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THE DEXTRAN ACCEPTOR REACTION OF DEXTRANSUCRASE FROM *Streptococcus mutans* K1-R

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ABSTRACT

Soluble dextransucrase activity(ies) was eluted with a solution of clinical dextran from the insoluble dextran–cell complex produced by *Streptococcus mutans* K1-R grown in the presence of sucrose. Studies of the dextran acceptor-reaction of the soluble enzyme-preparation indicate that it is highly specific for dextran of high molecular weight. Increased dextran synthesis in the presence of dextran acceptor and the apparent inhibition of this stimulation by higher concentrations of dextran result from product modification rather than a direct effect on the level of enzyme activity. The results demonstrate that the potentially water-insoluble structure synthesized by dextransucrase on exogenous, soluble dextran acts as a more-efficient acceptor than the soluble dextran. The role of the acceptor reaction in the biosynthesis of complex dextrans is discussed.

INTRODUCTION

The insertion mechanism of a single dextransucrase[1,2] may be responsible for the synthesis of the α-D-(1 → 6)-main chain- and α-D-(1 → 3)-branching-linkages of the dextran formed in cultures of *Leuconostoc mesenteroides* B 512-F, but "one enzyme–one dextran" is not likely to apply in the synthesis of more-complex dextran structures.

Dextrans, synthesized from sucrose by the extracellular dextransucrases of bacterial cultures, vary widely in their content of α-D-glucosyl linkage-types (1 → 2-; 1 → 3-; 1 → 4-; and 1 → 6-) and in their water-solubility, viscosity, and other hydrodynamic properties[3]. Some are highly branched and are synthesized as a mixture of dextrans that clearly differ in structure and properties[4]. The formation of branching linkages has been ascribed to (*a*) branching enzymes[5] analogous to those involved in synthesis of glycogen and amylopectin, (*b*) the ability of dextransucrases to transfer glucosyl residues from sucrose to carbinol groups of internal as well as terminal D-glucose moieties in a growing dextran chain[6,7], and (*c*) the aforementioned in-

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sertion mechanism, in which a linear dextran chain is first synthesized (propagation reaction) and then transferred (acceptor reaction) to form a branching linkage between its terminal hemiacetal group and a free carbinol of an internal D-glucose residue of a preformed dextran molecule[8,9]. Multiple transfers to a single acceptor then form a highly branched dextran molecule.

Mixtures of dextransucrases possessing different specificities are found in bacterial cultures[10,11], suggesting that several enzymes participate in synthesis of complex dextrans[10]. Thus, regardless of whether synthesis proceeds by an insertion or a transglucosylation mechanism, three important factors that will determine the structure of the product are (*i*) the α-D-glucosyl linkage-type synthesized by each participating dextransucrase, (*ii*) the relative activity of each dextransucrase, and (*iii*) the ability of each enzyme to use as acceptors those dextrans produced by, or in combination with, the other dextransucrase(s). Variation in the secretion of a small number of different dextransucrases would thus account for an almost infinite variation in the structure of the dextrans produced by bacteria.

The synthesis of complex, water-insoluble dextrans from sucrose assists a number of strains of *Streptococcus mutans* to colonize tooth surfaces and initiate dental caries. Enzyme preparations from some of these organisms synthesize, almost exclusively, a water-insoluble dextran from sucrose alone[12,13]. However, when soluble, clinical dextran is included as an acceptor in the reaction mixtures, the enzyme preparations synthesize less of the insoluble dextran[12] and synthesis of soluble dextran is stimulated to give a several-fold increase in the total dextran produced[14]. In view of its importance in synthesis of complex dextrans, the role of acceptor dextran was studied in the reaction catalyzed by an enzyme preparation from the cariogenic organism, *Streptococcus mutans* K1-R. Some of the results described here have been reported in a preliminary communication[15].

