Photoreaction Center of Photosynthetic Bacteria. 1. Further Chemical Characterization of the Photoreaction Center from *Rhodospirillum rubrum*†

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ABSTRACT: The photoreaction center from *Rhodospirillum rubrum* contains about 90% protein, 6% pigment, mere traces of lipids, and no cytochromes. It also contains at least 1 mol of ubiquinone and 1 iron atom per mol. Its three-component polypeptide chains were isolated by preparative electrophoresis, and their molar stoichiometry was established as 1:1:1. The amino acid composition of the photoreaction center from strain S1 and from its subunits is reported. The protein as a whole contains about 65% nonpolar residues, and the degree of hydrophobicity of its subunits is $\alpha < \beta < \gamma$. The minimal molecular weight based on the extinction coefficient and on the amino acid content is 90000. This corresponds to a half-cystine mole number of 6.

The first preparations of the photoreaction center from *Rhodospirillum* rubrum were obtained from the carotenoid-less strain G9 through the use of Triton X-100. Although they were stable and devoid of antenna bacteriochlorophyll, such preparations contained 10^6 g of protein and 1.8 mol of cytochrome per mol of P_{870} (Gingras & Jolchine, 1969).

The photoreaction center could not be extracted from wild-type strain S1 by the same procedure. Well purified preparations from this strain were obtained through the use of another detergent, dodecyldimethylamine *N*-oxide. These preparations were not only free of antenna bacteriochlorophyll and of cytochromes but also had a low minimal molecular weight of 87 000 ± 4000 [recalculated from the data of Noël et al. (1972) by using an ϵ_{868} of 143 mM^{-1} cm^{-1} as determined by van der Rest & Gingras (1974)]. $NaDodSO_4$–polyacrylamide gel electrophoresis showed a characteristic pattern of three polypeptide bands and no apparent contaminants (Noël et al., 1972; van der Rest et al., 1974).

The preparation procedure developed for the wild type could not be directly transposed to strain G9: for instance, we could not use ammonium sulfate fractionation, as Wang & Clayton (1973) also found. Since we were interested in the function of carotenoids (Boucher et al., 1977), we needed to have pure and stable preparations from both strains. The procedures that are described in this article have performed satisfactorily for several years in our laboratory.

The main purpose of the present work was to characterize these preparations more fully. This would facilitate comparison with photoreaction centers from other species. The main points that were studied were the overall protein, pigment, and lipid composition as well as the iron and ubiquinone contents. Amino acid composition, minimal molecular weight, and the stoichiometry of the polypeptide chains were also determined. Several of these results form a basis for the interpretation of a companion work on the size and on the quaternary structure of this chromoprotein (Vadeboncoeur et al., 1979).

Materials and Methods

Organisms and Growth Conditions. Rs. rubrum strain S1 (ATCC 11170) and carotenoid-less strain G9 were grown semianaerobically at 32 °C in the synthetic medium of Cohen-Bazire et al. (1957). For the cultivation of strain G9, the medium contained additionally 0.1% Bacto-Peptone (Difco Laboratories) and 0.1% Biocert yeast extract (Fisher Scientific Co). The cultures were carried out in 12-L cylindrical flasks (23-cm diameter) and illuminated by two 150-W photoflood lamps at a distance of 30 cm from the center of the flask. The cells were harvested after 5 days (late log phase) of illumination and washed with 0.5 volume of cold 50 mM Tris-HCl buffer (pH 7.6). The G9 cultures were left in the dark at 32 °C for 48 h prior to illumination.

Extraction of the 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 the Photoreaction Center. A modification of the method of Noël et al. (1972) was used for the isolation of the photoreaction center from the wild-type strain. All steps were carried out in darkness. The chromatophores (final A_{880} = 37.5) were suspended in 50 mM phosphate buffer (pH 7.0)–0.25% w/v LDAO[1] at 4 °C for 1 h. The LDAO concentration was then brought to 0.1% by dilution with the same buffer, and the preparation was centrifuged for 1.5 h at 105000*g*. The supernatant was carefully pipetted out and the pellet discarded. The supernatant was equilibrated at 20 °C and subjected to ammonium sulfate fractionation between 35 and 45% saturation. Although the original procedure called for the use of a saturated ammonium sulfate solution, addition of the solid salt (21 and 28% w/v) was preferred here since it leads to smaller final volumes and to higher yields. First, solid $(NH_4)_2SO_4$ (21% w/v) was added slowly with constant agitation of the solution, and the solution was allowed to stand for 30 min at 20 °C and centrifuged at 4 °C for an additional 30 min at 10000*g*. The $(NH_4)_2SO_4$ concentration was then increased to 28% w/v, and the solution was allowed to stand for 30 min at 20 °C and centrifuged as above. This operation

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[1] Abbreviations used: BSA, bovine serum albumin; DEAE, diethylaminoethyl; LDAO, dodecyldimethylamine *N*-oxide; $NaDodSO_4$, sodium dodecyl sulfate; PPO, 2,5-diphenyloxazole; dimethyl-POPOP, 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene.

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yielded a precipitate which tended to float, probably due to the presence of LDAO, and which was collected on filter paper (Schleicher & Schuell No. 595). The precipitate was resuspended in a minimal volume of 10 mM Tris-HCl (pH 8.0) and dialyzed for 20 h against 10 mM Tris-HCl (pH 8.0)–0.03% LDAO. About 20 mg of protein was then applied to a 16 × 300 mm column of DEAE-cellulose equilibrated with 10 mM Tris-HCl (pH 8.0)–0.05% LDAO. The column was washed successively with 2 volumes of this buffer and with at least 6 volumes of 25 mM NaCl–10 mM Tris-HCl (pH 8.0)–0.05% LDAO; the photoreaction center was eluted with 125 mM NaCl–10 mM Tris-HCl (pH 8.0)–0.05% LDAO. After elution, the preparation was immediately filtered on a column of Sephadex G-25 and dialyzed against 10 mM Tris-HCl (pH 8.0)–0.03% LDAO in order to prevent instability of the preparation which arises through the combined effect of high concentrations of salt and detergent. The yield of this preparation procedure was typically from 30 to 50%.

