Journal of Molecular Graphics and Modelling 28 (2010) 548–554

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Mechanical unfolding of bacterial flagellar filament protein by molecular dynamics simulation

Choon-Peng Chng [a,1], Akio Kitao [b,c,*]

[a] Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo, Japan
[b] Laboratory of Molecular Design, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
[c] Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Tokyo, Japan

ARTICLE INFO

Article history:
Received 20 August 2009
Accepted 30 November 2009
Available online 4 December 2009

Keywords:
Flagellin
Flagellar export
Force-denaturation
Protein mechanics
Multi-domain

ABSTRACT

Bacterial flagellum is a nano-scale motility device constructed by self-assembly. During construction of the cell-exterior filament (the 'propeller'), subunit proteins (called flagellin) are thought to be exported through the hollow flagellum to the growing filament tip in an unfolded state. To gain insight into the unfolded state preceding any force-spectroscopy experiments on flagellin, we employed force-probe molecular dynamics simulations. Two schemes to attain an unfolded state suitable for efficient transport were examined: (i) stretching flagellin along its length; (ii) unzipping flagellin from its adjacently placed termini. Atomic-level unfolding pathways and the mechanical efforts involved under each scheme were obtained for the four-domain flagellin from *S. typhimurium*. Flagellin appeared stiffer and required larger unfolding forces when stretched as the relative sliding of β-strands require the breaking of multiple hydrogen bonds at once. In contrast, unzipping requires lower unfolding forces as it mainly involves unraveling β-sheets by breaking hydrogen bonds one by one.

1. Introduction

The bacterial flagellum is a protein-based motility device. It consists of a micrometer-long screw-like tubular filament driven by a membrane-embedded rotary motor that is powered by a flow of protons or sodium ions. During self-assembly of the flagellum, proteins destined for the cell-exterior components have to be exported by the flagellar export apparatus after synthesis [1]. For filament construction, each of the subunit proteins known as flagellin has to be transported through a continuous channel to reach the site of assembly at the filament tip, where a capping protein complex assists in flagellin polymerization and prevents flagellin loss [2].

The flagellar channel diameter of 20 Å may be too narrow for a folded multi-domain flagellin to pass through: the complete 3D structure for *Salmonella typhimurium* flagellin obtained at atomic resolution [3] shows the presence of β-sheet rich domains which have cross-sections larger than 20 Å (Fig. 1). These so-called Hypervariable Region (HVR) domains are encoded by a stretch of amino-acid sequence in the middle of the flagellin sequence that varies greatly in composition and length among homologues [4]. It is likely for other homologues that contain HVR of similar length to encode similarly large domains. If the inner diameter of the filament could somehow expand in response to moving molecules, it would be possible to transport flagellin molecules in a largely folded state. However, to our knowledge there is no experimental evidence to support this possibility. Furthermore, hydrophobic interactions mediating coiled-coil formation between terminal helices of adjacent polymerized flagellin contributing to mechanical stability of the filament [3] might also limit how much the channel diameter can expand. MD simulations of a 44-flagellin segment of the filament showed relatively small fluctuations in the central channel diameter on the nanosecond time-scale [5]. Hence it is more likely for flagellin (and possibly other flagellar export proteins) to be transported in a partially if not completely unfolded form, as suggested by Namba and co-workers [3].

For flagellar proteins to be largely or completely unfolded during transport, they could have been either (i) maintained in an unfolded state after their synthesis or (ii) actively unfolded before transport. To date the only known chaperone of flagellin, FliS, binds to the partially structured C-terminal segment to inhibit cytoplasmic polymerization of flagellin while leaving the rest of the molecule folded [6,7]. Hence, flagellar proteins are likely to be largely folded in the cytoplasm while waiting to be exported. As proteins are unfolded mechanically for various processes such as membrane translocation or degradation [8], it is highly likely that

* Corresponding author at: Laboratory of Molecular Design, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Tel.: +81 3 5841 2297; fax: +81 3 5841 2297.
E-mail address: kitao@iam.u-tokyo.ac.jp (A. Kitao).
[1] Present address: Biophysics Team, A*STAR Institute of High Performance Computing, 1 Fusionopolis Way, #16-16 Connexis, Singapore 138632, Singapore.

1093-3263/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2009.11.007

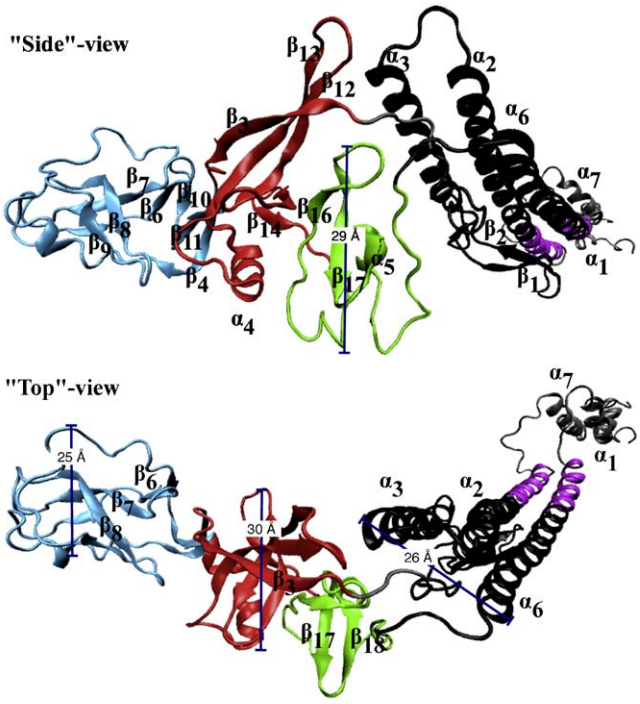

Fig. 1. Two views of the conformation of monomeric flagellin obtained from PDBid 1UCU by molecular dynamics simulation. Domains are colored as follows: D0 helices in gray, D1 in black and magenta, with proteolytic-resistant segment D_f1 in black, subdomain D2a in red and D2b in green, and D3 in light blue. Rough estimates of the cross-section of some domains are indicated.

the flagellar export apparatus also unfold flagellin mechanically though the origin of the force remains controversial [9].

