6A, 18B/2001G151

X-ray crystal analysis of α3β3γε sub-complex of F1-ATPase from a thermophilic bacterium

Yasuo SHIRAKIHARA*[1], Toshiharu SUZUKI[2], Aya SHIRATORI[1], Katsumi MAENAKA[1], Kazuyasu SHINDOH[1], Masasuke Yoshida[2]
[1] National Institute of Genetics, Mishima, Shizuoka 411-8540 Japan
[2] The Chemical Resources Laboratory, Tokyo Institute of Technology, Nagatsuta 4259, Yokohama 226-8503, Japan

Introduction

F1-ATPase, with a subunit composition of α3β3γδε is a catalytic sector of the membrane bound ATP synthase. The ATP synthase plays a central role in energy conversion, generating ATP from ADP and inorganic phosphate using energy derived from a trans-membrane electro-chemical potential. The rotational catalysis mechanism of F1 is accompanied by rotation of the rod-like γ subunit, which is thought to control the conformations of the three catalytic β subunits in a cyclic manner. Using the thermophilic F1-ATPase (TF1), we have been extending the structural study from the α3β3 sub-assembly to the α3β3γδε sub-assembly. In this extension, we aim to detect structural changes caused by different mode of nucleotide binding, which should provide with structural basis for understanding the rotational catalysis mechanism.

The α3β3γδε sub-assembly crystals, when experimented with rotating-anode source, diffracts to 4.5 A resolution at room temperature and 7 A at 100K. With synchrotron beam, we collected a data set to resolution of 4.5 A at 100K. From those figures, the current cooled crystals are thought to be unsatisfactory. While improvement in the cryo-cooling is in progress, we are pursuing to collect the diffraction data at room temperature. In the pervious experiment at BL44 in SP-ring8, we found that the beam (70um in diameter) had to be attenuated by a factor of 300 for avoiding the beam damage and that an IP detector had to be used for the required exposure time much over 1miniute (typically 3 minutes). The results of the experiment was puzzling and disappointing; in one orientation of the plate-shaped crystal, the data extended to only 8 A resolution, and in another orientation (orthogonal to that orientation) the data extended to 4-4.5 A resolution. Those data sets extend at best only marginally compared with the data set obtained in–house.

In this beam time, we made another attempt to collect data at room temperature using the milder beam available at BL18B. We could collect some data sets extending to 3.5 A resolution data, but found problems in collecting the complete data set.

Materials and methods

E. coli Over-expression system for the α3β3γε sub-complex was constructed to allow efficient purification of the sub-complex. The sub-complex was purified with Ni-NTA, DEAE Toyopearl, Phenyl-Toyopearl columns and with additional heat treatment. Crystals of α3β3γε sub-complex were grown at 25℃ by the sitting drop technique. The 5 μl drop contained 9-11 % PEG 6,000, 0.20 M sodium chloride, 0.05 M Tris-sulphate buffer (pH 8.0), 0.5mM ADP, 2mM DTT, 5mM CDTA, 10% (v/v) ethyleneglycol and 10 mg/ml protein, and the 1 ml reservoir contained 16 % PEG 6,000, 0.2 M sodium chloride and 0.05 M Tris-sulphate buffer (pH 8.0), 5mM CDTA, 10% (v/v) ethyleneglycol. Plate crystals (typical dimensions 0.4 mm × 0.3 mm × 0.15 mm) grew in 2 weeks. Crystals are tetragonal, space group I4122, with cell dimensions of a=b=233.3 Å, c=305.3 Å. For data collection at BL18B, the beam was not attenuated. The wavelength was 0.98A, and the ADSC CCD detector was placed at a camera distance of 300mm.

Results and discussions

The suitable exposure time was found to be 90-120sec, depending on crystals, for 1 degree rotation. The diffraction patterns were again more or less dependent on the orientation of the crystal, as observed before (see Introduction). But the dependency was less clear than in the previous experiment. When the x-ray hits the side of the plates, the patterns extended to 3.5-4.5A resolution (3 independent cases). However, when the incident x-ray hits the face of the plates (3 independent cases), the diffraction pattern extended to 4.5-5.0 A resolution. In both cases, the diffraction patterns were recorded covering 30-degree rotation before the radiation damage deteriorated the diffraction limit seriously. Overall, compared to the previous study, the data collection from the crystals at room temperature at BL18B is more practically feasible. The higher resolution of the data (3.5 A vs. 4 A), the less dependence of the resolution of the data on the orientation of the crystals and the more data collected from a single crystal (30 deg. vs. 3 deg.) constitute the reasons. However, the resolution of the data (the best 3.5 A, accompanied by much lower value at unfavorable orientations) is not much better than that obtained from the cooled crystals. We would need a breakthrough for data collection at room temperature.

*yshiraki@lab.nig.ac.jp