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THE MECHANISM OF ACCEPTOR REACTIONS OF *Leuconostoc mesenteroides* B-512F DEXTRANSUCRASE*†

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ABSTRACT

Reactions of dextransucrase and sucrose in the presence of sugars (acceptors) of low molecular weight have been observed to give a dextran of low molecular weight and a series of oligosaccharides. The acceptor reaction of dextransucrase was examined in the absence and presence of sucrose by using D-[^{14}C]glucose, D-[^{14}C]fructose, and ^{14}C-reducing-end labeled maltose as acceptors. A purified dextransucrase was pre-incubated with sucrose, and the resulting D-fructose and unreacted sucrose were removed from the enzyme by chromatography on columns of Bio-Gel P-6. The enzyme, which migrated at the void volume, was collected and referred to as "charged enzyme". The charged enzyme was incubated with ^{14}C-acceptor in the absence of sucrose. Each of the three acceptors gave two fractions of labeled products, a high molecular weight product, identified as dextran, and a product of low molecular weight that was an oligosaccharide. It was found that all three of the acceptors were incorporated into the products at the reducing end. Similar results were obtained when the reactions were performed in the presence of sucrose, but higher yields of labeled products were obtained and a series of homologous oligosaccharides was produced when D-glucose or maltose was the acceptor. We propose that the acceptor reaction proceeds by nucleophilic displacement of glucosyl and dextranosyl groups from a covalent enzyme-complex by a specific, acceptor hydroxyl group, and that this reaction effects a glycosidic linkage between the D-glucosyl and dextranosyl groups and the acceptor. We conclude that the acceptor reactions serve to terminate polymerization of dextran by displacing the growing dextran chain from the active site of the enzyme; the acceptors, thus, do not initiate dextran polymerization by acting as primers.

*Dedicated to Professor Dexter French, on the occasion of his 60th birthday and in recognition of his many contributions to the study of carbohydrate structure and the mechanisms of carbohydrase action.

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INTRODUCTION

Koepsell *et al.*[1] and Tsuchiya *et al.*[2] reported that sugars of low molecular weight added to dextransucrase–sucrose digests shifted the course of the reaction from the synthesis of a high molecular weight dextran to one of lower molecular weight, and they observed that large amounts of oligosaccharides were formed. The low molecular weight sugars were called glucosyl acceptors. Because of the synthesis of oligosaccharides that are not observed in the absence of low molecular weight acceptors, it was assumed that the acceptors competed with chain ends of native dextran and that dextransucrase, like glycogen phosphorylase[3], required a primer (native acceptor) to which D-glucosyl groups from sucrose were added to the nonreducing ends of the primer. It was thus assumed that the low molecular weight sugars were also acting as primers and were lowering the molecular weight of the dextran by providing many initiation-sites for the synthesis of dextran chains. A thorough study, however, failed to prove that dextransucrase required a native dextran primer[4]. Recently, a study of the activation of *Streptococcus mutans* dextransucrase by added dextran and by nonreducing-end modified dextrans showed that the activation was not the result of a required primer-reaction for dextran synthesis[5].

We have previously shown that the dextransucrase of *Leuconostoc mesenteroides* B-512F forms covalent D-glucosyl and dextranosyl complexes and that dextran is biosynthesized by dextransucrase from the reducing end of the dextran chain by an insertion mechanism[7]. It is difficult to envisage how the mechanism could require nonreducing chain-ends as primers.

Thus, because of the uncertainty of the mechanism of the acceptor reactions in lowering the molecular weight of dextran and in producing oligosaccharides, we have undertaken a study of the mechanism of the acceptor reaction by using ^{14}C-labeled D-glucose and D-fructose and ^{14}C-reducing-end labeled maltose.

EXPERIMENTAL

Materials. — Purified dextransucrase (53 U/mg) was obtained (method to be described[6]). Dextranase was obtained from Ferment AG, Basle, Switzerland. Exodextranase was prepared from *Arthrobacter globiformis* T6 according to the method of Sawai *et al.*[8]. [U-^{14}C]Sucrose, D-[U-^{14}C]glucose, and D-[U-^{14}C]fructose were purchased from New England Nuclear, Boston, MA. ^{14}C-Reducing-end labeled maltose was prepared by the *Bacillus macerans* transglycosylase coupling reaction between cyclohexaamylose and D-[U-^{14}C]glucose[9,10]. Bio-Gel P-6 was purchased from Bio–Rad Laboratories, Richmond, CA.

Paper chromatography. — Whatman 3 MM paper was used with two solvent systems: (*A*) 10:4:3 (v/v) ethyl acetate–pyridine–water, descending for 18 h at 40° on a 56-cm length of paper, and (*B*) 8:1:1:1 (v/v) nitromethane–acetic acid–abs. ethanol–boric acid-saturated water, descending for 18 h at 40° on a 56-cm length of paper[12]. Preparative paper-chromatography was conducted by streaking the sample

along the origin of a 23 × 56-cm paper sheet that was irrigated with system *A*. After irrigation, guide strips were cut out, and developed with silver nitrate[13] or, when the compounds were radioactive, radioautograms were prepared. Areas corresponding to each sugar were cut out, and eluted with distilled water. The resulting eluates were lyophilized or evaporated *in vacuo*.

