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学位論文内容の要旨

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学位論文題名

Structure-function relationship of GH13_31 α-glucosidase from *Bacillus* sp. AHU2216

（*Bacillus* sp. AHU2216株由来GH13_31α-グルコシダーゼの構造と機能に関する研究)

α-Glucosidase (AGase) hydrolyzes α-glucosidic linkage at the non-reducing end of substrates, and is involved in the amylolytic pathway of various organisms. It also catalyzes transglucosylation and is utilized in industrial production of oligosaccharides. AGases are classified mainly into glycoside hydrolase families (GHs) 13 and 31 based on their amino acid sequences. GH13 enzymes share a $(\beta/\alpha)_8$-barrel-folded catalytic domain A and two additional domains, B and C. This family includes various enzymes such as α-amylase, cyclodextrin glucanotransferase, and branching enzyme, and is subdivided into subfamilies. AGases belong to subfamily 17 (GH13_17), GH13_23, GH13_30, GH13_31, and GH13_40. In this study, GH13_31 AGase with high transglucosylation activity was found in *Bacillus* sp. AHU2216, and its functions and structure were investigated.

1. Characterization and structural analysis of AGase GH13_31 from *Bacillus* sp. AHU2216

Cell-free extract of *Bacillus* sp. AHU2216 showed transglucosylation activity producing maltotriose (G3) from maltose (G2) even at a low concentration (15 mM). The AGase gene (*Bsp*AG13_31A) was cloned based on the sequence of *BMWSH_3792* from *Bacillus megaterium* WSH_002, which is phylogenetically close to *Bacillus* sp. AHU2216. *Bsp*AG13_31A is composed of 555 amino acid residues with 98% identity to BMWSH_3792. Recombinant *Bsp*AG13_31A produced in *Escherichia coli* was specific to α-(1→4)-linked maltooligosaccharides as the substrate. The highest activity was shown in G2, whereas 0.190% relative activity was detected in sucrose. K_{TG}, a substrate concentration giving 50% of transglucosylation ratio, for G2 was 21.6 mM.

Crystal structures of *Bsp*AG13_31A in apo-form (wild type) and complexes (E256Q) with G2, G3, and G4 were determined at 2.5, 1.7, 1.7, and 1.6 Å resolution, respectively. The overall structure was similar to GH13_31 AGases. But the torsion angles of glucose residues in subsites +1 and +2 were less stable than the global minimum. His203 and Asn258, located on the β→α loops 4 and 5, respectively, formed hydrogen bonds with 2-O of the D-glucosyl residue at subsite +1, and these are thought to be important for specificity to α-(1→4)-linkage. Disaccharide specificity may have arisen from steric hindrance by Asn258 in subsite +2. Trp288 on flexible β→α loop 6 occupied the substrate-binding site in the apo-form, but was placed suitable for binding substrate in the complexes. Possible conformational change of this loop along with the reaction process may be important for transglucosylation by stabilizing the glucosyl enzyme intermediate and preventing water molecules from binding and acting as substrate.

Alternation of substrate specificity and transglucosylation of *Bsp*AG13_31A through site-directed mutagenesis at Asn258

Involvement of Asn258 in substrate specificity was examined through a mutation analysis. Two mutants, N258P and N258G, retained the regioselectivity of α-(1→4)-glucosidic linkage, but showed the trisaccharide preference. k_{cat1}/K_{m1} for G2 was drastically lower (23 and 30 times in Gly and Pro variants, respectively) than in the wildtype. However, k_{cat}/K_m values for G3 were 11-27 times higher in the mutants than k_{cat1}/K_{m1} values for G2, while the wild type exhibited decreased k_{cat}/K_m (26%) for G3. N258G showed higher transglucosylation activity with a very low K_{TG} (1.13 mM) for G2. N258P acted on sucrose as a good substrate to produce erlose (Glcα1-4Glcα1-2βFru) by transglucosylation. The crystal structures of inactive enzymes, E256Q/N258P and E256Q/N258G, in complex with G3 were solved at 1.7 and 1.5 Å resolution, respectively. The steric hindrance by Asn258 was eliminated and glucose residue in subsite +2 was in a relaxed form. A hydrogen bond between Met229 and 3-O of the glucose residue in subsite +2 was predicted in E256Q/N258G. The change in subsite +2 presumably stabilized the Michaelis complex and the transition states in the action on G3.