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DISPROPORTIONATION REACTIONS CATALYZED BY *Leuconostoc* AND *Streptococcus* GLUCANSUCRASES*

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ABSTRACT

Glucansucrases from *Leuconostoc mesenteroides* NRRL B-512F and *Streptococcus mutans* 6715 were found to utilize a number of D-gluco-oligosaccharides as D-glucosyl donors and as acceptors. These donors included isomaltotriose and its homologs, panose, maltotriose, and dextran. In each case, D-glucosyl groups were transferred from the donor to an acceptor sugar. When the donor sugar also acted as an acceptor, disproportionation reactions occurred. Isomaltotriose, for example, gave rise to isomaltose and isomaltotetraose initially, and to a series of isomalto-oligosaccharides eventually. In addition to forming α-D-(1→6) linkages in these reactions, dextransucrase from *S. mutans* 6715 was capable of forming α-D-(1→3)-linked products.

INTRODUCTION

Glucansucrases are D-glucosyltransferases that catalyze the synthesis of D-glucans from sucrose. *Leuconostoc mesenteroides* NRRL B-512F dextransucrase synthesizes a dextran that has 95% of α-D-(1→6) linkages and 5% of α-D-(1→3) branch linkages[1]. *Streptococcus mutans* 6715 produces two D-glucans from sucrose, a water-soluble dextran containing 73% of α-D-(1→6) linkages and 27% of α-D-(1→3) branch linkages[2], and a water-insoluble glucan ("mutan") that contains 93% of α-D-(1→3) linkages and 7% of α-D-(1→6) linkages[3]. The enzymes that synthesize the soluble and insoluble D-glucans are usually referred to as GTF-S and GTF-I, respectively[4,5].

Although sucrose is the usual D-glucosyl donor for these enzymes, other substrates can act as D-glucosyl donors. These include α-D-glucopyranosyl fluoride[6], α-D-glucopyranosyl α-L-sorbofuranoside[7], and 4^F-*O*-α-D-galactopyranosylsucrose[8].

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Reactions in which glycosyl groups are transferred from one saccharide donor to identical, or similar, saccharide acceptors are known as disproportionation reactions (*e.g.*, 2 maltose $\rightleftharpoons$ D-glucose + maltotriose), and have been observed for such enzymes as cellulase[9], amylase[10], glucoamylase[11], transglucosylase[12], glucodextranase[11], and isomaltodextranase[13]. Disproportionation reactions catalyzed by glucansucrases are, however, not well known, and have been overlooked by most investigators and reviewers[14,15].

In control studies on acceptor specificities of purified *L. mesenteroides* NRRL B-512F dextransucrase and *S. mutans* 6715 GTF-S, we have observed that a number of saccharides can act as D-glucosyl donors, as well as acceptors.

EXPERIMENTAL

Carbohydrates. — Isomalto-oligosaccharides were prepared by partial hydrolysis of commercial B-512F dextran with acid. A solution of dextran (Sigma Chem. Co., St. Louis, MO; 15 g) in 0.3M trifluoroacetic acid (500 mL) was heated on a steam bath for ~2–3 h, and individual oligosaccharides were isolated from the resulting mixture by chromatography on a column of charcoal[16]. Panose was prepared by the acceptor reaction of dextransucrase with sucrose and maltose[17]. Maltotriose was prepared by the method of French *et al.*[18]. Maltose was purchased from EM Laboratories, Elmsford, NY; T-10 dextran, from Pharmacia Fine Chemicals, Uppsala, Sweden; and methyl α-D-glucopyranoside, from Eastman Kodak Co., Rochester, NY, was recrystallized. Planteose[19] and isopanose[20] were obtained from the laboratories of the late Professor Dexter French. Turanose and melibiose were purchased from Sigma Chemical Co., and melezitose, from Nutritional Biochemicals Corp., Cleveland, OH.

Enzymes. — *Streptococcus mutans* 6715 was grown on the medium described by Ciardi *et al.*[21]. After removal of cells, and concentration of the filtrate in a Pellicon membrane, cassette filtration-system (Millipore Corp., Bedford, MA), the exocellular glucansucrases were separated, and purified, by the following, successive steps: Bio-Gel A-15m chromatography[2]; DEAE-cellulose chromatography[22] in 20mM sodium phosphate buffer, pH 6.8, by using a 0 to 0.2M gradient of sodium chloride; DEAE-Bio-Gel A chromatography[23], using the same buffer and gradient; and Sephadex G-50 affinity chromatography[23]. It contained 45 IU of GTF-S per mg of protein when assayed in the presence of 3.3 mg of T-10 dextran/mL.

Leuconostoc mesenteroides B-512F dextransucrase was purified by the method of Miller and Robyt[24]; briefly, this entailed chromatography on DEAE-cellulose, Sephadex G-200 affinity chromatography, and DEAE-Trisacryl chromatography. The specific activity was 76 IU/mg.

Chromatography. — Oligosaccharides were separated, and analyzed, by thin-layer chromatography on plates of Whatman K5F silica gel in one of the following solvents: (*A*) 5:2:4:4 (v/v/v/v) 1-propanol–nitromethane–acetonitrile–water, or (*B*) 17:3 (v/v) acetonitrile–water. The sugars were visibilized by spraying

the plates with 20% (v/v) sulfuric acid in methanol, followed by heating for 10 min at 120°.

