Antenna Holochrome B880 of *Rhodospirillum rubrum* S1. Pigment, Phospholipid, and Polypeptide Composition†

Rafael Picorel, Gilles Bêlanger, and Gabriel Gingras*

ABSTRACT: An antenna bacteriochlorophyll–protein complex (holochrome B880) was isolated from chromatophores of *Rhodospirillum rubrum*. The yield of chromatophore bacteriochlorophyll recovered as holochrome was about 90% in the initial crude fractions and about 75% after two chromatographic purification steps. The holochrome protein is estimated to account for approximately 60% of the total chromatophore proteins. According to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, this preparation contains two different polypeptides, designated α and β, of apparent M_r 7600 and 6400. Very likely, polypeptide α is the organic solvent soluble polypeptide earlier isolated and sequenced in other laboratories. According to their relative intensity of staining by Coomassie Brilliant Blue in polyacrylamide gels, polypeptides α and β are present in an equimolar ratio. Amino acid analysis of the holochrome and of the polypeptides α and β prepared from it shows the two polypeptides to have different amino acid compositions and agrees with a 1:1 mole ratio. Bacteriochlorophyll and spirilloxanthin, the pigments of the holochrome, are present in a 2:1 mole ratio. The minimal mass of protein (minimal molecular weight) associated with 2 mol of bacteriochlorophyll and 1 mol of spirilloxanthin is 23 000. The data indicate that the minimal oligomer has an $\alpha_2\beta_2$ structure. The holochrome preparation contains about 10% each of pigments and of phospholipids per unit weight of protein. These phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol; they are present in approximate mole ratios of 1.0, 0.5, and 0.5, respectively, per 23 000 g of protein.

Solubilization and fractionation techniques applied to chromatophore membranes from photosynthetic bacteria have led to the isolation and characterization of two classes of chromoproteins, the photoreaction center and the antenna bacteriochlorophyll complexes. The main function of the antenna is to collect light energy, which is subsequently transfered to the photoreaction center where it is converted to a chemical potential. Due to its prime importance in the photosynthetic mechanisms, the structure and function of the photoreaction center has received much attention in the last decade somewhat to the detriment of the antenna, whose structure has been more neglected.

In the Rhodospirillaceae so far studied, there appear to exist two groups of light-harvesting Bchl[1]–protein complexes or holochromes: holochrome B875 and holochrome B800–B850. In better studied Rhodospirillacea such as *Rhodopseudomonas sphaeroides* (Sauer & Austin, 1978; Broglie et al., 1980) and *Rhodopseudomonas capsulata* (Feick & Drews, 1978; Shiozawa et al., 1980), these holochromes appear to be composed of one or several copies of different polypeptides.

Rhodospirillum rubrum has a simpler antenna with a peak at about 880 nm in wild-type strain S1 and at about 870 nm in carotenoidless strain G9. In this species, the small 805-nm band is probably entirely due to absorbance by the photoreaction center (Picorel et al., 1980). This may be thought to indicate that a single holochrome is present in that species. Despite this apparent simplicity, the holochrome of this species has not been fully characterized. Photoreaction center depleted chromatophores showed a major band in the apparent M_r 10 000 region on $NaDodSO_4$–polyacrylamide gels, which was thought to be due to antenna Bchl-binding protein (van der Rest et al., 1974). Drachev et al. (1976) obtained a holochrome fraction from the wild type by successive LDAO extractions of the chromatophores but reported no further characterization. A similar preparation was obtained from the carotenoidless mutant strain G9 by Cuendet & Zuber (1977). In their method, chromatophores were first depleted of their photoreaction center by successive treatments with LDAO and deoxycholate, and the holochrome was solubilized with LDAO before precipitation with ammonium sulfate. This preparation displayed a chromatophore-like absorption spectrum but underwent a large blue shift of its far-red band upon resuspension in 0.1% LDAO. The preparation behaved as a single polypeptide with an M_r of about 12 000–14 000 as determined by several methods. This polypeptide was soluble in chloroform–methanol, and its amino acid composition was similar to that of the chloroform–methanol extract of Tonn et al. (1977). The chloroform–methanol extraction method developed by Tonn et al. (1977) and modified by Shiozawa et al. (1980) has led to the isolation of a polypeptide pure enough and in amounts sufficient for the determination of its complete amino acid sequence (Brunisholz et al., 1981).

Sauer & Austin (1978) have purified this holochrome from wild-type *Rs. rubrum* with Triton X-100 and sucrose density gradient centrifugation; their preparation appeared to be in the native state. It behaved like an M_r 420 000 particle on a molecular sieve and was resolved by $NaDodSO_4$–polyacrylamide gel electrophoresis into a single polypeptide of about M_r 10 000. On the basis of pigment content, it was suggested that 3 molecules of Bchl plus an undefined amount of carotenoids are associated with two copies of this polypeptide. According to Cogdell & Thornber (1979), this carotenoid is spirilloxanthin and is present in a Bchl:spirilloxanthin mole ratio of 2:1.

In this work, we isolated from wild-type *Rs. rubrum* a stable holochrome preparation apparently in its native state. This preparation is resolved into two different polypeptides, one of

† From the Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7. *Received November 15, 1982.* Supported by the Medical Research Council of Canada and by fellowships from the C.S.I.C. (Spain) to R.P. and from the Ministère de l'Education (Québec) to G.B.

[1] Abbreviations: Bchl, bacteriochlorophyll; DEAE, diethylaminoethyl; LDAO, dodecyldimethylamine *N*-oxide; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Spir, spirilloxanthin.

