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EFFECT OF FLUORESCAMINE MODIFICATION OF PURPLE MEMBRANES ON EXCITON COUPLING AND LIGHT-TO-DARK ADAPTATION

Eric Lam* and Lester Packer

Membrane Bioenergetics Group, Lawrence Berkeley Laboratory, and
Department of Physiology-Anatomy and *Department of Biophysics,
University of California, Berkeley, California 94720.

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SUMMARY

Purple membranes of Halobacterium halobium were modified with fluorescamine. At pH 8.8, with a molar ratio of fluorescamine to bacteriorhodopsin of 170, about 6 residues of lysine were modified while the arginines were not affected at all. Except for the appearance of the fluorescamine peak at 394 nm and some broadening of the chromophore peak at 570 nm, the absorption spectrum of bacteriorhodopsin was not significantly changed after modification. After fluorescamine modification, circular dichroism studies indicated loss of exciton coupling between bacteriorhodopsin molecules in the purple membrane. Rotational diffusion studies suggested enhanced mobility of the chromophore after modification. However, the spectral changes accompanying the light-to-dark adaptation of purple membranes were not prevented by fluorescamine modification. The implications of these findings are that exciton coupling between neighboring bacteriorhodopsin molecules in the purple membrane is not required for light-to-dark adaptation.

INTRODUCTION

Purple membranes are known to consist of hexagonal arrays of bacteriorhodopsin trimers (1). Bacteriorhodopsin is known to function as a light-activated proton pump and is composed of one single polypeptide chain with the pigment retinal bound to a lysine via a schiff base linkage (2). This protein has been subjected to intensive research since its initial discovery. However, relatively little is known about the relationship between the structure and function of bacteriorhodopsin in purple membrane. The negative-positive transition centered around the chromophore absorption peak (i.e., about 570 nm) in the CD spectrum

Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; BR, bacteriorhodopsin; EA, ethyl acetimidate.

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of purple membrane has been demonstrated to be due to exciton coupling between neighboring bacteriorhodopsin molecules (3). Recently, a study on the relationship between trimeric structure and the light-dark adaptation ability of purple membrane has been reported (4). It was suggested that protein-protein interaction is responsible for the stabilization of the light-adapted form of purple membrane. However, it is not clear whether interactions between the retinal of neighboring BR molecules, as manifested by the exciton coupling observed in CD spectra, are related to the light-dark adaptation ability of the purple membrane or not. In the present report, evidence is presented which suggests that exciton coupling between neighboring BR is not required for light-dark adaptation of the chromophore in purple membrane.

MATERIALS AND METHODS

Purple membranes from the S_9 strain of Halobacterium halobium were obtained as described previously (5). Fluorescamine is disolved in acetone and added to a rapidly vortexing aliquot of purple membrane via rapid injection at a molar ratio of Fluorescamine:BR = 170. Purple membranes for modification were suspended in 0.05 M sodium borate, 0.05 M NaCl at pH 8.8. The concentration of bacteriorhodopsin is 0.25 mg/ml using an extinction coefficient of 64,000 at 570 nm and a molecular weight of 26,000 (6). After addition of fluorescamine, the suspension is left at room temperature for about 5 minutes and then washed three times in cold distilled water. Modification by ethyl acetimidate was essentially as in (5) with 100 mM ethyl acetimidate during the incubation period. Amino acid analysis was carried out as described in (5) with the sample hydrolyzed in 6 M HCl. Absorption spectra were obtained with an Aminco DW-2 spectrophotometer.

Purple membranes were dark adapted by storage in the dark at 4° C overnight and light-adapted by illuminating for 5 minutes with intense light from a projector lamp.

Resonance Raman spectra at room temperature and 87° K were obtained as previously described (7).

Circular dichroism studies were carried out either with a JASCO J-20A spectropolarimeter or with a spectrometer described elsewhere (8). Rotational diffusion studies were carried out as described (9) with a flash photolysis set-up described previously (5). Changes in absorption at 568 nm were followed and the anisotropy (r) at a time t calculated by:

$$r(t) = \frac{A_{11}(t) - A_{1}(t)}{A_{11}(t) + 2A_{1}(t)}$$

Protein concentrations were also determined by the Lowry method (10) using native purple membrane and bovine serum albumin as standards. HEPES and fluorescamine were obtained from Sigma Chemical Co. All other reagents were of the best grade available.

