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

[1] Coskun Ü (2004) *J. Biol. Chem.* **279:** 38644–38648.
[2] Grüber G *et al.* (2008) *BioEssays* **30:** 1096–1109.
[3] Kumar A *et al.* (2010) *J. Mol. Biol.* **396:** 301–320.
[4] Kumar A *et al.* (2008) *Acta Cryst.* D**64:** 1110–1115.
[5] Kumar A *et al.* (2009) *Proteins* **75:** 807–819.
[6] Manimekalai MSS *et al.* (2009) *J. Struct. Biol.* **166:** 39–45.

doi:10.1016/j.bbabio.2010.04.115

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

Jonna Hakulinen[1], Jan Hoffmann[2], Luise Eckhardt-Strelau[1], Bernd Brutschy[2], Thomas Meier[1]
[1]*Department of Structural Biology, Max-Planck-Institute of Biophysics, Max-von-Laue Str. 3, 60438 Frankfurt/Main, Germany*
[2]*Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany*
E-mail: jonna.hakulinen@biophys.mpg.de

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.

doi:10.1016/j.bbabio.2010.04.116

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

V. Havlíčková, V. Kaplanová, H. Nůsková, Z. Drahota, L. Stibůrek, J. Houštěk
Department of Bioenergetics, Institute of Physiology and Center of Applied Genomics, Academy of Sciences of the Czech Republic
E-mail: vendula.havlickova@post.cz

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.

References

[1] Lai-Zhang *et al.* (2000) *Eur. J. Biochem.* **267:** 2409–2418.
[2] Stock D *et al.* (1999) *Science* **286:** 1700–1705.
[3] Guelin E *et al.* (1993) *J. Biol. Chem.* **268:** 161–167.
[4] Rak M *et al.* (2009) *Proc. Natl. Acad. Sci. U. S. A.* **106:** 18509–18514.

doi:10.1016/j.bbabio.2010.04.117

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

Makoto Fujisawa, Jun Liu, Oliver Fackelmayer, Terry A. Krulwich, David B. Hicks
Mount Sinai School of Medicine, Dept. of Pharmacology and Systems Therapeutics, USA
E-mail: david.hicks@mssm.edu

Proton uptake from the periplasm into the F_O sector of the F-type ATP synthase occurs through a putative half-channel in the a-subunit [1, 2]. Alkaliphilic *Bacillus* species have a lysine (residue 180 in *B. pseudofirmus* OF4) at the position equivalent to a key glycine in helix-4 of the *E. coli* subunit [3]. This lysine has been shown to play a role in ATP synthesis at high pH (pH 10.5)-low protonmotive force (*pmf*) and is hypothesized to play a role in proton binding and delivery to the *c*-subunit carboxylate. Here we mutated lysine, to glycine, alanine, cysteine, histidine or arginine, in the *B. pseudofirmus* OF4 chromosome. All mutants had ≥115% of the octylglucoside-stimulated ATPase activity of the wild-type enzyme. Only the mutants with Gly, Ala or His substitutions retained ATP synthase activity at pH 7.5. Assays of ATP synthesis in ADP + P_i-loaded alkaliphile vesicles showed that synthesis by these mutants was more sensitive to CCCP inhibition than the wild type, with the order of CCCP sensitivity being Ala > Gly > His > wild type. This is the first indication that the alkaliphile-specific lysine plays a role at low *pmf* even when the external pH is not highly alkaline. The *a*-subunit from the ATP synthase of alkaliphilic *B. pseudofirmus* OF4 is less tolerant of substitution of other residues for lysine-180 than the subunit from more moderately alkaliphilic thermophile *Bacillus* sp. TA2.A1. For example, substitution of lysine-180 by Arg resulted in an active enzyme in the *Bacillus* sp. TA2.A1 [4] but in an inactive enzyme in *B. pseudofirmus* OF4. Moreover, the alkaliphile enzyme supports ATP synthesis over a much broader pH range. Sequence comparisons suggested that the *a*-subunits of extreme alkaliphiles are distinct from that of *Bacillus* sp. TA2.A1. Mutations made in several additional positions of the *a*-subunit in which alkaliphiles diverge both from non-alkaliphiles and *Bacillus* sp. TA2.A1 show that the alkaliphile-specific residues in several of these positions are required for normal malate-growth at pH 10.5. This suggests that the more stringent requirement of the *B. pseudofirmus* OF4 for lysine-180 is part of a broadly adapted structure that allows function over a broad range of pH values, from 7.5 to > 10.5.

