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PRONOUNCED HYDROLYSIS OF HIGHLY BRANCHED DEXTRANS WITH A NEW TYPE OF DEXTRANASE

Mikihiko Kobayashi, Yasushi Mitsuishi and Kazuo Matsuda

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai 980, Japan

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SUMMARY: Two distinct dextranase activities, I and II, were isolated from the culture supernatant of a soil bacterium (strain M-73). Among the various α-glucans tested, only dextrans containing (1→2)-α-linkage at the branch points were susceptible to dextranase I. The hydrolyzate of the B-1299 dextran with this enzyme was constituted of glucose as a sole low molecular-weight product and limit dextrin. Therefore, it was suggested that dextranase I has a definite specificity to (1→2)-α-linkage at the branch points.

Fragmentation analysis based on enzymatic hydrolysis has been expected to provide useful information for the elucidation of the precise structure of dextran, since the fragment oligosaccharides obtained can be reconstructed according to the mode of substrate specificity of the hydrolyzing enzymes. However, hydrolysis of highly branched dextrans with common dextranases is restricted by the occurrence of non-(1→6)-α-linked branches in the molecule. This paper deals with the preliminary characterization of a novel type of dextranase having specificity to (1→2)-linkage of highly branched dextrans.

MATERIALS AND METHODS

A gram negative bacterium (strain M-73) was isolated from soil as a source of dextranase. The bacterium was grown in a medium containing yeast extract (0.3%), $(NH_4)_2HPO_4$ (0.2 %), KH_2PO_4 (0.1%), $MgSO_4 \cdot 7H_2O$ (0.05%), KCl (0.05%), $FeSO_4 \cdot 7H_2O$ (0.001%) and water-soluble dextran (0.5%) of Leuconostoc mesenteroides NRRL B-1299 (1) in deionized water at pH 7.0. Cultivation was carried out with shaking for 7 days at 30°.

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The culture supernatant was subjected to ammonium sulfate fractionation, and the precipitate at complete saturation was collected, redissolved in water, and dialyzed overnight. The dialyzate was applied to a DEAE-cellulose column (1.9 x 12 cm) pre-equilibrated with 0.01 M acetate buffer (pH 5.6). Major part of the dextranase activity was eluted with 0.2 M NaCl, and was used as a crude dextranase preparation.

From the results of preliminary experiments, the occurrence of two types of dextranases was demonstrated; dextranase I releases glucose from the B-1299 dextran and dextranase II releases isomaltotriose from various dextrans. A dextranase II-free preparation was prepared by the heat treatment of the crude enzyme for 10 min at 50°.

The dextranase activity was determined by incubating 0.5 ml of the diluted enzyme with 0.5 ml of 0.5% dextran solution in 0.05 M acetate buffer (pH 5.6) for 10 min at 40°. Reaction was stopped by adding 1.0 ml of the alkaline copper reagent, and the digest was assayed for reducing sugar by the Nelson-Somogyi method as described previously (2). One unit of the dextranase activity was defined as the amount of enzyme reguired to liberate 1 μmole of glucose per min under the conditions described above.

Acetolysis and methylation analysis were carried out as described previously (1,3). Dextran of L. mesenteroides NRRL B-1298 was prepared by the method of Jeanes (4). Endo-dextranase from Chaetomium gracile was kindly provided from Sankyo Co, (5).

RESULTS AND DISCUSSION

Isolation of the two dextranase activities. Dextrans elaborated by some strains of Leuconostoc mesenteroides such as NRRL B-1298 (6), B-1299 (3), and B-1397 (7) have highly branched structures in which most of the (1→2)-α-linkages (12 - 34%) constitute branch points. The water-soluble dextran of the strain B-1299 has been shown to contain 27.9 - 36.7% of (1→2)-α-linkages (3). Moreover, the analytical data from ^{13}C-n.m.r. spectra of the B-1299 soluble dextran have suggested that (1→2)-linked branches are short in length, mostly composed of one glucose unit or two (8).