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which appears to be identical with the organic solvent soluble fraction of Tonn et al. (1977). The holochrome contains bacteriochlorophyll and spirilloxanthin in a 2:1 mole ratio. We present evidence that the two polypeptides, in a 1:1 molar ratio, form an oligomer of 23 000 minimal molecular weight. This oligomer is thought to contain 2 mol of Bchl and 1 mol of spirilloxanthin plus 1 mol of phosphatidylethanolamine and approximately 0.5 mol each of phosphatidylglycerol and of diphosphatidylglycerol.

Materials and Methods

Organisms and Growth Conditions. *Rs. rubrum* strain S1 (ATCC 11170) was grown semianaerobically at 32 °C in the synthetic medium of Cohen-Bazire et al. (1957). The cultures were carried out in 12-L cylindrical flasks (23-cm diameter), which were illuminated by three 150-W photoflood lamps at a distance of 30 cm from the center of the flask. The cells were harvested after 4 days (late log phase) of illumination and washed with cold 50 mM phosphate buffer (pH 7.0). After harvest, the cells were kept at −20 °C.

Extraction of Chromatophores. The chromatophores were extracted by alumina grinding of the bacterial pellet and isolated by differential centrifugation as described elsewhere (van der Rest & Gingras, 1974). The chromatophores were washed twice in 50 mM phosphate buffer (pH 7.0) before further treatment.

Isolation of Antenna Holochrome. All steps were carried out in darkness. The chromatophores (final A_{880} = 37.5) were suspended for 1 h at 4 °C in 50 mM phosphate buffer (pH 7.0)–10 mM sodium ascorbate–0.5% w/v LDAO (method A) or 0.35% w/v LDAO (method B). The LDAO concentration was then brought to 0.1% w/v by dilution with phosphate (method A) or with phosphate–0.1% w/v Triton X-100 (method B), and the preparation was centrifuged for 1.5 h at 105000*g*. The supernatant containing the photoreaction center was decanted out, and the pellet was resuspended (final A_{880} = 25) in phosphate–10 mM ascorbate–0.4% or 0.35% LDAO (method A or B, respectively) for 1 h at 4 °C. The LDAO concentration was brought to 0.1% by dilution with buffer, and the suspension was centrifuged for 1.5 h at 105000*g*. The supernatant containing the antenna holochrome was pipeted out by suction. The pellet was extracted again with 0.35% LDAO as described above. The second supernatant obtained after a new centrifugation (1.5 h at 105000*g*) was pooled with the first one and 3 times dialyzed for 24 h in 4 L of 50 mM Tris-HCl (pH 8.0).

The preparation at this stage is fairly pure antenna holochrome as explained under Results. Nevertheless, it was further purified by chromatography on DEAE-cellulose as follows. The equivalent of 400 units of A_{880} was applied on a 16 × 200 mm column of DEAE-cellulose equilibrated with 50 mM Tris-HCl (pH 8.0) containing 0.1% w/v of either LDAO or Triton X-100. The column was washed with this buffer containing 25 mM NaCl until the red color reached the bottom of the column. The preparation was then eluted with the same buffer containing 250 mM NaCl. The first fractions were discarded since they were contaminated with pigments with an absorption peak at 770 nm due to monomeric Bchl. This contaminant was more abundant when using LDAO in the elution buffer. After elution, the preparation was dialyzed in the same buffer.

Absorption Spectroscopy. The absorption spectra were measured with a Cary 14R spectrophotometer at room temperature in 1-cm path-length cells.

Assays. Protein was assayed by the method of Lowry et al. (1951) with bovine serum albumin (Sigma) as the standard. Quantitative bacteriochlorophyll and spirilloxanthin assays were carried out on lyophilized samples according to van der Rest & Gingras (1974). We used the following extinction coefficients in acetone–methanol: for bacteriochlorophyll, ϵ_{771} = 65.3 $mM^{-1}\cdot cm^{-1}$ and ϵ_{525} = 1.5 $mM^{-1}\cdot cm^{-1}$; for spirilloxanthin, ϵ_{525} = 101.4 $mM^{-1}\cdot cm^{-1}$. In assessment of the amount of Bchl and of spirilloxanthin, account was taken of the overlap between the absorption bands of the two pigments at 525 nm (van der Rest & Gingras, 1974).

Phospholipid Determination. Total lipids were extracted by the method of Bligh & Dyer (1959). To 1.2–1.6-mL samples (~1 mg of protein) were added 3 mL of methanol and 1.5 mL of chloroform, the solutions were vortex mixed for 30 s, and another 1.5 mL of chloroform was added, followed by 30 s of vortex mixing. Finally, 1.5 mL of water was added, followed by 30 s of vortex mixing and by a 10-min centrifugation at 2000*g*. The chloroform phase containing the phospholipids and the pigments was removed and the aqueous phase extracted twice with 3 mL of chloroform each time. The chloroform extracts were pooled and evaporated under a stream of nitrogen gas, and the residue was redissolved in 2 mL of chloroform–methanol (2:1 v/v). Two fractions of 0.9 mL each were removed and evaporated again under a stream of nitrogen gas. One fraction was used for chromatography of phospholipids and the other for chromatography of neutral lipids. The residues were dissolved in about 10 drops of chloroform–methanol (2:1 v/v) and applied on thin-layer silica gel plates. Two types of plates were used, Whatman P4850820, type K5, and Analtech GHL, An 11011. The solvents were either chloroform–methanol–water (60:25:4) or chloroform–methanol–acetic acid–water (65:25:1:4) for phospholipids. For neutral lipids, the solvent was benzene–ether–ethanol–acetic acid (50:40:2:0.2). Chromatography was carried out in the dark along with phospholipid standards. The bands were stained by iodine vapors. The bands were scraped out of the gels, and phosphate was determined by a modification of the method of Broekhuyse (1967). For determination of total phospholipids, the origin of the neutral lipid plate was used since phospholipids do not migrate in this solvent. Phosphate recovery was 92.5%.

