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Crystallographic analysis of the sugar complexes of maltohexaose-producing amylase from alkalophilic *Bacillus* sp.707

Ryuta KANAI[1,2], Toshihiko AKIBA[2], Keiko HAGA[1], Kunio YAMANE[1], and Kazuaki HARATA[*,2]
[1]Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.
[2]Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan.

Introduction

G6-amylase (E.C.3.2.1.98) from alkalophilic *Bacillus* sp.707, which belongs to the glycoside hydrolase family 13, predominantly produces maltohexaose (G6) from starch and related α-1, 4-glucans. Previously we have found that Trp140 is a key residue for the G6 production. To elucidate the role of Trp140, crystal structures of the G6-amylase complexes with G5 and G6 were determined and compared with the structure of the previously reported pseudo-maltononaose complex [1, 2].

Results and Discussion

Preparation and crystallization

G6-amylase from alkalophilic *Bacillus* sp.707 was expressed by *Bacillus subtilis* 207-25. The enzyme was crystallized by the hanging drop vapor diffusion method using the reservoir solution containing 50% (v/v) 2-methylpentane-2, 4-diol, 100 mM Tris-HCl (pH 8.5) and 200 mM ammonium phosphate. Calcium chloride and sodium chloride were added to final 1 mM in the drop solution. Rod-like crystals were grown within 4 days at room temperature. The crystals of the complexes with G5 and G6 were prepared by soaking for 30 minutes in a solution containing 40 mM G5 or 75 mM G6.

Measurement of X-ray diffraction data

X-ray diffraction measurements for the crystals of the G6-amylase complexes with G5 and G6 were carried out at the BL-18B and AR-NW12 station, respectively. Both the crystals belong to the space group $P2_12_12_1$ and the unit cell dimensions are a = 47.5 Å, b = 82.5 Å, and c = 126.9 Å for the G5 complex and a = 47.6 Å, b = 82.7 Å, and c = 127.0 Å for the G6 complex. Respective intensity data were collected at 100 K to the resolution of 2.06 Å and 1.94 Å, and 31,896 and 40,560 unique reflections were obtained with the completeness of 97.8% and 98.7%, and R_{merge} of 16.8% and 4.8% for the complexes with G5 and G6, respectively.

Structure determination and refinement

The crystal structure of the G6-amyalse complex with G5 was determined by molecular replacement and refined at 2.3 Å resolution. The final R and R_{free} values were 116.8% and 21.3%, respectively. The structure of the G6 complex was determined by the same procedure using the native structure and refined at 1.9 Å resolution. The final R and R_{free} values were 15.5% and 18.4%, respectively. The structures of G5 and G6 were constructed based on 3*F*o-2*F*c and *F*o-*F*c electron density. In addition, several short sugar molecules were modeled on the molecular surface in the both crystals. Atomic coordinates have been deposited with Protein Data Bank (G5 complex: 2D3L, G6 complex: 2D3N).

Description of the structure and discussion

Our previous crystallographic study showed that G6-amylase has nine subsites, from –6 to +3, and pointed out the importance of the indole moiety of Trp140 in G6 production. G6-amylase has very low levels of hydrolytic activities for oligosaccharides shorter than maltoheptaose. In the active site of the G6-amylase/G5 complex, G5 is bound to subsites –6 to –2 while G1 and G6 are found at subsites +2 and –7 to –2, respectively, in the G6-amylase/G6 complex. This indicates that such a binding mode prevents G6 from being hydrolyzed to shorter oligosaccharides. In both structures, the glucosyl residue located at subsite –6 is stacked to the indole moiety of Trp140 within a distance of 4 Å. The sugar molecules are also stabilized by hydrogen bonds with several residues in the active site cleft. In addition to the sugar molecules bound to the active site, several sugar molecules are bound on the molecular surface; two G1 and one G2 in the crystal of G5 complex, and two G2, one G3, and one G4 in the crystal of G6 complex. The measurement of the activities of the mutant enzymes when Trp140 was replaced by leucine (W140L) or by tyrosine (W140Y) showed that the G6 production from short-chain amylose by W140L is lower than that by W140Y or wild type enzyme. The face-to-face short contact between Trp140 and substrate sugars is suggested to regulate the disposition of the glucosyl residue at subsite –6 and to govern product specificity for G6 production.

References

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* k-harata@aist.go.jp