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Molecular weight of bacteriorhodopsin solubilized in Triton X-100

(membrane proteins/sedimentation equilibrium/purple membrane/halobacteria)

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ABSTRACT **Bacteriorhodopsin from *Halobacterium halobium* has been solubilized in the nonionic detergent Triton X-100. The circular dichroic spectrum and hydrodynamic properties indicate that the structure of this protein in the detergent is not significantly altered from that of the native membrane-bound form. Bacteriorhodopsin is monomeric under the conditions of solubilization with a molecular weight of 24,250 ± 2,000 and binds approximately one micelle of Triton X-100.**

The purple membrane from *Halobacterium halobium* contains one protein, bacteriorhodopsin, that mediates light energy transduction (1, 2). Recent diffraction studies have suggested that this protein is a compact, globular macromolecule consisting of seven helical segments 35–40 Å long that are inserted into the phospholipid bilayer and oriented at right angles to the plane of the membrane (3, 4). A molecular weight of approximately 26,000 has been suggested based on composition (1) and sedimentation equilibrium in sodium dodecyl sulfate (5). The protein associates in the membrane to form a hexagonal lattice with three molecules of protein in the unit cell (6).

A 7-Å resolution map of the lattice shows three molecules of bacteriorhodopsin clustered around a 3-fold axis of the lattice, apparently in close contact and forming a trimer (7, 8). A negative circular dichroic band in the visible region has been interpreted to be the result of exciton coupling of the retinal chromophores in the trimer (9–12). Disappearance of this band has been taken as evidence of dissociation into monomers and has been observed in Triton X-100-solubilized bacteriorhodopsin as well as in partially reconstituted "bleached" purple membrane (10, 13). Because there are small differences between the spectra of the presumed monomers in the detergent and those obtained by reconstitution, we have investigated the degree of association of bacteriorhodopsin in Triton X-100. The results are also of general interest for the problems of membrane protein solubilization in which nonionic detergents are used to simulate the hydrophobic milieu of lipid bilayers (14, 15).

EXPERIMENTAL PROCEDURE

Purple membranes were prepared from *H. halobium* as described previously (16). Ten milligrams of membrane (7.6 mg of protein) was suspended in 0.4 ml of 5% (vol/vol) Triton X-100 in 0.1 M acetate buffer, pH 5.0, and allowed to stand at room temperature for 2 days. The suspension was centrifuged at 100,000 × *g* for 30 min and this supernatant was used in all subsequent experiments. Greater than 90% of the membrane protein was found in the supernatant.

Circular dichroism was measured on a Jobin-Yvon Dichrographe with 1-mm cells.

Molecular weights were determined by a procedure described in detail elsewhere (17) using sedimentation equilibrium in a Spinco model E ultracentrifuge equipped with a photoelectric scanner. The concentration of protein as a function of radial distance was determined by scanning the cell at 555 nm (the detergent absorbs at 280 nm). Sedimentation velocity measurements together with molecular weight determinations were used to calculate the frictional ratios of the protein in the detergent micelle.

RESULTS

Bacteriorhodopsin solubilized in Triton X-100 has an absorption maximum at 555 nm as compared to 570 nm in the membrane-bound state. Fig. 1 shows the circular dichroic spectrum of the protein–detergent complex. The depth of the negative trough at 208 nm was used to estimate an α-helix content of close to 70% by the method of Greenfield and Fasman (18), which is in excellent agreement with electron diffraction data for the native protein in the purple membrane (3).

The molecular weight of the complex was determined by sedimentation equilibrium measurements as a function of solvent density, with D_2O used to vary the solvent density (17). The experimental parameter is

$$\frac{d\ln c}{dr^2} = \frac{M(1 - \phi'\rho)\omega^2}{2RT} \qquad [1]$$

in which ϕ' = effective partial specific volume, M = molecular weight of the protein, ρ = solvent density, and ω = angular velocity. Also

$$M(1 - \phi'\rho) = M\left[(1 - \bar{v}_p\rho) + \sum_i \delta_i(1 - \bar{v}_i\rho)\right] \qquad [2]$$

in which $\bar{v}_p$ = partial specific volume of the protein, δ_i = g of bound component i per g of protein, $\bar{v}_i$ = partial specific volume of bound component.

