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Identification of a single and non-essential cysteine residue in dextransucrase of *Leuconostoc mesenteroides* NRRL B-512F

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Abstract
Amino acid analysis of purified dextransucrase (sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferase EC 2.4.1.5) from *Leuconostoc mesenteroides* NRRL B-512F was carried out. The enzyme is virtually devoid of cysteine residue there being only one cysteine residue in the whole enzyme molecule comprising over 1500 amino acid residues. The enzyme is rich in acidic amino acid residues. The number of amino acid residues was calculated based on the molecular weight of 188,000 (Goyal and Katiyar 1994). Amino sugars were not found, implying that the enzyme is not a glycoprotein. It has been shown earlier that the cysteine residue in dextransucrase is not essential for enzyme activity (Goyal and Katiyar 1998). The presence of only one cysteine residue per enzyme molecule illustrates that its tertiary structure is solely dependent on other types of non-covalent interactions such as hydrogen bonding, ionic and nonpolar hydrophobic interactions.

Keywords: *Dextransucrase,* Leuconostoc mesenteroides*, NRRL B-512F, cysteine, amino acid analysis*

Introduction

The product dextran synthesized by the enzyme dextransucrase has a variety of commercial applications [1]. Extensive work has been done on dextransucrase production from the commercial strain *Leuconostoc mesenteroides* NRRL B-512F [2]. The dextransucrases have been included in the glycoside hydrolase family and, based on their sequence homologies, have been grouped into 104 families (http://afmb.cnrs-mrs.fr/CAZY/). The enzyme dextransucrase belongs to the glycoside hydrolase family 70. Several reports are available on the purification of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F and they indicate that dextransucrase exists in either single or multiple forms having molecular weights (M_r) in the range 64,000–245,000 [3–9]. In the present study the dextransucrase was purified by PEG-fractionation and the molecular weight was determined as 188,000 [8]. The gene encoding a dextransucrase-like protein in *Leuconostoc mesenteroides* NRRL B-512F was cloned, expressed and characterized [10–12]. Although the enzyme has been cloned and expressed yet there are different molecular weights reported in the literature. The exact amino acid composition of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F is not clearly known as the gene contains the variable region and the role of this region is still not known. The analysis by SDS-PAGE revealed that different forms of recombinant dextransucrase (DSRS) from *Leuconostoc mesenteroides* NRRL B-512F were produced, corresponding to bands of 200, 180, 160 and 120 kDa [10]. The molecular weight of the enzyme was 180,000 and closer to the M_r values of 170,000 [13] and 184,000 [14]. The amino acid composition has been reported for dextransucrase from *Streptococcus mutans* [15,16] and *Streptococcus sanguis*

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[17]. A striking feature of the amino acid analysis of these dextransucrases is that they are virtually devoid of cysteine residues and are rich in acidic amino acid residues. In the present study, we carried out the total amino acid composition of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. We found similar results in that the dextransucrase is rich in acidic amino acid residues and that it contains only one cysteine residue.

Materials and methods

Material

The dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F used in the present study was purified by the method described previously [8]. Amino acids used as standard were from LKB. All other chemicals used were of highest purity grade commercially available.

Methods

Protein concentration determination. Protein concentration was estimated by the method of Lowry et al. [18] using bovine serum albumin as the standard.