RESULTS AND DISCUSSION

Spectral and Chemical Analysis of Fluorescamine Modified Purple Membranes - The absorption spectrum of purple membranes after modification by fluorescamine is shown in Figure 1. A slight broadening of the chromophore absorption peak at 570 nm was observed but there was no significant alteration in extinction coefficient. Moreover, a new absorption peak due to fluorescamine moiety appeared at 394 nm. There are 7 lysine residues in bacteriorhodopsin (11). Amino acid analysis showed that about 6 residues of lysine are lost after modification while arginines were not affected at all. These observations suggest that after maximum modification of the available lysines by fluorescamine, the chromophore structure and its environment are not drastically altered. This is further supported by resonance-raman studies of the light-adapted fluorescamine-modified purple membranes which showed that their resonance raman spectrum was identical to that of native purple membranes (Lam, E., Pande, J., Callender, R., and Packer, L., unpublished data). Therefore, the cis:trans ratio of modified sample is probably similar to that of native purple membrane after light adaptation. The slight broadening of the chromophore absorption peak upon modification is consistent with an increase in torsional motions in the chromophore by a less rigid fixation of the retinal in the opsin (12).

Circular Dichroism of Fluorescamine-Modified Purple Membranes - The CD spectrum of fluorescamine modified purple membrane is compared to that of the native membrane in Figure 2. The characteristic positive-negative transition around 570 nm in the CD spectrum of the native membrane was abolished by fluorescamine modification. A broad positive band at about 545 nm was observed while the negative band at 320 nm was drastically reduced. These features are similar to those observed upon solubilization of purple membranes by non-ionic detergents (13). A new negative band at 390 nm was observed which corresponded to the presence of fluorescamine labeled L-lysines as reported by Kovacs (14). Since the positive-negative transition observed in the native membrane has been attributed to exciton coupling between neighboring BR molecules (3,15), these observations are consistent

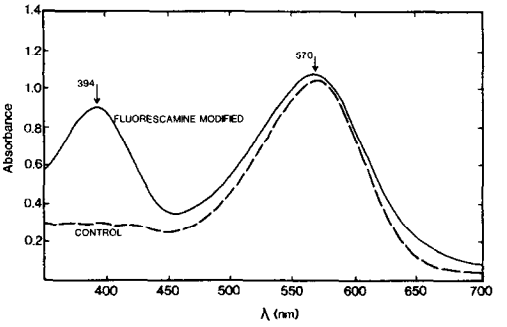

Figure 1. Light-Adapted Absorption Spectra of Native and Fluorescamine-Modified Purple Membranes. Fluorescamine-modified purple membranes and unmodified purple membranes were suspended in 2 mM HEPES, pH 7.0 at a concentration of about 0.45 mg protein/ml. Light adaptation carried out as described in Materials and Methods.

with the interpretation that fluorescamine modification interferes with the exciton coupling between neighboring chromophores in purple membrane. The decrease in the optical activity at 320 nm in the modified membrane suggests a loss of rigidity of the retinal-apoprotein interaction (15).

Rotational Diffusion Studies of Fluroescamine-Modified Purple Membranes - Since Triton X-100 treatment and fluorescamine modification both abolish the exciton coupling in CD spectra characteristic of the native bacteriorhodopsin

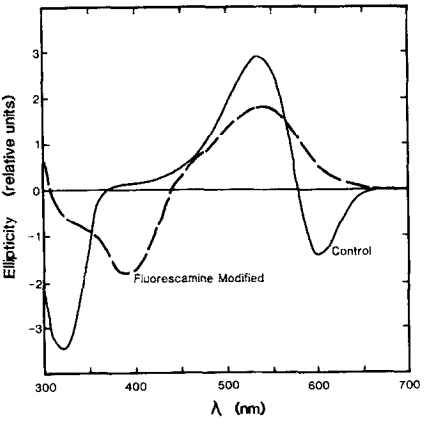

Figure 2. Circular Dichroism Spectra of Native and Fluorescamine-Modified Purple Membranes. Conditions as in Figure 1.