Charging of dextransucrase. — Dextransucrase was assayed by a radiochemical method previously described[6]; 1.5 ml having a total of 3.5 units was charged with nonlabeled sucrose by reaction with 120mM sucrose in 20mM acetate (pH 5) buffer for 30 min at 25°. The charging ensured the formation of the D-glucosyl and dextranosyl enzyme complex. The D-fructose resulting from the reaction, and any unreacted sucrose, were removed from the charged enzyme by passing the mixture through a 1 × 90 cm column of Bio-Gel P-6, which was eluted (27 ml/h) with 20mM (pH 5) acetate buffer containing 3mM calcium chloride. Fractions (3 ml) were collected and the void volume of the column, which contained the dextransucrase, was pooled and referred to as charged enzyme. D-Fructose and sucrose were completely removed from the enzyme by being retarded on the column and they emerged several fractions beyond the void volume. The charged enzyme was used in the sucrose-free, acceptor reactions.

Acceptor reactions with charged enzyme in the absence of sucrose. — Three labeled acceptors were used: D-[U-^{14}C]glucose (130,000 c.p.m./μg, 70,000 c.p.m./μl), D-[U-^{14}C]fructose (280,000 c.p.m./μg, 250,000 c.p.m./μl), and ^{14}C-reducing-end labeled maltose (20,000 c.p.m./μg, 7,000 c.p.m./μl). ^{14}C-Acceptor (0.3 ml) was added to 3 ml of charged enzyme (0.2 U/ml) and incubated for 24 h at 25°.

Acceptor reactions in the presence of sucrose. — Acceptor reactions were performed in the presence of 150mM sucrose by using the same amounts of dextransucrase (uncharged enzyme), ^{14}C-labeled acceptors, and the same conditions as just described for the acceptor reactions in the absence of sucrose.

Analysis of acceptor-reaction products. — Aliquots, containing about 50,000 c.p.m., were applied to 2 × 56 cm strips of Whatman 3 MM paper, which were irrigated with system *A*. The radioactive products were determined by cutting 1.5-cm pieces from the origin to the end of the paper; the papers were counted for radioactivity in a toluene cocktail in a liquid scintillation-counter.

The remainder of the mixtures was passed over 1 × 90 cm columns of Bio-Gel P-6, which were eluted with distilled water at a flow rate of 20 ml/h. Fractions (3 ml) were collected and the radioactivity was determined by counting 50 μl of each fraction. The labeled material migrating at the void volume of the column was designated Fraction I, and the labeled material retarded on the column was designated Fraction II. Fraction I was dextran and Fraction II was oligosaccharides plus the original, unreacted acceptor.

Structural studies on fraction I. — Aliquots of Fraction I from the three, labeled acceptors in the absence and presence of sucrose were subjected to reduction with sodium borohydride and hydrolysis with trifluoroacetic acid. Sodium borohydride (60–120 mg/ml) was added to 1–2 ml of an aqueous solution of [^{14}C]polysaccharide

(~500 c.p.m.). The reduction was allowed to proceed for 24 h at 25° and an additional 30 min at 95°. The excess of borohydride was decomposed with glacial acetic acid and the resulting borates were removed by extensive dialysis against distilled water. The reduced dextran was hydrolyzed by heating it for 75 min to 121° in M trifluoroacetic acid in a sealed glass ampoule. The products were chromatographed by using system *B*.

Fraction I (1 ml, 2000–5000 c.p.m.) from all three acceptors was treated with 0.3 ml of dextranase S (11 U/ml) at pH 5 at 37° for 30 h. The labeled products were isolated by preparative paper-chromatography. The products were reduced with sodium borohydride and hydrolyzed with trifluoroacetic acid as already described. The products were chromatographed with system *B*.

Structural studies on Fraction II. — Fraction II products from all three acceptors, in the presence and absence of sucrose, were subjected to preparative paper-chromatography. Each labeled oligosaccharide was reduced with sodium borohydride and hydrolyzed with trifluoroacetic acid as already described for Fraction I. The samples were neutralized, and borate was removed by passing the solutions through a 0.5 × 5 cm column of Amberlite MB-3 or by repeated evaporation in acidic methanol. They were then chromatographed with system *B*.

The oligosaccharides (Fraction II) from the [^{14}C]maltose and D-glucose reactions were subjected to hydrolysis by exodextranase and the products examined by paper chromatography with system *A*.

Relatively large amounts of unlabeled oligosaccharides resulting from the maltose acceptor-reaction were prepared by treating 225 mg of maltose with 225 mg of sucrose and 5 units of dextransucrase in 4.5 ml of 20mM pH 5 acetate buffer for 24 h at 25°. The resulting oligosaccharides were isolated by preparative paper-chromatography. Each oligosaccharide (d.p.$_3$–d.p.$_6$)* was treated with exodextranase: 0.5–4 mg of the oligosaccharide was incubated with 0.1 units of exodextranase in 1 ml of 20mM pH 5 acetate buffer at 37°; 0.3 ml aliquots were withdrawn at 10, 30, and 60 min, taken to dryness, and redissolved in 50 µl of water and chromatographed using system *A*.

