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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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(A_{850} = 37.5) were incubated in total darkness for 1 h at 4 °C in a 50 mM sodium phosphate buffer (pH 7.0) containing 0.25% (w/v) LDAO. The detergent concentration was then lowered to 0.1% by dilution, and the resulting solution was centrifuged for 105 min at 90000*g*. To the supernatant was added 277 g/L ammonium sulfate; the suspension was left at 4 °C for 30 min. The precipitate was then collected by centrifugation at 20000*g* for 30 min, followed by filtration on filter paper, and suspended in a minimal volume of 10 mM Tris-HCl (pH 8.0) containing 0.1% LDAO. This solution was dialyzed 16 h at 4 °C against the same buffer. Subsequent purification was by chromatography. The sample was adsorbed onto a DEAE-cellulose column equilibrated with 10 mM Tris-HCl (pH 8.0)–0.1% LDAO. The column was washed with the same buffer containing 25 mM NaCl, and the photoreaction center was eluted with the same buffer containing 125 mM NaCl. The active fractions were dialyzed against a 50 mM sodium phosphate buffer (pH 7.8) containing 0.05% LDAO and 1 mM EDTA and then chromatographed on a Sepharose 6B column equilibrated with this buffer.

Isolation of the βγ Dimer of the Photoreaction Center of Rp. sphaeroides 2.4.1. The method used was similar to that of Okamura et al. (1974), developed for the R-26 strain of *Rp. sphaeroides*. The photoreaction center was first concentrated on a small DEAE-cellulose column; elution was carried out with 50 mM Tris-HCl (pH 8.0) containing 0.03% LDAO and 1 M NaCl. The photoreaction center solution was then dialyzed against the same buffer without NaCl. An aliquot (0.2 mL) of solution ($A_{802} \simeq 5$) was placed on a 5–20% sucrose gradient (5 mL) prepared in 50 mM Tris-HCl (pH 8.0) containing 0.03% LDAO, 0.1% $NaDodSO_4$, and 1 mM EDTA. Centrifugation was for 20 h at 60000 rpm, 15 °C, in a SW 65 Ti rotor in a Beckman L2-65B ultracentrifuge. The gradients were fractionated into samples of 0.25 mL, the absorbance of which was determined at 280 nm.

Protein Concentration. An extinction coefficient based on pigment absorption at 802 nm was used to estimate protein concentration: $E_{802,1cm}^{1mg/mL} = 3.3$ and 3.13 for photoreaction centers from *Rs. rubrum* (G9) (Vadeboncoeur et al., 1979) and from *Rp. sphaeroides* (Steiner et al., 1974), respectively.

Determination of Detergent Content in the Photoreaction Center—Detergent Complexes. (a) Sodium Dodecyl Sulfate. The photoreaction center (1 mg/mL) was incubated for 30 min at 37 °C in a buffer containing 10 mM Tris–acetate (pH 8.15), 1% $NaDodSO_4$, and 1% β-mercaptoethanol. The solution was then dialyzed 24 h at room temperature against a buffer containing the same components except for a lower detergent concentration (0.1% $NaDodSO_4$). The $NaDodSO_4$ concentrations in both the dialysate and the solution containing the photoreaction center were measured by the colorimetric method of Ray et al. (1966), as modified by Reynolds & Tanford (1970), which involves extraction of a methylene blue–detergent complex in chloroform. The excess $NaDodSO_4$ concentration inside the dialysis bag represents binding to protein. A standard curve of absorbance at 655 nm vs. detergent concentration was prepared by using solutions of known detergent concentration. Binding of $NaDodSO_4$ to standard globular proteins (bovine serum albumin, lysozyme, and chymotrypsinogen A) was measured in the same way. In a set of control experiments, $NaDodSO_4$ binding to these proteins was measured after their complete denaturation with 6 M guanidine hydrochloride, as described by Reynolds & Tanford (1970).

(b) Triton X-100. The photoreaction center was equilibrated with Triton X-100 at room temperature in one of two ways. (1) A sample (1–1.5 mg of protein) in 10 mM Tris-HCl buffer (pH 7.5) containing 0.1% (w/v) Triton X-100 was adsorbed onto a DEAE-cellulose column (0.9 × 16 cm) fully equilibrated with the same buffer. The column was washed with 50 mL of this buffer, and the photoreaction center was eluted with the same buffer containing 0.3 M NaCl. Fractions of 0.5 mL were collected. (2) The second method, gel filtration on Sepharose 6B, permitted detergent equilibration at constant salt concentration of the elution buffer. Samples of about 5 mg of protein in 1 mL were applied to a column (1.6 × 40 cm) of Sepharose 6B equilibrated with the appropriate buffer containing 0.1% Triton X-100. Elution occurred at a flow rate of about 0.8 mL/h. Fractions of 2 mL were collected.

