Characterization of Reaction Centers from Photosynthetic Bacteria. I. Subunit Structure of the Protein Mediating the Primary Photochemistry in *Rhodopseudomonas spheroides* R-26†

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ABSTRACT: A photochemically active protein–bacteriochlorophyll complex (reaction center) was isolated from *Rhodopseudomonas spheroides* R-26 by a modification of an earlier procedure. In sodium dodecyl sulfate, the protein dissociates into three subunits that migrate in polyacrylamide gel with electrophoretic mobilities corresponding to molecular weights of 21,000, 24,000, and 28,000. The stoichiometry of the three subunits was determined to be 1:1:1. In a mixture of the detergents sodium dodecyl sulfate and *N,N*-dimethyllauryl-amine oxide, the two smaller subunits remain associated in a complex that is photochemically active at room temperature. This complex was separated from the third subunit by zonal centrifugation in a sucrose gradient. No significant amounts of carbohydrates or acid-labile sulfides were found in the reaction centers. Reaction centers were also prepared from *Rhodospirillum rubrum* G-9. Their subunit structure, optical spectrum, and electrophoretic mobilities were similar to those obtained from *R. spheroides* R-26 reaction centers.

The conversion of electromagnetic energy into chemical energy that can be utilized metabolically by photosynthesizing organisms is mediated in green plants by a protein–chlorophyll complex and in bacteria by a protein–bacteriochlorophyll complex. Emerson and Arnold (1932), in their pioneering work, suggested that the conversion was associated with a small fraction of the total chlorophyll molecules. Direct proof of the presence of specialized bacteriochlorophylls came from the observation of photoinduced optical absorbance changes (Duysens, 1962) that could be enhanced by the destruction of the bulk of the pigment either by photochemical (Clayton, 1963) or chemical (Loach *et al.*, 1963) means.

The particles containing these specialized bacteriochlorophylls, together with other chemical moieties that make up the photochemical machinery, are called *reaction centers*[1] (Clayton, 1963). They have played and are continuing to play an important role in the study of the primary photochemical processes in photosynthesis. These reaction center units in bacteria are located in the cell membrane. They have been isolated from a crude membrane fraction, the chromatophores, by a variety of procedures[2] (Reed and Clayton, 1968; Gingras and Jolchine, 1969; Thornber, 1970; Feher, 1971; Clayton and Wang, 1971; Reiss-Husson and Jolchine, 1972; Noël *et al.*, 1972). The size and composition of the reaction centers vary greatly depending on the procedures employed in their preparation. Consequently, the "reaction center" unit is not defined in absolute terms but only with respect to the particular preparation. It seems clear, however, that there must be a well-defined *minimum size unit* that still retains photochemical activity. Accordingly, we have undertaken to isolate this unit (*i.e.*, the "primary photosynthetic protein") and to characterize its physical and biochemical properties.

The minimum size of the "active unit" may depend on the definition of activity. In this work, we define photochemical activity as the reversible bleaching of the long-wavelength absorption band (called P_{865}) and the concomitant appearance of an electron paramagnetic resonance signal at an electronic g value of 2.0026. This definition, however, must be refined depending on whether the activity measurements are made at room temperature or at cryogenic temperatures. This distinction is necessary since the above criteria for activity are properties of the primary donor molecule only and are not dependent on the nature of the primary electron acceptor. At cryogenic temperatures, it is probable that the electron donor and acceptor are frozen in their physiological configuration. In this case, the kinetic behavior of the bleaching and recovery processes in isolated reaction centers should be the same as that in intact bacteria. This has, indeed, been shown to be the case (McElroy *et al.*, 1969, 1974). At room temperature, however, it is possible that the physiological acceptor is replaced by another (exogenous) acceptor (Feher *et al.*, 1972). In this event, the kinetic behavior at low temperatures would probably differ from that of the physiological donor–acceptor pair; in the limiting case such a complex might be inactive at cryogenic temperatures although retaining activity at room temperature. Accordingly, the minimum size unit required for activity with proper kinetics at cryogenic temperatures will probably contain the physiological donor–acceptor pair, and may be larger than the unit required for activity at room temperature, which need contain only the physiological donor.

The present study is concerned primarily with reaction centers prepared with the detergent LDAO[3] from the blue-

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‡ Recipient of National Institutes of Health Postdoctoral Fellowship No. GM-50549.

[1] Particles containing all of the chlorophylls are called photoreceptor units (Loach *et al.*, 1970).

[2] We have cited only a few representative procedures. Reaction centers obtained by different procedures have been reviewed by Sauer (1974).

[3] Abbreviations used are: LDAO, *N,N*-dimethyllaurylamine oxide; EDTA, ethylenediaminetetraacetic acid; TL buffer, 10 mM Tris-Cl (pH 8.0)–0.1% LDAO–1 mM EDTA; $A_{\lambda}^{1\text{ cm}}$, optical absorbance at wavelength λ for 1-cm path length; $OD V_{\lambda}$, the absorbance at wavelength λ (nanometers) in a 1-cm path length times the volume (milliliters) of the solution (it reflects the total amount of material).

green (carotenoidless) mutant of the photosynthetic bacterium, *Rhodopseudomonas spheroides* R-26. It has been shown in earlier work that these reaction centers are composed of protein and a number of prosthetic groups including bacteriochlorophyll, bacteriopheophytin, iron, and ubiquinone (Feher, 1971; Reed and Peters, 1972; Feher *et al.*, 1972; Straley *et al.*, 1973). In the present work, we describe some properties of the protein component of the reaction centers, in particular, its subunit structure and the stoichiometry of the subunits. In the accompanying paper (Steiner *et al.*, 1974), the amino acid composition of the reaction center protein and its subunits is reported.

In order to determine to what extent the physical and chemical characteristics of the reaction centers from *R. spheroides* are applicable to other organisms, we compared the subunit structure of reaction centers from *R. spheroides* with those obtained from the carotenoidless mutant of *Rhodospirillum rubrum* G-9.

Materials and Methods

Growth of Bacteria. Plates of 2% Bacto-agar in modified Hutner medium (DeKlerk *et al.*, 1965) were streaked with cells of *Rhodopseudomonas spheroides* R-26 (grown from an inoculum obtained from R. K. Clayton) or *Rhodospirillum rubrum* G-9 (supplied by R. G. Bartsch). The plates were incubated aerobically in the dark at 30° until single (green) colonies appeared. Screw-capped tubes (15 ml) containing modified Hutner medium were then inoculated with the single colonies. These cultures were grown anaerobically at 30° for 1 day in the dark (to consume oxygen in the solution) and for 8–10 days at a light intensity of about 1 mW/cm^2 (40-W showcase tungsten lamps, G. E. 40T8) as measured with a Model 65 radiometer (Yellow Springs Instruments). When the solutions reached an absorbance of $A^{1\,cm}_{865} = 1$, an inoculum of 5 ml was added to 1 l. of modified Hutner medium. The 1-l. bottles were kept in the dark for 1 day at 30° and then in a water bath at 30° illuminated with a light intensity of 1 mW/cm^2. After 3–4 days, cells were harvested by centrifugation and either used immediately or lyophilized and stored at 4°.

To prepare radiolabeled reaction centers, the growth medium was modified. For incorporation of ^{14}C-labeled amino acids, the usual sources of amino acids were excluded (yeast extract, peptone, and monosodium glutamate) and 100 μCi of mixed ^{14}C-labeled L-amino acids (International Chemical and Nuclear Corp.; specific activities of amino acids, 1–2 mCi/mg) were used per liter of medium. The specific activity of purified reaction centers containing ^{14}C was 2×10^4 cpm/ODV_{802}.[3] For incorporation of ^{59}Fe, the iron content of the medium was reduced to less than 0.1 ppm (w/w) of iron by leaving out the yeast extract and the inorganic iron source. To this medium, 150 μCi of ^{59}Fe (International Chemical and Nuclear Corp., sp act., 30 mCi/mg) and 0.5 ppm (w/w) of nonradioactive inorganic iron were added per liter of medium. The specific activity of purified reaction centers containing ^{59}Fe was 5×10^4 cpm/ODV_{802}.

