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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. 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 about 4A resolution, irrespective of nucleotide binding modes. Among those, we found that, in a novel single-nucleotide bound condition, F1 may present a novel structure where the inhibitory ε-subunit let two extended helices to penetrate into core of catalytic $\alpha 3\beta 3$ sub-assembly. However, we needed its higher resolution structure for us to be certain about the structure.

In our recent examination of freezing conditions of those crystals showed that diffraction limit could be extended from 4A to 3.7A, by increasing the precipitant concentration in a cryo buffer to 30 or 32.5 % (14% PEG in the mother liquor). Furthermore, we planned to improve the F1 structure (previously solved using molecular replacement (MR) only) by incorporating experimental MAD phase information. A severe radiation damage problem of the SeMet derivative crystals was got around by using the beam at BL6A. In the fiscal year 2007, we collected several MAD datasets at BL6A. We were lucky in that one MAD dataset was devoid of shaking of a cryo loop due to not optimized cold gas flow there. We used the dataset in the analysis described below. In the fiscal year 2008, we did no experiment on the beams lines, simply because no beam time was allocated when we applied for it (later we stopped applying because we thought the same could happen). This was ok, since the best MAD data were collected some time in the year 2007 as described, and further efforts in that year to get any better data were unsuccessful.

Results

The best MAD data (unit cell 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). In SCALA, scaling was done in the way that all equivalent frames at each wavelength join simultaneously in scaling.

We initially used SOLVE that allowed us to find 60 Selenium sites out of the expected 79. However, the map was not good enough. Then we used SHARP by feeding 79 Selenium positions expected from the molecular replacement solution, and SHARP confirmed 68 sites and refined them. The SHARP map was excellent, showing detailed features in density, which was missing in our previous 4A resolution MR maps. The subsequent SHARP residual analysis combined with external phase information from the 68 sites found new 9 sites (so a total of 78 sites being identified) and gave better heavy atom statistics, however, maps were not improved much.

We then computed maps using combined phases from both MR and MAD. Those maps were either accompanied by B-factor sharpening (-95) or without it. B-factor sharpening helped a lot to see many of side chain densities, but it was not perfect as this also brought some noises in maps. Both types of maps from the combined phases were, however, generally better than the previous 4 A resolution MR maps.

The best map was from MR phases to 3.7A resolution, combined with B-factor sharpening (-95). The used data were the peak's in our best MAD data set. The map presents side chain density for most of the 3300 residues and helped much especially in modelling C-terminus 50 residues containing two extended helices of the ε-subunit. That part had been quite difficult to model in our previous 4 A resolution MR map which lacked side chain features there. The two methionines in that part identified in SHARP help enormously to avoid register error in modelling. The C-terminus 50 residues have a key role in regulating activity of F1, which was evidenced by many biochemical and biophysical studies.

Although the use of combined phases did not work as we had anticipated, the surpassed resolution of the used peak data and positions of selenium atom did invaluable good to our structure analysis. Refinement of the model is now in progress.

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