale as the absorbance at 820 nm. (Bottom) Reaction center activity determined by measuring the light-induced absorbance changes at 605 nm with a saturating pulse of near-infrared irradiation.

Brunisholz et al. (1986), it was implied that the conformation of the N-terminal region of the protein was different with carotenoid present.

The next step is that of adding OG, which must be conducted very carefully to obtain a stable product in quantitative yield. As OG was added to a sample of EDTA–Triton-washed G-9 chromatophores, the far-red λ_{max} shifted from 873 to 820 nm (Figure 1). The amount of OG needed to effect this shift depended upon the concentration of the chromatophores and the age of the chromatophore preparation. In general, a higher percent OG was needed to shift a higher concentration of chromatophores, but this was not a linear relationship., The concentration of OG needed was somewhat above the cmc of OG [deGrip and Bovee-Geurts (1979) determined the cmc to be 0.68%]. An excess of OG caused the BChl λ_{max} to shift to 777 nm.

After observing that OG converted the LH complex to a form absorbing at a shorter wavelength by some 55 nm, reaction center (RC) activity was measured and found to be essentially unchanged under saturating light intensities. Thus, if the LH complex had dissociated into subunits, these should be small enough to separate from an intact RC by gel filtration chromatography. The results of the application of the OG-treated G-9 chromatophore material absorbing at 820 nm to a Sephadex G-100 column are shown in Figure 2. Reaction centers were eluted immediately behind the void volume and were usually nearly completely separated from the B820 complex. Reaction center polypeptides could not be detected

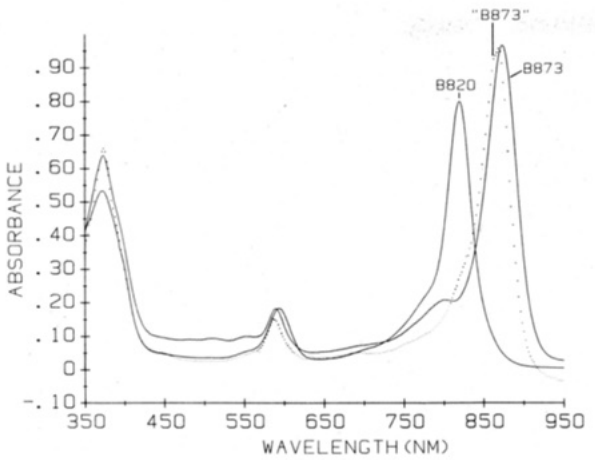

FIGURE 3: Absorbance spectra of EDTA–Triton-washed and benzene-extracted chromatophores of wild-type *R. rubrum* in 0.40% OG at room temperature (B873), the peak fraction from a G-100 column purification absorbing at 820 nm (0.80% OG), and the latter sample after cooling to 6 °C ("B873").

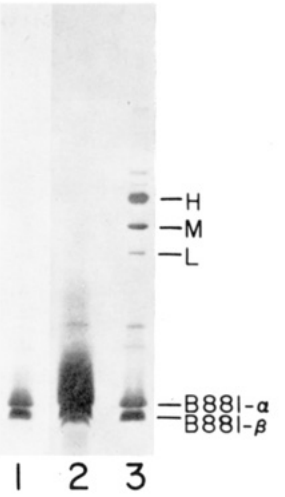

FIGURE 4: SDS–PAGE of B820 (lanes 1 and 2) compared with chromatophores (lane 3) of wild-type *R. rubrum*. The proteins were separated on an 8–20% acrylamide gradient gel and stained with Coomassie blue. The sample applied to lane 2 was identical with that applied to lane 1 except that the protein concentration was approximately 4× greater to test for the presence of other minor bands.

in B820 fractions by silver-stained or Coomassie blue stained SDS–PAGE. A small quantity of what was assumed to be free BChl (absorbing at 777 nm) eluted after the B820 complex, appearing as a tail on the main band. Identical separation behavior was found for OG-treated membranes from wild-type *R. rubrum*. Figure 3 shows the absorbance spectrum of B820 from the peak fraction after purification on a G-100 column.

The λ_{max} could be shifted from 820 nm to a longer wavelength (860–873 nm) by lowering the concentration of OG by dilution, dialysis, or gel filtration, or by lowering the temperature. The absorbance spectrum of the resultant complex was often only slightly blue-shifted from that exhibited before OG was added (Figure 3).

