学 位 論 文 審 査 の 要 旨

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学位論文題名

Catalytic mechanism of three α-glucosidases belonging to glycoside hydrolase family 31

(Glycoside hydrolase family 31に属する３つのα-glucosidaseの触媒機構に関する研究)

The thesis is composed of 100 pages, 30 figures, 19 tables, and 5 chapters with 1 reference dissertation.

α-Glucosidase originally catalyzes the hydrolysis of α-glucosidic linkage at the non-reducing end of substrate. The enzyme also catalyzes the formation of α-glucosidic linkage under the high substrate concentration. The latter reaction is called transglucosylation and often utilized for the production of the industrially valuable oligosaccharides, such as panose, isomaltooligosaccharides (IOSs), and nigerooligosaccharides (NOSs). α-(1→6)-Glucosyl oligosaccharides (panose and IOSs) display the prebiotic effect, and NOSs activate the immune system. Therefore, the elucidation of the relationship between structure and function of α-glucosidases contributes to the efficient production of these useful oligosaccharides and leads to developing the synthesis of novel oligosaccharides. This study mainly investigated the reaction mechanism of transglucosylation catalyzed by three microbial α-glucosidases (AgdA, AgdB, and McAG31) belonging to glycoside hydrolase family (GH) 31.

1. Structural element to regulate the transglucosylation specificity of AgdA

Aspergillus niger encodes genes of AgdA and AgdB in its chromosome. While AgdA is widely used for the industrial production of α-(1→6)-glucosyl oligosaccharides, little is known about the molecular mechanism of the transglucosylation. The study revealed that wild-type AgdA (WT) also catalyzed the formation of α-(1→4)-glucosidic

linkage as well as that of α-(1→6)-glucosidic linkage. Asn694 was selected as a candidate to regulate the transglucosylation specificity, and N694A/L/F/W was constructed. Kinetic parameters for the mutant enzymes toward a series of maltooligosaccharides (MOS) suggested that Asn694 involved in a formation of +1 and +2 subsites. The mutant enzymes except for N694A produced maltotriose from maltose more than WT at the early stage of the reaction. N694F/W accumulated larger amount of IOS than WT at the late reaction stage. The majority of final product by N694A/L was panose, which is different from that of the WT. These finding indicates that Asn694 is a structure element that regulates transglucosylation specificity of AgdA.

2. Characterization of function-unknown AgdB

The involvement of AgdB in starch utilization of *A. niger* had been suggested by the transcriptome analysis. However, its actual function remained obscure. A recombinant AgdB was produced in *Pichia pastoris*, generating two isoforms which was caused by varying degree of *N*-glycosylation at Asn354. Therefore, N354D constructed was used as parent enzyme for further study. N354D displayed higher hydrolytic activity toward α-(1→4)- and α-(1→3)-glucosidic linkages. N354D had an ability to synthesize NOS. These catalytic features are distinct from those of AgdA. In addition, AgdB has two times stronger transglucosylation ability than AgdA does.

3. Characterization of thermostable McAG31

Although the high thermostability is often favored by industrial oligosaccharide production, a research on heat-resistant GH31 α-glucosidase has not been much done. A gene encoding a GH31 enzyme was cloned from thermophilic microorganism and its recombinant protein (McAG31) was produced in *Escherichia coli*. The enzyme preferentially hydrolyzed MOS. McAG31 possessed the significant thermostable property, exhibiting the half-life of 10 h at 60°C. McAG31 catalyzed the formation of α-(1→3)- and α-(1→4)-glucosidic linkages through its transglucosylation. Approximately 80% of the total reaction proceeded as transglucosylation, when 100 mM maltose was used as substrate. These results suggest the promising possibility of McAG31 for industrial application to accomplish the high-yield production of oligosaccharide.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.