At $\rho = 1/\bar{v}_i$ the contribution from bound component i is zero, and the contribution of detergent to $M(1 - \phi'\rho)$ can thus be eliminated. Fig. 2 shows $M(1 - \phi'\rho)$ versus ρ for the protein–detergent complex. All plots of ln A_{555} versus r^2 were linear over the concentration range investigated (maximum concentration, 0.7 mg/ml).

The partial specific volume of Triton X-100 is 0.908 and therefore a rather long extrapolation is involved. Nevertheless, the data in Fig. 2 indicate unequivocally that bacteriorhodopsin is monomeric in this detergent. The original purple membrane preparation contained 0.315 g of lipid per g of protein. We have not directly measured lipid binding to the detergent–protein complex. However, in Table 1 we present molecular weights of the protein moiety, amounts of bound detergent, and frictional ratios obtained for the extremes of assumed lipid binding of zero and 0.315 g/g. It is apparent that the amount of bound lipid has no effect, within experimental error, on the results. Bacteriorhodopsin is monomeric, binds approximately one micelle of Triton X-100, and is a highly compact, globular particle.

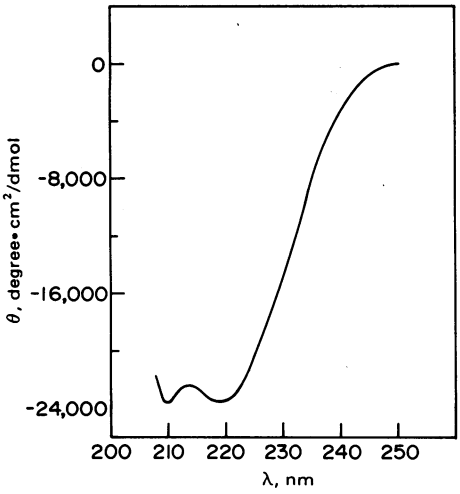

FIG. 1. Circular dichroic spectrum of bacteriorhodopsin in Triton X-100/0.1 M acetate buffer, pH 5.0.

DISCUSSION

Bacteriorhodopsin solubilized in Triton X-100 essentially maintains its native structure as evidenced by hydrodynamic and optical criteria; it also remains functionally intact, because its photoreaction cycle shows little if any change (R. H. Lozier and W. Stoeckenius, unpublished data). Because this is the only intrinsic membrane protein for which we have detailed information regarding structure in its native environment, it is important to note that no detectable structural and functional alterations occur when it is solubilized in detergent. This provides strong evidence that the premise of using detergents to mimic the hydrophobic environment of a lipid bilayer is sound (14).

The protein is monomeric in Triton X-100. This suggests that associative forces within the membrane must be weak. Because Triton contains a phenyl ring in its hydrocarbon region, it is not an ideal detergent for simulation of lipid alkyl chains. Several other nonionic detergents should be explored in order to ascertain whether or not bacteriorhodopsin is ever observed in oligomeric form in the solubilized state.

Binding of approximately one micelle of Triton X-100 to intrinsic membrane proteins has been observed in a number of systems other than bacteriorhodopsin. For example, both bovine rhodopsin (22) and cytochrome b_5 (23) interact with one micelle of this detergent.

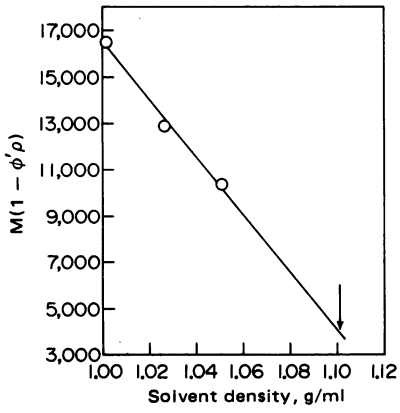

FIG. 2. Sedimentation equilibrium results for bacteriorhodopsin in Triton X-100. The arrow indicates $\rho = 1/\bar{v}_{Triton}$.

Table 1. Properties of protein-detergent complex

Assumed lipid: protein ratio, g/g	Mol. wt. of protein moiety*	Bound Triton X-100 g/g	Bound Triton X-100 mol/mol†	Stokes radius, Å	f/f_{min}
0	25,500	4.22	168	41	1.11
0.315	23,000	5.14	185	41	1.12

* $\bar{v}_{protein} = 0.750$; $\bar{v}_{Triton} = 0.908$; $\bar{v}_{lipid} = 0.98$.
† Mean aggregation number for Triton X-100 micelles is 140 ± 10 (19–21).

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