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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