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.

doi:10.1016/j.bbabio.2008.05.062

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.

doi:10.1016/j.bbabio.2008.05.063

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.

doi:10.1016/j.bbabio.2008.05.064

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.

doi:10.1016/j.bbabio.2008.05.065

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

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