X-ray crystallographic study of bacteriorhodopsin's reaction intermediates by the time-resolved Laue method

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Bacteriorhodopsin (bR) is a membrane protein that functions as a light-driven proton pump. When the retinal chromophore in bR absorbs light, bR undergoes a cyclic chemical reaction during which one proton is translocated across the membrane. For better understanding of the proton pumping mechanism of bR, it is crucial to obtain structural infomation of the photoreaction intermediates of bR. We have recetnly shown that a well-ordered 3D crystal is produced by successive fusion of the vesicular assemblies of bR. This new crystal is made up of stacked membranes, in each of which the bR trimers are arranged on a honeycomb lattice.

Using the new crystal, we made a feasibility study of structural analyses of bR by the time-resolved Laue diffraction method. Figure 1 shows a Laue photograph of a single crystal of bR. This image was obtained by 10-ms exposure of the light-adapted crystal to X-rays in the wavelength window from 0.4 to 2.0 Å. The streak of each diffraction spot is small, indicating that the crystal mosaicity is small enough to allow investigation of conformational changes of bR by the Laue method.

We also collected diffraction data of a frozen crystal in which a large fraction of bR molecules was trapped in the M intermediate (an intermediate having a blue-shifted absorption spectrum). Time-resolved absorption spectra indicated that the decay of M is inhibited in the crystal. Since its lifetime became as long as 1 sec at 20 ℃, the M intermediate easily accumulated in continuous visible light. For crystallographic analyses of the M intermediate, bR crystals were grown in the presence of 30% trehalose (cryo-protectant), and a single crystal with a thickness of 50 μ m was picked up with a cryo-loop, irradiated by orange light (570-700 nm; 110 mW/cm^2) for a few seconds and then rapidly cooled with liquid ethane. In this way, we prepared a frozen crystal in which the M intermediate accounts for 50 - 70% of the total protein. Diffraction data of the frozen crystal were collected with R-AXIS IV at the station BL44B2. The temperature of the crystal was maintained at 100 K using a cryostreem cooler and the content of M in the crystal was determined with a microspectrometer. The wavelength of X-rays was set at 1.0 Å and the crystal-to-detector distance was 350 mm. The reflections were indexed and integrated with Denzo. Completeness of data collection up to 2.8 Å was 79% with Rsym of 6.2%. The diffraction data thus obtained were analyzed together with those of the light-adapted crystal. From the difference electron density map between the light-adapted bR and the M intermediate, it was indicated that the tilt angle of the retinal chromophore with respect to the membrane normal increases upon the formation of M. This movement is accompanied by a local conformational change in the protein moiety (e.g., re-orientation of the side chain of Arg82), but little change is induced in the over-all protein structure.