For efficient transport and assembly, the multi-domain flagellin may have been evolutionarily designed to unfold easily under tension yet quick to refold from the denatured state. Using thermal unfolding simulations of flagellin, we have found suggestions that the (re)folding process might be accelerated with the formation of three-stranded Z-like β-sheets as folding nuclei in the β-rich HVR domains [10]. Would flagellin also unravel easily when pulled upon by the flagellar export system? As the mechanical response of protein molecules are known to be dependent on how they are deformed [11,12], could there be any preferred direction to unfold flagellin with minimal effort? For instance, Titin mutants showing less resistance to mechanical unfolding by Atomic Force Microscopy (AFM) are also more easily imported into mitochondria [13]. An understanding of the mechanical response of flagellin might thus provide insight into the export process. As no single-molecule force-spectroscopy experiments have been reported for flagellin, we wish to use computer simulations to gain some preliminary insights. Force-probe [14] or steered molecular dynamics (SMD) simulations [15] have shown some success at mimicking AFM action *in silico*. They also serve to complement AFM experiments by revealing the unfolding pathway in atomic detail [16,17].

In this study, we explored how to mechanically deform flagellin so as to attain a conformation suitable for transport through the narrow flagellar channel by means of force-probe MD simulations. Two conveniently chosen but also most extreme pulling schemes were investigated here. In the first scheme, termed *Unzip*, flagellin is unraveled from its adjacently placed termini into a linear polypeptide. In the second scheme, termed *Stretch*, flagellin is stretched along its major principal axis into a hairpin-like conformation (by virtue of its "fold-back" topology). We have quantified the associated mechanical efforts and identified the load-bearing elements encountered along the unfolding pathways in each scheme. These could help to interpret future single-molecule force-spectroscopy experiments on flagellin. We found that *Unzip* may be the more likely of the two schemes investigated here to produce a transport-capable flagellin.

2. Methods

2.1. Monomeric flagellin structures

We first explain the procedure to prepare equilibrated models of *monomeric* flagellin in explicit solvent before describing how starting structures for mechanical unfolding are generated.

The atomic coordinates of flagellin was taken from PDBid 1UCU [3], solved as a subunit of the bacterial filament of *Salmonella typhimurium* (*polymeric* flagellin). *S. typhimurium* flagellin has 494 residues contained in four domains, with terminal helical domains D0, D1 being highly conserved across bacterial species [4] as they form the inner and outer filament tubes responsible for filament structure integrity [3]. The remaining two outer domains (D2, D3) encoded by the mid portion of the amino-acid sequence are called Hypervariable Region (HVR) domains as they vary significantly in size and composition among homologues. D2 of *S. typhimurium* consists of subdomains D2a and D2b. We obtained the conformation of free (*monomeric*) flagellin in solution from the structure of *polymeric* flagellin. The procedure is outlined in our thermal unfolding study of flagellin [10]. The flagellin conformer after 1.4-ns of NPT production MD in explicit solvent was taken as the *monomeric* form of flagellin (Fig. 1) and denoted as *S1_pre*. For an alternative *monomeric* flagellin conformer, we used the 3.3-ns snapshot from the 8-ns NVE control simulation in our thermal unfolding study (denoted as *S2_pre*).

2.2. Force-probe simulations

Force-probe MD simulations initially were carried out in a very long explicit solvent box to accommodate the elongated flagellin molecule (~170 Å) but later found to be impractical. Implicit solvent models were then chosen for their much lower computation cost, despite the limitations (see Section 3). Specifically, the OBC model II variant [18] of the Generalized-Born/Surface-Area (GB/SA) model in AMBER 8 was chosen, which computes the polar component of the solvation free energy by the GB method and the non-polar component taken to be proportional to the solvent accessible surface area of the molecule with an experimentally obtained proportionality constant. The salt-concentration was set to a physiological value of 0.2 M. A large non-bonded cutoff of 25 Å for both electrostatics and Lennard-Jones interactions was used. Use of SHAKE [19] to constraint chemical bonds involving H-atoms allowed a time-step of 2-fs. After equilibrating *S1_pre* and *S2_pre* under the GB/SA model and using the Langevin thermostat to mimic solvent friction and stochastic effects at 300 K (using collision frequency of 1 ps^{-1}), the resultant structures (denoted *S1* and *S2*) served as starting points for our force-probe studies.

To carry out a force-probe MD simulation under constant-velocity, each group of atoms to be pulled (pull-group) has its center-of-mass restrained by a spring-like potential to some reference position. These positions or reference coordinates were then displaced successively by 1 Å along the pulling direction to apply a constant strain on the molecule. To *stretch* flagellin, the pulling direction "**CP**" lies along the line joining coordinate "**C**" in domain D3, being either the C_α atom of Gly211 ($\mathbf{C}_1$) or the C_α atom of Gly237 ($\mathbf{C}_2$), to coordinate "**P**" which denotes the center-of-mass of the termini C_α atoms (Fig. 2). The **CP** vector was initially oriented to the *x*-axis of the simulation system. To *Unzip* flagellin, the N- and C-terminal C_α atoms are displaced in opposite directions along a vector perpendicular to the *stretch* direction, either along *y*- or *z*-axis (see Table 1). The pulled atoms relax