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Designing of a novel dextransucrase efficient in acceptor reactions

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ABSTRACT

Dextransucrase is produced by *Leuconostoc, Streptococcus* and *Lactobacillus* Species. The enzyme synthesizes dextran and acceptor products some of which act as prebiotics that are increasingly used in such industries as food, medicine, and cosmetics. B-512F *Leuconostoc mesenteroides* dextransucrase (DSR-S) is the preferred enzyme in commercial production of dextran and prebiotics. In the present work, a novel dextransucrase which is efficient in prebiotics production was designed. The enzyme was produced at optimal conditions in *Escherichia coli* by truncation and fusion to glutathione S-transferase (GST) in the gene from *Leuconostoc mesenteroides* B-512 FMC. The novel enzyme (MW: 119 kDa) was active and carried out dextran biosynthesis and acceptor reactions effectively. The novel dextransucrase (fTDSR-S) was produced by truncating signal, variable, and the glucan-binding regions in the gene and fusion of gst gene at the 5′ end. fTDSR-S was characterized in detail and compared to the DSR-S. Truncation and fusion resulted in an increase in fTDSR-S biosynthesis in *E. coli* BL21 (DE3) by 35 fold. fTDSR-S leads to production of dextran as well as increased acceptor reactions. Due to GST fusion, it was possible to immobilize fTDSR-S covalently onto Eupergit C successfully. It was also found that the size of the active site of dextransucrase is 49 amino acids shorter than that reported previously in the literature.

1. Introduction

Leuconostoc mesenteroides NRRL B-512 FMC dextransucrase synthesizes dextran using the glucosyl part of sucrose, with fructose being formed as a byproduct. B-512 F *Leuconostoc mesenteroides* dextransucrase (DSR-S) is the only industrial enzyme used in production of commercial dextran. B-512 F dextran has 95% α-(1→6) and 5% α-(1→3) branch linkages.[1] Dextrans have many uses including production of Sephadex and blood plasma substitute. Hydrolysis products of dextrans, isomaltooligosaccharides, act as prebiotics. DSR-S catalyzes the formation of dextran from sucrose, but acceptor reactions take place when acceptors are added to sucrose–dextransucrase digests. In acceptor reactions, some of the glucosyl groups of sucrose molecules are transferred to the acceptors leading to the formation of oligosaccharides and decreased synthesis of dextran.[2,3]

DSR-S has 1527 amino acids (aa). This enzyme can be broken down into four different domains called signal peptide, variable, catalytic, and C-terminal glucan binding regions. The signal peptide region is 32–34 aa-long. Variable region is 123–129 aa-long and shows high amount of variability among glycosyltransferases. The catalytic region is approximately 1000 aa-long and responsible for sucrose binding, separation of fructose units, and glycosyl transfer. The C-terminal domain is approximately 500 aa-long and composed of a series of homologous directly repeating glucosyl binding units for glucan binding and chain growth reactions.[4–6]

Signal peptides of glucansucrases are typical gram-positive bacterial signal peptide.[7] There is an unprotected area immediately after the signal peptide, known as the high variable region.[8] It was found that truncation in the variable region of DSR-S from *L. mesenteroides* NRRL B-1299 did not cause any significant change in the enzymatic activity.[9] The function of this region in the enzymatic reaction has not yet been determined.[10] Studies have shown that dextran synthesis occurs in two different regions. The first one is responsible for catalyzing the transfer of glycosyl units (catalytic region) and the other one, found at the C-terminal, is responsible for glucan chain elongation (glucan binding region).[8,11–14] Consequently, truncating glucan binding region stops polymer growth without blocking glycosyl transfer.[10,11,13,15–17]

In the present study, a novel dextransucrase, truncated and fused with glutathione S-transferase (GST), was produced in *E. coli.* The enzyme produced, efficient in acceptor reactions, was characterized in detail.

