Characterization and possible application of the isomaltose-producing enzyme involved in Taka-amylase A induction in aspergilli

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Isomaltose is the most effective inducer for Taka-amylase A (TAA) synthesis among various α-linked oligosaccharides in *Aspergillus nidulans*. While TAA induction by maltose is completely inhibited by addition of castanospermine, a strong α-glucosidase inhibitor, induction by isomaltose is not affected by the inhibitor, suggesting that amylase induction by maltose requires α-glucosidases and that maltose would be converted to isomaltose by transglycosylation activity of α-glucosidases prior to amylase induction. We isolated an isomaltose-producing enzyme from *A. nidulans*, which turned out to be a novel α-glucosidase and was designated α-glucosidase B (AgdB). AgdB was a heterodimeric protein comprising 74 and 55 kDa subunits and possessed strong transglycosylation activity with preferential formation of α-1,6 glucosidic linkage.

Cloning and sequence analysis of the gene (*agdB*) encoding isomaltose-producing enzyme

Based on the chemically determined amino acid sequences, genomic and cDNA clones carrying the *agdB* gene were obtained. The *agdB* gene comprised 3,055 bp, interrupted by three short introns, and encoded a polypeptide of 955 amino acids. The deduced amino acid sequence contained the chemically determined N-terminal and internal amino acid sequences of the 74- and 55-kDa subunits. This implies that AgdB is synthesized as a single polypeptide precursor and then the precursor is processed to form the mature heterodimeric protein. The first 20 amino acids at the N-terminus of AgdB showed a typical feature of signal peptide. Taken together with the cellular distribution and glycosylation of the enzyme, AgdB appears to be an extracellular enzyme present in the cell wall.

AgdB was a member of glycosyl hydrolase (GH) family 31. It showed low but overall sequence identity of 20–30% to the GH family 31 enzymes. Phylogenetic analysis of the GH family 31 α-glucosidases revealed that AgdB was obviously distant from any other α-glucosidases, indicating that AgdB is a novel α-glucosidase that appears to have diverged from other α-glucosidases at an early stage of fungal evolution. Homologues of *agdB* were found in *A. oryzae* and *Neurospora crassa* genome databases. Enzymes similar to AgdB may be widely distributed in filamentous fungi.

Effects of the disruption of the α-glucosidase genes on amylase induction

The *agdB* disruptant (Δ*agdB*) was obtained by replacing the entire coding region with *N. crassa pyr4* gene. The effects of the *agdB* disruption on amylase induction were analyzed by using the Δ*agdB* strain carrying the TAA gene. Contrary to our expectation that the disruption of *agdB*, which is responsible for a major isomaltose-producing activity in *A. nidulans*, would impair the maltose-dependent amylase induction, the *agdB* disruptant inducibly produced significant amounts of amylase in the presence of maltose as well as isomaltose, reaching 4 to 7 times more activity than the wild type. One of the implications from these results is that AgdB is not the sole isomaltose-producing α-glucosidase in *A. nidulans*. To date, two α-glucosidase genes, *agdA* (α-glucosidase A-encoding) and *agdB*, have been cloned from *A. nidulans*. AgdA is a homologue of *A. niger* α-glucosidase that has been shown to possess a strong isomaltose-producing activity, suggesting that AgdA may also possess isomaltose-producing activity. Therefore, effects of *agdA* disruption on amylase induction were also examined. Disruption of the *agdA* gene did not affect the amylase induction at all in the wild type background. On the other hand, it significantly reduced the maltose-dependent induction in the Δ*agdB* background, although this double disruptant still inducibly produced amylase. Furthermore, isomaltose-dependent induction was slightly enhanced by the *agdA* disruption in the Δ*agdB* background. These results suggest that any α-glucosidases having isomaltose producing activity may be involved in the maltose-dependent amylase production.

In the *agdB* disruptant, consumption rate of isomaltose was greatly reduced, indicating that AgdB is involved not only in isomaltose formation but also in hydrolysis of isomaltose. Therefore, the extremely increased amylase production in the *agdB* disruptant may be caused by the decrease in isomaltose hydrolysis. This implies that prevention of isomaltose hydrolysis is more effective than enhancement of isomaltose forming activity to achieve high yield of amylase. Increase in amylase production in the Δ*agdB* strain was also observed when starch was used as an inducer. The *agdB* disruption would cause elevated amylase production under any inducing conditions, since the disruption reduce hydrolysis of isomaltose, the real physiological inducer.

Formation of variety of transglycosylation products by AgdB

AgdB has been shown to possess a strong transglycosylation activity at a rather low maltose concentration of 0.5%; approximately 50% of maltose was converted to transglycosylation products such as isomaltose and panose. Transglycosylation products from various oligosaccharides as substrates were analyzed by thin layer chromatography. AgdB produced a series of transglycosylation products from kojibiose, nigerose and isomaltose. Isomaltotriose and isomaltotetraose were the main transglycosylation products from isomaltose, indicating that AgdB preferentially forms α-1,6 glucosidic linkage. Several unidentified products were formed from kojibiose and nigerose. These would be kojibiose and nigerose derivatives having α-1,6 linked glucose unit(s) at their non-reducing ends. AgdB could also form transglycosylation products from relatively high molecular oligosaccharides such as maltotetraose and maltopentaose. Consequently, AgdB revealed strong transglycosylation activity toward variety of oligosaccharides, implying that it may be useful in production of rare oligosaccharides.