Purification of Reaction Centers. R. spheroides R-26. A modification of a purification procedure described previously was used (Feher, 1971). Crude reaction centers from *R. spheroides* R-26 were extracted from disrupted bacteria with the nonionic detergent LDAO (Onyx Chemical Co., Jersey City, N. J.) (see steps 1–6, Feher, 1971). All further operations were performed at room temperature unless otherwise stated. The floating pellet resulting from ammonium sulfate precipitation contained the crude reaction centers. These were resuspended in 10 mM Tris-Cl (pH 8.0) buffer to give $A^{1\,cm}_{802} = 5$. The solution was stirred slowly overnight, and clarified by centrifugation at 20,000*g* for 10 min. Celite (Johns-Manville) was added to the clear supernatant to a final concentration of 1 g/50 ODV_{802}. Ammonium sulfate (53%, w/v) was added while stirring to give a final concentration of 24% (w/v). This slurry was packed into a 2.5 × 10 cm column and washed with one bed volume of 24% ammonium sulfate in TL buffer.[3] The protein was eluted with an ammonium sulfate gradient (King, 1972); 400 ml of ammonium sulfate in TL buffer with a linearly decreasing concentration from 24 to 15% (w/v) was passed through the column at a rate of 3 ml/min. The reaction centers were eluted as a peak at 20% ammonium sulfate. They were then dialyzed against the TL buffer at 4° and applied to a 2.5 × 10 cm column of DEAE-cellulose (Whatman DE-52) equilibrated in this buffer. The column was washed with the same buffer containing 0.06 M NaCl until the reaction centers began to elute; the salt concentration was then increased to 0.12 M NaCl and the bulk of the reaction centers was eluted. In order to concentrate them, the eluted reaction centers were diluted 1:3 in TL buffer and reapplied to a 0.9 × 5 cm DEAE-cellulose column equilibrated with TL buffer. Elution with 1 M NaCl–0.1% LDAO yielded a reaction center solution with an optical density ($A^{1\,cm}_{802}$) of about 10. The reaction center yield of this procedure was approximately ODV_{802} = 30 per 10 g (wet weight) of bacteria.

For storage, the reaction centers were precipitated by dialyzing them at 4° against 10 mM Tris-Cl (pH 8.0) and then distilled water. The precipitated reaction centers are stable for weeks at 4° and have been stored for months at −80° without loss of activity. They are easily solubilized in 0.1% LDAO up to concentrations corresponding to $A^{1\,cm}_{802} = 100$–200.

R. rubrum G-9. Reaction centers from the G-9 mutant of *R. rubrum* were prepared by using a modification of the procedure used for *R. spheroides*. The main modifications were lower pH and lower LDAO concentration (Nöel *et al.*, 1972). Chromatophores were suspended at 4° in 10 mM phosphate buffer (pH 7.0) at a concentration corresponding to $A^{1\,cm}_{865} = 40$. LDAO was added to a final concentration of 0.4% (w/v) and the material was centrifuged for 60 min at 250,000*g* (Beckman 60 Ti Rotor). Celite was added as described above and the reaction centers were precipitated in 22% (w/v) ammonium sulfate in 10 mM phosphate (pH 7.0)–0.04% LDAO. The Celite containing the precipitated reaction centers was packed into a 2.5 × 10 cm jacketed column maintained at 4° and washed, first with 1 vol of 23% ammonium sulfate–0.04% LDAO–10 mM phosphate (pH 7.0) and then with 2 vol of 23% ammonium sulfate–10 mM Tris-Cl (pH 8.0) (without LDAO), until no material absorbing at 280 nm was eluted. The reaction centers were eluted from the Celite with an ammonium sulfate gradient decreasing from 24 (w/v) to 0% (10 mM Tris-Cl (pH 8.0), no LDAO).

Optical Spectroscopy. The optical spectra of the reaction centers were obtained with a Cary 14R spectrophotometer. Photobleaching of the long-wavelength band was accomplished by either operating in the IR II mode of the instrument or by cross-illuminating with actinic light obtained from a 1000-W tungsten iodide lamp. The photoactivity and kinetics at low temperature (77°K) were obtained by techniques described elsewhere (McElroy *et al.*, 1974).

The gels (see next section) were scanned either before or after staining, using a gel scanner of local design. The scanner

fits into the Cary cell compartment and could be operated at any wavelength within the range of the Cary 14. The monochromatic measuring beam passes through an auxiliary slit (width = 0.32 mm) resulting in a spatial resolution of 1.0 mm within the gels.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate. The method of Shapiro *et al.* (1967) was used with minor modifications. The gels were prepared in 8 × 0.6 cm quartz tubes and, unless otherwise specified, contained 10% acrylamide (w/v), cross-linked with methylene bisacrylamide (Eastman Ortec, 0.20 g of bis/7.5 g of acrylamide). In gels assayed for radioactivity, the cross-linker was ethylene diacrylate (K & K Laboratory, 0.30 ml of EDA/7.5 g of acrylamide) (Choules and Zimm, 1965). They were polymerized by the addition of 0.05% (v/v) *N,N,N',N'*-tetramethylethylenediamine (Bio-Rad) and 0.1% (w/v) ammonium persulfate (Bio-Rad). Both the electrode reservoirs and the gels contained 0.1% sodium dodecyl sulfate–50 mM Tris-Cl (pH 8.0). In the experiments in which the stability of the reaction center unit was investigated, different amounts of LDAO (up to 0.01%) were added to the gels and buffer containing 0.1% sodium dodecyl sulfate. Gels were pre-run for 3 hr at 60 V (to decrease the absorbance at 280 nm), and the reservoir buffers were then replenished. After electrophoresis, the gels were scanned optically and then fixed overnight in 50% methanol–5% acetic acid, stained with 0.25% (w/v) Coomassie Brilliant Blue R250 (Colab) in the same solvent for 3 hr, and destained in 10% methanol–10% acetic acid.

For the determination of the molecular weight and subunit structure, the reaction center sample was prepared in the following manner: to 1 vol of reaction centers $OD^{1\,cm}_{802} = 10$, 9 vol of acetone was added to extract the bacteriochlorophyll. The sample was centrifuged, the precipitate washed twice with 10 vol of acetone, then twice with 10 vol of water, and resolubilized in 0.5% sodium dodecyl sulfate–10 mM Tris-Cl (pH 8.0) to a concentration corresponding to $A^{1\,cm}_{280} = 5$. Dithiothreitol was added to a final concentration of 1% and the sample was heated at 65° for 30 min.

The molecular weights of the subunits were obtained by comparing their electrophoretic mobilities with those of known proteins (Shapiro *et al.*, 1967). For this purpose, split gels were used (Leboy *et al.*, 1964). To one side of the gel, 10 μl of the denatured reaction center protein, as described above, was applied; to the other side a mixture of "marker proteins" (~5 μg of each) was applied. These were first denatured by heating at 65° for 30 min in 0.5% sodium dodecyl sulfate–10 mM Tris-Cl (pH 8.0)–1% dithiothreitol. The following proteins were used and the molecular weights given by Weber and Osborn (1969) were assumed as noted below: horse heart cytochrome *c*, 11,700 (Schwarz/Mann); β-lactoglobulin, 18,400 (Sigma); rabbit IgG heavy chain, 50,000, and light chain, 23,500; bovine pancreatic chymotrypsinogen A, 25,700 (Schwarz/Mann); bovine erythrocyte carbonic anhydrase, 29,000 (Sigma); ovalbumin, 43,000 (Schwarz/Mann); bovine serum albumin, 68,000 (Schwarz/Mann).