Elution profiles were established by estimating protein content (absorbance at 802 nm) and the detergent concentration, which was determined by measurement of either radioactivity or absorbance. In the first case, the photoreaction center was first equilibrated with tritium-labeled Triton X-100 of known specific activity (1.036×10^3 Bq/g). After elution, a small aliquot (40 μL) of each fraction was added to 10 mL of a Triton X-100–toluene scintillation mixture (Patterson & Green, 1965). The radioactivity in each sample was counted 3 times for 5 min in a Packard liquid scintillation counter. All results were corrected for background. Triton X-100 was also assayed spectrophotometrically by using an experimentally determined extinction coefficient, $E_{280,1cm}^{\%}$, of 19 in 10 mM Tris-HCl (pH 7.5). This value is in agreement with those of Holloway (1973) and Clarke (1975). The assay was performed on the photoreaction center–Triton X-100 complex, the A_{280} of which is due to both of these components. In order to determine the detergent content, we had to take the intrinsic A_{280} of the photoreaction center into account. It was calculated from the value of A_{365}, to which Triton X-100 contributes only negligibly, multiplied by a constant (0.94) obtained from Figure 1 of the preceding article (Vadeboncoeur et al., 1979). Both methods were used in this work and they gave identical results.

Polyacrylamide Gel Electrophoresis. The method was the same as that described in the preceding article (Vadeboncoeur et al.,1979) except that the samples were dispersed in 10 mM Tris–acetate (pH 8.15) containing 0.1% $NaDodSO_4$ and dialyzed overnight at 20 °C against the same buffer. β-Mercaptoethanol (0.1%) was added to the sample just before electrophoresis. Those samples prepared by sucrose density gradient centrifugation were used directly as they were collected from the centrifuge tube.

Ultracentrifugation. Sedimentation studies were carried out with a Beckman Spinco Model E analytical ultracentrifuge equipped with a photoelectric scanning absorption system. Double-sector Epon cells were used. For sedimentation velocity experiments, the speed was controlled at 40000 rpm and temperature was controlled at 20 °C. The moving boundary was analyzed at 365 nm every 8 min. The observed sedimentation coefficient was corrected for solvent density and viscosity to standard conditions; the densities and viscosities of the various solutions were determined experimentally. For sedimentation equilibrium, the speed was 15000 rpm and the temperature was 20 °C. Equilibrium was attained after 24 h. Molecular weights were calculated from the slope of the usual plot of ln c as a function of r^2, where c is the protein concentration and r is the distance from the center of rotation.

Partial Specific Volume. The method of Edelstein & Schachman (1967) was used to determine the partial specific volume of the photoreaction center-Triton X-100 complex. The solvent contained 100 mM sodium phosphate (pH

7.5)–0.1% Triton X-100 (w/v) in H_2O or D_2O (New England Nuclear; 99.7% pure); for the experiment in D_2O, a 0.1-mL sample of the photoreaction center solution was diluted 10-fold with the D_2O-containing buffer and then dialyzed vs. 10 mL of D_2O-containing buffer with two changes during a 24-h period. The sedimentation equilibrium experiments were carried out simultaneously using two double-sector cells.

The method of Tanford et al. (1974) was used to calculate the buoyant density factor for use in interpreting sedimentation equilibrium data to yield the molecular weight of pure protein in a multicomponent system. The partial specific volume of the reaction center protein was calculated from the amino acid composition published in the accompanying paper and was found to be 0.732 mL/g. Tanford et al. (1974) have found this method, based on studies of Cohn & Edsall (1965) and Mc Meekin & Marshall (1952), to give a good approximation of $\bar{v}_p$. The partial specific volume of Triton X-100, measured pycnometrically, was found to be 0.94 mL/g.

Determination of Stokes Radius. Approximately 0.5 mg of the photoreaction center was applied to a Sepharose 6B column (1.5 × 80 cm) equilibrated with a buffer containing 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100 at 20 °C. The column was calibrated with standard proteins (ferritin, catalase, hemoglobin, and myoglobin); its void volume was measured with dextran blue, and the internal volume was measured with riboflavin. The Stokes radius, R_S, of the detergent–protein complex was determined from a plot of log R_S as a function of K_{av}, the distribution coefficient (Demassieux & Lachance, 1974). The values of R_S for the standards were taken from Siegel & Monty (1966) except for myoglobin, the Stokes radius of which was calculated from its diffusion coefficient.

Results

$NaDodSO_4$ Binding. To verify that the electrophoretic method for molecular weight determination is applicable to the photoreaction center, we have compared $NaDodSO_4$ binding to the photoreaction center and to those proteins used as molecular weight markers. Experimental conditions exactly duplicated those used for determining the molecular weight by polyacrylamide gel electrophoresis (Vadeboncoeur et al., 1979). While bovine serum albumin, lysozyme, and chymotrypsinogen were found to bind 0.61, 0.72, and 0.68 g of $NaDodSO_4$ per g of protein, respectively, the photoreaction center from *Rs. rubrum* strain G9 bound 3.9 g of $NaDodSO_4$ per g of protein. It is thus clear that the photoreaction center binds considerably more $NaDodSO_4$ than the standard proteins. This anomalous binding would be expected to lead to an underestimate of the molecular weight of the polypeptide chains of the photoreaction center.