RESULTS

Separation and formation of acceptor-reaction products. — The chromatography on Bio-Gel P-6 of the [^{14}C]glucose acceptor-reaction in the absence of sucrose (charged enzyme) is shown in Fig. 1A, and in the presence of sucrose in Fig. 1B. The void-volume fraction (Fraction I) is dextran, and the retarded fraction (Fraction II) is a mixture of oligosaccharides and unreacted acceptor. Fig. 1C shows the reaction of the acceptor with uncharged enzyme in the absence of sucrose; it serves as a control for the experiment of Fig. 1A. Similar results were obtained for D-[^{14}C]fructose and ^{14}C-reducing-end labeled maltose.

Table I gives the yields of Fraction I and Fraction II from the three acceptor-

*d.p.$_n$ refers to an oligosaccharide having a degree of polymerization of n D-glucose residues.

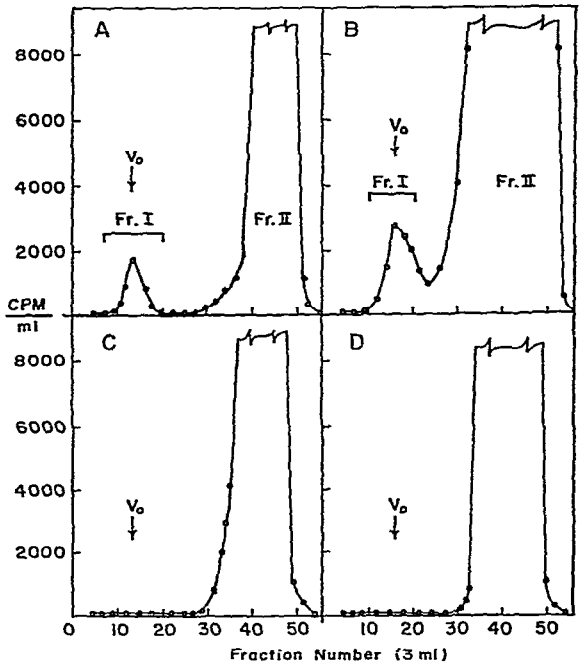

Figure 1. Chromatography on Bio-Gel P-6 of D-[^{14}C]glucose acceptor-reaction products: A, reaction with charged enzyme in the absence of sucrose; B, reaction in the presence of sucrose; C, reaction with uncharged enzyme in the absence of sucrose; D, acceptor control. Fr. I = Fraction I, Fr. II = Fraction II, V_0 = void volume.

TABLE I

PERCENTAGE OF THE TOTAL RADIOACTIVITY IN FRACTIONS I AND II OBTAINED FROM ACCEPTOR REACTIONS WITH D-[^{14}C]GLUCOSE, D-[^{14}C]FRUCTOSE, AND ^{14}C-REDUCING-END LABELED MALTOSE IN THE ABSENCE AND PRESENCE OF SUCROSE

Acceptor	*Sucrose*	*% of total radioactivity*	
		Fraction I[a]	*Fraction II*[c]
D-[^{14}C]Glucose[a]	—	0.05	0.2
D-[^{14}C]Glucose	+	0.3	10.2
D-[^{14}C]Fructose[b]	—	0.02	0.2
D-[^{14}C]Fructose	+	0.2	23.1
D-[^{14}C]Maltose[c]	—	0.2	5.4
D-[^{14}C]Maltose	+	0.7	58.9

[a]Percents based on 2.1 × 10^7 c.p.m. in the glucose acceptor. [b]Percents based on 7.4 × 10^7 c.p.m. in the fructose acceptor. [c]Percents based on 2.1 × 10^6 c.p.m. in the maltose acceptor. [d]Product at the void volume of the Bio-Gel P-6 column. [e]Product retarded on a Bio-Gel P-6 column (exclusive of the labeled acceptor).

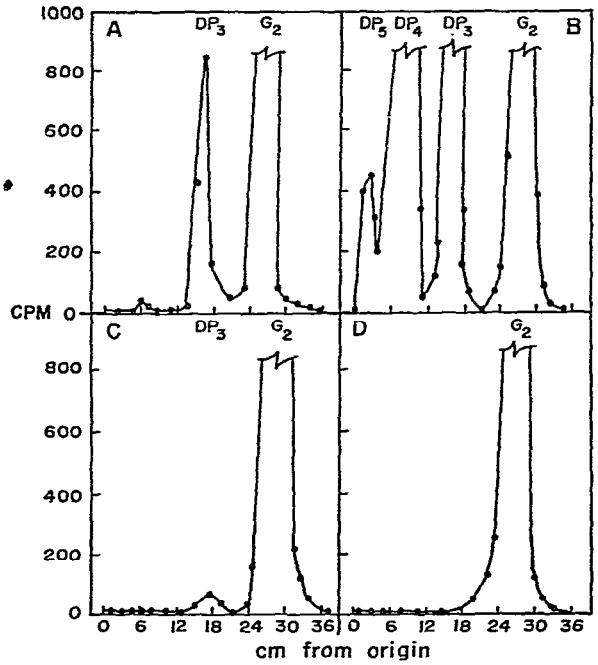

Fig. 2. Paper chromatography of the Fraction II [^{14}C]maltose acceptor-reaction products; A, reaction with charged enzyme in the absence of sucrose; B, reaction in the presence of sucrose; C, reaction with uncharged enzyme in the absence of sucrose; D, acceptor control. G_2 = maltose, d.p.3, d.p.4 and so on = trisaccharide, tetrasaccharide, and so on. The chromatography was carried out on 2 × 56 cm Whatman 3 MM strips using system A. The strips were sectioned into equal 1.5 cm pieces and counted.

