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, ε, ε′, δ′, δ″, a, b′, b″ 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, b′, and b″ 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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Abstracts

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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