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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