High-resolution powder diffraction from purple membrane

To understand the actions of proteins in living cells, knowledge in their structures and dynamics in their natural environment is required. X-ray diffraction can be used to study biological membranes and filamentous materials under physiological conditions. Acquiring high-resolution diffraction data of the samples of protein assemblies requires a small X-ray beam and a detector system with a high spatial resolution and a wide dynamic range. X-ray beams provided by third-generation synchrotron facilities have small diameters and low angular divergences. Imaging plate (IP) detectors with spatial resolution of about 100 μm and dynamic range of 10^5 are suitable for recording their diffraction patterns because stacked biological membranes and oriented sols of filamentous assemblies of proteins have long-range ordered structures and display wide-ranging intensity resolutions.

A Guinier camera was developed for powder diffraction experiments of microcrystalline materials with large unit cells [1] (Fig. 1). The camera has a maximum specimen-to-detector distance of 1000 m with the highest angular resolution of 0.024° when using a blue IP of 100 μm spatial resolution. The high angular resolution lends itself to X-ray diffractometry of biological membranes and/or sols of filamentous biological materials because many diffraction peaks at high angles could be too close to each other to be separated with low-resolution detectors.

Powder X-ray diffraction was applied to structural analysis of Purple Membrane (PM), which is a two-dimensional crystalline array of bacteriorhodopsin acting as a light-driven proton pump in *Halobacterium salinarium*. X-ray diffraction images of oriented films of PMs showed clear peaks up to a resolution of 7.0 Å. It was, however, difficult to separate diffraction peaks and to evaluate intensities beyond the resolution because diffraction peaks overlapped considerably. Thus, X-ray diffraction studies of PM on the structural changes during proton pumping have been limited to low resolution even in recent years.

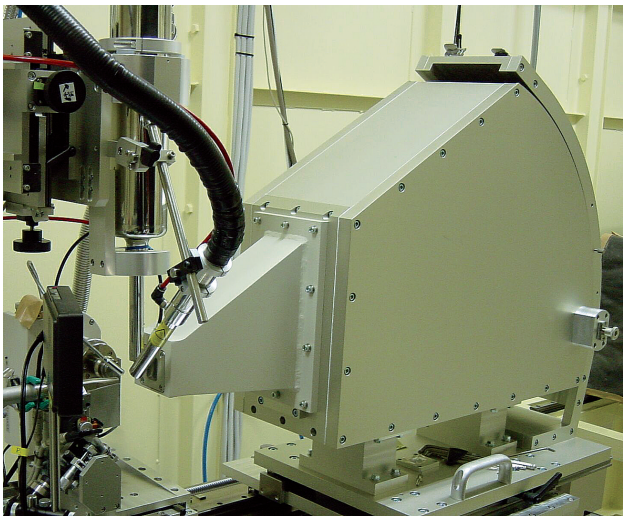

Fig. 1. Photograph of the Guinier camera.

Oriented films of PMs were measured with the Guinier camera [2]. Figure 2 shows a one-dimensional diffraction profile of the PM film reduced from the diffraction patterns on two IPs. The intensities of diffraction peaks decrease gradually with increasing S, but peaks are visible beyond 0.40 $Å^{-1}$ (2.5 Å resolution). The lattice constant of PM at around 110 K was calculated to be 61.0 Å from the positions of 47 sharp diffraction peaks located between 0.03 and 0.32 $Å^{-1}$. The expected positions of diffraction peaks from a hexagonal lattice correspond exactly to the observed peak positions in the diffraction profile (Fig. 2). As a result, 52 diffraction intensities of 55 expected reflections from (1 0) to (11 3) are integrated using the profile fitting method. An electron density map of BR projected normal to the membrane plane was calculated at a resolution of 4.2 Å (Fig. 3). The map clearly indicates electron density of seven transmembrane α-helices and its trimer structure. When the map is compared with those in previous studies obtained at a resolution of 7.0 Å, it displays characteristics to be observed in a projection map calculated at a resolution of around 3.5 Å by electron. Firstly, the four helices (A, E, F and G) located at the outer rim of the BR monomer have projection densities lower than the three helices (B, C and D) in the inner part. This characteristic comes from the tilting of the four helices from the membrane normal as demonstrated in a crystal structure of BR. Secondly, small but significant density peaks are visible in the map. In particular, a small peak is identified between helices C, D, E and F. Such a peak is invisible in the maps obtained at 7.0 Å. When referring to the crystal structure, the small peak plausibly corresponds to the electron densities of the β-ionone group of the chromophore retinal and the side chain of Trp189 adjoining the chromophore.

