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Designing of a novel dextransucrase efficient in synthesizing oligosaccharides

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ABSTRACT

Dextransucrase (EC2.4.5.1) from strain *Leuconostoc mesenteroides* 0326, which synthesizes dextran and oligosaccharides, which act as prebiotics, are popularly used in such industries as food and medicine. A novel dextransucrase efficient in synthesizing oligosaccharides was designed. We constructed the truncation mutant DSR-S1-ΔA (residues 1–3087 bp) by deleting the 1494 bp fragment of the C-terminal. The novel enzyme (MW: 110 kDa) loss activity, when sucrose was used as only substrate. After adding an acceptor, DSR-S1-ΔA was fully activated but with heavily impaired polysaccharide synthesis ability. Instead, the enzyme produced a large amount of oligosaccharides. DSR-S1-ΔA showed transglycosylation for synthesizing more oligosaccharides of lower degree of polymerization (DP) with different acceptors, and it also improved the selection range of dextransucrase acceptor response to acceptors. The enzyme developed in this study can be applied in glycodiversifcation studies.

1. Introduction

Dextransucrase (EC 2.4.1.5) is large multidomain glucansucrase enzyme usually produced by *Leuconostoc mesenteroides* and oral streptococci [1]. Dextransucrase is induced when strain *L. mesenteroides* 0326 is grown in sucrose-rich media. It is a large multidomain enzyme belonging to the CAZy family 70 of glycoside hydrolase enzymes (GH70) and a popular enzyme in the field of dextran [2]. Dextransucrase catalyzed the transfer of D-glucosyl units from sucrose to acceptor molecules [3,4]. Depending on the receptors, dextransucrase catalyzes two kinds of reactions, namely, hydrolysis, in which water is used as acceptor, and glycosyl transfer. Glycosyl transfer reaction can be classified into two kinds: In the first type, the main reaction is polysaccharide synthesis, in which the growing glucan chain is used as an acceptor. In the second type, the side effect is oligosaccharide synthesis, in which oligosaccharides (such as maltose) are used as acceptors [5].

The majority species of oligosaccharides, also known as functional oligosaccharides is beneficial to the human body. Some properties of oligosaccharides are similar to those of sugars. Thus, oligosaccharides can directly replace sugars sweet food ingredients. These compounds can reach the intestine, because they can not be degraded by human gastric acid and gastric enzyme or absorbed in the small intestine. Oligosaccharides have served as functional dietary carbohydrates for decades and have attracted growing interest worldwide [6]. Oligosaccharide is also used as a prebiotic, which can selectively promote the proliferation of the intestinal flora and *Lactobacillus* [7]. The high demand for but low available amount of oligosaccharides has led tothe catalytic synthesis of dextransucrase transfructosylation. The dextransucrase molecular structure is transformed to improve the ability of dextransucrase to catalyze the synthesis of oligosaccharides.

L. mesenteroides 0326 (dex-YG) has 1527 amino acids (aa). This enzyme can be broken down into the following four domains: signal peptide; N-terminal variable region; conserved catalytic domain; and C-terminal glucan-binding domain (GBD), which is composed of a series of tandem repeats [8]. The signal peptide is 35–38 aa long, in which lead extracellular enzymes are secreted outside the cell membrane. Two functional regions of the N-terminal decompose the sucrose donor and C- terminal binding dextran. The N-terminal variable region shows a strong variable in the glycosyltransferase. The conserved catalytic domain is responsible for binding to sucrose and decomposition of sucrose into glucose and fructose. The C-terminal domain is responsible for sugar chain extension.

The signal peptides of glucansucrases are typical Gram-positive bacterial signal peptides [9]. An unprotected area, known as the high-variable region, immediately exists after the signal peptide

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[10]. Studies have shown that dextran synthesis occurs in two different regions. The first region is responsible for catalyzing the transfer of glycosyl units (catalytic region), and the other one, which is found at the C-terminal, is responsible for glucan chain elongation (glucan binding region) [11–13]. Monchois [14] performed the first truncation study on the C-terminal end of DSR-S. Deletions of the last YG repeats of the C-terminal end had no evident effect on enzymatic activities. Truncation of domain V of GTF180 completely impaired polysaccharide-synthesizing ability [15]. Consequently, truncating the glucan binding region stops polymer growth without blocking glycosyl transfer and promotes the production of oligosaccharides [9,13,16,17].

In the present study a novel dextransucrase, which was truncated at the C-terminal ends, was produced in *Escherichia coli* BL21 DE3 Star. The reactions and product specificities of the truncation mutant were compared with UR-enzyme to investigate effects of truncated strains on receptor response.

2. Materials and methods

2.1. Materials, bacterial strains, medium and plasmid

In the previous study, a strain of BL21 (DE3)/Pet28-dexYG (DE3) was obtained from an industrial production of *L. mesenteroides* 0326 [1]. *E. coli* DH5α (Phabagen, Utrecht, The Netherlands) was used as host for cloning. *E. coli* BL21 DE3 star (Invitrogen, Carlsbad, CA, USA) was used to express mutant dextransucrase. The plasmid pEASY-T1 (Trans) was used for cloning and plasmid pET28a$^+$ (Novagen) was used to express the truncation mutant in *E. coli* BL21 DE3 Star. *E. coli* strains were grown aerobically at 37 °C in Luria–Bertani (LB) medium [18]. *E. coli* strains containing recombinant plasmids were cultivated in LB medium with 100 μg/mL ampicillin at 240 rpm and 37 °C for 24 h.

