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Structure biology of thermophilic F1-ATPase

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Introduction

ATP synthase is responsible for ATP production in living cells, and is a membrane protein located in the energy conversion membrane. ATP synthase consists of a channel Fo portion (about 100,000 dalton, subunit composition of ab2c8-12) and a large soluble catalytic F1 portion (380,000 dalton, $\alpha 3\beta 3\gamma\delta\varepsilon$). The unique rotational catalysis mechanism of F1 includes rotation of the rod-like γ subunit, which is thought to control the conformations of the three catalytic β-subunits in a cyclic manner by its rotation.

F1-ATPase is a catalytic sector of the membrane bound ATP synthase. We have been working on structural study of thermophilic F1-ATPase. We have been looking at structural changes caused by different modes of binding of nucleotides that are catalytic or non-catalytic. This approach should provide with structural basis for understanding the rotational catalysis mechanism.

Previously, we had collected data from various crystals of F1 that are different in number and species of bound nucleotides. Those crystals diffracted to 4A resolution, irrespective of various nucleotide binding modes. However, our recent examination of freezing conditions of the crystals showed that diffraction limit could be extended from 4A to 3.8A, by increasing the precipitant concentration considerably (however, the exact conditions had not been established). At the same time, we planned to improve the previous F1 structures solved only using molecular replacement, by incorporating experimental MAD phase information. The selenomethionine derivative of F1 was successfully made in our hands, and was crystallized as the native protein. In the preliminary diffraction experiment, however, those crystals were much more radiation sensitive than the native crystals.

For three beam times available in 2007, we prepared selenomethionine F1 crystals that were formed in the absence of added nucleotide, and then were flash-frozen in the cryo buffer containing 25% glycerol and a number of different concentration of PEG (24% to 40%; 14% PEG was in the mother liquor). The beam at BL6A was just good for those radiation-sensitive F1 crystals; for example the beam without any attenuation at NW12 was too strong to collect 90 degree slice of data for those I4122 crystals. In our experiences, the BL6A allows to collect 90 degree slice of data(1~2min exposure time per 1 degree frame) over three wavelengths.

In the course of experiment at the beam line, we noticed that some of the crystals did not look stationery some time, and we suspected that the movement was real, not the artefact due to density fluctuation of the cry beam through which we observe the crystal. We checked over the matter by looking at the correlation of crystal images and diffraction statistics of all the available crystals.

Results

All the data (unit cell is a=b=233.3 A c=305.6 A, space group of I4122, were processed with MOSFLM and CCP4 program suite (SCALA and other programs).

Firstly, for seeing effect of possible shaking of crystals, we thought the difference between the resolution of single image (typically the first image) and the conventional resolution of the data set. We expect shaking will make the difference larger. We find that usual cases (without apparent shaking in the image; 5 cases) had differences of about 0.2A,. however, that three conspicuous cases had differences of 0.6~1.1A. We concluded that the cry stream made some of, but no all of, our cryo loops unstationery.

Secondly, we find that the PEG concentration in the cryo solution of 30 or 32.5 % gave the best resolution of data sets (3.8A). Our previous PEG concentration of 16% gave 4.1A. When the PEG concentration is increased, the resolution steadily gets better, reaching the optimum value above. When the PEG concentration is further increased, the resolution gets rapidly worse with eg 4.25 A at PEG concentration of 40%.

'Solve' analysis of the best MAD data obtained with the PEG concentration of 32.5% set found 60 Selenometionine sites out of expected 72. Compared with various softwares available for MAD analysis, the combination of solve and SHARP gave the best results i.e. the best density map and the best accompanied statistics. Further structural analysis by combination of molecular replacement and MAD phases is in progress.

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