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The interaction of Triton X-100 with purple membrane. Effect of light-dark adaptation

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The effects of Triton X-100 on purple membrane have been examined for dark- and light-adapted membrane suspensions. Bacteriorhodopsin is more readily solubilized from dark-adapted than from light-adapted preparations, while light-dark adaptation does not influence phospholipid solubilization. Surfactant-induced changes in the absorption spectrum and retinal isomer distribution of bacteriorhodopsin are also illumination-dependent. These results are discussed in the light of structural data.

Bacteriorhodopsin, the only protein in *Halobacterium* purple membrane [1], may exist in the light-adapted and dark adapted forms, characterized by absorption maxima at about 570 nm and 560 nm respectively [2–4]. The light-adapted purple membrane contains only all-*trans*-retinal as the bacteriorhodopsin prosthetic group, while the dark-adapted form contains a mixture of all-*trans*- and 13-*cis*-isomers [5]. Bacteriorhodopsin may be obtained in micellar solution with the aid of detergents [6–8]; it is commonly used in this form for reconstitution and physical studies [9–12]. Although there are many reports of bacteriorhodopsin interaction with Triton X-100, the effect of light-dark adaptation on the solubilization process has not been examined in detail, and constitutes the object of the present report.

The methods have been described previously [8]. Briefly, purple membrane was isolated from *Halobacterium halobium* [13]; dark- and light-adaptation were achieved, respectively, by storing overnight a membrane suspension (0.33 mg protein/ml 20 mM Tris-maleate buffer (pH 5.0)) either in the dark or under constant white light illumination (≈ 30 μmol photons/s per m^2) at 22 ± 1°C. Membranes were incubated with the appropriate amounts of Triton X-100 for an additional period of 24 h, under the same conditions of darkness or illumination. Detergent-treated suspensions were centrifuged (200000 × *g*, 1 h, 4°C) and the supernatants were considered to contain the solubilized fraction; protein [14] and phospholipid [15] were assayed in these supernatants. Retinal isomers were analyzed following closely the procedure described by Scherrer et al. [16]; all precautions mentioned by these authors were scrupulously kept. Turbidity was measured as absorbance at 450 nm. Spectra were recorded with a Uvikon 860 Kontron spectrophotometer, equipped with continuous stirring, and interfaced to an IBM PC computer. Control experiments demonstrated that the procedure for spectral recording did not modify the conditions of light or dark adaptation of our samples.

After detergent incubation, solubilization of membrane protein and phospholipid was assessed, as shown in Fig. 1. Solubilization starts above 10^{-4} M Triton X-100. Differences are observed in the proportion of solubilized bacteriorhodopsin under each condition; in general, dark adaptation appears to favour solubilization when compared to light-adapted samples. However, no differences are observed in the solubilization of phospholipids from dark- or light-adapted membranes.

The visible absorption spectrum of purple membrane is blue-shifted, and its molar absorbance decreased, in the presence of Triton X-100, and the spectral changes parallel membrane solubilization [8]. This is observed irrespective of dark-light conditions, but with peculiarities in each case. Fig. 2A shows the change in maximum absorption wavelength of the purple membrane visible spectrum, as a function of Triton X-100 concentration. Spectral patterns were similar to those published previously [8]; shifts are not due to appearance of new

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bands. The extent of spectral shifts is, respectively, of 14 nm (light), and 10 nm (dark). The surfactant-induced decrease in absorbance under light and dark conditions is shown in Fig. 2B. In the sublytic range of detergent concentrations (below $7.5 \cdot 10^{-4}$ M) the decrease in absorbance is more pronounced in the light than in the dark. Moreover, in the presence of solubilizing concentrations of Triton X-100, all membrane preparations show a marked decrease in absorbance, with no clear differences between them. Changes in membrane suspension turbidity have also been used to monitor bacteriorhodopsin solubilization [8]. In our case (Fig. 2C) a large decrease in turbidity occurs when the membrane is dispersed into lipid-detergent-protein mixed micelles, e.g., near 10^{-3} M Triton X-100. Above this concentration, both samples are indistinguishable from the point of view of turbidity. Differences are seen, however, in the sublytic region. In particular, the turbidity of light-adapted membrane suspensions is higher than that of the dark-adapted samples. The reason for this is obscure, but the observed difference constitutes a good explanation for the changes in 'absorbance' observed in Fig. 2B at sublytic surfactant concentrations.

The all-*trans*- and 13-*cis*-isomers of retinal have been extracted and separated by HPLC under each condition. The proportion of all-*trans*-retinal for each preparation, as a function of Triton X-100 concentration, is shown in Fig. 3. The dark-adapted samples fail to show any change in the approximately equimolar distribution of isomers upon addition of detergent. This is also the case for the light-adapted suspensions in the presence of

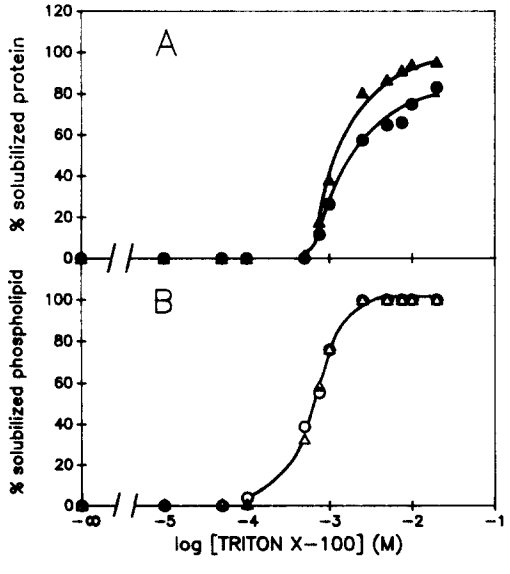

Fig. 1. The effect of light-dark adaptation on the solubilization of purple membrane by Triton X-100. Solubilization is expressed (in percentage) as a function of surfactant concentration. 100% corresponds to the total amount of protein or phospholipid in the detergent-treated sample. (A) Protein. (B) Phospholipid. Circles, light-adapted; triangles, dark-adapted. Average values of three determinations.