Amino Acid Analysis. Protein samples (about 120 μg) were dialyzed for 48 h in deionized water and lyophilized. All operations, beginning with lyophilization, were carried out in the same tube. To each sample were added 0.5 mL of HCl (constant boiling; Pierce Chemical Co.) and one drop of phenol. The tubes were then sealed under vacuum and kept at 110 °C for 24, 48, 72, or 102 h. The hydrolysates were dried in vacuo and dissolved in 100 μL of 0.2 M sodium citrate (pH 2.2), mixed, and centrifuged at 2000*g* for 5 min. Cysteine and methionine were determined on HCl hydrolysates after oxidation with performic acid according to Moore (1963) with omission of HBr. For determination of tryptophan, the samples were hydrolyzed in methanesulfonic acid according to Mahoney & Hermodson (1979). The amino acid analyses were carried out in a Durrum Model 1 D500 amino acid analyzer.

Analytical Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed at 20 °C in a Pharmacia gel electrophoresis apparatus, Model GE-2/4LS. The method used was that of Laemmli (1970). The gel slabs were 0.7 mm thick and 200 mm long. The separation gel contained 18% acrylamide and 0.48% *N,N'*-methylenebis(acrylamide). The samples were incubated in the electrophoresis Tris-HCl buffer containing 2% sodium dodecyl sulfate, 4% 2-mercaptoethanol,

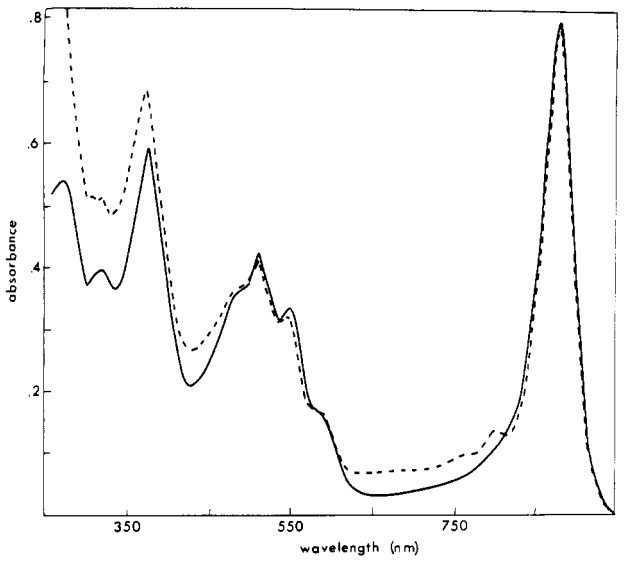

FIGURE 1: Absorption spectrum of chromatophores and of purified antenna holochrome from *Rs. rubrum* S1. Both holochrome (—) and chromatophores (---) were in 50 mM Tris-HCl (pH 8.0). Holochrome was extracted and purified with LDAO according to method A under Materials and Methods.

and 7% sucrose for 30–45 min at room temperature and applied immediately onto the gel. Electrophoresis was carried out for 8 h at 25 mA. Staining (Coomassie Brilliant Blue G250) and destaining were according to Fairbanks et al. (1971). Molecular weight markers were trypsin inhibitor, cytochrome *c*, aprotinin, the β chain of insulin (all from Boehringer Manheim), and myoglobin (Sigma Chemical Co.). Optical scanning of the gels at 550 nm was with a Zeiss spectrophotometer equipped with a scanning attachment.

Separation of Polypeptides α and β. Preparative NaDodSO$_4$ gel electrophoresis (Vadeboncoeur et al., 1979) was sometimes used to separate the subunits of the antenna holochrome. We also made use of the selective solubility of the two polypeptides in organic solvents as follows. Samples of purified antenna holochrome in 50 mM Tris-HCl (pH 8.0) were twice dialyzed for 24 h against 4 L of deionized water and then lyophilized. The lyophilizate was resuspended in 10 mL of chloroform–methanol (1:1 v/v) (Tonn et al. 1977), homogenized, and centrifuged for 10 min at 10000*g*. The supernatant was removed and evaporated and the residue resuspended in 5 mL of chloroform–methanol (1:1 v/v) and centrifuged at 10000*g* for 10 min. The supernatant was evaporated, and the residue was resuspended in chloroform–methanol (1:1 v/v) and chromatographed at 4 °C on Sephadex LH-60 preequilibrated with chloroform–methanol (1:1 v/v) containing 0.1 M ammonium acetate. This procedure allows the separation of pure α polypeptide without any contaminating pigments or lipids (Tonn et al., 1977). The residue of the first centrifugation was extracted again 4 times with chloroform–methanol as described above. The final pellet was found to contain polypeptide β free of polypeptide α (see Results).