Yields of the B820 complex were consistently higher than 80% (based on the absorbance of the band at either 588 or 820 nm of the sample applied to the G100 column multiplied by the volume of the sample and compared with the corresponding absorbance of pooled B820 fractions multiplied by its volume). For example, the yields of both B820 and the RC in the preparation shown in Figures 1 and 2 were greater than 95%. When conducted carefully, losses of BChl in the EDTA–Triton wash and benzene extraction steps were very small (2–5%), and the OG titration was quantitative.

Polypeptides of B820. On SDS–PAGE, two polypeptides were found in the B820 complex, and they migrated to the same location as B873-α and B873-β, the light-harvesting polypeptides of *R. rubrum* chromatophores (Figure 4). Assuming that the extinction coefficients for Coomassie blue staining of B873-α and B873-β were identical at the concentrations assayed (Picorel et al., 1983), the ratio of B873-α to B873-β was 1.20.

Amino acid analyses were performed on samples of the isolated B820 complex. Results of such an analysis are presented in Table I along with analysis of the B880 holochrome purified by Picorel et al. (1983) and the amino acid composition of B873 predicted from the sequences of B873-α (Gogel et al., 1983) and B873-β (Brunisholz et al., 1984). The results are consistent with the expectation that B873-α and B873-β are present in the B820 complex in a 1:1 stoichiometry. They are also in very close agreement with those from the B880 holochrome of Picorel et al. (1983). The glycine content was found to be high, probably due to contamination. Isoleucine, leucine, and phenylalanine were somewhat low, most likely because of incomplete hydrolysis of the protein. Serine and threonine were not corrected for loss during hydrolysis.

Table I: Amino Acid Analyses of B873 from *R. rubrum*

amino acid	B820 OPA[a]	B820 PTC[b]	predicted from sequence[c]	B880 holochrome (Picorel et al., 1983)
Asx	5.2	ND[d]	3	4.2
Glx	12.5	ND	11	9.8
Ser[e]	5.9	6.6	8	7.1
Gly	8.7	8.9	5	8.1
His	3.2	2.4	3	1.7
Arg	3.4	3.1	4	4.2
Thr[e]	8.7	5.0	6	5.4
Ala	8.7	8.6	8	9.2
Pro	ND	6.0	5	4.7
Tyr	1.0	1.0	1	1.1
Val	ND	7.8	8	7.9
Met	ND	1.0	1	1.2
Ile	6.0	5.5	7	6.4
Leu	11.9	10.6	14	12.0
Phe	8.4	9.0	10	8.3
Lys	4.0	3.5	4	3.9
Cys	ND	ND	0	0.3
Trp	ND	ND	6	4.1

[a] Analysis performed by using *o*-phthalaldehyde precolumn derivation of amino acids (see Materials and Methods), 72-h hydrolysis. Numbers are in moles of amino acid found per mole of protein assuming there are 1.0 Tyr and 4.0 Lys. [b] Analysis performed by using phenylthiocarbamyl precolumn derivatization of amino acids (see Materials and Methods), 48-h hydrolysis. Numbers are in moles of amino acid found per mole of protein assuming there are 1.0 Tyr and 1.0 Met. [c] Amino acid composition predicted (number of residues per mole of protein) from a 1:1 stoichiometry of B873-α and B873-β primary sequences (Brunisholz et al., 1981, 1984a, Gogel et al., 1983). [d] ND = not determined. [e] Not corrected for loss during hydrolysis.

A procedure was developed for separating the α- and β-polypeptides on HPLC. When a B820 preparation was subjected to analysis using this procedure, two polypeptides were obtained at the locations expected for α and β (see Figure 5). For about half of the assays, the α-polypeptide split into two very close peaks as shown in Figure 5. The isolated α-polypeptide also sometimes split into two very close peaks. Analysis by SDS–PAGE of each peak from the HPLC column gave a single band appropriate for α or β. No evidence was found for other polypeptides. From calibration of the HPLC column with B873-α and B873-β standards, the percent recovery of each was 90% and 30%, respectively. Using this calibration,

