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Investigations of a Useful α-Glycosidase for the Enzymatic Synthesis of Rare Sugar Oligosaccharides

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Construction of various rare sugar oligosaccharides by glycosidase-catalyzed transglycosylation reaction may require α-glycosidases that possess unique glycon specificity. In order to obtain such α-glycosidase, we carried out two studies to: 1) investigate unknown glycon specificities of several α-glycosidases using various types of rare sugar containing glycosides as substrates, and 2) change the glycon specificities of the α-glucosidase from *Geobacillus stearothermophilus* by site-specific mutagenesis. Through the former studies, several α-glycosidases were found to possess hydrolytic activities towards specific glycon monodeoxy analogs of *p*-nitrophenyl (*p*NP) α-D-glycopyranosides. Using *Aspergillus niger* α-glucosidase that showed activity towards 2-deoxy glucoside and jack bean α-mannosidase that showed activity towards 6-deoxy mannoside (α-D-rhamnoside), the glycon 2-deoxy derivative of isomaltoside (ethyl 2-deoxy-α-D-*arabino*-hexopyranosyl-1,6-β-D-thioglucopyranoside) and α-D-rhamnodisaccharide derivative (ethyl α-D-rhamnopyranosyl-1,2-α-D-thiorhamnopyranoside) were prepared by their transglycosylation reaction in good yields. For the latter studies, fifteen mutant enzymes of *Geobacillus stearothermophilus* α-glucosidase were prepared and their hydrolytic activities towards the maltose, eight diastereomers of *p*NP α-D-aldohexopyranoside, and possible monodeoxy- and mono-*O*-methyl analogs of *p*NP α-D-gluco, -manno and -galactopyranosides were elucidated. For these mutant enzymes, there were differences

between the specificities for *p*NP α-D-glucopyranoside and those for maltose, while significant changes were not confirmed in the specificity for other *p*NP α-D-aldohexopyranosides or the partially modified analogs of *p*NP α-D-glycopyranosides.

Key words: α-glycosidase, glycon specificity, oligosaccharide synthesis, rare sugar, site-specific mutagenesis