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RELATIVE, QUANTITATIVE EFFECTS OF ACCEPTORS IN THE REACTION OF *Leuconostoc mesenteroides* B-512F DEXTRANSUCRASE*

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ABSTRACT

The acceptor reaction of dextransucrase consists of the transfer of D-glucosyl groups from sucrose to other carbohydrates, and occurs at the expense of dextran synthesis. In the present study, solutions of [^{14}C]sucrose and of each of seventeen acceptor sugars were digested with highly purified *Leuconostoc mesenteroides* B-512F dextransucrase. The products were separated by paper chromatography, and quantitated by liquid scintillation counting. Maltose was the most effective acceptor; its products, members of an isomaltodextrinyl-maltose series (d.p. 3 to 6), accounted for >75% of the D-glucosyl groups of sucrose. Other acceptors giving rise to a similar series of oligosaccharide products were (in order of decreasing effectiveness): isomaltose, nigerose, methyl α-D-glucoside, 1,5-anhydro-D-glucitol, D-glucose, turanose, methyl β-D-glucoside, cellobiose, and L-sorbose. Lactose, raffinose, melibiose, D-galactose, and D-xylose each gave a single, mono-D-glucosylated product; D-fructose and D-mannose each gave a pair of mono-D-glucosylated (disaccharide) products. Another series of digests contained sucrose and various proportions of maltose. As the level of maltose increased, the size of the largest oligosaccharide acceptor-product decreased, and less dextran was produced. The virtual absence of high-d.p. (8 to 13) oligosaccharide products in all acceptor digests is interpreted as evidence against a role for acceptors as primers of dextran synthesis.

INTRODUCTION

Dextransucrase polymerizes the D-glucosyl moiety of sucrose, to give the α-(1→6)-linked polysaccharide, dextran. When carbohydrates other than sucrose are added to sucrose–dextransucrase digests, the D-glucosyl group of sucrose is diverted from the synthesis of dextran and is added to the carbohydrate[1]. This carbohydrate has been called an acceptor[1–4]. When the acceptor is a sugar of low

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molecular weight, such as a monosaccharide, disaccharide, or trisaccharide, the product is also of low molecular weight, increased only by the molecular weight of the added D-glucosyl group. Some of these acceptor products are also acceptors[1], and, in these cases, a series of homologous acceptor-products results[1-4]. One such series is that of the methyl α-isomaltosides that result when methyl α-D-glucoside is the acceptor[5]. With other acceptors, the first acceptor product (*i.e.*, the product in which one D-glucosyl group has been added to the acceptor) is a poor acceptor or a nonacceptor, and a single acceptor product preponderates. An example of this is the reaction of D-fructose as an acceptor, to give leucrose[1] (5-*O*-α-D-glucopyranosyl-D-fructopyranose).

It has been observed that many different carbohydrates will act as acceptors. A total of twenty-six different acceptors has been recognized[6], and the structures of most of the products have been determined[1-7]. Initially, the acceptor was considered to be a primer or dextran-chain initiator[3,8]. The addition of low-molecular-weight acceptors, however, diverts the D-glucosyl group of sucrose away from making dextran, and the amount of dextran and its molecular weight are actually decreased[1,4]. The mechanism of the acceptor reactions has been studied[1]; it was found that the acceptor interacts with a covalent enzyme–D-glucosyl or enzyme–dextranosyl intermediate to release the D-glucosyl or dextranosyl units from the enzyme active-site, with formation of a covalent linkage between D-glucose or dextran and the acceptor[1]. The acceptor reaction thus terminates the polymerization of dextran by releasing it from the active site, and thereby decreases the amount and molecular weight of the dextran. The amount and size of the dextran is also decreased by the release of the D-glucosyl unit, which would otherwise be incorporated into the dextran molecule.

Some of the acceptor products reported have been formed by the addition of the acceptor to growing cultures of *Leuconostoc mesenteroides*[7], or by the use of dextransucrase in a cell-free, culture supernatant liquor[3,5,9]. Although Ebert and Schenk[2] classified some of the acceptors as "strong", "intermediate", and "weak", there has not been a systematic, quantitative study of the relative effectiveness of the different acceptors. We therefore studied the relative reactions of seventeen acceptors by using ^{14}C-labeled sucrose, consistent conditions of temperature, pH, substrate and acceptor concentrations, and highly purified dextransucrase from *L. mesenteroides* B-512F. We determined the number of products formed for each acceptor, their amounts, and the amount of dextran formed. The relative effectiveness of the different acceptors was determined by comparing the percentage of D-glucose incorporated into the various oligosaccharide-acceptor products.