Sephadex was purchased from Pharmacia Fine Chemicals; Bio-Gel, from Bio-Rad Laboratories (Richmond, CA); DEAE-cellulose, from Sigma Chemical Co; and DEAE-Trisacryl, from LKB Instruments, Inc. (Gaithersburg, MD).

Reaction conditions. — All reactions were conducted at 30° in 50mM sodium acetate buffer, pH 5.5, containing 0.02% of sodium azide, unless stated otherwise.

Oligosaccharide disproportionation-reactions were performed by treating 0.1M saccharide (0.1 mL) with 0.1 mL of enzyme that contained ~5 IU of *S. mutans* 6715 GTF-S/mL, or 10 IU of *L. mesenteroides* B-512F dextransucrase/mL. Mixtures for reactions in the presence of sucrose contained 0.1M oligosaccharide (0.1 mL), 90 μL of one of the enzymes described, and 1.0M sucrose (10 μL). The reactions between dextran and methyl α-D-glucoside contained enzyme (116 μL), 0.6M methyl α-D-glucoside (17 μL), and 67 μL of a solution containing 150 mg of T-10 dextran/mL.

RESULTS

Both *Streptococcus mutans* 6715 GTF-S and *Leuconostoc mesenteroides* B-512F dextransucrase were found to disproportionate isomalto-oligosaccharides.

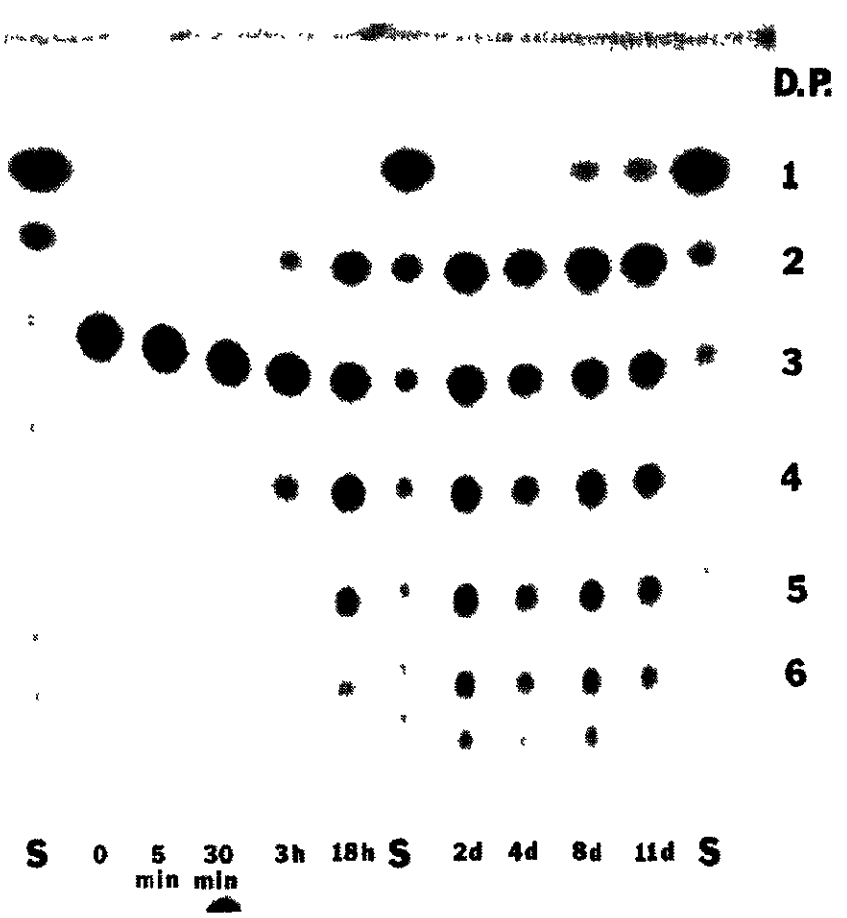

Fig. 1. Thin-layer chromatogram of products arising from the action of *Streptococcus mutans* 6715 GTF-S on isomaltotriose. [Two ascents in solvent *A*, at 37°. S indicates a series of isomalto-oligosaccharides, used as standards. D.p. refers to the degree of polymerization of each of the isomalto-oligosaccharides. Reaction times, from left to right: t = 0, 5 min, 30 min, 3 h, 18 h, 2 d, 4 d, 8 d, and 11 d. Five μL of each reaction mixture was chromatographed.]

