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SIZE AND SHAPE OF DETERGENT-SOLUBILIZED PHOTOCHEMICAL REACTION CENTERS FROM TWO STRAINS OF *RHODOPSEUDOMONAS SPHAEROIDES*

A SOLUTION X-RAY SCATTERING STUDY

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Solubilized reaction centers purified from *Rhodopseudomonas sphaeroides* (wild type and R26 strains) were studied in a nondenaturing detergent, dodecyldimethylamine *N*-oxide, by solution X-ray scattering. Some thermodynamic parameters were also obtained by coupling the results of this study with sedimentation equilibrium data previously obtained (Rivas, E., Reiss-Husson, F and le Maire, M. (1980) Biochemistry 19, 2943–2950). The particle weight of both types of reaction centers was found to be about 160 000 Da, corresponding to a protein molecular weight close to 90 000. Both hydrodynamic and solution X-ray scattering experiments suggest that the complexes have a globular shape, with a maximal chord of about 90 Å as indicated by the autocorrelation function. This maximal dimension is probably created by the binding of detergent to the solubilized complex. The approach followed in this study to investigate the shape of detergent-protein complexes involved a comparison of the Stokes' radii and the radii of gyration of various proteins.

Introduction

Reaction centers purified from *Rhodopseudomonas sphaeroides* (wild type and R26 strains) have been characterized in detergent solutions (Triton X-100 and dodecyldimethylamine *N*-oxide) by sedimentation equilibrium and sedimentation velocity studies [1,2]. These complexes are multimolecular structures (for a review see Ref. 3) composed of: (1) three polypeptide chains which are integral membrane proteins, hereafter referred to as the 'protein'; (2) a fixed number of associated molecules: four bacteriochlorophyll, two bacteriopheophytin, two ubiquinone Q-10, one Fe, one spheroidene and a few phospholipid molecules (the last two components are only present in the wild-type strain); (3) a fixed number of bound detergent molecules maintaining the complexes in solution in a monodisperse and native-like state ('native' in the sense of being capable of performing the primary photochemical reaction). In a previous work [2], we found by sedimentation equilibrium that the molecular weight of the protein moiety of the two complexes is about 84 000, and we suggested on the basis of hydrodynamic studies that the overall shape of the entire complexes (protein + associated molecules + detergent) is globular. However, because of the great complexity of the detergent-solubilized reaction centers, any physico-chemical study is bound to be dif-

Abbreviation: Tes, *N*-tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid.

ficult and the results obtained are necessarily less precise than in comparable studies performed on water-soluble proteins devoid of prosthetic group. Further study is therefore warranted, and we decided to pursue our investigation on the size and shape of the reaction center complexes by the small-angle X-ray scattering technique. We took the opportunity to combine the sedimentation equilibrium and solution X-ray scattering results as was recently done in the case of the Ca^{2+}-ATPase solubilized in deoxycholate and the 50 S subunit of the *Escherichia coli* ribosome [4,5]. In the present study this approach has been somewhat extended by a comparison of the Stokes' radius and the radius of gyration. Together with information recently published by other laboratories on reaction centers in membranous form [6–12], we propose a low-resolution model for the dodecyldimethylamine *N*-oxide-reaction center complex, based on all the structural information that we have gathered from our sedimentation equilibrium, sedimentation velocity and solution X-ray scattering experiments.

Materials and Methods

Preparation of solubilized reaction centers. Wild-type (strain Y) and mutant (strain R26) *R. sphaeroides* were prepared in 1 mg/ml dodecyldimethylamine *N*-oxide, 10 mM Tes (pH 7.5), 50 mM NaCl as previously described [2]. Ion-exchange (hydroxyapatite or DEAE-cellulose) chromatography allowed the elution of a concentrated peak of reaction center complex [2], and, when needed, the sample was further concentrated by centrifugation for 22 h at $150\,000 \times g$. Dialysis against the above buffer was then performed except when a combined ion-exchange-gel filtration chromatography was used [2,13].

