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X-ray crystallographic studies of V-type H^+-ATPase

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Introduction

V-type H^+-ATPases (V_oV_1-ATPase) and F-type H^+-ATPases (F_oF_1-ATPase) are two subclasses of the ATPase/ATP synthase superfamily. In eukaryotic cells, F_oF_1-ATPases are responsible for ATP synthesis and present in the inner membranes of mitochondria and thylakoid membranes of chloroplasts. In prokaryotic cells, V_oV_1-ATPases 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.

Thermus thermophilus is an aerobic thermophilic eubacterium, which has an optimum growth temperature of 343K. It was shown that the plasma membrane H^+-translocating ATPase of *T. thermophilus* belongs to V_oV_1-ATPase. V_oV_1-ATPase from *T. 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. The catalytic sites are located on the A subunits, which are homologous to the β subunits of F_1-ATPases, and has nucleotide binding Walker motifs.

The three-dimensional structures of F_1-ATPases have already been determined at atomic resolution. However, no three-dimensional structures of V_oV_1-ATPases are available. Therefore, the structure determination at high resolution is indispensable for understanding of V_oV_1-ATPases; furthermore, the structural comparison between F_oF_1-ATPases and V_oV_1-ATPases is expected to elucidate the common structural features as biological energy conversion machinery, which couples ATP synthesis/hydrolysis reaction with proton translocation across a membrane.

Results and Discussion

V_1-ATPase from *T. thermophilus* was purified as described [1]. Crystallization was performed by the sitting drop vapor diffusion technique. The crystals of V_1-ATPase appeared under conditions using Ammonium Sulfate and Dioxane as precipitants. The optimized crystallization condition is as follows; 2-4 μl of a solution mixture of 20 mg/ml protein in a buffer solution of 10 mM Tris-Cl, pH 8.0, and the same volume of a reservoir solution were equilibrated against 500 μl of the reservoir at 293 K for 2 weeks. The reservoir solution consisted of 1.7 M Ammonium Sulfate, 10% (w/v) Dioxane, and 100 mM MES-NaOH, pH 6.0. Well-shaped hexagonal crystals with dimensions of $0.7 \times 0.5 \times 0.5$ mm^3 were obtained. Crystals were sealed in thin glass capillaries.

X-ray diffraction studies were performed at room temperature at the Photon Factory (BL6A and BL18B). Diffractions from these crystals extend to 6.0 Å resolutions. The data were processed using program HKL2000. These crystals belong to the space group $P321$ (or $P3_121$, $P3_221$). 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.

References

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