Structural analyses of isomaltooligosaccharide-producing enzymes

Zui Fujimoto and Mitsuru Momma

Biomolecular Research Unit

The four enzymes that totally produce isomaltooligosaccharides from starch were structurally characterized using X-ray crystallography. The three-dimensional structures revealed the catalytic mechanisms of the enzymes. We also succeeded in increasing the production of isomaltooligosaccharides with more than nine degrees of polymerization of glucoses.

Keywords: isomaltomegalosaccharide, starch, X-ray crystallography, catalytic mechanism

Background

Isomaltooligosaccharides are 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 hydrophobic surface, they appear to be novel bionanomaterials with wide range of applications in various bioindustries. However, the function and characteristics of isomaltomegalosaccharides with high degrees of polymerization (≥10) have not been fully elucidated. We determined the three-dimensional structures of enzymes involved in the production of isomaltooligosaccharide and analyzed their catalytic mechanisms in order to increase the isomaltooligosaccharide productivity.

Results and Discussion

1. The three-dimensional structure of the four enzymes, namely, (1) synthase that hydrolyzes the α-1,4-glucosidic bond of starch and produces dextran-type saccharides containing α-1,6-glucosidic bond, (2) cyclase that produces cycloisomaltooligosaccharides from dextran, (3) hydrolase that degrades dextran into isomaltooligosaccharides, and (4) transferase that transfers glucose moiety of isomaltooligosaccharides to produce α-1,6-linked isomaltomegalosaccharides, were determined by X-ray crystallography and their catalytic mechanisms were analyzed (Fig. 1).
2. Dextranase from *Streptococcus mutans* (SmDEX) consisted of catalytic domain comprising of $(\beta/\alpha)_8$-barrel and two β-domains in both the N-and C-terminal end of the catalytic domain. Complex structure analyses with isomaltotriose and inhibitor revealed its catalytic substrate-binding mechanisms and the structural factors important for thermostability.
3. The core structure of *Bacillus circulans* T-3040 cycloisomaltooligosaccharide glucanotransferase (BcCITase) that produce cycloisomaltooligosaccharides from dextran was similar to that of SmDEX. In addition to the core structure, the inserted loop in the catalytic domain also formed an extra β-domain (CBM35). The crystal structure of the catalytically inactive mutant of BcCITase 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.
4. Mutant BcCITase altered in CBM35 resulted in reduction of the main product cycloisomaltooctasaccharide (CI8) and enhancement of the production of cycloisomaltooligosaccharides 2.6 times (Fig. 2).

Future prospects

1. Crystal structures and catalytic mechanisms enable the molecular engineering of four enzymes that might lead to efficient production and wide application of isomaltooligosaccharides.

2. Molecular engineering of BcCITase facilitates to the control of the enzyme's product specificity, since CBM35 is involved in determination of product size.

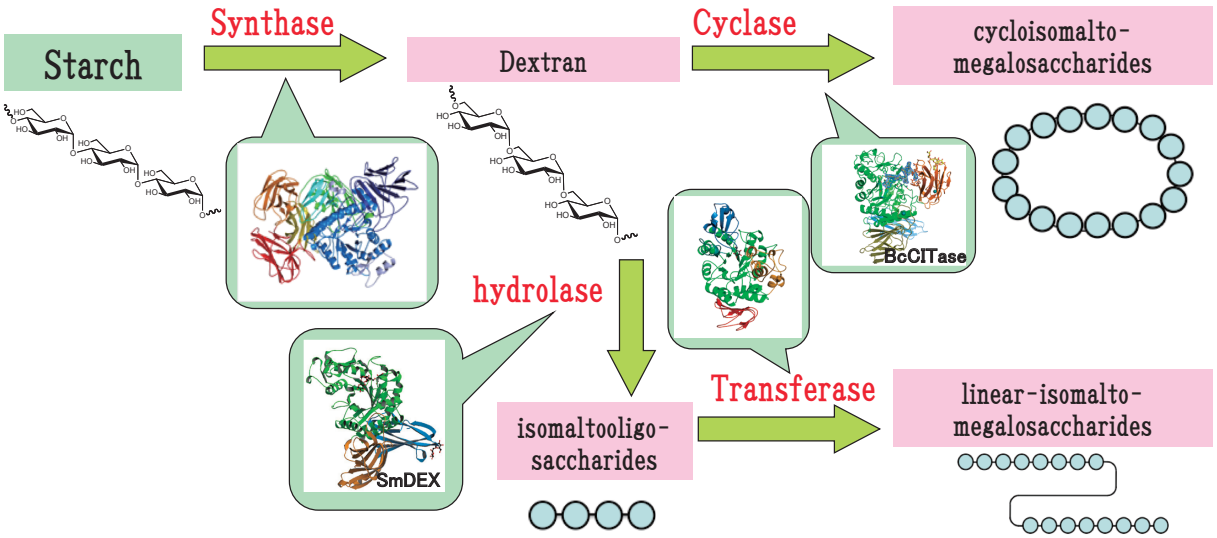

Fig. 1. Production scheme of isomaltomegalosaccharides from starch. The crystal structures of the four enzymes used for isomaltomegalosaccharides are shown. The catalytic mechanism of each enzyme was elucidated from the enzyme–sugar complex structure.

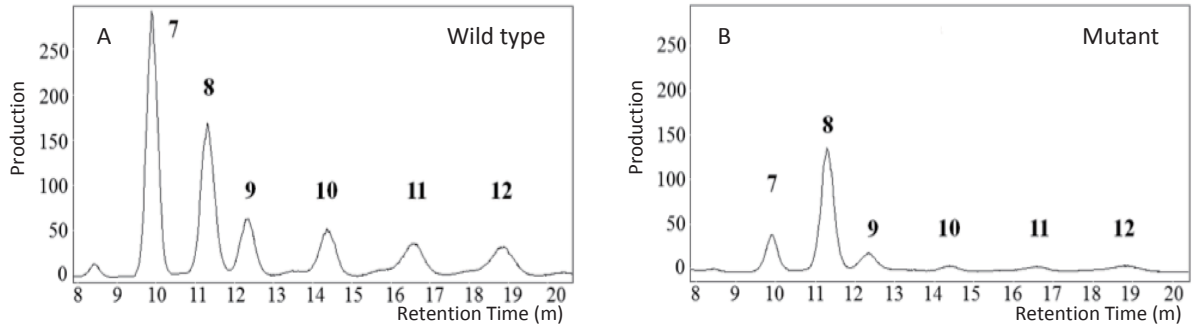

Fig. 2. Isomaltomegalosaccharide production. Numbers indicate the degrees of polymerization of glucose in the produced cycloisomaltooligosaccharides. (A) Wild-type enzyme, (B) Mutant enzyme

Collaborators

Funane Kazumi (National Food Research Institute), Atsuo Kimura (Hokkaido University)

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