Polyacrylamide Gel Electrophoresis in LDAO. Polyacrylamide gels containing 0.1% LDAO–50 mM Tris-Cl (pH 8.0) were prepared by a procedure similar to that described for sodium dodecyl sulfate gels, except that the acrylamide concentration was 5%. A nylon net was used at the end of the tubes to support the gel. The reservoirs contained 0.1% LDAO–50 mM Tris-Cl (pH 8.0). Reaction center samples were applied in 10% sucrose without pretreatment. Electrophoresis was performed at room temperature in the dark at 60 V (4–5 mA/tube) for 5–10 hr.

Sucrose Gradient Centrifugation. The subunits of reaction centers were separated by centrifugation in a sucrose density gradient. Reaction centers of *R. spheroides* R-26 (0.2 ml; $A^{1\,cm}_{802} = 5$; 1% sodium dodecyl sulfate–0.6% LDAO–50 mM Tris-Cl (pH 8.0)–1 mM EDTA) were centrifuged in a sucrose gradient (5–20%, w/v) containing the same detergent and buffer composition as the sample. The centrifugation was performed at 20° for 20 hr at 300,000*g* (65,000 rpm in a SW 65L Ti rotor, Beckman L2-65B, centrifuge). After centrifugation the tubes were punctured at the bottom and 0.25-ml fractions were collected. The optical absorbance at 280 and 802 nm and the radioactivity of the fractions were determined. The subunit composition of selected fractions was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Before electrophoresis, the fractions were dialyzed against 1% sodium dodecyl sulfate–10 mM Tris-Cl (pH 8.0)–1 mM EDTA–0.01% dithiothreitol and then heated at 65° for 30 min. Incomplete removal of LDAO resulted in artifacts in the sodium dodecyl sulfate gels.

Determination of Radioactivity. After electrophoresis of the radiolabeled proteins, the gels were optically scanned (see previous section), frozen, and sliced into 0.7-mm sections with a Mickle gel slicer (Brinkmann Instruments). The gel slices were placed into scintillation vials containing 1 ml of ammonium hydroxide. The closed vials were incubated for 4 hr at 50° and opened and the ammonia evaporated in a hood. Scintillation fluid was added (10 ml containing five parts of Beckman Ready-Solv IV and one part Beckman Bio-Solv BBS-3) and the vials were counted in a Beckman scintillation counter (LS-100). The ^{14}C counting efficiency was determined with a standard [^{14}C]toluene solution to be 70 ± 1%. Samples containing ^{59}Fe were counted with the variable Isoset unit (Beckman) set between the limits of 300 and 700.

The specific activities of the amino acids of the ^{14}C-labeled reaction centers were obtained by amino acid analysis and counting of the effluent fractions (Steiner *et al.*, 1974).

Carbohydrate Analyses. Two independent methods were used. (a) The phenol–sulfuric acid method (Dubois *et al.*, 1956) was applied to reaction centers from which the pigments had been extracted with acetone. D-Glucose was used as a standard. (b) Reaction centers were hydrolyzed in 2 N trifluoroacetic acid at 121° for 2 hr. Alditol acetate derivatives were prepared and analyzed by gas chromatography according to the method of Niedermeier (1971).

Iron Analysis. The iron content was determined with an atomic absorption spectrophotometer (Varian, Techtron AA-5) utilizing the carbon rod technique. In the case of bacteria labeled with ^{59}Fe the specific activity was determined, and the radioactivity was used as a convenient assay for iron in subsequent reaction center preparations.

Labile Sulfide Analysis. One-half milliliter of reaction centers $A^{1\,cm}_{800} = 14$ was placed in a 5-ml erlenmeyer flask. The sample was acidified by adding 0.5 ml of 1 M HCl and quickly covered with parafilm and shaken for about 10 sec. The sample was then analyzed for the characteristic smell of hydrogen sulfide. The sensitivity of the olfactory detectors was calibrated using a series of two different standard samples: (1) Na_2S dissolved in 1 mM EDTA (pH 8.0) and analyzed by standard iodometric titration and (2) high-potential iron–sulfur protein, which has four labile sulfurs. The concentration of the latter protein was determined from the optical absorption assuming an extinction coefficient for the reduced species of $\epsilon^{M}_{388} = 16 \times 10^3\ M^{-1}\ cm^{-1}$ (Dus *et al.*, 1967). The average sensitivity of the olfactory system was found to be

the same with both solutions. It was determined to be approximately 2×10^{-10} mol of sulfide (four subjects tested).

Results

Purity and Composition of Reaction Centers. In preparing the reaction centers, the ratio of the absorbance at 280 nm to that at 802 nm served as a useful index of relative purity. As contaminating proteins were eliminated, this ratio decreased until a value of 1.22 ± 0.03 was reached. The ratio was not improved by repeated passage through DEAE-cellulose or by preparative electrophoresis in polyacrylamide gels containing LDAO. When the reaction center preparation was analyzed by electrophoresis in polyacrylamide gels containing LDAO, two bands were obtained. The minor band corresponded to about 10% of the total protein and traveled 1.5 times faster than the major band. The optical spectrum, photochemical activity, and the subunit compositions of the proteins eluted from each band were found to be the same. Evidently both bands contain closely related proteins; the difference in mobility may be related to differences in aggregation or charge.

The purity of the reaction centers was also evaluated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. The resulting electrophoretograms are shown in Figure 1. Three major bands are seen. These correspond to the subunits of the reaction center protein (see later section). Occasionally, minor components were detected (*e.g.*, near 2 cm in Figure 1), probably representing aggregates of the major components. These results indicate that the purity of the reaction centers is at least 95%.

Antisera were prepared against the purified reaction centers (Steiner *et al.*, 1974). When these were allowed to react with the antigen in double diffusion in agar containing LDAO, a single precipitin band was obtained. This is consistent with the homogeneity of the reaction-center preparation.

Analysis of the purified reaction center for carbohydrates by the phenol–sulfuric acid method revealed less than 0.1% (w/w) neutral sugars. The gas chromatographic method gave less than 0.1% (w/w) fucose, mannose, galactose, glucosamine, and galactosamine, but showed the presence of 0.2% (w/w) glucose. In view of the results of the phenol–sulfuric acid analysis, we ascribe the presence of glucose to an accidental contamination. In addition, we determined that there was less than 0.01 of an acid-labile sulfide moiety per reaction center particle.