The following method was used with strain G9. All steps were carried out in centrifuge tubes compatible with the rotor, Model 35 (Beckman Instrument, Inc.). To each tube was added 8.0 mL of chromatophores (A_{870} = 75) in 50 mM sodium phosphate (pH 7.0) and 8.0 mL of 0.55% (w/v) LDAO in deionized water. After standing 1 h on ice, each tube received 32 mL of 60 mM phosphate (pH 7.0)–0.1% (w/v) Triton X-100 and was then centrifuged for 90 min at 90000*g*. The supernatants were collected by pipet and were pooled and dialyzed against 4 L of 10 mM Tris-HCl (pH 7.5) for 4 h. This solution ($A_{865} \simeq 0.4$) was then mixed with preequilibrated DEAE-Sephadex A-50 (equivalent to 1.2 g of dry Sephadex A-50 per 100 mL of supernatant of A_{870} = 0.4). After 10 min, the suspension was filtered on Whatman No. 4 paper. The green cake was resuspended in 100 mL of 10 mM Tris-HCl (pH 7.5)–100 mM NaCl–0.1% (w/v) Triton X-100. After 10 min of contact, the suspension was filtered as above. The green cake was then resuspended in a minimal volume (20–40 mL) of 10 mM Tris-HCl (pH 7.5)–1 M NaCl–0.1% (w/v) Triton X-100. The blue-green eluate was collected by filtration as above and dialyzed overnight in 10 mM Tris-HCl (pH 7.5). The preparation was next purified by two successive chromatographic separations on DEAE-cellulose. The resin in a 16 × 200 mm column was first equilibrated with 500 mL of 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100. The preparation was adsorbed on this column and washed with 100 mL of the same buffer containing 20 mM NaCl–0.1% Triton X-100. This step removed varying amounts of material with a near-infrared absorption maximum at 755 nm. After complete elution of this contaminant, the photoreaction center was eluted with 10 mM Tris-HCl (pH 7.5)–0.1% (w/v) Triton X-100–125 mM NaCl. [Some yellowish material with an absorption peak at 408 nm remains bound at the top of the column. This material, which we designate as P_{408}, is spectrally similar to C407 recently described by Clayton & Clayton (1978).] The photoreaction center (ca. 30 mL) was immediately dialyzed overnight against 4 L of 10 mM Tris-HCl (pH 7.5). It was then applied on top of another DEAE-cellulose column equilibrated with 10 mM Tris-HCl (pH 7.5)–0.1% (w/v) Triton X-100. The column was washed as described above with 100 mL of 10 mM Tris-HCl (pH 7.5)–0.1% (w/v) Triton X-100–20 mM NaCl, and the photoreaction center eluted as a sharp band ($A_{865} \simeq$ 5) with 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100–1 M NaCl. NaCl was immediately removed by dialysis. The yield in P_{870} from the chromatophores was typically from 30 to 50%.

Absorption Spectroscopy. The absorption spectra were measured with a Cary 14R spectrophotometer at room temperature in 1.0-cm path length cells. Photochemical activity was assayed at 870 nm with the same instrument under cross illumination from a 650-W tungsten halogen lamp filtered by a Corning No. 9782 colored glass filter. Schott RG9 filters were placed in front of the photocell in the blank and in the sample compartment.

Assays. Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin (Sigma Chemicals) as the standard. Heme was determined on acetone–methanol (7:2 v/v) residues by the pyridine hemochromogen method described by Bartsch (1963).

Assay for Lipid Content. The lipids were extracted according to the method of Kates et al. (1964). The photoreaction center protein (5 mg) from strain G9 was lyophilized and suspended in 10 mL of chloroform–methanol (1:2 v/v). The solution was shaken vigorously and kept overnight at room temperature. After addition of 3 mL of water and 3 mL of chloroform and vigorous shaking, the phases were separated by low-speed centrifugation, and the chloroform was collected. The extraction was repeated 5 times. After partial evaporation in vacuo, one-third of the extract was applied on three thin-layer plates (Merck; silica gel G) for chromatography in three different solvents: (1) chloroform–acetone–methanol–acetic acid–water (50:70:10:10:5 v/v); (2) acetone–acetic acid–water (100:2:1 v/v); (3) chloroform–methanol–water (65:25:4 v/v). After development, the plates were dried and sprayed with Rhodamine 6G (Eastman Kodak Co). The lipid concentration was estimated by using known amounts of lecithin as a standard.

Iron Determination. A modification of the spectrophotometric method of Woiwod (1947) was used. To 1.0 mL of 10 mM Tris-HCl (pH 8.0)–0.1% Triton X-100 containing from 1.0 to 10 nmol of the photoreaction center was added 0.1 mL of 11 N HNO_3 (Ultrex; J. T. Baker Chemical Co., Phillipsburg, NJ). After 20 min, 0.8 mL of 100 mM sodium acetate buffer (pH 4.8) containing 1% NH_2OH was added, and the pH was adjusted to 4.8 through the addition of 11 N NaOH. The solution was clarified by centrifugation for 10 min at 20000*g*. Iron was revealed with 25 μL of 10% α,α-bipyridyl in methanol. Color developed in 20 min. The assay was carried out, with proper controls, at 522 nm in a Cary 14R spectrophotometer. The calibration curve was established with $Fe(NO_3)_3 \cdot 9H_2O$. The accuracy of the method was tested with cytochrome *c*, myoglobin, and hemoglobin. The average of 16 determinations was 0.95 ± 0.04 of theoretical expectations. Assays on photoreaction center preparations containing known amounts of added $Fe(NO_3)_3$ were also according to expectations.