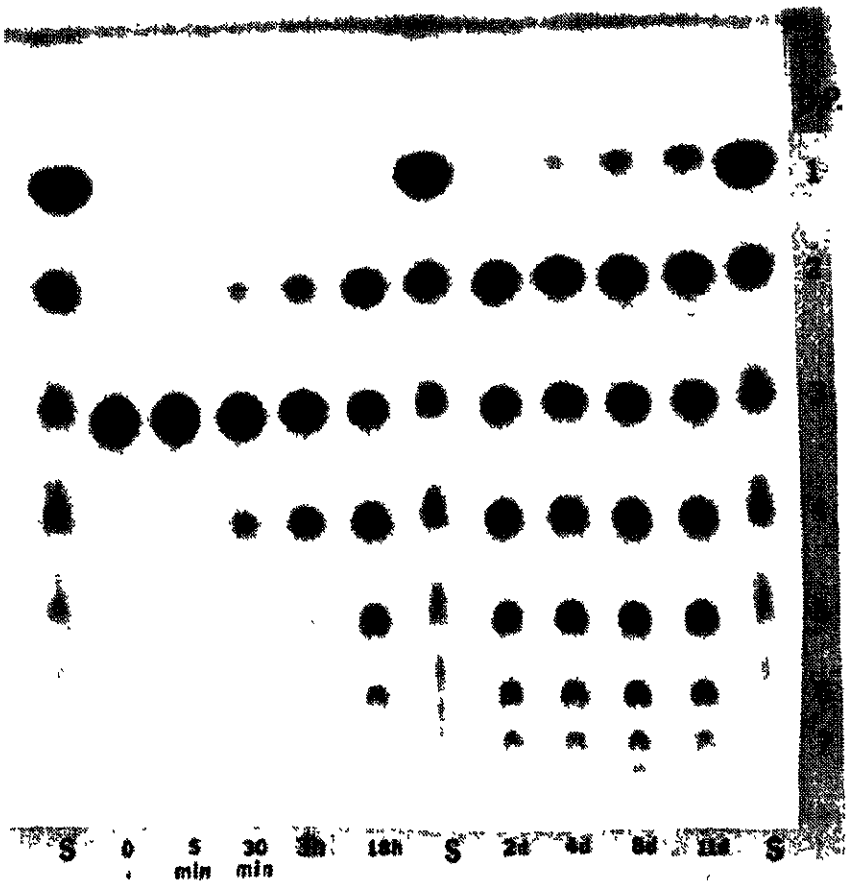

Fig. 2. Thin-layer chromatogram of products arising from the action of *Leuconostoc mesenteroides* B-512F dextransucrase on isomaltotriose [For details, see Fig. 1]

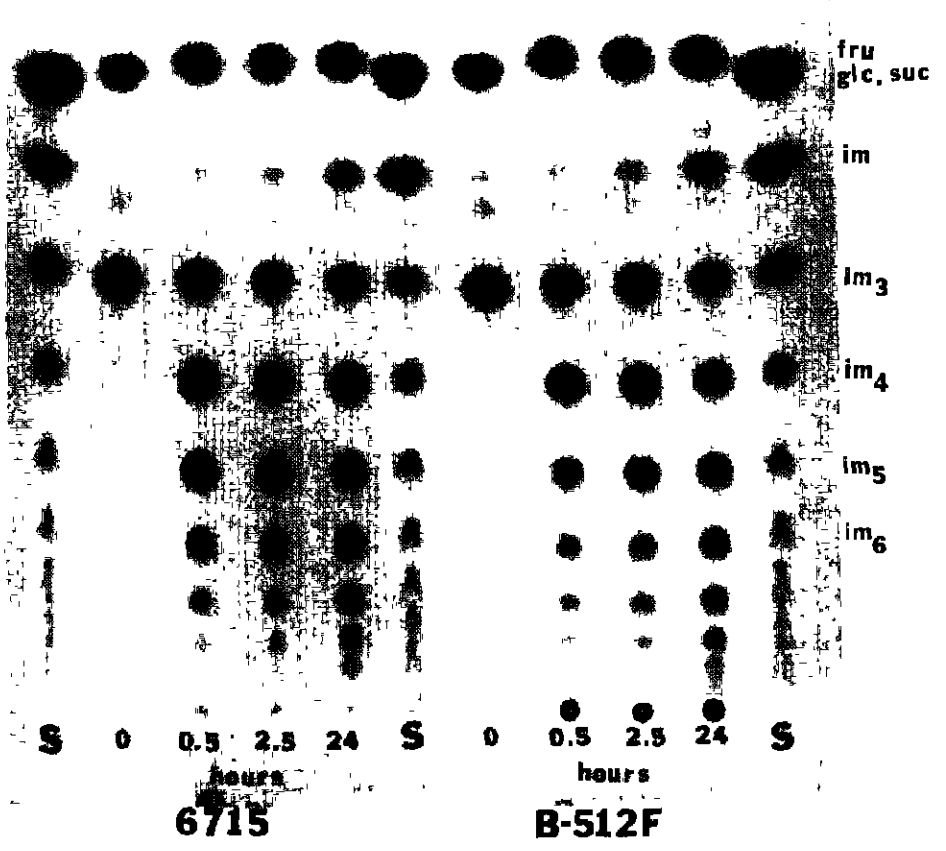

Fig. 3. Thin-layer chromatogram of products arising from the action of dextransucrases on isomaltotriose in the presence of sucrose. [S refers to isomalto-oligosaccharide standards. Left side of plate: *Streptococcus mutans* 6715 GTF-S reaction mixture; time points taken at t = 0, 0.5, 2.5, and 24 h. Right side of plate: *Leuconostoc mesenteroides* B-512F dextransucrase action on isomaltotriose in the presence of sucrose. Time points same as for *S. mutans*. Five μL of each mixture was chromatographed for two ascents at 37° in solvent *A*.]

Figs. 1 and 2 show that the first products to appear from the reaction with isomaltotriose are isomaltose and isomaltotetraose. After further reaction, higher-d.p. isomalto-oligosaccharides appear that differ from one to the next by a single D-glucosyl unit. Eventually, after prolonged reaction-times, D-glucose is also produced in small proportion. Fig. 3 shows that the products formed in the presence of sucrose are the same as those formed in the absence of sucrose, but that the formation of these products is much faster when sucrose is the D-glucosyl donor. It should be noted that the two enzymes form the same products (isomalto-oligosaccharides), even though the D-glucans formed by each differ significantly in their structures. It was, however, observed that, after much longer incubation times, the *S. mutans* GTF-S produced small amounts of other products which may contain linkages other than α-D-(1→6).