Results

Absorption Spectra and Yield of Extraction. Figure 1 shows an absorption spectrum of the antenna holochrome prepared from strain S1 with LDAO as the sole detergent (method A) as compared to a chromatophore suspension from the same organism. Such preparations typically have a ratio $A_{280}/A_{880} = 0.67$; they can be kept in the cold for a few weeks without any important shift in their absorption spectrum.

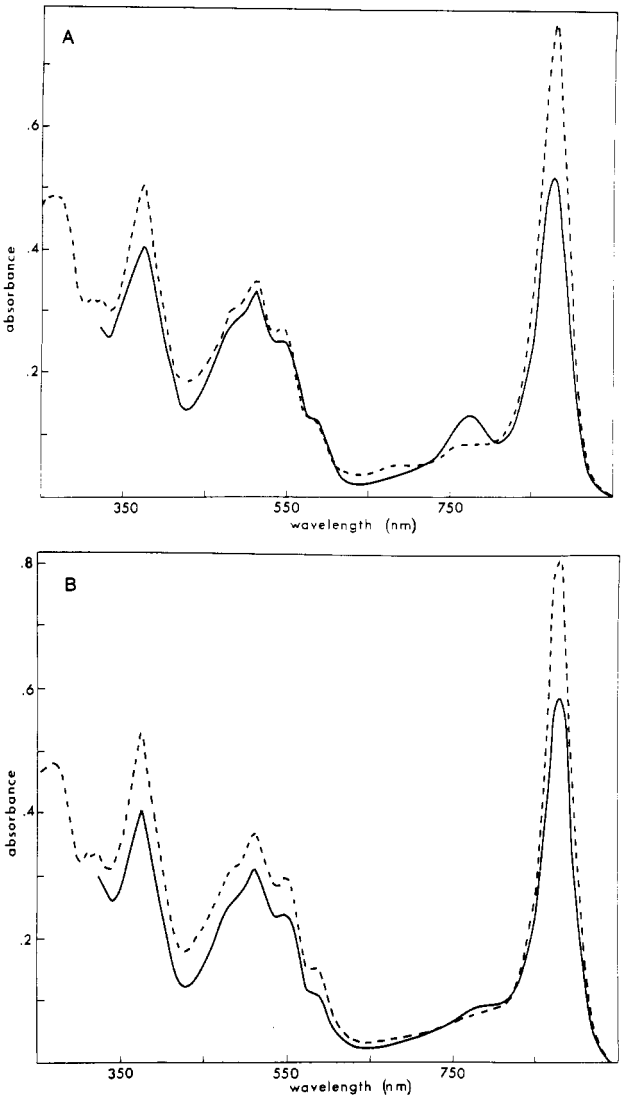

FIGURE 2: Effect of an extensive dialysis on absorption spectra of two preparations of antenna holochrome from *Rs. rubrum* S1. (A) The preparation was obtained solely with LDAO (method A) and redispersed in 50 mM Tris-HCl (pH 8.0) without (—) or with (---) an extensive dialysis in the same buffer. (B) The preparation was obtained with a combination of LDAO and Triton X-100 (method B) and was redispersed in 50 mM Tris-HCl without (—) or with (---) an extensive dialysis in the same buffer.

Since Triton X-100 strongly absorbs in the ultraviolet, method A was used here for illustrative purposes. However, our method of choice is method B, which employs a combination of LDAO and Triton X-100. The yield of extraction with this procedure was determined on the basis of the Bchl content of the starting chromatophore material and of the purified antenna-holochrome preparation. Bchl was determined by acetone–methanol extraction as described under Materials and Methods. The yield before chromatography on DEAE-cellulose was typically about 90% and decreased to about 75% after chromatography in the presence of 0.1% Triton X-100 and to a somewhat lower value with 0.1% LDAO. Figure 2 shows that the absorption spectra of antenna holochrome prepared with LDAO alone and LDAO plus Triton X-100 are similar. One remarkable fact observed with all our prepartions whether they were obtained by method A or B is that they show a band at 770 nm that we attribute to monomeric Bchl. The 770-nm band is most clearly seen (Figure 2A) with preparations made with LDAO as the sole detergent (method A). After an extensive dialysis, this band

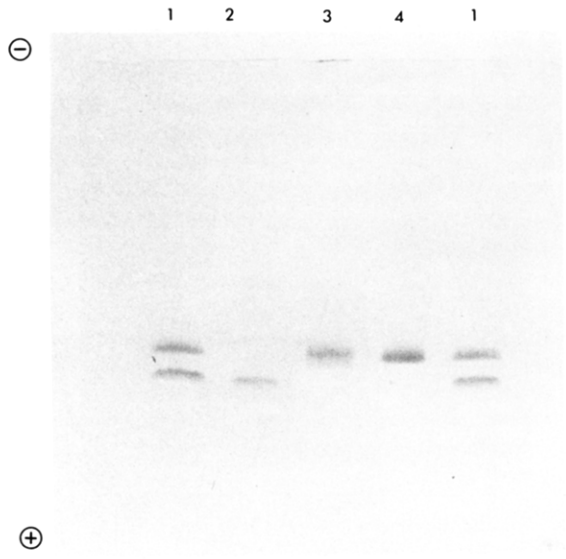

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of *Rs. rubrum* S1 antenna holochrome and of polypeptides isolated therefrom. Electrophoresis was carried out in an 18% polyacrylamide gel slab acording to Laemmli (1970). (Lanes 1) Purified antenna holochrome; (lanes 2 and 3, respectively) polypeptides β and α isolated by organic solvent extraction; (lane 4) organic solvent soluble polypeptide supplied by Dr. P. A. Loach (Northwestern University, Evanston, IL).

disappears simultaneously with an increased absorption especially noticeable at 365 and at 880 nm. This phenomenon may be due to reversible disruption of Bchl–Bchl interaction induced by high salt and LDAO concentration.