Amino-acid composition analysis of dextransucrase. The amino acid composition of purified dextransucrase was determined by hydrolyzing the lyophilised enzyme (10 mg protein) with 6N HCl under nitrogen in sealed tubes at 110°C for 24–70 h according to the procedure of Moore and Stein, 1963 [19]. After hydrolysis, HCl was removed under vacuum and the residue thus obtained was dissolved in sodium citrate buffer (pH 2.2), filtered and applied to the column of the amino acid analyzer after necessary dilution. The amino acid analysis was carried out on an amino acid analyzer (Model, LKB 4101) according to the procedure of Salnikow et al. [20]. The column was packed with Ultrapack II, a cation exchange resin. The column temperature was 50°C for the first 45 min and then 70°C till the end. A 0.2 M sodium citrate buffer of pH 3.25 was used for the first 10 min, followed by the same buffer of pH 4.25 for the next 45 min and finally with the same buffer of pH 6.45, till the end of the analysis. A solution of ninhydrin in ethylene glycol, containing sodium citrate buffer pH 5.5 was used for colour development, at 100°C for 30 min. A mixture of standard LKB amino acids (50 nmol each) was used as the standard reference. The quantification was done by calculating the peak area of a particular amino acid and comparing with the standard reference, according to the method of Moore and Stein [19]. Spectroscopic estimation of tryptophan was carried out by the method described by Goodwin and Morton [21].

Results and discussion

Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F was purified to homogeneity, by fractionation using polyethylene glycol 400, as described previously by Goyal and Katiyar [8]. The amino acid analysis of purified dextransucrase was carried out as described in "Methods". The number of amino acid residues was calculated based on the molecular weight of 188,000 [8]. The results are summarized in Table I. The enzyme dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F is virtually devoid of cysteine residue. There is only one cysteine residue in the whole enzyme molecule comprising over 1500 amino acid residues. The enzyme is rich in acidic amino acid residues. Similar results have been reported for dextransucrase from *Streptococcus mutans* [15,16] and *Streptococcus sanguis* [17] where either no cysteine or only one cysteine residue for the enzyme was reported. It should be pointed out here that no constituents other than amino acids were detected. Specifically, amino sugars were not observed and this implies that the enzyme in not a glycoprotein.

A fluorogenic and bi-functional reagent, *o*-phthaladehyde, is a chemical modifier of enzymes and is used for identification and location of cysteine and lysine residues at the active site. This reagent specifically binds to the sulfhydryl group of cysteine and the amino group of lysine and results in the formation of a fluorescent, isoindole derivative. Formation of isoindole derivative is possible, only when these two functional groups are in close proximity to each other and are not more than 3A° apart. This provides vital information about the distance and orientation of these residues in an enzyme.

Table I. Amino acid composition of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F.

Amino acid	Average number of residues per molecule of enzyme of molecular weight 188,000
Aspartic acid	150 (± 4)
Threonine	94 (± 2)
Serine	82 (± 2)
Glutamic acid	113 (± 3)
Proline	41 (± 2)
Glycine	428 (± 7)
Alanine	221 (± 5)
Cysteine	1.3 (± 0.3)
Valine	106 (± 2)
Methionine	16 (± 1)
Isoleucine	63 (± 2)
Leucine	91 (± 2)
Tyrosine	22 (± 1)
Phenylalanine	42 (± 2)
Histidine	28 (± 1)
Lysine	102 (± 2)
Arginine	50 (± 1)
Tryptophan	7 (± 1)

In our previous studies we showed that the stoichiometry of the reaction of dextransucrase with *o*-phthalaldehyde indicated the formation of only one isoindole derivative, which involves one cysteine and one lysine residue [22,23], and that the cysteine residue is present in close proximity to an essential lysine residue that is present at the active site of the enzyme [22,23]. Chemical modification of the cysteine residue by cysteine specific inhibitors, such as 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and *p*-chloromercuricphenyl sulphonic acid (PCMS) and N-ethyl maleimide (NEM), did not result in the inactivation of dextransucrase [23] which showed that the cysteine is not an essential residue for the catalytic activity of the enzyme. All these experimental findings conform to the conclusion that dextransucrase of *Leuconostoc mesenteroides* NRRL B-512F has a single cysteine residue that is non-essential for enzyme activity. The presence of only one cysteine residue eliminates the possibility that disulfide bonds are responsible for retention of tertiary structure and rather illustrates that its tertiary structure is dependent on other types of non-covalent interactions such as hydrogen bonding, ionic and nonpolar hydrophobic interactions.

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