MATERIALS AND METHODS

Materials. — Sucrose was purchased from Fisher Scientific Company and was dialyzed through a cellophane membrane to remove traces of contaminating dextran. [U-^{14}C]Sucrose, [^{14}C]sucrose ([U-^{14}C]glucosyl), and [^{3}H]sucrose ([1-^{3}H]fructosyl) were purchased from New England Nuclear Corporation, Boston, Mass. Clinical dextran (molecular weight, 60,000–90,000) and amylose (average degree of polymerization, 300) were purchased from Nutritional Biochemicals Corporation. Methyl α-D-glucopyranoside, glycogen (type II), and amyloglucosidase (Grade II) were purchased from Sigma Chemical Company. Panose was prepared as by Smith and Whelan[16], and laminaran and nigeran were gifts from Dr. J. J. Marshall, Department of Biochemistry, University of Miami. Dextranase (Merck) and cultures of *Streptococcus mutans* strains K1-R and BHT were obtained from Dr. R. Cowman of the Dental Research Unit, Veterans Administration Hospital, Miami, Florida. Concanavalin A was prepared from jack-bean (*Canavalia ensiformis*) meal as described by Sumner and Howell[17].

Analyses. — Concentrations of dextran and oligosaccharide solutions were determined by the phenol–sulfuric acid method[18] and protein was determined as by Lowry[19]. Total reducing-sugar was measured by a modified Nelson procedure[20] and D-glucose by a specific D-glucose oxidase method[21]. Fructose in dextransucrase digests was estimated from the difference between the total reducing sugar and glucose released. The interactions of dextrans with concanavalin A was measured turbidimetrically, as described by Goldstein, Hollerman, and Merrick[22].

Enzyme assays. — Dextransucrase and levansucrase activities were determined by monitoring the incorporation of radioactivity into polysaccharides from [^{14}C]-sucrose ([U-^{14}C]glucosyl) and [^{3}H]sucrose ([1-^{3}H]fructosyl), respectively.

Dextransucrase assay-mixtures (0.25 ml) containing an appropriate amount of enzyme solution, clinical dextran (50 μg/ml), radioactive sucrose (6.7 mg/ml, 3.3 μCi/ml) and 50mM sodium phosphate buffer (pH 6.0) were incubated at 37°. The radioactivity incorporated into ethanol-insoluble polysaccharide was determined by spotting samples onto filter-paper squares (1 cm), twice washing the paper in 67% ethanol, and measuring the radioactivity retained on the papers as described previously[23]. Levansucrase was assayed similarly except that [^{3}H]sucrose ([1-^{3}H]fructosyl) was used. One International Unit (IU) of dextransucrase is that amount of enzyme that incorporates one μ mol of D-glucose into ethanol-insoluble polysaccharide per minute.

The invertase activity of dextransucrase preparations was determined from the difference between the amount of reducing sugars released (glucose plus fructose) and the amount of radioactive glucose and fructose incorporated from [U-^{14}C]sucrose into ethanol-insoluble polysaccharide. Dextranase activity was determined from the release of reducing sugars in solutions containing enzyme, clinical dextran (2 mg/ml), and 50mM sodium phosphate buffer pH 6.0.

Preparation of soluble fraction of dextransucrase ("soluble" enzyme). — *S. mutans* K1-R cells were grown for 18 h at 37° under 95% nitrogen–5% carbon dioxide in broth (3 L) containing Trypticase (20 g/L), yeast extract (4 g/L), sodium carbonate (4 g/L), cysteine (100 mg/L), and sucrose (5 g/L). The broth was inoculated with 10% (by volume) of a stationary-phase culture of the cells. After growth, the insoluble dextran-cell aggregate was collected by centrifugation at 13,000 × *g*, washed twice with 50mM sodium phosphate buffer pH 6.0 (3 L) and suspended in the same buffer (280 ml). The suspension, containing 47 IU of dextransucrase, was incubated with clinical-dextran solution (20 ml, 1.5 mg/ml) for 30 min at 37° with occasional gentle stirring. Centrifugation at 37,000 × *g* for 10 min and filtration of the supernatant solution through glass wool gave a clear solution (218 ml) containing 13 mg of protein and 19 IU of dextransucrase activity.

