2P.16 3D-reconstruction and overall topology of the dimeric mitochondrial ATP synthase of the colorless alga *Polytomella* sp

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Mitochondrial F_1F_O-ATP synthase of chlorophycean algae like *Chlamydomonas reinhardtii* and *Polytomella* sp. is a stable dimeric complex of 1,600,000 Da. It contains the conserved subunits of the rotor and catalytic sectors (α, β, γ, δ, ε, OSCP, a and c) but lacks the classic subunits that constitute the peripheral stator-stalk, regulatory elements, and the polypeptides involved in the dimerization of the complex (b, F6, A6L, IF_1, h, and g). Instead, it contains nine polypeptides of unknown evolutionary origin named ASA1 to ASA9. Therefore, the algal enzyme seems to have modified the structural features of its peripheral scaffold, while conserving almost intact the structure of its rotor and catalytic subunits. The isolated enzyme exhibits a very low ATPase activity (0.03 U/mg), that increases upon heat treatment or by incubation in the presence of low concentrations (0.01% w/v) of several non-ionic detergents. The detergent-activated enzyme is fully sensitive to oligomycin. In this work, we readdressed the overall topology of the enzyme with different experimental approaches: dissociation of the enzyme into subcomplexes, detection of close vicinities between subunits based on cross-linking experiments, and inference of subunit stoichiometry based on cysteine residue labelling. Monomer–monomer interactions seem to be mediated by the membrane-bound subunits ASA6 and ASA9, while ASA4, ASA2 and ASA7 appear to be closely-associated forming an important structural element of the peripheral stalk. In addition, three-dimensional structural features of the algal dimeric F_1F_O-ATP synthase were obtained using different experimental approaches: small angle X ray scattering with the enzyme in aqueous solution, and electron microscopy image reconstruction from single particle images.

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2P.17 How to survive and thrive without mitochondrial DNA: A protozoan's guide to ATP synthase modification

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Trypanosoma brucei is a single cellular eukaryotic parasite which normally has a complex host–vector life cycle. This organism undergoes extensive physiological modification in order to survive and proliferate within the very different host/vector organisms. Amongst other adaptations, ATP generation shifts from mitochondrial oxidative and substrate level phosphorylation in the insect vector to glycolysis in the mammalian bloodstream form. In spite of this, mitochondrial function and mitochondrial DNA gene expression are still essential in the bloodstream form. The procyclic forms utilize ATP synthase (complex V) and the proton gradient to generate ATP from ADP. Bloodstream forms, however, possess an incomplete respiratory chain and use ATP synthase in reverse in order to maintain the essential membrane potential of the mitochondrion; metabolizing ATP and pumping protons out of the organelle in the process. In trypanosomes, only one subunit of the F_O moiety of ATP synthase, A6, is encoded in the mitochondrial DNA. Despite the fact that mitochondrial DNA replication and gene expression in *T. brucei* are normally essential processes, so called dyskinetoplastic forms, which lack mitochondrial (=kinetoplast) DNA have been induced in the lab and also occur in nature. Thus, the dyskinetoplastic forms must have developed mechanisms to compensate for the essential mitochondrial gene product(s), reminiscent of petite-negative yeasts [1]. Indeed, we have previously identified a candidate mutation in the nuclearly encoded F_1 subunit γ of a laboratory induced dyskinetoplastic strain [2] and similar sequence polymorphisms have been identified in γ subunits of naturally occurring dyskinetoplastic forms [3]. We are currently testing the hypothesis that the identified mutations in the nuclearly encoded γ subunit of ATP synthase can allow the loss of the mitochondrial genome in *T. brucei*. Evidence will be presented indicating that a single point mutation in this subunit is indeed necessary and sufficient to permit survival of bloodstream *T. brucei* in the absence of mitochondrial DNA. Interestingly, this mutation appears to render the ATP synthase complex insensitive to oligomycin, suggesting that it results in uncoupling of ATP hydrolysis from proton transport across the inner mitochondrial membrane.

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2P.18 Crystallographic insight into the catalytic mechanism of subunit A of the A-ATP synthase and the P-loop switch in evolution

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In archaea type ATP synthases (A-ATP synthases), subunits A and B alternate in an A_3:B_3 hexamer, forming the A_1 headpiece. The hexamer is attached by a central and two peripheral stalks to a membrane-embedded ion-translocating part known as A_O, and a collar-like structure [1]. The central stalk is made of subunits C, D and F. ATP is proposed to be synthesized or hydrolyzed in the interface of A and B subunits and the energy provided for or released during that process is transmitted to the membrane-bound A_O domain, consisting of subunits *a* and *c*. The energy coupling between the two active domains occurs via the stalk part [2]. Crystallographic structures of the nucleotide-binding subunits A and B of A-ATP synthases reveal, that they are composed of the N-terminal β barrel, the α-β domain, and the C-terminal α-helical bundle [3–5]. Most recently, transition position of ADP and ATP could be described in crystallographic structures of subunit B, providing information on the ATP traversing pathway to the final binding pocket [6]. However, the mechanism of nucleotide-binding and ATP synthesis in subunit A of A-ATP synthases still remains a puzzle. Here we describe the crystal structure of subunit A from *P. horikoshii* OT3 A-ATP synthase in the absence and presence of AMP-PNP as well as ADP at 2.47 Å and 2.4 Å resolutions, with defined features of the nucleotide-binding sites and

