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The role of charged residues in C-terminus of PomA in V. alginolyticus
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Bacterial flagellar motor uses energy from specific ion gradient to drive rotation of the flagellar filament. The rotor-stator interaction coupled by ion flow generates torque. The electrostatic interactions between charged residues of the stator protein MotA and the rotor protein FlIG are shown to be important for torque generation. The charged residues are conserved in the components, PomA and FlIG, of the Na+-driven motor of V. alginolyticus, however, mutational studies suggested that they were not important for Na+-driven motor rotation. We speculate that PomA contains the other important charged residues than the conserved residues of E. coli MotA. We made eight charge-reversing mutants in the residues of C-terminus in PomA and measured the effects on swimming and swimming. Besides, the PomA mutants were examined using chimeric motor, which can work as sodium type motor, also in E. coli. Mutility was eliminated by PomA-K203E, R215E, D220K either of motor in V. alginolyticus or of chimeric motor in E. coli. The R215E and D220K mutants impaired the motility for wild-type motor of V. alginolyticus. The R207E mutant conferred the motility for motor in V. alginolyticus but not for the chimeric motor in E. coli. The E211K and R232E protein seems to be functional almost same as the wild-type PomA in Vibrio but greatly reduced in E. coli. The amount of the E211K protein was reduced as compared with that of wild-type PomA. We could show that the charged residues K203, R215, D220 appeared to be important for rotation.

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A reversibility parameter for Markovian steppers
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Biological molecular motors shares the features that they have a unit step and the steps stochastically occurs either to forward or backward direction along a filament. Recent experimental studies [1] on kinetic rates of steps revealed that they appears to violate the local detailed-balance (or microscopic reversibility) condition (LDB), which relates the kinetic rates to the change in free energies before and after a transition [2]. In this paper, in order to account for the violation of LDB, we present a simple Langevin model that involves a "hidden" internal degree of freedom along the "accessible" spatial degrees of freedom. Although this model satisfies LDB if all the degrees of freedom arc considered, we show that this model appears to violate LDB if observation is restricted on the spatial degrees of freedom. Using this model, we show the violation of LDB is closely related to the structure of kinetic pathways in a state space. When the kinetic pathways make a linear chain, the LDB is not violated if the observation is restricted. In contrast, when the kinetic pathways make a branched structure, the LDB appears violated by the restricted observation. In the present paper, we present a mechanism of this fictive violation of LDB and its implication on the mechanism of molecular motors.


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Localization of the stator of the Na+-driven flagellar motor dependent on Na+ ion
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Vibrio aliginolyticus has a Na+-driven flagellum at its cell pole. Its motor consists of the rotor and stator and rotation is driven by the rotor-stator interaction that couples to the Na+ ion in flux through the motor. PomA and PomB are believed to be stator proteins for the Na+-driven flagella of Vibrio alginolyticus. Fukuda et al. (J. Mol. Biol. 351, 707-717(2005)) constructed GFP fusions of the stator proteins. GFP-PomA or GFP-PomB is localized at a cell pole in the presence of the rotor. In this study, we first investigated the effect of polar localization by the mutation of PomA or PomB. As the result, GFP-PomA was not localized at a cell pole when co-expressed with the mutant PomB that has a mutation at putative sodium binding site (D24C). We thought the ion flux through the stator might affect its polar localization of the stator. When GFP-PomB was expressed in the Na+-free buffer, it was not localized at the cell pole. However, when those cells were transferred in Na+ 500mM buffer, GFP-PomB was restored to the cell pole in several cells. We observed that the fraction of the polar localization of GFP-PomB reduced in 100mM buffer or less. On the other hand, GFP-FlIG, which is one of the components of the rotor, was localized at the cell pole independent of Na+ concentration. These may show that the Na+ ion is required not only for torque generation but also for assembly of the stator. We speculate that Na+ ion induces the conformational change of the stator protein and the stator can associate with rotor to assemble the motor.

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Inter-modular Communication among Multiple Nucleotide-binding/hydropysis AAA+ Modules of Cytotplasmic Dynein
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An AAA+ superfamily member dynein heavy chain is a microtubule-based minus-ended motor protein that contains at least four putative nucleotide binding sites. To detect motions of the tail domain coupled with ATPase cycle at AAA1 module of the motor domain, GFP-based FRET was applied. The dynein motor domain adopted at least two conformational states defined by the FRET signals: State I (low FRET efficiency state occurs in the absence of ATP and in the presence of ADP) and State II (high FRET efficiency state occurs in the presence of ATP). Kinetic analyses of transitions between the FRET-based conformational states in a wild-type protein and its Walker B motif mutants indicated that catalytic sites located at AAA1 and AAA4 modules indeed hydrolyze ATP and that normal cycle of ATPase at AAA1 module depends on what the nucleotide states of these two modules are. Furthermore, non-nucleotide-hydrolyzing AAA2 module may bind ADP to play a regulatory role on dynein activity.