2. Materials and methods

2.1. Cell strains and vectors

Mutant *L. mesenteriodes* B-512FMC-13 Glc has been provided by Dr. J.F. Robyt (Iowa State University, Department of Biochemistry,

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Biophysics, and Molecular Biology, Ames, IA, USA). This mutant produces dextransucrase which biosynthesizes dextran, similar to commercial B-512 F dextran. *E. coli* DH5α and *E. coli* BL21 (DE3) bacterial strains were provided by Promega (Fitchberg, WI, USA) and Invitrogen (Carlsbad, CA, USA), respectively. Carrier Vector (pGEM-T Easy) and expression vector (pGEX-4T I) were provided by Promega and GE Healthcare (Little Chalfont, United Kingdom), respectively.

2.2. Chemicals and enzyme

Fructose, lactose, maltose, glucose, sucrose, glycine, Bacto tryptone, yeast extract, peptone, Coomassie Brilliant Blue, and α-naphthol were obtained from Fluka (Buchs SG, Switzerland). Calcium acetate, isopropyl β-D-1-thiogalactopyranoside (IPTG), phenylmethanesulfonyl fluoride (PMSF), sulfuric acid, ethanol, acetonitrile, bromphenol blue, sodium chloride, sodium azide, potassium diphosphate, disodium phosphate, calcium chloride, Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membranes were purchased from Merck (Darmstadt, Germany). *N*–*N*–*N*–*N*-tetramethylendiamine, sodium dodecyl sulfate (SDS), lysozyme, methanol, tris base, and agarose were purchased from Sigma–Aldrich (St. Louis, MO, USA). Molecular weight markers (Mw: 4000–250,000) were purchased from Invitrogen. Thin layer chromatography (TLC) plates (Whatman K5 and Merck TLC silica gel 60) were purchased from Whatman (Clifton, NJ, USA) and Merck KGaA (Darmstadt, Germany), respectively. Protein reagent for the Bradford dye-binding assay, restriction and modification enzymes were obtained from Thermo Scientific (Waltham, MA, USA). DNA purification and PCR procedure kits were purchased from Thermo Scientific. MRS medium, LB medium, and agar were obtained from Fluka Laboratories.

2.3. Genomic DNA isolation from *L. mesenteroides* B-512FMC-13 Glc

Prior to genomic DNA isolation, *L. mesenteroides* B-512FMC-13 glc was produced in MRS broth by incubating at 23 °C at 130 rpm for 48 h. Genomic DNA isolation was carried out using DNA purification kit from Thermo Scientific.

2.4. Construction of plasmid

Plasmid (pGEM T Easy) bearing fragment of B512-F dextransucrase gene (*fmdsr-s*) was constructed as follows: a pair of primers (5′-ATGCCATTTACAGAAAAAGTAATGCG-3′, and 5-AAAACGGCCGTG CTGACACAGCATTTCCATTATTATC-3, *Xma III* restriction site) was synthesized to amplify a 4591 bp fragment containing the entire *fmdsr-s* by polymerase chain reaction (PCR). The primers were based on the sequence determined by Wilke-Douglas et al.[18] PCR was carried out as follows: one cycle at 95 °C for 3 min; 35 cycles at 94 °C for 20 s, 58 °C for 60 s, and 72 °C for 4 min; and one final cycle at 72 °C for 10 min. PCR products were analyzed using bioanalyzer (Agilent Technologies, California, USA). *fmdsr-s* was cloned into vector pGEM T Easy using AT cloning, and the plasmid DNA was transformed into the *E. coli* DH5 strain. The plasmid was isolated by QIAprep Spin Miniprep kit (QIAGEN, Hilden, Germany). DNA sequencing was carried out by Refgen (Ankara, Turkey). DNA homology search of GenBank was performed by using the BLAST program (NCBI, Bethesda, MD, USA).

