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Crystal structure of *Bacillus circulans* T-3040 Cycloisomaltooligosaccharide Glucanotransferase

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1 Introduction

Cycloisomaltooligosaccharides are cyclic oligomers of glucose molecules linked by α-1,6-glucosidic bonds, and exhibit an ability to form inclusion-complexes with various hydrophobic molecules and anti-plaque activity. Since they are highly water-soluble and have a central hydrophobic cavity, they appear to be novel bionanomaterials with wide range of applications in various bioindustries. However, the function and characteristics of cycloisomaltooligosaccharides (CIs) have not been fully elucidated. We determined the three-dimensional structures of cycloisomaltooligosaccharide glucanotransferase from *Bacillus circulans* T-3040 (BcCITase) involved in the production of CI and analyzed its catalytic mechanism in order to increase the CI productivity.

2 Experiment

BcCITase was expressed in *Escherichia coli* and purified to homogeneity. BcCITase was crystallized by the sitting-drop vapor diffusion method at 293 K and plate-shaped crystals appeared within two weeks [1]. X-ray diffraction experiments for crystals were conducted at the Photon Factory and the Photon Factory Advanced Ring (PF-AR), High Energy Accelerator Research Organization, Tsukuba, Japan. For the sugar ligand complex structural analyses, the catalytic inactive mutant was used and sugars were soaked into the crystals before the X-ray experiments. The structure was determined by the multiple-wavelength anomalous dispersion method using the selenomethionine-derivative crystal data [2].

3 Results and Discussion

The core structure of BcCITase that produce cycloisomaltooligosaccharides from dextran consisted of catalytic domain comprising of $(\beta/\alpha)_8$-barrel and three β-domains in the N-and C-terminal ends and the middle part of the catalytic domain (Fig. 1). The core structure was similar to that of *Streptococcus mutans* dextranase (SmDEX), in which the middle β-domain was not conserved [3]. The catalytic domain contained the catalytic groove where the soaked isomaltooligosaccharides were observed. In addition to the catalytic site, the sugar ligands were observed in the middle β-domain. This domain was turned out to be the carbohydrate-binding domain that belonged to the family 35 (CBM35). The crystal structure of BcCITase mutant in complex with isomaltooctasaccharide (IG8) showed that IG8 bound in the catalytic domain and extended to the sugar-binding site of CBM35, indicating that CBM35 plays an important role in determining the product specificity.

Another isomaltooctasaccharide was observed in the different side of CBM35 domain. This indicates that CBM35 plays the canonical sugar-binding ability in promoting the catalytic activity.

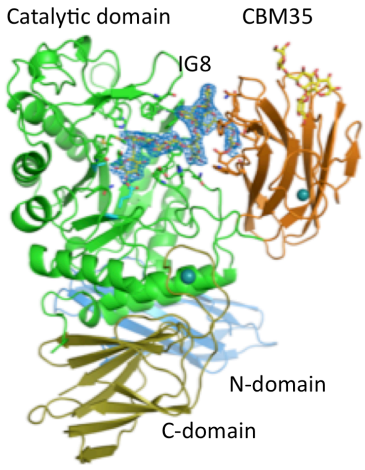

Fig. 1: Crystal structure of BcCITase in complex with IG8. Two IG8 molecules in yellow sticks. The IG8 in the catalytic groove is shown with its electron density map.

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References

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