Monodispersity and native state of the samples. Despite all the precautions taken, not all the highly concentrated samples (up to 30 mg/ml protein) that we prepared for solution X-ray scattering could be used: out of a dozen preparations four had to be discarded, since they were either found to be aggregated or had lost the visible spectra typical of an intact reaction center [14,15]. The state of aggregation was ascertained by analytical ultracentrifugation as described in Ref. 2, or directly by solution X-ray scattering, in which case the Guinier plots of the aggregated samples were nonlinear, showing an upward bend near the origin. We cannot explain why some samples behave differently from the rest. However, factors favoring aggregation were exposure to light or room temperature, and the presence of oxidizing impurities in the dodecyldimethylamine *N*-oxide [2]. Visible and ultraviolet spectra [14,15] were recorded before and after solution X-ray scattering with no noticeable differences.

Chemical characterization and physical parameters needed for solution X-ray scattering. We have used the composition of the dodecyldimethylamine *N*-oxide-solubilized wild-type and R26 strain given in Table I of Rivas et al. [2]. This composition is generally agreed upon in the literature (see, e.g., Ref. 3). The sum of the molecular weights of the nonprotein and nondetergent components of the complexes can be calculated to be 12428 (wild type) and 7209 (R26). In Refs. 2 and 13 we have paid particular attention to the measurements of dodecyldimethylamine *N*-oxide/protein binding ratios and we have used the values reported in these studies (0.58 g/g for the wild type and 0.67 g/g for R26) throughout this work because they pertain to the same experimental conditions as in the present work. To our knowledge, there is only one other reported value for the dodecyldimethylamine *N*-oxide/protein binding ratio (to the R26 strain). It is cited by Feher and Okamura in Ref. 3 but the experimental conditions are unpublished. Protein concentrations [16] were corrected as described [2,17]. Tables I and II of Ref. 2 and the amino acid composition [17] allow the calculation of the partial specific volumes of the complexes (v_2, in cm^3/g), their electron partial specific volumes (ψ_2 in $Å^3/e$) and the ratios of electrons to molecular weight of the complexes ($\mu_2 = m_2/M_2$), where m_2 and M_2 are the number of electrons and molecular weight of the solubilized complex, respectively. The values of μ_2 are 0.5457 for the wild type and 0.5467 for R26.

Equipment and theoretical treatments. The equipment, experimental procedures, notations and equations necessary for the interpretation of the solution X-ray scattering or the coupled used of solution X-ray scattering and sedimentation equilibrium are described elsewhere [4,5]. Briefly, the

experimental quantity measured by solution X-ray scattering, the normalized intensity at the origin, $i_n(0,\rho_0)/c_{e_2}$, is given by:

$$i_n(0,\rho_0)/c_{e_2} = m_2(1-\rho_0\psi_2)^2$$

$$= M_2\mu_2\left(1-\frac{\rho_0 v_2}{N\mu_2 10^{-24}}\right)^2 \quad (1)$$

where c_{e_2} is the concentration expressed as the ratio of number of electrons of the complex to the number of electrons of solution, ρ_0 the electron density (e/Å^3) of the solvent and N Avogadro's number. M_2 can thus be calculated if v_2 is known or if the same sample is subjected to sedimentation equilibrium analysis [4,5]. The protein molecular weight, M_P, is calculated from:

$$M_2 = M_P(1+\delta_D) + \Sigma n_i M_i \quad (2)$$

where δ_D is the amount of detergent bound in g/g protein, and $\Sigma n_i M_i$ the sum of the molecular weights of the nonprotein and nondetergent components of the complexes.

The value of the radius of gyration, calculated from the Guinier plots, is defined by the following equation:

$$R_G^2 = \int[\rho(\vec{r})-\rho_0]r^2 d\vec{v_r}/\int[\rho(\vec{r})-\rho_0]d\vec{v_r} \quad (3)$$

where $\rho(\vec{r})$ is the electron density of the elementary volume $d\vec{v_r}$ at point $\vec{r}$. The origin of $\vec{r}$ is chosen to be the center of mass of $[\rho(\vec{r})-\rho_0]$. Thus, in solution X-ray scattering, R_G depends both on the shape of the particle and on the distribution of the electron density contrast. Consequently, R_G is not necessarily identical to the radius of gyration of the volume V of the particle which depends solely on its shape:

$$R_V^2 = \int_V r^2 dV \quad (4)$$

When the electron density is uniform within the particle $R_V = R_G$; if not, a relation between R_V and R_G exists and R_V can be calculated from R_G by the procedure of solvent electron density variation [18–21].