In powder diffraction studies, as the diffraction angle becomes larger, the number of peaks increases

and peaks become wider and weaker. As a result, it is difficult to distinguish each diffraction peak separately at a high diffraction angle. Owing to the high angular resolution of the large Guinier camera, observed peaks became sharp up to a high resolution (Fig. 2) and peak widths became less than half of that obtained in an earlier experiment. As a result, intensities were evaluated up to a spacing of 4.2 Å. The present experimental data for PM suggest the possibility of X-ray diffraction experiments for structural studies of intact biological membranes and macromolecule assemblies.

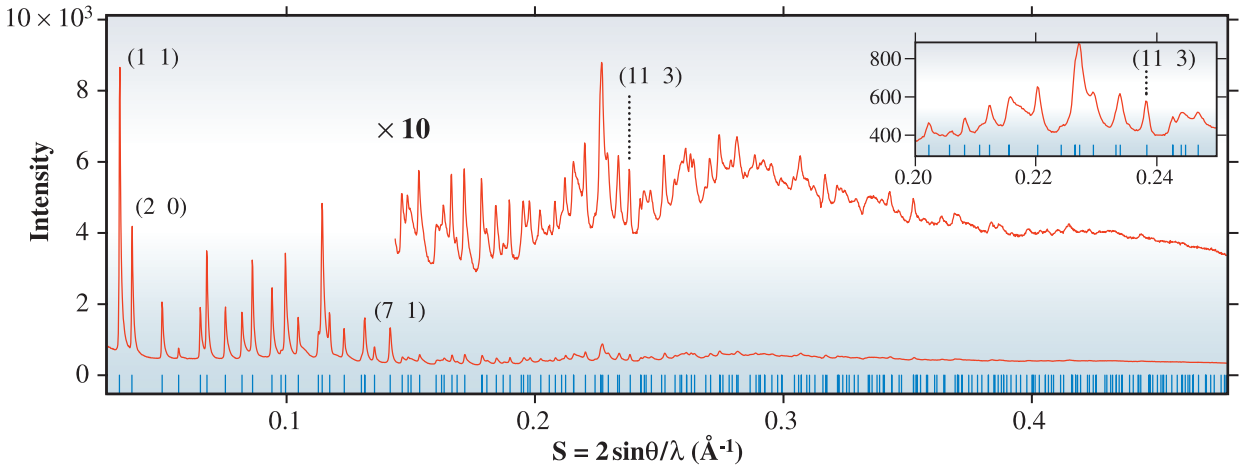

Fig. 2. X-ray diffraction profile of PM. Bars below the diffraction profile are the expected positions of diffraction peaks from a hexagonal lattice of a plane group *p3*. The inset shows the magnified profile.

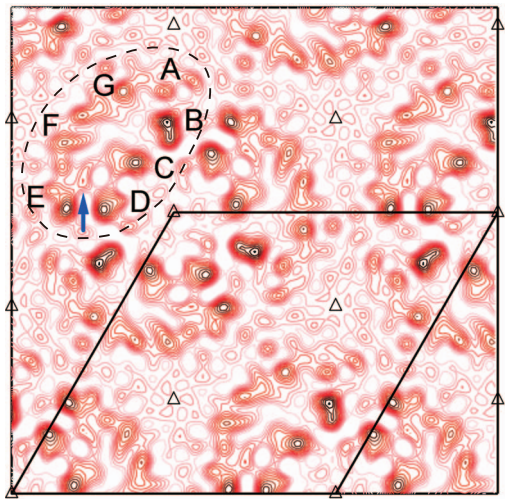

Fig. 3. Electron density map of PM projected onto a membrane plane. The dashed line shows the border of one BR monomer. An arrow indicates a small peak between helices C, D, E and F.

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References

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[2] *T. Oka, K. Miura, K. Inoue, T. Ueki and N. Yagi: J. Synchrotron Rad.* ***13*** *(2006) 281.*