Stator Assembly and Activation Mechanism of the Flagellar Motor Revealed by the Crystal Structure of the Periplasmic Region of MotB

The bacterial flagellar motor is a supramolecular nanomachine powered by the electrochemical potential difference of ions across the cell membrane and spins flagellar filaments to drive cell motility [1]. In the *Salmonella* motor, torque is generated by rotor-stator interactions coupled with proton translocation through the channel within the $MotA_4MotB_2$ stator complex. To produce a fully functional motor, multiple stator units have to be incorporated into appropriate positions around the rotor and anchored to the peptidoglycan (PG) layer by the peptidoglycan-binding (PGB) domain of MotB. The motor complex is not a rigid structure; the stators dynamically assemble to and disassemble from the functional motor. Moreover, overproduction of the MotA/MotB complex does not affect cell growth, suggesting that the proton-conducting activity of the MotA/MotB complex is tightly coupled to its incorporation around the rotor. To elucidate the molecular mechanisms of the stator assembly and activation, we determined the 1.75 Å resolution structure of a C-terminal fragment of *Salmonella* MotB ($MotB_C$) that contains the PGB domain and covers the entire periplasmic region essential for motility using X-ray diffraction data collected at beamline **BL41XU** [2].

$MotB_C$ (residues 99-276) appears as a single domain structure with a long N-terminal α-helix (α1) protruding from the domain (Fig. 1). The core of the domain has a typical OmpA-like structure and shows considerable structural similarities to other PGB domains such as the C-terminal regions of PAL, RmpM, and MotY. Although the N-terminal region of $MotB_C$ shows a relatively low sequence similarity among MotB proteins from various bacterial species, secondary structure prediction using the PSIPRED server suggests that α1, α2 and β1 are common structural elements. $MotB_{C2}$ forms a dimer through the interaction between the PGB domains. Mutation analyses of the residues on the dimer interface indicated that dimerization through the PGB domain is crucial to the motor function.

Superposition of the structure of PAL bound to the PG precursor [3] on the $MotB_{C2}$ structure allowed us to predict the PGB site of $MotB_C$ (Fig. 2(a)). The identified PGB sites are present on the top surface of the $MotB_{C2}$ dimer and located opposite to α1. Since $MotB_{\Delta L}$, which is a mutant MotB whose internal fifty residues (Ser51 to Lys100) were deleted, can form a functional stator with MotA, the $MotA/B_{\Delta L}$ complex must be anchored to the PG layer around the rotor. However, the size of the $MotB_C$ dimer is too small to reach the PG layer. Therefore, a large conformational change is required for anchoring the stator. Because the PGB core forms a conserved compact domain, the N-terminal region of $MotB_C$ is the most plausible candidate for the conformational change (Fig. 2(b)). Mutation in α1 (L119P or L119E) of $MotB_{\Delta L}$ affected the cell growth when overproduced, and mutant cells showed significantly better motility than wild-type $MotB_{\Delta L}$ under non-inducing conditions, supporting the idea that the mutations alter the structure of the $MotA/B_{\Delta L}$ complex to an active conformation for proton translocation across the cell membrane as well as for the functional motor assembly.

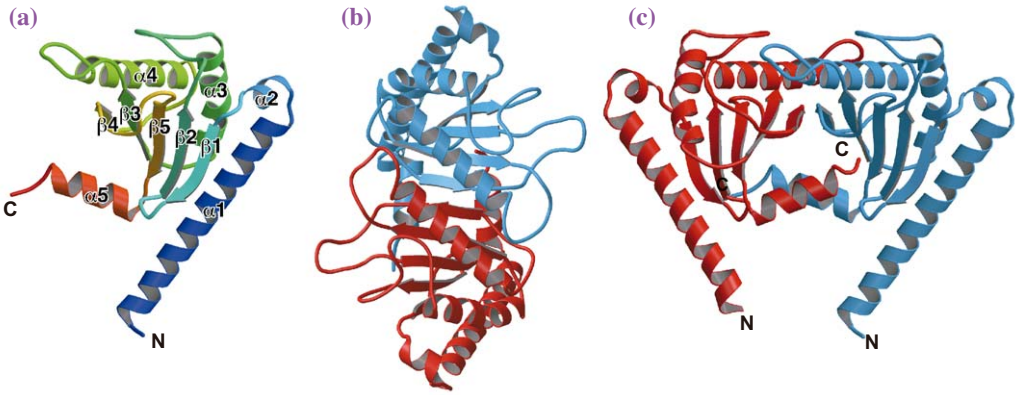

Fig. 1. Structure of the C-terminal fragment of MotB and its dimer. **(a)** Cα ribbon drawing of $MotB_C$, color coded from blue to red from the N- to the C-terminus. $MotB_C$ is composed of a typical common PGB core domain (α3, α4, β2, β3, β4 and β5) and N- and C-terminal sub-structures (α1, α2, α5 and β1). **(b)** and **(c)** Cα ribbon representation of the $MotB_C$ dimer along and perpendicular to the 2-fold axis. Two subunits are colored cyan and red.