One may define, as suggested in Ref. 22, a minimum radius of gyration of the volume, $R_{V\min}$, which is the radius of gyration that the particle (in this case, the complex) would have if it were a dry solid sphere:

$$R_{V\min} = \sqrt{\tfrac{3}{5}}\left(\frac{3M_2 v_2}{4\pi N}\right)^{1/3} \quad (5)$$

The ratio $R_V/R_{V\min}$ is a parameter containing at the same time a hydration and an asymmetry term; the closer it is to unity, the more likely the particles are to have a spherical shape. In an analogous way, $R_S/R_{\min}$, numerically equivalent to the frictional ratio $f/f_{\min}$ [22], is the ratio of the Stokes' radius (R_S) over the minimum radius of the particle assuming no water bound and a spherical shape ($R_{\min}$). This is expressed by the following equations:

$$R_{\min} = \left(\frac{3M_2 v_2}{4\pi N}\right)^{1/3} \quad (6)$$

$$R_S = \frac{M_2(1-v_2 d_0)}{6\pi\eta s N} \quad (7)$$

where d_0 is the density of the solvent (g/cm^3), η its viscosity, and s the sedimentation coefficient of the particle; the quantity $M_2(1-v_2 d_0)$ is directly determined by sedimentation equilibrium. Alternatively, the Stokes' radius can be calculated from the diffusion coefficient [22].

Results and Discussion

Thermodynamic parameters

X-ray experiments were made at several protein concentrations and no dependence was detectable in the range of protein concentration used (from 5 to 27 mg/ml). A representative Guinier plot for the wild-type reaction center (Y strain) is shown in Fig. 1A. From this and other experiments, values of $m_2(1-\rho_0\psi_2)^2$ and radii of gyration were calculated for both types of reaction center (Table I). The molecular weights of the complexes (M_2) which can be calculated from the scattering data are highly dependent on v_2 (see Materials and Methods). We have recently introduced a new method to calculate simultaneously the values of M_2 and v_2 by combining the data obtained by the scattering and sedimentation equilibrium methods [4,5]. From Table II, it can be seen that the values

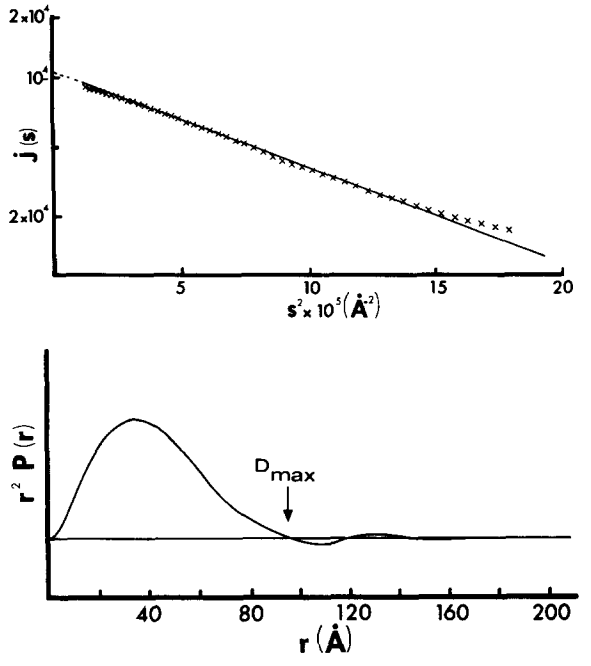

Fig. 1. (A) X-ray scattering of wild-type reaction center at 27 mg/ml: Guinier plot – logarithm of intensities plotted on an arbitrary scale as a function of scattering angle, s. $J(s)$ is the unsmoothed experimental intensity after background substraction and before collimation correction. (B) Autocorrelation function $r^2P(r)$ of mutant reaction center as a function of r. In this experiment the maximal dimension of the particle (D_{max}) was found to be 92 Å.