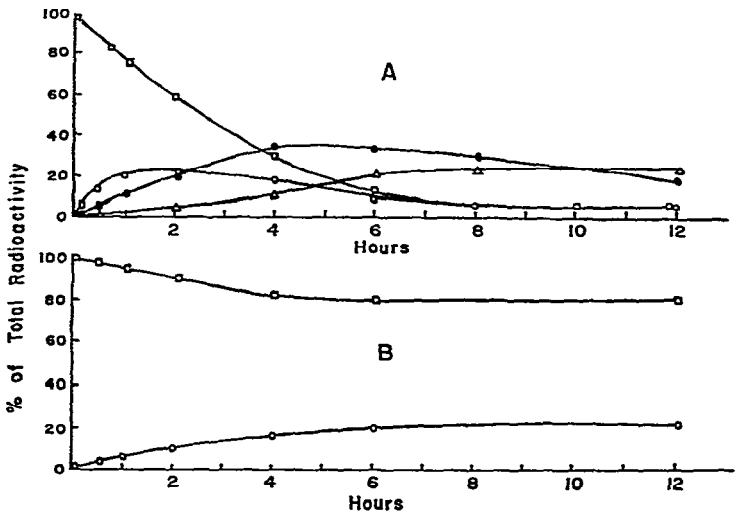

Fig. 3. Formation of labeled products from acceptor reactions with [^{14}C]maltose and D-[^{14}C]fructose in the presence of sucrose: A, [^{14}C]maltose (—□—); d.p.3 (—○—); d.p.4 (—●—); d.p.5 (—△—). B, D-[^{14}C]fructose (—□—); leucrose (—○—).

reactions in the absence and presence of sucrose. The data show that maltose is the best of the three acceptors. The yields in the presence of sucrose are higher than in its absence because the enzyme is continuously being charged in the presence of sucrose.

Fig. 2 shows the paper-chromatographic analysis of the labeled acceptor-products resulting from the reaction of ^{14}C-reducing-end labeled maltose. Essentially only a single oligosaccharide, a trisaccharide, results from the reaction with charged enzyme in the absence of sucrose (Fig. 2A). In the presence of sucrose, however, a series of oligosaccharides is observed (Fig. 2B). Reaction with uncharged enzyme (Fig. 2C) gave a small amount of trisaccharide, which constituted 5% of the amount obtained with charged enzyme. D-[^{14}C]glucose and D-[^{14}C]fructose gave similar results, with the major exception that D-[^{14}C]fructose in the presence of sucrose gave only a single disaccharide (leucrose*), whereas D-[^{14}C]glucose and [^{14}C]maltose gave a series of labeled oligosaccharides.

Fig. 3 shows the formation (mol percent) as a function of time of labeled products from acceptor reactions with [^{14}C]maltose and D-[^{14}C]fructose in the presence of sucrose.

Structural studies on fraction I. — Aliquots of each of the Fraction I products, from the three labeled acceptors in the absence and presence of sucrose, were treated with sodium borohydride and then hydrolyzed with acid. The polysaccharides were evidently not reduced readily, as a variable amount of D-glucitol was formed (0–35%

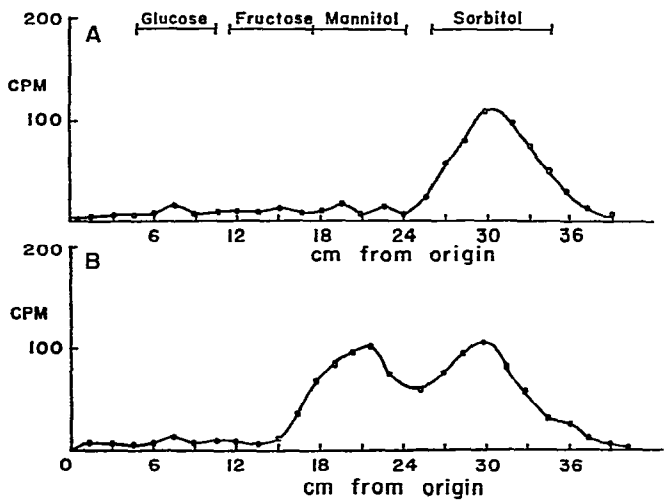

Fig. 4. Paper chromatography of the reduced and subsequently hydrolyzed products derived from action of dextranase on Fraction I: A, reduced and hydrolyzed [^{14}C]isomaltose from action of dextranase on the [^{14}C]glucose acceptor, Fraction I product; B, reduced and hydrolyzed ^{14}C-high molecular weight product from dextranase action on the D-[^{14}C]fructose acceptor Fraction I product. The migration positions of D-glucose, D-fructose, D-mannitol, and D-glucitol are indicated on this Fig. Chromatography was performed on 2 × 56 cm Whatman 3 MM strips and solvent system *B*. The strips were sectioned into equal 1.5-cm pieces and counted.