Dissociation of Reaction Centers into Three Subunits. Reaction centers were treated with acetone to extract bacteriochlorophyll and bacteriopheophytin and were analyzed by electrophoresis in polyacrylamide gels. The gels were scanned immediately after running. Three components with an absorption maximum at 280 nm were observed (Figure 1, upper trace) and have been designated light (L), medium (M), and heavy (H) in order of decreasing mobility. The absorption of the H component at 280 nm was less than that of the other two. The gels were then stained with Coomassie Blue and scanned at 650 nm. Three components were again detected but the H component was now more prominent (Figure 1, lower trace). The weak absorbance of the H component at 280 nm is due to its low tryptophan content (Steiner *et al.*, 1974), as well as to selective losses during electrophoresis (see later section). The spectrum of the dye bound to each of these components was found to differ. The absorbance maxima of the two faster components occurred at 561 nm and that of the slowest at 556 nm. This spectral difference provides

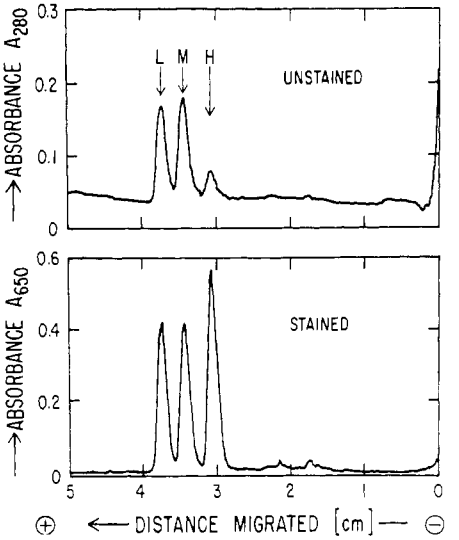

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of reaction centers from *R. spheroides* R-26. Results before and after staining with Coomassie Blue are presented. The molecular weights of the L, M, and H subunits were determined to be 21,000, 24,000, and 28,000.

a convenient method for identifying the subunits and their aggregates in the gel. For this purpose, the ratios of absorbance at 620 nm to that at 525 nm were determined to be 1.17, 1.15, and 0.84 for the L, M, and H components, respectively. The H component can also be identified by its more intense fluorescence (Clayton and Haselkorn, 1972).

No significant change in the distribution of the protein components was observed when the reaction centers, containing bacteriochlorophyll, were incubated at 65° in 1% sodium dodecyl sulfate, 1% dithiothreitol, and 8 M urea, and then run on polyacrylamide gels containing 1% sodium docecyl sulfate. However, an additional component with an absorption peak at 760 nm was found to migrate just ahead of the three protein bands. This additional band is due to bacteriochlorophyll that dissociated from the reaction center protein. The distribution of components was also not significantly altered when the proteolytic enzyme inhibitor phenylmethylsulfonyl fluoride (1.1×10^{-3} M) was added to the chromatophores before the addition of LDAO and during the ammonium sulfate fractionation step. When the Tris-Cl buffer (pH 8.0) was replaced by sodium phosphate buffer (pH 7.5), the three bands were not resolved in polyacrylamide gels containing 0.1% sodium dodecyl sulfate (see, for example, Feher, 1971, Figure 2). In the same phosphate buffer, but with 1% sodium dodecyl sulfate, the three bands were again resolved.

The *molecular weights* of the three major components were obtained by comparing their electrophoretic mobilities, in 10% polyacrylamide gels, with those of a set of marker proteins. The values thus determined were: L, 21,000 ± 1000; M, 24,000 ± 1000; H, 28,000 ± 1000. The statistical errors quoted were estimated from the results of several runs. They do not take into account possible factors other than molecular weight that may affect mobility (Banker and Cotman, 1972). The apparent molecular weights varied slightly with the acrylamide concentration; in 7.5% gels the molecular weights were approximately 5% lower, whereas in 12.5% they were 5% higher than the values quoted above. Increasing the sodium dodecyl sulfate concentration in the gel to 1% did not affect the relative mobilities.

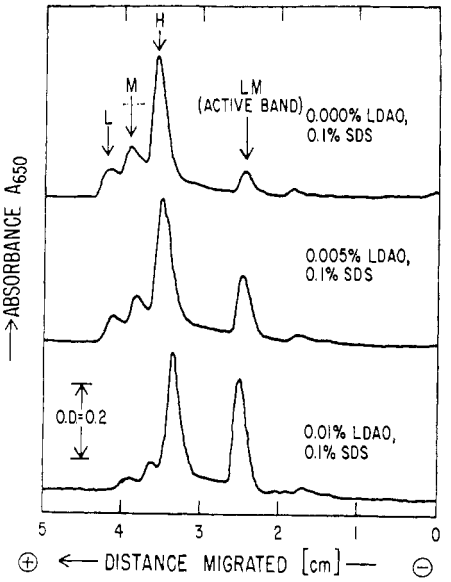

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of reaction centers from *R. spheroides* R-26 with different amounts of LDAO. These samples were not exposed to sodium dodecyl sulfate before application to the gel. The active, pigmented band (LM) is protected by increasing amounts of LDAO in the gel.

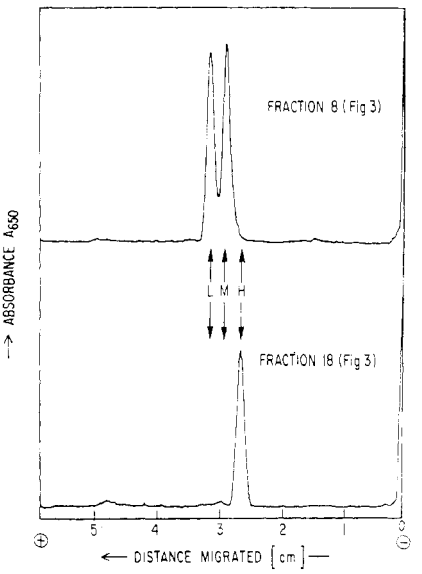

FIGURE 4: Sodium dodecyl sulfate–polyacrylamide gel electrophoretograms (stained) of peak fractions from sucrose gradient centrifugation of reaction centers of *R. spheroides* R-26 (see Figure 3). These results indicate that there is very little cross-contamination between the LM and H units.

Dissociation of Reaction Centers into Two Subunits. When native reaction centers (*i.e.*, without removal of pigment and without pretreatment with sodium dodecyl sulfate) were run on polyacrylamide gels containing 0.1% sodium dodecyl sulfate, a pigmented band was observed to migrate behind the L, M, and H components (see Figure 2). Furthermore, the intensity of the L and M bands relative to the H band was diminished. When a small amount of LDAO was added to the gel, the additional pigmented band was more prominent and there was a corresponding decrease in the L and M bands. With a further increase in LDAO concentration, the L and M components were virtually absent and the new component was yet more prominent (see Figure 2). Evidently, in a mixture of LDAO and sodium dodecyl sulfate, the L and M components remain associated in a unit we have called LM.

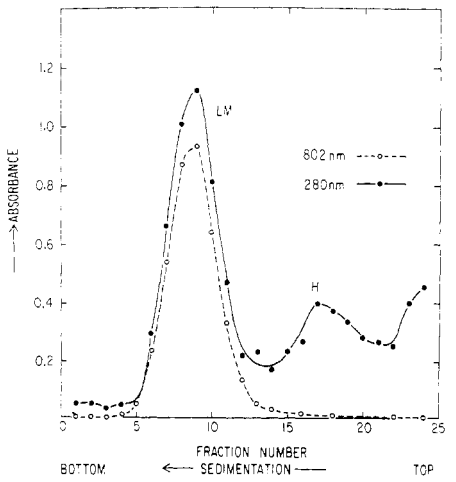

FIGURE 3: Sucrose gradient centrifugation of reaction centers from *R. spheroides* R-26 in 1% sodium dodecyl sulfate 0.6% LDAO. The pigmented LM band showed reversible photochemical activity at room temperature. The increase in absorbance at 280 nm near the top of the gradient is probably due to scattering from mixed sodium dodecyl sulfate–LDAO micelles. Fractions 22, 23, and 24 contained no protein.

When unstained gels were scanned, it was found that the spectrum of the LM band resembled that of native reaction centers. Moreover, its long-wavelength peak could be bleached reversibly at room temperature by actinic light.

