Enzyme and Microbial Technology 102 (2017) 26–34

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/enzmictec

Research paper

Functional analysis of truncated and site-directed mutagenesis dextransucrases to produce different type dextrans

Chao Wang, Hong-bin Zhang*, Meng-qi Li, Xue-qin Hu, Yao Li

School of Biological and Medical Engineering, Hefei University of Technology, 193# Tunxi Road, Hefei 230009, Anhui Province, P. R. China

ARTICLE INFO

Keywords:
Dextransucrase
Dextran
Truncated mutation
Site-directed mutation
Product specificity
Enzyme activity

ABSTRACT

Dextrans with distinct molecular size and structure are increasingly being used in the food and pharmaceutical industries. Dextran is produced by dextransucrase (DSR, EC2.4.5.1), which is produced by *Leuconostoc mesenteroides*. DSR belongs to glycosyl hydrolase family (GH70) and synthesizes branched α-glucan (dextran) with both 5% α(1–3) and 95% α(1–6) glycosidic linkages. The DSR gene *dex-YG* (Genebank, Accession No. DQ345760) was cloned from the wild strain *Leuconostoc mesenteroides* 0326. This study generated a series of C-terminally truncated variants of dextransucrase and substituting the amino-acid residues in the active site of DSR. With shorter length of DSR, its polysaccharide-synthesizing capability was impaired heavily, whereas oligosaccharide (acting as prebiotics)-synthesizing capability increased significantly, efficiently producing special sizes of dextran. All truncated mutant enzymes were active. Results demonstrated that the catalytic domain dextransucrase was likely in 800 aa or less. Based on the three-dimensional structure model of dextransucrase built through homology modeling methods, the DSR and its mutants with the acceptor substrate of maltose and donor substrate of sucrose were studied by molecular-docking method. Substituting these amino-acid residues significantly affected enzyme activities. Compared with the wild-type dextran, mutant enzymes catalyzed the synthesis of a-glucan with 1–9% α(1–3) and 90–98% α(1–6) branching linkages. Some mutants introduced a small amount of α(1–4) linkages and α(1–2) linkages. This strategy can be effectively used for the rational protein design of dextransucrase.

1. Introduction

Dextrans are widely used in several fields, such as in food and pharmaceutical industries and in chromatographic analysis, because of their outstanding physicochemical properties [1–3]. Dextransucrase is a glucansucrases belonging to CAZy family 70 of glycoside hydrolase enzymes (GH70). To date, 64 GS enzymes have been biochemically characterized and three-dimensional structures are available for only four glucansucrases. These structures were obtained by crystallization of recombinant truncated forms of GS from *Lb. reuteri* 180 (GTF180-ΔN; PDB: 3KLK), *S. mutans* (GTF-SI; PDB: 3AIE), *Ln. mesenteroides* NRRL B-1299 (ΔN123-GBD-CD2; PDB: 3TTQ) and *Lb. reuteri* 121 (GTFA-ΔN; PDB: 4AMC). Together with GH13 and GH77 enzymes, GH70 GSs belong to the α-amylase superfamily enzymes and are classified in clan GH-H [4]. Glucansucrases (GS) catalyze the formation of both linear and branched α-D-glucans with various types of osidic linkages, namely α(1–2), α(1–3), α(1–4) and α(1–6) glucosidic bonds. The α-glucans are classified into four types according to the dominant linkage type in the main chain, namely, dextran, mutan, alternan, and reutern [5].

Three-dimensional structures have been reported for the N-terminally truncated GTF-180 enzyme of *L. reuteri* 180. The crystal structure of GTF180-ΔN revealed that the polypeptide chain followed a U-shape course to form five domains. The catalytic core of dextransucrase consists of three domains, which resemble the A, B, and C domains in glycoside hydrolase family 13 (GH13) enzymes. Two other domains, called IV and V, are attached to the catalytic core [6]. The catalytic residues of GH70 family are highly conserved and employs a conserved catalytic triad, namely, a nucleophile (aspartate), an acid as acid/base (glutamate), and a transition state stabilizer (aspartate) as revealed by a sucrose bound structure of GTF-180 The mechanism of the reaction have been extensively studied in GH13enzymes. In a double-displacement mechanism, first the glycosidic linkage of the substrate is cleaved between subsites −1 and +1, resulting in a covalent glucosyl-enzyme intermediate; In the second half-reaction the glucosyl moiety is transferred to an acceptor with retention of the −anomeric configuration. The mechanism of GH70 was proposed to be similar [6,7].

