TIME-RESOLVED X-RAY DIFFRACTION EXPERIMENTS OF PURPLE MEMBRANE

Bacteriorhodopsin (BR) is a proton transporting membrane protein, driven by the energy of absorbed photons. During the first step of proton transport, the absorption of a photon by retinal, located inside BR, induces a conformational change in the molecule from an all-trans to a 13-cis configuration. A sequence of reactions follows to facilitate the transport of protons by BR. Several intermediate states have been characterized by difference spectra in the visible range as J, K, L, M, N, and O. Reprotonation of the retinal Schiff base is an integral reaction in BR proton pump function; the Schiff base receives a proton from D96 during the M-N transition in BR photocycle. Because the pK of D96, located within a hydrophobic microenvironment, is unusually increased, D96 has a protonated form in the unphotolyzed state. Upon the release of the proton, the pK of D96 is lowered by an additional reaction. Therefore, a critical event enabling the lowering of the D96 pK must occur at the M-N transition.

To study the structural transition from the M to the N intermediate, we performed time-resolved X-ray diffraction of purple membrane at the SAXS station of **BL45XU** (Fig. 1) [1]. Stacked purple membranes (two-dimensional crystals of BR) placed on a mylar sheet, relaxed at pH9 and excited at 10 °C by a xenon flash, immediately following the start of diffraction acquisitions. By exposing to X-rays, perpendicular to the membrane plane, stacked purple membranes diffract X-rays circularly in a manner similar to powder diffraction. Diffracted images were averaged circularly. To decompose the component in this reaction, the data were analyzed by singular value decomposition (SVD) (Fig. 2 and Fig.3). Three components are distinguishable from the SVD analysis. U spectra give either the diffraction profiles or the difference profiles of independent components; V spectra display the time course. U1 is similar to the original diffraction pattern of the purple membrane, except for its negative sign, which is influenced by the sign

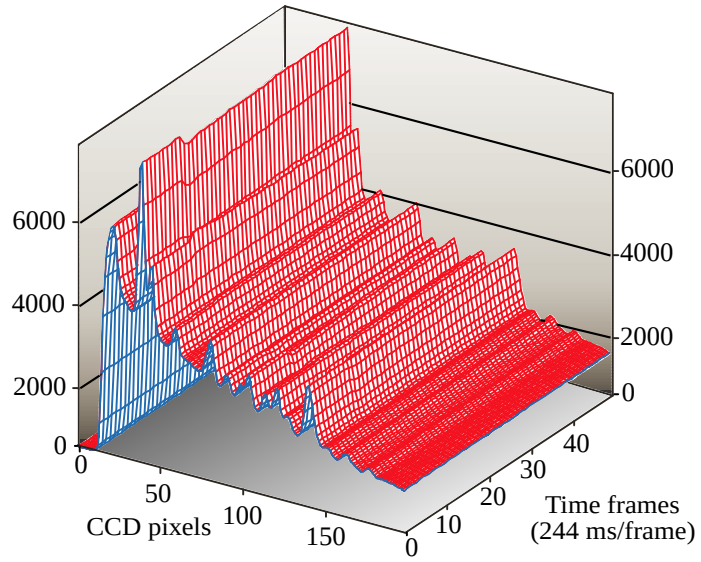

Fig. 1. Time-resolved X-ray diffraction pattern of purple membrane (pH9, 10 °C) before and after xenon flash lamp excitation. Bragg peaks of a magnitude as great as 7 Å are observed, utilizing a time resolution of 244 ms. BR was illuminated at the beginning of the 11th frame.

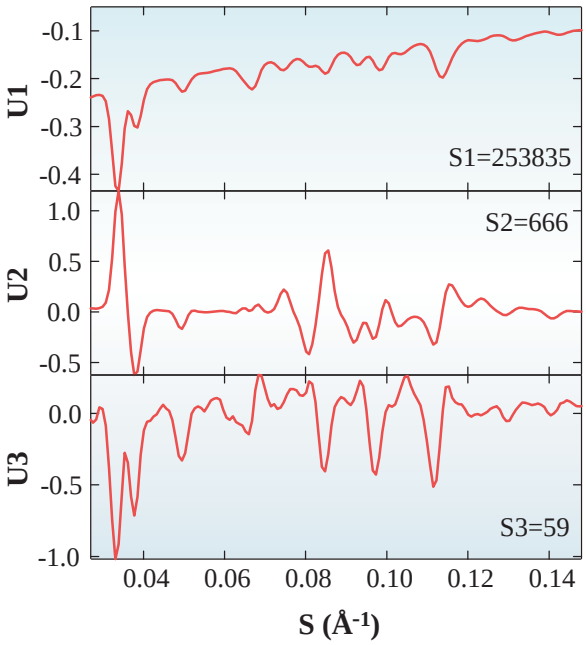

Fig. 2. The U spectra of wild-type BR (pH9, 10 ℃) obtained by SVD analysis for weighted diffraction data. This figure displays the three major components with the singular values. U spectra are either the diffraction profiles or the difference profiles of independent components.

respectively. Utilizing difference Fourier analysis, we examined the two difference diffraction profiles to identify the domains undergoing large conformational changes (Fig. 4). The projections, mapping onto the membrane plane of the two components, demonstrate changes characteristic to the M and N intermediates, respectively. The slow component contains changes near F and G helices; the positive peak on the outside of the F helix becomes higher than the positive peak of the G helix, a characteristic property of the N intermediate. The analysis of the fast component revealed that the positive peak on the outside of the F helix reaches a position as high as the positive peak of the G helix, indicating the F helix alters its structure in this manner during the M-N transition.