of v_2 obtained by this method agree with the calculated values of v_2, based on the known chemical composition of the complexes and assuming additivity of partial specific volumes of the individual components. This leads to M_2 and M_P values (Table II) which are nearly identical for the wild type and R26 strain, whatever method is used for the calculation (solution X-ray scattering, sedimentation equilibrium or a combination of both). The v_2 values obtained by sedimentation equilibrium in water isotopes (Table II) were about 2% lower, leading to lower M_2 and M_P: we do not know the reasons for this slight difference but it should be noted that this method, although it is expected to give precise results, rests on the assumption that in $H_2^{18}O$, the partial specific volume v_2 remains the same as in H_2O. In conclusion, our scattering results lead to a protein molecular weight for both reaction centers of about 93 000. This is a value which is slightly higher than previously proposed (approx. 84 000) on the basis of sedimentation equilibrium studies in $H_2O/H_2^{18}O$; it is also definitely higher than the sum of the molecular weights of the polypeptides chains as previously estimated by SDS gel electrophoresis (approx. 73 000) [17] which is still commonly used in the literature (see, e.g., Ref. 11). However, a recent report indicated that the previous SDS gel electrophoresis values were erroneous and suggested a protein molecular weight of 90 000–96 000, based on amino acid composition [23].

TABLE I

EXPERIMENTAL PARAMETERS OF THE SOLUTION X-RAY SCATTERING STUDY

Values are means ± S.D.

	Wild-type Y reaction center	R26 strain reaction center
$m_2(1-\rho_0\psi_2)^2$	890 ± 130 (5)	840 ± 100 (3)
R_G (Å)	29.5 ± 1.5	30 ± 1.5
D_{max} (Å)	87 ± 10 [a]	90 ± 10 [a]

[a] If the maximal dimension of the complex includes a layer of detergent surrounding the protein, the true value of D_{max} could be slightly higher, due to fluctuations in electron density within the detergent molecule.

Radius of gyration

The interpretation of the radii of gyration given in Table I (29.5–30 Å) in terms of molecular dimensions is somewhat difficult. The reason is that, unlike water-soluble proteins or the membrane protein Ca^{2+}-ATPase solubilized in deoxycholate [4], we cannot assume that the reaction center-dodecyldimethylamine *N*-oxide complex has a uniform electron density. Therefore, the measured radius of gyration, R_G, is not necessarily identical to the radius of gyration of the volume, R_V, which is meaningful parameter for a structural description of the object (see Materials and Methods and Refs. 18–21). However, we may cautiously proceed with the analysis by including auxiliary data, obtained on other membrane proteins. For the rhodopsin-dodecyldimethylamine *N*-oxide complex, Sardet et al. [19] measured both R_G and R_V and found that R_V is about 23% lower than R_G in water. The difference is mainly attributable to the effect of bound dodecyldimethylamine *N*-oxide.

TABLE II

PARTICLE WEIGHTS, PARTIAL SPECIFIC VOLUMES AND PROTEIN MOLECULAR WEIGHTS

Technique used	Wild-type Y reaction center			R26 strain reaction center		
	M_2	v_2 (cm^3/g)	M_P	M_2	v_2 (cm^3/g)	M_P
Combination of sedimentation equilibrium and solution X-ray scattering	160000 ± 23000	0.880 ± 0.017	94000 ± 17000	161000 ± 15000	0.886 ± 0.012	92000 ± 10000
Solution X-ray scattering in water	158000 ± 23000	(0.881 ± 0.005) [a]	92000 ± 16000	166000 ± 22000	(0.888 ± 0.005) [a]	95000 ± 15000
Sedimentation equilibrium in water [b]	161700 ± 9900	(0.881 ± 0.005) [a]	94500 ± 8500	163900 ± 11900	(0.888 ± 0.005) [a]	93800 ± 9500
Sedimentation equilibrium in water isotopes [b]	140700 ± 2100	0.863 ± 0.001	81200 ± 2100 87900 ± 1700	138900 ± 6100	0.868 ± 0.002	78900 ± 3600 86100 ± 3400

[a] Values in parentheses have not been measured but are calculated assuming additivity of partial specific volumes of individual components of the reaction centers

[b] From Ref. 2. It should be noted that in Ref. 2 we have used the symbols M^* and $\bar{v}^*$ instead of M_2 and v_2.