Preparation of ammonium sulfate fractions of dextransucrase. — ("Crude" enzyme). *S. mutans* strains K1-R and BHT were cultured as just described except that D-glucose (1% by weight) was substituted for sucrose. After growth, cell-free cultures were brought to 50% saturation with ammonium sulfate at 4°. Precipitates were centrifuged at 13,000 × *g* for 10 min, dissolved in 50mM sodium phosphate buffer

pH 6.0 (4 ml), and dialyzed against three changes of the same buffer (1 L). Enzyme assays showed that 50% of the transglycosylase in the BHT preparation had levansucrase activity, and so dextran synthesis by both preparations was measured by using [^{14}C]sucrose ([U-^{14}C]glucosyl).

Preparation of acceptor dextrans modified by action of "soluble" enzyme. — Clinical dextran (10 mg/ml) was incubated at 37° with [U-^{14}C]sucrose (10 mg/ml) and a suitable amount of dextransucrase in 10 mM sodium phosphate buffer, pH 6.0. Reactions were stopped at different times by heating the mixtures for 10 min at 100°. The increases in molecular weight of the acceptor dextran calculated from the [^{14}C]glucose incorporated into polysaccharide and from the release of fructose in the reaction mixtures were in good agreement.

Gel-filtration chromatography of ^{14}C-labelled, modified dextrans. — Clinical dextran (10 mg) and a heat-inactivated reaction mixture (1.0 ml), in which "soluble" enzyme had incorporated [^{14}C]glucose (3% by weight) into clinical dextran (10 mg), were applied separately to a column (1.6 × 64 cm) of Biogel P-150 previously equilibrated with 0.02% sodium azide solution. The column was irrigated with the same solution at a flow rate of 25 ml/h. The carbohydrate content of fractions (2 ml) was determined by the phenol–sulfuric acid method and radioactivity was measured by adding 0.2 ml of the fractions to a toluene-based scintillation solution containing