Ubiquinone Determination. Ubiquinone was extracted as follows. To known amounts (10–20 nmol) of the photoreaction center in 1.0 mL of 10 mM Tris-HCl (pH 8.0)–0.1% (w/v) Triton X-100 or 0.03% (w/v) LDAO was added 0.26 nmol (7.6 Bq) of [^{14}C]ubiquinone as a tracer in 5.0 μL of ethanol. The mixture was kept at 20 °C for 25 min and then pipetted into 10.0 mL of acetone–methanol (1:1 v/v) at −78 °C.[2] It was then kept for 15 min in a water bath at 22 °C under constant agitation and centrifuged at 5000*g* for 10 min. The supernatant was then extracted by successive volumes of 10.0, 8.0, and 8.0 mL of petroleum ether. The petroleum ether phase was decanted and mixed with 1.0 mL of spectroscop-

[2] Comparative experiments in which $FeCl_3$ was added (Morrison et al., 1977) or not to the acetone–methanol extracts showed no significant effect of this addition on ubiquinone determination.

ic-grade ethanol, and the mixture was evaporated to ca. 2.0 mL in vacuo. The residue was quantitatively transferred to a small test tube and was evaporated further to ca. 0.1 mL under a stream of N_2 gas.

The sample was next spotted on a plate coated with silica gel (Merck; HR ultrapure) (0.5 mm thick; 20 cm long) that had been prewashed with ethanol. A sample of synthetic ubiquinone-6 (Sigma Chemicals) was run in parallel on the same plate for approximate R_f determination ($R_f \simeq 0.3$). The chromatograms were developed with a mixture of petroleum ether (boiling range 60–110 °C)–ethanol, 96:4 (v/v), for photoreaction center extracts from *Rhodopseudomonas sphaeroides* and from *Rhodospirillum rubrum* strain G9 or with a mixture of petroleum ether (boiling range 60–110 °C)–acetone, 90:10 (v/v), for photoreaction center extracts from *Rs. rubrum* S1. The silica gel was scraped off the plate in a 1-cm^2 circle around the ubiquinone spot. It was collected and extracted 3 times with small volumes (2.0, 1.0, and 1.0 mL) of spectroscopic-grade ethanol. The combined extracts were cleared by centrifugation at low speed, evaporated to dryness under a stream of N_2 gas, and redissolved in 1.0 mL of spectroscopic-grade ethanol.

Ubiquinone was assayed by difference spectroscopy between its reduced and oxidized forms with potassium borohydride as a reducing agent using an ϵ_{275}^{red-ox} of 12.5 mM^{-1} cm^{-1} (Crane & Barr, 1971). The ubiquinone contents reported here were based on this assay corrected for the overall loss of tracer [^{14}C]ubiquinone. Radioactivity was measured in a Packard Tricarb Model 3320 scintillation counter. The scintillating solutions contained 4 g of PPO and 0.1 g of dimethyl-POPOP per 1 L of toluene. Appropriate corrections were made for counting yield and background noise.

Biosynthesis of [^{14}C]Ubiquinone. [^{14}C]Ubiquinone was obtained by growing *Rs. rubrum* (strain S1) in the presence of *p*-hydroxybenzoic acid labeled uniformly in the ring with ^{14}C (a gift of Dr. Paul A. Loach, Northwestern University). In this experiment, 13.3×10^5 Bq of *p*-hydroxybenzoic acid in 1.0 mL of ethanol was added to 1 L of the growth medium at the time of inoculation of the culture. The bacteria were grown as described above and collected after 5 days of culture. The cells were thoroughly washed with water, and ubiquinone was extracted by the method of Takamiya & Takamiya (1969) except that $FeCl_3$ was added to the water–acetone–methanol mixture prior to partioning with petroleum ether (Morrison et al., 1977). The petroleum ether extract was streaked on two plates of silica gel (20 × 20 cm) and chromatographed as described above. A total of 4.3 μmol (12.2×10^4 Bq) of [^{14}C]ubiquinone was thus recovered.

The purity of this material was tested by thin-layer chromatography. Radioactive ubiquinone (50 Bq) was spotted on silica gel plates (Merck; HR ultrapure) and developed both in a mixture of petroleum ether–ethanol (96:6 v/v) in a mixture of benzene–chloroform (1:1 v/v) according to Morrison et al. (1977). The plate was divided into 11 bands (1.5 cm) which were scraped into scintillation vials. Ninety-five percent of the radioactivity was recovered as one spot with an R_f value of 0.3 in either system.

Amino Acid Analysis. Protein samples were precipitated with 90% (final volume) cold acetone and lyophilized. All these operations were carried out in the same tube. To each sample was added 0.5 mL of HCl (constant boiling; Pierce Chemical Co.) and 1 drop of phenol. The tubes were then sealed under vacuum and kept at 110 ± 3 °C for 24 h. The hydrolysates were then diluted with deionized water, filtered on No. 1300 Millipore filters, and dried under a gentle stream of N_2 gas. The hydrolysates were then dissolved in 0.2 M sodium citrate (pH 2.2) buffer to a final concentration equivalent to 1 mg/mL protein. The amino acid analyses were performed with a Durrum Model D500 amino acid analyzer.

Cysteine was determined on HCl hydrolysates after oxidation with performic acid according to Moore (1963) with omission of HBr. In these cases, samples of 200 μg of protein were analyzed. For determination of tryptophan, the samples were hydrolyzed for 50 h at 110 ± 3 °C in 5 N NaOH. After hydrolysis, the samples were neutralized with HCl and filtered and dried as indicated above.

Analytical Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed with slight modifications of a method described before (Noël et al., 1972). The reservoir and gel buffer was 10 mM Tris–5 mM acetate (pH 8.15) containing 0.1% sodium dodecyl sulfate. The gel contained 10% acrylamide and 0.28% *N,N'*-methylenebis(acrylamide). Before applying the samples to the gels, the latter were submitted to preelectrophoresis for 2 h at a current density of 4 mA/tube. The reservoir buffer was replenished before electrophoresis proper. The samples were incubated in the Tris–acetate buffer containing 1% sodium dodecyl sulfate for 30 min at 40 °C and applied immediately onto the gels. Electrophoresis was carried out for 2.5 h at a current density of 2.5 mA/gel. Staining and destaining were according to Fairbanks et al. (1971). Molecular weight markers were bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome *c* (all from Sigma Chemicals). After destaining, the gels were scanned at 550 nm with a Zeiss spectrophotometer equipped with a gel scanning attachment and a recorder.