As shown in Fig. 1a, the B-1299 soluble dextran was appreciably hydrolyzed with the crude dextranase preparation. Two main components eluted at Fr.no. 59-67 and 75-80 were identified by paper chromatography as isomaltotriose and glucose, respectively. Thermal stability test of the crude enzyme showed that dextran-

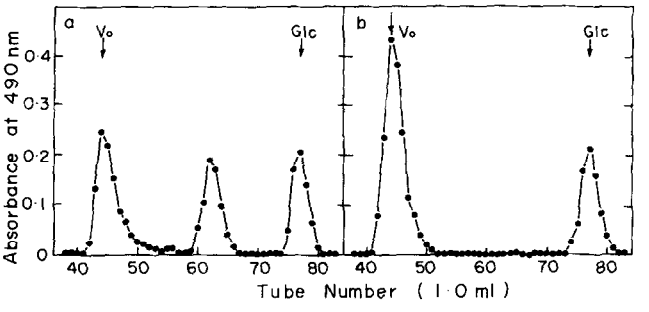
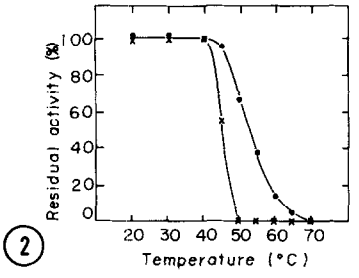


Fig. 1. Elution pattern of the dextranase digests of the B-1299 dextran from a Sephadex G-15 column. Each digest (2.5 mg/0.5 ml) was applied to the column (1.5 x 84 cm) and eluted with deionized water. (a) digest with the crude dextranase; (b) digest with dextranase I.

ase II, assayed with clinical dextran as the substrate, was completely inactivated by heat treatment for 10 min at 50°, whereas 67% of dextranase I survived (Fig. 2). The end-products by the action of dextranase I on the B-1299 dextran were only glucose and limit dextrin (Fig. 1b).

Characteristics of dextranase I. The optimum pH and temperature of dextranase I were pH 5.6 - 6.0 and 40 - 45°, respectively. The hydrolysis of the B-1299 dextran with this enzyme reached a maximum in 6 hr and the degree of hydrolysis (D.H.) was 28.1%. The substrate specificity of dextranase I was examined by incubating the enzyme with α-glucans containing various types of linkage for 20 hr. As shown in Table I, D.H. values of dextran from the strains B-1298 and B-1299 were 25.6% and 28.1%, respectively. On the contrary, α-glucans containing no (1→2)-linkage could not be hydrolyzed by this enzyme. Thus, dextranase I was shown to have a strict specificity to (1→2)-α-glucosidic linkage. However, dextranase I could not hydrolyze disaccharides such as isomaltose, kojibiose and nigerose.

Dextrans from the strains B-1298 and B-1299 were hydrolyzed



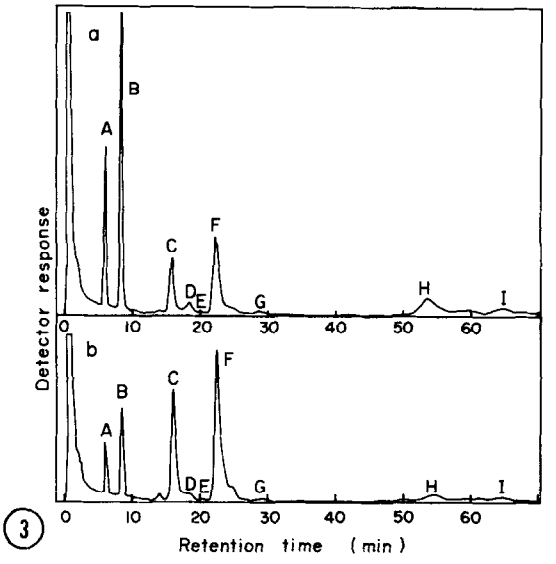


Fig. 2. Thermal stability of the crude dextranase. After the incubation for 10 min at the temperature indicated, the remaining activity was assayed for dextranase I (●) and II (x) using the B-1299 and clinical dextrans, respectively, by the standard method.

