S1.P24

Subunit F of A-ATP synthases is an ATPase stimulating subunit
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Archaeal A-ATP synthases are composed of 9 different subunits in the proposed stoichiometry of A₃B₃C:D:F:(EH)₂:αc, where the soluble domain A₃ has a hexameric head made up of A₂B₃. Subunits C, D, and F form the central stalk, and a heterodimer of subunits E and H forms the peripheral stalk [1]. The membrane embedded integral A₀ domain contains the subunits α and c, which are responsible for ion-translocation. Genes encoding the subunits A–F have been synthesized to express the complexes A₃B₃D and A₃B₃DF of the chemically-driven motor A-ATP synthase from Methanosarcina mazei Gö1. A new purification protocol has been developed to generate highly pure proteins, which were used for 2D-classification and 3D-reconstructions of negatively stained EM-images. The 3D-reconstruction of the A₃B₃D- and A₃B₃DF-complexes employed a complementary approach of solution X-ray scattering and NMR spectroscopy to determine the solution structures of M. tuberculosis subunit ε and its C-terminal domain, respectively [1]. Based on the physiological role of ε regulation in the ATP synthase, bacteria show variations in length and composition of the amino acid sequence of this C-terminus. Here we used mutational studies to map the critical epitopes and residues of the coupling and regulatory subunit ε from M. tuberculosis F-ATP synthase.

Reference

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S1.P26

Subunit ε of Mycobacterium tuberculosis F-ATP synthases is critical in ATPase inhibition
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Functional F-ATP synthase is critical for the viability of pathogenic bacteria such as Mycobacterium tuberculosis. The mycobacterial F₀F₁ ATP synthase consists of the ATP synthesizing F₁ section (α₃β₃γεδc) and the proton-translocating F₀ part (subunits α₁β₂γ₁c₉). Recently we have employed a complementary approach of solution X-ray scattering and NMR spectroscopy to determine the solution structures of M. tuberculosis subunit ε and its C-terminal domain, respectively [1]. Based on the physiological role of ε regulation in the ATP synthase, bacteria show variations in length and composition of the amino acid sequence of this C-terminus. Here we used mutational studies to map the critical epitopes and residues of the coupling and regulatory subunit ε from M. tuberculosis F-ATP synthase.

Reference

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S1.P25

Single-molecule approach to compare the rotation of F₁-ATPase with the archaeal A₃-ATPase
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The rotary ATP synthase is a unique molecular machine consisting of two rotary nanomotors, which work as a generator/motor and can synthesize ATP by utilizing an electrochemical potential of H⁺/Na⁺ over the membrane, or vice versa. The soluble complex that is capable of hydrolyzing ATP only consists of subunits α₃γεδc. For the bacterial F₁-ATPase a single-molecule domain A₁ has a hexameric head made up of A₃:B₃:subunits C, D, and F which form the central stalk, and a heterodimer of subunits E and H forms the peripheral stalk [1]. The membrane embedded integral A₀ domain contains the subunits α and c, which are responsible for ion-translocation. Genes encoding the subunits A–F have been synthesized to express the complexes A₃B₃D and A₃B₃DF of the chemically-driven motor A-ATP synthase from Methanosarcina mazei Gö1. A new purification protocol has been developed to generate highly pure proteins, which were used for 2D-classification and 3D-reconstructions of negatively stained EM-images. The 3D-reconstruction of the A₃B₃D- and A₃B₃DF-complexes employed a complementary approach of solution X-ray scattering and NMR spectroscopy to determine the solution structures of M. tuberculosis subunit ε and its C-terminal domain, respectively [1]. Based on the physiological role of ε regulation in the ATP synthase, bacteria show variations in length and composition of the amino acid sequence of this C-terminus. Here we used mutational studies to map the critical epitopes and residues of the coupling and regulatory subunit ε from M. tuberculosis F-ATP synthase.

