A Mechanical Switch Found in the Bacterial Flagellar Protofilament Structure

The bacterial flagellum consists of a rotary motor and a helical propeller by means of which bacteria swim. The flagellar motor is at the base of the flagellum and drives its rotation at around 300 Hz. The long helical propeller is called the flagellar filament, and it grows up to around 15 μm by polymerization of a single kind of protein, flagellin. The filament is not simply a rigid helical propeller but switches its helical form from a left-handed to right-handed ones upon quick reversal of the motor rotation, by which a bundle of several filaments formed during a straight run falls apart quickly, and this makes the bacterial cell tumble and change its swimming direction for chemotaxis or thermotaxis.

It is not a simple question to answer how chemically identical molecules can build curve and twisted tubular structures. It was proposed by Asakura [1] that various helical forms of the flagellar filament are produced by two types of protofilaments with distinct repeat distances and lateral packing modes in each of the 11 protofilaments that form the tubular structure. Electron cryomicroscopic structure analysis of two types of straight flagellar filaments, the L- and R-type, each made of either L- or R-type protofilaments, showed that the overall structure of the flagellin subunit does not change much in the two packing modes [2]. X-ray fiber diffraction revealed that the L- and R-type protofilaments have a subunit repeat distance of 52.7 Å and 51.9 Å, respectively [3]. By simple mechanical simulation to build a tube with a mixture of two types of protofilaments, this difference of 0.8 Å and a small change in the lateral packing explained the curvature and twist of various helical forms of the filament observed by dark-field optical microscopy, demonstrating that this subtle difference is physically meaningful [3].

We then carried out X-ray crystallographic structure analysis of monomeric flagellin to find out what structural changes would be responsible.

Crystallization of flagellin, however, was impossible because flagellin has a strong tendency to polymerize into filaments. We therefore cleaved off 52 NH_2- and 44 COOH-terminal residues from 494 amino acid residues of *Salmonella* flagellin to suppress the strong polymerization ability. This 41 kDa fragment named F41 formed crystals but they were only about 5 μm thick. It would have been impossible to collect diffraction data from such a thin crystal without a highly brilliant X-ray beam

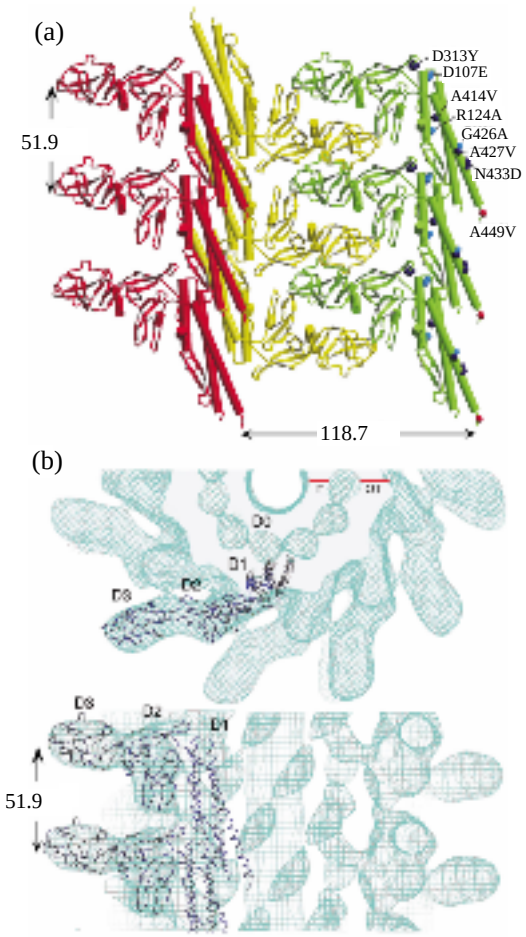

Fig. 1. Crystal structure of the flagellin fragment F41 ***(a)*** *and docking of the protofilament model into an EM density map* ***(b)****.*

from SPring-8 beamlines. Annealing of frozen crystals was also essential for collecting high-resolution data. We collected a set of multiple anomalous diffraction data to 2.0 Å resolution at one of the RIKEN beamline **BL45XU**, which was designed based on the trichromatic concept [4].

The structure of F41 had three domains. The one with an elongated shape and made of three α-helices and a β-hairpin formed an axial array with very intimate interactions in the crystal packing. The repeat distance was 51.9 Å, exactly the same value as that of the R-type protofilament (Fig. 1a). The atomic model of this array of F41 fitted very well when docked into a density map of the R-type filament deduced by electron cryomicroscopy (Fig. 1b), indicating that this is the atomic model of the protofilament [4].

Because the flagellar protofilament is a kind of mechanical switch that switches between the two states with distinct repeat distances, we carried out a computational simulation of extending the protofilament model to identify the switch region within the molecular structure. We treated three axially aligned subunits as a protofilament model, and while fixing Cα atoms of the top subunit, we translated the bottom subunit by 0.1 Å in the direction extending the protofilament model and did energy minimization of the whole model with Cα atoms of the top and bottom subunits all fixed. We repeated this procedure up to an extension of 6 Å. Up to an extension of 4.5 Å, the middle subunit showed only gradual elongation in every portion of domain D1, but from 4.5 to 4.7 Å, the β-hairpin in domain D1 showed an abrupt jump in its conformation (Fig. 2). We thus identified that this β-hairpin structure, which pairs with three long α-helices to form domain D1, is the mechanical switch in flagellin to produce the two distinct protofilament states with slightly different repeat distances [4].

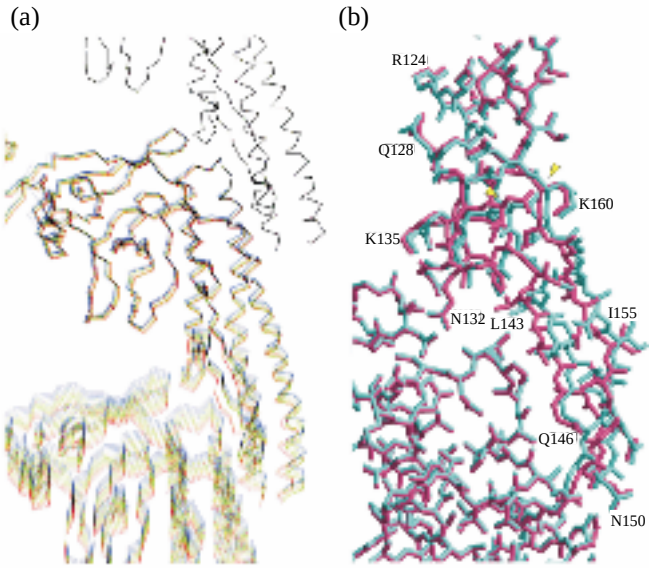

Fig. 2. Simulated extension of the protofilament model. ***(a)*** *Superposition of different stages at every 0.5 Å extension.* ***(b)*** *Magnified image of the β-hairpin portion of domain D1. Colors represent 4.5 Å (cyan) and 4.7 Å (pink) extension.*

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