Lacking of available crystal structure of dextransucrase, previous truncation studies attempt to assign functional roles to the different

* Corresponding author.
E-mail address: hbzhang@hfut.edu.cn (H.-b. Zhang).

http://dx.doi.org/10.1016/j.enzmictec.2017.03.011
Received 3 November 2016; Received in revised form 21 March 2017; Accepted 23 March 2017
Available online 25 March 2017
0141-0229/

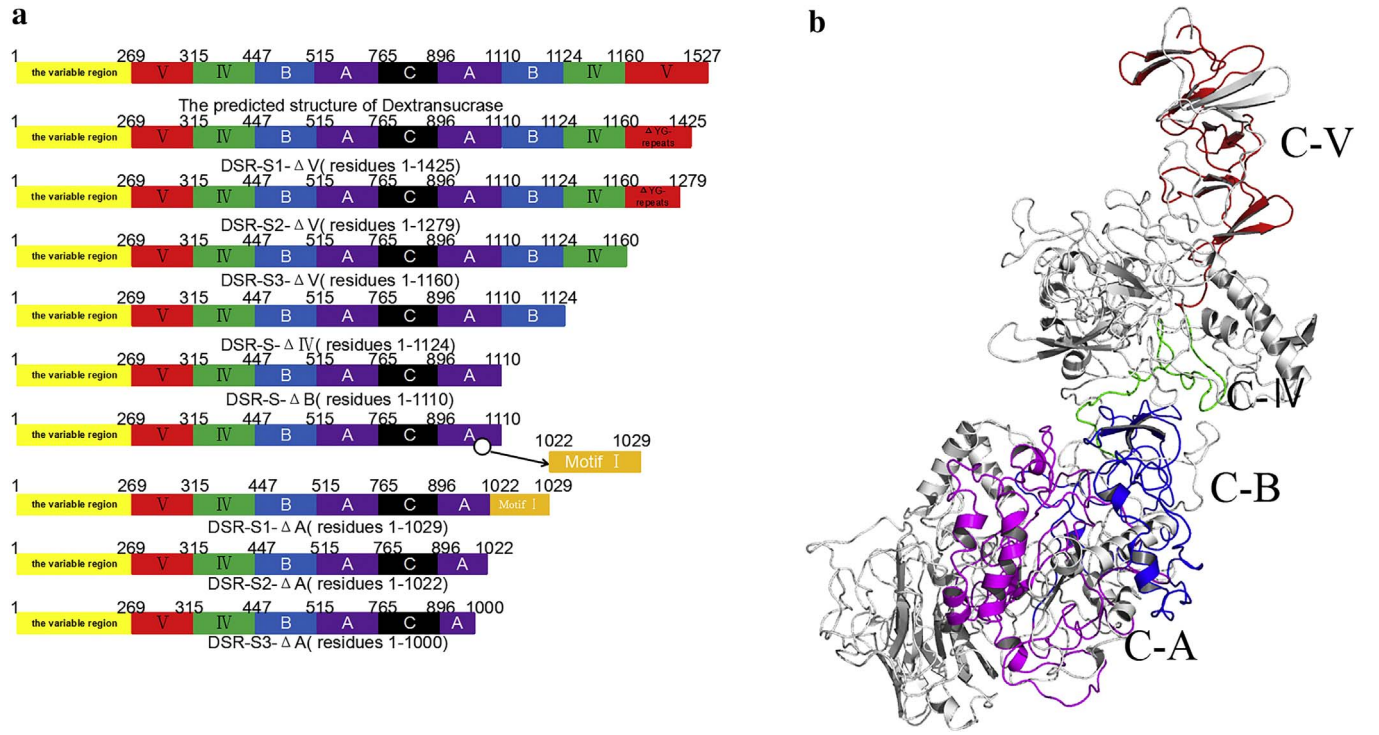

Fig. 1. (a) Linear schematic representation of the predicted domain organization of dextransucrase and the truncated mutation enzymes (a series of different length of truncation on C-terminal end of dextransucrase). The number of amino-acid residues of different domains is indicated in dextransucrase and truncated mutation enzymes. Orange region 1022–1029aa has homology with motif. (b) 3D model of dextransucrase created through Modeller using the structure of the N-terminally truncated glucansucrase GTF180-ΔN (PDB accession code: 3KlK) as restraint. Different truncated domains on C-terminal end of dextransucrase are highlighted in corresponding colors to linear schematic. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dextransucrase regions were based on the assumption of a linear domain organization. The N-terminal variable region and C-terminal glucan-binding domain contain different amino acid sequence repeat units. Truncation of those repeats lead to decrease of enzyme activity [8,9]. The deeper truncation therefore has not been performed before. The functional role of dextransucrase regions was not well understood. Here, according to the model of dextransucrase from *Leuconostoc mesenteroides* 0326, the truncation of present studies designed a rational and accurate scheme (Fig. 1a). The studies investigate the functional role of conserved catalytic domain, and C-terminal glucan-binding domain of dextransucrase. The different product specificities of these glucansucrases are reflected in their different binding cleft shapes. However, the mechanism of dextransucrase that controls the type of glycosidic linkage is still unclear. The determinants of GS specificity have thus not been completely described. Most studies focused on a few separated residues. Compared to previous mutagenesis studies, the study aimed to choose targeted site according to the simulation parameters. Guided by parameters of molecular simulation of dextransucrase, mutants were constructed to yield dextrans with different linkage type efficiently and intentionally.