the role of the P-loop residue Ser238 in phosphate-binding. The structures display novel conformations in the P-loop which are believed to represent important intermediates on the catalytic pathway. Comparison of the wild type structure of subunit A with the mutant S238A reflects its central role in the unique arched P-loop structure of A in A-ATP synthases and suggests an important evolutionary switch in P-loop and thereby in nucleotide recognition and mechanism of ATP synthesis and/or ATP hydrolysis of the biological machines A-, F-ATP synthases and V-ATPase.

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2P.19 Biochemical and structural investigations of the *Ilyobacter tartaricus* F_O ATP synthase

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Adenosine triphosphate (ATP) synthase catalyzes the synthesis of ATP from ADP and phosphate by the dissipation of a transmembrane electrochemical ion gradient, which can be created by the respiratory chain complexes. The enzyme consists of two main subcomplexes F_1 and F_O that both function as the rotary motors. The water-soluble F_1 consists of subunits $\alpha_3\beta_3\gamma\delta\varepsilon$ and harbours the three nucleotide catalytic binding-sites. In bacteria the membrane-embedded F_O subcomplex consists of a ring of 10–15 c-subunits, which rotates against the neighbouring stator a- and b_2-subunits, thereby conducting ions across the membrane. Details for the ion translocation and mechanism and torque generation in the F_O motor are available on the basis of biochemical data and structures of the c-ring but structural data on the a-subunit is completely missing. In the bacterium *Ilyobacter tartaricus*, a-subunit is a hydrophobic protein of about 32 kDa size, consisting of five or six transmembrane α-helices. It is proposed to be part of the water-accessible access pathways to and from the rotor ion binding sites and to provide a key arginine, which forms reversible contacts with glutamates on the c-subunits of the rotor ring during the ion translocation. The aim of this work is the biochemical and structural characterization of the F_O subcomplex from *I. tartaricus* F_1F_O-ATP synthase. The whole enzyme was heterologously expressed in *Escherichia coli* host cells. Either the whole enzyme or the F_O subcomplex, after separation from F_1, was purified by affinity chromatography from the solubilized membrane fraction. Size-exclusion chromatography and Blue Native polyacrylamide gel electrophoresis confirm that both complexes (F_1F_O and F_O) are intact and fully assembled. The correct mass and subunit composition of the holo-enzyme (F_1F_O) and of the isolated F_O-subcomplex was furthermore determined and confirmed by laser-induced liquid bead ion desorption mass spectrometry (LILBID-MS). The purified F_O-subcomplex was successfully reconstituted into lipid vesicles and first structural investigations by electron microscopy are presented.

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2P.20 Down-regulation of F_1 ε subunit in HEK293 cells

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The mammalian subunit ε is the smallest and functionally less characterized subunit of F_1 catalytic part of ATP synthase. The mammalian subunit ε encoded by *ATP5E* gene is a 5.8 kDa protein that lacks a cleavable import sequence. Compared to other F_1 subunits, ε is the only one without a homolog in bacteria or chloroplasts. Complementation studies confirmed that the yeast and mammalian ε are structurally and functionally equivalent [1]. F_1 subunits γ, δ and ε together with c-subunits oligomer form the rotor of ATP synthase [2]. Disruption of the *ATP15* gene encoding ε subunit in yeast resulted in no detectable oligomycin-sensitive activity, decreased content of γ, δ and F_O subunits and in F_1 instability [3]. It was also associated with accumulation of a/b dimer [4]. Here we report that silencing of ATP5E gene leads to a decrease of activity and protein content of mitochondrial ATP synthase complex and ADP-stimulated respiration in mammalian HEK293 cell to approximately 40% of the control. Decreased amount of ε subunit in *ATP5E* silenced cell lines was accompanied by a decreased content of the F_1 subunits α and β and as well as the F_O a- and d-subunits, while the content of F_O c-subunit was not affected. We found the accumulated c-subunit to be present in fully assembled ATP synthase complex and in subcomplexes of 200–400 kDa, which contained neither F_1 subunits α and β, nor the F_O subunits a, b or d. Our study shows that ε subunit is necessary for assembly and/or stability of the F_1 catalytic part of the mammalian ATP synthase and it is also important for incorporation of the hydrophobic subunit c into F_1-c oligomer during ATP synthase biogenesis.

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2P.21 Adaptations of the ATP synthase a-subunit to support synthesis at low protonmotive force at both pH 7.5 and 10.5 may underpin the more stringent requirement for lysine-180 in TMH-4 by alkaliphilic *Bacillus pseudofirmus* OF4 than by more modestly alkaliphilic thermoalkaliphile *Bacillus* sp. TA2.A1

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