EXPERIMENTAL

Enzyme. — Highly purified *L. mesenteroides* B-512F dextransucrase (76 IU/mg), containing about 0.2 μg of carbohydrate/IU, was obtained by the method of Miller and Robyt[10]. Enzyme activity was determined by a radiochemical assay

using [^{14}C]sucrose[11], and is given in International Units (IU), that is, in μmol of D-glucose incorporated per min into dextran at pH 5.3 and 22°.

Carbohydrates. — [U-^{14}C]Sucrose was obtained from New England Nuclear, Boston, MA. D-Xylose, D-fructose, D-galactose, D-glucose, D-mannose, methyl β-D-glucoside, L-sorbose, cellobiose, lactose, melibiose, raffinose, and turanose were obtained commercially. Methyl α-D-glucoside and maltose were obtained commercially, and recrystallized. 1,5-Anhydro-D-glucitol was synthesized from D-glucose[12]; isomaltose was prepared by hydrolysis of B-512F dextran with endodextranase and was purified by chromatography on a column of silica gel; nigerose was prepared by acetolysis of *L. mesenteroides* B-1355 glucan-S (alternan)[13], and was purified by chromatography on a column of silica gel.

Acceptor-reaction digests. — Dextransucrase acceptor-reaction digests (75 μL) contained 80mM [U-^{14}C]sucrose (3.7 MBq; 1 μCi), 80mM acceptor sugar, 20mM pyridine–acetic buffer (pH 5.3), 2mM calcium chloride, 0.01% sodium azide, and 120 mIU of dextransucrase. The reactions were conducted for 3 h at 27°, and all of the sucrose was consumed. Aliquots (25 μL) were spotted onto Whatman 3MM paper (23 × 57 cm) for descending chromatography at 37° with (*a*) 10:4:3 (v/v/v) ethyl acetate–pyridine–water (14 h) or (*b*) 2:1 (v/v) 1-propanol–water (24 h). Both chromatographic sovents were used for all digests. They are complementary in their separating properties: solvent (*a*) resolves various monosaccharides (*e.g.*, D-glucose, D-fructose, and D-galactose), whereas solvent (*b*) resolves oligosaccharide series up to d.p. 8, and moves oligosaccharides of up to d.p. 13 from the origin. Labeled products were located by autoradiography (8 days exposure). The individual labeled compounds were cut out from the chromatogram, and the papers were added to toluene scintillation cocktail (20 mL), and counted in a liquid scintillation spectrometer.

Acceptor reaction as a function of acceptor concentration. — Various concentrations of maltose acceptor (0–200mM) were added to a constant concentration (80mM) of [^{14}C]sucrose under the same conditions as in the other acceptor reactions already described. The amount of [^{14}C]dextran formed (measured as methanol-insoluble material) was determined by adding aliquots (25 μL) to 1.5-cm squares of Whatman 3MM paper, which were then subjected to six 10-min washes in methanol (100 mL). The ^{14}C label remaining on the paper was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

The quantitative incorporation, by B-512F dextransucrase, of D-glucose from sucrose into products, in the presence of each of 17 acceptors, is summarized in Fig. 1 and Table I. Ten of the acceptors gave a series of homologs, which were formed from each of the preceding acceptor-products; *i.e.*, d.p. 3 gave d.p. 4, which gave d.p. 5, *etc.* Each higher homolog was produced in decreasing amounts. In no case did oligosaccharides having a d.p. of 8 to 13 contain more than 2% of the total D-glucosyl groups transferred.

