HOKKAIDO UNIVERSITY

Title	Structure and function of dextran-related enzymes from Streptococcus mutans [an abstract of dissertation and a summary of dissertation review]
Author(s)	Klahan, Patcharapa
Citation	北海道大学. 博士(農学) 甲第13323号
Issue Date	2018-09-25
Doc URL	http://hdl.handle.net/2115/71851
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/4.0/
Type	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Patcharapa_Klahan_review.pdf (審査の要旨)

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

学 位 論 文 審 査 の 要 旨

博士の専攻分野名称　博士（農学）　氏　名 Patcharapa Klahan

審査担当者　　主　査　　教　授　木村　淳夫
副　査　　教　授　森　　春英
副　査　　講　師　奥山　正幸
副　査　　助　教　田上　貴祥

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)

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 α-isomaltooligosaccharides, indicating 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.

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