On the other hand, the products formed by the disproportionation of panose (4-*O*-α-isomaltosyl-D-glucose) differ between the two enzymes. Fig. 4 shows that *L. mesenteroides* B-512F dextransucrase forms a series of 4-*O*-α-isomaltodextrinyl-D-glucose oligosaccharides from panose, by transferring α-D-glucosyl units one at a time; maltose is also released as a result of the removal of the nonreducing (terminal) D-glucosyl group from panose. The products formed in the presence of sucrose (see Fig. 5) are the same as those formed in the absence of sucrose.

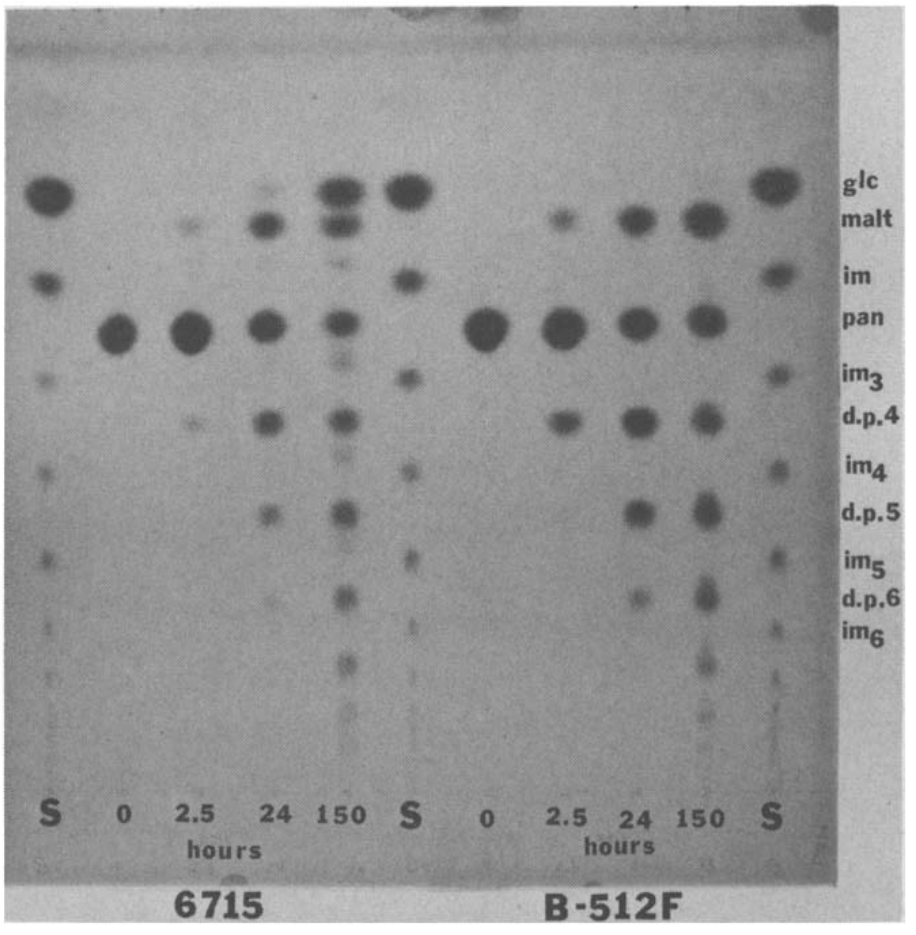

Fig. 4. Thin-layer chromatogram of products arising from the action of dextransucrases on panose. [S refers to isomalto-oligosaccharide standards. Left side of plate: *Streptococcus mutans* 6715 GTF-S reaction mixture; time points taken at t = 0, 2.5, 24, and 150 h. Right-side of plate: *Leuconostoc mesenteroides* B-512F dextransucrase reaction mixture; same time points as for *S. mutans*. Five μL of each mixture was chromatographed for two ascents in solvent *A* at 37°.]

