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

[1] Mitchell P (1961) *Nature* **191:** 144–148.

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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

[1] Smith JB, Sternweis PC (1980) *Biochemistry* **19:** 526–531.

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

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