Proton uptake from the periplasm into the F_O sector of the F-type ATP synthase occurs through a putative half-channel in the a-subunit [1, 2]. Alkaliphilic *Bacillus* species have a lysine (residue 180 in *B. pseudofirmus* OF4) at the position equivalent to a key glycine in helix-4 of the *E. coli* subunit [3]. This lysine has been shown to play a role in ATP synthesis at high pH (pH 10.5)-low protonmotive force (*pmf*) and is hypothesized to play a role in proton binding and delivery to the *c*-subunit carboxylate. Here we mutated lysine, to glycine, alanine, cysteine, histidine or arginine, in the *B. pseudofirmus* OF4 chromosome. All mutants had ≥115% of the octylglucoside-stimulated ATPase activity of the wild-type enzyme. Only the mutants with Gly, Ala or His substitutions retained ATP synthase activity at pH 7.5. Assays of ATP synthesis in ADP + P_i-loaded alkaliphile vesicles showed that synthesis by these mutants was more sensitive to CCCP inhibition than the wild type, with the order of CCCP sensitivity being Ala > Gly > His > wild type. This is the first indication that the alkaliphile-specific lysine plays a role at low *pmf* even when the external pH is not highly alkaline. The *a*-subunit from the ATP synthase of alkaliphilic *B. pseudofirmus* OF4 is less tolerant of substitution of other residues for lysine-180 than the subunit from more moderately alkaliphilic thermophile *Bacillus* sp. TA2.A1. For example, substitution of lysine-180 by Arg resulted in an active enzyme in the *Bacillus* sp. TA2.A1 [4] but in an inactive enzyme in *B. pseudofirmus* OF4. Moreover, the alkaliphile enzyme supports ATP synthesis over a much broader pH range. Sequence comparisons suggested that the *a*-subunits of extreme alkaliphiles are distinct from that of *Bacillus* sp. TA2.A1. Mutations made in several additional positions of the *a*-subunit in which alkaliphiles diverge both from non-alkaliphiles and *Bacillus* sp. TA2.A1 show that the alkaliphile-specific residues in several of these positions are required for normal malate-growth at pH 10.5. This suggests that the more stringent requirement of the *B. pseudofirmus* OF4 for lysine-180 is part of a broadly adapted structure that allows function over a broad range of pH values, from 7.5 to > 10.5.

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2P.22 Direct observation of steps in c-ring rotation of *Escherichia coli* F_OF_1-ATP synthase

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F_OF_1-ATP synthase (F_OF_1) is a complex of two rotary motors, and ATP synthesis/hydrolysis reaction catalyzed by water-soluble F_1 motor is coupled with the proton transport through membrane-embedded F_O motor via mechanical rotation. Direct observations of F_OF_1 rotation driven by ATP hydrolysis were carried out previously, but steps (pauses) in rotation rate-limited by F_O motor (proton transport) have not been directly resolved yet. Although single-molecule fluorescence resonance energy transfer has been applied to detect the rotation steps of F_O rotor c-ring recently, it is not a direct method visualizing rotation. In this study, with a goal of direct observation of steps in the rotor c-ring of F_O, mutation was introduced into the putative proton channel in the stator a-subunit (aE219H) to slow down proton transport through F_O motor. For direct observation of rotation driven by ATP hydrolysis and coupled with proton transport, F_OF_1 was reconstituted into the supported membrane formed on the NTA-modified agarose, and immobilized by histidine-tags introduced into the c-ring of F_O. Rotation was observed by 200 nm latex bead attached to the β subunits of F_1. At high ATP concentration which rotation steps rate-limited by F_1 motor are not visible, rotation rate of the aE219H mutant (9.1 ± 2.4 Hz) was lower than that of wild-type (20.0 ± 10.1 Hz), and showed good agreement with the value expected from ATPase activity in biochemical assay ($40\ s^{-1}/3 = 13$ Hz). On the other hand, in wild-type, rotation rate was much lower than the expected value ($285\ s^{-1}/3 = 95$ Hz). These results indicated that viscous drag of water against the bead is the rate-limiting of the rotation in wild-type, but in the mutant the rate-limiting factor is not the bead but an intrinsic property of the mutant. When the rotation of the mutant was analyzed in detail, many small steps were found. Distribution of rotary angle showed many peaks spaced by about 36°. Analysis of the pair-wise angle distance also indicated the minimal step size of about 36°. This step size was consistent with the previous cross-linking experiment reporting 10 protomers in a rotor c-ring of *E. coli* F_O motor. Furthermore, during observation the mutant F_OF_1 showed frequent backward 36° steps. This result indicates a low free energy barrier per step, and supports the notion that the Brownian ratchet is the rotary mechanism of the F_O motor.

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2P.23 ATP binding to the epsilon subunit of thermophilic ATP synthase is essential for coupling ATPase and H^+-pumping

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In our previous study, ATP binding to the epsilon subunit of F_OF_1-ATP synthase (TF_OF_1) from thermophilic *Bacillus* PS3, has been shown to shift the equilibrium between ATPase-inactive and active forms of F_1-ATPase (TF_1), a soluble part of TF_OF_1, by stabilizing ATPase-active form. In the present study, how the ATP binding to the epsilon subunit may concern with ATPase and H^+-pumping activities of the holo-enzyme, TF_OF_1, was examined by using a mutant TF_OF_1 containing epsilon subunit deficient in ATP binding. As for the ATPase activity, the mutant TF_OF_1 showed lower ATPase activity than the wild type indicating that the situation was similar to that with TF_1. Unexpectedly, however, the mutant had *no* H^+-pumping activity even under the conditions where the mutant had comparable ATPase activity to the wild type. Elimination of 14 basic amino acid residues in the C-terminal domain of the epsilon subunit by replacement with alanines recovered coupling although the mutant was also deficient in ATP binding. From these results, it was hypothesized that the bulky conformation of the C-terminal domain of the epsilon subunit, due to the repulsion of positive charges in the C-terminal domain in the absence of bound ATP may affect the coupling between F_1 and F_O.

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