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Crystal Structure of ChiL, a Chitinase from *Chitiniphilus shinanonensis*

Miruku Ueda, Makoto Shimosaka, and Ryoichi Arai*
Fac. of Tex. Sci. Tech., Shinshu Univ., Ueda, Nagano 386-8567, Japan

1 Introduction

Chitin, a linear polysaccharide consisting of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc), is widely distributed in nature, such as in the exoskeletons of crustaceans and insects, and in the cell walls of fungi. Chitin and its deacetylated derivative, chitosan, have attracted considerable interest because of their biological properties, and are widely used in various fields including the health care, food, agriculture, chemical, and environmental engineering industries. GlcNAc oligomers prepared from chitin have useful biological activities, such as immunostimulation and induction of plant defense responses. Additionally, GlcNAc can be utilized as a sweetener and nutritional supplement. Chemical hydrolytic reactions involving strong acids have been used for production of GlcNAc and its oligomers on an industrial scale, but costs associated with treatment of waste products to prevent environmental pollution are expensive. Hence, microbial chitinolytic enzymes have been investigated extensively for their potential use in the eco-friendly enzymatic production of GlcNAc and its oligomers.

Chitiniphilus shinanonensis SAY3^T is a strong chitinolytic bacterium, originally isolated from the moat water in Ueda, Japan [1]. The chitinolytic enzymes from this strain are potentially useful for efficient production of GlcNAc and its oligomers from native chitin. ChiL is a chitinase from *C. shinanonensis* SAY3^T [2], which has a weak transglycosylation activity. To reveal the enzymatic reaction mechanism and utilize it for further enzyme engineering, we have solved the crystal structure of ChiL.

2 Experiments

The catalytic domain (41-406) of ChiL was expressed with His_6 tag in *Escherichia coli*, and purified by the following steps: immobilized-metal affinity chromatography, cleavage of His_6 tag by TEV protease, anion exchange chromatography, and gel filtration chromatography.

ChiL was crystallized at 20ºC using the sitting drop or hanging drop vapor diffusion method. ChiL was mixed with the same volume of reservoir solution (0.2 M ammonium sulfate, 0.1M Tris pH 8.0, 25% PEG3350). X-ray diffraction data collections were performed at KEK Photon Factory AR NW12A at 95 K with reservoir solution added to 25% PEG 400 as a cryoprotectant. The structure was solved by molecular replacement method using Phaser with model structures of chitinase A1 from *Bacillus circulans* WL-12 (PDB: 1ITX) [3]. The crystal structure was refined using COOT and REFMAC5.

3 Results and Discussion

The ChiL crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit cell constants of a = 69.19 Å, b = 81.55 Å, c = 130.01 Å, and contains two protein molecules per asymmetric unit. The structure was refined to high resolution 1.25 Å (R_{work} = 14.4%, R_{free} = 18.4%). The crystal structure shows that the catalytic domain of ChiL comprises an monomeric $(\alpha/\beta)_8$-TIM-barrel and a small β-domain. The TIM-barrel structure has a deep cleft for binding the substrate, chitin.

In the active site pockets of ChiL, the diagnostic DxDxE motif residues (155-159) are highly conserved in family 18 chitinases. The catalytic residue, Glu204 of the chitinase A1, correspond to Glu159 of ChiL, suggesting their common mechanism of enzymatic reaction.

In the present study, the high resolution structure of ChiL provides useful structural information. To understand the catalytic mechanism of ChiL in detail, further study is currently in progress.

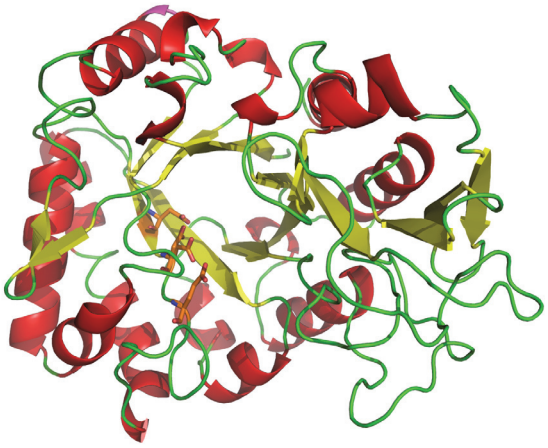

Fig. 1: The crystal structure of *C. shinanonensis* ChiL.

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*rarai@shinshu-u.ac.jp