Molecular structures and catalytic mechanisms of glucosyltransferases from *Leuconostoc mesenteroides* and dextranase from *Lipomyces starkeyi*

Hee-Kyoung KANG KOSEF - 10316

Researcher,
Faculty of Chemical Engineering, Chonnam National University

Japanese Advisor : Atsuo KIMURA
Professor, Hokkaido University

Levansucrase (EC 2.4.1.10, GH family 68) and dextransucrase (EC 2.4.1.5, GH family 70) of *Leuconostoc mesenteroides* synthesize levan and dextran from sucrose, respectively. Because of the industrial importance of levan, dextran and oligosaccharide as well as the need to produce them in large quantities, much has to be known about the expression and regulation of levansucrase and dextransucrase genes from industrially interesting producer, *L. mesenteroides*. On the other hand, dextranase (EC 3.2.1.11) catalyses the hydrolysis of the α-1,6-glucosidic linkage of dextran. Commercially available dextranase is produced by fermentation with either *Penicillium* spp. or *Chaetomium* spp. However, since these fungi also produce various antibiotics and toxic metabolites, it is difficult to obtain an FDA approval. Harmless yeast, *Lipomyces*, possesses the ability to synthesize dextranase, one of valuable candidates for industrial enzyme.

An *m1ft* (levansucrase gene) of *L. mesenteroides* B-512 FMC was found to be composed of 1,272 bp nucleotides and to code a protein of 424 amino acid residues with calculated molecular mass of 47.1 kDa. Levan formation was confirmed by NMR analysis. Recombinant M1FT (rM1FT) converted 150 mM sucrose to levan (18%), 1-kestose (17%), nystose (11%) and 1,1,1-kestopentaose (7%) with the liberation of glucose. rM1FT produced erlose as an acceptor product from sucrose and maltose. The optimum temperature and pH for levan formation were 37℃ and pH 6.0, respectively. Enzyme activity was completely abolished by 1 mM Hg^{2+} or Ag^{2+}. The K_m and k_{cat} values were calculated to be 31.0 mM and 81.5 s^{-1}, respectively. Three Asp and one Glu are candidates of catalytic amino acids, since these residues are completely conserved in Glycoside Hydrolase (GH) family 68 enzymes. Replacement of D53, D210 or E294 completely lost the enzyme activity, indicating that theses three residues were essential for catalytic reaction.

A *dsrB742CK* (dextransucrase gene) was obtained from the ultrasoft X-ray-mutated *L. mesenteroides* B-742CB, followed by the expression of a dextransucrase (rDSRB742CK) in *Escherichia coli*. The rDSRB742CK differed in several aspects from DSRB742 of a parent dextransucrase from B-742CB strain: the former had 2.32 times higher activity (29.2 U/mg) and synthesized a highly (15.6%) α-(1·3)-branched dextran. Fourteen nucleotides of the parent gene

(*dsrB742*) were changed in the DNA sequence. But, the replacement of nucleotides had no influence on amino acid substitution: there were one nucleotide difference at the structural gene (silent mutation), three nucleotides difference at the promoter region, and three nucleotides deletion, suggesting that unknown modification of rDSRB742CK occurring in *E. coli* may change the enzyme function.

L. mesenteroides B-1299CB4 utilizes sucrose for synthesis of a dextran with only α-(1·6) glucosidic linkages. Comparison of the amino acid sequence of DSRBCB4 (a dextransucrase of B-1299CB4 strain) with glucansucrases from *Streptococcus* and *Leuconostoc* identified conserved amino-acid residues in the catalytic core critical for enzyme activity. Replacement of D530, E568 or D641 completely lost the enzyme activity, indicating that three residues were essential for catalytic reaction. Further mutation works in the catalytic center elucidated the structural element to determine the product specificity as follows. To mimic the reuteransucrase sequence, one double mutant (V534P/V537I), one triple mutant (S642N/E643N/V644S) and their combined mutant (V534P/V537I/S642N/E643N/V644S) were constructed. S642N/E643N/V644S influenced the product specificity to convert the formation of an α-(1·6) linear dextran to that of a branched glucan [α-(1·3) and α-(1·4)]. The subsequent introduction of mutation V534P/V537I resulted in synthesis of an α-glucan containing a far increased percentage of α-(1·4) glucosidic linkage. This change in glucosidic linkage specificity was also observed in the acceptor reaction to elevate the productivity of maltose-sucrose and isomaltose-sucrose. The amino acids most likely contribute to binding of acceptor substrates to acceptor site of glucansucrase.

A cDNA encoding dextranase (LSD1) from *L. starkeyi* KSM22 was isolated and expressed heterogeneously, and the resultant product (rLSD1) was characterized. The 2,052 bp DNA fragment harboring the dextranase gene exhibited one ORF composed of 1,824 bp flanked by a 41 bp 5'-UTR and a 184 bp 3'-UTR, including a 27 bp poly(A) tail. The LSD1 gene in chromosome contains no intron. The ORF encoded a 608 amino acid polypeptide with a 67.2 kDa of molecular mass. The primary structure exhibits the distant similarity to those of GH family 49 enzymes that comprise *Penicillium* dextranase. The optimum pH of rLSD1 was 6.0 and the optimum temperature was 37℃. rLSD1 activity was decreased by exposure to 1 mM Hg^{2+}, Ag^{3+} and Mn^{2+}. rLSD1 exhibited high hydrolyzing activity toward dextran (100%) as well as toward soluble starch (5%) and mutan (8%).

This research contributes to an understanding of the relationship between structure and function of industrially important enzymes, two glycansucrases (levansucrase and dextransucrase) and a glucanhydrolase (dextransucrase).