A set of control experiments demonstrated that, when the molecular weight marker proteins are first denatured in 6 M guanidine hydrochloride, $NaDodSO_4$ binding attains the level of 1.4 g of $NaDodSO_4$ per g of protein, as reported by Reynolds & Tanford (1970). The lower values presented above presumably result from incomplete equilibration under standard conditions for gel electrophoresis. Pitt-Rivers & Impiombato (1968) and Nelson (1971) have, in fact, shown that prolonged exposure of native proteins to $NaDodSO_4$ and β-mercaptoethanol is often required in order to reach true equilibrium.

Sedimentation Analysis of Photoreaction Center–Detergent Complexes. The photoreaction center sediments as a homogeneous material with a sedimentation coefficient $s_{20,w}$ of 4.89 S [0.1 M sodium phosphate (pH 7.5)–0.1% Triton X-100]. Since the moving boundary was analyzed at 365 nm, we cannot, with these results alone, exclude a contamination by other proteins which absorb only at 280 nm; analysis at this wavelength is impossible due to the presence of Triton X-100. However, the electrophoretic results of the preceding article (Vadeboncoeur et al., 1979) show the photoreaction center to be very pure.

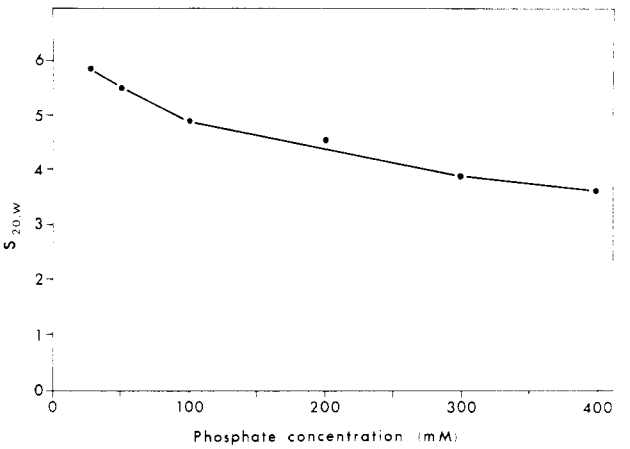

FIGURE 1: Variation of the sedimentation coefficient $s_{20,w}$ of the photoreaction center (*Rs. rubrum* strain G9) as a function of phosphate buffer concentration. For these experiments, the photoreaction center was dialyzed into sodium phosphate buffers (pH 7.5) of various concentrations containing 0.1% (w/v) Triton X-100.

When the molarity of the solvent is increased at constant pH and detergent concentration, the sedimentation coefficient of the photoreaction center decreases (Figure 1). This behavior was observed for both phosphate and Tris-HCl buffers. In all cases, the sedimenting boundary was symmetrical. Such behavior could indicate a progressive dissociation of the photoreaction center, a change in solvation (Aune & Timasheff, 1970), or a change in detergent binding as a function of salt concentration.

The sedimentation equilibrium method of Edelstein & Schachman (1967) makes it possible to determine both the partial specific volume and the molecular weight of a protein from the data of a single experiment. For the photoreaction center, this method yielded linear plots of ln c vs. r^2 in both H_2O and D_2O. Using an isotope exchange constant for protein, $K = 1.0155$ (Edelstein & Schachman, 1967; Hvidt & Nielsen, 1966), and neglecting isotope exchange with the detergent, we calculated a partial specific volume of 0.87_4 mL/g from the data; the corresponding molecular weight was 190000. This experiment was conducted in 10 mM sodium phosphate (pH 7.5)–0.1% Triton X-100. These values of partial specific volume and molecular weight are probably valid only for these conditions, since sedimentation velocity experiments reported above suggest a change in the properties of the particle as a function of salt concentration. These values may, however, be compared with those of Reiss-Husson & Jolchine (1972) for the photoreaction center of *Rp. sphaeroides* in a similar solvent. Their value of 0.87_7 mL/g for the partial specific volume is nearly identical but their molecular weight (150000) is significantly lower. This unusually high value of the partial specific volume for a protein (normally between 0.70–0.75 mL/g) must be due to the binding of a considerable amount of detergent since the photoreaction center is virtually lipid free (Vadeboncoeur et al., 1979).