Fig. 3. GLC pattern of methyl glycosides from the methylated B-1299 dextran (a) and its limit dextrin (b). A,B; 2,3,4,6-tetra-O-methyl-D-glucose, C,F; 2,3,4-tri-O-methyl-D-glucose, D,F; 3,4,6-tri-O-methyl-D-glucose, E,G; 2,4,6-tri-O-methyl-D-glucose, H,I; 3,4-di-O-methyl-D-glucose.

with dextranase I for 20 hr. The elution patterns of the digests from a Sephadex G-15 column showed that the final products from both dextrans were only glucose (25.6 - 28.1%) and limit dextrin (71.9 - 74.7%).

Structure of the dextranase I limit dextrin. Methylation analysis of the limit dextrin from B-1299 dextran produced by the action of dextranase I was carried out to determine the molar proportions of the various glucosidic linkages. The relative amount of 2,3,4,6-tetra-O-methyl-glucose in the fully methylated limit dextrin was significantly decreased as compared with that of the original B-1299 dextran (Fig. 3). The difference of the

TABLE I Substrate specificity of dextranase I

Substrate	Major linkage	D.H. (%)
B-1299 dextran	(1→6), (1→2)*, (1→3)	28.1
B-1298 dextran	(1→6), (1→2)*, (1→3)	25.6
B-1307 dextran	(1→6), (1→3)*	0
Clinical dextran	(1→6), (1→3)*	0
Soluble starch	(1→4), (1→6)*	0
Glycogen	(1→4), (1→6)*	0
Pseudonigeran	(1→3)	0
Mutan IG-1[a]	(1→3)*, (1→6)	0
Lentinus α-glucan[b]	(1→3), (1→4)	0

[a,b] See details ref. (9) and (10), respectively.

* Most of these linkages except Mutan IG-1 constitute branch points.

average chain length of these two glucans, the original B-1299 dextran (CL 3) and its limit dextrin (CL 4), indicated that about one fourth of the branch points through (1→2)-α-linkage were cleaved by this enzyme. Thus, the limit dextrin has a less branched structure and seems to be more susceptible to endo-dextranase. In fact, this was confirmed by the hydrolytic pattern of limit dextrin with endo-dextranase of Chaetomium gracile (5). As shown in Table II, the D.H. value of the limit dextrin (33.0%) was by far higher than that of the B-1299 dextran (4.1%). Moreover, the D.H. of the B-1299 dextran (51.3%) obtained by the combined use of dextranase I and endo-dextranase was as high as that of clinical dextran (55.6%).

TABLE II Effects of endo-dextranase on the dextranase I action

Substrate	Enzyme used		D.H. (%)
	dextranase I	endo-dextranase	
B-1299 dextran	+	-	30.4
B-1299 dextran	-	+	4.1
B-1299 dextran	+	+	51.3
B-1299 limit dextrin[a]	-	+	33.0
Clinical dextran	+	-	0
Clinical dextran	-	+	55.6
Clinical dextran	+	+	54.8

[a] Prepared from the hydrolyzate with dextranase I as described in the text.

Whereas acetolysis of the original B-1299 dextran gave glucose (53.6%) and kojibiose (46.4%) almost in equal proportion, acetolyzate of the limit dextrin consisted predominantly of glucose (71.8%) and a lesser amount of disaccharides (19.7%) composed of about equal portions of isomaltose and kojibiose. Molar ratio of 3,4,6-tri-O-methyl-glucose in the two methylated glucans were almost constant (9.1 - 10.1%). Therefore, (1→2)-linkage in the limit dextrin was distributed predominantly in the linear chains of the dextran. Moreover, the limit dextrin was excluded from a Sepharose 6B column and seems to have a molecular weight of the same order of the original dextran. This indicates that (1→2)-α-linkage in the linear chains is not hydrolyzed with this enzyme, since no significant decrease of molecular weight of the limit dextrin has occurred.

These results from the structural analyses of the limit dextrin suggest that dextranase I can hydrolyze only branched (1→2)-α-linkages in dextran probably by exo-type of action. A more definite conclusion must be drawn from the results using purified enzyme and various oligosaccharides as substrates.

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