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X-ray structure of *Clostridium perfringens* pili protein B N-terminal domain

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1 Introduction

Sortase-mediated pili are flexible rod proteins composed of major and minor/tip pilins, playing important roles in the initial adhesion of bacterial cells to host tissues. The pilus shaft is formed by covalent polymerization of major pilins, and minor/tip pilin is covalently attached to the tip of the shaft involved in adhesion to the host cell. The Gram-positive bacterium *Clostridium perfringens* has major pilin, and minor/tip pilin (CppB) with the collagen-binding motif. Bioinformatic analysis predicted that CppB has five domains (D1 - D5) of an Ig-like fold, and collagen-binding assays of CppB domain variants showed that N-terminal two consecutives domains of CppB (CppB-D1D2) has a high collagen-binding activity. X-ray structure of CppB-D1D2 were determined, and only 64% of partial structure of D1 could be built in the resultant electron due to a disordered structure. In order to obtain the complete structure of D1, X-ray structure determination of N-terminal domain of CppB (CppB-D1) was performed.

2 Experiment

Crystals of CppB-D1 (Glu33 - Tyr164) were grown at 20°C in a droplet mixed with 1 μL of protein solution (110 mg/mL) and 1 μL of reservoir solution against 50 μL of the reservoir solution (50 mM bis-tris pH 6.5, 50 mM ammonium sulfate, 30% v/v pentaerythritol ethoxylate (15/4 EO/OH)), using the sitting drop vapor diffusion method. Crystal was flash-cooled in liquid nitrogen at 100 K using 25% v/v glycerol in reservoir solutions as cryoprotectants. X-ray diffraction data were collected on PF-BL5A in KEK (Tsukuba, Japan). Using the partial model of D1 (64%) and a model derived from the program AlphaFold2, the structure of CppB-D1 was solved by molecular replacement with the program MOLREP, and structure was refined using the programs Refmac5 at 1.55 Å resolution (PDB ID: 8GSY) [1].

3 Results and Discussion

The overall structure is shown in Fig. 1A. CppB-D1 is comprised of two antiparallel β-sheets and a helix. The β-sheet S1 consisting of B5, B8, B3, and B1 faces the β-sheet S2 consisting of B2, B10, B9, B4, B7, and B6, in which a short β-strand B2 is irregularly parallel to the neighboring β-strand B10. Most of the loops between β-strands lie across the two β-sheets, except B6-B7 and B9-B10 loops. A short helix, H1, locates on the B3-B4 loop. To generate a complete structure of CppB-D1D2, the structure of CppB-D1 is introduced into the structure of CppB-D1D2 (Fig. 1B). The structure adopts an L-shaped structure considered to be in open form by a structural comparison with *Staphylococcus aureus* collagen-binding adhesin in complex with the synthetic collagen peptide (PDB: 2F6A).

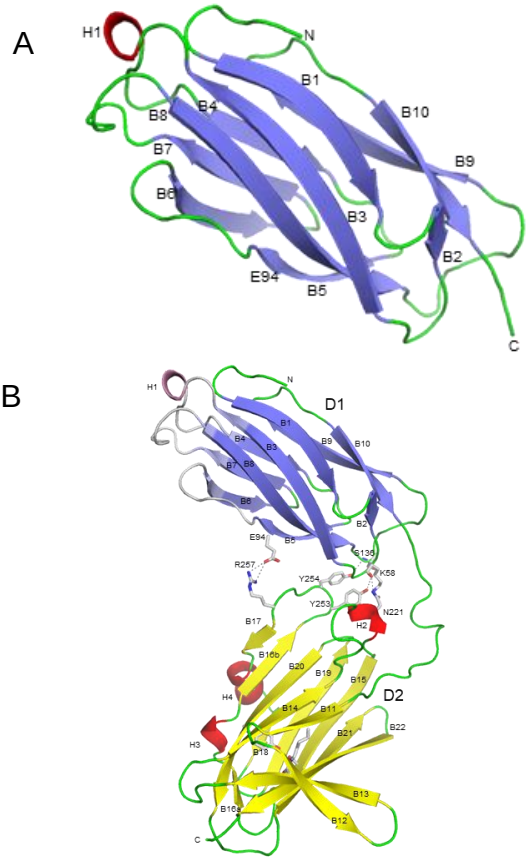

Fig. 1: Structures of CppB-D1 and CppB-D1D2. (A) Overall structure of CppB-D1 is illustrated with blue β-strands, green loops, and red α-helix. (B) Overall structure of CppB-D1D2 is illustrated. The disordered regions in D1 are shown in light gray. The amino acids involved in the interactions between domains are shown by a stick model.

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References

[1] E. Tamai *et al.*, *FEBS Letters* **597**, 1317 (2023).

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