A simple analysis of the partial specific volume found for the complex makes it possible to approximate the amount of detergent bound. The partial specific volume of the complex, $\bar{v}^*$, can be expressed in terms of $\bar{v}_P$, partial specific volume of pure protein, δ_D, the bound detergent/protein weight ratio, and

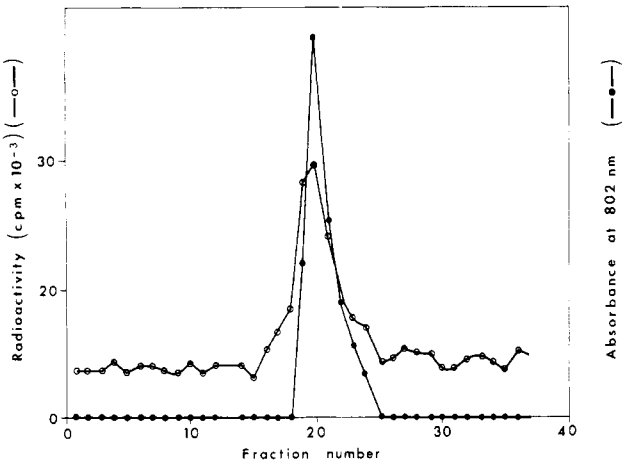

FIGURE 2: Equilibration of the photoreaction center (*Rs. rubrum* strain G9) in 10 mM Tris-HCl (pH 7.5) containing 0.1% (w/v) [^{3}H]Triton X-100 on a column of DEAE-cellulose. The column was washed with 100 mL of this buffer. This was sufficient to ensure that the radioactivity leaving the column was constant. The photoreaction center was eluted with the same buffer containing [^{3}H]Triton X-100 and 0.3 M NaCl. Radioactivity (●). Absorbance (○).

$\bar{v}_D$, partial specific volume of detergent when bound to protein (Tanford et al., 1974):

$$\bar{v}^* = \frac{\bar{v}_P + \delta_D \bar{v}_D}{1 + \delta_D} \quad (1)$$

Using the experimental values of $\bar{v}^*$ and $\bar{v}_D$ and the calculated value of $\bar{v}_P$, we find δ_D equal to 2 g of detergent per g of protein. Thus, the molecular weight of protein in the photoreaction complex must be about 190 000/3 = 63 000.

The method of Edelstein & Schachman (1967) is strictly applicable to the photoreaction center in detergent solution only if detergent binding is the same in the H_2O and D_2O buffers. We have no direct data on this. But others (Simons et al., 1973; Clarke, 1975) have shown that some membrane proteins do indeed bind different amounts of detergent in H_2O and in D_2O. Thus, the results of this section must be verified by an independent method.

Binding of Triton X-100 to the Photoreaction Center. To prepare the photoreaction center containing a constant amount of bound detergent, we established reproducible equilibration methods. Since Triton X-100 crosses dialysis membranes very slowly, chromatography appeared more suitable than dialysis. The quantity of bound Triton X-100 was determined as described under Materials and Methods.

The first experiments were carried out on a DEAE-cellulose column. A typical elution profile is shown in Figure 2: the detergent peak always slightly preceded the protein peak on this type of column. Such behavior has also been noted by Osborne et al. (1974) for bovine rhodopsin in Triton X-100 solutions during chromatography on ECTEOLA–cellulose. This could be due to variation in detergent binding as a function of salt concentration, as may be inferred from the sedimentation velocity experiments above. Measurements of the quantity of protein and of detergent in the peak fraction of this chromatography and of two similar experiments showed that the photoreaction center binds 1.2 ± 0.2 g of detergent per g of protein (Table I).

A second set of chromatography experiments making use of Sepharose 6B as chromatographic support permitted variations in salt concentrations (profile not shown). The Triton X-100 micelles were only slightly separated from the protein under the experimental conditions, and this limits the accuracy of the method. Only the peak fraction (A_{802}) was used for analysis. Results are summarized in Table I. Experimental variation was greater here than in the experiments with DEAE-cellulose; for example, in experiments with 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100, δ_D, the bound detergent/protein weight ratio, varied between 1.8 and 2.3. However, the results obtained by either method fell within the same range. It is also clear that detergent binding depends on salt concentration: δ_D varied from 1.2 to 2.3 as the salt concentration decreased. This undoubtedly explains the increase of $s_{20,w}$ at low salt concentration.

Table I: Characterization of Photoreaction Center–Triton X-100 Complexes

equilibration method	buffer	no. of expt	av detergent binding[a]	M_r of protein moiety
DEAE-cellulose	10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100–300 mM NaCl	3	1.2	58 000
Sepharose 6B	10 mM sodium phosphate (pH 7.5)–0.1% Triton X-100	2	2.3	56 000
Sepharose 6B	10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100	3	2.1	59 000
Sepharose 6B	100 mM Tris-HCl (pH 7.5)–0.1% Triton X-100	1	1.6	57 000
Sepharose 6B	200 mM Tris-HCl (pH 7.5)–0.1% Triton X-100	1	1.3	58 000

[a] In g/g of protein.

