achieved and complexes were isolated using Native PAGE and gel filtration chromatography. Pull-down results confirm the stoichiometric association of A and the annotated smaller B subunits in the presence of nucleotides to form higher order complexes that appear stable at high temperature. The complex is consistent with the formation of a hetero-hexameric A_3B_3 structure, and so the annotation of the putative small B subunit appears correct. We have scaled up this expression system for structural study and are also attempting to determine whether the reconstituted A_3B_3 complex can associate with the purified D subunit to form a functional A1 complex. Preliminary results suggest that the *N. equitans* A1A0 ATPase is, like other genes in its genome, representative of an archaic, minimalistic, yet functional form.

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16P4

The membrane modulates internal proton transfer in cytochrome *c* oxidase

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Reconstitution of membrane proteins into lipid bilayers often affects their function (compared to the detergent solubilized protein). In the present study we have investigated proton transfer during reaction of the reduced aa_3 cytochrome *c* oxidase (Cyt*c*O), from *Rhodobacter sphaeroides*, with oxygen in liposomes. The data indicate that the pH-dependence profile of the proton-transfer rate through one of the two proton pathways (the D pathway) changes upon reconstitution of the Cyt*c*O into lipid membranes [1]. While with the wild-type Cyt*c*O in detergent solution a pK_a of 9.4 was observed in this pH dependence, upon membrane reconstitution the proton-uptake rates were slowed in the pH range 6.5–9.5, consistent with an apparent pK_a of 6.8. This pK_a is interpreted to reflect the pK_a of Glu286, close to the catalytic site, but also the equilibrium constant between two possible conformers of the side chain. According to this model, the introduction of the membrane around Cyt*c*O may either change the equilibrium constant between the two positions of Glu286 or affect the actual pK_a values of these two states.

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16P5

The charged amino acid residues of two different stators of the flagellar motor in *Bacillus subtilis* are important for motility

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Many bacteria can swim by using flagella, filamentous organelles that extend from the cell surface. A flagellum consists of three parts, the filament, the hook, and the basal body. Intensive genetic and biochemical studies of the flagellum have been conducted in *Salmonella* and *Escherichia coli*, and more than 50 gene products are known to be involved in the flagellar assembly and function. The flagellar motor is energized by either a H^+ or Na^+ motive force. MotAB-type stators use protons, while MotPS-type stators and PomAB-type stators use sodium ions as coupling ions. The MotAB-type stator flagellar motor torque in *E. coli* is considered to be generated by electrostatic interactions at the interface between rotor and stator as indicated by previous studies. There are conserved charged amino acid residues (arginine 90 and glutamic acid 98) in the cytoplasmic loop between the second and third transmembrane segments of MotA, which probably interact with the conserved charged amino acid residues (lysine 264, arginine 281, glutamic acid 288, glutamic acid 289 and arginine 297) of the C-terminal domain of rotor protein FliG. However, it is not clear whether the electrostatic interaction between the rotor and the sodium ion-type stator PomAB is critical. In this study, we studied a flagellar motor that consists of two different stators, MotAB and MotPS, in *Bacillus subtilis* and tried to identify critically charged residues for torque generation in each MotA and MotP subunit. We selected the conserved charged amino acid residues in the cytoplasmic loop between the second and third transmembrane segments of MotA and MotP by using multiple sequence alignment with ClustalW. We identified charged amino acid residues that were conserved at two positions in MotA or MotP. *B. subtilis* with mutations in conserved charged amino acid residues and several other charged amino acid residues were measured for swarming and stator subunit protein expression levels. These charged amino acid residues in the cytoplasmic loop of stators can be divided into types important to torque generation by interaction between rotor and stators, and stabilization of the structure of stators. Therefore, important charged residues for motility in the H^+-driven MotAB and Na^+-driven MotPS were identified in this study.

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