Reference

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S1.P27

Tmem70 gene knockout alters biogenesis of ATP synthase and leads to embryonal lethality in mice
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Tmem70 is a 21 kDa transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of the eukaryotic ATP synthase. Tmem70 mutations are responsible for isolated deficiency of ATP synthase leading to a severe, often fatal neonatal encephalocardiomyopathy in patients. To better understand the function of this ancillary biogenetic factor, we generated Tmem70 knockout mice by embryonic stem cell technology and verified the lack of the Tmem70 expression in the homozygous embryos by quantitative RT-PCR. While the heterozygous mice were viable, the homozygous embryos revealed profound growth retardation and died at the stage of approximately 8.5–9.5 days post coitum. More detailed morphological analysis indicated the disturbance of cardiovascular system. Blue native electrophoresis demonstrated isolated decrease of ATP synthase complex in homozygous embryos similarly to the samples of fibroblasts from human patients. The content of fully assembled F₁F₀ ATP synthase detected by WB was decreased in homozygous embryos to less than 25% compared to that in wild type embryos, while the F₁ subcomplex of ATP synthase in the homozygous embryos increased. Similarly, ATPase in-gel hydrolytic activity revealed identical changes. In addition, transmission electron microscopy showed disturbed mitochondrial cristae morphology in Tmem70 knockout embryos compared to wild type embryos. Our results thus demonstrate that Tmem70 ablation in the mouse model has lethal effects on the formation of ATP synthase and affects embryonic development.
consequences that are analogous to Tmem70 dysfunction in human and prove the critical role of this factor in biosynthesis and assembly of mammalian ATP synthase. This work was supported by the Grant Agency of the Czech Republic (grant P303/11/0970).

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S1.P28

Heterologous expression, purification and crystallization on central stalk and peripheral stalk of F1F0 ATP synthase of Aquifex aeolicus

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Aquifex aeolicus is a hyperthermophilic eubacterium, and its genome has been sequenced [1]. The F1F0 ATP synthase has been purified from this organism, which contains nine subunits α, β, γ, δ and ε of F1 part and a, b1, b2 and c of F0 part [2]. Two stalks connecting F1 and F0 could also be shown clearly by electron microscopic single particle analysis [2]. This project is mainly focused on the two stalks: central stalk and peripheral stalk of F1F0 ATP synthase from A. aeolicus. The central stalk includes subunits γ and ε. These two subunits had been successfully expressed in Escherichia coli and verified by western blot and mass spectrometry, respectively. ε subunit was purified and the molecular weight was determined as 17.34 kDa by size exclusion chromatography. The crystals of ε subunit have been obtained, and they are diffracting at a resolution of 2.3 Å. Initial purification of γ subunit by Ni-NTA affinity chromatography showed that the protein was unstable and easy to form aggregations. Co-expression of ε and γ subunits was successful. The subcomplex was stable at pH 6.4. Vectors for co-expression subunits a, b1 and b2 of F0 part have been constructed. All three subunits had been expressed in E. coli and verified by western blot and mass spectrometry. The initial results showed that the three subunits could form a subcomplex. Further experiments are in progress.

References

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S1.P29

Isolation and characterization of the F1F0 ATP synthase from Heliobacterium modesticaldum

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The heliobacteria (Heliobacterium modesticaldum) are anoxygenic photosynthetic bacteria, which genome has been completely sequenced. Their photosynthetic electron transfer chain contains a unique type-I photosynthetic reaction center, which catalyzes light-driven electron transfer that produces the electro-chemical gradient for the synthesis of ATP by the ATP-synthase [2]. While most of the functional and structural investigations have so far been focused on the energy metabolism and the reaction center [2,3], the ATP-synthase from this organism has not yet been studied in detail [5]. The proton-ATP synthase (F1F0) is one of the most important enzymes on earth because of its universal role in the ATP synthesis that drives the energy metabolism of the cells. This multi-subunit membrane-bound enzyme is composed of two coupled domains with different structural and functional properties: the hydrophilic F1 domain, which acts as the catalytic site of ATP synthesis and the hydrophobic F0 domain, which acts as a proton-driven rotor and proton channel [1]. The major aim of this project is to establish a defined procedure to isolate functional active ATP synthase (F1F0) in high purity with good yield from H. modesticaldum, and to characterize its catalytic activity, regulation and biochemical features, as oligomeric state and subunit composition. These data provide essential pre-requisites for further experiments to grow 2D crystals (a lipid monolayer at the water–fluid interface technique) and nano-crystals of the intact enzyme with the final goal to determine its structure with serial femtosecond nanocrystallography [4].

References

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