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Structural Analysis of α-Xylosidase from *Escherichia Coli*

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Introduction

α-Xylosidase activity was detected from the product of *yicI* gene in *Escherichia coli*. The α-xylosidase has the ability to catalyze the release of α-xylose from the non-reducing terminal side of α-xyloside.

Based on sequence similarity, YicI has been assigned to glycoside hydrolase (GH) family 31. The enzymes classified into GH-31 are family II of α-glucosidase, glucoamylase, sucrase-isomaltase, α-xylosidase, α-glucan lyase, and isomaltosyltransferase. Three-dimensional structure of YicI was determined, recently. This structural information and a previous study of *Schizosaccharomyces pombe* α-glucosidase belonging to the same family indicated that two acidic residues corresponding to Asp416 and Asp482 of YicI are candidates as catalytic residues. In order to reveal the details of the substrate recognition, we tried to determine the structure of YicI substrate complex.

Experiments and Results

YicI mutant D482A was expressed and purified. The crystals of D482A mutant enzyme were grown in 100mM MES buffer (pH5.6) containing 10% (v/v) PEG20000, 2% (w/v) glycerol, 2% (v/v) isopropanol, and 10mM isoprimeverose (6-*O*-α-xylopyranosyl-glucopyranose) by hanging-drop vapour diffusion method. The dataset to a resolution of 2.5Å was collected at 100K the beamline BL-6A, under cryogenic condition. The data was processed using HKL2000 suite. The data processing statistics are given in Table 1. The crystals belonged to the space group $P2_12_12_1$ with unit cell parameters a = 160.5Å, b = 174.6Å and c = 209.9Å. Refinement and calculation of electron density maps were performed by CNS using a wild type coordinate (1WE5) as an initial model. However, the electron density corresponding to the substrate was not observed.

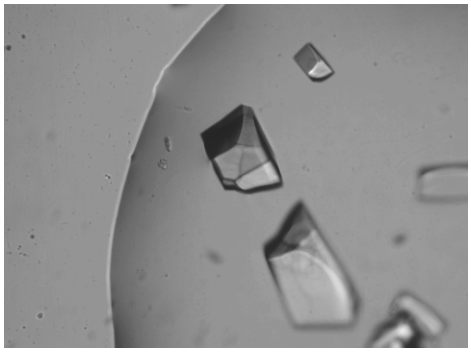

Fig 1. The crystals of YicI mutant

Table 1: Diffraction data statistics

Dataset	SusB native
Beamline	BL6A
Wavelength (Å)	0.9779
Space group	$P2_12_12_1$
Unit cell parameters	a = 160.5 Å, b = 174.6 Å, c = 209.9 Å
Resolution (Å)	50-2.50 (2.59-2.50)
Observed reflections	1,449,778
Unique reflections	202,629
Completeness (%)	99.7 (100.0)
Redundancy	7.2 (6.8)
$I/\sigma(I)$	34.3 (6.05)
R_{merge}	0.084 (0.430)

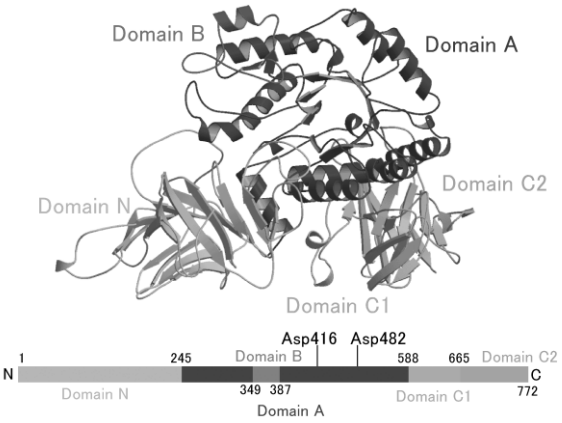

Fig 2. Structure of YicI

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