Molecular Weight of Protein in the Photoreaction Center–Detergent Complexes. Sedimentation Equilibrium. Sedimentation equilibrium experiments were carried out on the same chromatographic fractions that were analyzed for detergent binding. The data were interpreted by using the approach outlined by Tanford et al. (1974). The molecular weight, M_r, of pure protein is related to the experimentally determined protein concentration, c, as a function of radial position, r, by the relation

$$2RT/\omega^2[\mathrm{d} \ln c/\mathrm{d} r^2] = M_r(1 - \phi'\rho) \quad (2)$$

where ω is the radial velocity of rotation, ρ is the density of the solution, and ϕ is the effective partial specific volume. The right-hand side of eq 2 may be equated to eq 3, which then

$$M_r(1 - \phi'\rho) = M_r[(1 - \bar{v}_P\rho) + \delta_D(1 - \bar{v}_D\rho)] \quad (3)$$

permits calculation of the buoyant density factor. Pigments which account for less than 6% of the total weight and lipids (Vadeboncoeur et al., 1979) were considered negligible in this analysis.

The final column of Table I presents the various molecular weight values calculated from sedimentation equilibrium and detergent binding data. The values vary from 56 000 to 59 000, depending on the conditions of buffer and salt concentration. Experimental error in the determination of detergent binding does not drastically affect the molecular weight values. For example, variation in δ_D between 1.0 and 1.4 in 10 mM Tris-HCl (pH 7.5)–300 mM NaCl and between 1.8 and 2.3 in 10 mM Tris-HCl (pH 7.5) yields respective molecular weight values of 61 000–55 000 and 61 000–56 000.

The average molecular weight of all 10 experiments reported in Table I is 57 800 ± 1135. This value is far from the minimal molecular weight of 87 000. In fact, in order to obtain that

value from the sedimentation equilibrium data, using eq 2 and 3, we must assign detergent binding, δ_D, a value of zero. This is clearly in contradiction of the experimental data on detergent binding and can be considered an unacceptable hypothesis.

Molecular Weight of Protein in Photoreaction Center–Detergent Complexes Using $s_{20,w}$ and R_S. An alternate method of determining protein molecular weight in protein–detergent complexes, proposed by Tanford et al. (1974), combines the sedimentation coefficient, s, and the Stokes radius, R_S:

$$s = \frac{M_r(1 - \phi'\rho)}{6\pi\eta N R_S} \quad (4)$$

where η is the viscosity of the solvent. The Stokes radius is a property of the whole macromolecular particle, in this case the protein–detergent complex. It can be determined by gel filtration. The sedimentation coefficient and the value of ϕ' must be determined in the solvent used for determining R_S. In 10 mM Tris-HCl (pH 7.5)–0.1% Triton X-100, the photoreaction center–Triton X-100 complex has a Stokes radius of 42 Å. The sedimentation coefficient in this solvent is 5.34 S; the average value of δ_D is 2.1 g of Triton X-100 per g of protein. Inserted into eq 3 and 4, these data yield a molecular weight value of 65 000, close to that obtained by sedimentation equilibrium.

Symmetry of the Photoreaction Center–Triton X-100 Particle. From these data, we can obtain a general idea of the shape of the photoreaction center–Triton X-100 particle in solution. This can be done by comparing the experimentally measured Stokes radius, R_S, to the radius, R_{min}, of an anhydrous spherical particle of the same molecular weight and binding the same amount of detergent (Tanford et al., 1974)

$$R_{min}^3 = \frac{3M_r(\bar{v}_P + \delta_D\bar{v}_D)}{4\pi N} \quad (5)$$

The ratio R_S/R_{min} is a measure of the sphericity of the particle. Because of water of hydration, values of $R_S/R_{min} \leq 1.25$ are considered as characteristic of globular particles, while higher values indicate more elongated shapes (Tanford et al., 1974). In the present case, we find a value of 1.03. The photoreaction center–Triton X-100 complex appears therefore to be a spherical particle with little or no water of hydration, in contrast to other hydrophobic protein–Triton X-100 complexes reported to be asymmetrical (Cuatrecasas, 1972; Clarke, 1975).

Sedimentation Studies of the Photoreaction Center from Rp. sphaeroides. In order to clarify the apparent discrepancy between minimal molecular weight (87 000) and molecular weight determined by sedimentation equilibrium (57 800) for *Rs. rubrum*, we have carried out sedimentation studies on the photoreaction center from *Rp. sphaeroides*, which has the same minimal molecular weight.

Purified photoreaction center from this organism was equilibrated with Triton X-100 by DEAE-cellulose chromatography, and detergent concentration in the fraction of maximum absorption at 802 nm was measured by spectrophotometry, as explained under Materials and Methods. The photoreaction center bound 1.3 g of Triton X-100 per g of protein, a value similar to that determined for the preparation from *Rs. rubrum* strain G9 in the same experimental conditions (Table I).

