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X-ray crystallographic study of V_1-ATPase

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Introduction

V-type ATPase belongs to a class of ATP-driven proton pumps responsible for acidification of intracellular compartments in the cells. Although it had been believed that all H^+-translocating ATPases in prokaryotic cells are F_0F_1-ATPases, some kinds of eubacteria as well as most archea have been found to hold V_0V_1-ATPases whose role is ATP synthesis coupled with proton flux across membranes. V_0V_1-ATPases consist of two functional sectors (as does the F_0F_1-ATPase) designated as a peripheral V_1 moiety and an integral membrane V_0 moiety.

The proton translocating ATPase found in the plasma membrane of an aerobic thermophilic eubacterium, *Thermus thermophilus*, belongs to the class of V_0V_1-ATPases. The V_1 moiety from *T. thermophilus* HB8 consists of four subunits with a stoichiometry of $A_3B_3\gamma_1\delta_1$. The molecular masses of the individual subunits are approximately 66 (*A*), 55 (*B*), 30 (γ) and 11 kDa (δ), respectively[1].

A structural comparison at high resolution between V- and F-type ATPases would elucidate the common features involved in biological energy by which ATP synthesis/hydrolysis reaction is coupled to proton translocation across a membrane.

Experimental

Prior to the crystallization, the protein solution was incubated in the presence of 5 mM Mg-ADP at 328 K for 10 minutes. Crystallization conditions were screened by use of the sparse-matrix approach. The hanging drop vapor diffusion method was employed throughout the crystallization study. The crystallization procedure was as follows; 2 µl of a solution containing 16.2 mg/ml *Thermus* V_1-ATPase in 20 mM Tris-HCl pH 7.2, and the same volume of a reservoir solution were equilibrated against 500 µl of reservoir at 298 K for 2-3 weeks. The reservoir solution contained 100 mM sodium acetate, 2 M sodium formate, and 5 mM Mg-ADP, adjusting pH to 5.5.

The obtained crystals were mounted in cryo-loops followed by rapid dipping through a buffer containing the same components as the reservoir solution plus 30 % glycerol as a cryo-protectant. Intensity data were collected at 105 K using synchrotron radiation at the BL-6A. Oscillation photographs were taken by the ADSC Quantum 4R CCD detector. The data were processed using DPS/MOSFLM and programs from the CCP4 suite.

Results

Crystals of *Thermus* V_1-ATPase with approximate dimensions of 36 × 36 × 143 µm were frequently obtained. Larger sized crystals were rare and was probably due to the tendency of V_1-ATPase to unfold at the interfaces, forming a film of unstructured polypeptides that inhibited further equilibration.

Preliminary intensity data were collected in which the diffraction extended beyond 3.4 Å resolution. The data indicated that the crystals belong to the trigonal space group *P*3. The unit cell dimensions were determined as a = b = 89.0 Å, c = 179.2 Å, and γ = 120°. Assuming that the unit cell contains one molecule of V_1-ATPase with a molecular mass of 404 kDa, the V_m value is calculated as 3.0 $Å^3$/Da, and corresponds to a solvent content of 59.6 %.

The initial processing of data has been restricted to the diffraction range of 20 - 6.5 Å, due to the anisotropic nature of the diffraction data obtained. There still remain several possibilities to assign the space group within the hexagonal system, such as *P*6, $P6_3$, $P6_322$, as well as to the trigonal system *P*3. The peripheral portion (hexagon) composed of the large subunits, *A* and *B*, may strongly dominate the diffraction data, especially if the small γ and δ subunits are located with some disorder, as has been described for the atomic model of mitochondrial F_1[2]. Recognizing that the *A* and *B* subunits are highly homologous but not identical, and that there are only single copies of the γ and δ subunits in the V_1 moiety, we have to be aware that the (pseudo-) symmetrical axis just suggested may be limited only to the low and middle resolution range. I thus conclude that the space group of *P*3 is the one that most likely describes the current resolution range (20 - 6.5 Å)[3]. The unit cell dimensions seem reasonable from the view of the molecular size of V_1-ATPase, and from a comparison with the previously reported dimensions for the crystals of F_1-ATPases[2, 4].

According to the report[1], the crystals that I obtained and described above are likely to contain V_1-ATPase molecules in the Mg-ADP-inhibited form. Thus, the structure analysis of this crystal form may help to understand the manner in which Mg-ADP associates with and inactivates V_1-ATPase during ATP hydrolysis. The crystals obtained under these condition can be used for structure determination at higher resolution. The further optimization of crystallization conditions is in progress, along with intensity data collection and structure determination.

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