*Leucrose is *O*-α-D-glucopyranosyl-(1 → 5)-D-fructopyranose.

of that theoretically possible) in the experiments with D-glucose and maltose. The fractions were treated with an endodextranase (dextranase S): the D-[^{14}C]glucose Fraction I gave two labeled products, isomaltose and a fraction of high molecular weight; [^{14}C]maltose and D-[^{14}C]fructose gave only a product of relatively high molecular weight (> d.p.$_5$). Samples of the high molecular weight, labeled products and the labeled isomaltose were reduced and hydrolyzed with acid.

The labeled isomaltose from the D-[^{14}C]glucose acceptor-reaction gave exclusively ^{14}C-D-glucitol (see Fig. 4) and the high molecular weight fraction from D-[^{14}C]fructose acceptor reaction gave equal amounts of D-[^{14}C]mannitol and D-[^{14}C]glucitol, as would be expected for the reduction of a ketose (see Fig. 4). Likewise, the fraction of high molecular-weight from the D-[^{14}C]glucose and the ^{14}C-reducing end-labeled maltose gave exclusively D-[^{14}C]glucitol.

Structural studies on Fraction II oligosaccharides. — Each of the purified oligosaccharides from the three acceptor-reactions was reduced and hydrolyzed by acid. The disaccharide from the D-[^{14}C]fructose acceptor-reaction gave exclusively D-[^{14}C]mannitol and D-[^{14}C]glucitol; all of the oligosaccharides from the D-[^{14}C]-

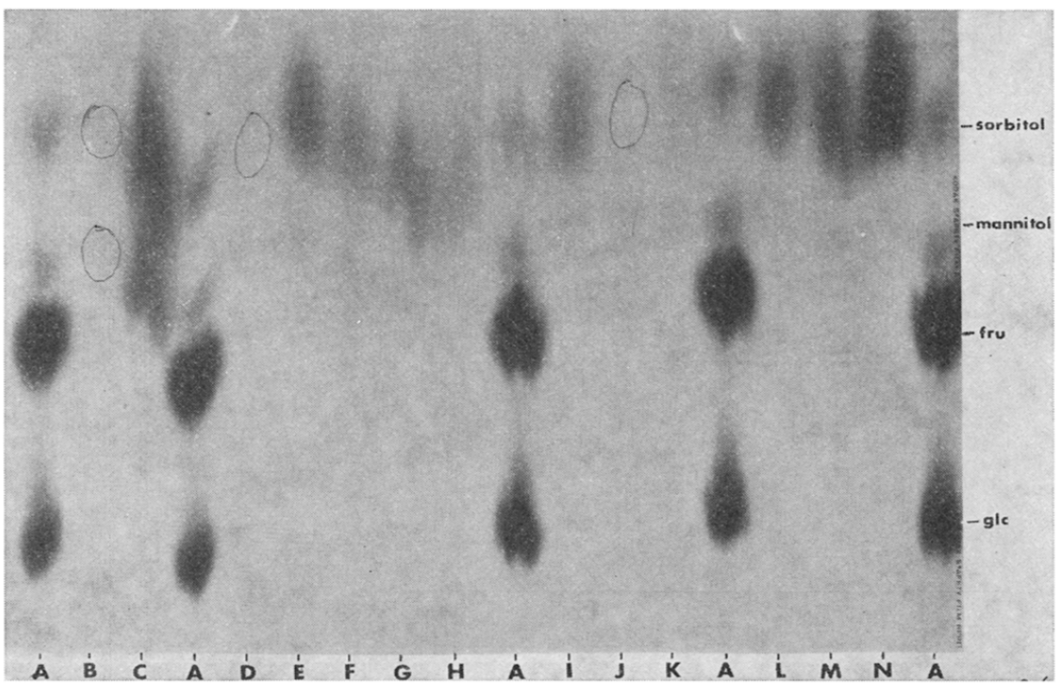

Fig. 5. Radioautogram of the products from the reduction and subsequent acid hydrolysis of the labeled Fraction II acceptor-products: A, D-[^{14}C]glucose, D-fructose, D-glucitol, and mannitol standards; B, d.p.$_2$ from [^{14}C]fructose in the absence of sucrose; C, d.p.$_2$ from [^{14}C]fructose in the presence of sucrose; D, d.p.$_2$ from [^{14}C]glucose in the absence of sucrose; E–H, d.p.$_2$ to d.p.$_5$ from [^{14}C]glucose in the presence of sucrose; I, d.p.$_3$ from [^{14}C]reducing end labeled maltose in the absence of sucrose; J, d.p.$_4$ from ^{14}C-reducing-end labeled maltose in the absence of sucrose; K–N, d.p.$_3$ to d.p.$_6$ from ^{14}C-reducing end-labeled maltose in the presence of sucrose. The chromatogram was irrigated with system *B*.

