The coupling mechanism of ATP synthase depends on the peripheral stalk or stator (consisting of single copies of subunits OSCP, F_6, b and d) connecting the F_1-ATPase domain to subunit a in the F_O domain, and holding them static relative to the rotation of the central stalk and attached c-ring. In this study, the structure of F_1-ATPase containing a sub-complex of the peripheral stalk, consisting of the OSCP, F_6, residues 99–214 of subunit b, and residues 1–118 of subunit d has been determined by X-ray crystallography to 3.2 Å. The structure of the core of the bovine peripheral stalk (most of subunits b, d and F_6) is an elongated α-helical structure. It extends from the "top" of the F_1-ATPase domain to the inner surface of the inner mitochondrial membrane, where interactions with subunits of the F_O domain occur. The C-terminal domain of the OSCP subunit interacts with the N-terminal region of subunit b, and its N-terminal domain interacts with the N-terminal region of an α-subunit. Hydrophobic residues in residues 6–19 of the α-subunit interact with hydrophobic surfaces in helices 1 and 5 of the N-terminal domain of the OSCP. However, no other extensive interactions between the peripheral stalk and the F_1-ATPase are thought to occur. This structure has provided new information about how the peripheral stalk and F_1-ATPase interact with each other, and about how the peripheral stalk fulfils its roles in the catalytic mechanism of the ATP synthase.

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S1.17 Function of epsilon subunit in bacillus PS3 F_oF_1-ATP synthase: C-terminal helices of epsilon conduct the substrate specificity toward nucleoside triphosphates

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F_oF_1-ATP synthase (F_oF_1) catalyzes synthesizing ATP in cells by the energy of the electrochemical potential of H^+. As a reverse reaction, the enzyme also functions translocating H^+ by the energy of ATP hydrolysis to establish membrane potential. The two energy terms are indispensable for cells and therefore, sophisticated regulatory mechanism is expected to conduct it. Epsilon subunit would be one of the candidates to achieve it. Epsilon has been suggested to regulate the F_oF_1 function by changing the conformation of the C-terminal helices in response to ATP/ADP ratio and membrane potential. In the present study, the C-terminal helices were eliminated from *Bacillus* PS3 FoF_1, and the effect on recognizing several nucleoside triphosphates (NTPs) was investigated. Interestingly, at high NTP condition (>100 μM), the mutated F_oF_1 showed significantly higher activities toward GTP and UTP, while no change in ATPase. This means that C-terminal helices suppress only GTPase and UTPase, leading to improving the substrate specificity toward ATP. To know the molecular mechanism, two charged residues (Glu83 and Atr92) in epsilon, which are important for the direct binding of epsilon with ATP, were substituted by alanine. The mutated F_oF_1 lost the ATPase activity, suggesting that the specific hydrolysis of ATP is ascribed to releasing the inhibitory effect by the direct binding between epsilon and ATP. The high substrate specificity toward ATP may be effective for sensing cellular ATP concentration from the pool of NTPs to regulate the F_oF_1 function.

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S1.18 Crystal structure of A_3B_3 of *Thermus thermophilus* V-ATPase

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The crystal structure of A_3B_3 of vacuole-type ATPase/synthase (prokaryotic V-ATPase) from *Thermus thermophilus* was determined to be of 2.8 A resolution. The structure has three folds symmetry, and contained no nucleotide. The catalytic sites, which were identified in the interface between A and B subunits, had similar conformation to open form of b subunit in F_1-ATPase. Although structure of non-catalytic B subunit mostly identical to F_1-a, catalytic A subunit had an additional region (bulge domain) between N-termini b barrel domain and nucleotide binding domain. Also A subunit had an additional a helical domain at C termini. Studies of site directed mutagenesis based on the structure were carried out. Function of unique motif in V_1 domain, including the bulge domain, will be discussed with both the structural information and biochemical analysis.

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S1.19 Observation of rotation of subunit c in the membrane integrated EF_0F_1 by single molecule fluorescence

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F_0F_1-ATP synthases catalyze ATP formation from ADP and phosphate. Internal rotation of subunits $\gamma\varepsilon c_{10}$ versus $\alpha_3\beta_3\delta ab_2$ couples the chemical reaction at the nucleotide binding sites in F_1 to the proton translocation through F_0. In EF_0F_1 the γ- and the ε-subunits rotate in 120° steps during catalysis with opposite directions for ATP synthesis and ATP hydrolysis. It is not known, whether the c-ring rotates in 120° steps or in a different way, e.g. in 36° steps. In order to study the movement of the c-ring relative to the ε-subunit cysteines were introduced in the c-subunit (cL45C) and the ε-subunit (εH56C). The isolated ε-mutant of F_1 was selectively labelled with Cy5 and the isolated c-mutant with Atto532. To obtain functional F_0F_1, c-subunits (1:50 mixture of c45-Atto532 and c wild type) were reconstituted together with ab_2 in liposomes and the labelled F_1 part was bound. The success of the procedure was checked by ATP-synthesis measurements using an artificial generated pH gradient. Single pair FRET experiments during ATP hydrolysis showed photon bursts with one constant FRET-level as well as transitions between different FRET levels. The data indicates that the c-ring does not rotate with the γ- and ε-subunits in 120° steps during ATP-hydrolysis.

