S1.13 The external stalk of the F_OF_1-ATPase: Three-dimensional structure of the subunit *b*-dimer as determined by site-specific spin labeling, ESR and molecular modeling

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The structure of the external stalk of the F_oF_1-ATP synthase and its function during the catalytic mechanism remains one of the important and open questions in the field. The external stalk has been proposed to be either a rigid stator that binds and holds F_1 to F_o or an elastic structural element that may be used to store and transmit energy during rotational catalysis. We have employed proteomics, sequence-based structure prediction, molecular modeling and electron spin resonance spectroscopy using site-directed spin labeling to understand the structure and interfacial packing of the *E. coli b*-subunit homodimeric and the *Synechocystis* sp. PCC 6803 *bb′* heterodimeric external stalks of ATP synthases. While comparisons of bacterial, cyanobacterial and plant *b* and *b′* subunits demonstrated little sequence similarity, structure prediction algorithms suggested that all of the compared *b* and *b′*-sequences have extensive heptad repeats. This finding suggests that these proteins may favorably pack as left-handed coiled coils. Molecular modeling followed by energy minimization procedures showed that b_2 from *E. coli* as well as *bb′* from cyanobacterial (*Synechocystis*) ATP synthase were able to pack into low energy left-handed coiled coils. Thirty-eight amino acid substitutions to cysteines in soluble homodimeric *b*-constructs (*E. coli*) were generated and thirty-nine intra- and inter-molecular double cysteine pairs in heterodimeric *bb′*‘ (*Synechocystis*) were prepared that allowed specific introduction of spin labels and the determination of inter- and intra-subunit distances by ESR spectroscopy. Comparison of the inter- and intra-molecular interspin distances that we obtained by ESR experiments with the distances that we derived from molecular modeling of both *b*- and *bb′*-dimers as left-handed coiled coils strongly support our proposition that the bacterial *b*-dimer external stalks of ATP synthases indeed form left-handed coiled coils as their low energy structures. Initial ESR experiments using the complete *E. coli* F_oF_1-ATP synthase and where we introduced and spin-labeled specific cysteine pairs in the *b*-dimer of an otherwise cysteine-less enzyme indicate that the inter-subunit packing of the dimer changes during catalytic turnover. This change in the overall winding may be a mechanism by which the *b*-dimer elastically couples the different rotational processes of the enzyme.

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S1.14 Regulatory transitions of subunit epsilon in ATP synthase from thermophilic *Bacillus* PS3

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In bacterial and chloroplast ATP synthase subunit epsilon inhibits ATP hydrolysis; its alpha-helical C-terminal domain is responsible for the inhibition. In *Bacillus* PS3 the C-terminal domain undergoes large conformational changes. In one (inhibitory) conformation the domain is extended along subunit gamma into F_1, while in the contracted (non-inhibitory) conformation this domain is folded as a hairpin close to F_0. We investigated this conformational transition using FRET between Cy3 fluorophore attached to the subunit gamma N-terminus and Cy5 fluorophore attached to the C-terminus of epsilon. Without nucleotides epsilon was in the extended conformation (high FRET) both in F_1 and F_OF_1. ATP induced transition to contracted state (low FRET). ATP concentration dependence of the transition in F_1 and F_OF_1 F_0F_1 was markedly different: the apparent K_d were ~20 and ~200 μM, respectively. ADP had no effect on both conformations, but slowed down the transition induced by ATP. We also studied the role of DELSEED region in subunit beta on epsilon transitions. The negatively charged residues in βDELSEED are important for epsilon inhibitory effect. When the negative residues were substituted to alanines (AALSAAA), much lower ATP concentration induced the transition to contracted state (apparent K_d ~2.5 μM). Moreover, ADP also induced the same transition (although much slower). A scheme for regulatory transition of subunit epsilon is proposed.

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S1.15 The mechanism of inhibition of bovine F_1-ATPase by the inhibitor protein IF_1

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The structure of bovine F_1-ATPase in complex with residues 1–60 of the inhibitor protein, bovine IF_1, has been solved at 2.2 Å resolution. In this structure, the resolved region of I1-60His from residues 8–50 consists of an extended structure from residues 8–13, followed by two α-helices from residues 14–18 and 21–50, linked by a turn from residues 19–20. The long helix of I1-60His (residues 21-55) is 42 Å long. Its binding site in F_1-ATPase is complex. It involves five subunits of the F_1-ATPase, the most significant contributors being subunits β_{DP}, α_{DP} and γ, with more limited contributions from the β_{TP}- and α_E-subunits. In order to understand which residues in IF_1 are important for inhibition, many of the residues in the long helix have been mutated and the effects of the mutations on inhibitory potency have been studied by determination of association and dissociation constants. Most of the binding energy comes from hydrophobic interactions with the β_{DP}-subunit and from an ionic interaction between E30 of IF_1 and R408 in β_{DP}. Many charged residues of IF_1 that are conserved across a wide range of species are not directly involved in binding interactions. Their role appears to be to guide and orient the inhibitor appropriately into its binding site.

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S1.16 Interaction of the peripheral stalk of the bovine ATP synthase with the F_1 domain

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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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