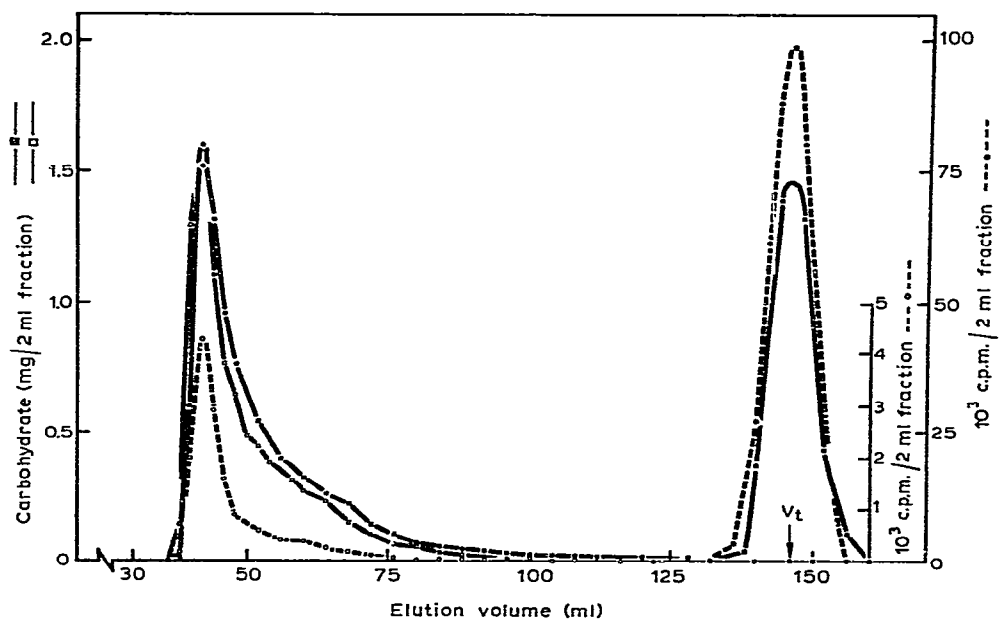

Fig. 1. Elution profiles of gel filtration on Bio-Gel P-150 of unmodified clinical dextran (□) and of dextransucrase products formed in the presence of soluble acceptor-dextran and [^{14}C]sucrose (■, ○, ●). A mixture (1 ml) comprising soluble enzyme solution (0.05 IU), clinical dextran (10 mg), U-^{14}C-labelled sucrose (10 mg, 0.4 μCi) and 10mM sodium phosphate buffer (pH 6.0) was incubated for 60 min at 37°, heated for 15 min at 100°, cooled, and applied to the column as described in Materials and Methods.