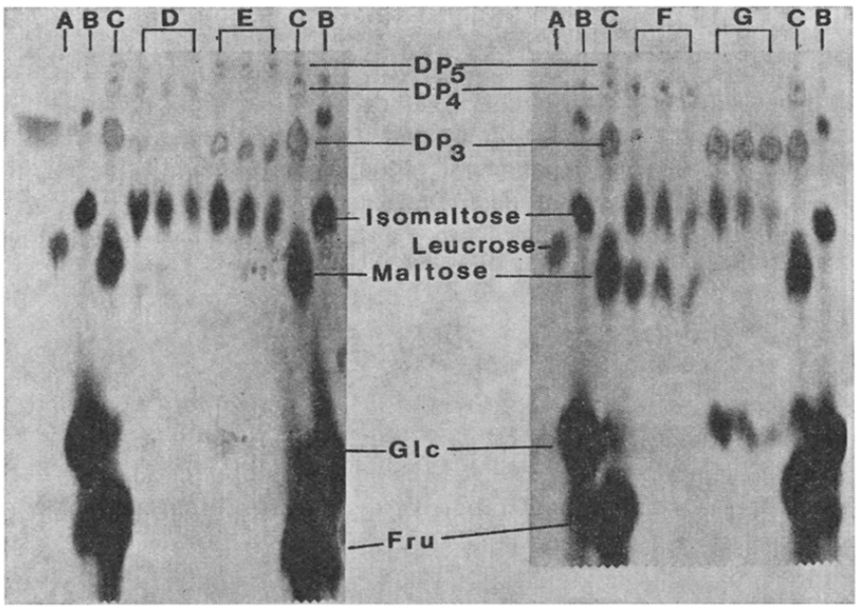

Fig. 6. Paper-chromatographic analysis of the exodextranase action on d.p.$_3$ to d.p.$_6$ products from the maltose acceptor-reactions in the presence of sucrose. A = leucrose standard; B = isomalto-oligosaccharide standards; C = maltose acceptor-reaction mixture; D = d.p.$_6$ hydrolysis from left to right, 60, 30, and 10 min hydrolyzates; E = d.p.$_5$ hydrolysis at 60, 30, and 10 min.; F = d.p.$_4$ hydrolysis at 60, 30, 10 min; G = d.p.$_3$ hydrolysis at 60, 30, and 10 min. The chromatograms were irrigated with system *A*.

glucose acceptor-reaction gave exclusively [^{14}C]glucitol; and all of the oligosaccharides from the ^{14}C-reducing-end labeled maltose gave D-[^{14}C]glucitol (see Fig. 5). Samples E, F, and G (d.p.$_2$, d.p.$_3$, and d.p.$_4$) of Fig. 5 show a small proportion of D-[^{14}C]-mannitol, formed as a result of a limited amount of alkaline isomerization during reduction.

The chromatographic mobilities and hydrolysis products with exodextranase and endodextranase of the D-glucose acceptor-oligosaccharides indicated that they were isomalto-oligosaccharides.

The large-scale preparation of oligosaccharides (d.p.$_3$–d.p.$_6$) obtained as products from maltose as acceptor in the presence of sucrose was hydrolyzed with exodextranase (see Fig. 6): d.p.$_3$ gave isomaltose and glucose (the action of exodextranase on panose gives the same products, because the exodextranase bypasses the nonreducing-terminal α-D-(1 → 6) linkage and hydrolyzes the α-D-(1 → 4) linkage[8]); d.p.$_4$ gave maltose and isomaltose; d.p.$_5$ gave isomaltose and d.p.$_3$; and d.p.$_6$ gave isomaltose and d.p.$_4$, which eventually gave maltose and isomaltose. From the foregoing data, a consideration of the chromatographic mobilities, and the specificity of the exodextranase, d.p.$_3$ is panose (6^2-α-D-glucosyl-maltose) and the other oligosaccharides are isomalto-oligosaccharides terminated with a maltose group at the reducing end to give a panose structure.

DISCUSSION

Acceptor reactions with D-[^{14}C]glucose, D-[^{14}C]fructose, and ^{14}C-reducing-end labeled maltose were carried out with *L. mesenteroides* B-512F dextransucrase in the absence of sucrose (charged enzyme) and in the presence of sucrose. Fig. 1A shows that a labeled dextran (Fraction I) is released from the charged enzyme by D-[^{14}C]glucose acceptor in the absence of sucrose. Fig. 2A shows that a labeled trisaccharide (Fraction II) is released from the charged enzyme by [^{14}C]maltose in the absence of sucrose. These results demonstrate, contrary to previous views[2,14], that the acceptor reactions can occur in the absence of sucrose. The results also confirm our hypothesis[7] that dextransucrase forms covalent intermediates with D-glucose and dextran, and that these are released from the enzyme by the acceptor.

In the absence of sucrose, all three of the acceptors released two types of product from the charged enzyme, a dextran of high molecular weight (Fraction I) and an oligosaccharide of low molecular weight (Fraction II). In the presence of sucrose, a series of homologous oligosaccharides was produced by D-glucose and by maltose, with a concomitant decrease in the production of dextran. The rates of appearance and disappearance of oligosaccharides in the reaction with maltose (see Fig. 3) indicate that the trisaccharide is formed first from maltose and that the tetrasaccharide is derived from the trisaccharide, the pentasaccharide from the tetrasaccharide, and so on. However, D-fructose gave only a disaccharide either in the presence or in the absence of sucrose. The disaccharide was identified as leucrose (*O*-α-D-glucopyranosyl-(1 → 5)-D-fructopyranose). Because a series of oligosaccharides was not produced by D-fructose in the presence of sucrose, leucrose must be a very poor acceptor, if one at all, and thus the reaction terminates with this disaccharide. Leucrose occurs as a normal reaction-product of dextransucrase, because D-fructose is a normal product of the dextran polymerization-reaction from sucrose. When the concentration of D-fructose increases, the fructose acts as an acceptor, giving rise to leucrose and a D-fructose-terminated dextran.