TABLE III

COMPARISON OF THE ASYMMETRY-HYDRATION PARAMETERS OF VARIOUS PARTICLES AS MEASURED BY HYDRODYNAMIC OR SCATTERING TECHNIQUES [a]

	R_S/R_{min}	R_V/R_{Vmin}
Water-soluble proteins and complexes [b]		
Human low-density lipoprotein	1.09	1.12
Catalase	1.25	1.28
β-lactoglobulin (A and B forms)	1.25	1.26
Lysozyme	1.32	1.24
Bovine serum albumin	1.35	1.42
Myosin	3.53	11.4
Solubilized membrane proteins with bound detergent [b]		
ATPase-deoxycholate	1.60	1.4
Rhodopsin-dodecyldimethylamide *N*-oxide	1.11	1.26
Reaction center dodecyldimethylamide *N*-oxide		
Wild-type Y	1.28	1–1.26 [c]
R26 strain	1.11	1–1.28 [c]

[a] The respective importance of the asymmetry and of the hydration is not necessarily the same for the hydrodynamic (R_S/R_{min}) or the scattering (R_V/R_{Vmin}) techniques (see text).

[b] Values were calculated from this work and Refs. 4, 19, 27, 28, 29 and 30 or found directly in Refs. 2, 22 and 24. The measurements of R_V/R_{Vmin} for the human low-density lipoprotein and the rhodopsin-dodecyldimethylamine *N*-oxide complex implied solvent electron density variations.

[c] The averages of M_2v_2 obtained by combined sedimentation equilibrium and solution X-ray scattering and sedimentation equilibrium in water isotopes were used: the range expresses the most likely extreme values of R_V/R_{Vmin} calculated from experimentally determined R_G (see text).

For the reaction center less dodecyldimethylamine *N*-oxide is bound (0.58 g dodecyldimethylamine *N*-oxide/g protein for the wild type and 0.67 g/g for R26) than in the case of rhodopsin (0.92 g/g). We therefore consider that for the reaction center the difference between R_V and R_G, which can be positive or negative depending on the respective position of the detergent and the protein, is unlikely to be more pronounced, i.e., $0.77R_G \leqslant R_V \leqslant 1.23R_G$. On the other hand, R_V is limited on the lower side by R_{Vmin}, the minimum radius of gyration of the volume, which is the radius of gyration that the complexes would have if they were dry, solid spheres ($R_{Vmin} \leqslant R_V \leqslant 1.23R_G$). From Eqn. 5, R_{Vmin} for the wild-type and R26 reaction centers is calculated to be 28.9 and 28.8 Å, respectively. Thus, if we compare these values with the experimentally determined R_G (Table I), one has $0.96R_G \leqslant R_V \leqslant 1.23R_G$.

Parameters which are most important for an evaluation of particle asymmetry are the R_S/R_{min} and R_V/R_{Vmin} ratios (Materials and Methods). In Table III, we have listed for various proteins a number of these values which were either found directly in the literature or which were calculated on the basis of literature data or from the present work (Tables I and II). As previously discussed [22], R_S/R_{min} and R_V/R_{Vmin} contain both a hydration and a asymmetry term which raise these ratios to values above unity. It should be noted that the importance of the hydration and of the asymmetry is not necessarily the same for the hydrodynamic (R_S/R_{min}) or the scattering (R_V/R_{Vmin}) experiments. Indeed, in hydrodynamic experiments (Eqn. 7) more bound water could be moved along with the particle than what is measured in solution X-ray scattering. Furthermore, specific shapes might affect R_S and R_G differently. However the question of deciding the respective importance of these two factors (asymmetry or hydration) only becomes crucial if R_S/R_{min} and R_V/R_{Vmin} are greater than 1.25 as most water-soluble globular proteins have an R_S/R_{min} value close to 1.25 [22] (Table III). An overall look at Table III, and in particular a comparison between the data obtained for the reaction centers and the rhodopsin-dodecyldimethylamine *N*-oxide complexes, leads to the conclusion that both of these are globular particles like typical water-soluble proteins. On the other hand, Ca^{2+}-ATPase in deoxycholate has higher R_S/R_{min} and R_V/R_{Vmin} values. Since we have measured the amount of bound water and found it to be low in this case (0.26 g/g complex, see Ref. 4), we can conclude that it has a more asymmetrical shape than the other complexes. It should be noted that these differences pertain to the whole complex and not only to its protein part; in fact, detergent could play a significant role in the overall shape (see later).

