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X-ray structures of *Clostridium perfringens* sortase B and its mutant form

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1 Introduction

The pathogenesis and infectivity of Gram-positive bacteria are mediated by many surface proteins that are covalently attached to peptidoglycans of the cell wall. The covalent attachment of these proteins is catalyzed by sortases (Srts), a family of cysteine transpeptidases, which are classified into six classes, A – F, based on their amino acid sequences and biological roles. *Clostridium perfringens*, one of the pathogenic clostridial species, has a class B sortase (CpSrtB) with 249 amino acid residues. X-ray structures of CpSrtB and its inactive mutant form with the replacement of the catalytic Cys232 with Ser (CpSrtB_C232S), were determined at 2.2 Å and 1.8 Å resolutions, respectively. [1]

2 Experiment

Crystals of CpSrtB were grown at 293 K in a droplet mixed with 1 µl of protein solution (18.4 mg/ml in 20 mM Tris-HCl, pH 7.5) and 1 µl of reservoir solution (200 mM sodium nitrate, 20% (w/v) PEG3350, pH6.8) with 50 µl of the reservoir solution, using the sitting drop vapor diffusion method. Crystals of CpSrtB_C232S were prepared by using protein solution (30.2 mg/ml in 20 mM Tris-HCl, pH 7.5) and reservoir solution (200 mM ammonium phosphate monobasic, 20% (w/v) PEG3350, pH4.6). X-ray diffraction data for CpSrtB and CpSrtB_C232S were collected at 100 K using an ADSC QUANTUM 315R area detector system on the PF BL-5A beam line in the KEK (Tsukuba, Japan).

3 Results and Discussion

CpSrtB was crystallized in space group $P2_12_12$, and the structure was refined to R-factor of 0.227 at 2.2 Å resolution. Crystals of CpSrtB_C232S were obtained in space group $C2$, giving a higher resolution of data, and the structure was refined to R-factor of 0.184 at 1.8 Å resolution. The overall structure of CpSrtB_C232S is shown in Fig. 1 with a topological diagram of secondary structure elements. CpSrtB_C232S adopts a typical sortase-protein fold with eight β-strands and seven helices. There is a distorted β-barrel at the center of the protein. The two α-helices (H1 and H2) form the α-bundle at the N-terminal site. The connected loop between H1 and H2 in CpSrtB_C232S is invisible due to the highly disordered structure. A long loop including a short 3_{10} helix (H3) is inserted between B2 and B3. The 3_{10} helix (H4) and α-helix (H5) between B4 and B5 are across the β-barrel. The two α-helices (H6 and H7) between B6 and B7 are arranged in an anti-parallel manner, contacting the β-barrel. The conserved catalytic residues His136, Ser232 (Cys) and Arg240 are located at the loop between B4 and H4, the end of B7, and the beginning of B8, respectively. A groove for substrate-binding is formed by three β-strands (B4, B7 and B8) and two helices (H3 and H6) creating the sidewalls of the groove.

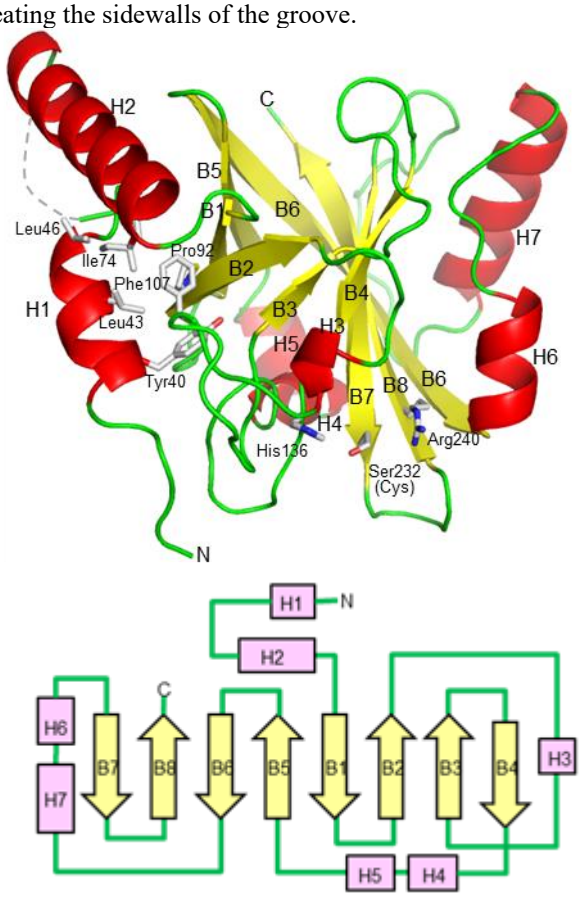

Fig. 1: The overall structures of CpSrtB_C232S and the topological diagram of the secondary elements. Three catalytic residues and hydrophobic residues involved in the formation of the hydrophobic cluster with H1 and H2 are shown with a stick model.

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Reference

[1] E. Tamai *et al.*, *Biochem Biophys Res Commun.* 493, 1267 (2017). doi: 10.1016/j.bbrc.2017.09.144.

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