Preparative NaDodSO$_4$ Gel Electrophoresis. This technique was used to isolate the polypeptide subunits of the photoreaction center. Electrophoresis was carried out in a homemade glass column with an upper portion (12 cm long; 3.5 cm i.d.) which served as the cathode compartment and a lower portion (25 cm long; 2.5 cm i.d.) which contained the polyacrylamide gel. The column plunged in a 1.0-L beaker which served as the anode compartment. The cathode compartment was connected to a 1.0-L reservoir, and the buffer was continuously circulated from one to the other by means of a persistaltic pump. The electrodes were made of platinum wire. Electrophoresis was carried out at room temperature.

The gels (a 4 cm long stacking gel and a 20 cm long separating gel) and buffers were prepared according to Laemmli (1970). The protein (4 mg/mL) sample was first incubated at 40 °C for 2 h in 10 mM Tris-HCl (pH 8.0)–2% NaDodSO$_4$–5% β-mercaptoethanol, cooled to 20 °C, mixed with sucrose (10% final concentration), and applied at the surface of the stacking gel under the electrode buffer. The electrode current was set at 6 mA, and the cathode reservoir buffer flow was 0.2 mL/min. Migration in the stacking gel took about 4 h for a sample of 4 mL. The electrode current was then brought to 12 mA, and the flow rate of the cathode reservoir buffer was brought to 1.5 mL/min. After 22 h, the fastest moving polypeptide was at about 1.5 cm from the lower end of the gel. The protein bands were easily localized on the gel because of their different refractive indexes. After completion of the electrophoresis, the polypeptide bands were cut out, and the slices were crushed with a spatula and laid on top of a gel 1.5 cm long. This gel had the same composition as the stacking gel just described and was maintained at the end of a 30 × 1.5 cm (i.d.) glass column by a piece of nylon

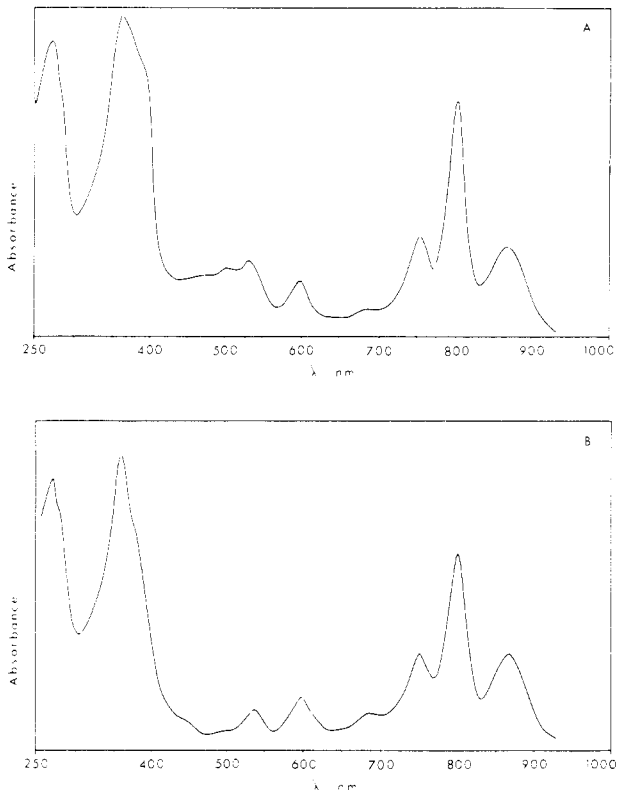

FIGURE 1: Absorption spectra of the photoreaction center from *Rs. rubrum* strain S1 (A) and strain G9 (B). Both preparations were dispersed in 10 mM Tris-HCl (pH 8.0) containing 0.03% LDAO.

stocking and a rubber band. The electrophoresis buffer was 10 mM Tris-HCl (pH 8.0)–2% $NaDodSO_4$. Electrophoresis was allowed to run for 4 h at 15 mA. The protein was collected in a dialysis bag containing 2.0 mL of the electrophoresis buffer and dialyzed for 24 h in this buffer. Before amino acid analysis, it was precipitated with 9 volumes of cold acetone and washed twice with acetone and twice with distilled water.

Results and Discussion

Figure 1 shows absorption spectra of the photoreaction center prepared from strain S1 (Figure 1A) and from strain G9 (Figure 1B). For illustrative purposes both preparations were dispersed in 10 mM Tris-HCl (pH 7.5)–0.03% LDAO, although that from strain G9 is normally kept in 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100. Such preparations typically have ratios of $A_{280}/A_{365} = 0.91$. They can be kept for a few weeks in the cold without loss of photochemical activity. However, the preparation from strain G9 is more light sensitive than its wild-type counterpart which contains spirilloxanthin (Boucher et al., 1977).

Overall Composition. The two main components of these preparations are protein and detergent. The amount of detergent bound to the protein can be quite high (Vadeboncoeur et al., 1979). Because of its low rate of dialysis, Triton X-100 is almost impossible to remove entirely by dialysis. However, LDAO dialyzes more rapidly, and a long dialysis can remove most of it. The overall composition of three preparations from strain S1, initially in 0.3% LDAO, was established after an 8-day dialysis against deionized water at 4 °C. Dry weight and protein content (Lowry et al., 1951) were determined. Pigment content was calculated from the known amount of P_{870} present (van der Rest & Gingras, 1974). Protein was found to account for 90.3% and pigments for 5.8% of the dry weight. A total of 3.9% of the dry weight was unaccounted for. How much of this is due to residual LDAO is unknown.

Table I: Ubiquinone and Iron Content of Photoreaction Center Preparations from *Rs. rubrum* Strains S1 and G9 as a Function of the Number of Chromatographic Steps on DEAE-cellulose[a]

	crude prepn	no. of chromatographic steps 1	2	3	4
		Ubiquinone[b] Content			
strain S1	2.1 ± 0.3	1.8 ± 0.4	1.1 ± 0.1	1.1 ± 0.1	
strain G9	4.8 ± 2.0	2.3 ± 0.3	1.3 ± 0.1	1.2 ± 0.1	
		Iron[c] Content			
strain S1	7.0 ± 3.3	4.6 ± 2.0	2.8 ± 1.6	1.6 ± 0.7	1.0 ± 0.1
strain G9	5.7 ± 1.9	3.2 ± 2.3	1.5 ± 0.7	1.3 ± 0.3	1.0 ± 0.1
strain S1[d]	1.9	1.1	1.0	1.0	

[a] Means and standard deviations in moles of ubiquinone and in gram-atoms of iron per mole of P_{870}. [b] Analysis of five different preparations from each strain. [c] Analysis of seven different preparations from each strain. [d] Analysis of a single preparation.