In order to isolate larger quantities of the LM unit, a separation method using zonal centrifugation in a sucrose gradient in the presence of LDAO and sodium dodecyl sulfate was developed. A pigmented band was observed to sediment ahead of a second nonpigmented band. The absorbance of fractions taken from these bands at 280 and 802 nm is shown in Figure 3. Their composition was determined by analyzing the various fractions by electrophoresis in sodium dodecyl sulfate–polyacrylamide gels. The electrophoretograms of the peak fractions (8 and 18 in Figure 3) are shown in Figure 4. Evidently, the pigmented band was composed of the L and M units, whereas the unpigmented band was composed mainly of the H component (Figure 3). In addition, the unpigmented band contained a small amount (~5%) of the L and M units, arising from the partial decomposition of the pigmented moiety.

The optical spectrum of the pigmented (LM) band (see Figure 5) resembled that of the intact reaction centers, except that the ratio A_{280}/A_{802} was 1.08 ± 0.03 (instead of 1.22 ± 0.03 for the reaction centers). This decrease is expected from the loss of the unpigmented unit. In addition, the long-wavelength absorption maximum was shifted from 865 to 855 nm and the band at 530 nm was narrowed. The optical spectrum of the LM unit could be bleached reversibly at *room temperature* by actinic light, but the recovery in the dark was much slower than that observed in untreated reaction centers. The LM unit was also more prone to irreversible loss of its absorbance at 802 nm after prolonged illumination with actinic light. In contrast to the behavior of intact reaction centers, the photochemical activity of the LM unit at low temperatures (T = 80°K) was only a few per cent of its activity at room temperature. Moreover, the electron paramagnetic resonance spectrum of the LM unit differed markedly from that of intact reaction centers, resembling the characteristics of modified reaction centers low in iron (Loach and Hall, 1972;

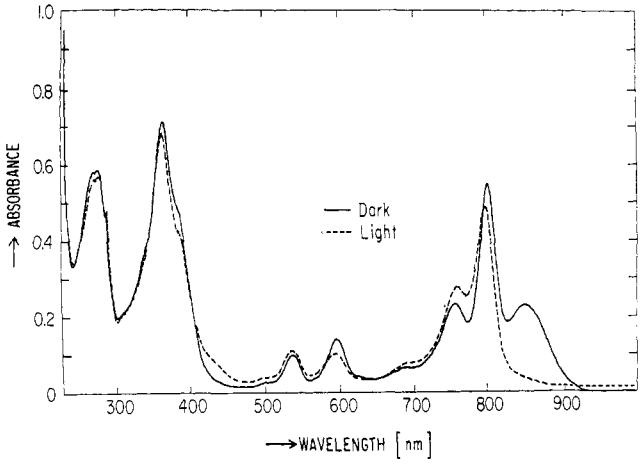

FIGURE 5: Optical absorption spectrum of the LM band (fraction 8 of Figure 3) from sucrose gradient centrifugation of reaction centers. The spectrum resembles that of intact reaction centers. Reversible photochemical activity was observed at room temperature with actinic light.

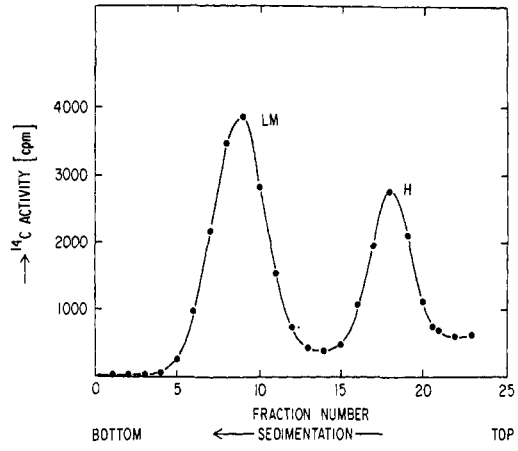

FIGURE 6: Sucrose gradient centrifugation of ^{14}C-labeled reaction centers in 1% sodium dodecyl sulfate–0.6% LDAO. From the ratio of radioactivity (^{14}C) in the two bands, the stoichiometry of the L, M, and H subunits was determined (see Table I).

Feher *et al.*, 1972). Indeed, the mole fraction of iron in the LM unit was determined to be less than 0.1.

An alternate method of preparing the LM unit is centrifugation of the reaction centers in the chaotropic agent 1 M lithium thiocyanate with 0.5% LDAO (pH 8). The optical spectra of these LM units at room temperature and at low temperature resemble those of the LM units prepared with sodium dodecyl sulfate and LDAO, but they seem to retain more activity at cryogenic temperatures.

Stoichiometry of Subunits. In the preceding section we have shown that the reaction center particle is made up of three subunits, L, M, and H. In this section we discuss the experimental results leading to the determination of the stoichiometry of these subunits. As will be shown later, the method of sodium dodecyl sulfate–polyacrylamide gel electrophoresis could not be used to determine the stoichiometry. Furthermore, the amino acid analysis was not accurate enough to determine the stoichiometry with certainty (Steiner *et al.*, 1974). Instead, the stoichoimetry was determined with radioactively labeled reaction centers that were dissociated into their LM and H constituents by centrifugation in a sucrose gradient containing sodium dodecyl sulfate and LDAO. The ^{14}C label was distributed in two bands, as shown in Figure 6. In order to relate the total radioactivity in each band to the stoichiometry, two minor corrections were made. (1) The contribution of the radiolabeled tetrapyrrole pigments was subtracted from the LM fraction. This contribution (6% of the total radioactivity) was obtained by measuring the radioactivity of the acetone extractable fraction. (2) The contribution to the radioactivity of the H band made by the small amount of dissociated L and M units was subtracted and added to the LM fractions. This contribution (8.5% of the radioactivity in the H band) was estimated from the intensity of the stained bands after electrophoresis of the fractions in sodium dodecyl sulfate–polyacrylamide gels (Figure 4). The recovery of radioactivity in the LM and H bands in four experiments was greater than 90%.

The ratio of the corrected radioactivity (counts per minute) in the LM and H bands is given by

$$\frac{(\text{cpm})_{LM}}{(\text{cpm})_{H}} = \frac{l(\text{MW})_L(\text{SA})_L + m(\text{MW})_M(\text{SA})_M}{h(\text{MW})_H(\text{SA})_H} \quad (1)$$

where l, m, and h are the stoichiometric coefficients (moles of subunit per mole of reaction center), (MW) is the molecular weight of the subunit (*i.e.*, 21,000, 24,000, and 28,000), and (SA) is the relative specific activity (cpm/g of protein) normalized to the L subunit. The specific activities of the three subunits were found to be in the ratio: $(\text{SA})_L:(\text{SA})_M:(\text{SA})_H = 1.00:0.98:0.97$. The values were obtained from the amino acid composition (Steiner *et al.*, 1974) and the measured specific activity of each amino acid in the reaction centers.

Table I shows the theoretical ratios of radioactivity of the LM and H fractions for different stoichiometries of the subunits and the experimentally determined ratio. The observed ratio of 1.67 ± 0.10 corresponds to a stoichiometry of 1:1:1 for the L, M, and H subunits.

An alternate way of obtaining the stoichiometry would be to determine the distribution of either radioactivity or stain among the subunits separated by electrophoresis in sodium dodecyl sulfate–polyacrylamide gels. We have found that these methods are subject to certain experimental limitations. The recovery in the three bands of the radioactive material

TABLE I: Ratios of Radioactivity (^{14}C) of the LM and H Fractions of Reaction Centers for Different Stoichiometries of the Subunits.

Subunit Stoichiometry[a]			$(\text{cpm})_{LM}/(\text{cpm})_H$	
l	m	h	Calcd[b]	Obsd[c]
1	1	2	0.78	
2	1	2	1.21	
1	2	2	1.25	
1	1	1	1.64	1.67 ± 0.10
1	2	1	2.50	
2	1	1	2.42	
2	2	1	3.28	

[a] The stoichiometric coefficients l, m, and h are expressed in moles of subunit per mole of reaction center. [b] Calculated from eq 1. [c] Average of four runs (see Figure 6). Corrections were applied for tetrapyrrole content and decomposition of LM fraction as explained in the text. Quoted error is the standard deviation.