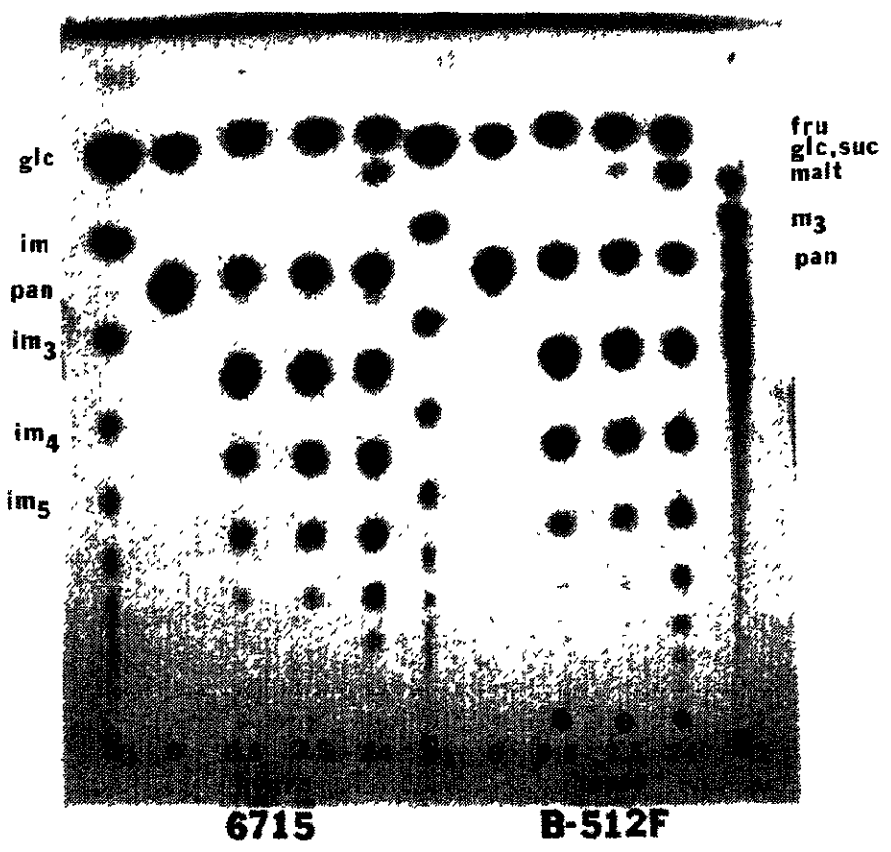

Fig. 5. Thin-layer chromatogram of products arising from the action of dextransucrases on panose in the presence of sucrose. [S_1 refers to isomalto-oligosaccharide standards, and S_2 refers to malto-oligosaccharide standards. Left side of plate: *Streptococcus mutans* 6715 GTF-S reaction mixture, time points taken at t = 0, 0.5, 2.5, and 24 h. Right side of plate: *Leuconostoc mesenteroides* B512F reaction mixture; same time points as for *S. mutans*. Five μL of each mixture was chromatographed for two ascents in solvent *A* at 37°.]

The products formed by *S. mutans* 6715 GTF-S, however, differ from those formed by B-512F dextransucrase. Fig. 4 shows that, in addition to maltose and 4-*O*-α-isomaltodextrinyl-D-glucose oligosaccharides, smaller amounts of other oligosaccharides are also formed. These other products are not, however, so prominent when sucrose is present (see Fig. 5). The identities of these oligosaccharides are not known at present, but we presume that they contain α-D-(1→3), as well as α-D-(1→6), linkages.

The ability of dextransucrase to utilize panose as a D-glucosyl donor suggests a certain amount of flexibility in the substrate-binding site of the enzyme. Further evidence for this is provided by the observation that both of the enzymes studied are capable of acting on maltotriose (see Fig. 6). Fig. 6 also shows that the two enzymes differ in their specificity of action on maltotriose. *S. mutans* 6715 GTF-S acts on this substrate to give D-glucose, maltose, and a higher-d.p. oligosaccharide of unknown structure, whereas *L. mesenteroides* B-512F dextransucrase forms maltose and a higher-d.p. oligosaccharide which differs from that produced by GTF-S. After a longer reaction-time, a second, minor product also appears and, in t.l.c., migrates slightly ahead of the major, higher-d.p. product from B-512F dextransucrase. It is important to note that maltotriose alone does not give rise to a series of higher isomaltodextrinyl oligosaccharides.

The major products of the action of *S. mutans* 6715 GTF-S on maltotriose are

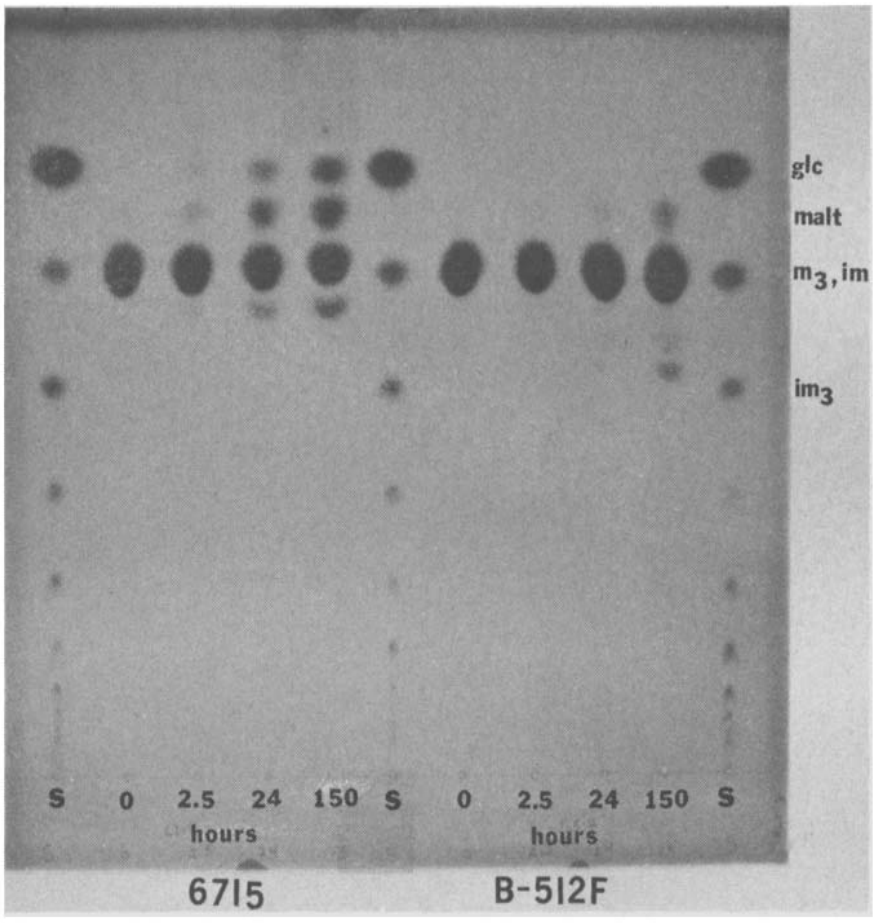

Fig. 6. Thin-layer chromatogram of products arising from the action of dextransucrases on maltotriose. [S refers to isomalto-oligosaccharide standards. Left side of plate *Streptococcus* 6715 GTF-S reaction mixture; time points taken at t = 0, 2.5, 24, and 150 h. Right side of plate: *Leuconostoc mesenteroides* B-512F dextransucrase reaction mixture; time points same as for *S. mutans*. Five μL of each mixture was chromatographed for two ascents in solvent *A* at 37°.]

