NE3A/2010G125

Crystallographic Analysis of Prokaryotic V-type ATPase

Nobutaka Numoto[1], Akiko Kita[1], Kazuki Takeda[2] and Kunio Miki[2,*]
[1]Research Reactor Institute, Kyoto University, Kumatori, Osaka 590-0494, Japan
[2]Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

1 Introduction

V-type ATPases (V-ATPases) synthesize or hydrolyze ATP through the rotary catalytic mechanism in a similar manner to well-known F-type ATPases (F-ATPases), and both enzymes belong to the rotary ATPase/synthase superfamily. V-ATPases occur in the membranes of acidic organelles, such as lysosomes and endosomes in eukaryotic cells, maintaining acidic pH by pumping protons coupled with ATP hydrolysis. In prokaryotic cells, V-ATPases exist in the plasma membrane and are primarily responsible for ATP synthesis. V-ATPases consist of cytosolic component (V_1-ATPase) and membrane embedded component. V_1-ATPase shows ATPase activity and its structural composition is A_3B_3DF.

Although V_1-ATPase is almost homologous to F_1-ATPase ($\alpha_3\beta_3\gamma\delta\varepsilon$) from the viewpoint of overall structures and catalytic mechanisms, detailed analyses of the rotation kinetics of V_1-ATPase have revealed divergence in the torque and rotation steps between V_1- and F_1-ATPases. Our structural study of whole V_1-ATPase from *Thermus thermophilus* at 4.5 Å resolution [1] strongly suggests that V_1-ATPase and F_1-ATPase utilize different rotation mechanisms due to different manners in inter-subunit interactions and in tertiary structural transitions of the catalytic subunits. To elucidate more abundant structural basis on the rotation mechanism of V_1-ATPase and a common essence of the two rotary ATPases, we have addressed a crystallographic analysis of prokaryotic V_1-ATPase at higher resolution.

2 Experimental

Purified V_1-ATPase was crystallized under high magnetic forces fields (gradient magnetic fields) from a superconducting magnet system designed for the protein crystallization. The protein solution containing 30 mg/ml of V_1-ATPase and final concentrations of 10 mM of Mg-ADP and 1 mM AlFx were prepared for the crystallization experiments. To compare the structures, crystallization was also preformed without the magnetic forces fields. All crystallization experiments were performed using the vapour-diffusion method with a reservoir solution containing 1.6-1.8 M ammonium sulphate, and typical dimensions of the obtained crystals were $0.3\times0.3\times0.2$ mm^3. X-ray diffraction experiments were performed at the NE3A beamline using an ADSC Q270 detector. The wavelength, beam size, camera length, oscillation range, and exposure time were 1.0000 Å, 100 $\times$100 μm^2 (with no attenuator), 457.9 mm, 0.5 degree, and 5.0 sec, respectively.

3 Results

The crystals grown in the high magnetic forces fields and the reference condition diffracted up to 6.0 Å resolution. The diffraction data were processed using programs HKL2000 and CCP4i, and the data collection statistics are given in Table 1. Slight improvements in the values of overall R and crystal mosaicity were observed for the crystal obtained in the high magnetic forces fields. These facts suggest that crystals grown in the high magnetic forces fields could exhibit an enhanced crystal quality and could be useful for the data collection at higher resolution.

Electron densities for the bound nucleotides were clearly observed in the difference Fourier map at two of three catalytic sites of V_1-ATPase. Further analyses are in progress.

Table 1: Data collection statistics

Crystal	Magnetic forces fields	Reference
Wavelength (Å)	1.0000	1.0000
Oscillation range (deg.)	0.5	0.5
Exposure time (sec)	5.0	5.0
Space group	*P*321	*P*321
Cell *a*/*c* (Å)	379.6 / 147.2	381.0 / 147.3
Resolution (Å)	50-6.00 (6.21-6.00)	50-6.00 (6.21-6.00)
No. of observed	340,862	342,312
No. of unique	30,813	31,049
Redundancy	11.1 (10.7)	11.0 (10.6)
Completeness (%)	99.8 (100)	99.9 (100)
$I/\sigma(I)$	34.8 (2.5)	24.2 (1.5)
Mosaicity	0.35-0.57	0.41-0.61
R_{sym} (%)	8.7 (>100)	9.8 (>100)

Values in parentheses are for the outermost resolution shell.

References

[1] N. Numoto *et al*., EMBO Rep., **10,** 1228 (2009).

* miki@kuchem.kyoto-u.ac.jp