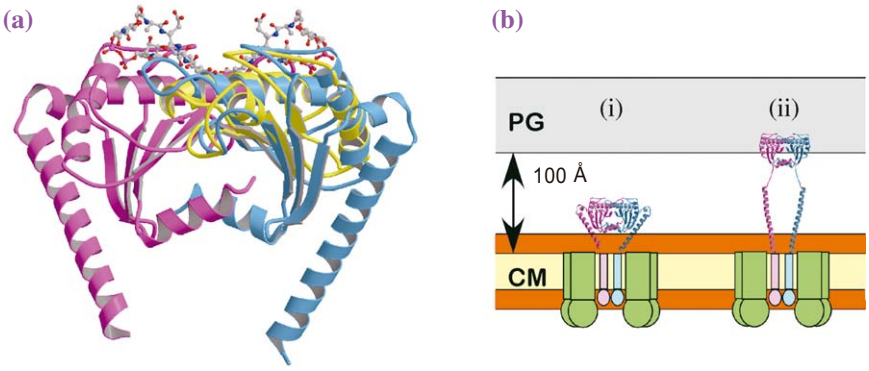

Fig. 2. Conformational changes of PEM required for anchoring the stator. **(a)** Possible PG binding site of $MotB_C$. The structure of *Haemophilus influenzae* Pal (yellow) in complex with a PG precursor (ball-and-stick) is superimposed on subunit A (cyan) and subunit B (magenta) of the $MotB_C$ dimer. Note that only the PG precursor is shown for the Pal complex superimposed on subunit B. **(b)** Plausible models of $MotB_{\Delta L}$ in a freely diffusing, inactive form (i) and in a PG layer-anchored, active stator form assembled into the motor with the proton channel opened (ii). The cytoplasmic segments and transmembrane helices of $MotB_{\Delta L}$ are displayed as rods and ellipsoids colored in pink and cyan. The green boxes and balls represent MotA subunits. The hydrophilic surface and hydrophobic core layers of the cell membrane (CM) are shown in orange and yellow, respectively. The relative sizes of $MotB_{\Delta L}$, PG, and CM are shown in the correct scale.

On the basis of these results, we propose the assembly and activation mechanism of the stator (Fig. 3). Before associating with the rotor, the MotA/B complex would dynamically diffuse in the cytoplasmic membrane. When the MotA/B complex is incorporated into an appropriate position around the rotor, the association signal triggers the conformational changes of the N-terminal region of $MotB_C$ to allow the PGB domain to anchor to the PG layer as well as to open the proton channel to activate the motor. Our study thus unveiled a novel activation mechanism of the stator coupled with its own assembly.

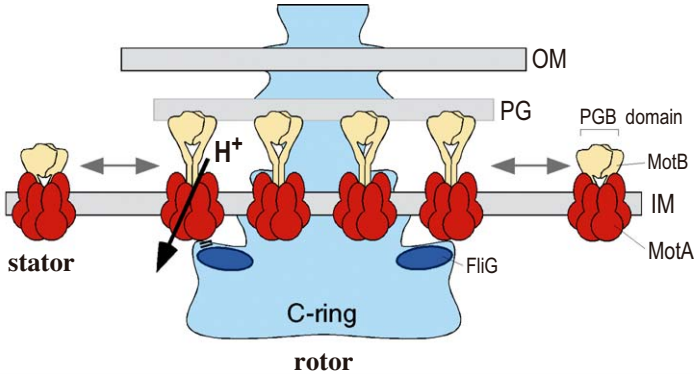

Fig. 3. Proposed model for activation of the proton channel by association with the motor. The MotA/MotB stator complex diffuses through the cytoplasmic membrane with inactive form for proton conduction. When incorporated, the conformational change in the N-terminal region of $MotB_C$ is induced to open the proton channel and allow the PGB domain to anchor the PG layer at the appropriate position near the rotor.

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