Rotation Measurement of Na⁺-driven Chimeric Flagellar Motor with Tandem PomA

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Four PomA and two PomB, homologs of MotA and MotB in the H⁺-driven flagellar motor of Escherichia coli, form a stator complex in the Na⁺-driven flagellar motor of Vibrion alginoticus. The motor torque is generated by the interaction between the cytoplasmic domain of PomA and the C-terminal domain of FlgH, a component of the rotor. It was shown that a tandem fused PomA is functional as a torque generator in V. alginoticus (Sato and Homma, JBC 2000). Furthermore, a chimeric stator (which consists of PomA and the chimeric fusion protein PotB) works as a Na⁺-driven flagellar motor in E. coli (Asai et al., JMB 2003). In the last annual meeting, we demonstrated that tandem PomA dimer was expressed as a single polypeptide in E. coli and swimming speed of E. coli cells with tandem PomA was about half of that with the monomeric PomA, suggesting that tandem PomA functions as a torque generator in E. coli.

Here, in order to characterize the details of tandem PomA, we carried out rotation measurements of single flagellar motors in E. coli cells expressing tandem PomA using 1 μm beads attached to truncated flagellar filaments. Maximum motor speed with tandem PomA was 80 Hz, similar to that with monomeric PomA. However, the motor speed with tandem PomA distributed broadly compared to that with monomeric PomA. We also measured motor speeds with tandem PomA with several combinations of mutants in the cytoplasmic charged residues, which are thought to be crucial for torque generation. Details of these results will be discussed.

The functional role of the charged residues of two different stators: MotA subunit and MotB subunit in B. subtilis flagellar motor

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The flagellar motor is energized by either H⁺ or Na⁺ motive force. MotAB-type stators use H⁺, while MotPS-type and PomAB-type stators use Na⁺ 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 stator and rotor as indicated by previous studies. There are conserved charged residues in the cytoplasmic loop of MotA, which probably interact with the conserved charged residues of the C-terminal domain of rotor FlgH. However, it is not clear whether the electrostatic interaction between the rotor and the Na⁺-type stator PomAB is required. Here we studied a flagellar motor that consists of two different stators, MotAB and MotPS, in B. subtilis and tried to identify critical charged residues for torque generation in each MotA and MotB subunit. We identified the conserved charged residues in the cytoplasmic loop of MotA and MotP. B. subtilis with mutations in conserved charged residues and several other charged residues were measured for swimming and stator subunit protein expression levels. These charged residues in the cytoplasmic loop of stators can be divided into two types: those important to torque generation by interaction between rotor and stators, and those involved in stabilization of the structure of stators. Thus, important charged residues for motility in the H⁺-driven MotAB and Na⁺-driven MotPS were identified in this study.

Structural and functional analyses of a periplasmic region of Fltp, a component of the flagellar protein export apparatus

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Most of flagellar proteins are transported by a specific export apparatus from the cytoplasm to the growing distal end of the flagellum where they self-assemble. The export apparatus consists of a water-soluble ATPase complex and a proton-driven export gate made of six membrane proteins FlhA, FlhB, FlhC, Flf, Flip, and FlQ. The ATPase complex escorts flagellar proteins from the cytoplasm to the export gate. It has been reported that FlhA plays a role in energy transduction along with the ATPase complex. However, the structure and function of other gate proteins still remain unknown. Flip is the only protein that has a signal peptide in its N terminus. Flip has a molecular weight of about 26 kDa and is predicted to have four transmembrane helices (TM). Flip has a relative large periplasmic domain (Flipp) with ca. 8.5 kDa between TM-2 and TM-3. A deletion of FlipP results in a loss-of-function phenotype. We focused on FlipP to elucidate the role of Flip in flagellar protein export. An alanine-substitution of highly conserved residues of FlipP indicated that Gln178 is critical for the export function. We constructed an E. coli expression system of FlipP from Thermotoga maritima for structural analysis. The results of size exclusion chromatography, static light scattering and analytical ultracentrifugation indicated that purified FlipP forms homo-tetramer. Far-UV CD spectra showed that the secondary structure is mostly α-helix. Crystallization for X-ray structural analysis is now in progress.