2.2. Molecular techniques

General procedures for cloning, *E. coli* transformation, DNA manipulation, and agarose gel electrophoresis were as described by Sambrook [19]. Restriction endonuclease digestion and ligation with T4 DNA ligase were performed as recommended by the enzyme suppliers. Primers were synthesized from Eurogentec, Seraing, Belgium. Sequencing was performed as described by Kralj [20].

2.3. Construction of plasmid

The plasmid (pEASY T1 + dex-YG) bearing fragment of dex-YG dextransucrase genewas constructed as follows: two primers [sense (5′-CGCGGATCCATGCCATTTACAGAAAAAGT-3′) *Bam*HI restrictionsite and antisense (5′-CCCAAGCTTTTATGCTGACACAGCATTT-3′) *Hind*III restriction site] were designed toamplify the gene by PCR. The primers were based on the sequence of dex-YG (Genbank Accession No. DQ345760). PCR was performed as follows:one cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 5 min; and one final cycle at 72 °C for 10 min. PCR products were analyzed by nucleotide sequencing. Dex-YG was cloned into vector pEASY T1 using at cloning. Moreover, the plasmid DNA was transformed into the *E. coli* DH5α strain. The plasmid was isolated by Easy Pure Plasmid Kit (Trans, Beijing, China). DNA homology search of the Genebank was performed using the Blast program (NCBI, Bethesda, MD, USA). *E.coli* transformations, restriction enzyme digestion, and agarose gel electrophoresis were performed by standard procedures.

2.4. Truncation of dex-YG dextransucrase gene

Dex-YG dextransucrase gene truncation is performed by PCR mutation method in which f 5′-CGCGGATCCGATGCCATTTACAGAAAAAGT-3′ (*Bam*HI): r 5′-CCCAAGCTTTTGGTCCGGCACCCAATCGG-3′ (*Hind*III) primers were used. PCR was performed as follows: one cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 5 min; and one final cycle at 72 °C for 10 min. PCR products were identified by nucleotide sequencing. PCR products and pET28a$^+$ vector were cloned using *Bam*HIand *Hind*III restriction endonuclease enzymes. Cloning vector was transferred to *E.coli* BL21 DE3 star.

2.5. Dextransucrase production and purification

Recombinant wild-type dextransucrase and mutant enzyme were expressed in *E. coli* BL21 DE3 star. The cultures were grown at 37 °C and shaken at 240 rpm for 16–18 h *E. coli* strains were grown aerobically in LB (pH 7.0) supplemented with 100 μg/mL kanamycin. The cultures were grown at 37 °C and 240 rpm for 16–18 h. Then, according to the inoculation amount of 1%, 2 mL of the LB seed medium was inoculated into 200 mL of culture medium A, which is composed of tryptone (2.34 g), KNO_3 (2.34 g), $NaHPO_4.12H_2O$ (3.42 g), KH_2PO_4 (0.6 g), NH_4Cl (0.2 g), $MgSO_4{\cdot}7H_2O$ (0.0492 g), glycerol (1 g) and glucose (1 g) (pH 7.0), and grown at 37 °C and 260 rpm to a ultraviolet absorption rate of 2.0–2.2 at 600 nm. Subsequently, the enzyme expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG, final concentration of 0.1 mM), and the cultures were incubated at 25 °C and 240 rpm for 4–6 h. Cells were collected by centrifugation (10 min, 4 °C, 10,000*g*) and were suspended in acetic acid–calcium acetate buffer (40 mL, pH 5.4). The cells were broken by sonication (2 s burst with 4 s of cooling on ice between bursts for 15 min). The supernatant was obtained after centrifugation (15 min, 4 °C, 6000*g*) of the lysed cell. The enzymes were purified by Ni^{2+} nitrilotriacetic acid (NTA) affinity chromatography. Purity enzyme was analyzed by SDS-PAGE, and enzyme concentrations were determined by Bradford's method [21].

2.6. Carbohydrate analysis

The carbohydrates were analyzed by high-performance liquid chromatography (HPLC) in a CXDH-3000 chromatographic work station with a Series III pump, refraction index detector (Model ProStar 355 RI), and column oven (Timberline). Separation was achieved using a Luna 5 μm NH_2 (250 mm × 4.6 mm) column at 40 °C. The samples (20 μL) were run through the column with an eluent composed of acetonitrile: water (80:20, V:V) at a flow rate of 1 mL/min. Maltose, sucrose (Acros Organics, Geel, Belgium), fructose and glucose (Merck) were used as standards.

2.7. Enzymatic activity assays

Enzymatic activity was divided into transferase and hydrolysis activities. The various enzymatic activities were determined by measuring release of glucose and fructose from sucrose conversion. Total activities were determined by measuring the release of fructose (V_F) from sucrose. Hydrolytic activities were determined by measuring the release of glucose (V_G) from sucrose in time. Transferase activities were calculated by subtracting hydrolytic activities from total activities ($V_F - V_G$). One unit of enzymatic activity was defined as the release of 1 μmol of monosaccharide per hour. The reactions were performed for 1 h at 25 °C in 3 mM acetic acid–calcium acetate buffer (pH 5.4), containing 30 nM purified enzyme. The reaction was stopped by incubation at 100 °C for