TABLE II: Distribution of Radioactivity (^{14}C), Absorbance, and Bound Dye in Reaction Center Subunits after Electrophoresis in Sodium Dodecyl Sulfate–Polyacrylamide Gels.[a]

		Integ Radioact	Integ Absorb.	Recovery of Protein[b]	Sp Absorb.[c] (Integ Absorb. per g of Protein)
Unstained	L	1.00	1.00	1.00	1.00
gels	M	0.97 ± 0.05	1.07 ± 0.05	0.87 ± 0.04	1.08 ± 0.06
λ 280 nm	H	0.68 ± 0.08	0.26 ± 0.03	0.53 ± 0.06	0.37 ± 0.03
Stained	L	1.00	1.00	1.00	1.00
gels[d]	M	1.06 ± 0.06	1.00 ± 0.10	0.95 ± 0.05	0.92 ± 0.10
λ 560 nm	H	0.91 ± 0.15	1.41 ± 0.10	0.70 ± 0.12	1.50 ± 0.14

[a] Reaction centers were precipitated in acetone to remove bacteriochlorophyll and pheophytin and were heated for 30 min at 65° in 0.5% sodium dodecyl sulfate and 1% dithiothreitol before application to the gel. All values are expressed relative to those of the L subunit. [b] Obtained by dividing the integrated radioactivity (first column) by the specific activity of the subunit (1.00: 0.98:0.97 for L–M–H, respectively) and multiplying by the ratio of the molecular weight of L to that of the appropriate subunit. [c] Obtained by dividing the integrated absorbance by the integrated radioactivity and multiplying by the specific activity. The division was carried out for each individual gel experiment (duplicate runs of five different loads). This procedure eliminates the effect of variable protein recovery on the specific absorbance. [d] Fixed overnight in methanol–acetic acid, stained with Coomassie Blue for 4 hr, and destained for 4 days.

applied to the gels was only 60–80%. The loss of radioactivity may not be shared uniformly by the three subunits; this would cause a large error in the determination of the stoichiometry. In addition, the intensity of staining with Coomassie Blue was found not to be proportional to the distribution of the radiolabel, indicating that the three subunits have different affinities for the stain. Nevertheless, since we have already determined the stoichiometry, we can quantitate the selective loss of subunits in the gels (as well as their individual "staining efficiencies"). We can then utilize sodium dodecyl sulfate gel electrophoresis to obtain additional information about the subunits, *e.g.*, the distribution of specific amino acid residues (Steiner *et al.*, 1974).

Table II shows the results of electrophoresis of radiolabeled reaction centers in sodium dodecyl sulfate–polyacrylamide gels. Five different loads, ranging from 7 to 35 μg of protein, were run in duplicate. The gels were scanned and gel slices were analyzed for radioactivity. Duplicate gels were stained, scanned, and also sliced for radioactivity measurements. The integrated radioactivity and optical absorbance of the bands were found to be linearly dependent on the load. The values in the first and second columns are the integrated radioactivity and absorbance of the three bands in the gels, expressed relative to the L component. The third column shows the relative recoveries of the three components from the gels, based on the recovery of radioactivity in each band and the assumption that the stoichiometry is 1:1:1 (as determined in the centrifugation experiment summarized in Table I). The fourth column lists the relative specific absorbances of the three components. These values will be used in the accompanying paper for the determination of the relative content of tryptophan and half-cystine in the subunits.

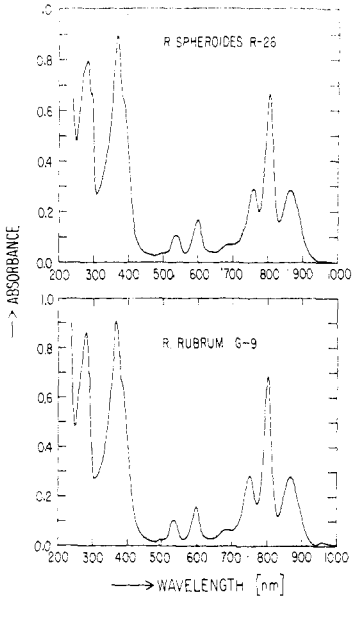

FIGURE 7: Optical absorption spectra of reaction centers from *R. spheroides* R-26 and *R. rubrum* G-9 (T = 23°).

Comparison of Reaction Centers from R. rubrum G-9 and R. spheroides R-26. Reaction centers were prepared from *R. rubrum* by procedures slightly modified from those used with *R. spheroides*. Selective solubilization of the *R. rubrum* reaction centers from chromatophores was more effective at a lower pH and was carried out in phosphate buffer at pH 7. The stability of the reaction centers at this pH was increased by carrying out all procedures up to DEAE chromatography at 4° and at a lower concentration of LDAO.

Figure 7 shows the spectrum of reaction centers from *R. rubrum* compared with that of reaction centers from *R. spheroides*. The two spectra are very similar; the ratio A_{280}/A_{802} for the *R. rubrum* reaction centers is 1.25 ± 0.03. The stability of the two preparations differed markedly. Reaction centers from *R. spheroides* may be stored for several months at 4° in 0.1% LDAO–10 mM Tris-Cl (pH 8), without a change in the optical spectrum. However, reaction centers from *R. rubrum* deteriorated in several hours under these conditions, as detected by a decrease in the absorbance at 802 nm and an increase in absorbance at 760 nm. By reducing the LDAO concentration, the stability of the *R. rubrum* reaction centers could be increased manyfold.

The subunit structure of reaction centers in *R. rubrum* and *R. spheroides* was compared by electrophoresis of the two samples in sodium dodecyl sulfate–polyacrylamide gels. As shown in Figure 8, the reaction centers of *R. rubrum* are also

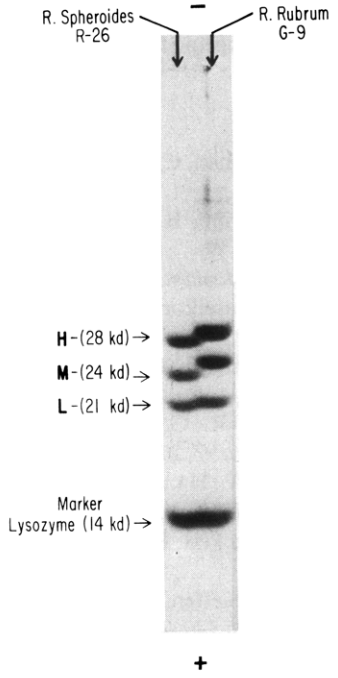

FIGURE 8: Comparison of the subunit structure of reaction centers from *R. spheroides* R-26 and *R. rubrum* G-9 by electrophoresis on a split sodium dodecyl sulfate–polyacrylamide gel stained with Coomassie Blue. Egg-white lysozyme was used as a marker.

composed of three subunits; their mobilities, however, are a few per cent lower than those of the subunits of *R. spheroides*.

Summary and Discussion

Photochemically active reaction centers were isolated from chromatophores of *R. spheroides* R-26 using a modification of an earlier procedure (Feher, 1971; Clayton and Wang, 1971). It has been shown previously that these reaction centers are mainly composed of protein with four bacteriochlorophylls, two pheophytins (Straley *et al.*, 1973; Reed and Peters, 1972), and approximately one iron (Feher, 1971), one ubiquinone (Feher *et al.*, 1972), and less than one phosphorus per active unit (Feher, 1971). We have now extended this analysis and have shown that no significant amounts of carbohydrates or labile sulfide are present.