The trisaccharide produced in the maltose reaction was identified as panose (6^2-α-D-glucopyranosylmaltose), and the other homologs as isomalto-oligosaccharides terminated at the reducing end with maltose in a panose structure. The use of dextransucrase–sucrose digests, in the presence of relatively high concentrations of D-fructose or maltose, has been reported for the respective preparations of leucrose[15] and panose[16].

The location of the acceptor in the acceptor-reaction products has been determined by reduction and acid hydrolysis of Fraction I and Fraction II products. Although the label in the Fraction I products was not readily reduced with sodium borohydride, hydrolysis of Fraction I with endodextranase gave products whose label was completely reducible. It is impossible to picture how an endodextranase hydrolysis (essentially a random hydrolysis) could yield exclusively reducing-end labeled products if the label had not originally been at the reducing end. Thus, we conclude that Fraction I is labeled exclusively at the reducing end but, because of the secondary

and tertiary structure of the dextran, the reducing end is buried and unavailable for reaction with sodium borohydride until the molecular size of the dextran has been decreased by dextranase hydrolysis. Hydrolysis of Fraction I by exodextranase did not release any labeled isomaltose and gave a labeled dextran limit-dextrin, further indicating that the label was located at the reducing end.

The label in Fraction II products, however, was completely reducible (see Fig. 5). Thus, we conclude that the labeled acceptors are incorporated into the Fraction I and Fraction II acceptor-products exclusively at the reducing end. This result was the same whether the products were produced from charged enzyme in the absence of sucrose, or from enzyme in the presence of sucrose.

In consequence, a mechanism for the acceptor reactions requires that (*a*) the reaction be capable of occurring in the absence of sucrose, (*b*) the acceptor be incorporated covalently at the reducing end of the product, (*c*) the products be dextran and a low molecular weight oligosaccharide(s), and (*d*) as the ratio of the acceptor to sucrose increases, there is a concomitant decrease in the amount of dextran formed and an increase in the amount of oligosaccharide(s) formed.

To explain these facts, we propose that the acceptors act as nucleophiles in which a hydroxyl group (C_6-OH for D-glucose and maltose, and C_5-OH for D-fructose) attacks C-1 of the glucosyl or dextranosyl groups in the enzyme complex. Fraction I products (dextrans) are obtained by the displacement of the dextranosyl group, and Fraction II product (oligosaccharide) is obtained by displacement of the D-glucosyl group (see Fig. 7). When the acceptor reactions occur in the presence of sucrose, the glucosyl and dextranosyl groups are continuously regenerated at the active site of the enzyme and are displaced by the acceptor, thus giving rise to yields of acceptor products higher than when the reaction occurs in the absence of sucrose. Furthermore, when the concentration of the first acceptor-product becomes sufficiently high, it too acts as an acceptor, giving rise to the next higher homolog which in turn will act as an acceptor and thus give a series of homologous oligosaccharides. When the ratio of the concentration of the acceptor to sucrose is sufficiently high (such as 10:1)[16], the acceptor continuously displaces the D-glucosyl group from the enzyme, thus preventing D-glucose from being incorporated into dextran and thus decreasing or preventing dextran biosynthesis.

The proposed mechanism fulfils all of the aforementioned experimental criteria, and is consonant with the insertion mechanism for dextran biosynthesis proposed by Robyt *et al.*[7] and the mechanism for forming α-D-(1 → 3) branch linkages proposed by Robyt and Taniguchi[17]. The acceptor reaction provides a mechanism for terminating dextran-chain polymerization, and, by the release of dextran from the active site of the enzyme, provides a mechanism for the biosynthesis of new dextran chains.

This latter aspect answers a criticism of the insertion mechanism in which it was argued that the mechanism is untenable on the grounds that the number of dextran molecules *must* equal the number of active sites on the enzyme[18].

The structure of the acceptor products and the location of the acceptor in the products are the same, whether the acceptor reaction takes place with charged

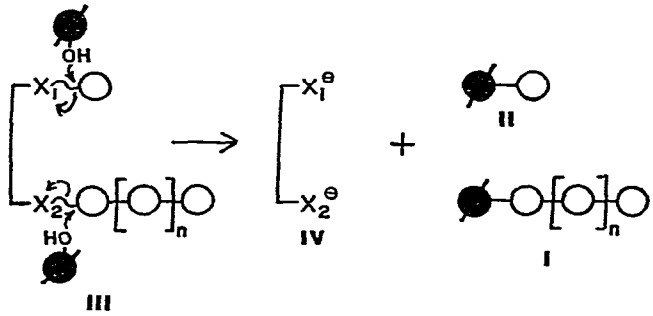

Fig. 7. Mechanism for the acceptor reaction with charged dextransucrase in the absence of sucrose. X_1 and X_2 represent nucleophilic groups at the active site of the enzyme; −O−O− represents two glucose residues units linked by a (1 → 6) α-D-glucosidic bond; $\overset{OH}{\bullet}$ denotes a ^{14}C-labeled acceptor. I is a Fraction I, reducing-end labeled dextran acceptor-product; II is a Fraction II reducing-end labeled oligosaccharide acceptor-product; III is labeled acceptor displacing glucosyl and dextranosyl groups from the charged enzyme; and IV is nascent enzyme.