The results of the sedimentation equilibrium experiments carried out on this photoreaction center are shown in Figure 3. From the slope of the straight line relating ln c to r^2 (closed circles) and the buoyant density factor calculated according to eq 2 and 3, we find a molecular weight of 87 000, very close

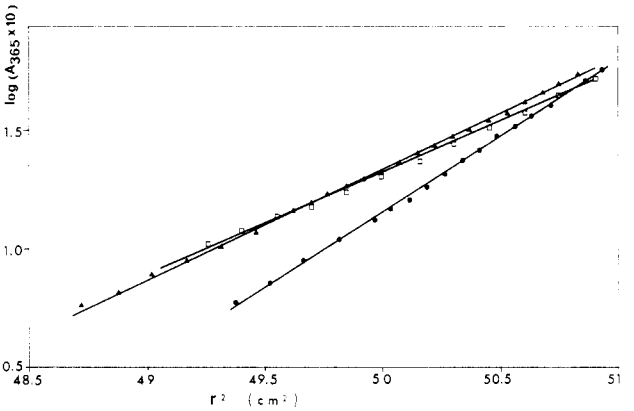

FIGURE 3: Sedimentation equilibrium of the photoreaction centers from *Rs. rubrum* strain G9, *Rp. sphaeroides* strain 2.4.1, and the dimer $\beta\gamma$ isolated from the latter. Samples were equilibrated with Triton X-100 by DEAE-cellulose chromatography; the buffer for sedimentation equilibrium contained 10 mM Tris-HCl (pH 7.5)–300 mM NaCl–0.1% (w/v) Triton X-100. Other experimental conditions are presented under Materials and Methods. (□) Photoreaction center from *Rs. rubrum* strain G9. (●) Photoreaction center from *Rp. sphaeroides* strain 2.4.1. (▲) $\beta\gamma$ dimer from the photoreaction center of *Rp. sphaeroides* strain 2.4.1.

to the minimal molecular weight of 90 000 reported by Steiner et al. (1974). Here, in contrast to the case of *Rs. rubrum* strain G9, the minimal molecular weight and the molecular weight determined by sedimentation equilibrium agree.

Sedimentation Studies on the $\beta\gamma$ Dimer from the Photoreaction Center of Rp. sphaeroides. In a mixture of $NaDodSO_4$ and LDAO, the two smaller subunits (β and γ) of this photoreaction center remain associated and retain their pigments; they may be separated from the third subunit, α, by sucrose density gradient centrifugation (Okamura et al., 1974). It appeared plausible to us that the difference between minimal and experimentally determined molecular weight for the photoreaction center of *Rs. rubrum* strain G9 could be explained on a similar basis: in the buffers used for sedimentation equilibrium, the photoreaction center might dissociate into a dimer, $\beta\gamma$, bearing pigments and thus absorbing at 365 nm and a separate polypeptide, α, without pigments. Repeated attempts to separate the putative $\beta\gamma$ dimer from chain α by gel filtration, in both Tris-HCl and phosphate buffers of varying ionic strength containing 0.1% (w/v) Triton X-100, were unsuccessful. In all cases, a single peak was observed, and this peak was found, by polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ and β-mercaptoethanol, to contain each of the three chains α, β, and γ. This result suggests that, if chain α is indeed separated from the $\beta\gamma$ dimer in solution, it must self-aggregate to form an oligomer of dimensions comparable to those of the $\beta\gamma$ dimer.

We thus turned to experimental conditions adapted from Okamura et al. (1974) in order to separate the $\beta\gamma$ dimer from chain α. However, the presence of 0.1% $NaDodSO_4$ in the solvent caused the photoreaction center of *Rs. rubrum* (both strains S1 and G9) to lose photochemical activity, in contrast to the case of the photoreaction center of *Rp. sphaeroides* which retains activity in these conditions. Hence we elected to continue our study with the $\beta\gamma$ dimer from the photoreaction center of *Rp. sphaeroides*.

The purified photoreaction center (*Rp. sphaeroides*) was treated as described under Materials and Methods to separate $\beta\gamma$ from α. Figure 4 shows the sucrose density gradient profile and the $NaDodSO_4$–polyacrylamide gels of the protein pools made from the two gradient bands. Material from the upper

