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# X-ray Structure of full-function catalytic domain of the novel autolysin Acd24020 from *Clostridioides difficile* as a lytic enzyme

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## 1 Introduction

Autolysin is a lytic enzyme that hydrolyzes peptidoglycans of the bacterial cell wall, with a catalytic domain and cell wall-binding domains, to be involved in different physiological functions that require bacterial cell wall remodeling. We identified a novel autolysin, Acd24020, from *Clostridioides* (*Clostridium*) *difficile* (*C. difficile*), with an endopeptidase catalytic domain belonging to the NlpC/P60 family and three bacterial Src-homology 3 domains as cell-wall binding domains. The catalytic domain of Acd24020 (Acd24020-CD) exhibited *C. difficile-specific* lytic activity equivalent to full-length Acd24020, indicating that Acd24020-CD has full-function as a lytic enzyme by itself. To elucidate the specific peptidoglycan-recognition of Acd24020-CD, X-ray structure determination and a modeling study of the enzyme/substrate complex were performed.

## 2 Experiment

Crystals of Acd24020-CD were grown at 20°C in a droplet mixed with 1 µL of protein solution (125 mg/mL in 20 mM Tris-HCl, 50 mM NaCl, pH 7.5) and 1 µL of reservoir solution (200 mM ammonium citrate dibasic, 20% (w/v) polyethylene glycol 3,350, pH 5.1). X-ray diffraction data were collected at 100 K using the PILATUS3 S 2M pixel detector system on the PF BL5A beam line in KEK (Tsukuba, Japan). Diffraction data were processed using the program XDS, and the CCP4 program suite. The structure of Acd24020-CD was solved by molecular replacement with the program MOLREP using the structure of BcYkfC (PDB code: 3H41) as a probe model. Further model building was performed with the program Coot, and the structure was refined using the programs Refmac5, to $R_{cryst}$ = 0.201 ($R_{free}$ = 0.258) using 1.56 Å resolution data.

## 3 Results and Discussion

Acd24020-CD has an hourglass-shaped substrate-binding groove with a length of 28 Å across the molecule, into which the peptide side chain of *C. difficile* peptidoglycan could be well-fitted (Fig. 1A). A characteristic feature of *C. difficile* peptidoglycan is the direct 3-4 cross-linking of peptide side chains, and it is recognized at the narrowest point of the groove in a modeling structure (Fig.1A). An hourglass-shaped substrate-binding groove may be a reason that Acd24020-CD has full-function as a lytic enzyme by itself. Based on the X-ray structure and modeling study, we propose a dynamic Cys/His catalyzing mechanism, in which the catalytic Cys299 and His354 residues dynamically change their conformations to complement each step of the enzyme catalytic reaction (Fig.1B).

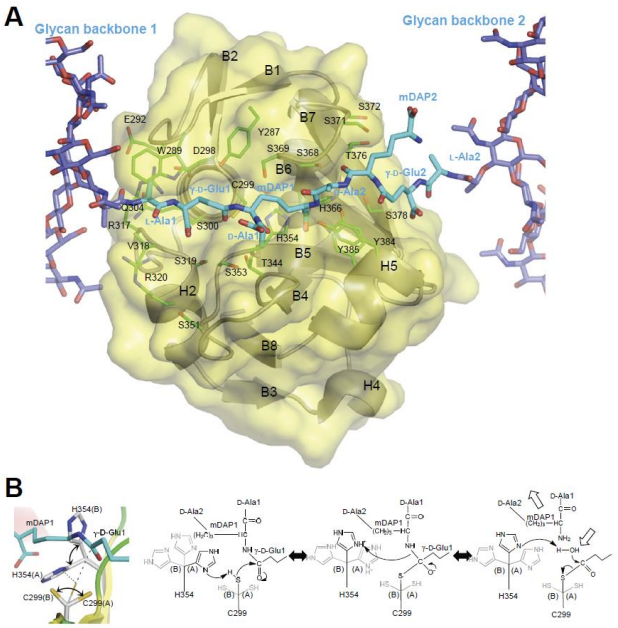


Fig.1: Modeling structure of Acd24020-CD/peptidoglycan complex and proposed catalytic reaction mechanism. (A) The hourglass-shaped substrate-binding groove of Acd24020-CD with the modeled peptide side chains cross-linking two glycan backbones is illustrated. (B) The catalytic Cys299 and His354 residues in two forms (Form-A and Form-B) are illustrated. Cys299 in the intermediate position to be activated is shown by a thin bond (left). The proposed catalytic reaction mechanism (dynamic Cys/His catalyzing mechanism) of Acd24020 is shown (right).

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References

[1] H. Sekiya *et al*., *Mol. Microbiol.* **115**, 684 (2021).

Research Achievements

1. A graphical result of this research was selected as a journal cover illustration. doi: 10.1111/mmi.14636.

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