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Improvement of ATP synthase crystals

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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.

Starting from elucidation of the $\alpha 3\beta 3$ sub-assembly structure of the thermophilic F1, we have been moving up to the sub-assemblies with higher levels. The $\alpha 3\beta 3\gamma$ sub-assembly was difficult to crystallize, but the $\alpha 3\beta 3\gamma\varepsilon$ sub-assembly gave crystals that allowed to see a novel conformation of F1. We are now dealing with the holo-enzyme, ATP synthase, a membrane protein that is distinct from the previous subassemblies.

The membrane protein ATP synthase is still a challenging target for a structural study, in view of relatively few solved structures of the membrane proteins so far. Though three years ago we were able to record the first diffraction patterns from ATP synthase crystals to about 7 Å, we obviously had to improve quality of the crystals and that has been done over these years.

Firstly, we have continued intensive examination of the protein preparation by changing detergents species, PS3 culture batches, kinds of nucleotide present in the extraction step and various purification parameters. Quality of each preparation was evaluated by its crystallization capability and by diffraction capability of the resultant crystals, if crystallized. This procedure established the following. Decyl-maltoside and dodecyl-maltoside, both of which had been useful for crystallization but indistinguishable between the two, were shown to have their own features. Though dodecyl-maltoside was much better than decy-maltoside in a number of respects, we have encountered a problem that crystals from dodecyl-maltoside were produced less frequently.

Secondly, we did a laser experiment in a hope to get better crystals, because it was shown that a well defined application of laser beam to crystallization setups is useful for inducing crystallization and sometimes for getting better ordered crystals. Although the laser beam clearly induced crystallization in the experiment, the resultant crystals were no better than the previous best crystals.

Thirdly, we made further efforts to analyze the diffraction patterns obtained those years. The analysis had been hampered by incorrect beam position parameters supplied and a high mosaicity of crystals. Data were recollected in a way that should allow diffraction analysis,

Results

Firstly, as described above for last year, we still had a serious problem that crystals from dodecyl-maltoside, the best detergent, were produced less frequently. We have therefore continued intensive examination of the protein preparation focusing on its variability over PS3 culture batches. This was after finding that dodecyl-maltoside from some, but not all, of the manufacturers were usable and that columns had to be carefully checked for its lifetime. Still, there was a lot of variability of quality of the preparations over batches, however, all the recent preparations have been inferior to the best preparations made two years ago. We are checking about a possible problem in storage of the stocks of cultured PS3 cells.

Secondly, we made further efforts to analyze the diffraction patterns obtained so far. The analysis had been hampered as described above, and our current view for processing the collected data sets is: (1) use plural frames in autoindexing stage (no single image was effective because one axis is almost parallel to beam). (2) use the orientation matrix from that stage in processing the rotation data. This view is realized best with adoption of mosflm in processing. For autoindexing, we wrote a script to check lots of possible (BeamX, BeamY) parameters against position data of spots picked from 90 degree apart stills (some were hand-picked), because of ambiguous beam positions. The obtained parameters allowed integration of about 20 images for the first time. However, it is apparent we have to make further efforts for complete integration of the obtained images (120 images).

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