D-glucose, maltose, and a tetrasaccharide (see Fig. 6). The formation of D-glucose occurs when water acts as an acceptor instead of maltotriose, to give hydrolysis rather than disproportionation. This is not the case when the enzymes are incubated with both sucrose and maltotriose. Fig. 7 shows that different products are formed when sucrose acts as the D-glucosyl donor. In addition to the two saccharides that are formed when sucrose is absent, *L. mesenteroides* B-512F dextransucrase reacts with sucrose and maltotriose to give a homologous series, which ostensibly are isomaltodextrinyl saccharides having maltotriose at the reducing end. No maltose was detected.

The GTF-S reaction with maltotriose and sucrose gives, in addition to the products noted in the absence of sucrose, a series of oligosaccharides that appear to be the same as those produced by B-512F dextransucrase. Maltose, however, is also released by GTF-S. It should be noted that the relative amounts of the higher saccharides differ between the two enzymes, with GTF-S giving more of the higher-d.p. products just ahead of the origin, whereas B-512F dextransucrase gives lesser amounts of these, compared to greater amounts of those of d.p. ~6 to 8 (see Fig. 7).

Perhaps most notably, it was found that both of these glucosyltransferases were capable of transferring D-glucosyl groups from a predominantly α-D-(1→6)-

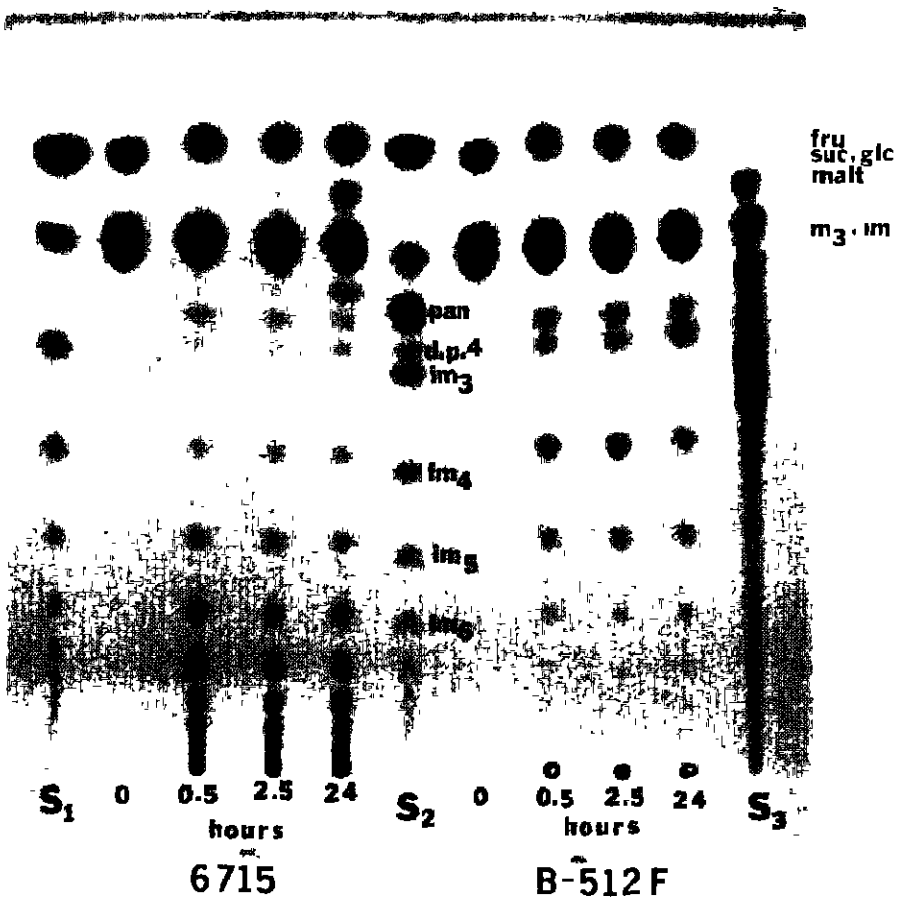

Fig. 7. Thin-layer chromatogram of products arising from the action of dextransucrases on maltotriose in the presence of sucrose [S_1 refers to isomalto-oligosaccharide standards, S_2 to the following standards (top to bottom): D-glucose, isomaltose, panose, 4^2-*O*-α-isomaltosylmaltose, isomaltotriose, and higher-d.p. isomalto-oligosaccharides, and S_3 to malto-oligosaccharide standards. Left side of plate: *Streptococcus mutans* 6715 GTF-S reaction mixture; time points taken at t = 0, 0.5, 2.5, and 24 h. Right side of plate: *Leuconostoc mesenteroides* B512F dextransucrase reaction mixture; time points same as for *S. mutans*. Five μL of each mixture was chromatographed for two ascents in solvent *A* at 37°.]

linked dextran to an acceptor sugar. Fig. 8 shows that both glucansucrases transfer single D-glucosyl groups from a B-512F clinical-sized dextran (average molecular weight ~10,000) to methyl α-D-glucopyranoside. B-512F dextransucrase gives rise to methyl α-isomaltoside in 24 and 150 h, as judged by mobility in t.l.c. *S. mutans* 6715 GTF-S reacts more slowly, to give the same products, as well as another, faster-moving disaccharide, most probably methyl α-nigeroside (see Fig. 8). On prolonged incubation, D-glucose and higher-d.p. products were also observed. D-Glucose was identified by its chromatographic mobility and by detection in t.l.c. by means of orthanilic acid[25], which did not reveal the methyl α-glycosides.

