Changes in linkage pattern of glucan products induced by substitution of Lys residues in the dextransucrase

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Received 6 June 2005; revised 16 July 2005; accepted 19 July 2005

Available online 8 August 2005

Edited by Stuart Ferguson

Abstract **Dextransucrase S (DSRS) is the only active glucansucrase that has been found in *Leuconostoc mesenteroides* NRRL B-512F strain. Native DSRS produces mainly 6-linked glucopyranosyl residue (Glc*p*), while *Escherichia coli* recombinant DSRS was observed to produce a glucan consisting of 70% 6-linked Glc*p* and 15% 3,6-Glc*p*. Lys residues were introduced at the N-terminal end of the core domain by site-directed mutagenesis. In glucans produced by the one-point mutants T350K and S455K, the amount of 6-linked Glc*p* was increased to about 85% of the total glucan produced, more similar in structure to native B-512F dextran. The double mutant T350K/S455K produced adhesive, water-insoluble glucan with 77% 6-linked Glc*p*, 8% 3,6-linked Glc*p* and 4% 2,6-linked Glc*p*. The T350K/S455K mutant exhibited a 10-fold increase in glucosyltransferase activity over those of the parental DSRS-His_6 and its T350K and S455K mutants. This is the first report demonstrating a change in the properties of a dextransucrase or a related glucosyltransferase through simple site-directed mutagenesis to create 2,6-linked Glc*p*.**

Keywords: Dextransucrase; Dextran; Glucansucrase; Glucan; Glucosyltransferase; Lysine residue

1. Introduction

Glucansucrases [EC 2.4.1.5] are produced by several kinds of *Leuconostoc*, *Streptococcus*, and *Lactobacillus* bacterial strains to catalyze the synthesis of α-D-glucan from sucrose. Glucansucrases that produce primarily α -1,6-D-glucan are called dextransucrases. *Leuconostoc mesenteroides* NRRL B-512F produces a high percentage (95%) of α-1,6-dextran. Frequently, different glucosidic linkages are found in glucan products; i.e., *Leuconostoc* strains produce glucans with α-1,6-linkages, frequently α-1,3-, and sometimes α-1,2- or α-1,4-linkages. [1]. *Streptococcus* strains produce glucan with α-1,6-linkages and/or α-1,3-linkages [2]. *Lactobacillus reuteri* 121 was reported to produce glucan with mainly α-1,4- and α-1,6-linkages [3]. The glucosidic linkage pattern of the glucan product differs depending on the kind of glucansucrase. However, the mechanisms as to what kinds and what proportions of the linkages are produced are unclear.

Glucansucrases are approximately 160 kDa in size and belong to the glycoside hydrolase family 70 (see http://afmb.cnrs-mrs.fr/CAZY/). A typical glucansucrase amino acid sequence includes a signal sequence, followed by a variable stretch of approximately 200 amino acids, conserved core region of about 900 amino acids (N-terminal catalytic core domain), and a series of direct repeating units of about 400 amino acids (C-terminal glucan-binding domain) [4]. In the highly-conserved core region, about 700 amino acids make up a predicted circularly permuted $(\beta/\alpha)_8$ barrel [5–7]. Catalytic Asp [7–9] and essential substrate-binding Gln [6] exist in this region. The N-terminal end of about 200 amino acids out of this $(\beta/\alpha)_8$ barrel are highly conserved among glucansucrases, but have not been found in other enzymes or proteins. The properties and functions of this region (glucansucrase specific-conserved region, GSC region) are unclear. In *Streptococcus downei* glucansucrase GTFI, Trp344 and His355, both of which are in the GSC region, were reported to be involved in the enzyme reaction [10]. It was also suggested that His355 plays a role in the binding to the subsite necessary for glucan and oligosaccharide elongation [10]. This region has been reported to contain an essential Asp511 residue at the border between the GSC region and the $(\beta/\alpha)_8$ barrel in the dextransucrase S (DSRS) of the *L. mesenteroides* NRRL B-512F strain, which could function to bind a sucrose molecule [11,12]. In one of our previous studies, a chemical modification of the Lys residues was performed on the native DSRS of the B-512F strain with *o*-phthalaldehyde in the presence and absence of sucrose, the sucrose analog sucrose monocaprate, and clinical dextran [13]. The results suggested the GSC region contained sucrose- and dextran-binding Lys-residues. Lys residues in the GSC region may have some role in substrate binding, but it is unclear whether or not some of the Lys residues contribute to the enzymatic reaction. The GSC regions of some glucansucrases from *Leuconostoc*, *Streptococcus*, and *Lactobacillus* are aligned, as shown in Fig. 1. Lys residues are indicated in white letters with a black background. Many of the Lys residues are not conserved in this region, but there are a few Lys residues conserved in most of the glucansucrases. In another one of our previous studies, DSRS was incubated with a glucan prepared from a *Streptococcus mutans* culture, and a glucan-binding peptide fragment was detected in the GSC region [14].