E. coli transformations, restriction enzyme digestion, and agarose gel electrophoresis were performed by standard procedures.[19] Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE, 12% acrylamide) was carried out as previously described.[1]

2.5. Truncation of B-512F dextransucrase gene

B-512F dextransucrase gene truncation is performed by PCR mutation method in which f 5′-AAAAGGATCCTCTGGTGTGATTG-3′ (*BamH* I): r 5′-AGAGCCACTCGAGTACTTAGCCG-3′ (*Xho* I) primers were used. PCR was carried out as follows: one cycle at 95 °C for 2 min; 35 cycles at 94 °C for 20 s, 58 °C for 60 s, and 72 °C for 2 min; and one final cycle at 72 °C for 5 min. PCR products were analyzed using bioanalyzer. PCR product and pGEX-4T I vector were cloned using *BamH* I and *Xho* I restriction endonuclease enzymes. Cloning vector was transferred to *E. coli* BL21 (DE3).

2.6. Dextransucrase production

Enzyme production in *E. coli* BL21 (DE3) was carried out in modified Studier auto-induction medium (1 L) which is composed of casein digest peptone (20 g), yeast extract (5 g), NaCl (5 g), Na_2HPO_4 (6 g), KH_2PO_4 (3 g), glucose (0.5 g), lactose (2 g), glycerol (6 mL), tris base (12.1 g), sucrose (0.0–2.0 g), and $CaCl_2$ (0.05 g). The recombinant *E. coli* BL21 (DE3) were cultured overnight in LB medium (5 mL) containing ampicillin (5 μL, 1 g/mL) at 200 rpm at 37 °C. Cultures (0.2–4 mL), added into modified Stadier auto-induction medium [pH(6.0–7.2), sucrose (0.0–0.4 g) (200 mL)] containing ampicillin (200 μL, 1 g/mL), were grown at 200 rpm at temperatures (18–28 °C) for 12 h during which cell growth and enzyme activities were determined at 1 h intervals.

The cells were collected by centrifugation (8000*g*, 15 min) and were suspended in sodium acetate buffer (100 mM, pH 5.5) containing $CaCl_2$ (1 mM), NaN_3 (0.02%), dextran (0.1 mM), and PMSF (1 mM). The cells were lysed by sonication (8 × 30 s bursts with 1 min of cooling on ice between bursts) followed by treating with Triton X-100 [0.5% (v/v)] and lysozym (0.2 mg/mL) at 4 °C for 12 h. Then, the supernatant was obtained after centrifugation (8000*g*, 30 min) of the cell lysed. In concentration of dextransucrase, Amicon ultra-15 centrifugal filter unit with ultracel-100 membrane was used. Protein determinations were carried out using the Bradford method.[20]

2.7. Carbohydrate analysis

Thin Layer Chromatography (TLC) was used for quantitative analysis of carbohydrates.[12] In determination of fructose, TLC plates (Whatman K5 or Merck Silica gel 60) were developed using 2 ascents of acetonitrile/water [85:15 (v/v)]. In determination of acceptor reaction products, TLC plates were run using 3 ascents of nitromethane/water/1-propanol [2:3:5 (v/v)] up to two thirds of the plate, and then 2 ascents of acetonitrile/water [85:15 (v/v)] to the top of the plate. Carbohydrates on TLC plates were developed by dipping the plates into sulfuric acid [5% (v/v)] in ethanol containing α-naphthol [0.5% (w/v)], followed by heating on a hot plate at 110 °C for 10 min. TLC-imaging densitometer, Bio-Rad GS-670, was used for quantitative determination of carbohydrates.[21–23]

2.8. Determination of dextransucrase activity

The assay mixture (20 mL) contained enzyme solution (1 mL) and sucrose (200 mM) in sodium acetate buffer (50 mM, pH 5.5) containing $CaCl_2$ (18 mM). The reaction mixture was incubated at 25 °C with stirring at 200 rpm for 30 min. Aliquots (0.2 mL) from the reaction mixture were added to distilled water (0.8 mL) and boiled for 10 min. The amount of fructose formed during the reaction was determined using TLC-densitometer technique. The activity unit (IU) is defined as the amount of enzyme catalyzing the formation of 1 μmol of D-fructose from sucrose (200 mM) in