Polypeptide Composition. $NaDodSO_4$–polyacrylamide gel electrophoresis shows this preparation to be composed of two different polypeptides (Figure 3, lanes 1) that we designate as α and β. These polypeptides were resolved from the antenna holochrome both by $NaDodSO_4$ preparative gel electrophoresis and by differential extraction with chloroform–methanol followed, in the case of polypeptide α, by chromatography on Sephadex LH-60 (Materials and Methods). While both methods yielded well-resolved α and β subunits, the organic-solvent method allowed the preparation of larger amounts of protein. A further advantage of the second method is that it is much less laborious than the first. The samples on lanes 2 and 3 (Figure 3) were polypeptides β and α, respectively, isolated from antenna holochrome. The sample on lane 4 was the organic solvent soluble polypeptide of Tonn et al. (1977). It was obtained from the laboratory of Dr. P. A. Loach (Northwestern University, Evanston, IL). Polypeptide α clearly comigrates with this protein. Moreover, both proteins react with antibodies prepared against α (G. Bêlanger and G. Gingras, unpublished results). We feel this is strong evidence that both are the same protein. This is also indicated by the amino acid composition of these proteins (see below).

Apparent M_r. Polypeptides α and β were submitted to electrophoresis on 18% polyacrylamide gel slabs along with suitable molecular weight markers. By this method, the apparent M_rs for the antenna-holochrome polypeptide are α = 7600 and β = 6400.

Stoichiometry of Polypeptide Subunits. To determine the stoichiometry of the two subunits, we relied on the extent of staining of each subunit by Coomassie Brilliant Blue as determined by optical scanning of polyacrylamide gels after electrophoresis in the presence of $NaDodSO_4$. For this method to be quantitative (Vadeboncoeur et al., 1979), it is necessary that the specific intensity of staining first be determined for at least one of the two subunits. In this case, increasing amounts of isolated subunits α and β were applied to polyacrylamide gels and stained with Coomassie Brilliant Blue. After destaining, the gels were scanned, and the relative area under the peaks was obtained by weighing the paper delineated by each curve on a photocopy. Background absorbance of the gel was compensated by taking as the base line regions of the gel without polypeptide bands. Figure 4 shows the calibration curve obtained in this manner for each polypeptide. The corresponding slopes, expressed in relative area per milligram of protein, were found to be 1.0 for α and 1.9 for β. The different affinities of the polypeptides for Coomassie Brilliant Blue may reflect different primary structures. A similar operation was next performed on three different holochrome preparations (about 5 μg of protein) in order to obtain the relative area corresponding to each polypeptide. When these values were divided by the apparent molecular weights of the subunits, molar stoichiometries of 0.9 for α and 1.1 for β were found.

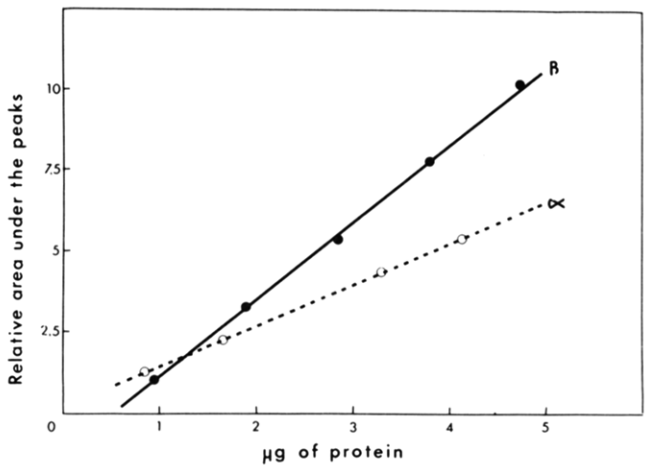

FIGURE 4: Relative intensity of staining by Coomassie Brilliant Blue G250 of holochrome polypeptides α and β as a function of amount of protein applied to a $NaDodSO_4$–18% polyacrylamide gel. The intensity of staining was determined from the relative areas under the peaks of electrophoretograms after optical scanning at 550 nm.

Amino Acid Composition. The holochrome protein and subunits α and β isolated from it by organic-solvent extraction (Materials and Methods) were hydrolyzed in 6 N HCl for 24, 48, 72, and 102 h. For subunit α and for the holochrome protein, the mole percents of Leu, Phe, Val, and His were observed to increase with time, indicating incomplete hydrolysis of a hydrophobic stretch, as noted by Brunisholz et al. (1981). The Ser mole percent did not decrease linearly with time whereas the Thr mole percent decreased in a more nearly linear fashion. This behavior was not observed for subunit β, whose hydrolysis was essentially complete after 24 h.

Table I gives the mole percent amino acid composition of the holochrome protein and of its two subunits according to a 72-h hydrolysis. The data are uncorrected except for Thr and Ser, whose values were extrapolated to initial time. As indicated above, this correction is not very accurate for Ser in the holochrome and in subunit α. The amino acid composition of subunit α is comparable with that reported for the organic solvent soluble polypeptide (Tonn et al., 1977; Cuendet & Zuber, 1977; Brunisholz et al., 1981). The amino acid composition of polypeptide α is clearly different from that of β: particularly noteworthy are the Leu content (about twice higher in α than in β) and the Tyr content (absent in α but present as 1 mol/chain in β). According to our analysis, both chains contain one residue each of His and Met. Only one Arg residue is found in β.