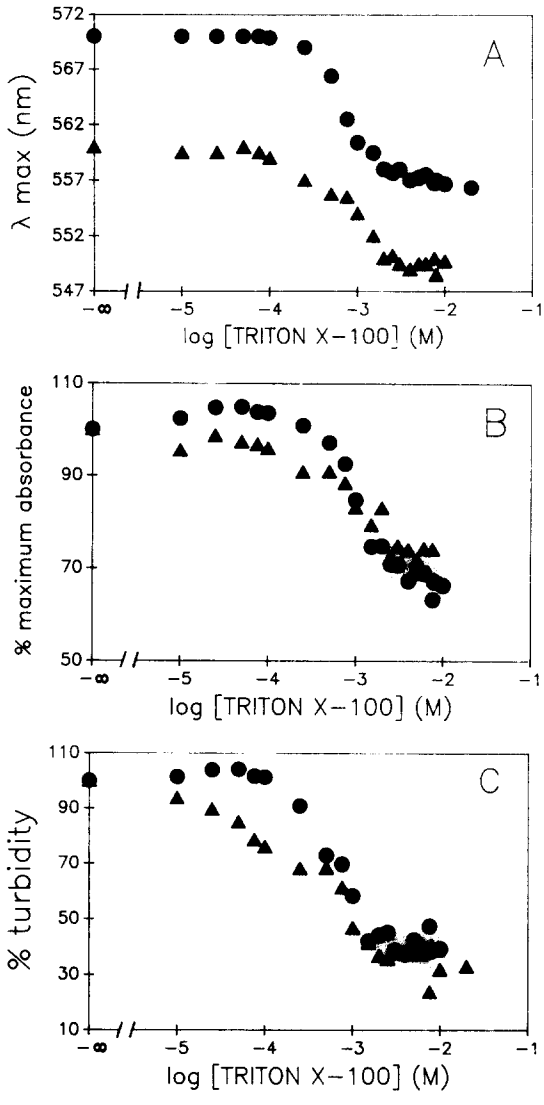

Fig. 2. The effect of light-dark adaptation on the spectral properties of purple membrane in the presence of Triton X-100. (A) The maximum absorption wavelength as a function of surfactant concentration. (B) the absorbance at the maximum absorption wavelenght expressed in percentage (100% being the value in the absence of surfactant) as a function of surfactant concentration. (C) Turbidity (A_{450}) expressed in percentage (100% being the turbidity in the absence of detergent) as a function of Triton X-100 concentration. Circles, light-adapted; triangles, dark-adapted. Average values of three experiments.

sub-lytic surfactant concentrations. However, membrane solubilization under light conditions produces a very marked decrease in the proportion of all-*trans*-retinal, from virtually 100% to about 65%. The consensus view that light-adapted membranes contain only all-*trans*-retinal, while in dark-adapted ones approximately equimolar mixtures of 13-*cis*- and all-*trans*-isomers are found has been recently challenged by Scherrer and co-workers [16] who, using an improved extraction method, find a 2 : 1 mole ratio of 13-*cis*- and all-*trans*-retinal in dark-adapted membranes. We have followed as closely as possible their procedure but still the pro-

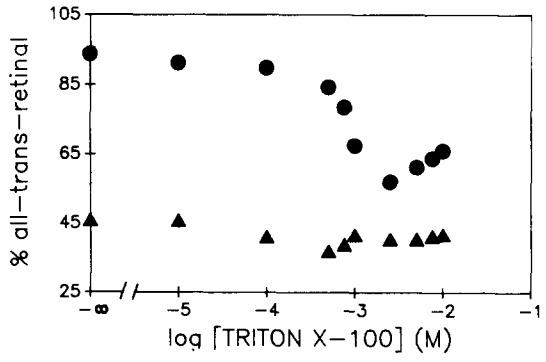

Fig. 3. The effects of light-dark adaptation on the retinal isomer composition of purple membrane, and its variation in the presence of Triton X-100. The proportion of all-*trans*-retinal is plotted versus surfactant concentration. Circles, light-adapted; triangles, dark-adapted. Average values of four measurements.

portion of all-*trans*-retinal in our dark-adapted preparations is 46 ± 1%. While the reasons for this discrepancy are difficult to ascertain at the present time, our data clearly show that the detergent does not modify the proportion of isomers in dark-adapted preparations, while it promotes the appearance of 13-*cis*-retinal in light-adapted samples (Fig. 3), as suggested by previous studies for selected detergent concentrations [9,16].

A correlation between the observed light-dark differences in bacteriorhodopsin solubilization and the effect of these parameters on purple membrane structure is difficult to establish because of the scarcity of structural data. The phenomenon of light-dark adaptation has been extensively studied, but mainly from the physiological point of view [4,17–20]. Apart from retinal isomerization, localized conformational changes of the protein had been proposed [17]; however, recent structural investigations by neutron diffraction [21,22] have shown that there are no localized conformational changes of the protein during light-dark adaptation. Therefore we lack, at present, the structural correlate to explain the fact that bacteriorhodopsin from light-adopted membranes is more resistant to solubilization than that from dark-adapted ones (Fig. 1). The fact that phospholipid solubilization is not affected by light-dark conditions suggests a direct link between changes in bacteriorhodopsin and protein solubility in the presence of detergent.

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