The dextransucrase gene dex-YG (Genebank, Accession No. DQ345760) was cloned from Leuconostoc mesenteroides 0326 [10]. C-terminal and N-terminal structure of dextransucrases are symmetric as described [6]. To understand the structure and function of dextransucrases, as well as to produce different sizes of dextrans, the study constructed various C-terminal truncation mutants of dextransucrase and several site-directed mutants The molecule-docked complexes, that the acceptor substrate maltose or donor substrate sucrose bound in the catalytic site of mutant enzymes, showed significant diversity to the wild-type DSR. The enzymatic activities and products were characterized in detail. This study provides a strategy for rational protein design and expands the application of dextransucrase and dextran products.

2. Materials and methods

2.1. Bacterial strains, plasmid, enzyme, and growth conditions

Escherichia coli DH5α (Phabagen, Utrecht, Netherlands) was used as the host for DNA manipulation. *E. coli* BL21 DE3 star (Invitrogen, USA) was used to express various mutant enzymes. *E. coli* strains were cultured in Luria-Bertani (LB) (10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl, pH 7.2) medium with 100 μg/ml kanamycin at 37 °C and 240 rpm. Vectors pEASY-T1 and pET-28a-(+) were provided by Beijing TransGen Biotech.

General procedures for cloning, *E. coli* transformations, and DNA isolation were as described [11]. *BamH*I and *Hind*III (Takara Bio Inc., Japan) restriction endonuclease enzymes and ligation with T4 DNA ligase (TransGen Biotech, China) were performed as recommended by enzyme suppliers. Primers were synthesized and sequencing was performed by Genewiz (Suzhou, China).

2.2. Construction of plasmids

Plasmid (pEASY-T1 + *dex-YG*)-bearing fragment of dextransucrase gene (*dex-YG*) was constructed as follows: a pair of primers (5′-CGCGGATCC**ATG**CCATTTACAGAAAAAGT-3′, *BamH*I restriction site, 5′-CCCAAGCTTTTATGCTGACACAGCATTT-3′, *Hind*III restriction site) was synthesized through polymerase chain reaction (PCR) to amplify a 4602 bp fragment containing the entire *dex-YG*. The primers were based on the sequence of *dex-YG*. PCR was carried out as follows: 1 cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 5 min with TransStart FastPfu DNA Polymerase; and 1 final cycle at 72 °C for 10 min. PCR products were identified by nucleotide sequencing(Genewiz, Suzhou, China). The *dex-YG* was cloned into vector pEASY T1, and the plasmid DNA was transformed into the *E. coli* DH5α strain. The plasmid was isolated through EasyPure Plasmid Kit (TransGen Biotech, China). DNA homology search in the Genebank