An important outcome of the amino acid analysis is that the nearest integer number of amino acids in the holochrome

Table I: Amino Acid Composition of Antenna Holochrome from *Rhodospirillum rubrum* S1[a]

	polypeptide α			polypeptide β		holochrome	
	mol %	no. of residues[b]	no. of residues from sequence[c]	mol %	no. of residues[b]	mol %	no. of residues[c]
Asp	4.6	2.5 (2)	2	4.9	2.2 (2)	4.2	4.2 (4)
Thr[d]	7.0	3.6 (4)	4	4.3	2.1 (2)	5.4	5.4 (5)
Ser[d]	8.1	4.2 (4)	3	5.7	2.9 (3)	7.1	7.1 (7)
Glu	11.2	6.0 (6)	5	10.6	4.9 (5)	9.8	9.8 (10)
Pro	4.2	2.3 (2)	2	5.4	2.5 (3)	4.7	4.7 (5)
Gly	5.7	3.0 (3)	2	10.6	4.9 (5)	8.1	8.1 (8)
Ala	8.3	4.5 (4)	4	9.9	4.6 (5)	9.2	9.2 (9)
$^1/_2$-Cys	0.0	0.0 (0)	0	0.4	0.2 (0)	0.3	0.3 (0)
Val	6.4	3.5 (3)	3	8.3	3.8 (4)	7.9	7.9 (8)
Met	2.0	1.1 (1)	1	1.1	0.52 (1)	1.2	1.2 (1)
Ile	5.4	2.9 (3)	3	6.5	3.0 (3)	6.4	6.4 (6)
Leu	15.6	8.5 (9)	10	8.7	4.0 (4)	12.0	12.0 (12)
Tyr	0.2	0.05 (0)	0	2.1	1.0 (1)	1.1	1.1 (1)
Phe	8.7	4.7 (5)	5	7.7	3.6 (4)	8.7	8.7 (9)
His	1.0	0.6 (1)	1	2.4	1.1 (1)	1.7	1.7 (2)
Lys	2.6	1.4 (1)	1	4.9	2.3 (2)	3.9	3.9 (4)
Arg	4.8	2.6 (3)	3	2.8	1.3 (1)	4.2	4.2 (4)
Trp	5.3	2.9 (3)	3	3.3	1.4 (1)	4.1	4.1 (4)

[a] Average of analyses from three different 72-h hydrolysates. [b] Number of residues calculated from average residue M_r of 115, 109, and 112.5 and M_r of 6106, 5142, and 11 248 for polypeptides α and β and for the holochrome, respectively. [c] From Brunisholz et al. (1981). [d] Corrected by extrapolation to initial values.

Table II: Pigment Composition and Minimal Molecular Weight of Antenna Holochrome from *Rhodospirillum rubrum* at Different Stages of Purification

fraction	Bchl:Spir mole ratio[a]	g of protein/2 mol of Bchl	g of protein/1 mol of Spir
chromatophores	1.94 (3)	35 300[b]	33 900[b]
supernatant (method A)	1.88 (6)	25 800[b]	22 700[b]
supernatant (method B)	1.89 (4)	25 500[b]	24 000[b]
first chromatography on DEAE-cellulose	1.79 (4)	26 300[b]	23 300[b]
second chromatography on DEAE-cellulose	1.93 (6)	26 300,[b] 21 000[c]	23 800,[b] 20 000[c]

[a] Average of n experiments. [b] Protein determined by the method of Lowry et al. (1951). [c] Protein determined by summing the contents of the individual amino acid residues in 6 N HCl 72-h hydrolysates.

and in subunits α and β is in excellent agreement with a 1:1 stoichiometry of the subunits.

Pigment Composition. The pigment composition was established on the acetone–methanol extracts (7:2 v/v) of different lyophilized preparations according to van der Rest & Gingras (1974). Spectroscopic analysis in this solvent showed peaks at 365, 600, and 771 nm due to Bchl and at 446, 494, and 525 due to spirilloxanthin. We found no evidence for the presence of other components, such as bacteriopheophytin, in any significant amount.

Table II shows the mole ratio of Bchl to spirilloxanthin at different stages of the purification procedure of the antenna holochrome. Within experimental error, this ratio is found to be 2:1. We also determined the ratio of protein to Bchl and spirilloxanthin as a means of checking the purity of the preparation. Protein was assayed by the method of Lowry et al. (1951) for all the preparation steps. It was also assayed by taking the sum of the amino acid contents in three purified samples of known pigment content. Table II shows the ratio of protein mass per 2 mol of Bchl or 1 mol of spirilloxanthin. This is equivalent to measuring the minimal molecular weight as a function of purification. The data indicate that the main purification step is extraction from the chromatophores and that DEAE-cellulose chromatography does not significantly improve purity as expressed on a minimal molecular weight basis. However, we routinely use at least one DEAE-cellulose chromatography in the course of this preparation because it eliminates some minor protein contaminants apparent on $NaDodSO_4$–polyacrylamide gels (not shown). Two important results can be deduced from this experiment: the minimal molecular weight of the holochrome and the amount of holochrome present in the chromatophores. The minimal molecular weight, on the basis of the presence of 1 mol of spirilloxanthin/mol of protein, is between about 26 000 and 20 000 for the purest samples analyzed. This range appears to correspond to a systematic error inherent in the protein assay methods, the higher value being obtained with the method of Lowry et al. (1951) and the lower by amino acid analysis. For the rest of this paper, we will take the average between these extremes and assume a minimal molecular weight of 23 000.

