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 crystalize the V_1-ATPase and obtained several crystals.

In this project, we examined the X-ray diffraction patterns of the crystals. We obtained several crystal forms, plate-like, coffine-like, cubic, and needle-like forms. High resolution was obtained using the plate-like crystals. The symmetry was C_2, the lattice constants were 241.6, 141.1, 239.1 Å, and the resolution was 2.4 Å. We are still trying to get better crystals for determination of three dimensional structure.

We found that an enolase from *Enterococcus hirae* was co-purified with our V_1-ATPase. We further purified this enzyme and succeeded in crystallization. We obtained coffine-like and plate-like crystals. High resolution diffraction was obtained, symmetry being I_4 and lattice constants being 153.5, 153.5, 90.6 Å, 90°, 90°, 90° with 2.2 Å resolution. An asymmetry unit contained 8 enolase molecules. We are now determining the three dimensional structure of it by molecular displacement.

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

Protein Crystallography by MAD Method Using Xe or Cs

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Xenon, a rare gas, can be introduced into many protein crystals with no handling but pressurization. Cesium is an alkali metal and soluble up to high concentration in water as ion. These atoms are candidates for the heavy atom of routine phase determination by multi-wavelength anomalous dispersion (MAD) technique. However, *K*-absorption edges of these atoms are located at so short wavelength region (~0.35Å) that these atoms had not been used as heavy atom on MAD. Until now, BL41XU is an only beamline for macromolecular crystallography in SPring-8 that we can perform MAD experiment using such short wavelengths.

To evaluate beam strength and detection ability of the detector (marCCD 165) at such short wavelengths, we collected reflection data of the crystals of extracellular chaperonin, LolA at 1.0000 Å (Data1) and 0.3585 Å (Data2) from one crystal. Experimental conditions and data statistics are listed in Table 1. Lattice constants determined from Data2 are 0.3% smaller than that of Data1.

By XAFS measurements of NH_4I, Xe and CsCl, wavelengths of *K*-absorption edges were determined as 0.3731Å, 0.3580Å and 0.3438Å, respectively. These values are about 0.2 % shorter than the values previously reported.

Disagreement of the lattice constants between two data sets and the shift of absorption edges may suggest that we must calibrate wavelengths at this short wavelength region.

Three data sets of the Xe derivative of LolA were collected for MAD phasing to establish this technique using short wavelengths. Data analysis is now in progress.

Table 1

	Data1	Data2
Wavelength (Å)	1.0000	0.3585
Camera length (mm)	120	300
Exposure time (sec/degree)	5	25
Temperature (K)	100	100
Space group	I222	I222
a (Å)	55.83	55.66
b (Å)	75.27	75.06
c (Å)	99.28	98.97
Resolution (Å)	25.0-1.80	25.0-2.20
(last shell)	(1.86-1.80)	(2.28-2.20)
Completeness (%)	99.7	99.0
(last shell)	(99.8)	(94.6)
Rsym	0.047	0.077
(last shell)	(0.193)	(0.281)
<I/sigma>	27.5	12.5
(last shell)	(5.5)	(3.2)