2P.10 Dual-axis cryo-electron tomography on intact mitochondria reveals the association of ATP synthase into oligomeric chains

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Cryo-electron tomography (CET) is an emerging electron microscopic technique for reconstructing a 3D volume representing large non-periodic objects, such as organelles, under cryogenic "life-like" conditions by incrementally tilting the specimen. Until recently only single-axis CET was possible due to technical limitations, but recently a rotation stage was developed allowing to collect two orthogonal tilt series in a cryo-protected way. Dual-axis tilting reduces the missing information of sampling originating from single-axis tilting over restricted tilt angles. In spite of that up to now most of the work on whole mitochondria is done by chemical fixation and plastic embedding. We implemented dual-axis cryo-electron tomography on an FEI Polara microscope and use it to generate tomograms from the frozen-hydrated mitochondria at about 6 nm resolution. The overall organisation of mitochondrial proteins remains a puzzle, therefore we initiated our work on intact mitochondria from *Bos taurus* and *Polytomella*, a green alga related to *Chlamydomonas*. We have obtained 3D reconstructions of intact mitochondria by cryo-ET and averaged subvolumes of oligomeric ATP synthase at 5.7 nm resolution [1]. This revealed the 3D arrangement of rows of ATP synthase dimers within the cristae membranes. Specific known details of monomeric ATP synthase, such as the connection of the stator to the OSCP subunit, were revealed for the first time in intact organelles. Previously performed single particle image analysis on isolated dimeric ATP synthases revealed a 70° angle between monomers, which strongly pointed out their functional role in the curvature of the inner mitochondrial membrane. The measured angle of 70° between the two F_1F_O ATP synthases in intact mitochondria supports this assumption. In fragmented bovine mitochondrial membranes dimers are flexible and the angle ranged from 55° to 95° [1]. Rendering of the membrane surfaces clearly demonstrated rows of ATP synthases located mostly in the curved region of the lamella cristae in bovine mitochondria and along the whole surface of tubular cristae in *Polytomella*. Likely other OXPHOS complexes are located between the rows of ATP synthases in *Polytomella* whereas the less highly curved regions are rather empty, as it was proposed for mammalian mitochondria [2].

References

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doi:10.1016/j.bbabio.2010.04.107

2P.11 What is the mechanism of MgADP-inhibition in F_OF_1-ATP synthase?

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Inhibition of F_OF_1 ATPase activity by MgADP trapped in a catalytic site in the absence of phosphate seems to be a universal feature of ATP synthase from all organisms. Although the phenomenon has been well-studied on the biochemical level, its molecular mechanism is still unknown. W. Allison et al. suggested that MgADP-inhibited state occurs when an arginine residue (α365Arg in *Bacillus* PS3 F_1 (TF_1), also known as the "Arg finger" essential for multisite catalysis) side-chain changes location from nucleotide phosphates towards the ribose moiety. As a first step of probing the molecular basis of MgADP inhibition we tried to check this hypothesis by mutating the nearby Val (α363Val) residue in thermophilic *Bacillus* PS3 enzyme. We found that introduction of Arg or Asn instead of the α363Val prolonged the lag in the onset of ATPase activity in isolated TF_1. In contrast, introduction of Ile completely abolished this lag. Pre-incubation of the sample with MgADP restored the lag in ATPase onset activity in αVal363Ile mutant, suggesting that the effect was caused by release of the inhibitory ADP. The steady-state rate of ATP hydrolysis, however, was not significantly influenced by any of the three mutations.

The mutations also affected the stimulation of ATPase activity by detergent LDAO that is known to relieve MgADP inhibition. In αVal363Ile mutant LDAO stimulated the activity slightly more than in the wild type TF_1. In contrast, in αVal363Arg and αVal363Asn mutants LDAO stimulating effect decreased by more than two-fold. The results suggest that the region on the αβ-interface near residues αVal363 and αArg365 might be involved in LDAO binding. We also found that αVal363Arg TF_1 was less sensitive to inhibition of ATPase activity by azide. The mutations (especially αVal363Arg) also somewhat diminished the suppression of ATPase activity by 10 mM phosphate. A possible role of these and other residues in MgADP inhibition is being discussed.

doi:10.1016/j.bbabio.2010.04.108

2P.12 The structure with bound nucleotides of a thermoalkaliphilic F_1-ATPase from *Bacillus* TA2.A1

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The structure of the F_1-ATPase lacking the δ-subunit ($F_1\Delta\delta$) from the thermoalkaliphile *Bacillus* sp. TA2.A1 has been determined at 3.0 Å resolution. The TA2 $F_1\Delta\delta$ sub-complex was over-expressed in *E. coli* C41(DE3) from the expression vector pTrc99a containing the gene *atpAGDC*. The purified TA2 $F_1\Delta\delta$ subcomplex was crystallized by microbatch in the presence of 1 mM ADP and 2 mM $MgCl_2$. A complete X-ray diffraction data set was collected from a single crystal, which belonged to the $P2_1$ space group, with two $F_1\Delta\delta$ sub-complexes in the asymmetric unit. MgADP is bound to the β_{DP}- and β_{TP}-subunits, and the β_E-subunit has no bound nucleotide. TA2 $F_1\Delta\delta$ has a canonical "ground state" structure, very similar to that described for F_1-ATPase from bovine and yeast mitochondria [1–2]. The γ-subunit does not have the truncated structure described in the structure of nucleotide free *Bacillus* $F_1\Delta\delta$ [3], and, as usual, its C-terminal α-helix penetrates into the collar region of the $\alpha_3\beta_3$ sub-complex. The C-terminal α-helices of the ε-subunit are held in the "down" position by an ATP molecule bound between the helices and the N-terminal β-sandwich domain, as observed in the isolated ε-subunit of F_1-ATPase from *Bacillus* PS3 [4].