The other interesting point is the amount of holochrome present in chromatophores, which is found to be surprisingly high: about 60% on the basis of the protein assay method of Lowry et al. (1951). This question is more extensively treated under Discussion.

Phospholipid Composition. According to phosphate analysis of chloroform–methanol extracts, our preparation contains approximately 0.1 mg of total phospholipid/mg of protein, taking an average M_r of 770 for phospholipids. A more detailed analysis was carried out by thin-layer plate chromatography in three different systems. System A (for experimental conditions, see Materials and Methods) showed only two phospholipid spots, one due to phosphatidylethanolamine and the other to a mixture of phosphatidylglycerol and of diphosphatidylglycerol. Systems B and C resolved three phospholipid spots that migrated as phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol.

Each spot was scraped off the plate and eluted, and its phosphate content was determined. The results are given in

Table III: Phospholipid Composition of Antenna Holochrome from *Rhodospirillum rubrum* S1[a]

chromatographic system[b]	total phospholipid[c]	phosphatidyl-ethanolamine	phosphatidyl-glycerol plus diphosphatidyl-glycerol	phosphatidyl-glycerol	diphosphatidyl-glycerol
A	116	49	60		
B	120	48		33	46
C	122	49		29	44
av of results with A, B, and C	119	49		31	45

[a] Moles of phospholipid per 23 000 g of protein: phosphatidylethanolamine, 1.1; phosphatidylglycerol, 0.7; diphosphatidylglycerol, 0.5. Composition values are expressed as nanomoles of PO_4 per milligram of protein. [b] (A) Whatman TLC plate P4850820 type K5 developed with chloroform-methanol-acetic acid-water (65:25:1:4); (B) same TLC plate developed with chloroform-methanol-water (65:25:4); (C) Analtech An 11011 silica gel plate developed with chloroform-methanol-acetic acid-water (65:25:1:4). [c] Determined on the eluates of the origin of the corresponding plates after development with benzene-ether-ethanol-acetic acid (50:40:2:0.2) as described under Materials and Methods.

Table III. On a weight basis, phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol, respectively, account for 39, 24, and 37% of the phospholipid content. Taking the minimal protein molecular weight of the holochrome as 23 000, one finds the following respective phospholipid:protein mole ratios for phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol: 1.1, 0.7, and 0.5. The protein population, then, is heterogeneous with respect to the nature of its associated phospholipid. One might suggest that each protein oligomer is associated with 1 molecule of phosphatidylethanolamine and with 1 molecule of either phosphatidylglycerol or diphosphatidylglycerol.

Discussion

From *Rs. rubrum* S1, we were able to obtain purified antenna holochrome, apparently in its native state. This can be deduced from a comparison of its absorption spectrum with that of the chromatophores from which it was extracted (Figure 1). With a combination of two detergents, LDAO and Triton X-100, the yield of extraction is as high as 90%, and it could probably approach 100% with one or two more extraction cycles.

In an attempt to find out the specific pigment content of the holochrome, we determined the Bchl and spirilloxanthin to protein ratio as purification progressed (Table II). This led us to a minimal protein molecular weight of 23 000 for the purified preparations. These experiments also showed a clear decrease of the protein to Bchl ratio between the chromatophores and the supernatant.

These results can also be used to estimate the fraction of the total chromatophore protein that can be accounted for by the holochrome. We will assume that the photoreaction center and the holochrome are the only chromatophore proteins that bind Bchl. Our calculation is based on the known minimal molecular weight of these proteins, their Bchl contents, and the size of the photosynthetic unit. The photosynthetic unit in *Rs. rubrum* is generally thought to contain 30 molecules of antenna Bchl/mol of photoreaction center (Aagaard & Sistrom, 1972; Picorel et al., 1980), although photosynthetic unit sizes as low as 21 have been reported (Loach, 1980).

The protein weight fraction of holochrome or of photoreaction center in the chromatophore is given by the number of photosynthetic units in a given chromatophore sample times the total mass of that specific protein in a photosynthetic unit divided by the mass of protein in the chromatophore sample. In other terms, the protein weight fraction, F, is given by

$$F = (NM/U)/C$$

where M and N are respectively the molecular weight of the protein and its number of copies per photosynthetic unit, U is the total mole number of Bchl (antenna plus photoreaction center) in a photosynthetic unit, and C is the chromatophore protein to Bchl ratio in grams of protein per mole of Bchl. M is 90 000 for the *Rs. rubrum* photoreaction center (Vadeboncoeur et al., 1979) and 23 000 for the holochrome. From Table II, C is 17 650. According to whether a photosynthetic unit size of 30 or of 21 is considered, N for holochrome is $^{30}/_{2}$ or $^{21}/_{2}$ and U is 34 or 25. From these considerations, F is calculated to be 0.58 or 0.54 for the holochrome and 0.15 or 0.2 for the photoreaction center, in the first and second hypothesis, respectively. An F value of 0.15 for photoreaction center is in excellent agreement with a previous estimate based on photochemical activity (van der Rest et al., 1974). In the light of the present evidence that indicates that the holochrome represents approximately 55–60% of the chromatophore protein, the earlier estimate of 50% for the organic solvent soluble polypeptide alone (Tonn et al., 1977) was probably too high.