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Abbreviations: DSRS, dextransucrase S; *dsrS*, a gene encodes DSRS; GTF, glucosyltransferase; Glc*p*, glucopyranosyl residue; GSC region, glucansucrase specific-conserved region

0014-5793/$30.00
doi:10.1016/j.febslet.2005.07.050

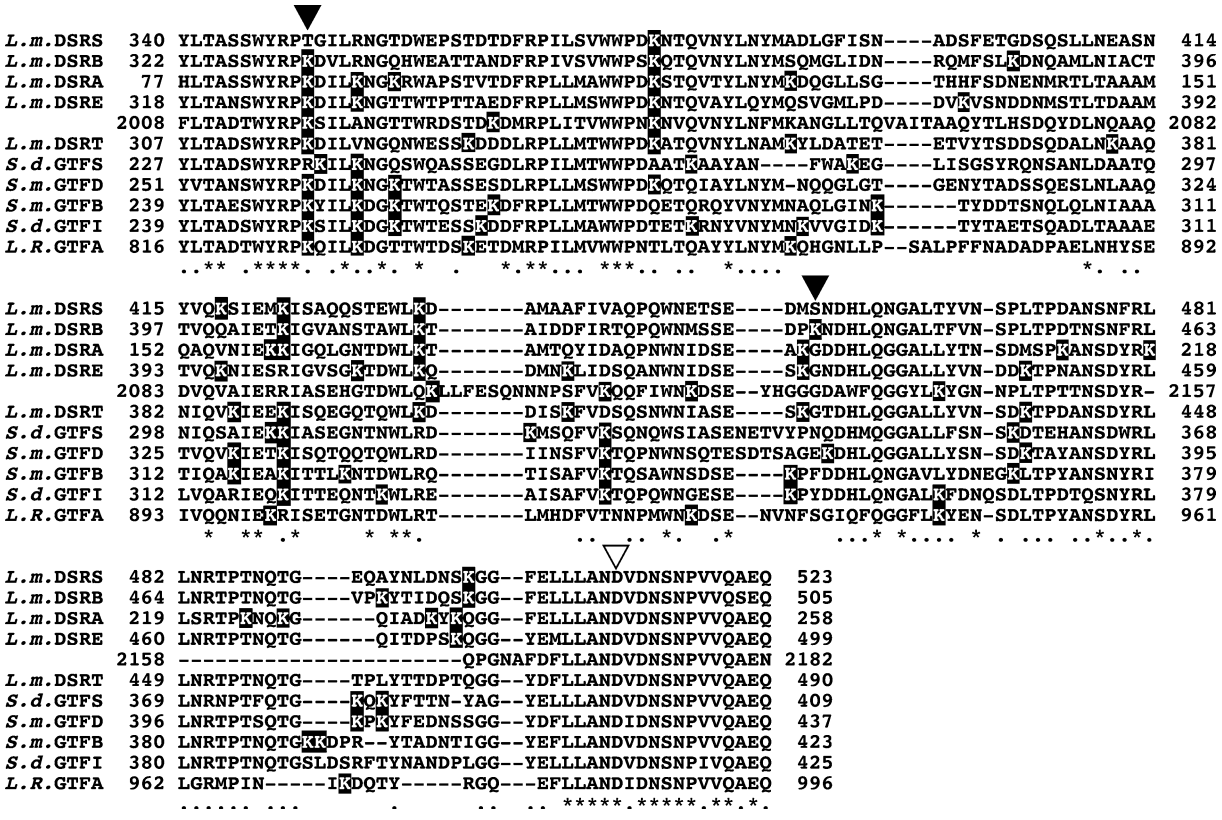

Fig. 1. Alignments of amino acid sequences and positions of Lys residues in the GSC region of various *Leuconostoc* dextransucrases and streptococcal glucosyltransferases. The deduced amino acid sequences of the GSC regions of *L. mesenteroides* dextransucrases are DSRS from the gene *dsrS* (I09598) [12,34], DSRB from *dsrB* (AF030129) [23], DSRA from *dsrA* (U38181) [35], DSRE from *dsrE* (AJ430204) [32] and DSRT from *dsrT* gene (AB020020) [18]. The streptococcal glucosyltransferase are GTFS from *gtfS* from *S. downei* (M30943) [36], GTFD from *S. mutans gtfD* (M29296) [37], GTFB from *S. mutans gtfB* (M17361) [38] and GTFI from *S. downei gtfI* (M17391) [39]. The *Lactobacillus* glucansucrase is GTFA from *L. reuteri gtfA* (AY697435) [3]. All were aligned by the GENETYX version 11.2 program (Software Development Co., Tokyo) as described in Section 2. DSRB produces α-1,6-glucan [23], DSRA produces α-1,6- and α-1,3-glucans [35] and DSRE produces α-1,2-branched linkage-containing α-1,6-glucan [32]. GTFS [36] and GTFD [37] produce water-soluble α-1,6-glucan. GTFI [39] and GTFB [38] produce water-insoluble α-1,3-glucan. GTFA [3] produce water-soluble α-1,4- and α-1,6-glucan. The positions of the Lys residues are indicated by white letters with a black background. Solid arrowheads are the positions of substituted Lys residues in the DSRS protein. The open arrowhead is an essential Asp residue [11,12].

Additionally, in our most recent study, chimeric enzymes constructed from two different recombinant dextransucrases that were exchanged their GSC regions each other produced glucans different from those of their parental enzymes [15]. It should be noted, however, that the positions of the glucan-binding Lys residues in the GSC region have not yet been identified, nor have the roles of the Lys residues in the GSC region been clarified. The positively charged amino acid residues of His, Arg, and Lys can potentially bind with sugars or other carbohydrates via hydrogen bonding. The Lys residue was reported to be one of the essential residues for sugar binding in a lectin from the freshwater Indian gastropod *Balamyia bengalensis* [16] and for carbohydrate binding in bovine β-lactoglobulin [17].