enzyme in the absence of sucrose, or whether it takes place in the presence of sucrose. The mechanism for the acceptor reaction should thus be identical in the presence or the absence of sucrose. The acceptors, therefore, appear not to be primers as previously postulated[2,18,19], but in fact are chain terminators rather than chain initiators.

Many different molecules[14,20] have been reported as acceptors having various degrees of effectiveness. They range from several monosaccharides, such as the common ones D-glucose[2], D-fructose[2], and D-mannose[21], to disaccharides such as maltose[2], isomaltose[2], and cellobiose[22], and to such alditols as glycerol[23], D-glucitol[23], and riboflavin[24]. Dextransucrases from many species and strains of *Leuconostoc* and *Streptococcus* have been observed to carry out the acceptor reactions[2,20–26]. As all of these reactions follow similar patterns, it is logical to conclude that they have the same mechanism as has been proposed here for *L. mesenteroides* B-512F dextransucrase.

REFERENCES

1 H. J. KOEPSELL, H. M. TSUCHIYA, N. N. HELLMAN, A. KAZENKO, C. A. HOFFMAN, E. S. SHARPE, AND R. W. JACKSON, *J. Biol. Chem.*, 200 (1952) 793–801.
2 H. M. TSUCHIYA, N. N. HELLMAN, H. J. KOEPSELL, J. CORMAN, C. S. STRINGER, S. P. ROGOVIN, M. O. BOGARD, G. BRYANT, V. H. FEGER, C. A. HOFFMAN, F. R. SENTI, AND R. W. JACKSON, *J. Am. Chem, Soc.*, 77 (1955) 2412–2419.
3 C. F. CORI AND G. T. CORI, *J. Biol. Chem.*, 131 (1939) 397–398.
4 C. S. STRINGER AND H. M. TSUCHIYA, *J. Am. Chem.*, 80 (1958) 6620–6625.
5 J. F. ROBYT AND A. J. CORRIGAN, *Arch. Biochem. Biophys.*, 183 (1977) 726–731.
6 J. F. ROBYT AND T. F. WALSETH, *Carbohydr. Res.*, submitted.
7 J. F. ROBYT, B. K. KIMBLE, AND T. F. WALSETH, *Arch. Biochem. Biophys.*, 165 (1975) 634–640.
8 T. SAWAI, K. TORIYAMO, AND K. YANO, *J. Biochem. (Tokyo)*, 75 (1974) 105–112.
9 D. FRENCH, M. L. LEVINE, E. NORBERG, P. NORDIN, J. H. PAZUR, AND G. M. WILD, *J. Am. Chem. Soc.*, 76 (1954) 2387–2390.
10 J. H. PAZUR, *J. Am. Chem. Soc.*, 77 (1955) 1015–1018.

11 D. FRENCH, J. L. MANCUSI, M. ABDULLAH, AND G. L. BRAMMER, *J. Chromatogr.*, 19 (1965) 445–447.
12 J. F. ROBYT, *Carbohydr. Res.*, 40 (1975) 373–374.
13 E. F. L. J. ANET AND T. M. REYNOLDS, *Nature*, 174 (1954) 930–931.
14 K. H. EBERT AND G. SCHENK, *Adv. Enzymol.*, 30 (1968) 179–221.
15 F. H. STODOLA, E. S. SHARPE, AND H. J. KOEPSELL, *J. Am. Chem. Soc.* 78 (1956) 2514–2518.
16 M. KILLEY, R. J. DIMLER, AND J. E. CLUSKEY, *J. Am. Chem. Soc.*, 77 (1955) 3315–3318.
17 J. F. ROBYT AND H. TANIGUCHI, *Arch. Biochem. Biophys.*, 174 (1976) 129–135.
18 E. J. HEHRE, *J. Polym. Sci., Part C*, 23 (1968) 239–244.
19 G. J. WALKER, *Carbohydr. Res.*, 30 (1973) 1–10.
20 R. L. SIDEBOTHAM, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
21 Y. IRIKI AND E. J. HEHRE, *Arch. Biochem. Biophys.*, 134 (1969) 130–138.
22 F. YAMAUCHI AND Y. OHWADA, *Agr Biol. Chem.*, 33 (1969) 1295–1299.
23 K. H. EBERT AND G. SCHENK, *Z. Naturforsch., 23b* (1968) 788–797.
24 Y. SUZUKI AND K. UCHIDA, *J. Vitaminol.*, 11 (1965) 313–316.
25 W. R. FIGURES AND J. R. EDWARDS, *Carbohydr. Res.*, 48 (1976) 245–253.
26 E. NEWBRUN AND J. CARLSSON, *Arch. Oral Biol.*, 14 (1969) 461–468.