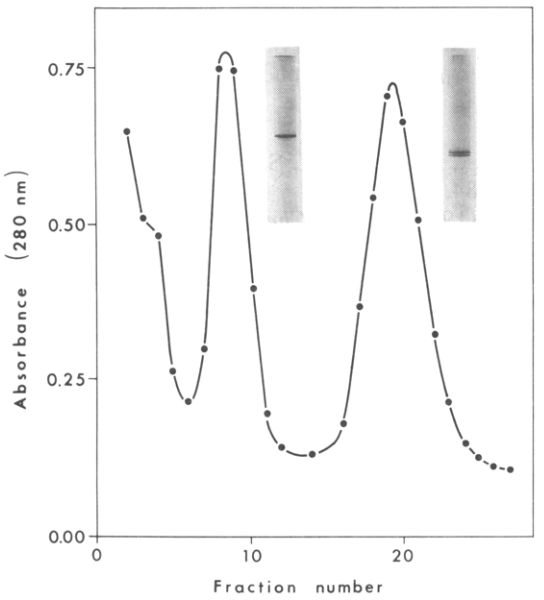

FIGURE 4: Separation of dimer $\beta\gamma$ from chain α of the photoreaction center from *Rp. sphaeroides* strain 2.4.1 by sucrose density gradient centrifugation. The experimental details are presented under Materials and Methods. Sedimentation is from left to right. Polyacrylamide gel electrophoresis was carried out on pools from fractions 8–11 (upper colorless band) and from fractions 15–20 (lower pigmented band).

colorless band shows a single protein which migrates as α. Material from the lower colored band shows two proteins which migrate, on $NaDodSO_4$–polyacrylamide gels, to positions characteristic of β and γ: the absorption spectrum of this material showed the absorption bands associated with sphaeroidene, bacteriochlorophyll, and bacteriopheophytin. The uppermost fractions (1–5) contain no protein. We think that their optical density is probably due to light scattering by detergent micelles.

The $\beta\gamma$ dimer was equilibrated with Triton X-100 by DEAE-cellulose chromatography, and detergent concentration in the fraction of maximum absorption at 802 nm was measured by spectrophotometry. Protein concentration in that fraction was estimated by using an $E_{802,1cm}^{1mg/mL}$ of 3.13 divided by 2/3 to correct for the absence of polypeptide α. Dimer $\beta\gamma$ was found to bind 2 g of Triton X-100 per g of protein.

Sedimentation equilibrium results for dimer $\beta\gamma$ (closed triangles) and for photoreaction center preparations from *Rp. sphaeroides* (closed circles) and from *Rs. rubrum* (open squares) are shown in Figure 3. As expected, dimer $\beta\gamma$ from *Rp. sphaeroides* and the photoreaction center from *Rs. rubrum* have comparable molecular weights, both of which are lower than that of the intact photoreaction center from *Rp. sphaeroides*. Insertion of proper data into eq 2 and 3 yields molecular weight values of 57 000 for dimer $\beta\gamma$, 58 000 for the photoreaction center from *Rs. rubrum*, and 87 000 for the photoreaction center from *Rp. sphaeroides*.

Discussion

The major goal of this investigation was to establish the molecular weight of the photoreaction center from *Rs. rubrum* and thereby to obtain information about its quaternary structure in solution. This is not a trivial problem since this protein is solubilized as a complex with detergent.

The sum of the apparent molecular weights of the three subunits determined by $NaDodSO_4$–polyacrylamide gel electrophoresis is 76 000 (Noël et al., 1972; Okamura et al., 1974; Vadeboncoeur et al.,1979). Our data show that the photoreaction center binds considerably more $NaDodSO_4$ than the molecular weight marker proteins used to establish the calibration curve relating mobility to molecular weight. As a consequence, the electrophoretic method underestimates the molecular weight of the photoreaction center. We feel that this accounts for the discrepancy between electrophoresis results and the minimal molecular weight of about 90 000.

The other paradox is the molecular weight value of about 150 000, obtained by sedimentation equilibrium and by molecular sieving (Reiss-Husson & Jolchine, 1972; Noël et al., 1972).

However, the results reported here for the sedimentation equilibrium method of Edelstein & Schachman (1967), although yielding a particle weight of 190 000 for the photoreaction center, gave a partial specific volume of 0.87 mL/g. In the virtual absence of lipids, such a high specific volume immediately suggested that the photoreaction center forms a complex with Triton X-100. From the estimated complex composition of 2 g of Triton X-100 per g of protein, it appeared that the true molecular weight of the protein might be 63 000.

Because of uncertainty concerning the applicability of the method of Edelstein & Schachman (1967) to the photoreaction center, we turned to direct analysis of detergent binding. The photoreaction center binds a considerable amount of detergent, varying from 1.2 to 2.3 g of Triton X-100 per g of protein, according to salt concentration. Under salt conditions identical with those used to measure partial specific volume of the complex, we found detergent binding to be 2.3 g/g of protein, in close agreement with the estimate of binding derived from the partial specific volume. This suggests that the method of Edelstein & Schachman (1967) is indeed applicable to the photoreaction center.

Tanford et al. (1974) have outlined and validated an approach for evaluation of the molecular weight of a protein in detergent solution. Their approach uses a calculated buoyant density factor to analyze the results of sedimentation equilibrium. Following this approach, we have determined the molecular weight of the pure photoreaction center protein in 0.1% Triton X-100 solutions to be 58 000. Another method we have used is the combination of sedimentation coefficient and Stokes radius in an equation which again makes use of the calculated buoyant density factor. We obtained 65 000.

