Structure determination of sodium-translocating ATPase from *Enterococcus hirae*

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Ion-translocating ATPases are divided into three types: P-ATPase, F_0F_1-ATPase (F-ATPase) and V_0V_1-ATPase (V-ATPase). P-ATPase is found in plasma membranes. F-ATPase functions as an ATP synthase in mitochondria, chloroplasts and oxidative bacteria. V-ATPase is a proton pump in acidic organelles, plasma membranes of eukaryotic cells and bacteria. V- and F-ATPases resemble each other both structurally and functionally. The X-ray crystal structure of the F_1-ATPase was determined by John Walker's group. He was awarded with the Nobel prize. The structures of V-ATPases have not been obtained yet.

A V-ATPase transports Na^+ or Li^+ in an eubacterium *Enterococcus hirae*. The enzyme, consisting of nine subunits encoded by a Na^+-responsive operon (designated *ntp*), has been purified in large-scale using the cloned genes, and characterized (1). We have also established the large-scale purification of the head part (V_1) of the Na^+-ATPase. Then we tried to crystallize the V_1-ATPase and obtained several crystals.

We have already collected diffraction data to 7.0 Å resolution [2001B0025-NL-np]. The data analysis is now in progress; however, it was difficult to determine the starting model structure by molecular replacement, because of the insufficient quality of the data with high mosaicity. Therefore, we tried to obtain better crystals by growing under different conditions from the previous one. Cryoprotection condition was also examined with various reagents and concentrations.

We obtained several crystal forms, plate-like and needle-like forms. X-ray diffraction measurements were carried out under cryogenic condition (100 K) using flash-cooling technique on the BL40B2 station of SPring-8. The wavelength, the camera length and the oscillation range were 1.0 Å, 300 mm and from −180° to 180°. The oscillation angle and exposure time per frame were 1.0 degree and 10.0 sec, respectively. The crystals diffracted to 10.0 Å resolution.

1) T. Murata et al., J. Biol. Chem. 272, 24885-24890 (1997)

X-ray Fiber Diffraction Measurements of Native Collagen Fiber Obtained from Kangaroo Tail Tendon

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Collagen is most abundant protein in mammals and represents the major structural protein in the extracellular matrix. The basic structural motif of collagen is the triple helix. Each of the three strands has a left-handed polyproline II type 3/1-helical conformation. Such three strands wind around a common axis in a right-handed fashion. Among the structural models proposed for native collagen, the Rich and Crick model (10/3-helical model) and the Okuyama model (7/2-helical model) have been generally accepted. Although the latter was derived from the structure analysis of the model peptide, $(Pro\text{-}Pro\text{-}Gly)_{10}$ single crystal, this could explain the fiber diffraction pattern of native collagen. Recently, structural studies on other model peptides, such as $(Pro\text{-}Hyp\text{-}Gly)_{10}$, $(Pro\text{-}Hyp\text{-}Gly)_4$-$(Pro\text{-}Hyp\text{-}Ala)$-$(Pro\text{-}Hyp\text{-}Gly)_5$ and so on, have revealed the overall structures of peptides to be in accordance with the Okuyama model. We are currently investigating a series of the crystal structures of collagen model peptides (2002A0089-NL1-np). In addition, we also try to collect high resolution fiber diffraction data of collagen using synchrotron radiation source in order to elucidate the details of collagen structure and confirm our model.

Specimens for X-ray measurements were dissected from kangaroo tail tendon which was provided from Tama Zoo in Tokyo, Japan. We collected about 10 fiber diffraction patterns of native collagen by using R-AXIS IV^{++} detector with 300 seconds exposure at room temperature and low temperature. Diffraction data were processed using XFIX and FTOREC programs (CCP13). X-ray intensity distribution of native collagen along each layer line is shown in Fig. 1. Structure analysis is now in progress.

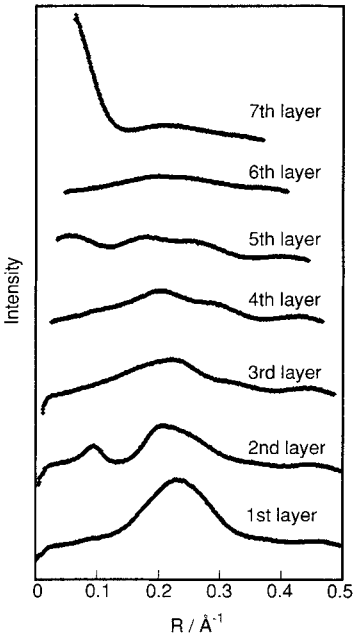

Fig. 1 X-ray intensity distribution of native collagen from kangaroo tail tendon.