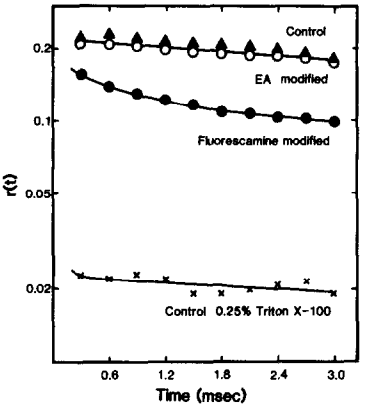

Figure 3. Rotational Diffusion Studies of Native, Modified, and Solubilized Purple Membranes. Samples were suspended in 2 mM HEPES, pH 7.0 at about 0.5 mg protein/ml. (-▲-▲-) native purple membrane; (0-0-0-) EA modified purple membrane; (-●-●-) fluorescamine modified purple membrane; and (-X-X-) purple membrane solubilized in 0.25% Triton X-100.

trimers in purple membranes, it was of interest to study their effects on the relative mobility of bacteriorhodopsin by rotational diffusion (9). As shown in Figure 3, the value of r, the anisotropy, was substantially decreased after fluorescamine modification, although not as much as in the case of Triton X-100 solubilization. Assuming that the membrane fragments are approximately equal in size to those of the native membrane, this observation suggests that after modification, the bacteriorhodopsin molecules became more mobile. Modification by ethyl acetimidate did not cause any significant change when about 80-90% of the available lysines were reacted, strongly suggesting that the addition of the fluorescamine moiety causes the effects on the rotational diffusion measurement.

Modification of purple membranes by EA did not affect either the absorption spectrum or the CD spectrum (data not shown) even at conditions when 80-90% of the available lysines were reacted. Thus, it is reasonable to suggest that the structural effects we observed upon fluorescamine modification are due to

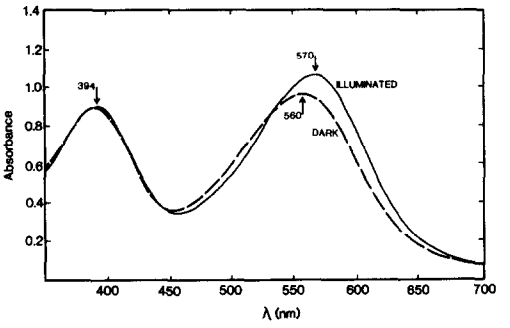

Figure 4. Light- and Dark-Adapted Absorption Spectra of Fluorescamine-Modified Purple Membranes. Conditions as in Figure 1.

the presence of the fluorescamine moiety and not to the modification of primary amino groups.

Light-Dark Adaptation Studies of Fluorescamine - Modified Purple Membranes - When the spectra of light- and dark-adapted fluorescamine-modified purple membranes are compared (Figure 4), it is observed that the 560 nm peak of the dark-adapted modified membrane was shifted to about 570 nm upon light adaptation and was increased substantially in extinction. These characteristics are similar to those of native purple membranes as reported by others (4).

The results of fluorescamine modification of purple membrane lead us to the following tentative conclusions: First, since the exciton coupling was altered drastically, as manifested by the loss of positive-negative transition in the chromophore absorption region of the CD spectrum, while few effects were observed on the absorption spectrum, the resonance raman spectrum, or the light-dark adaptation process, we suggest that the observed chromophore absorption at 570 nm of the native membrane is due mostly to the interactions and specific structural features of the retinal-apoprotein complex. Interactions between neighboring chromophores probably are not primary factors, if involved at all, in the "opsin-shift" observed upon addition of the retinal to the bacterioopsin (12). Secondly, our studies indicate that exciton coupling between neighboring bacteriorhodopsin is not required for light-to-dark adaptation of purple membrane.

Thirdly, the presence of the fluorescamine moiety induces an enhancement of the chromophore mobility, as shown by the rotational diffusion studies, and a decrease in the rigidity of the retinal-opsin interaction, as suggested by the CD and absorption data.

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