Thus, three independent approaches have yielded values of about 60 000. Yet the minimal molecular weight is 87 000. In an attempt to explain this difference, we first examined the photoreaction center from *Rp. sphaeroides*, known to be rather similar to that of *Rs. rubrum*. However, the molecular weight of this protein, measured by sedimentation equilibrium, proved to be 87 000, a value close to the minimal molecular weight of 90 000 reported by Steiner et al. (1974). We then found that the $\beta\gamma$ dimer from *Rp. sphaeroides* and the photoreaction center from *Rs. rubrum* strain G9 have very similar molecular weights, when measured by sedimentation equilibrium.

This indicates that the photoreaction center from *Rs. rubrum* strain G9, at least under our experimental conditions, dissociates in solution into a dimer composed of the two lighter subunits β and γ and into a free α subunit. Since the latter is nonpigmented, we were unable to estimate its state of aggregation in the presence of Triton X-100, which precludes optical scanning at 280 nm. (Our attempts at using Schlieren optics were unsuccessful because of the relative insolubility of the photoreaction center.) However, the gel filtration experiments mentioned earlier suggest that chain α forms an aggregate which must be of a size comparable to that of the $\beta\gamma$ dimer. Cross-linking experiments with dimethyl suber-

imidate, followed by $NaDodSO_4$ gel electrophoresis, gave results which, although unexpected, are in agreement with this model (Vadeboncoeur, 1978). They show chain α to exist as an oligomer in which the chains are readily cross-linked; no $\alpha\beta$ or $\alpha\gamma$ dimers were observed. Surprisingly, no $\beta\gamma$ dimers were found, but this may be due to their relatively lower lysine contents. In contrast, under the same experimental conditions, the cross-linked photoreaction center from *Rp. sphaeroides* strain 2.4.1 yielded $\alpha\gamma$ dimers [see also Feher & Okamura (1978)], showing the α chain to be intimately associated at least with chain γ in this protein. This correlates well with the sedimentation equilibrium results and indicates that this photoreaction center is a trimer composed of three subunits, α, β, and γ.

The molecular weight obtained by sedimentation equilibrium may be compared to values calculated from other parameters. If the photoreaction center from *Rp. sphaeroides* strain 2.4.1 is indeed an oligomer composed of a single copy of each of the three basic polypeptide chains, its molecular weight should be the sum of its minimal molecular weight plus its weight in pigments; i.e., 90 000 (Steiner et al., 1974) + 6000 = 96 000. This is in fair agreement with 87 000 obtained by sedimentation equilibrium.

The same assumptions would lead to a molecular weight of 93 000 for the photoreaction center from *Rs. rubrum* strain G9. This is in complete disagreement with our experimental observations. However, if the particle analyzed by ultracentrifugation is the $\beta\gamma$ dimer, its molecular weight (M_r) may be arrived at by the equation

$$M_r = M_{r(p)} + (M_{r(a)}{}^{\beta} + M_{r(a)}{}^{\gamma})K$$

where $M_{r(p)}$ is the weight of 4 mol of bacteriochlorophyll + 2 mol of bacteriophaeophytin + 1 mol of spirilloxanthin = 6000; $M_{r(a)}{}^{\beta}$ and $M_{r(a)}{}^{\gamma}$ are the apparent molecular weights of subunits β and γ, respectively; and K is the ratio of minimal molecular weight to the sum of the apparent molecular weights of subunits α, β, and γ, i.e., 87 000/76 000.

The calculated result is 58 000, in excellent agreement with the value obtained by sedimentation equilibrium (57 800).

This work gives some clues as to the quaternary structure of the photoreaction center in the chromatophore membrane. We feel that the weight of the evidence indicates that in situ the oligomer is of the type $\alpha_1\beta_1\gamma_1$. This is consistent with the stoichiometry of $\alpha/\beta/\gamma$ = 1:1:1 for the isolated photoreaction centers from *Rp. sphaeroides* and from *Rs. rubrum* (Okamura et al., 1974; Vadeboncoeur et al., 1979) as well as with the molecular weight data on the preparation from *Rp. sphaeroides*. By analogy, we suppose that the photoreaction center of *Rs. rubrum* is also of the $\alpha_1\beta_1\gamma_1$ type, in situ. We feel that it is only during or after its extraction from the membrane that it dissociates into subunit α and dimer $\beta\gamma$.

This leaves open the question of the role of subunit α. It may be of interest, however, that, among 300 known proteins listed by Klotz et al. (1975), only 10 have an odd number of subunits and that, among these, only bovine procarboxypeptidase A, a bifunctional zymogen, has a subunit structure of the type $\alpha_1\beta_1\gamma_1$ like the photoreaction center.

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