F_oF_1-ATP synthase is the major ATP supplier in aerobic cells. F_1-ATPase is the catalytic domain of F_oF_1-ATP synthase. In this presentation, we will introduce three different topics; one is the temperature-sensitive reaction intermediate state of F_1-ATPase. This state was found as an intervening pause in the rotation assay of F_1-ATPase at low temperature. The intermediate reaction step occurs at the ATP-binding angle, and that it is not relevant to ATP-binding but to ADP-releasing step. The second topic is the correlation between the rotational substeps of F_1-ATPase found in single molecule studies and the chemical state in the crystal structures of F_1-ATPase. To directly clarify the question which rotational pausing states the crystal structure of F_1-ATPase represent, we crosslink the β and γ subunits of F_1-ATPase to fix the conformational state in the crystal structure during the single molecule rotation assay. The last topic is the development of a fluorescent ATP sensor. The recent studies show that the ε subunit of F_1-ATPase undergoes a large conformational change upon ATP binding. By conjugating CFP and YFP at the N- and C-termini of the ε subunit respectively, we developed the fluorescent ATP sensor which largely enhances the efficiency of FRET from CFP to YFP. We will show some movies of intracellular ATP imaging during several cellular activities such as apoptosis and cell division.

doi:10.1016/j.bbabio.2008.05.066

S1.29 Exploring rotary ATP synthase by electron tomography of two-dimensional crystals

Christoph Gerle[a], Christopher P. Arthur[b], Ken Yokoyama[c], Yoshinori Fujiyoshi[a], Kaoru Mitsuoka[d]
[a]*Department of Biophysics, Faculty of Science, Kyoto University, Oiwake, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan*
[b]*The Scripps Research Institute, Department of Cell Biology, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*
[c]*ATP System Project, 5800-3 Nagatsuta, Midori-ku, Yokohama 226-0026, Japan*
[d]*JBIRC, 2-41-6 Aomi, Koto-ku, Tokyo 135-0064, Japan*
E-mail: cgerle@em.biophys.kyoto-u.ac.jp

The aim of this study was to examine the feasibility of employing electron tomography to analyze two-dimensional crystals of the rotary H^+-ATP synthase from *T. thermophilus*, not fit for crystallographic analysis. Two-dimensional crystals of the intact ATP synthase were grown in a three step dialysis procedure and stained with uranyl acetate. Images were taken on a dual axis from −70° to +70° in one degree increments and tomograms reconstructed using the IMOD software package. Examination of several tomograms revealed an upside-down packing of H^+-ATP synthase dimers in the crystal. Furthermore, point defects and bended rows clearly demonstrate the causes underlying the low quality of crystal order that prevent crystallographic structure analysis. However, the apparent conformity of dimers in the crystal indicates specific contacts between molecules in each dimer, limiting structural heterogenity. Therefore, it is conceivable that, by avoiding the pitfalls of structural and orientational heterogenity of single particle analysis on H^+-ATP synthase in solution, single particle analysis of three-dimensional volumes of dimers extracted from several tomograms could yield informative structural information on the intact complex.

doi:10.1016/j.bbabio.2008.05.067

S1.30 The F_0F_1-ATPase activity and molecular hydrogen production by *Rhodobacter sphaeroides*

Lilit Gabrielyan, Armen Trchounian
Department of Biophysics, Yerevan State University, Yerevan, Armenia
E-mail: gabrielli@ysu.am

In the present work, ATPase activity and molecular hydrogen (H_2) production by *Rhodobacter sphaeroides* different strains D-3 and A-10, isolated from spring waters in Armenia, are studied, the effects of *N,N′*-dicyclohexylcarbodiimide (DCCD) and NaN_3 on these processes are shown. *Rh. sphaeroides* were capable of growing and producing H_2 in anaerobic conditions at illumination in 1500 lx, pH 7.0–7.2 (28–30 °C) using succinate and lysine as the carbon and nitrogen sources: H_2 production was a higher in A-10 than D-3 strains. This process was suppressed at the presence of the F_0F_1-ATPase inhibitors — DCCD and NaN_3. In addition, H_2 production disappeared with a protonophore — carbonyl cyanide *m*-chlorophenylhydrazone. Membrane vesicles of *Rh. sphaeroides* demonstrated marked ATPase activity, determined by the liberation of inorganic phosphate in the reaction with ATP. Incubation of membrane vesicles of *Rh. sphaeroides* in the presence of DCCD (0.1 mM) led to significant (~60%) inhibition in ATPase activity. After treatment of membrane vesicles of both strains with NaN_3 (1 mM), ATPase activity decreased by ~80 and 100%, respectively.

In view of that used inhibitors suppress proton-translocating systems of bacterial membrane, and in anaerobic conditions the activity of the F_0F_1-ATPase at *E. coli* and the other bacteria, it is possible to admit the role of this ATPase in H_2 production by *Rh. sphaeroides*.

doi:10.1016/j.bbabio.2008.05.068

S1.31 Special adaptations of the ATP synthase F_0 sector are required for alkaliphile oxphos at high pH

David B. Hicks, Jun Liu, Makoto Fujisawa, Terry A. Krulwich[a]
Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY, USA
E-mail: david.hicks@mssm.edu

We here detail the dependence of ATP synthesis at high pH by alkaliphile *B. pseudofirmus* OF4 upon alkaliphile-specific residues in the a- and c-subunits. The lysine in helix 4 (K180) of the a-subunit, two helical turns from the essential arginine, was mutated to alanine, cysteine, histidine, and arginine in the native alkaliphile host. The alanine, cysteine, and arginine mutations resulted in poor or no non-fermentative growth on malate at either pH 7.5 or 10.5. The histidine mutant grew to near wild type levels on malate at pH 7.5 but much less well at pH 10.5. The GXGXGXG motif found in helix 1 of the c-subunit of many ATP synthases is, instead, AXAXAXA in *B. pseudofirmus* OF4. Strains with single alanine mutations to glycine grew slightly less than the wild type, while changing 2 or 3 alanines caused a 75% inhibition in malate growth at pH 10.5. Strains with all 4 alanines switched showed virtually no malate growth or ATP synthesis pH 10.5, although oxidative phosphorylation in vesicles was observed at pH 7.5. The c-ring has been selectively extracted from the purified holoenzyme so we can determine the effect of growth pH and c-subunit mutations in two alkaliphile-specific motifs on stoichiometry.

doi:10.1016/j.bbabio.2008.05.069