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

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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. To elucidate the reaction mechanism of G6-amylase, crystal structures were determined for the native enzyme and its complex with pseudo-maltononaose [1].

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. Crystals of the complex with pseudo-maltononaose were obtained by soaking the crystal for 3 days in the crystallization solution containing 10 mM acarbose and 10 mM maltotriose.

Measurement of X-ray diffraction data

X-ray diffraction measurements for the crystals of native G6-amylase and its pseudo-maltononaose complex were carried out at the BL-6A and AR-NW12 station, respectively. Both the crystals belong to the space group $P2_12_12_1$ and the unit cell dimensions are a = 47.6 Å, b = 82.8 Å, and c = 127.2 Å for the native crystal and a = 47.4 Å, b = 82.5 Å, and c = 126.9 Å for the complex. Intensity data were collected at 100K to the resolution of 1.94 Å, and 32,772 and 39,475 unique reflections were obtained with the completeness of 98.7% and 98.8%, and R_{merge} of 4.8% and 5.1% for the native and sugar complex crystals, respectively.

Structure determination and refinement

The crystal structure of native G6-amyalse was determined by molecular replacement and refined at 2.1 Å resolution. The final R and R_{free} values were 16.6% and 21.0%, respectively. The structure of the pseudo-maltononaose complex was determined by the same procedure using the native structure and refined at 1.9 Å resolution. On the electron density maps, the sugar molecule was initially constructed as an α-1, 4-linked chain of nine 6-deoxy-D-glucoses, and then the structure was corrected to Acv-Glc-Glc-Glc-Acv-Glc-Glc (Acv, acarviosine, disaccharide analogue unit; Glc, glucose). The final R and R_{free} values were 17.2% and 20.7%, respectively. Atomic coordinates have been deposited with Protein Data Bank (native structure: 1WP6, complex structure: 1WPC).

Description of the structure and discussion

G6-Amylase consists of three domains A, B and C. Domain A (5 –105, 208 – 396) forms a $(\beta/\alpha)_8$ barrel like the other enzymes of α-amylase family, domain B (106-207) is attached on the domain A, and domain C consists of β-strands. The backbone structure of G6-amylse is very similar to those of some liquefying α-amylases with an average difference of less than 1.0 Å in their equivalent Cα position. G6-amylase contains the Ca^{2+}-Na^{+}-Ca^{2+} metal ion triad at the interface between domain A and domain B.

The crystal structures revealed that Asp236 is a nucleophilic catalyst and Glu266 is a proton donor/acceptor. The enzyme has three and six subsites at reducing- and non-reducing-end side, respectively. The pseudo-maltononaose molecule was observed at the subsites –6 to +3 in the active site cleft like an enzyme-substrate complex. The structure of the pseudo-maltononaose, Acv-Glc-Glc-Glc-Acv-Glc-Glc, suggests that it is derived from acarboses and maltotrioses by intermolecular transglycosylation and hydrolysis. There are a few direct and water-mediated contacts between the sugar residues and subsites –2 and –3. The indole moiety of Trp140 is closely stacked on the cyclitol and 4-amino-6-deoxyglucose residues located within 4 Å distance at subsites –6 and –5, respectively. Such a face-to-face short contact indicating strong hydrophobic interaction may regulate the disposition of the glucose residue at subsite –6 to dominantly produce maltohexaose.

References

[1] R. Kanai et al., Biochemistry, 43, 14047 (2004).

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