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.

References


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2P.19 Biochemical and structural investigations of the Ilyobacter tartaricus F0 subunit in HEK293 cells

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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 α3β3γδε 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 α- 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 α-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 F1:F0-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 (F1:F0 and F0) are intact and fully assembled. The correct mass and subunit composition of the holoenzyme (F1:F0) and of the isolated F0-subcomplex was further determined and confirmed by laser-induced liquid bead ion desorption mass spectrometry (LLIBID-MS). The purified F0-subcomplex was successfully reconstituted into lipid vesicles and first structural investigations by electron microscopy are presented.

References


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2P.21 Adaptations of the ATP synthase a-subunit to support synthesis at low protonotive 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 OP4 than by more modestly alkaliphilic thermoalkaliphilic Bacillus sp. TAZA1

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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 ATP5S gene encoding ε subunit in yeast resulted in no detectable oligomycin-sensitive activity, decreased content of γ, δ and F1 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.