We would like to mention at this point that we have attempted to measure R_V by the ususal procedure of solvent electron density variation [5,18–21]. However, the addition of the low molec-

ular weight electron-dense compound that we have used (sucrose) produced aggregation of the reaction center-dodecyldimethylamine *N*-oxide complexes according to sedimentation equilibrium; this led to meaningless solution X-ray scattering results (unpublished data). Since this appeared peculiar in the light of the previous similar study on rhodopsin [19], we performed detergent-binding experiments and found that, at high sucrose concentration (approx. 40% w/w), the amount of dodecyldimethylamine *N*-oxide bound was only about 0.15 g/g reaction center, i.e., about 5-times less than the binding ratio measured in the absence of sucrose [2]. The decrease in detergent binding is likely to be the explanation for the observed aggregation.

Autocorrelation function

The Fourier transform of the intensity curve (Fig. 1A) allows the calculation of the autocorrelation function, $r^2P(r)$. An example of such a function obtained with the mutant reaction center (R26 strain) is shown in Fig. 1B. A complete interpretation of the autocorrelation function is not possible here because the complexes under study do not have a uniform electron density (see above or Refs. 18–21 for a detailed discussion). However, one important parameter, D_{max}, can be obtained from this function: it is the maximal chord of the particle (including bound detergent, water, etc.) which represents the point beyond which the autocorrelation function vanishes (Fig. 1B). For both reaction centers the maximum dimension of the protein-dodecyldimethylamine *N*-oxide complex is about 90 Å (Table I). It is interesting at this point to consider other information on membranous *R. sphaeroides* reaction centers. There is a large amount of literature data pertaining to the membrane topography of the reaction centers' polypeptide chains (see, for example, Refs. 6–8, 11 and 12]. From these data it seems clear that at least one and maybe all the three polypeptide chains cross the membrane, with the proteins probably not extending far outside the lipid bilayer. This view is also supported by neutron and X-ray diffraction data [9,10]. In the model presented by Pachence et al. [10] the reaction center extends about 6 Å on each side of the membrane, resulting in a total length of the protein in that orientation of about 60 Å. The maximal chord (diagonal) which can be deduced from the overall shape of this protein model is about 70 Å. This dimension is smaller than our D_{max} values, which may therefore reflect the presence of bound detergent. It is generally assumed that in a protein-detergent complex, the detergent covers the hydrophobic portion of the protein which, in the membrane, is protected by lipids [25]. Thus, with the model of Pachence et al. [10] in mind, the dimension which could have increased after solubilization would be the one previously embedded in the bilayer. The solubilized complex could be a rather globular object, for example, approximated by an oblate ellipsoid of axes 90, 90 and 70 Å. It should be noted that such an object would have a volume compatible with the molecular size experimentally determined in Table II (average of wild-type and R26 strains) assuming about 0.3 g H_2O bound per g complex [4,19], a D_{max} compatible with our experimental results (90 Å, Table I) and a radius of gyration of 32.5 Å. R_S/R_{min} and R_V/R_{Vmin} of this ellipsoid would be 1.11 and 1.12, respectively, in good agreement with the experimentally determined values (Table III). Interestingly, by freeze-fracture electron microscopy, the wild-type reaction centers in dodecyldimethylamine *N*-oxide also seem to be gobular objects with D_{max} values, after substraction of the layer of shadowing materials, of about 100 Å [26].

In conclusion, in the present study we have obtained independent confirmation of the molecular weights of the reaction centers and of their monodispersity in detergent solution. By comparison of available data on three solubilized membrane proteins we are able to conclude that the rhodopsin and the reaction center-dodecyldimethylamine *N*-oxide complexes have a globular shape, whereas the Ca^{2+}-ATPase deoxycholate complex is definitively more asymmetical.

Future studies by solution X-ray scattering or neutron scattering might allow separate focusing on the size and shapes of the different polypeptide chains of the reaction centers.

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