

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* F₀ 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 F₁ and F₀ that both function as the rotary motors. The water-soluble F₁ consists of subunits $\alpha_3\beta_3\gamma\delta\epsilon$ and harbours the three nucleotide catalytic binding-sites. In bacteria the membrane-embedded F₀ subcomplex consists of a ring of 10–15 c-subunits, which rotates against the neighbouring stator a- and b₂-subunits, thereby conducting ions across the membrane. Details for the ion translocation and mechanism and torque generation in the F₀ 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 F₀ subcomplex from *I. tartaricus* F₁F₀-ATP synthase. The whole enzyme was heterologously expressed in *Escherichia coli* host cells. Either the whole enzyme or the F₀ subcomplex, after separation from F₁, was purified by affinity chromatography from the solubilized membrane fraction. Size-exclusion chromatography and Blue Native polyacrylamide gel electrophoresis confirm that both complexes (F₁F₀ and F₀) are intact and fully assembled. The correct mass and subunit composition of the holo-enzyme (F₁F₀) and of the isolated F₀-subcomplex was furthermore determined and confirmed by laser-induced liquid bead ion desorption mass spectrometry (LILBID-MS). The purified F₀-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 F₁ ϵ subunit in HEK293 cells

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The mammalian subunit ϵ is the smallest and functionally less characterized subunit of F₁ 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 F₁ 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]. F₁ 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 F₀ subunits and in F₁ 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 F₁ subunits α and β and as well as the F₀ a- and d-subunits, while the content of F₀ 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 F₁ subunits α and β , nor the F₀ subunits a, b or d. Our study shows that ϵ subunit is necessary for assembly and/or stability of the F₁ catalytic part of the mammalian ATP synthase and it is also important for incorporation of the hydrophobic subunit c into F₁-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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