When D-fructose was present in enzyme digests wherein dextran or isomalto-oligosaccharides served as D-glucosyl donors, leucrose was formed (data not shown), just as it is when sucrose acts as the D-glucosyl donor[26].

A number of other sugars were also tested as D-glucosyl donors, but they were found to be relatively unreactive compared to the previously described substrates. Planteose, turanose, and melezitose gave only very slight traces of products after prolonged incubation with large proportions of enzyme, and melibiose, isopanose, and 6,6′-dideoxy-6,6′-difluorosucrose[27] gave no products at all.

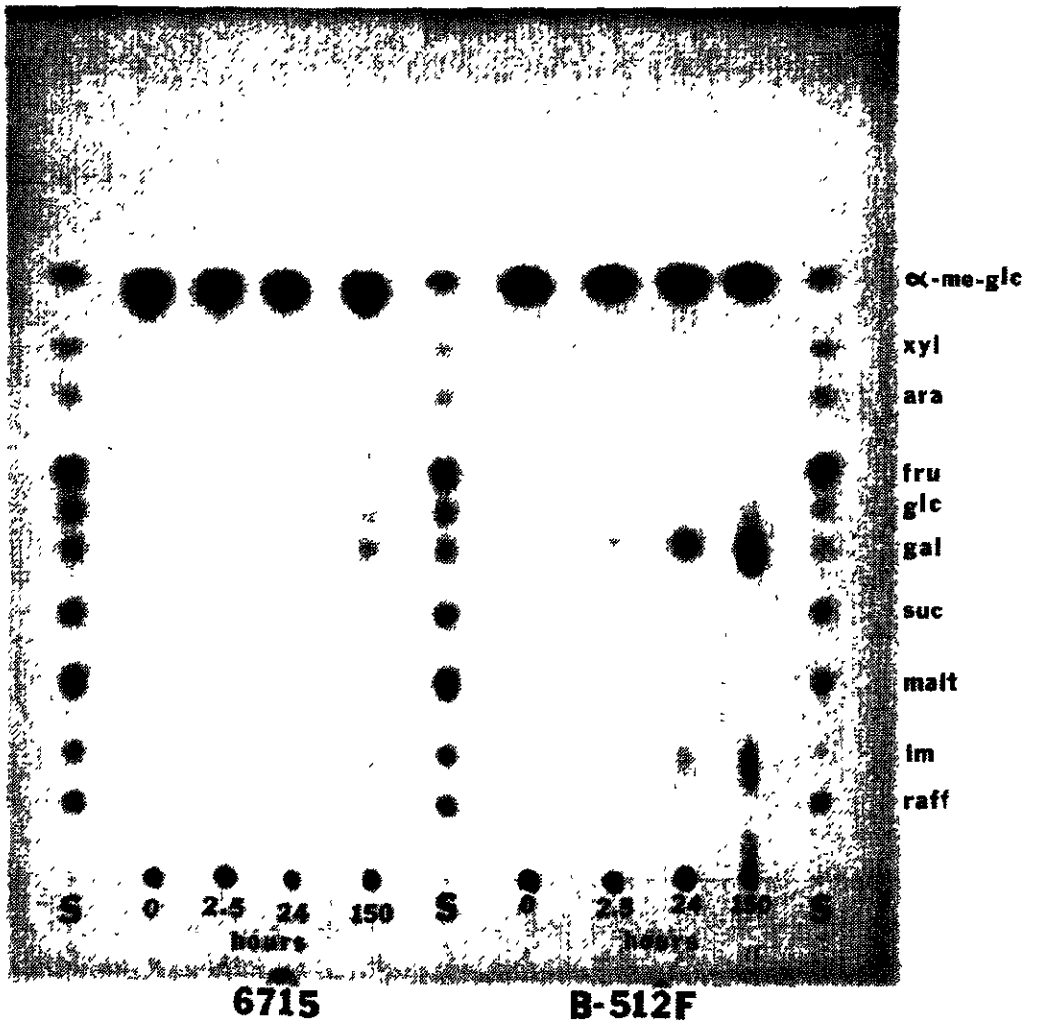

Fig. 8. Thin-layer chromatogram of products arising from the action of dextransucrases on B-512F clini-cal dextran in the presence of methyl α-D-glucoside. [S refers to the following standards (top to bottom): methyl α-D-glucoside, D-xylose, L-arabinose, D-fructose, D-glucose, D-galactose, sucrose, maltose, iso-maltose, and raffinose. Left side of plate: *Streptococcus mutans* 6715 GTF-S reaction mixture; time points taken at t = 0, 2.5, 24, and 150 h. Right side of plate: *Leuconostoc mesenteroides* B512F dextransucrase reaction mixture; time points same as for *S. mutans*. Five μL of each mixture was chro-matographed for three ascents in solvent *B* at 25°.]

