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X-ray structures of *Clostridium perfringens* sortase C with C-terminal cell wall sorting motif of LPST

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1 Introduction

The pathogenesis and infectivity of Gram-positive bacteria are mediated by many surface proteins covalently attached to the bacterial cell wall that surrounds their cytoplasmic membranes. The covalent attachment of surface proteins is catalyzed by sortases (Srts), a family of cysteine transpeptidases, which have attracted attention as potential therapeutic targets. The pathogenic bacterium *Clostridium perfringens* sortase C (CpSrtC) catalyzes the polymerization and attachment of pilin proteins to form pili, flexible rod proteins associated with the bacterial cell surface, that play important roles in the initial adhesion of bacterial cells to host tissues. CpSrtC recognizes the cell wall sorting motif of LPSTG of pilin proteins for the formation of pili. To identify the structure of CpSrtC with the bound LPSTG, a mutant form of CpSrtC with a sequence of LPST at the C-terminal site (CpSrtC-LPST) was prepared, and its X-ray structure was determined.

2 Experiment

CpSrtC-LPST was crystallized in two forms: trigonal ($P3_221$) and monoclinic ($C2$) forms. Data collections were performed on the PF-BL5A beam line in KEK (Tsukuba, Japan), and using a Rigaku R-AXIS VII imaging plate system on a Rigaku RA-Micro7HF rotating anode. The data were processed using XDS and the CCP4 program suite. The initial phase was determined by the molecular replacement method using the program MOLREP, with the previously determined structure of CpSrtC (PDB code: 6IXZ) as a search model. Model building was performed with the program Coot, and the structures were refined using the programs Refmac5, to an R-factor of 0.246 at a 2.38 Å resolution ($P3_221$ form), and an R-factor of 0.158 at a 1.68 Å resolution ($C2$ form).

3 Results and Discussion

In the $P3_221$ form, a molecule is in an asymmetric unit, designated as Mol-A, and two molecules (Mol-A and Mol-A') related by a crystallographic 2-fold symmetry form a novel intermolecular substrate-enzyme complex, in which the attached C-terminal loop of AGFSLPST (CWSS loop) bind to each other (Fig. 1). In the $C2$ form, there are two molecules in an asymmetric unit related by non-crystallographic 2-fold symmetry, and the CWSS loops of both molecules were invisible in the electron density map, which are disordered in crystals.

CpSrtC-LPST adopts a typical sortase-protein fold with eight β-strands and six helices (Fig.2). There is a distorted β-barrel at the center of the molecule, which is surrounded by helices and loops. The conserved catalytic residues of His149, Cys211, and Arg220 form the catalytic triad, and the substrate-binding groove is formed along B4, B7, and B8. The CWSS loop of another molecule binds to the groove formed by the H1, H2, H2-B1 loop, and H4. Since the CWSS loop is far from the catalytic triad (21 Å), this site is expected to act as a subsite for substrate-binding. The subsite likely plays a role in recruiting the CWSS of pilin proteins, so that they can easily approach the catalytic site of CpSrtC for polymerization.

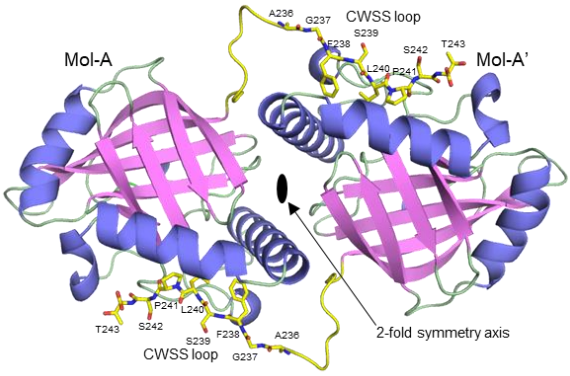

Fig. 1: Intermolecular substrate-enzyme complex by two molecules of CpSrtC-LPST in the $P3_221$ form.

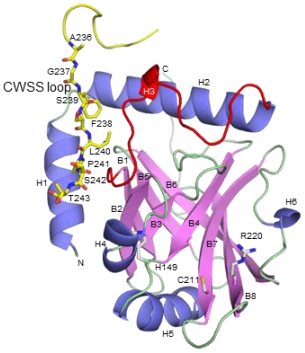

Fig. 2: The overall structure of CpSrtC-LPST in the $P3_221$ form. Three catalytic residues are shown with a stick model.

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References

[1] E. Tamai, H. Sekiya, H. Nariya, S. Katayama and S. Kamitori *Biochem. Biophys. Res. Commun.* **554**, 138 (2021).

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