X-ray Crystallographic Studies of V-type Proton-ATPase

Nobutaka Numoto (0004712)[1] and Kunio Miki (0003192)[1,2]※

[1] Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[2] RIKEN Harima Institute / SPring-8, Koto 1-1-1, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan

V-type proton-ATPases (V_oV_1-ATPase) are found in the plasma membranes of some archaea and eubacteria. Their physiological role is ATP synthesis coupled with proton flux across the plasma membranes. V_oV_1-ATPase from *Thermus thermophilus* consists of two functional sets of subunits, a peripheral V_1 moiety (V_1-ATPase) and a membrane integrated V_o moiety. The V_1-ATPase has an ATP synthesis/hydrolysis activity and is composed of four subunits, A, B, γ and δ, with a stoichiometry of $A_3B_3\gamma_1\delta_1$. The molecular weight of the V_1-ATPase is estimated to be 400,000.

We have succeeded in the crystallization of the V_1-ATPases from *Thermus thermophilus*. Well-shaped hexagonal crystals with dimensions of 0.7×0.5×0.5 mm^3 were obtained within two weeks at 20 °C from a protein solution of 20 mg/ml, 1.7 M ammonium sulfate and 10% (v/v) dioxane in 100 mM MES-NaOH buffer at pH 6.0. Crystals were sealed in thin glass capillaries. X-ray diffraction experiments were performed at the BL41XU beamline at room temperature. The wavelength, the camera length, and the oscillation range were 1.000 Å, 350 mm, and 1.0 degree, respectively. Diffractions from crystals extend to 6.0 Å resolutions (Figure).

The data were processed using program HKL2000. Crystals belong to the space group $P321$ ($P3_121$, $P3_221$). The unit cell dimensions were determined as a = 389.9 Å and c = 156.9 Å. Assuming that asymmetric unit contains two, three or four molecules of V_1-ATPase, the V_M value is calculated as 4.2, 2.8 or 2.1 Å^3/Da, respectively.

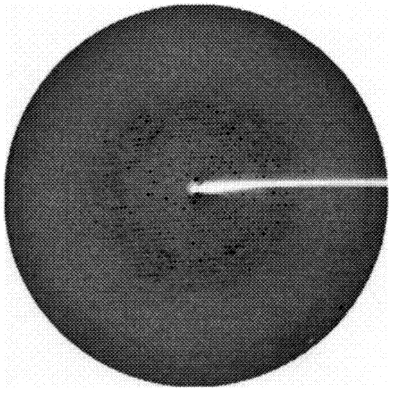

Figure. X-ray diffraction photograph of V_1-ATPase crystals.

Crystallographic study of a 32K fragment of flagellar hook protein

Katsumi Imada* **0003386**
Keiichi Namba **0003335**
Fadel Samatey **0003388**
Shigehiro Nagashima **0003379**

Namba Protonic NanoMachine Project, ERATO, Japan Science and Technology Corporation

Bacteria flagellum consists of a long filament, a short curved segment called hook and a basal body. The hook is constructed from subunits of a single protein, FlgE, which is also called hook protein. The hook acts as a universal joint. The torque generated by the motor in the basal body is transmitted to the filament smoothly through the hook. To understand the universal joint mechanism of the hook, we are analyzing the atomic structure of FlgE using X-ray crystallographic method.

FlgE tends to polymerize into filament under normal crystallization condition, therefore, a 32 K dalton fragment (H32) was produced for crystallization. Orthorhombic crystals (a = 48.9 Å, b = 96.7 Å, c = 128 Å) were grown from a solution containing PEG2K and copper acetate by the hanging drop vapor diffusion method.

Cupper acetate is necessary for the crystallization of H32, therefore, Cu was expected as a good candidate for a MAD experiment. We collected multiple anomalous data near the Cu K edge. Although Bijvoet difference Patterson maps indicated specific binding of Cu, we could not obtain good phases enough for structure analysis.

This time, we prepared Pt derivative crystals using cisplatin or K_2PtCl_4 and collected diffraction data. Three wavelengths, 1.0722 Å, 1.0725 Å and 1.0662 Å, were selected for the MAD experiment based on the XAFS measurement.

Diffraction data were recorded with a Mar CCD detector. The exposure time was 30 sec for 1° oscillation. An aluminum attenuator with a thickness of 0.4mm was inserted during the measurement to avoid radiation damage. The diffraction data were processed with HKL2000. Results of the data collection are summarized in Table 1. The structure analysis using these data is underway.

Table 1 Summary of data collection

cisplatin derivative

	peak	edge	remote
Resolution	2.5Å	2.5Å	2.5Å
Rm	8.0%	8.9%	9.0%
Completeness	99.5%	99.0%	98.0%

K_2PtCl_4 derivative

	peak	edge	remote
Resolution	2.5Å	2.5Å	2.5Å
Rm	7.5%	6.7%	7.7%
Completeness	99.0%	97.0%	99.0%