2P.29 Aqueous access channels in subunit a of sodium transporting F_OF_1-ATP synthase

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F_OF_1-ATP synthase (F_OF_1) is the enzyme that synthesizes ATP from ADP and phosphoric acid by using the electrochemical potential gradient of the ion (H^+ or Na^+) between inside and outside of the membrane. The F_O works as an ion channel in the membrane. The ion channels are thought to be composed of the acidic residue of *c*-ring located at the center of the membrane and two half-channels. It has been reported that both periplasmic and cytoplasmic sides of the H^+ half-channels in H^+-transporting F_OF_1 exist in *a* subunit. In contrast, it has been proposed that the periplasmic side of the Na^+ half-channels in Na^+-transporting F_OF_1 exists in *a* subunit and the cytoplasmic side exists in *c*-ring. However it is unclear where the Na^+ half-channels are because the cytoplasmic side of the Na^+ half-channels is not found from the structure of *c*-ring. In this study, we examined aqueous accessibility of a transmembrane helix of Na^+-transporting F_O by reactivity of cysteine substituted residue using a hybrid F_OF_1 (F_1 from thermophilic *Bacillus* PS3 and Na^+-transporting F_O from *Propionigenium modestum*). We predicted that both periplasmic and cytoplasmic sides of the Na^+ half-channels in Na^+-transporting F_OF_1 existed in *a* subunit as the H^+ half-channels. Then, Cys residues were introduced into *a* subunit from *a*I211 to *a*V236 except *a*R226. After the modification of Cys of the mutant F_OF_1 with N-ethylmaleimide (NEM), the labeling yield of Cys by NEM and the ATP synthesis activity were examined. When Cys of G215C, K219C and N230C was labeled at 50% labeling yield, 40% of ATP synthesis activity was lost. These amino acid residues were accessible from outside of the membrane. In these mutants, ATP synthesis activity was inhibited because the modified Cys with NEM blocked Na^+ transport. Therefore, it was suggested that G215C and K219C in the cytoplasmic side and N230C in the periplasmic side were the amino acid residues that formed the Na^+ half-channels. It was suggested that both periplasmic and cytoplasmic sides of the Na^+ half-channels in Na^+-transporting F_OF_1 existed in *a* subunit as the H^+ half-channels.

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2P.30 On the rotary mechanism of F_1F_O-ATP synthases

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The mechanism of the F_1F_O ATP synthase couples the downhill membrane translocation of H^+ or Na^+ to the rotation of an oligomeric ring of c-subunits (c-ring) in the F_O motor. The torque is transduced into the F_1 motor, which causes sequential conformational changes in the catalytic centers, finally resulting in the generation of ATP. The design of the c-ring rotor provides the ion binding specificity and contributes to the translocation of the ions through the membrane during enzyme operation. The crystal structure of the c_{15} ring of the F_1F_O-ATP synthase from *Spirulina platensis* has been solved at 2.1 Å resolution [1]. The way the proton is bound to this c-ring proposes that all ion binding sites of the c-ring remain in the *proton-locked* conformation while exposed to the membrane, whereas exposure to a more hydrophilic environment can unlock the ion binding site and promote ion release. This model is supported by combined structural, biochemical and *in silico* generated data of the proton binding site.

Reference

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2P.31 The crystal structure of bovine mitochondrial F_1-ATPase, grown in the presence of phosphonate reveals a new intermediate in the catalytic cycle

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The ground state structure of yeast F_1-ATPase has a phosphate ion bound in the β_E-subunit, whereas the ground state structure of bovine F_1-ATPase does not [1]. In order to try and gain structural information about the phosphate binding site in the bovine enzyme, crystals were grown in the presence of ADP, magnesium ions and the phosphate analogue, phosphonate. The structure solved to 2.5 Å resolution reveals surprisingly that ADP is bound in the nucleotide binding domains of all three catalytic subunits. However, the ADP molecule bound in the β_E-subunit does not have an associated magnesium ion, whereas a magnesium ion is bound in the β_{DP}- and β_{TP}-subunits, as in other structures of F_1-ATPase. In these respects, the structure is similar to that of yeast F_1-ATPase inhibited with residues 1–52 of yeast IF_1 [2]. This latter structure has been interpreted as representing a post-hydrolysis state in which the magnesium ion has been released from the catalytic site before ADP. No electron density was observed that could be interpreted as bound phosphonate, and its probable role appears to be to chelate divalent metal cations, including magnesium ions, rather than acting as a phosphate analogue.

