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Crystal structure of *Aspergillus niger* ATCC9642 isopullulanase

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Introduction

Enzymes hydrolyze pullulan are useful in the production of various oligosaccharides. Isopullulanase (IPU) from *Aspergillus niger* ATCC9642 was originally reported to be an enzyme which hydrolyzes specific sites of pullulan to produce isopanose (Glc-α-(1→4)-Glc-α-(1→6)-Glc). Although IPU does not hydrolyze starch, the enzyme hydrolyzes substrates containing panose (Glc-α-(1→6)-Glc-α-(1→4)-Glc) motif, which is also found in the structure of starch. Due to the unique properties, IPU has been assigned its own EC number (EC 3.2.1.57).

Except for IPU, pullulan-hydrolyzing enzymes, such as pullulanases, *Thermoactinomyces vulgaris* α-amylases, and neopullulanases, are classified into glycosyl hydrolase family (GH) 13, known as the α-amylase family. In contrast, IPU is the sole enzyme which is classified into GH 49 among these pullulan-hydrolases, and no homology between IPU and α-amylase family enzymes has been found. Interestingly, IPU does not hydrolyze dextran at all, while all other GH 49 enzymes are dextran-hydrolyzing enzymes, such as endo-dextranase and isomaltotrio-dextranase. In this report, we describe the crystal structure of IPU.

Materials and Methods

Since the active IPU was not obtained from recombinant *E. coli* cells, *Pichia pastoris* was used for the heterologous expression of IPU [1]. The purification of IPU was performed using a hydrophobic column. IPU produced in *P. pastoris* was highly glycosylated. In many cases, glycosylation is an obstacle of crystallization, thus the purified IPU was deglycosylated using endoglycosidase Hf, and further purified using an ion-exchange column [2]. Crystals of IPU were grown at 20 °C using the hanging drop vapor-diffusion method, with a well solution of 20 % (w/v) PEG 8000 in 100 mM sodium acetate buffer (pH 4.6) and a protein solution of 14 mg/ml. The diffraction data was collected under cryogenic condition, and the structure was solved by molecular replacement using *Penicillium minioluteum* dextranase (1OGM) as the search model.

Results and Discussion

The asymmetric unit contains two molecules of IPU. The structure of IPU is composed of N-terminal and C-terminal parts, which are designated domains N and C (Fig. 1). Domain N is composed of a β-sandwich. Domain C has a right-handed parallel β-helical fold. Three β-sheets form a coil, and the β-helix contains 10 coils. The amino acid sequence of IPU has 15 potential glycosylation sites, and the electron densities of *N*-acetylglucosamine residues were seen for the 11 sites. The overall structures of IPU and *P. minioluteum* dextranase resemble each other, while the shapes of their catalytic clefts are different.

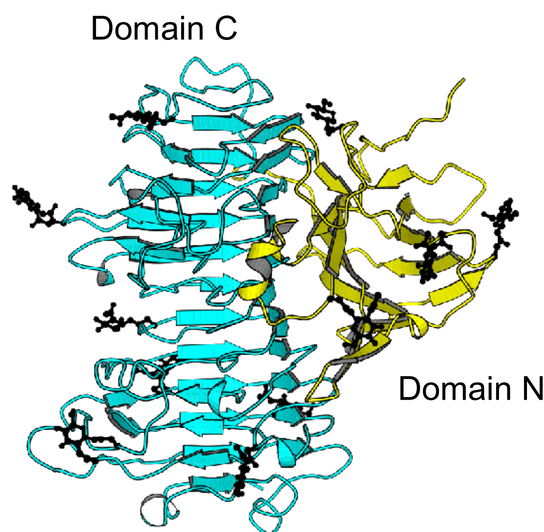

Fig. 1. Structure of IPU. *N*-acetylglucosamine residues are indicated.

References

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[2] H. Akeboshi et al., Eur. J. Biochem. 271, 4420 (2004).

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