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Structure Study of a Novel Type of Chitinase from Archaea

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1 Introduction

Chitinase D (designated as *Pc*-ChiD) was found in a hyperthermophilic archaeon *Pyrococcus chitonophagus* (previously described as *Thermococcus chitonophagus*), isolated from media containing only chitin as carbon source [1]. *Pc*-ChiD displays chitinase activity and is thermostable at temperatures up to 95°C, raising its potential for industrial use. *Pc*-ChiD has a secretion signal peptide and two chitin-binding domains (ChBDs) in the N-terminal domain. However, the C-terminal domain shares no sequence similarity with previously known saccharide-degrading enzymes, and does not contain the DXDXE motif conserved in the glycoside hydrolase (GH) 18-family chitinases. To elucidate its overall structure and reaction mechanism, we tried to determine the first crystal structures of *Pc*-ChiD, both in the ligand-free form and in complexes with substrates.

2 Experimental

We used *Pc*-ChiD(ΔBD), a truncated form of *Pc*-ChiD which consists only of the C-terminal putative chitinase catalytic domain based on primary structure and was previously shown to exhibit chitinase activity. The target proteins were overexpressed with *Escherichia coli*. They were purified with a heat treatment, an anion exchange column, a hydrophobic interaction column, and a gel filtration column. Crystals of the ligand-free form were obtained using a reservoir solution containing $(NH_4)_2SO_4$. Crystals of the substrate-complex were obtained by the co-crystallization method using a reservoir solution containing $NH_4H_2PO_4$. X-ray diffraction data were collected at the BL-1A and AR-NW12A beamlines at the Photon Factory, and were processed with the HKL2000 package. The structures of *Pc*-ChiD(ΔBD) were determined by the single isomorphous replacement method, using the platinum derivative.

3 Results and Discussion

We determined three types of *Pc*-ChiD crystal structures, one ligand-free form and two substrate-complex forms, respectively. Structure analyses revealed that Pc-ChiD(ΔBD) consists of a third ChBD (ChBD3), which cannot be predicted from the amino acid sequence, and a catalytic domain similar to those found in not the GH18 family but the GH23 family (Fig. 1). Based on the similarity with GH23-family chitinase, the catalytic residues of *Pc*-ChiD were proposed and confirmed by mutagenesis analyses.

Sequence alignment in this family based on Dali server results indicated that *Pc*-ChiD has a unique C-terminal extension. The C-terminal helix is positioned between ChBD3 and the catalytic domain and fixes ChBD3 next to the catalytic domain. Moreover, the *Pc*-ChiD structures in complexes with ligands revealed that the two ligands are bound in a straight line, and two aromatic side chains Trp391 (in ChBD3) and Tyr798 (in the C-terminal extension) are located at an extension of this line (Fig. 2). These residues may contribute to the binding with the long chitin chain. These findings indicate that the C-terminal extension functions not only for structure rigidity of the protein, but also for chitin-chain binding. Our findings reveal the structure of a unique archaeal chitinase that is distinct from previously known members of the GH23 family.

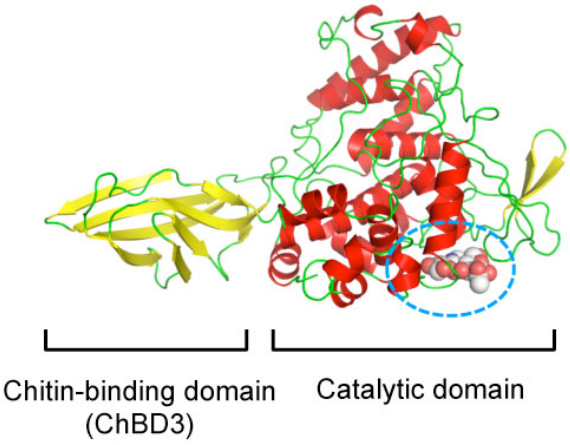

Fig. 1: Overall structure of Pc-ChiD(ΔBD).

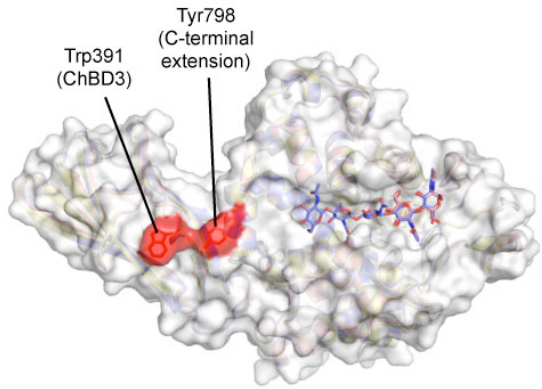

Fig. 2: Proposed chitin-chain binding site.

References

[1] A. Horiuchi *et al.*, *Appl. Environ. Microbiol.* **82**, 3554 (2016).

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