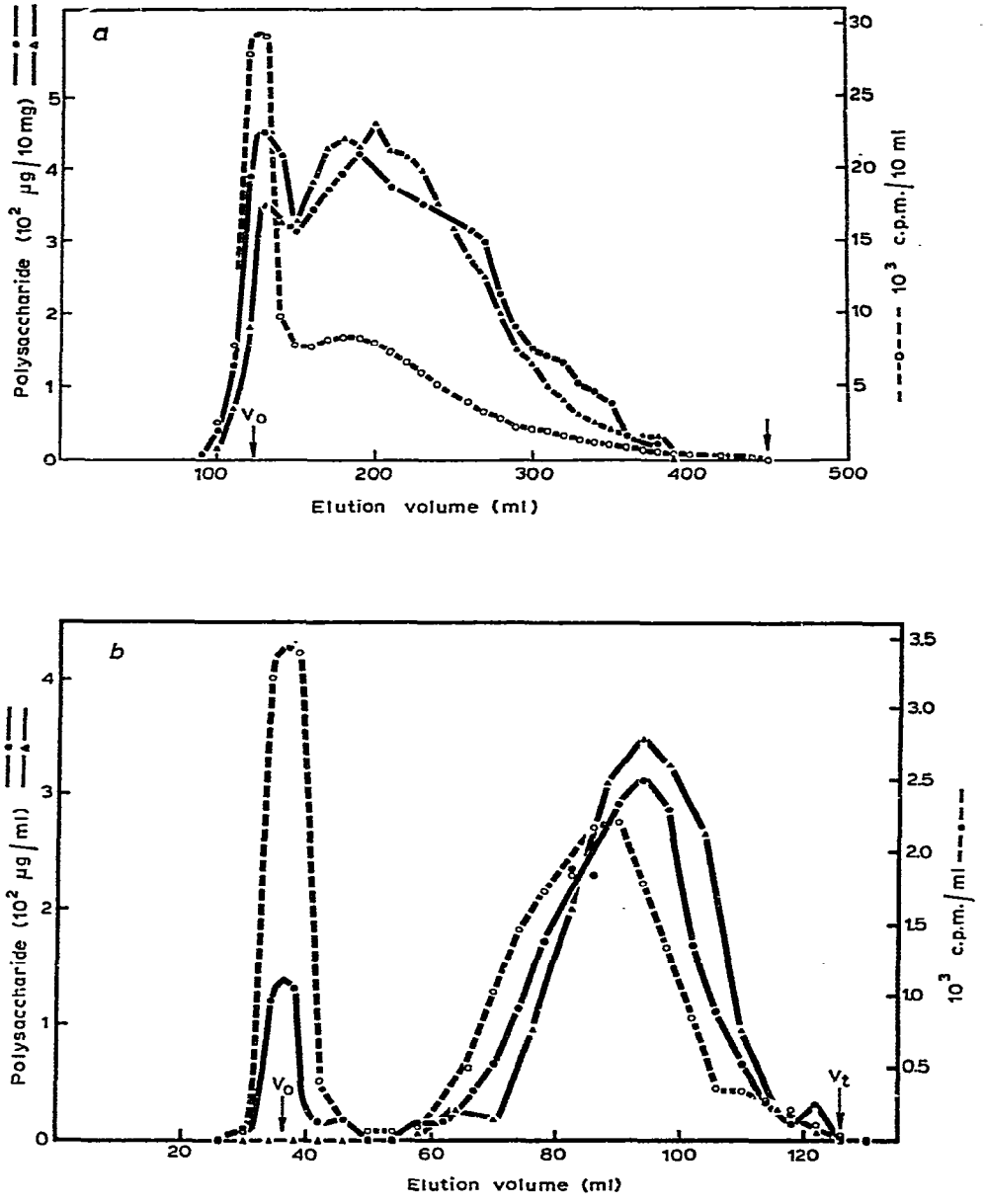

Fig. 2. Elution profiles of gel filtration of acceptor dextran before and after modification by the action of the soluble fraction of dextransucrase. Solutions (10 mg/5 ml) of unmodified clinical dextran (▲) or ^{14}C-labelled (modified) clinical dextran (● ○) were prepared and fractionated as described in Materials and Methods. (*a*) Fractionation on Bio-Gel P-300 (2.5 × 95 cm bed) of unmodified clinical dextran and acceptor dextran containing 6% incorporated [^{14}C]glucose. (*b*) Fractionation on Sepharose 4B (1.6 × 56 cm bed) of unmodified clinical dextran and acceptor dextran containing 25% of incorporated [^{14}C]glucose.

10% Scintisol (Fig. 1). Modified clinical dextrans containing 6 and 25% by weight of [^{14}C]glucose were prepared with "soluble" enzyme and exhaustively dialyzed against distilled water to remove unreacted [U-^{14}C]sucrose, and were then lyophilized. Modified dextran (10 mg) containing 6% of [^{14}C]glucose (4.35×10^5 c.p.m.) was applied in 5 ml to a Bio-Gel P-300 column (2.5×95 cm). The column was irrigated with 0.02% sodium azide solution at 40 ml/h and 10-ml fractions were collected (Fig. 2a). Modified dextran (10 mg) containing 25% (by weight) of [^{14}C]glucose (1.8×10^5 c.p.m.) was fractionated on a column (1.6×56 cm) of Sepharose 4B. The column was irrigated with sodium azide solution at 25 ml/h and 2-ml fractions were collected (Fig. 2b). Carbohydrate concentration in fractions from the Bio-Gel P300 and Sepharose 4B columns was determined by the phenol–sulfuric acid method and radioactivity by the filter-paper assay-method. Unmodified clinical dextran was fractionated on both columns for comparison with the elution profiles of the modified dextrans (Fig. 2a and 2b). The exclusion (V_0) and total (V_t) volumes of the Bio-Gel and Sepharose columns were determined from the elution volumes of glycogen (V_0) and glucose (V_t).

Preparation of amyloglucosidase limit-dextran. — Clinical dextran (4 g) and amyloglucosidase (1 g) were dissolved in 100 mM sodium acetate buffer (pH 5.0, 200 ml) and the solution was dialyzed at 23° against 20 volumes of the acetate buffer. At intervals, the outside buffer was replaced with fresh buffer and the total D-glucose that had diffused out of the mixture was determined. When no more D-glucose was released (96 h), the mixture was heated for 5 min at 100°, dialyzed against distilled water, centrifuged to remove insoluble material, and then lyophilized. The amounts of D-glucose released (1.2 g) and of residual limit-dextran (2.6 g) indicated that about 33% degradation had occurred. The limit dextran was resistant to further degradation by amyloglucosidase.

Preparation of partially hydrolyzed dextrans with dextranase. — Clinical dextran (100 mg) and dextranase (10 mg) dissolved in 50mM phosphate buffer, pH 6.8 (2 ml) were incubated for 5 min at 37° and then heated for 30 min at 100° to inactivate dextranase. Mixtures also were incubated for 10 and 30 min. The extent of dextran hydrolysis in each reaction was determined from the release of reducing sugars and expressed as percent conversion into glucose equivalents.

