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X-ray structure of *Clostridioides difficile* autolysin CdCwlT33800 catalytic domain

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1 Introduction

Clostridioides difficile is a major pathogen of pseudomembranous colitis, and new antimicrobial agents are needed for its treatment. Autolysins are peptidoglycan-degrading enzymes that can kill bacteria by bacteriolysis, and have potential as novel therapeutic agents for the treatment of infectious diseases. We identified an autolysin genes in the genome of *Clostridioides difficile* strain 630, *cdCwlT33800* with a lysozyme-like domain and endopeptidase domain. The entire region and each domain of the protein were expressed, purified, and assayed for bacteriolytic activity. Only the endopeptidase domain variants exhibited bacteriolytic activity against *Clostridioides difficile.* To elucidate the catalytic and substrate-recognition mechanisms of the endopeptidase domain, the X-ray structure of the CdCwlT33800 endopeptidase domain (CdCwlT33800CD2) was determined.

2 Experiment

The crystals were grown at 20°C in a droplet mixed with 1 µL of a protein solution (23.2 mg/mL) and 1 µL of a reservoir solutions (3.5 M sodium formate and 100 mM sodium acetate/HCl, pH 4.6), against 50 µL of the reservoir solution, using the sitting drop vapor diffusion method. Data collection was performed on the PF-BL5A beam line in KEK (Tsukuba, Japan) at 100 K. Diffraction data were processed using the program XDS and the CCP4 program suite. The structure was solved by molecular replacement with the program MOLREP using a model derived from AlphaFold2, and refined using the programs Refmac5 and Phenix Refine at a resolution of 1.45 Å (PDB ID: 9UBF).

3 Results and Discussion

CdCwlT33800CD2 has a spherical shape with five helices and eight β-strands (Fig. 1A). There is a large β-sheet comprising six anti-parallel β-strands (B3, B4, B5, B6, B7, and B8) at the center of the molecule. An α-bundle-like structure with three helices (H1, H2, and H3), and a small β-sheet formed by B1 and B2 are located on one side of a large β-sheet, and two helices (H4 and H5) are located on the opposite side. There is a large substrate-binding groove at the center of molecule, and a large β-sheet form the bottom of the groove. The catalytic residue Cys242 and His296 are located at the center of the groove, facing each other, and Cys242 is oxidized to cysteic acid (Fig. 1B). B1-B2 loop and B6-B7 loop form the sidewall of the substrate-binding groove, and B4-B5 loop form another sidewall. H1, H2, and H9 are embedded in the molecule to stabilize the structure of the substrate-binding groove.

Based on the X-ray structure, we have constructed a model structure of CdCwlT33800CD2 complexed with a peptidoglycan. CdCwlT33800CD2 has a substrate-binding groove across the molecule with a length of 28 Å (Fig. 1C), in which B1-B2 loop, B4-B5 loop, and B6-B7 loop extend from both sides to cover the substrate. The peptide side chains cross-linking two glycan backbones adopts an almost extended conformation, and is fitted to the substrate-binding groove. The hydrolyzing site is thought to be between γ-D-Glu and mDAP (Fig. 1C, a black arrow).

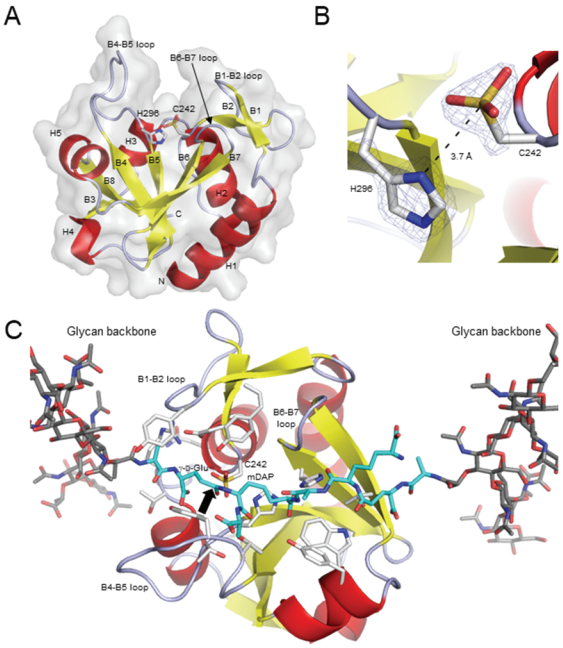

Fig. 1: Structure of CdCwlT33800CD2**.** (A) The overall structure is illustrated with the secondary structure element labels. (B) The catalytic Cys242 and His296 residues are shown with the electron density of omit map at the 3.0 σ contour level. (C) Model structure of CdCwlT33800CD2 complexed with a peptidoglycan is shown. The proposed hydrolyzing site is indicated by a black arrow.

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References

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