

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 F₁F₀ 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].

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2P.11 What is the mechanism of MgADP-inhibition in F₀F₁-ATP synthase?

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Inhibition of F₀F₁ 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 (α 365Arg in *Bacillus* PS3 F₁ (TF₁), 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 (α 363Val) residue in thermophilic *Bacillus* PS3 enzyme. We found that introduction of Arg or Asn instead of the α 363Val prolonged the lag in the onset of ATPase activity in isolated TF₁. In contrast, introduction of Ile completely abolished this lag. Pre-incubation of the sample with MgADP restored the lag in ATPase onset activity in α Val363Ile 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 α Val363Ile mutant LDAO stimulated the activity slightly more than in the wild type TF₁. In contrast, in α Val363Arg and α Val363Asn mutants LDAO stimulating effect decreased by more than two-fold. The results suggest that the region on the α β -interface near residues α Val363 and α Arg365 might be involved in LDAO binding. We also found that α Val363Arg TF₁ was less sensitive to inhibition of ATPase activity by azide. The mutations (especially α Val363Arg) 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.

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2P.12 The structure with bound nucleotides of a thermoalkaliphilic F₁-ATPase from *Bacillus* TA2.A1

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The structure of the F₁-ATPase lacking the δ -subunit (F₁ $\Delta\delta$) from the thermoalkaliphile *Bacillus* sp. TA2.A1 has been determined at 3.0 Å resolution. The TA2 F₁ $\Delta\delta$ sub-complex was over-expressed in *E. coli* C41(DE3) from the expression vector pTrc99a containing the gene *atpAGDC*. The purified TA2 F₁ $\Delta\delta$ subcomplex was crystallized by microbatch in the presence of 1 mM ADP and 2 mM MgCl₂. A complete X-ray diffraction data set was collected from a single crystal, which belonged to the P2₁ space group, with two F₁ $\Delta\delta$ 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 F₁ $\Delta\delta$ has a canonical “ground state” structure, very similar to that described for F₁-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* F₁ $\Delta\delta$ [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 F₁-ATPase from *Bacillus* PS3 [4].

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2P.13 ATP synthesis by the isolated and reconstituted monomeric mitochondrial H⁺-ATP synthase from yeast

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The H⁺/ATP synthase from yeast mitochondria, MF₀F₁, was isolated, purified and reconstituted into liposomes prepared from phosphatidylcholine and phosphatidic acid. Analysis by mass spectrometry revealed the presence of all known subunits of the yeast enzyme ($\alpha_3\beta_3\gamma\delta\epsilon$ 45ad89₁₀Hfjge) with the exception of the K-subunit. The MF₀F₁ liposomes were energized by acid base transitions and K⁺/valinomycin diffusion potentials and high rates of ATP synthesis are observed. Titration of the number of MF₀F₁ per liposome indicates that the monomeric enzyme is able to catalyze high rates of ATP synthesis similar to that observed under physiological conditions. ATP synthesis was abolished by addition of uncouplers, as well as by the specific inhibitor oligomycin. The rate of ATP synthesis was measured as a function of pH_{out}, pH_{in} and the phosphate concentration. Maximal rates (turnover number) of approx. 100 s⁻¹ are observed at a transmembrane pH difference of 3.2 U (at pH_{in}=4.8 and pH_{out}=8.0), in the presence of a superimposed transmembrane electric potential difference of 133 mV (Nernst potential). The apparent K_M for P_i depends on the pH_{out}.

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2P.14 IF₁ influences on energy metabolism not only under energy restricted condition but also under normal condition

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IF₁ is a mitochondrial protein coded in nuclear genome [1, 2]. It is established that IF₁ binds mitochondrial F₀F₁-ATP synthase and inhibits its ATP hydrolysis activity. On the other hand, IF₁ does not influence ATP synthesis reaction. According to such properties, IF₁ is assumed to play a role in suppression of wasteful consumption of ATP by inhibiting the reverse reaction of F₀F₁-ATP synthase. However, physiological function of IF₁ remains poorly understood. We, here, studied it by using HeLa cells whose IF₁ expression is knocked down by short hairpin RNA (IF₁-KD cells). In the IF₁-KD cells, the ATP level decreased faster than that in the mock-treated cells under chemical ischemia as previously reported [3]. We show that IF₁ influences on

energy metabolism under normal condition; the metabolic rate of IF₁-KD cells was higher than that of the mock cells under normal medium cultivation to compensate wasteful hydrolysis of ATP by F₀F₁-ATP synthase. In addition, we will report that IF₁ has a wide and long-range effect on energy metabolism.

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2P.15 A possible role for the shift between inner and outer membranes in the rotor ring of F- and V-ATPases

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F- and V-ATPases fulfil their energy transforming function by a rotary mechanism. A tenet of rotary catalysis by F- and V-ATPases is that their transmembrane stator stays tightly connected to the adjacent rotor ring during rotation. It is still not clear how the rotor and stator complex interact in order to stabilize the rotary complex even during a relative rotation of the two domains with a frequency up to 500 Hz [1]. A remarkable feature of their proton or sodium transporting rotor ring is that they contain an inner lipid membrane. An even more remarkable feature is a relative shift of the inner lipid membrane towards the periplasmic side. This shift between inner and outer membranes was observed in high resolution structures of rotor rings from evolutionary distant rotary ATPases and thus appears to be a general feature [2–4]. According to the half-channel model of torque generation in F₀/V₀ rotary motors, a water filled half-channel permits access to ion binding sites of the rotor ring from the periplasmic side. The ion-binding sites are situated close to the center of the outer membrane hydrophobic core. Therefore, the difference in height of the outer and inner membranes brings the periplasmic half-channel in close proximity to the water filled cytoplasmic inside of the rotor ring. The electric potential between the periplasmic and cytoplasmic sides is expected to fall-off where the insulation is thinnest. I speculate that the shift between the inner and outer membranes in rotor rings results in a close proximity of the water filled periplasmic half-channel and bulk water of the cytoplasmic inside of the rotor ring. The expected distance of less than 12 Å is far shorter than the diameter of a typical membrane. This should lead to a radial potential fall-off between the stator and rotor ring. The resulting electric field between the stator and rotor ring could stabilize the complex during rotation. Importantly, an increased rotational speed due to a higher transmembrane potential would be accompanied by a stronger attraction.

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