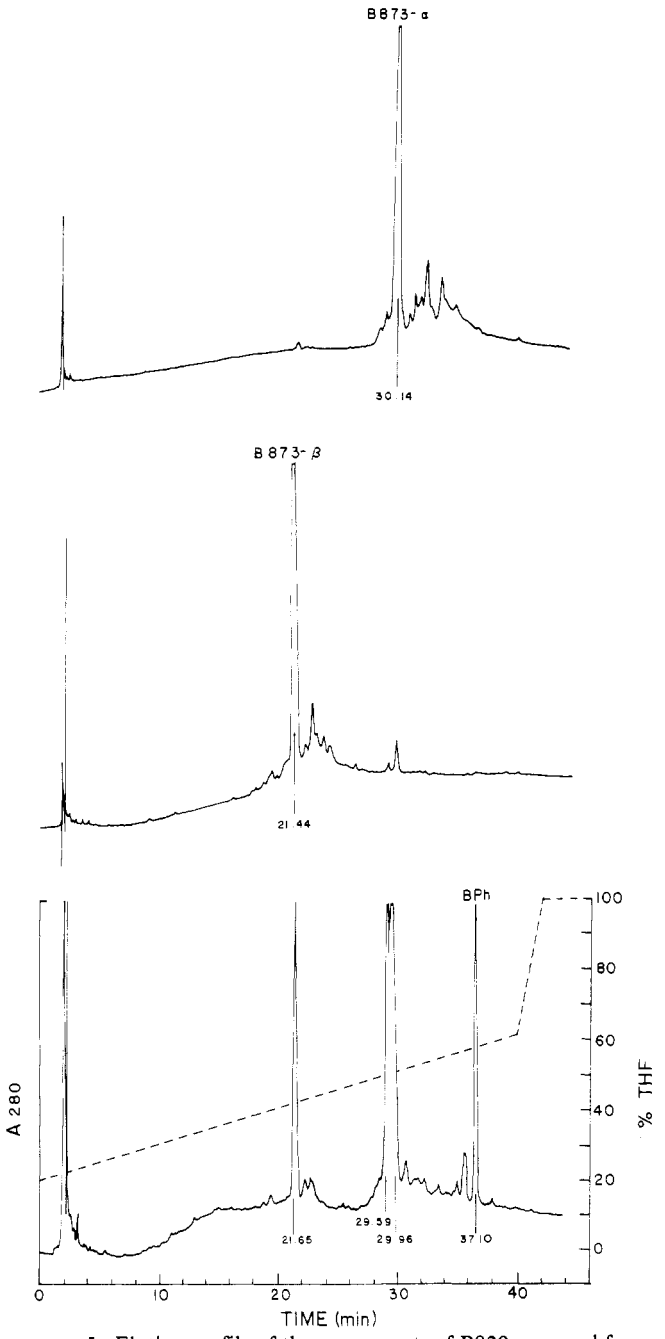

FIGURE 5: Elution profile of the components of B820 prepared from wild-type *R. rubrum* as observed in reverse-phase HPLC. The B820 sample was dissolved in 60% formic acid and subjected to an increasing gradient of tetrahydrofuran in 30% formic acid as indicated. The flow rate was 0.7 mL/min at room temperature. The BPh band was identified by its absorbance spectrum. The B873-α and B873-β polypeptides were identified by their UV absorbance spectra and their behavior on SDS–PAGE.

α and β were present in B820 samples in a ratio of 0.85.

BChl:αβ Ratio. The BChl:$\alpha\beta$ ratio was determined by using several different procedures (see Materials and Methods). There are a variety of problems that can occur during these analyses. Older samples, and fresh samples that had been exposed to light (even a few minutes of room light), had lower ratios because of BChl degradation. Also contributing to low BChl was incomplete extraction, although this was a relatively insignificant amount. The amount of protein could have been underestimated because B873-α is slightly to significantly soluble in most solvents used to extract BChl. For analysis, the procedure used for protein determination which involves measuring its absorbance at 290 nm is simple, but it was necessary to estimate the contribution to absorbance due to scattering. Alternatively, the Pierce protein assay was also simple, but the standard curve of B873-α differed greatly from the usual bovine serum albumin standard. Therefore, the standard curve of B873-α was used to determine the protein concentration in B820 samples. Because it was assumed that B873-β would respond to the assay identically with B873-α, some error may have resulted since even though both have very similar stretches of hydrophobic amino acids, B873-β has more charged groups (Brunisholz et al., 1984). Aside from this concern, the Pierce colorimetric assay was quite reproducible and has many advantages, among which are its low sensitivity to OG and insensitivity to 1% SDS, the latter of which is an excellent solvent for the B820 protein. All experimental results obtained by using a variety of methods (total of 19 determinations) gave an average value of 1.7 ± 0.3.