The same experiment was not performed with the preparation from strain G9. In this case, however, the lipid content was analyzed by thin-layer plate chromatography as described under Materials and Methods. Only a faint spot of lipid material was revealed after spraying with Rhodamine 6G. The area of this spot was smaller than that due to 20 μg of lecithin. Since the extract applied to each thin-layer plate came from 1.66 mg of photoreaction center protein, we estimate this unknown material to represent less than 12 μg/mg of protein (1.2%). We did not further investigate the nature of this material.

Cytochrome Content. As reported before (Noël et al., 1972), no heme could be detected in the photoreaction center preparations from strain S1. This also holds true for the preparation from strain G9.

Ubiquinone Content. Previous experiments were thought to indicate that ubiquinone could be almost entirely removed from the preparations along with the extensive exchange of LDAO which occurs during dialysis (Noël et al., 1972). In the present work, the bound detergent was allowed to exchange with the free detergent by adsorbing the preparation on a DEAE-cellulose column and washing it with a large volume of buffer containing either LDAO or Triton X-100 (Materials and Methods). The ubiquinone content was followed as a function of the number of such chromatographic steps. This was determined by extraction of the photoreaction center preparation with acetone–methanol, followed by thin-layer chromatography of the extracts in order to eliminate the pigments, detergents, or other contaminants which were found to interfere with the spectroscopic assay of ubiquinone. [^{14}C]Ubiquinone (UQ) was added in tracer amounts (maximum molar ratio exogenous/endogenous UQ = 3%) to the preparations before their extraction with acetone–methanol to allow calculation of the overall recovery of ubiquinone. The average recovery of ubiquinone after elution from the chromatogram was 62 ± 9% (35 experiments).

Table I shows the ubiquinone/P_{870} molar ratio for the preparations from strain S1 and from strain G9. Crude preparations from strain S1 contained close to 2 mol of ubiquinone per mol of P_{870} with a small standard deviation whereas crude preparations from strain G9 contained a significantly higher level of ubiquinone with a large standard deviation. This difference is probably a reflection of the preparation procedures employed. As the preparations underwent repeated chromatography, their ubiquinone/P_{870} ratio decreased to an asymptotic value of 1.0. The discrepancy with earlier results is attributed to the greater selectivity and sensitivity of the assay method used in the present work. Only ubiquinone could be detected in this manner. Rhodoquinone,

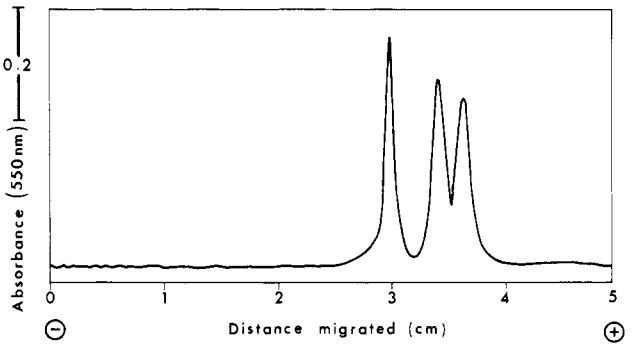

FIGURE 2: Electrophoretogram of the photoreaction center from *Rs. rubrum* strain G9 after staining with Coomassie blue. Polyacrylamide gel electrophoresis was in the presence of $NaDodSO_4$.

a component of the chromatophores, was not detected in the isolated photoreaction center.

The photoreaction center prepared from wild-type (strain 2.4.1) *Rp. sphaeroides* behaved similarly (not shown). Moreover, when we treated these preparations with 10 mM *o*-phenanthroline in 10 mM Tris-HCl (pH 8.0)–4% LDAO, as described by Okamura et al. (1974), they lost their reversible P_{870} activity simultaneously with ubiquinone. The same result was obtained when LDAO was replaced with 1% Triton X-100. P_{870} activity was restored upon addition of ubiquinone. However, the preparations from *Rs. rubrum* either were irreversibly denatured by such treatments (in 4% LDAO) or lost neither their firmly bound ubiquinone nor their P_{870} activity (in 1% Triton X-100). In spite of this, our results are felt to be consistent with an obligatory role for ubiquinone in the photoreaction center from *Rs. rubrum* such as demonstrated in *Rp. sphaeroides* by the experiments of Cogdell et al. (1974) and of Okamura et al. (1975). An obligatory role for ubiquinone in the primary photochemistry of chromatophores from *Rs. rubrum* was also strongly indicated by the experiments of Morrison et al. (1977). However, our results do not support the ubiquinone/P_{870} ratio of 0.5 found by these authors but confirm the value of 1.0 reported by Okamura et al. (1975) for the photoreaction center from *Rp. sphaeroides*.

Iron Content. The iron content of preparations from both strain S1 and strain G9 varied rather widely from culture to culture. As shown in Table I, the iron/P_{870} molar ratio decreased to a limiting value of 1.0 as the preparations underwent repeated chromatography on DEAE-cellulose. This is perhaps better illustrated (Table I) by a single preparation from strain S1 which had a low iron content from the beginning. In this case, the molar ratio remained at unity for the last three chromatographic steps.

Polyacrylamide Gel Electrophoresis. Figure 2 shows the scan of a polyacrylamide gel after electrophoresis of the photoreaction center from strain G9 in the presence of $NaDodSO_4$ and staining with Coomassie blue. The results are identical with those obtained with preparations from strain S1 (not shown). Both preparations contain only the three characteristic polypeptide bands. The apparent molecular weights of the polypeptides from the G9 photoreaction center were found to be 30 500 for α, 24 500 for β, and 21 000 for γ in close agreement with the results of Noël et al. (1972) for the photoreaction center of strain S1 and with those of Okamura et al. (1974) for an analogous preparation from strain G9.

