学位論文内容の要旨

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学位論文題名

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)

Streptococcus mutans extracellularly produces dextran-based glucans, one factor to cause tooth decay, so that it is of interested to study the depolymerizing enzymes from *S. mutans*. This bacterium generates dextranase (SmDex), which randomly hydrolyzes α-(1→6)-glucosidic linkages of the glucans 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 indispensable for such research fields as biochemistry and food science, but commercially available IGs has been quite difficult to be obtained in recent years. The goal of this study is the production of IGs with the various degrees of polymerization (DPs) using the wild-type SmDex and its engineered enzymes. SmDG can be also used for the production of IGs because the enzyme catalyzes not only hydrolysis but also transglucosylation, which forms α-glucosidic linkage. Thus, SmDG was analyzed to clarify the relationship between structure and function. While SmDex and SmDG, both of which cooperate to degrade dextran, are encoded in distant positions in *S. mutans* genome, an enzyme (SmTre13) similar to oligo-1,6-glucosidases, which catalyze the hydrolysis of IGs, is encoded in the genome of *S. mutans*. It is thus likely that SmTre13 is also associated with the dextran degradation together with SmDex and SmDG. However, the characteristics of this enzyme has not been elucidated yet. This study investigated the substrate specificity of SmTre13.

1. Dextranase (SmDex)

The enzymatic properties and reaction specificity of SmDex were investigated. From 4 $mg \cdot mL^{-1}$ dextran, IGs with DP 3 (IG3; isomaltotriose) and DP 4 were generated at the initial stage of the reaction, followed by production of glucose and isomaltose (IG2) at the final phase of the reaction. An increase in the dextran concentration (40 $mg \cdot mL^{-1}$) yielded IGs with higher DP. Mutations T558H, W279A/T563N and W279F/T563N increased the proportion of IGs with higher DP in the hydrolysate when 4 $mg \cdot mL^{-1}$ dextran was the substrate. T558H

especially produced IG with DP 5 at a high rate under this reaction conditions. In order to analyze bond-cleavage frequency of the wild type and its derivatives, a series of *p*-nitrophenyl α-isomaltooligosaccharides (PNP-IGs) were enzymatically synthesized. The analyses using PNP-IGs indicated that these mutations affected an affinity at the −4 subsite.

2. Dextran glucosidase (SmDG)

SmDG hydrolyzes α-(1→6)-glucosidic linkage at non-reducing end of substrates, but not α-(1→4)-glucosidic linkage. A previous study demonstrated that Val195, Lys275, and Glu371 were important residues for recognizing α-(1→6)-linkage. Further amino acid candidate for α-(1→6)-recognition is found by sequence comparison with α-(1→4)-specific homologs. Therefore, the replacement of this candidate with residue of α-(1→4)-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 α-(1→6)-substrates but increased those for α-(1→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 α-(1→6)-glucosidic substrate, whereas double mutant did both of α-(1→6)- and α-(1→4)-substrates, and quadruple mutant did only α-(1→4)-substrate.

3. Trehalose-6-phosphate hydrolase (SmTre13)

Recombinant SmTre13 displayed superior and moderate specificity toward trehalose 6-phosphate and *p*-nitrophenyl α-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 α-(1↔1)-α 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 α-(1↔1)-α specificity of SmTre13.