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X-ray structure of *Clostridium perfringens* major pilin

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1 Introduction

Gram-positive bacteria possess a thick cell wall, a mesh polymer of peptidoglycans, that surrounds their cytoplasmic membranes and provides physical protection. The pathogenesis and infectivity of Gram-positive bacteria are mediated by many surface proteins that are covalently attached to the cell wall. Pili formed by the covalent polymerization of major pilins are flexible rod proteins associated with the bacterial cell surface, that play important roles in the initial adhesion of bacterial cells to host tissues and their colonization [1]. Here, we report X-ray structure of major pilin from *Clostridium perfringens* strain 13 (CppA-St13).

2 Experiment

Crystallization condition of CppA-St13 is as follows: protein concentration of 22 mg/ml, reservoir solution of 100 mM imidazole/MES buffer pH 6.5, 10 mM sodium formate, 20 mM ammonium acetate, 20 mM sodium citrate tribasic dehydrate, 20 mM sodium potassium tartrate tetrahydrate, 20 mM sodium oxamate, 20% v/v ethylene glycol, 10% w/v PEG8000. X-ray diffraction data was collected on the PF-BL5A KEK (Tsukuba, Japan), and diffraction data were processed using the program XDS. The structure of CppA-St13 was solved by molecular replacement with the program MOLREP using the structure of domain truncated form of CppA-St13 (PDB code: 5XCC). The structure of CppA-St13 was refined to R_{cryst} = 0.230 (R_{free} = 0.273) using 2.48 Å resolution data.

3 Results and Discussion

In a crystal of CppA-St13, there are two molecules (Mol-A and Mol-B) in an asymmetric unit, with r.m.s. deviations for main chain atoms of 1.29 Å. The structures of Mol-A and Mol-B include 453 amino acid residues (Thr30 - Thr482). The overall structure of CppA-St13 (Mol-A) is shown in Fig. 1A. CppA-St13 adopts an elongated structure with a length of 120 Å, including 34 β-strands, three α-helices (H3, H4, and H5), and two short 3_{10} helices (H1 and H2). The molecule can be divided into three domains: domain 1 (D1, Met29 - Pro173), domain 2 (D2, Lys174 - Tyr336), and domain3 (D3, Thr337 - Ala475), and the C-terminal tail region including CWSS (Gly476 - Thr482) protrudes from D3. D2 and D3 are linearly arranged, and D1 is displaced from the principal axis of D2/D3, giving the molecule an arc-shape. In a crystal, a molecule (Mol-A) captures the C-terminal tail region of another molecule (Mol-A') through crystallographic 2_1 screw symmetry (Fig. 1B), and this dimer is continuously arranged along the *c*-axis to form a polymeric structure. It is thought that this polymeric structures of CppA-St13 provide the most practical model of the pilus fiber structure.

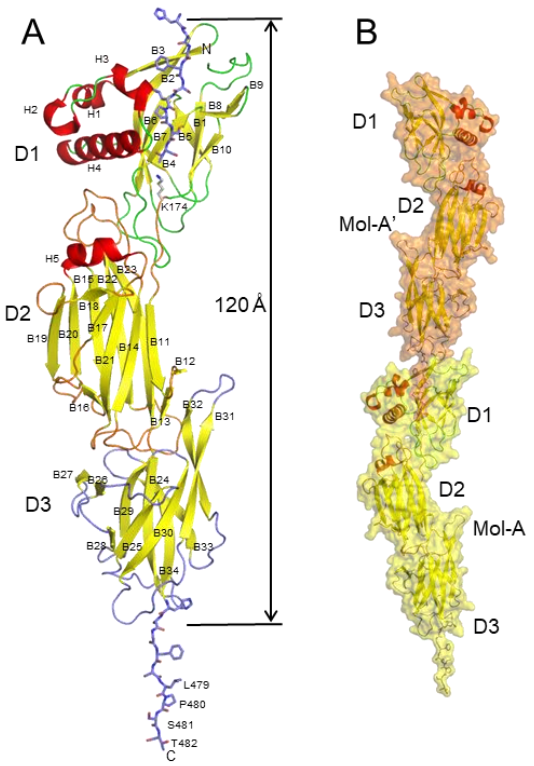

Fig. 1: Structure of CppA-St13. (A) The overall structure of CppA-St13 is illustrated with the labels of secondary structure elements. Helices and β-strands are shown in red and yellow, respectively. Loops in D1, D2, and D3 are green, orange, and blue, respectively. The C-terminal tail region is represented by a stick model. The C-terminal tail region of another molecule in D1 is also shown in a stick model. (B) Structure of Mol-A (yellow) and Mol-A' (orange) operated by crystallographic 2_1 screw symmetry is illustrated by surface representation.

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References

[1] H. Ton-That, H. and O. Schneewind (2004). *Trends Microbiol.* **12**, 228 (2004).

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