In this study, Lys residues were substituted in a recombinant C-terminal polyhistidine-tagged DSRS dextransucrase (DSRS-His_6) through site-directed mutagenesis. A possible role of the GSC region is speculated based on the changes in the linkage patterns of the glucan products of the DSRS-His_6 enzymes of the Lys-substituted mutant. The contributions of the Lys residues in the GSC region in terms of enzyme reactions and the determination of the kinds of glucosyl linkages made are discussed in this report.

2. Materials and methods

2.1. Construction of the DSRS-His_6 gene and the introduction of mutations

The plasmid pDSRS, carrying the complete *dsrS* gene in the form of pET23d, was constructed as described before [18]. To introduce the $(His)_6$-Tag at the C-terminal of the recombinant DSRS protein, an *Eco*RI/*Xho*I fragment of PCR-amplified DNA was used with pDSRS as a template with the primers 5′-GCCATGCTGTCACTGGTTTC-3′ and 5′-GCCTCGAGTGCTGACACAGC-3′, which were replaced in pDSRS to construct pDSRSH. Site-directed mutagenesis was carried out on pDSRSH with a Mutan-Super Express Km Kit (Takara).

2.2. Expression of pDSRSH and the purification of the gene product DSRS-His_6 and its mutants in E. coli *BL21(DE3)*

DSRS-His_6 and its mutant enzymes were produced as described before [18,19]. Cultured *E. coli* BL21(DE3) cells carrying pDSRSH or mutated plasmids were suspended in 20 mM Na-acetate (pH 5.2) containing 30% glycerol. The recombinant proteins were then extracted by sonication. The supernatant was obtained by centrifugation at $10\,000 \times g$ for 10 min, to which 6 M guanidine–HCl, 0.5 M NaCl, and 15 mM imidazole were added. The enzyme solution was put on a HiTrap™ 1 ml Chelating HP (Amercham Bioscience) column previously loaded with 0.1 M $NiSO_4$ and equilibrated by a 20 mM Na-acetate, 30% glycerol, 0.5 M NaCl, 6 M guanidine–HCl, 15 mM imidazole solution (pH 7.5). The column was washed with the same solution to