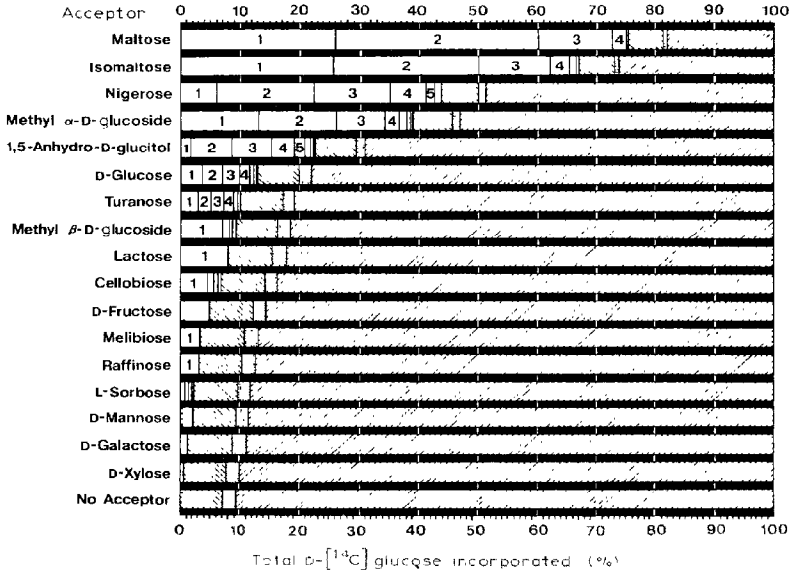

Fig. 1. Distribution of D-[^{14}C]glucose from sucrose in acceptor reaction-products. (Members of oligosaccharide acceptor product series are shown in order of increasing d.p. from left to right, with the number of added D-glucosyl units shown where possible. D-Fructose and D-mannose each gave two mono-D-glucosylated products. Key: ▭, added sugar acceptor products; ▨, free D-glucose ▭, D-fructose acceptor products; and ▨, dextran.)

In the synthesis of dextran from sucrose by B-512F dextransucrase, leucrose [*O*-α-D-glucopyranosyl-(1→5)-D-fructopyranose] and isomaltulose [*O*-α-D-glucopyranosyl-(1→6)-D-fructofuranose] are always observed as minor products; they result from acceptor reactions of the D-fructose released from sucrose in the polymerization reaction of the D-glucosyl moiety[14]. In addition, free D-glucose is produced, apparently by the acceptor action of water, which releases the D-glucosyl unit from the active site[15,16]. We found that, when no acceptor, or a poor acceptor, was added to the digest, the two D-fructose acceptor products and free D-glucose accounted for ~2–2.5% and ~7–7.5% of the total D-glucose incorporated; in the presence of a good acceptor (*e.g.*, any of the first five in Table I), these levels dropped to 0.6–1.5% and 5.9–6.9%, respectively.

In the present study, the molar ratio of acceptor to sucrose was 1:1 for all of the acceptors. Maltose was the most effective acceptor, diverting 75% of the D-

TABLE I

PERCENT OF THE TOTAL D-GLUCOSE FROM SUCROSE IN PRODUCTS OF *Leuconostoc mesenteroides* B-512F DEXTRANSUCRASE ACCEPTOR REACTIONS

Acceptor	*Dextran*	*Leucrose and iso-maltulose*	*Free D-glucose*	*Acceptor products*									*Percent of acceptor in-corporated*	*Relative acceptor efficiency*
				Total	*d.p. 2*	*d.p. 3*	*d.p. 4*	*d.p. 5*	*d.p. 6*	*d.p. 7*	*d.p. 8*	*d.p. >8*		
Maltose	18.0	0.6	5.9	75.5	—	26.1	34.0	12.6	2.5	0.3	0	0	48.0	**100.0**
Isomaltose	26.2	0.8	6.0	67.0	—	25.7	24.4	12.0	3.3	1.1	0.5	0	43.0	88.7
Nigerose	48.7	1.1	6.3	43.9	—	6.3	16.3	12.7	6.0	1.6	[a]	1.0	20.5	58.1
Methyl α-D-glucoside	53.1	1.3	6.7	38.9	13.3	12.9	8.1	2.1	1.2	0.6	0.3	0	23.4	51.5
1,5-Anhydro-D-glucitol	69.0	1.5	6.9	22.6	1.9	6.8	6.8	3.8	1.6	1.0	0.5	0.2	9.1	29.9
D-Glucose	77.9	2.1	7.1	12.9	3.7	3.4	2.8	1.7	0.7	0.4	[a]	0.2	6.9	17.1
Turanose	80.7	1.9	7.3	10.1	—	3.1	2.1	2.2	1.6	0.6	0.4	0.1	5.5	13.4
Methyl β-D-glucoside	81.5	2.2	7.0	9.3	7.2	0.9	0.7	0.5	0	0	0	0	8.0	12.3
Lactose	82.1	2.3	7.5	8.1	—	8.1	0	0	0	0	0	0	8.1	10.7
Cellobiose	83.6	2.1	7.5	6.8	—	4.5	1.1	0.7	0.5	0	0	0	5.4	9.0
D-Fructose	85.6	2.2[b]	7.4	4.8	4.2, 0.6[c]	0	0	0	0	0	0	0	4.8	6.4
Raffinose	86.8	2.4	7.5	3.3	—	—	3.3	0	0	0	0	0	3.3	4.4
Melibiose	87.4	2.2	7.2	3.2	—	3.2	0	0	0	0	0	0	3.2	4.2
L-Sorbose	88.2	2.2	7.2	2.4	0.8	0.7	0.5	0.4	0	0	0	0	1.4	3.2
D-Mannose	88.3	2.0	7.2	2.2	2.0, 0.2[d]	0	0	0	0	0	0	0	2.2	2.9
D-Galactose	88.9	2.5	7.3	1.3	1.3	0	0	0	0	0	0	0	1.3	1.7
D-Xylose	89.9	2.2	7.5	0.4	0.4	0	0	0	0	0	0	0	0.4	0.5
None	90.6	2.1	7.3	—	—	—	—	—	—	—	—	—	—	—

