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 Vibrio alginolyticus. The motor torque is generated by the interaction between the cytoplasmic domain of PomA and the C-terminal domain of FliG, a component of the rotor. It was shown that a tandem fused PomA is functional as a torque generator in V. alginolyticus (Sato and Homma, JBC 2000). Furthermore, a chimeric stator (which consists of PomA and the chimeric fusion protein PolB) 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 stalks: MotA subunit and MotP subunit in B. subtilis flagellar motor

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The flagellar motor is energized by either H^+ or Na^+ motive force. MotAB-type stalks use H^+, while MotPS-type and PomAB-type stalks use Na^+ as coupling ions. The MotAB-type stalk 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 residues in the cytoplasmic loop of MotA, which probably interact with the conserved charged residues of the C-terminal domain of rotor FliG. However, it is not clear whether the electrostatic interaction between the rotor and the Na^+-type stalk PomAB is required. Here we studied a flagellar motor that consists of two different stalks, MotAB and MotPS, in B. subtilis and tried to identify critical charged residues for torque generation in each MotA and MotP 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 stalks 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 stalks. Thus, important charged residues for motility in the H^{-}-driven MotAB and Na^+-driven MotPS were identified in this study.