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S1.20 Divalent metal binding to bovine heart F1 ATPase: An FT-ESEEM study

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The divalent metal binding sites of beef heart mitochondria F1ATPase were studied by FT-ESEEM spectroscopy, using Mn(II) as a paramagnetic probe, which replaces the naturally occurring Mg(II) and maintains the enzyme catalytic activity. Purified F1ATPase still containing three endogenous tightly bound nucleotides, named MF1 (1,2), was obtained under mild conditions, whereas a harsher treatment gave a fully nucleotide depleted enzyme, named MF1 (0,0). When MF1(1,2) was loaded with Mn(II) in 1:0.8 ratio, the spectrum showed evidence of a nitrogen interacting with the metal, while this interaction was not present in the spectrum of MF1(0,0) loaded with Mn(II) in the same ratio. However, when MF1(0,0) was loaded with 2.4 Mn(II), the spectrum showed metal-nitrogen interaction resembling that of MF1(1,2) loaded with Mn(II) in 1:0.8 ratio. When MF1(1,2) was loaded with 2.4 Mn(II) the metal-nitrogen interaction signal remained and a phosphorous coordination to the metal was also evident, indicating a binding of Mn^{2+} to a site containing a tightly bound nucleotide but metal free. These results strongly support the role of the metal alone in structuring the catalytic sites of the enzyme while ESEEM technique appears to be a sensitive and suitable spectroscopic method for conformational studies of MF1 with the advantage of using proteins in frozen solution.

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S1.21 Thermodynamic constraints in the reversal of adenine nucleotide translocase during the reversal of F0–F1 ATP synthase caused by respiratory chain inhibition: Critical role of substrate-level phosphorylation

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Mitochondria are the main ATP producers in the cell. However, during respiratory chain inhibition mitochondria become ATP consumers due to reversal of the F0–F1 ATP synthase. The current belief is that under these conditions, extramitochondrial ATP is carried into the matrix chiefly through the reversal of the ANT. For this, the mitochondrial membrane potential must reach values more positive than both the reversal potential of the ATP synthase (Erev-ATPase) and that of the ANT (Erev-ANT). Here we show that in mitochondria capable of substrate-level phosphorylation, inhibition of the respiratory chain shifts the membrane potential to a range bracketed by the Erev-ATPase and the Erev-ANT, the latter being more negative than the former. As a consequence of this, reversal of the ATP synthase generates a sufficient membrane potential to oppose the ANT from operating in reverse mode, for as long as substrate-level phosphorylation is maintained. During respiratory chain inhibition the ANT can only be reversed by a concomitant uncoupling when the membrane potential attains values more positive than the Erev-ANT, or by incapacitating substrate-level phosphorylation. The latter maneuver shifts the Erev-ANT towards more negative values than the prevailing mitochondrial membrane potential.

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S1.22 Determination of unidirectional H^+ transport in F-type ATPases

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F-type ATP synthases are ubiquitous enzymes, which can either synthesize ATP using an electrochemical gradient or hydrolyze ATP to generate a membrane potential. We have investigated the properties of these two working modes in the Na^+ dependent enzyme from *Propionigenium modestum* and the H^+-dependent enzyme from *E. coli*. We found that ΔpNa and $\Delta\psi$ are required for efficient ATP synthesis in the Na^+-dependent enzyme. In accordance with this, we were able to show that both driving forces are capable of energizing Na^+-transport through F_0, when the enzyme was driven in synthesis direction. Interestingly, ΔpNa alone could not stimulate ion transport in the reverse direction as found during hydrolyis, whereas a $\Delta\psi$ served efficiently as driving force. The two directions are therefore kinetically not equivalent. Furthermore, we observed different K_D values for Na^+ for either transport direction. Whereas the K_D for Na^+ in hydrolysis direction is in the range of 1 mM, it was determined to be 15 mM during ATP synthesis. To test whether this asymmetry is present in H^+ dependent ATP syntheses as well, we developed a fluorescent assay for monitoring transport in either synthesis direction or in hydrolysis direction through the F_0 part of *E. coli* and chloroplast ATP synthase. The assay allowed quantitative determination of initial transport rates for H^+ dependent ATP synthases from different organisms in a well defined *in vitro* system.

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S1.23 The H^+/ATP ratio of H^+-ATPsynthases from chloroplasts, *E. coli* and mitochondria

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The H^+/ATP ratio is an important parameter for energy balance in cells and for the mechanism of coupling between proton transport and ATP synthesis. Rotational catalysis predicts that the H^+/ATP coincides with the ratio of the c-subunits to the β-subunits, implying that a value of 4.7 is expected in the chloroplast ATPsynthase and a value of 3.3 is expected in the mitochondria and *Escherichia coli* enzyme. This ratio can be determined based on the energetics given by the chemiosmotic theory. The isolated enzymes were reconstituted into liposomes. The internal phase of the liposomes was equilibrated with the acidic medium during reconstitution, so that the internal pH could be measured with a glass electrode. An acid-base transition was carried out and the initial rates of ATP synthesis or ATP hydrolysis were measured with luciferin/luciferase as a function of ΔpH at constant $Q=[ATP]/([ADP][P_i])$. From the shift of the equilibrium ΔpH as a function of Q, the standard Gibbs free energy for phosphorylation and the H^+/ATP ratios were determined.

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S1.24 Effect of Pi and ADP on the intrinsic uncoupling in the isolated and reconstituted ATPsynthase of *E. coli*

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