References

[1] Angevine C, Fillingame R (2003) *J. Biol. Chem.* **278:** 6066.
[2] Angevine C, Fillingame R (2003) *Proc. Natl. Acad. Sci.* **100:** 13179.
[3] Wang Z *et al.* (2004) *J. Biol. Chem.* **279:** 26546.
[4] McMillan D, Keis S, Dimroth P, Cook G (2007) *J. Biol. Chem.* **282:** 17395.

doi:10.1016/j.bbabio.2010.04.118

2P.22 Direct observation of steps in c-ring rotation of *Escherichia coli* F_OF_1-ATP synthase

Ryota Iino[1], Khek-Chian Tham[2], Kazuhito V. Tabata[1], Hiroshi Ueno[1], Hiroyuki Noji[1]
[1]*Osaka University, The Institute of Scientific and Industrial Research, Japan*
[2]*Erasmus Medical Center, Dept. of Cell Biology and Genetics, Netherlands*
E-mail: iino@sanken.osaka-u.ac.jp

F_OF_1-ATP synthase (F_OF_1) is a complex of two rotary motors, and ATP synthesis/hydrolysis reaction catalyzed by water-soluble F_1 motor is coupled with the proton transport through membrane-embedded F_O motor via mechanical rotation. Direct observations of F_OF_1 rotation driven by ATP hydrolysis were carried out previously, but steps (pauses) in rotation rate-limited by F_O motor (proton transport) have not been directly resolved yet. Although single-molecule fluorescence resonance energy transfer has been applied to detect the rotation steps of F_O rotor c-ring recently, it is not a direct method visualizing rotation. In this study, with a goal of direct observation of steps in the rotor c-ring of F_O, mutation was introduced into the putative proton channel in the stator a-subunit (aE219H) to slow down proton transport through F_O motor. For direct observation of rotation driven by ATP hydrolysis and coupled with proton transport, F_OF_1 was reconstituted into the supported membrane formed on the NTA-modified agarose, and immobilized by histidine-tags introduced into the c-ring of F_O. Rotation was observed by 200 nm latex bead attached to the β subunits of F_1. At high ATP concentration which rotation steps rate-limited by F_1 motor are not visible, rotation rate of the aE219H mutant (9.1 ± 2.4 Hz) was lower than that of wild-type (20.0 ± 10.1 Hz), and showed good agreement with the value expected from ATPase activity in biochemical assay ($40\ s^{-1}/3 = 13$ Hz). On the other hand, in wild-type, rotation rate was much lower than the expected value ($285\ s^{-1}/3 = 95$ Hz). These results indicated that viscous drag of water against the bead is the rate-limiting of the rotation in wild-type, but in the mutant the rate-limiting factor is not the bead but an intrinsic property of the mutant. When the rotation of the mutant was analyzed in detail, many small steps were found. Distribution of rotary angle showed many peaks spaced by about 36°. Analysis of the pair-wise angle distance also indicated the minimal step size of about 36°. This step size was consistent with the previous cross-linking experiment reporting 10 protomers in a rotor c-ring of *E. coli* F_O motor. Furthermore, during observation the mutant F_OF_1 showed frequent backward 36° steps. This result indicates a low free energy barrier per step, and supports the notion that the Brownian ratchet is the rotary mechanism of the F_O motor.

doi:10.1016/j.bbabio.2010.04.119

2P.23 ATP binding to the epsilon subunit of thermophilic ATP synthase is essential for coupling ATPase and H^+-pumping

Fumitaka Kadoya[1], Shigeyuki Kato[1], Kei Watanabe[1], Toshiharu Suzuki[2], Masasuke Yoshida[2,3], Yasuyuki Kato-Yamada[1]
[1]*Department of Life Science, Rikkyo University, Japan*
[2]*ICORP ATP Synthesis Regulation Project, JST, Japan*
[3]*Faculty of Engineering, Kyoto Sangyo University, Japan*
E-mail: katoyama@rikkyo.ac.jp

In our previous study, ATP binding to the epsilon subunit of F_OF_1-ATP synthase (TF_OF_1) from thermophilic *Bacillus* PS3, has been shown to shift the equilibrium between ATPase-inactive and active forms of F_1-ATPase (TF_1), a soluble part of TF_OF_1, by stabilizing ATPase-active form. In the present study, how the ATP binding to the epsilon subunit may concern with ATPase and H^+-pumping activities of the holo-enzyme, TF_OF_1, was examined by using a mutant TF_OF_1 containing epsilon subunit deficient in ATP binding. As for the ATPase activity, the mutant TF_OF_1 showed lower ATPase activity than the wild type indicating that the situation was similar to that with TF_1. Unexpectedly, however, the mutant had *no* H^+-pumping activity even under the conditions where the mutant had comparable ATPase activity to the wild type. Elimination of 14 basic amino acid residues in the C-terminal domain of the epsilon subunit by replacement with alanines recovered coupling although the mutant was also deficient in ATP binding. From these results, it was hypothesized that the bulky conformation of the C-terminal domain of the epsilon subunit, due to the repulsion of positive charges in the C-terminal domain in the absence of bound ATP may affect the coupling between F_1 and F_O.

Reference

[1] Kato S *et al.* (2007) *J. Biol. Chem.* **282:** 37618–37623.

doi:10.1016/j.bbabio.2010.04.120