Isolation of the Polypeptide Subunits. The subunits of the photoreaction center from strain S1 were isolated by preparative polyacrylamide gel electrophoresis in the presence of

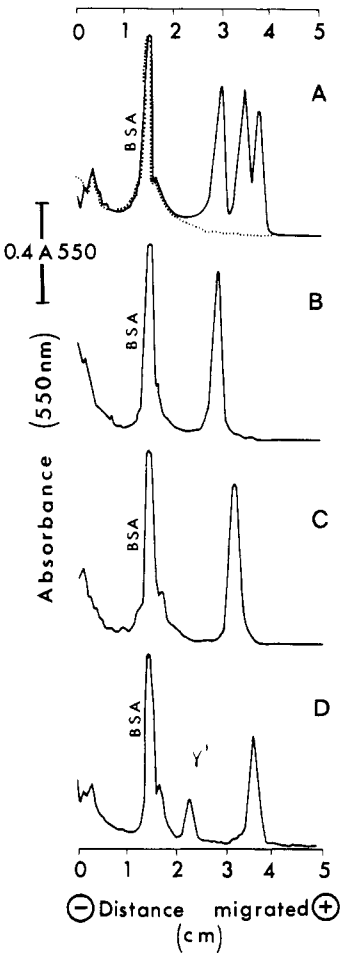

FIGURE 3: Electrophoretograms of the photoreaction center from *Rs. rubrum* strain S1 and of its isolated subunits. Polyacrylamide gel electrophoresis was in the presence of $NaDodSO_4$. Bovine serum albumin (BSA) was used as an internal marker. Gels were stained with Coomassie blue. (A) Dotted line: BSA alone. Continuous line: photoreaction center and BSA. (B) Isolated chain α and BSA. (C) Isolated chain β and BSA. (D) Isolated chain γ and BSA. γ' corresponds to a dimer of chain γ.

$NaDodSO_4$ as described under Materials and Methods. Typically, one run yielded ~2.5 mg of each polypeptide.

Before pursuing analytical work on these polypeptides, their purity was checked by analytical polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$. Since the starting material was pure and since only portions of the preparative gels corresponding to polypeptides α, β, and γ were eluted, this electrophoresis confirmed the absence of cross contamination among the subunits. Figure 3 shows an optical scan of such analytical gels. For alignment purposes bovine serum albumin was introduced as a marker with the purified polypeptides. The dotted line on Figure 3A corresponds to an electrophoretogram of BSA alone. The peaks corresponding to polypeptides α and β are shown to be free of significant contamination. Under our experimental conditions, isolated polypeptide γ had a strong tendency to aggregate (see the peak labeled γ' in Figure 3D). In separate experiments, polypeptide γ was sometimes found as a monomer (M_r ~21 000), dimer (M_r ~42 000), trimer (M_r ~63 000), and even as higher aggregates. When the monomer band was cut out, eluted, and reelectrophoresed, nearly all of the material was found at the top of the gel.

Stoichiometry of the Polypeptide Subunits. It has been suggested by Clayton & Haselkorn (1972) and demonstrated by Steiner et al. (1974) that the three polypeptide subunits

Table II: Amino Acid Composition of the Photoreaction Center from *Rs. rubrum* (Strain S1)

amino acid	subunit α[a] mol %	subunit α[a] no. of residues	subunit β[a] mol %	subunit β[a] no. of residues	subunit γ[a] mol %	subunit γ[a] no. of residues	sum of residues in subunits α, β, and γ	photoreaction center[b] mol %	photoreaction center[b] no. of residues
Asp	8.4	27	5.0	13	5.4	13	53	6.4	53
Thr	6.5	21	5.6	15	5.7	14	50	6.1	51
Ser	6.4	21	5.5	14	5.8	14	49	5.5	46
Glu	7.2	24	7.2	19	4.8	11	54	6.5	54
Pro	5.5	18	4.6	12	4.8	11	41	5.2	43
Gly	10.1	33	11.4	30	12.3	29	92	10.4	86
Ala	12.4	40	10.4	27	8.6	21	88	10.7	89
$^1/_2$-Cys								0.75	6
Val	7.2	23	5.3	14	7.0	17	54	6.4	53
Met	1.6	5	2.6	7	2.2	5	17	2.2	18
Ile	5.9	19	6.2	16	7.3	17	52	6.5	54
Leu	8.0	26	10.8	28	10.8	26	80	10.0	83
Tyr	3.7	12	3.0	8	4.7	11	31	3.7	31
Phe	3.2	10	9.5	25	7.2	17	52	6.6	55
His	0.7	2	2.3	6	3.1	7	15	2.1	17
Lys	5.1	17	2.1	5	2.4	6	28	3.2	27
Arg	6.4	21	4.8	13	2.7	6	40	4.8	40
Trp	1.3	4	3.4	9	4.7	11	24	3.0	25
polarity	0.407		0.325		0.299			0.346	
$H\phi_{av}$ (cal/residue)	1128		1295		1359			1266	

[a] Average of five analyses. The number of residues was calculated on the basis of the following molecular weights: 36 120 for α; 29 010 for β; 24 870 for γ (see the text). [b] Average of 10 analyses. The number of residues was calculated on the basis of a protein molecular weight of 90 000.

of the photoreaction center isolated from *Rp. sphaeroides* (strain R26) are in a 1:1:1 ratio. This has not been demonstrated so far for the photoreaction center from *Rs. rubrum*. Such a verification was important inasmuch as we show elsewhere that the oligomer obtained from the latter organism is smaller than that from *Rp. sphaeroides* (Vadeboncoeur et al., 1979).