The reaction center preparation, when analyzed by sodium dodecyl sulfate–polyacrylamide electrophoresis, was found to be composed of three subunits and less than 5% of other protein. The apparent molecular weights of the three subunits (termed L, M, and H), obtained from the relative electrophoretic mobilities in sodium dodecyl sulfate–polyacrylamide electrophoresis, were 21,000, 24,000, and 28,000, respectively. These findings are in substantial agreement with previous reports (Feher *et al.*, 1971; Clayton and Haselkorn, 1972; Okamura *et al.*, 1973), but are in disagreement with the conclusions of Jolchine and Reiss-Husson (1972). The latter workers based their conclusions on the results of polyacrylamide gel electrophoresis in sodium phosphate buffer containing 0.1% sodium dodecyl sulfate. We have found that the subunits do not dissociate under these conditions, but that higher concentrations of sodium dodecyl sulfate are required to obtain dissociation in sodium phosphate buffer. Accordingly, analyses of subunit composition and molecular weights obtained in the low sodium dodecyl sulfate–sodium phosphate system are probably not correct.

The validity of the sodium dodecyl sulfate–polyacrylamide gel method for determining molecular weights has been established for many hydrophylic proteins (*e.g.*, Weber and Osborn, 1969; Dunker and Rueckert, 1969). However, its applicability to highly hydrophobic proteins, such as the reaction center protein, remains to be verified. A necessary condition for estimation of molecular weights by sodium dodecyl sulfate–polyacrylamide gel electrophoresis is that the same results are obtained for several concentrations of acrylamide and/or different cross-linking (Banker and Cotman, 1972). We found this to be approximately the case, although a small increase in apparent molecular weight of the subunits with increasing concentration of polyacrylamide was observed. Such an increase is consistent with the reaction center protein binding more sodium dodecyl sulfate than the marker proteins and would lead to an underestimate of the reported molecular weights. Since all three subunits contain a large proportion of nonpolar residues (see Steiner *et al.*, 1974), the relative molecular weights are probably more accurate than their absolute values.

The stoichiometry of the three subunits was determined, by zonal centrifugation of radiolabeled reaction centers in a sucrose gradient, to be 1:1:1 (see Table I). Since, in expression 1, only the relative molecular weights are of importance, possible errors in their absolute values will not be seriously reflected in their stoichiometry. The amino acid composition of the three subunits is also consistent with the 1:1:1 stoichiometry (Steiner *et al.*, 1974). Clayton and Haselkorn (1972) had tentatively arrived at the same stoichiometry from a comparison of the integrated areas of a sodium dodecyl sulfate–polyacrylamide gel electrophoretogram stained with Coomassie Blue. We have found that the staining efficiency (integrated absorbance per gram of protein) of the H subunit is 1.5 times higher than that of the L subunit (see Table II). However, this is offset by the lower recovery of the H subunits in the gel giving approximately (and fortuitously) the correct stoichiometry. It is interesting to note that the staining efficiencies of the subunits correlate very well with the total amount of the basic amino acid residues lysine, histidine, and arginine (Steiner *et al.*, 1974).

The molecular weights of the subunits and their stoichiometry determine the minimum molecular weight of the reaction center protein. This value, 73,000 (*i.e.*, 21,000 + 24,000 + 28,000), is in fair agreement with the value of 90,000 obtained from the dry weight (Feher, 1971) together with the molar extinction coefficient (Reed and Peters, 1972; Straley *et al.*, 1973). The molecular weight obtained from the amino acid composition and extinction coefficient is discussed in the following paper. It should be noted that all these methods determine minimum molecular weights and do not exclude the possibility that active reaction centers occur as dimers or higher aggregates. Evidence that such associations are not required for activity was provided by the sodium dodecyl sulfate–LDAO–polyacrylamide gel electrophoresis experiments (Figure 2) in which the active unit migrated with a mobility that corresponds approximately to the minimum value. The higher molecular weights (140,000–150,000) obtained for reaction centers from gel filtration and sedimentation experiments (Reiss-Husson and Jolchine, 1972; Noël *et al.*, 1972) may be due to aggregation or to the presence of detergent molecules associated with the protein.

One of the aims of the investigation was to define the minimum size unit that retains photochemical activity as defined in the introduction. A pigmented component of the reaction

centers containing two of the subunits (L and M) was separated by sucrose density centrifugation in the presence of sodium dodecyl sulfate and LDAO. This unit, with an estimated minimum mol wt of 45,000 (*i.e.*, 21,000 + 24,000), retained full activity at room temperature. However, in contrast to the intact reaction centers, insignificant activity was observed at cryogenic temperatures (T = 80°K). Moreover, the LM unit was devoid of iron, a moiety that has been postulated to play an important role in the primary reduction (Feher, 1971; Leigh and Dutton, 1972). A possible explanation of these findings is that at room temperature an exogenous (nonphysiological) acceptor can diffuse to the donor site. It is, nevertheless, possible that the lack of activity of the LM unit at low temperatures may be the result of the particular preparative procedure and does not prove that the H subunit is essential. Indeed, preliminary experiments indicate that when the LM unit is prepared with chaotropic agents (in the absence of sodium dodecyl sulfate), it retains at least partial activity at 80°K. We are at present experimenting with alternate preparative procedures in an effort to maximize the activity at cryogenic temperatures. Since few proteins have an odd number of subunits (Klotz *et al.*, 1970), it would be unusual if all three subunits were essential for the primary photochemical act. It is interesting to note that in mutants of *R. spheroides* that are blocked in the synthesis of bacteriochlorophyll, the L and M subunits were reported to be absent, while the H subunit continues to be synthetized (Takemoto and Lascelles, 1973).

We have compared the optical spectra and subunit structure of reaction centers obtained from another photosynthetic bacterium, *R. rubrum* G-9. The optical spectra of the two preparations were nearly identical. The reaction centers from *R. rubrum* were also composed of three subunits with electrophoretic mobilities in sodium dodecyl sulfate–polyacrylamide gels a few per cent lower than those from *R. spheroides* (see Figure 8). We conclude, therefore, that reaction centers in these two species are similar but not identical. This conclusion is also supported by analyses of the amino acid composition and the antigenic properties of these proteins as discussed in the accompanying paper (Steiner *et al.*, 1974).

Acknowledgments

We thank L. C. Ackerson for his capable technical assistance, in particular, in connection with the preparation of reaction centers from *R. rubrum*, E. Moskowitz for the preparation of radiolabeled reaction centers, R. A. Isaacson for help in the design of the gel scanner, and A. D. Lopes for carrying out amino acid analyses of the radiolabeled reaction centers. We are indebted to K. Keegstra for the carbohydrate analysis by gas chromatography, to R. G. Bartsch for supplying the high potential iron–sulfur protein, and to M. Kamen and his group for the use of their large scale bacterial growth facilities.

Added in Proof

By using lithium perchlorate at low LDAO concentrations instead of lithium thiocyanate, we were able to separate LM units with an H contamination of less than 10%. The LM units, prepared in this way, exhibited full reversible photobleaching of the long wavelength absorption band at 80°K. We are at present investigating the correlation between the iron and ubiquinone content of the LM units and the photochemical kinetics at cryogenic temperatures.

References

Banker, G. A., and Cotman, C. W. (1972), *J. Biol. Chem. 247*, 5856.

Choules, G. L., and Zimm, B. H. (1965), *Anal. Biochem. 13*, 336.

Clayton, R. K. (1963), *Biochim. Biophys. Acta 75*, 312.

Clayton, R. K., and Haselkorn, R. (1972), *J. Mol. Biol. 68*, 97.

Clayton, R. K., and Wang, R. T. (1971), *Methods Enzymol. 23*, 696.

