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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 higher sub-assembly. 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.

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.

In an initial crystallization trial using ATP synthase extracted with dodecylmaltoside from the PS3 membrane, and then purified with an array of Q-sepharose high performance column, Superdex prep grade 200 column and the second Q-sepharose high performance column, we found that the preparation gave small crystals in polyethylene glycol solution containing MgADP as an ATP synthase specific additive. The preparation procedure was a conventional one. ATP synthase in our crystals contains all the 8 subunits, in contrast to yeast ATP synthase that lacks a and b subunits in crystals.

Encouraged by this initial success, we did further experiments that included improvement of the preparations, improvement in crystallization conditions and examination of resultant crystals using synchrotron beams. Last year, we did the following:

1. We identified the most promising detergents: dodecylmaltoside and decylmaltoside.
2. Among various columns examined, useful ones were found to be Q-sepharose high performance and Superdex prep grade 200 columns.
3. Factors in crystallization such as temperature, kind and concentration of monovalent salts, divalent salts and specific ligands have been optimized.
4. Crystals of dodecylmaltoside-extracted ATP synthase formed in presence of MgADP allowed us to record diffraction patterns to a resolution of about 7 A with a beam of BL5A. Crystals of decylmaltoside-extracted ATP synthase formed in similar conditions gave diffraction patterns also to a resolution of about 7 A with a beam of BL6A. From the PF experiments, we had an impression that crystals formed in presence of nucleotide other than ADP were worse than those formed in presence of ADP, irrespective of detergents.

Results

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. The problem was cured partially by finding better PS3 cell stocks, examining dodecyl-maltoside from different manufacturers and knowing properties of the used chromatographic materials better.

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, they were small compared with crystals grown without the laser beam application. We could not record protein diffraction pattern from either of the crystals. Further efforts to optimize the target crystallization conditions may be necessary for a successful laser experiment.

Thirdly, we made further efforts to analyze the diffraction patterns obtained last year. 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, and an analysis is going with our refined procedures.

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