Molecular Weights of B820 and "B873". The molecular weights of the B820 complex and the re-formed "B873" complex were estimated by gel filtration. It had been hypothesized that the B820 form was a subunit form and "B873" was a reassociated form of the light-harvesting antenna (Loach et al., 1985). By use of a gel filtration column and appropriate standards (see Materials and Methods), the molecular weights were determined to be between 42K and 65K for B820 and >200K for "B873", which supports the hypothesis. It is unknown how small a light-harvesting complex could be prepared and still maintain the 820-nm absorbance. It should be kept in mind that these molecular weights may not be very accurate because detergent was used in running the column and it is unlikely that the standards and the B820 and "B873" complexes bind the same amount of detergent (on a weight basis), nor is it likely that they have identical shapes. The more hydrophobic B820 complex almost certainly binds more detergent than the hydrophilic standard proteins used. Also, the OG concentration had to be lowered for the "B873" complex in order to ensure that the far-red λ_{max} remained near 873 nm. It is of interest, however, that the size of the reaction center complex, determined from the elution volume of the 870-nm-absorbing material from the G-100 column, was 100K which is the same size as determined from its composition. In controls, free BChl in 0.8% OG eluted substantially behind B820, indicating that BChl which may have dissociated from B820 should have been readily separated.

Other Detergents and B820 Formation. Partly because of the expense of OG, the possible use of other detergents for the preparation of B820 was evaluated. In early work with Triton X-100, LDAO, and SDS, we found the dissociation of B873 too readily proceeds to solubilized BChl absorbing at 770 nm without forming a stable B820 species. Three nitrogen analogues of OG recently available from Oxyl-GmbH (Bobingen, West Germany) were examined. These were the octanoyl, nonanoyl, and decanoyl derivatives of *N*-methylglucamide. An increasing effectiveness was found as the hydrocarbon chain increased from octanoyl to decanoyl. A conversion of about 80% of B873 to B820 could be obtained by using the latter detergent, but a substantial amount of 777-nm material also formed. Thus, OG was by far the most useful detergent tested.

Fluorescence Properties. When the LH complex of *R. rubrum* is associated with the RC in vivo, the fluorescence yield is quite low (<1%). Upon conversion to subunit form, the emission was at 822 nm, and the yield was much higher, about 30% that of detergent solutions of free BChl which absorbed at 777 nm and emitted at 780 nm (data not shown).

Circular Dichroism (CD) Spectra. CD is a sensitive probe of the structural organization of LH complexes (Sauer, 1978).

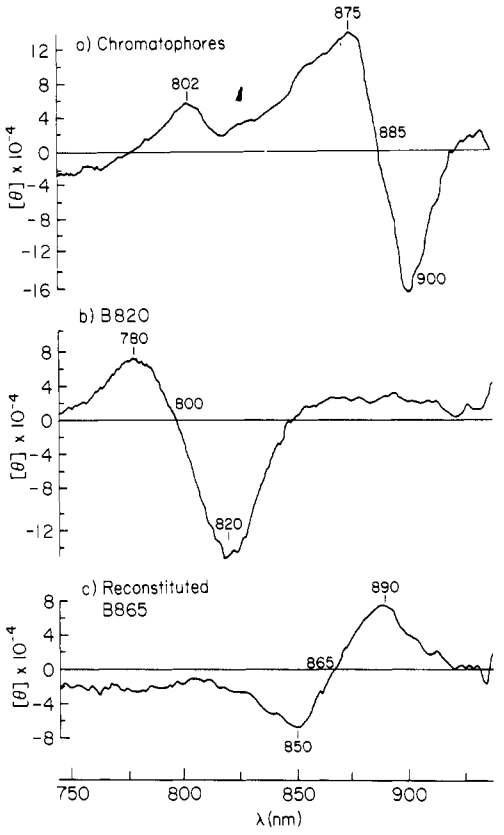

FIGURE 6: Circular dichroism spectra of the LH complex prepared from wild-type *R. rubrum*. (a) Chromatophores; (b) B820 at pH 7.5 in 0.05 M phosphate buffer containing 0.75% OG; (c) "B873", here absorbing at 865 nm, in 0.37% OG. All samples were prepared to be at the same concentration as chromatophores. These measurements were made at the University of Montreal in the laboratory of G. Gingras.

