Why Is the Purple Membrane a Two-dimensional Crystal?

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The purple membrane is an elegant yet simple structure. It contains only a single M_r 26,000 protein, bacteriorhodopsin (bR), which is arranged in a two-dimensional hexagonal lattice. Bacteriorhodopsin is a light-driven proton pump which converts the energy of photons into an electrochemical gradient ($\Delta\mu$). Each bR molecule contains a single highly dichroic chromophore, retinal. The conformation of retinal in bR and the well known seven α-helix structure of bR have been examined by biophysicists many times over the years. The information gathered by these studies and the more recent site-directed mutagenesis work of molecular biologists have been combined to provide much detailed information on the molecular mechanism of this proton pump (1). We have learned a great deal about what goes on *within* the individual bR molecule during the photocycle. We know considerably less, however, about possible interactions *between* bR molecules during the photocycle. Since individual bR monomers can pump protons (2), what is the purpose of arranging bR molecules in such a precise and highly organized manner in the purple membrane? Does the crystalline lattice provide a mechanism for bR molecules to interact in a highly specific and cooperative manner during the photocycle? The work done by Wan and Johnson (3) provides strong evidence that bR molecules can undergo light-induced reorientations within the purple membrane. This conclusion has important implications for understanding the function which resulted in the structure of the purple membrane.

The conformation and orientation of the chromophore within bR is obviously crucial to understanding the molecular mechanism of this proton pump since the chromophore is responsible for capturing the photon. Polarized absorption spectroscopy has been used to determine the orientation of the transition dipole moment of retinal in ground state bR by several investigators. A consensus has emerged that the transition dipole moment of retinal in ground state bR is oriented approximately 20° out of the membrane plane. Linear dichroism measurements made on hydrated multilayer purple membrane films indicated no change in the tilt of the chromophore out of the membrane plane following the conversion to the M photocycle intermediate (4). A more recent linear dichroism study which examined three-dimensional crystals of bR rather than purple membranes of bR, concluded that there was only a small ($2.2 \pm 0.5°$) reorientation of the chromophore in the M intermediate (5). The prevailing view which has emerged from these and other studies is that the chromophore transition dipole moment does not reorient significantly within an individual bR protein molecule and the bR protein must remain immobilized in the membrane during the photocycle. (A recent neutron diffraction study involving purple membranes regenerated with deuterated retinal appears to conflict with this hypothesis, however, since this study indicated a chromophore reorientation of 11° during the formation of the M intermediate (6).) Although most studies have indicated that the chromophore does not reorient within bR, they should not be interpreted to rule out chromophore reorientations resulting from bR protein rotations *within* the purple membrane. These studies involved immobilizing the purple membrane in 5 hydrated multilayer films or polyacrylamide gels which could significantly inhibit or alter protein rotations within the purple membrane.

The time-resolved photo-induced dichroism measurements of Wan and Johnson were done on randomly oriented aqueous purple membrane suspensions. The key observation was the remarkable dependence of the photo-induced dichroism on the wavelength at which the absorbance was measured. For example, the anisotropy measured at 570 nm rapidly decayed within a few milliseconds at room temperature, while the anisotropy at 410 nm was constant during the lifetime of the photocycle. These apparently contradictory results cannot be explained by simple rotational diffusion of the purple membrane fragments. Furthermore, cross-linking the purple membranes with glutaraldehyde eliminated this unusual wavelength dependence of the photodichroism. In order to interpret their data, Wan and Johnson had to develop a method to analyze the complex problem of photo-induced dichroism for systems like the bR photocycle where several different photocycle intermediates would be present in the sample. Each photocycle species might have a different extinction coefficient and average chromophore dipole orientation. The analysis was based on comparing the time dependence of the time-resolved linear dichroism, $D(t) = \Delta A_{\parallel} - \Delta A_{\perp}$, with the transient absorbance, $\Delta A(t) = \Delta A_{\parallel} + 2\Delta A_{\perp}$. Chromophore reorientations must occur when the time dependence of $D(t)$ is significantly different from that of $\Delta A(t)$. The dichroism data is most consistent with an intramembrane chromophore reorientation model that involves photo-induced reorientations of nonexcited or "spectator" chromophores by about 8°. The chromophore reorientations probably are the result of "spectator" bR protein motion, since large reorientations of the retinal transition dipole moment within the nonexcited bR protein seem unlikely. The protein motion might result from rotations of entire bR molecules, or just segments of the protein. Whatever the structural changes responsible for these chromophore reorientations, these changes are not absolutely required for the photocycle, since treatments which eliminated the reorientations, such as protein cross-linking with glutaraldehyde, did not eliminate the photocycle.

Recent x-ray, neutron, and electron diffraction studies provide additional evidence for significant protein structure changes within the purple membrane structure during the photocycle.

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In a remarkable set of electron diffraction experiments, Subramaniam et al. (7) have produced 3.5-Å resolution Fourier projection maps of the difference between ground state bR and the M intermediate. The M intermediate was "trapped" by rapidly freezing the sample following room temperature flash illumination. The difference maps show relatively large structure changes in the vicinity of helices F and G. It would be very interesting to determine whether these structural changes occur in purple membranes cross-linked with glutaraldehyde and how this procedure effects proton pumping. This study indicates that relatively large changes may occur in the structure of the purple membrane during the photocycle. These structural changes may be responsible for the chromophore reorientations Wan and Johnson observe.

What is the function of these chromophore rotations? Wan and Johnson suggest that the rotations may be associated with cooperativity within the purple membrane during the photocycle. The motions may be the mechanism for a photocycling bR to influence the photocycle and/or the proton pumping efficiency of a neighbor bR in the crystalline lattice. In this way the proton pumping activity might be controlled by the light intensity. Cooperativity is generally used to provide feedback control. Light intensity seems to control the light-dark adaptation state of the purple membrane in a cooperative manner, but there are probably other elements in the control loop. Proteins such as hemoglobin, use cooperativity to provide a control "switch" to turn functional activity on and off in response to metabolic requirements. The metabolic function directly coupled to the purple membrane activity is $\Delta\mu$, as pointed out by Westerhoff and Dancsházy (8) and others. Therefore, it seems reasonable that purple membrane activity be under the control of $\Delta\mu$. The purple membrane may be able to control proton pumping activity in response to changes in $\Delta\mu$, by altering its structure in a cooperative manner. The chromophore reorientations and structural changes observed in the purple membrane may be an incomplete glimpse of this proton pumping control mechanism. Future studies should focus on whether the structural changes within the purple membrane during the photocycle are controlled by $\Delta\mu$. Wan and Johnson have helped to establish that potential mechanisms for cooperative control of function exists within the purple membrane lattice. It's up to future investigators to determine what control loop elements are activating these mechanisms.

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