That such a high proportion of the chromatophore protein is accounted for by this holochrome and by the photoreaction center makes it unlikely that another holochrome species would coexist with it in any sizeable amount. This consideration is strengthened by the fact that we could extract the near totality of this holochrome without any electrophoretic or spectroscopic evidence for the presence of another species.

The antenna holochrome is constituted of two different polypeptide chains that we designate as α and β. They have apparent M_r of 7600 and 6400, respectively, on 18% NaDodSO$_4$–polyacrylamide gels. α is almost certainly the same polypeptide that was first extracted and characterized by Tonn et al. (1977), further studied by Cuendet & Zuber (1977), and finally sequenced by Brunisholz et al. (1981). This statement is based on the following evidence: (1) from purified holochrome, we were able to isolate polypeptide α by chloroform–methanol extraction followed by chromatography on Sephadex LH-60; (2) when applied to NaDodSO$_4$–polyacrylamide gels either in the purified holochrome or in isolated form, polypeptide α comigrates with the organic solvent soluble polypeptide isolated by Loach and co-workers; (3) both α and the polypeptide prepared by Loach and co-workers react with antibodies prepared against α (G. Bélanger and G. Gingras, unpublished results). Polypeptide β is clearly different from α as indicated by its electrophoretic behavior and by its amino acid composition. According to its amino acid sequence, α has a molecular weight of 6106 (Brunisholz et al., 1981); we find 7600 by NaDodSO$_4$–polyacrylamide gel electrophoresis. If one assumes the same error factor of 6106/7600 for β, the latter would have a molecular weight of 5140 instead of 6400.

The molar stoichiometry of these subunits in the holochrome is 1:1. This is shown by two types of experiments. In the first one, the amount of each polypeptide was assayed by its staining

intensity on an electrophoretogram of the holochrome. For this method to be quantitative, the staining intensity with Coomassie Brilliant Blue is first calibrated as a function of the amount of the isolated polypeptides (Vadeboncoeur et al., 1979). In the present instance, polypeptide β was stained about twice as intensely as polypeptide α (Figure 4). In the second line of evidence, the number of amino acid residues in the holochrome and in its isolated subunits was determined by amino acid analysis, assuming respective M_r for α, β, and holochrome of 6106, 5142, and 11 248 (Table I). This experiment also indicates a 1:1 stoichiometry. Note that the amino acid analysis indicates a 1:1 stoichiometry irrespective of the exact M_r values adopted for α and β, provided that the ratio (based on electrophoretic mobility) of M_r^{α}/M_r^{β} is kept at 7600/6400.

The Bchl:spirilloxanthin mole ratio of 2:1 that we find in the holochrome is the same as that reported by Cogdell & Thornber (1979) for an otherwise undescribed preparation from *Rs. rubrum*. In our preparations, we find that the mass of protein that is associated with 2 mol of Bchl and 1 mol of spirilloxanthin is 23 000. In other words, 23 000 is the minimal M_r of the preparation. We suggest, therefore, that the minimal holochrome oligomer has an $\alpha_2\beta_2$ structure. This oligomer may well aggregate to form larger structures such as those reported by Sauer & Austin (1978).

For each milligram of protein, the preparation contains 0.1 mg of pigments and 0.1 mg of phospholipids. The phospholipid content is about 50% of that reported by Cuendet & Zuber (1977) and by Sauer & Austin (1978) for analogous preparations from *Rs. rubrum* G9 and S1, respectively. This may be due to different culture conditions or, more likely, to different preparation procedures.

Besides 2 molecules of Bchl and 1 molecule of spirilloxanthin, the oligomer carries 1 molecule of phosphatidylethanolamine plus, apparently, 1 molecule of either phosphatidylglycerol or diphosphatidylglycerol. The complex certainly has binding sites for the noncovalent binding of the pigments. It may quite possibly also have binding sites for these phospholipids. However, more work will be required before we can exclude the possibility that they are undistinct membrane fragments carried over with the holochrome.

From the pigment content of a purified sample of known absorbance, a molar extinction coefficient for the holochrome can be deduced if one assumes that each mole contains 2 mol of Bchl and 1 mol of spirilloxanthin. Because of its potential usefulness, we quote here the value that we obtained with well-dialyzed preparations: $\epsilon_{880} = 125\ mM^{-1}\cdot cm^{-1}$. However, we feel that this value should be used with caution because of its evident dependence on the state of the preparation. As shown most clearly immediately after elution from DEAE-cellulose with LDAO (Figure 2B), the preparation displays a peak at 770 nm. Upon extensive dialysis against buffer, this 770-nm peak undergoes marked hypochromism accompanied with hyperchromism of the 356- and 880-nm bands. This is suggestive of Bchl reversibly leaving its fixation site during elution with high concentrations of LDAO and salt. Under these conditions, the interaction between the Bchl molecules (Sauer & Austin, 1978) may possibly be disrupted with a concomitant diminution of the extinction coefficients at 365 and at 880 nm. This phenomenon is reminiscent of the reversible binding of Bchl observed in the antenna-holochrome B800–B850 of *Rp. sphaeroides* (Clayton & Clayton, 1981). An alternative explanation might be that the 770-nm band disappears because a small amount of monomeric Bchl—representing at most 10% of total Bchl—is released from the protein and dialyzed out as mixed Bchl–detergent micelles. At the same time, the extinction coefficient at 880 nm would increase as detergent is removed from the protein. We cannot decide at present which hypothesis is correct.

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