As shown in Figure 6, the B820 complex has a unique CD spectrum in which there is a positive lobe centered at 780 nm and a negative lobe centered at 820 nm. At the concentration of BChl in B820, the CD of free BChl in 0.8% OG is barely detectable. Because of the high molar ellipticity of B820, the data suggest that a single complex exists containing at least two interacting BChl molecules. Also shown in Figure 6 are the CD spectra of chromatophores from *R. rubrum* and that of a sample of reassociated "B873". While the spectrum of chromatophores agrees very well with the earlier data of Sauer and Austin (1978), and the reassociated "B873" spectrum also has a conservative pattern, the latter is inverted relative to the CD spectrum of chromatophores.

Discussion

The key step in the method presented here for isolation of the LH complex from *R. rubrum* was the use of OG to effectively convert the LH complex into a subunit form (B820) which could be separated from the RC by gel filtration chromatography. The B820 complex consists of equal quantities of the polypeptides B873-α and B873-β and contains BChl in a ratio of 2 BChl per $\alpha\beta$ pair. The molecular weight of B820 seemed to be in the range of 40K–65K. However, it is unclear whether this indicates the existence of aggregates of a simple $\alpha\beta$·2BChl subunit (e.g., $\alpha_2\beta_2$·4BChl, $\alpha_3\beta_3$·6BChl, or $\alpha_4\beta_4$·8BChl) or variable size micelles of $\alpha\beta$·2BChl and $\alpha_2\beta_2$·4BChl with large amounts of detergent surrounding them. These integral membrane complexes might be expected to bind an amount of detergent approximately equal to their mass. If so, the $\alpha_2\beta_2$·4BChl structure would have a molecular weight of about 56K.

On the basis of the known properties of B820 and B873 complexes, we have suggested that in the B873 LH complex a specific association of subunits occurs to form BChl dimers (Loach et al., 1985) having a structure similar to the dimer in the RC that is the primary electron donor (Deisenhofer et al., 1984). In Loach et al. (1985), a BChl molecule was proposed to be bound at each end of an $\alpha\beta$ pair so that in the associated B873 form two sets of close dimers were formed near opposite surfaces of the membrane. Scherz and Parson (1986) suggested a similar model with two sets of interacting dimers to account for the CD and absorbance properties of the LH complex.

It is interesting, and of possibly great utility, that the B820 form can be quantitatively reassociated to re-form a complex absorbing near the original 873 nm. However, it is clear from the CD spectrum of "B873" that the reassociated structure may be somewhat different from the original B873. In our preliminary studies, it did not seem to matter whether "B873" was re-formed by dilution, dialysis, gel filtration, or lowering of the temperature. Perhaps this is because the method of reassociation used in these cases allowed formation of an aggregate in three dimensions. Thus, a more controlled reassociation, for example, in liposomes which would allow only a two-dimensional association to occur, may be required to more exactly re-form the original B873 structure. It is also possible that the RC must also be present in a lipid bilayer matrix for appropriate reconstitution. The result that B820 can be quantitatively prepared and reversibly reassociated to perhaps re-form the native B873 will be very useful in structure–function studies (Callahan et al., 1985).

Acknowledgments

We express our appreciation to Dr. Gabriel Gingras of the University of Montreal for use of his instrument and for his help in making the CD measurements, to Dr. Larry Morrison of Standard Oil in Naperville, IL, for measurements of fluorescence yield, and to Leslye Meyer for some of the evaluation of detergents other than octyl glucoside. Meredith Mamoda is also gratefully acknowledged for her assistance in determinations of the bacteriochlorophyll to protein ratios and the conversion of the 820-nm complex to a longer wavelength form. We are also grateful to Mark Gyure of our laboratory for amino acid analyses and some SDS–PAGE determinations and to Gregory S. Martin and Dr. Lyman G. Armes of Dr. Margoliash's laboratory at Northwestern University for other amino acid analyses.

Registry No. OG, 29836-26-8.

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