Our method was based on the extent of staining of each subunit by Coomassie blue as determined by optical scanning of the polyacrylamide gels after electrophoresis in the presence of $NaDodSO_4$. In order for this method to be quantitative, the specific intensity of staining had to be determined for each one of the isolated subunits. Increasing amounts of the isolated subunits were therefore applied to polyacrylamide gels, submitted to electrophoresis, and stained with Coomassie blue. After destaining, the gels were scanned, and the relative area under each peak was obtaind by weighing the paper delineated by each curve on a photocopy. Background absorbance of the gel was compensated for by taking as the base line regions of the gel without polypeptide bands.

For polypeptide γ, the sum of the areas under the different states of aggregation was used. Figure 4 shows the calibration curves obtained in this manner. For subsequent work, only the linear portion of these curves was used. The corresponding slopes expressed in relative area per milligram of protein were found to be 0.97 for α, 1.04 for β, and 1.83 for γ.

A similar operation was next performed on electrophoretograms of eight different photoreaction center preparations from strain S1 (from 0.06 to 0.2 nmol) in order to obtain the relative area corresponding to each polypeptide. When these values were divided by the apparent molecular weights of the subunits, the molar stoichiometry was found to be 1.0 for α, 1.0 for β, and 1.0 for γ with a standard deviation of 0.05.

The same stoichiometry is obtained for preparations from strain G9, assuming the same relative amounts of Coomassie blue bound per milligram of subunit protein as found in preparations from strain S1.

Amino Acid Composition. The photoreaction center was subjected to 24, 48, and 72-h acid hydrolyses. From this experiment were derived the following correction factors which were applied to subsequent 24-h hydrolysates: Ser, 1.10; Thr, 1.03; Val, 1.10; Ile, 1.07. A correction factor of 1.06 was applied to Cys (Moore, 1963).

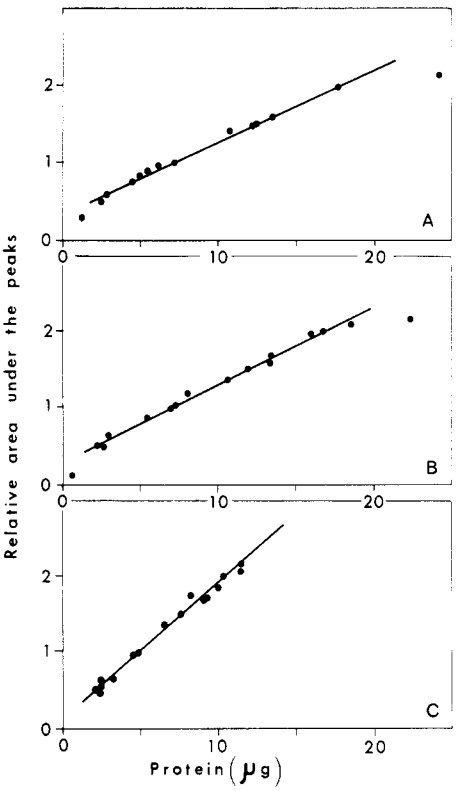

FIGURE 4: Relative intensity of staining by Coomassie blue of each of the three polypeptide chains as a function of the amounts of protein applied to $NaDodSO_4$–polyacrylamide gels. The intensity of staining was determined from the relative area under the peaks of electrophoretograms after optical scanning at 550 nm. Protein was assayed by the method of Lowry et al. (1951). (A) Subunit α. (B) Subunit β. (C) Subunit γ.

Such analyses were performed on known molar quantities of the photoreaction center as determined by the absorbance at 868 nm using an ϵ_{868} of 143 mM^{-1} cm^{-1} (van der Rest &

Gingras, 1974). Summation of the molar contents of all the amino acids led to minimal molecular weight values of 90 000 and 86 000 for the preparations from strains S1 and G9, respectively. The small difference of 4.5% is not considered significant.

Table II gives the mole percent amino acid composition and the mole number of amino acid residues for the photoreaction center from strain S1 and for its subunits. The composition of the preparation from strain G9 is indistinguishable within experimental error and is in good agreement with the data of Steiner et al. (1974). The mole number of amino acid residues of each subunit was calculated from the relationship $N_a = (M^{\%}{}_a M_{r(e)} 90\,000)/\bar{M}_{r(r)} 76\,000)$. N_a is the number of residues of a given amino acid in the subunit, $M^{\%}{}_a$ is the mole percent content of this residue, $M_{r(e)}$ is the apparent molecular weight of the polypeptide as determined by gel electrophoresis in the presence of $NaDodSO_4$, $\bar{M}_{r(r)}$ is the average molecular weight of all the residues of this polypeptide, 90 000 is the minimal molecular weight of the photoreaction center, and 76 000 is the sum of the $M_{r(e)}$ of the three polypeptide subunits. In this calculation, the apparent molecular weight of each subunit is assumed to be too low by a factor of 90 000/76 000, the ratio of minimal to apparent molecular weight of the whole protein. The difference between apparent and minimal molecular weight of the photoreaction center as a whole is probably explained by the greater affinity of $NaDodSO_4$ for the protein than for proteins used as molecular weight markers (Vadeboncoeur et al., 1979).

As can be seen from Table II, the photoreaction center contains a high proportion of hydrophobic amino acids. Its amino acid content, as calculated from the sum of the amino acid residues found in the subunits, assuming a 1:1:1 molar ratio, is in good agreement with its experimentally determined composition.

A more quantitative expression of the polarity of a protein may be based on the definition of Capaldi & Vanderkooi (1972): it is the sum of the residue mole percentage of Asp, Glu, Lys, Ser, Thr, Arg, and His. "Polarity" was calculated for the three isolated subunits as well as for the oligomeric photoreaction center. A less empirical expression, the "average hydrophobicity", $H\phi_{av}$, was defined by Bigelow (1967) as the sum of the free energies of transfer from water to ethanol of each residue divided by the total number of residues. Polarity and $H\phi_{av}$ values for the protein and the three subunits are given in Table II. By these two parameters, subunit α is the most polar of the three, $\alpha < \beta < \gamma$ being the order of increasing hydrophobicity. Subunits β and γ are both substantially more hydrophobic than subunit α, as was also observed for the photoreaction center of *Rs. sphaeroides* (Steiner et al., 1974). This is in line with subunit α being more accessible from the outside of the isolated chromatophore membrane (Feher & Okamura, 1976; Zürrer et al., 1977).

