Structure and function of dextran-related enzymes from Streptococcus mutans

(Streptococcus mutans由来デキストラン関連酵素の構造と機能に関する研究)

The thesis is composed of 121 pages, 45 figures, 16 tables, and 5 chapters with 1 reference dissertation.

Streptococcus mutans extracellularly produces dextran to cause tooth decay, so that it is of interested to study the depolymerizing enzymes from S. mutans. This bacterium generates dextranase (SmDex), which hydrolyzes α-(1→6)-glucosidic linkages of dextran to produce isomaltooligosaccharides (IGs). IGs are incorporated into the cells and hydrolyzed to glucose by dextran glucosidase (SmDG). IGs, known to have a prebiotic effect, are utilized for biochemistry and food science. The goal of this study is the production of IGs with the various degrees of polymerization (DPs) using the wild-type or engineered SmDex. SmDG can also catalyze the production of IGs by transglucosylation, necessary to analyze its mechanism. Furthermore, a function-unknown enzyme (SmTre13) having sequence similarity to oligo-1,6-glucosidases, which catalyze the hydrolysis of IGs, is encoded in the genome of S. mutans. This study also elucidated the substrate specificity of SmTre13.

1. Dextranase (SmDex)

At the initial reaction stage, SmDex generated IGs with DP 3 (IG3) and DP 4 from 0.4% dextran, followed by producing glucose and isomaltose (IG2). Reaction on 4% dextran yielded IGs with higher DP (IGh), which provides DP-regulating production of IGs. Mutations T558H, W279A/T563N and W279F/T563N increased IGh from 0.4% dextran. T558H especially produced IG with DP 5 at a high rate under this reaction conditions. Bond-cleavage frequency of the wild type and its derivatives was analyzed
using synthesized $p$-nitrophenyl $\alpha$-isomaltooligosaccharides, indicating that these mutations affected an affinity at the $-4$ subsite.

2. Dextran glucosidase (SmDG)

SmDG hydrolyzes $\alpha-(1\rightarrow 6)$-glucosidic linkage at non-reducing end of substrates, but not $\alpha-(1\rightarrow 4)$-glucosidic linkage. A previous study demonstrated that Val195, Lys275, and Glu371 were important residues for recognizing $\alpha-(1\rightarrow 6)$-linkage. Further amino acid candidate for $\alpha-(1\rightarrow 6)$-recognition is found by sequence comparison with $\alpha-(1\rightarrow 4)$-specific homologs. Therefore, the replacement of this candidate with residue of $\alpha-(1\rightarrow 6)$-specific group was conducted using V195A/K275A/E371A of SmDG as a parent enzyme, meaning the generation of quadruple mutant. Another comparison with sucrose-acting homologs also finds that one amino acid of SmDG is distinct, so that alternation of this residue was done using V195A as a parent enzyme, forming a double mutant. Both mutants decreased the activities on $\alpha-(1\rightarrow 6)$-substrates but increased those for $\alpha-(1\rightarrow 4)$-substrates. The double mutant exhibited higher affinity to sucrose than the wild type. Those alternations also reflected substrate specificity of transglucosylation: wild type displayed the transferring activity on $\alpha-(1\rightarrow 6)$-glucosidic substrate, whereas double mutant did both of $\alpha-(1\rightarrow 6)$- and $\alpha-(1\rightarrow 4)$-substrates, and quadruple mutant did only $\alpha-(1\rightarrow 4)$-substrate.

3. Trehalose-6-phosphate hydrolase (SmTre13)

Recombinant SmTre13 displayed superior and moderate specificity toward trehalose 6-phosphate and $p$-nitrophenyl $\alpha$-glucoside (PNP-Glc), respectively, and slight or no activity on other substrates containing IG2, IG3 and sucrose, thereby indicating that SmTre13 is a trehalose-6-phosphate hydrolase. To understand the recognition mechanism of the phosphate group of substrate, two basic residues were mutated. Both single mutants decreased activities on trehalose 6-phosphate but did not alter those on PNP-Glc, suggesting the phosphate group recognition by two basic residues. To elucidate $\alpha-(1\leftrightarrow 1)-\alpha$ specificity of SmTre13, the mutant enzymes were generated by replacing two neutral residues individually. Both mutants lost the activity toward trehalose 6-phosphate, while their activities displayed moderate reduction for PNP-Glc and increased for sucrose. The results suggest that the mutated residues are involved in $\alpha-(1\leftrightarrow 1)-\alpha$ specificity of SmTre13.

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