References

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2P.32 The structure at 2.5 Å resolution of the complex of F_1-ATPase from *Saccharomyces cerevisiae* inhibited with yeast IF_1

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F_1-ATPase from *Saccharomyces cerevisiae* was inhibited under conditions of ATP hydrolysis with a fragment of yeast IF_1 consisting of residues 1–53 (known as yI1–53). The complex was crystallised in the presence of 0.5 mM ADP and 1.5 mM ATP. In the inhibited structure, residues 17–40 of yI1–53 form an α-helix, of which residues 17–35 are bound in a cleft between the C-terminal domains of the α_{DP}- and β_{DP}-subunits, and residues 36–40 of this α-helix extend beyond the external surface of the F_1-domain. The α-helix interacts mainly with the β_{DP}-subunit, but also with subunits α_{DP} and β_{TP}. Residues 1–16 of yI1–53 form a loop from residues 6 to 16 held together by a salt bridge (residues R9 and D15) and a hydrogen-bonding network involving residues S4, R9 and D15 and residue R9 of the γ-subunit. The N-terminal region from residues 1 to 5 extends into the central aqueous cavity of the enzyme around the central stalk and makes contacts with the α_E-, β_{DP}- and γ-subunits. Many aspects of this structure are similar to those of the structure of bovine F_1-I1–60 [1]. However, the structures differ in several significant respects. First, the α-helix of yI1–53 is tilted more steeply (relative to the central stalk) than its counterpart in the bovine structure, and so the detailed interactions that contribute to binding differ in some respects. Second, the loop structure in residues 6–16 of yI1–53 replaces a second short α-helix from residues 13 to 17 of bovine I1–60. Third, the structures differ in the nucleotide occupancies of catalytic subunits; bovine F_1-I1–60 contains inorganic phosphate bound to the P-loop in the β_E-subunit whereas the β_E-subunit in the yeast complex contains ADP, but no magnesium. In both structures, the β_{DP}- and β_{TP}-subunits are occupied by magnesium ADP. The bovine structure was interpreted as representing a post-hydrolysis "dead-end" state [1], whereas the yeast structure appears to represent a post-hydrolysis, pre-product release intermediate that precedes the "ground state" structure of the enzyme in the hydrolytic cycle.

Reference

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2P.33 Kinetic equivalence of membrane potential and pH difference across membrane in ATP synthesis by *Bacillus* PS3 F_OF_1-ATP synthase

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F_OF_1-ATP synthase synthesizes ATP by using proton motive force (*pmf*) that consists of transmembrane gradients of electrical potential ($\Delta\psi$) and proton concentration (ΔpH). The two terms are thermodynamically equivalent according to the chemiosmotic theory of P. Mitchell [1]. But their kinetic equivalence to drive F_O has been reported to be varied by the source of F_OF_1s and preparations. Here, using *Bacillus* PS3 F_OF_1 (TF_OF_1) with a mutation lacking inhibitory effect of ε, we have developed simple and highly reproducible procedures to prepare active proteoliposomes and to analyze kinetics of ATP synthesis which was driven by acid–base transition and K^+/valinomycin diffusion potential. TF_OF_1 showed maximum rates of ATP synthesis of 18 s^{-1} at 30° with K_ms for ADP and Pi, 19 and 500 μM, respectively. Then, the rates of ATP synthesis were determined under several combinations of $\Delta\psi$ and ΔpH. The rates were highly correlated to the *pmf* value calculated from $\Delta\psi$ and ΔpH, although small deviation still remains. Osmotic imbalance between inside and outside of proteoliposomes has only little effect on the kinetics. Importantly, when K^+ concentration inside proteoliposomes is decreased below about 3 mM, it appears that Nernst equation tends to overestimate the valinomycin-induced $\Delta\psi$, which is one of the reasons of the deviation. Taking these results into consideration, we propose that rates of ATP synthesis are solely dependent on the magnitude of *pmf* but not on each of $\Delta\psi$ and ΔpH.

Reference

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2P.34 Isolation of the H^+-ATP synthase from *E. coli* and the stability of its subcomplexes

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The H^+-ATP synthase from *E. coli* ($EFOF_1$) and the hydrophilic part (EF_1) are isolated. The enzyme detergent micelles are purified either by sucrose density centrifugation or by ion exchange chromatography followed by gel permeation chromatography. For activity measurements $EFOF_1$ is reconstituted into phosphatidylcholine/phosphatidic acid liposomes and a transmembrane pH-difference is generated by an acid base transition. The initial rate of ATP synthesis is measured with luciferin/luciferase. The activity of EF_1 is measured by ATP hydrolysis with an ATP regenerating system. Analysis of the different fractions obtained after gel permeation chromatography reveals that the highest ATP synthesis activities and the highest ATP hydrolysis activities are found in different fractions, although the SDS-PAGE does not reveal significant differences in subunit composition of these fractions. Since the e-subunit is known to act as inhibitor of ATP hydrolysis without a significant effect on ATP synthesis, the dissociation of this subunit from the different subcomplexes was investigated. The dissociation constant of e was determined as described in [1]. The following dissociation constants are found: $K_D = 5.2$ nM for EF_1, $K_D = 0.7$ nM for $EFOF_1$ micelles, and $K_D = 0.1$ nM for $EFOF_1$ liposomes.

Reference

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2P.35 Biochemical and single-molecule analyses of human F_1-ATPase

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