To summarize, these photoreaction center preparations from *Rs. rubrum* contain 90.3% protein, 5.8% pigment, and, perhaps, traces of lipid material. Each mole equivalent of P_{870} is associated with 4 mol of bacteriochlorophyll *a*, 2 mol of bacteriopheophytin *a*, 1 mol of spirilloxanthin (the latter in strain S1), and at least 1 g-atom of iron and 1 mol of ubiquinone. The protein moiety is composed of three polypeptides in a 1:1:1 ratio. The minimal molecular weight of these preparations is about 90 000 whether protein is assayed by the method of Lowry et al. (1951) or by the sum of its individual amino acid residues. Interestingly, we find exactly 6 half-cystine residues per 87 000 g of protein. These values are nearly the same as those reported for the preparations from *Rp. sphaeroides* (Okamura et al. 1974; Steiner et al., 1974). The reported number of amino acid residues (and possibly also the stoichiometry) of the subunits may have to be revised when the true molecular weights of the subunits are known. This applies to photoreaction centers from both *Rs. rubrum* (this work) and *Rp. sphaeroides* (Okamura et al., 1974; Steiner et al., 1974).[3]

The preparations from *Rs. rubrum* and *Rp. sphaeroides* appear as basically similar on several points: their contents of pigments, iron, and ubiquinone, their minimal molecular weights, amino acid compositions, and subunit stoichiometry. Yet they are different in other respects: they show no immunological cross-reactivity, the apparent molecular weights of their protein subunits are slightly different (Okamura et al., 1974; Steiner et al., 1974), and the photoreaction center from *Rs. rubrum* will not withstand high concentrations of LDAO or of $NaDodSO_4$ in contrast to its counterpart from *Rp. sphaeroides*. It seems, moreover, that these two preparations may have different quaternary structures in solution (Vadeboncoeur et al., 1979).

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[3] According to a recent report [Rosen et al. as cited in Feher & Okamura (1978)], the molecular weight of the γ subunit is 28 000 rather than 21 000 in the photoreaction center from *Rp. sphaeroides*.

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Photoreaction Center of Photosynthetic Bacteria. 2. Size and Quaternary Structure of the Photoreaction Centers from *Rhodospirillum rubrum* Strain G9 and from *Rhodopseudomonas sphaeroides* Strain 2.4.1†

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ABSTRACT: The photoreaction center from *Rhodospirillum rubrum* strain G9 binds about 6 times as much sodium dodecyl sulfate as certain proteins commonly used as molecular weight markers for sodium dodecyl sulfate–polyacrylamide gel electrophoresis. This presumably explains the apparent discrepancy between the molecular weight of the photoreaction center determined by electrophoresis (76 000) and its minimal molecular weight (87 000). The molecular weight of the photoreaction center solubilized with Triton X-100 was determined by three different methods: conventional sedimentation equilibrium, a combination of sedimentation velocity and gel filtration measurements, and sedimentation equilibrium in H_2O and in D_2O. Each technique required a determination of the amount of bound detergent. All three methods gave molecular weight values close to 60 000. A similar molecular weight was found for the photoactive $\beta\gamma$ dimer obtained from the photoreaction center of *Rhodopseudomonas sphaeroides* strain 2.4.1 which, as a whole, had a molecular weight of 87 000. These results indicate that the photoreaction center from *Rp. sphaeroides* is an oligomer of the type $\alpha_1\beta_1\gamma_1$. In contrast, the photoreaction center from *Rs. rubrum* appears to be dissociated, in solution, into a photoactive $\beta\gamma$ dimer and a free polypeptide α.

The photoreaction center isolated from *Rhodospirillum rubrum* (strain G9) is composed of bacteriochlorophyll, bacteriopheophytin, ubiquinone, and iron associated with three polypeptide chains. The minimal molecular weight of the protein moiety is 87 000 (Vadeboncoeur et al., 1979). However, $NaDodSO_4$[1]–polyacrylamide gel electrophoresis yields apparent molecular weights for the three component chains of 30 500, 24 500, and 21 000 (α, β, and γ, respectively) and, thus, of 76 000 for their sum. Furthermore, sedimentation equilibrium and gel filtration studies of intact photoreaction centers from *Rp. sphaeroides* (strain Y) (Reiss-Husson & Jolchine, 1972) and from *Rs. rubrum* (strain S1) (Noël et al., 1972) indicate a particle weight of about 150 000. On the basis of these results, Noël et al. (1972) suggested that the photoreaction center preparation could be either a micellar complex composed of protein, membrane lipids, and detergent or a protein containing two copies of each of the three basic subunits.

We now know that the preparation from *Rs. rubrum* (strain G9) is virtually lipid free (Vadeboncoeur et al., 1979). However, the photoreaction center, being a hydrophobic membrane protein, probably binds detergent in amounts which cannot be considered negligible in any quantitative interpretation of the results obtained by the methods cited above. For this reason, we have determined the detergent content of the solubilized photoreaction center and have used methods outlined by Tanford et al. (1974) to obtain accurate information on the molecular weight and state of aggregation of this molecule of considerable biological interest.

Materials and Methods

Detergents. LDAO, Triton X-100, and $NaDodSO_4$ were from the same sources cited in the preceding article (Vadeboncoeur et al., 1979). [^{3}H]Triton X-100 ($10.36.10^6$ Bq/g) was a gift from Rohm and Haas Co.

Growth of Bacteria. Cells of *Rs. rubrum* (strain G9) and of *Rp. sphaeroides* (strain 2.4.1) were grown under conditions described in the preceding article (Vadeboncoeur et al., 1979).

Purification of Photoreaction Centers. Purification of the photoreaction center of *Rs. rubrum* strain G9 was carried out as described previously (Vadeboncoeur et al., 1979).

A combination of the methods of Noël et al. (1972) and Jolchine & Reiss-Husson (1974) was used to purify the photoreaction center of *Rp. sphaeroides*. Chromatophores

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[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; LDAO, dodecyldimethylamine *N*-oxide; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; ECTEOLA, epichlorohydrintriethanolamine.

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