We have recently shown that the H^+/ATP ratio can significantly decrease during ATP hydrolysis by the ATPsynthase of *Rb. capsulatus*, when the concentration of either ADP or Pi is maintained at a low level. This same phenomenon has then been observed in isolated membranes of *E. coli*. We have now purified the ATPsynthase of *E. coli* and reconstituted it into liposomes, in order to verify whether the same behavior could be observed in the isolated enzyme. The ATP hydrolysis and proton pumping activity were measured under the same experimental conditions. The hydrolysis was measured either with the colorimetric pH indicator Phenol Red or with an ATP regenerating enzymatic assay, and the proton pumping was evaluated by a calibrated ACMA assay. The hydrolysis activity was inhibited by Pi with an apparent K_d of 400 μM, while the steady state ΔpH was stimulated up to 200 μM Pi and was only slightly inhibited up to 1000 μM Pi. Both the inhibition of ATP hydrolysis and the stimulation of proton pumping as a function of Pi were lost upon ADP removal by an ADP trap. We conclude that the isolated and reconstituted ATPsynthase of *E. coli* can vary its degree of coupling as a function of Pi and ADP.

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S1.25 Immobilization of the H^+-ATPsynthase on glass surface and single molecule fluorescence spectroscopy

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During ATP synthesis the rotor subunits of the H^+-ATPsynthase $\gamma\varepsilon c_{10}$ rotate versus stator subunits $\alpha_3\beta_3$ δab_2. This rotation has been shown by single pair Fluorescence Resonanz Energy Transfer (spFRET). The double labeled enzyme was integrated into liposomes and the diffusion time through the confocal volume of the microscope limited the observation time. In order to extend the observation time the proteoliposomes were immobilized on a glass surface. To immobilize the proteoliposomes two techniques have been used. First, the enzyme was reconstituted into liposomes containing biotinylated lipids. They are immobilized with streptavidin on a biotinylated surface. Second, the H^+-ATPsynthase carried a his-tag on the β-subunits. After modification of the surface with BSA or Silan proteoliposomes were immobilized via the his-tagged enzyme. With this method the observation time of a single enzyme was significantly increased, however also photobleaching of the organic fluorophores was increased. Therefore, quantum dots (QD_{580} and QD_{600}) with hydrophilic shells have been used as fluorescence donors. They were bound covalently to the b-subunits of the H^+-ATPsynthase and Atto 647N was used as acceptor. This allows the observation of a single enzyme in the second to minute time range.

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S1.26 Structure of the C-terminal domain of the ε subunit of chloroplast-type F_1

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The chloroplast F_1-ATPase ε subunit inhibits ATP hydrolysis with ATP-independent manner, whereas the bacterial ε is ATP-dependent. To understand the structure–function relationship of ε, we prepared the chimera ε subunit combining the N-terminal domain from *Thermosynecoccus elongatus* and the C-terminal α-helical domain from spinach CF_1 (ε_{NB_CC}) or EF_1 (ε_{NB_CE}). The ε_{wt} from *T. elongatus* and ε_{NB_CC} inhibited the ATP hydrolysis activity of cyanobacteria F_1 to a similar extent, whereas ε_{NB_CE} was less potent. The solution structures of ε_{wt} and ε_{NB_CC} solved by NMR were so-called "retracted-state", which was similar to that of EF_1-ε or TF_1-ε. However, the length of α-helices in the C-terminal domain of these CF_1-type ε was longer than that of the bacterial ε. Interestingly, the loop between two helices of the ε_{NB_CC} was formed by only four residues and the retracted position of the helices was quite different from the previous reports. Significance of the unique conformation of the C-terminal α-helices is discussed.

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S1.27 Step size of proton-driven c ring rotation in single F_oF_1-ATP synthase by FRET

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A mean ratio of 4.0 protons transported per synthesized ATP has been determined for the *E. coli* F_oF_1-ATP synthase recently. However, the F_o part likely contains 10 *c* subunits corresponding to 3.3 H^+/ATP. Synthesis of ATP is performed by a stepwise internal rotation of subunits in F_oF_1. Sequential conformational changes of single enzymes are monitored in real time by fluorescence resonance energy transfer, FRET. Therefore two different fluorophores have to be attached to those protein domains, which move during function. We investigated the step size of proton-driven *c* subunit rotation in F_oF_1 by single-molecule FRET between *a* and *c*. 'Duty cycle optimized alternating laser excitation' minimized FRET artefacts. Rotary movements with stochastic single step sizes between 36° and 144° were determined by Hidden Markov Models. As the two coupled motors of F_oF_1 showed apparently different step sizes, contributions of rotor and stator subunits for transient energy storage can be located using FRET. Monitoring *c* rotation we identified the action mode of the allosteric inhibitor aurovertin B, which modulates single F_oF_1 activity by slowing down rotation upon ATP hydrolysis, but acts differently during ATP synthesis.

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S1.28 Mechanochemical coupling of F_1-ATPase and intracellular ATP imaging

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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.

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

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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.

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

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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*.

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

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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.

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