DeKlerk, H., Bartsch, R. G., and Kamen, M. D. (1965), *Biochim. Biophys. Acta 97*, 275.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Anal. Chem. 28*, 350.

Dunker, A. K., and Rueckert, R. R. (1969), *J. Biol. Chem. 244*, 5074.

Dus, K., DeKlerk, H., Sletten, K., and Bartsch, R. G. (1967), *Biochim. Biophys. Acta 140*, 291.

Duysens, L. N. M. (1962), Thesis, University of Utrecht.

Emerson, R., and Arnold, W. (1932), *J. Gen. Physiol. 16*, 191.

Feher, G. (1971), *Photochem. Photobiol. 14*, 373.

Feher, G., Okamura, M. Y., and McElroy, J. D. (1972), *Biochim. Biophys. Acta 267*, 222.

Feher, G., Okamura, M. Y., Raymond, J. A., and Steiner, L. A. (1971), *Biophys. Soc. Abstr. 11*, 38a.

Gingras, G., and Jolchine, G. (1969), *Progr. Photosyn. Res., Proc. Int. Congr. 1968, 1*, 209.

Jolchine, G., and Reiss-Husson, F. (1972), *Biochem. Biophys. Res. Commun. 48*, 333.

King, T. P. (1972), *Biochemistry 11*, 367.

Klotz, I. M., Langerman, N. R., and Darnall, D. W. (1970), *Annu. Rev. Biochem. 39*, 25.

Leboy, P. S., Cox, E. C., and Flaks, J. G. (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 1367.

Leigh, J. S., and Dutton, P. L. (1972), *Biochem. Biophys. Res., Commun. 46*, 414.

Loach, P. A., Androes, G. M., Maksim, A. F., and Calvin, M. (1963), *Photochem. Photobiol. 2*, 443.

Loach, P. A., and Hall, R. L. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 786.

Loach, P. A., Sekura, D. L., Hadsell, R. M., and Stemer, A. (1970), *Biochemistry 9*, 724.

McElroy, J. D., Feher, G., and Mauzerall, D. C. (1969), *Biochim. Biophys. Acta 172*, 180.

McElroy, J. D., Mauzerall, D. C., and Feher, G. (1974), *Biochim. Biophys. Acta 333*, 261.

Niedermeier, W. (1971), *Anal. Biochem. 40*, 465.

Noël, H., Van der Rest, M., and Gingras, G. (1972), *Biochim. Biophys. Acta 275*, 219.

Okamura, M. Y., Moskowitz, E., McElroy, J. D., Feher, G., and Steiner, L. (1973), *Biophys. Soc. Abstr. 13*, 270a.

Reed, D. W., and Clayton, R. K. (1968), *Biochem. Biophys. Res. Commun. 30*, 471.

Reed, D. W., and Peters, G. A. (1972), *J. Biol. Chem. 247*, 7148.

Reiss-Husson, F., and Jolchine, G. (1972), *Biochim. Biophys. Acta 256*, 440.

Sauer, K. (1974), *in* Bioenergetics of Photosynthesis, Govindjee, Ed., New York, N. Y., Academic Press, Chapter 3.

Shapiro, A. L., Viñuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.

Steiner, L. A., Okamura, M. Y., Lopes, A. D., Moskowitz, E., and Feher, G. (1974), *Biochemistry 13*, 1403.
Straley, S. C., Parson, W. W., Mauzerall, D. C., and Clayton, R. K. (1973), *Biochim. Biophys. Acta 305*, 597.
Takemoto, J., and Lascelles, J. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 799.
Thornber, J. P. (1970), *Biochemistry 9*, 2688.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.

Characterization of Reaction Centers from Photosynthetic Bacteria. II. Amino Acid Composition of the Reaction Center Protein and Its Subunits in *Rhodopseudomonas spheroides* R-26†

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ABSTRACT: The amino acid composition of the reaction center protein from *Rhodopseudomonas spheroides* R-26, and of its three subunits, was determined. The protein, which is extracted from the bacterial membrane by detergent, contains a large proportion of apolar residues. The two smaller subunits are especially hydrophobic in composition and are probably associated with the lipid part of the membrane. The amino acid composition of reaction centers from *Rhodospirillum rubrum* G-9 was similar but not identical with that of reaction centers from *R. spheroides* R-26. Immunochemical tests showed that reaction centers from these two bacterial species are antigenically distinct.

The photochemically active reaction center, isolated from chromatophores of *Rhodopseudomonas spheroides* R-26, is composed of protein and a number of associated molecules including bacteriochlorophyll, ubiquinone, and iron. In the accompanying paper (Okamura *et al.*, 1974), we have presented evidence that the protein moiety consists of three subunits, present in equimolar amounts, with a composite molecular weight of about 70,000. Two of these subunits appeared to be essential for activity; removal of the third resulted in extensive inactivation at cryogenic temperatures but not at room temperature (Okamura *et al.*, 1973, 1974).

The present paper is concerned with a more detailed analysis of the reaction center protein and its subunits. Our principal objectives have been to determine whether this protein, derived from the bacterial membrane, has an unusual amino acid composition, whether the two "essential" subunits are independent, and whether they differ in overall composition from the third subunit. In addition, since iron has been postulated to be the primary electron acceptor (Feher, 1971; Leigh and Dutton, 1972), it was particularly important to establish whether the intact protein, and the individual subunits, contain residues of cysteine that might function as binding sites for iron. Finally, we hoped that these studies would provide an independent estimate of the minimum molecular weight and of the stoichiometry of the subunits.

The reaction center protein from the related photosynthetic bacterium, *Rhodospirillum rubrum* G-9, is also composed of three subunits (Noël *et al.*, 1972; Clayton and Haselkorn, 1972; Okamura *et al.*, 1974). In order to obtain additional information on the properties of the photochemically active proteins from the two species, we have compared their overall amino acid composition and their antigenic properties.

Materials and Methods

Preparation of Reaction Centers. The preparation of reaction centers from *R. spheroides* R-26 and *R. rubrum* G-9 is described in the accompanying paper (Okamura *et al.*, 1974). Reaction centers containing either radiolabeled half-cystine or tryptophan were obtained from bacteria grown in modified Hutner medium without yeast extract, peptone, or glutamate, but including either 100 μCi of L-[^{35}S]cystine/l. (Schwarz/Mann, sp act. 25 mCi/mmol) plus 200 mg of L-methionine/l. or 200 μCi of DL-[^{14}C]tryptophan/l. (International Chemical and Nuclear Corp., ring-2 labeled, sp act. 3 mCi/mmol). In each case, the other amino acids were included at a concentration of 10 mg/l.

Preparative Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate. The procedure for preparative gel electrophoresis was essentially the same as that for analytical gel electrophoresis, discussed in the preceding paper, except that the load per gel was about 50 μg. After electrophoresis the gels were marked with a reference bristle and scanned at 280 nm. Sections corresponding to the protein bands were excised from 20 gels with a taut wire and template and macerated in a volume of buffer (50 mM Tris-Cl (pH 8.0)–0.1% sodium dodecyl sulfate) equal to 1.5 times the volume of the gel slices. The macerated gel sections were then placed into electrophoresis tubes (12 × 0.8 cm) sealed at the bottom with a 0.5 cm long plug of 10% polyacrylamide supported with a nylon net. The tubes were placed into concentric cylinders (2 × 2 cm) that were sealed at one end with a dialysis membrane and that contained the same buffer solution. The protein was eluted electrophoretically into these cylinders (15 mA/gel for 4 hr) and concentrated by vacuum dialysis in collodion bags (Schleicher and Schuell). The purity of the separated

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‡ Recipient of National Institutes of Health Postdoctoral Fellowship No. GM-50549.