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Structure of ATP synthase

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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 polyethylelene glycol solution containing MgADP as an ATP synthase specific additive. The preparation procedure was a conventional one.

Encouraged by this initial success, we proposed a further crystallization experiment that included improvement of the preparations, improvement in crystallization conditions and examination of resultant crystals using synchrotron beams.

Results

First a number of detergents have been examined for good extraction capability in the preparation procedure. In addition to dodecylmaltoside previously identified, decylglucoside and decylmaltoside have been shown to be promising candidates. Using those promising candidates, we examined preparation procedure and crystallizability of each of the obtained preparations carefully.

Detergents such as decylmaltoside, undecyl-maltoside, dodecyl-maltoside, tridecyl-malltoside were good in preparation. But others like alkyl glucosides were not. For example, dectylglucoside showed good extraction capability but gave an unusual elution profile in the Q-sepharose high performance column chromatography.

Among various columns examined, useful ones were Q-sepharose high performance, Superdex prep grade 200 columns. An array of Q-sepharose high performance, Superdex prep grade 200 and the second Q-sepharose high performance columns yielded a preparation of crystallization grade, but a smaller array of Superdex prep grade 200 and Q-sepharose columns only was indistinguishable from the first array.

Initial tiny crystals are now replaced by crystals with typical dimensions of 0.2mm, 0.2mm, 0.01mm after an extensive and systematic crystallization condition search. Among the detergents mentioned above, only decy-maltoside and dodecyl-maltoside gave diffracting crystals, though all of the four detergents gave similar-looking crystals.

ADP is the most favourite ligand, as others like AMPPNP, an ATP analogue, gave crystals that diffracted poorly. Other factors such as temperature in crystallization, kind and concentration of monovalent salts and those of divalent salts have been optimized.

ATP synthase in our crystals contains all the 8 subunits, in contrast to yeast ATP synthase that lacks a and b subunits in crystals.

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. Crystals from undecyl-maltoside or tridecyl-malltoside extracted ATP synthase diffracted significantly worse than the two. In 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. Further efforts are being made to get better diffracting crystals.

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