We have also found that *S. mutans* 6715 GTF-I ("mutansucrase") is capable of catalyzing types of transfer reactions similar to those described for GTF-S, but we have not investigated them in detail.

DISCUSSION

Disproportionation reactions catalyzed by dextransucrase were observed in our laboratory during a study of acceptor reactions with oligosaccharides of d.p. ≥3. These reactions were observed with both glucansucrase preparations, regard-less of purity. The fact that the reaction products were the same as those produced by acceptor reactions suggested that this phenomenon was due to glucansucrase it-self.

Over two decades ago, Tsuchiya[28] reported similar observations while work-ing with a relatively crude preparation of dextransucrase from *Leuconostoc mesen-teroides* B-512F. His results have been greeted with some degree of scepticism since

then, and have been interpreted by some as evidence of the presence of a contaminating, glycosidase activity. The issue was further clouded by Tsuchiya's own observation that his preparation also contained levansucrase activity[28]. Since then, no other reports have appeared which confirm his findings. In fact, Kobayashi and Matsuda[29] reported that their highly purified dextransucrase from *L. mesenteroides* B-512F did not act on isomaltotetraose, although it did act on raffinose. These findings differ from ours, but this may be due to the fact that they examined a dextransucrase fraction that did not bind to Sephadex, whereas our enzyme binds tightly to Sephadex and could be released by 3M urea[24]. Walker[30] described a dextransucrase preparation from *Streptococcus mutans* OMZ 176 which she reported as having no hydrolytic action on isomalto-oligosaccharides, but she did not state how this was determined. It should be realized that, if a reducing-value assay was employed, disproportionation itself would not give any increase in reducing value, except after very long reaction-times, during which a significant amount of D-glucose would eventually be produced.

It is unlikely that the type of D-glucosyl transfer that we have observed is due to an endodextranase. *S. mutans* endodextranase is incapable of transferring single D-glucosyl units[31–34]. Instead, the initial products of the action of *S. mutans* endodextranase on dextran are isomaltotriose, isomaltotetraose, and isomaltopentaose[4,31–34]. Streptococcal endodextranase is also incapable of hydrolyzing maltotriose[35]; compare this with Fig. 6. The patterns of products seen with our glucansucrase preparations are not consistent with those that are characteristic of endodextranase action.

It is also unlikely that these reactions are due to exodextranase or α-D-glucosidase activities, as these enzymes are predominantly endocellular in these particular organisms[33,36], whereas our enzyme preparations are derived from the exocellular culture-fluid. In addition, had an exodextranase or α-D-glucosidase been present, isomaltose would have been hydrolyzed faster than dextran or isomaltodextrins[33,37], but just the opposite was found. Also, the endocellular glycosidases would have been expected to act on such saccharides as isopanose, turanose, melezitose, planteose, and melibiose, but only a trace of such activity was observed. Furthermore, *S. mutans* 6715 GTF-S prepared as described was reported to lack any detectable contaminating activity of this sort[2,22–23]. Likewise, invertase action could not account for these reactions, as *S. mutans* invertase is a β-D-fructofuranosidase[38,39].

The mechanism proposed for the disproportionation reaction observed is the formation of an enzyme–D-glucosyl intermediate from the substrate, and the subsequent displacement of the D-glucosyl group by an acceptor, to give disproportionation of the substrate, as follows.

$$\text{Isomaltotriose} + \text{enzyme} \rightarrow \text{D-glucosyl-enzyme} + \text{isomaltose}$$

$$\text{D-Glucosyl-enzyme} + \text{isomaltotriose} \rightarrow \text{enzyme} + \text{isomaltotetraose}$$

Although the disproportionation reactions are slow compared to D-glucosyl transfer from sucrose, they are sufficiently rapid to be of concern in many instances. For example, our results with isomaltotriose indicate that this saccharide can undergo disproportionation in the presence of sucrose to yield isomaltose, which is produced even before all of the sucrose has been consumed. Thus, the rate of formation and the distribution of certain products depend not only on the transfer of D-glucosyl groups from sucrose, but also on the transfer of D-glucosyl groups to and from the products themselves. The transfer of D-glucosyl groups from one dextran chain to another, although not directly observed, may be of considerable interest if it is found that this does, indeed, occur. This is especially interesting in light of our findings that branching in dextrans can occur by acceptor reactions[40,41].

The findings described herein support those of Tsuchiya[28], namely, that dextran and α-(1→6)-linked D-glucose oligosaccharides can act as D-glucosyl donors for B-512F dextransucrase. We have also shown that these reactions can be catalyzed by a streptococcal glucansucrase, as well as by *L. mesenteroides* B-512F dextransucrase. These reactions show that, in addition to acting as acceptors, a rather wide variety of D-gluco-oligosaccharides can also act as D-glucosyl donors. This adds further evidence to support the concept that the active site of dextransucrase is flexible enough, or nonspecific enough, to bind to, and catalyze transfer to and from, a number of different substrates. This was first suggested for dextransucrase by Neely[42], who found that partially denatured *L. mesenteroides* B-512F dextransucrase was capable of disproportionating maltose into D-glucose and a trisaccharide, probably panose.

It is not yet known how these reactions would affect the findings of others[43–45] with respect to the specificity of dextransucrase action in the presence of isomalto-oligosaccharides, but the question warrants consideration.

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