Although there is disagreement concerning structural changes during the M-N transition [2], our research reveals that BR changes its structure

of V1. V1 demonstrates little time dependence, indicating that U1 does not change throughout the photocycle. Thus, the first component is composed primarily of the time-independent, basic structure of BR. V2 and V3, however, change dramatically during the flash excitation at time 0, indicating that two unique intermediate conformations exist in the decay process of wild-type BR following flash excitation. As the M and N intermediates only were detected spectrophotometrically in this time period, the two intermediate conformations can be assigned as these intermediates. The three components, derived from the SVD analysis, were reconstituted from global fit analysis to create diffraction profiles of the three states. Absorption and FTIR measurements indicate that the reconstituted profiles are likely to correspond to the unphotolyzed state, the absorption difference between N and the unphotolyzed state and the difference between M and the unphotolyzed state,

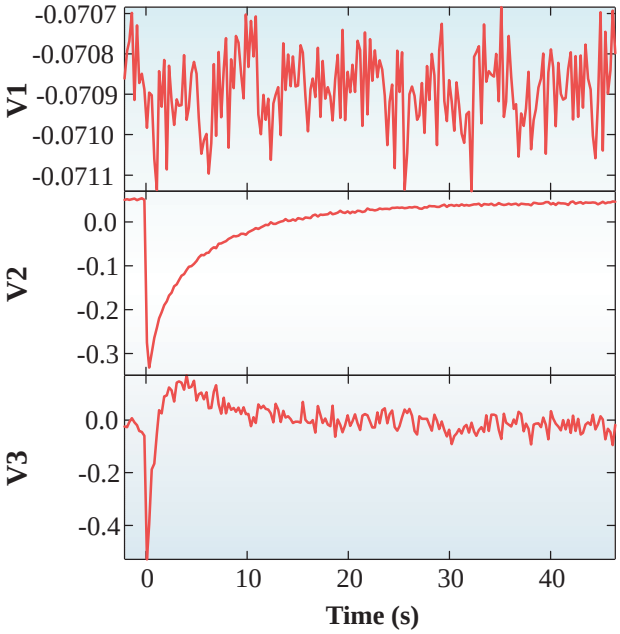

Fig. 3. V spectra of wild-type BR (pH9, 10 ℃) obtained by SVD analysis for weighted diffraction data. V spectra indicate the time course of the corresponding U spectra.

primarily in the M intermediate [3]. Our data demonstrates that global structural changes also occur during the M-N transition. The observed structural change at the F helix likely increases the accessibility of the Schiff base and D96 to the cytoplasmic surface, facilitating the proton transfer reactions beginning with the decay of the M state. We conclude that a large, two-step structural change is integral in the proton pumping activity of BR.

Toshihiko Oka

SPring-8 / RIKEN

E-mail: oka@spring8.or.jp

References

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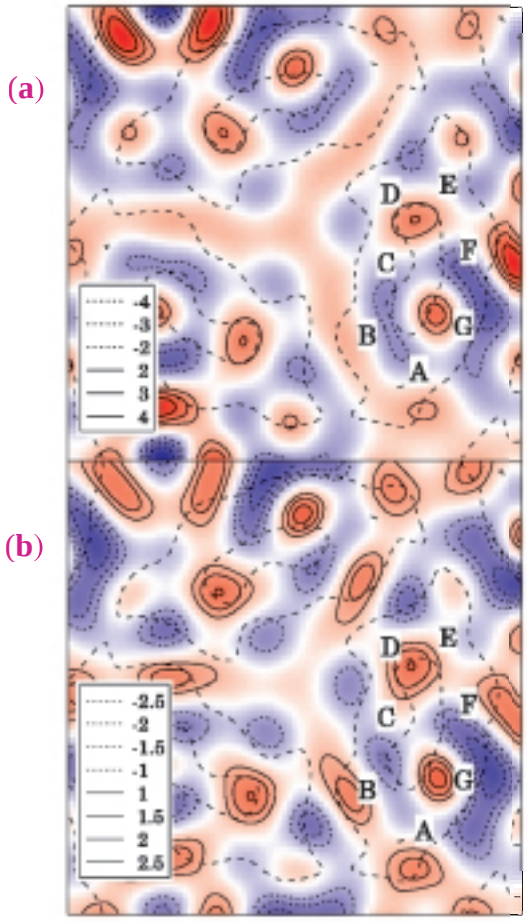

*Fig. 4. Difference Fourier maps projected onto the membrane plane of purple membrane samples (pH9, 10 °C). **(a)** Difference map of the N intermediate and the unphotolyzed state. **(b)** Difference map of the M intermediate and the unphotolyzed state. The dashed line indicates the outline of the BR trimer. Capital letters indicate the rough positions of the seven BR helices.*