[a]Included with d.p. >8 products. [b]Acceptor products of the D-[^{14}C]fructose released from sucrose only, not the products of added acceptor D-fructose; calculated from the average amounts of D-fructose acceptor products in other digests. [c]Leucrose and isomaltulose, respectively; from added D-fructose only (see footnote *b*). [d]α-D-Glucopyranosyl β-D-mannopyranoside and unknown disaccharide, respectively.

glucosyl groups of sucrose away from dextran and into a series of homologs of panose [*O*-α-D-Glc*p*-(1→6)-*O*-α-D-Glc*p*-(1→4)-D-Glc*p*] containing one to five transferred D-glucosyl units (d.p. 3 to 7). Higher homologs, of d.p. 8 to 13, were absent (see Fig. 1 and Table I). The next five most effective acceptors (isomaltose, nigerose, methyl α-D-glucoside, 1,5-anhydro-D-glucitol, and D-glucose) each gave a series of homologs in which D-glucopyranosyl and isomaltodextrinyl units were attached to O-6 of the acceptor sugars[3,5,17]. For isomaltose and nigerose, as with maltose, the attachment was to O-6 of the nonreducing residue. A series of homologs was also observed for turanose and methyl β-D-glucoside, the seventh and eight best acceptors, but the structures of these compounds have not yet been determined. Presumably, the series is composed of isomaltodextrins attached to O-6 of the D-glucosyl group in both cases.

Lactose and cellobiose, the ninth and tenth most effective acceptors, are both known to form acceptor products at O-2 of the (reducing-end) D-glucose residue[7]. In the present study, cellobiose was also observed to form a series of acceptor products, d.p. 3 to 6, which are, most probably, 2-*O*-isomaltodextrinyl-cellobioses. In contrast, lactose, an isomer of cellobiose having a D-galactopyranosyl instead of a D-glucopyranosyl group at the nonreducing end, gave only a single, mono-D-glucosylated product (see Fig. 1 and Table I). It is interesting that the other D-galactose-containing saccharides studied, raffinose [β-D-Fru*f* *O*-α-D-Gal*p*-(1→6)-α-D-glucopyranoside] and melibiose [*O*-D-Gal*p*-(1→6)-D-Glc*p*], also gave only a single mono-D-glucosylated acceptor-product. The raffinose product is known to have a D-glucopyranosyl group attached α-(1→2) to the D-glucoside residue[18], and presumably, the melibiose acceptor-product has a similar structure, with a D-glucopyranosyl group attached α-(1→2) to the (reducing) D-glucose residue.

D-Fructose gave two monoglucosylated products, leucrose (4.2% of incorporated D-glucose) and isomaltulose (0.6%). D-Mannose also gave two products. The major product (2% of incorporated D-glucose) is an unusual, nonreducing disaccharide, α-D-glucopyranosyl β-D-mannopyranoside[9]. The trace (0.2%) of a mono-D-glucosylated product is of unknown structure. D-Galactose gave a single acceptor-product (1.7% of incorporated D-glucose), namely, the nonreducing disaccharide α-D-glucopyranosyl β-D-galactofuranoside[9]. D-Xylose, which had previously been reported to be a nonacceptor, gave a trace (0.4% of incorporated D-glucose) of a mono-D-glucosylated product. α,α-Trehalose, as previously reported[7], did not give any acceptor products.

The amount of D-glucose diverted from dextran into acceptor products is dependent on the ratio of the concentration of the acceptor to that of the sucrose. Fig. 2 shows the effect on the formation of dextran when various concentrations of maltose (0–200mM) were added to digests containing 80mM [^{14}C]sucrose. The amount of dextran dropped rapidly as the maltose concentration was increased to 60mM; at higher concentrations, the amount of dextran decreased more slowly, but, even at 200mM (a maltose to sucrose ratio of 2.5:1), dextran was still produced at ~9% of the amount produced in the absence of maltose. At this ratio, only the first four

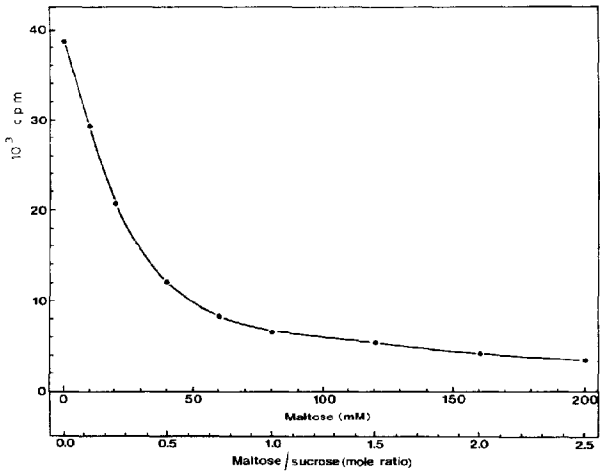

Fig. 2. Incorporation of D-[^{14}C]glucose from sucrose into dextran, as a function of maltose concentration. (The initial sucrose concentration was 80mM.)

TABLE II

MOLAR DISTRIBUTIONS OF ACCEPTOR PRODUCTS

Acceptor	*Percent of total moles of acceptor product*							
	d.p. 2	*d.p. 3*	*d.p. 4*	*d.p. 5*	*d.p. 6*	*d.p. 7*	*d.p. 8*	*d.p. >8*
Maltose	—	54	36	9	1	0.2	0	0
Isomaltose	—	60	28	9	2	<0.5	<0.2	0
Nigerose	—	31	40	20	7	1	[a]	<1
Methyl α-D-glucoside	57	28	11	3	1	<0.5	0	0
1,5-Anhydro-D-glucitol	20	37	25	10	3	2	1	<2
D-Glucose	53	25	13	6	1	1	[a]	<2
Turanose	—	56	20	12	7	2	2	<2

[a]Included with d.p. >8 products.

oligosaccharide homologs were detected in t.l.c. (on plates of Whatman K5F silica gel irrigated twice with 5:2:4:4 (v/v/v/v) 1-propanol–nitromethane–acetonitrile–water). Further increases in the concentration ratio to 10:1 have been reported to give mainly a single D-glucosylated product, namely, panose[17].

With regard to the possible priming-mechanism of the acceptor for dextran synthesis, it is seen that all of the acceptors giving rise to a homologous series gave decreasing amounts of oligosaccharide acceptor-products with increasing d.p. (see

Table II). Even with the best acceptor, namely, maltose, we found a discontinuous distribution of label into low-molecular-weight acceptor-products and dextran; intermediate acceptor-oligosaccharides of d.p. 7 to 10 are present at very low levels, if at all. If the acceptors were acting as primers for dextran synthesis, it would be expected that the reverse would be observed, *i.e.*, the amounts of oligosaccharide intermediates would increase with increasing d.p., and would merge with the material of higher d.p., namely, dextran. Furthermore, it would be expected that, as the concentration of the so-called primer is increased, there would be a stimulation of higher oligosaccharide and dextran synthesis due to the increase in the concentration of priming sites. The opposite is, however, observed; as the concentration of the acceptor is increased, there is a decrease in the amount of dextran (see Fig. 2), and there are no intermediate oligosaccharides of d.p. 7 and higher. The mechanism of action of the acceptors is thus, as has previously been shown[1], not one of priming dextran synthesis, but one of terminating dextran synthesis by the release of D-glucose and dextran from the active site of dextransucrase to form acceptor products.

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