Complete hydrolysis of dextrans with dextranase. — Samples of clinical dextran or clinical-dextran fractions containing [^{14}C]glucose incorporated by the dextransucrase acceptor-reaction, were incubated at 37° in solutions containing dextran (2.5 mg/ml), dextranase (1.25 mg of crude powder/ml) and 25 mM sodium phosphate, pH 6.8. Glycogen and clinical dextran controls were hydrolyzed 22 and 36% respectively in 30 min, with D-glucose as the preponderant product from glycogen. Dextran yielded less than 2% of glucose. After 17 h, there was no further hydrolysis of dextran (40% reducing sugars) and so the solutions were heated for 10 min at 100° to inactivate dextranase. The cooled solutions were twice treated with mixed-bed resin in the carbonated form [AG 501-X8(D)] and then lyophilized. The residues were dissolved in a small volume of water and applied to Whatman 3 MM chromato-

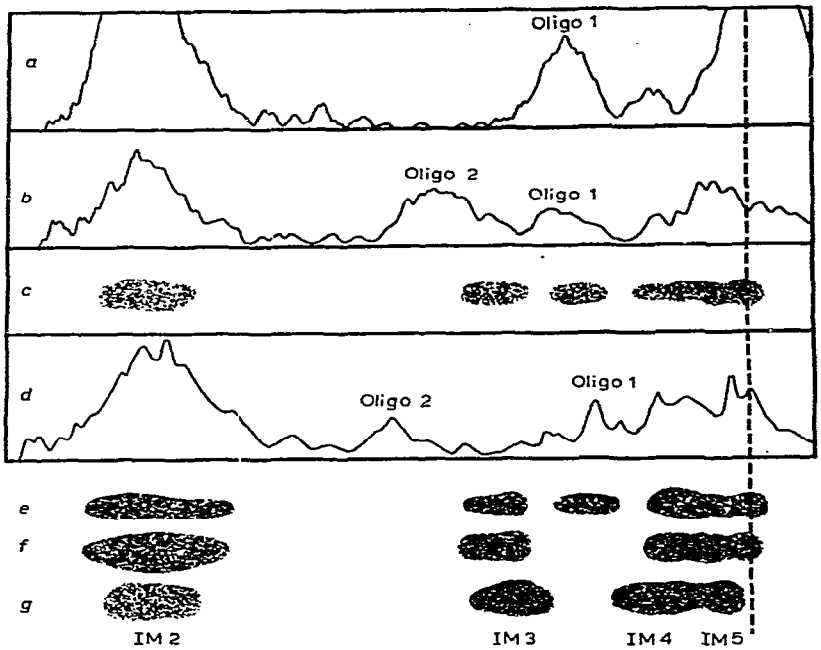

Fig. 3. Chromatographic separation of products of dextranase digestion of ^{14}C-labelled acceptor-dextrans. Sections (*a*), (*b*), and (*d*) are traces showing the distribution of ^{14}C on chromatograms, as determined by scanning with a Baird-Atomic Radiochromatogram Scanner, Model RSC-363. Sections (*c*), (*e*), (*f*), and (*g*) show distribution of carbohydrates on the chromatograms. Section (*a*), ^{14}C-labelled dextran excluded from Bio-Gel P-300 (Fig. 2a); (*b*) and (*c*), ^{14}-labelled dextran retarded by Bio-Gel P-300 (Fig. 2a); (*d*) and (*e*), unfractionated [^{14}C]dextran containing 6% incorporated [^{14}C]glucose; (*f*), clinical dextran; (*g*) isomalto-oligosaccharide standards: isomaltose (IM2), isomaltotriose (IM3), isomaltotetraose (IM4), and isomaltopentaose (IM5). The locations of oligosaccharides 1 and 2 are indicated by the notations "Oligo 1" and "Oligo 2". The chromatographic solvent was 10:4:3 ethyl acetate–pyridine–H_2O, and staining was by silver nitrate–sodium hydroxide.

graphy papers, which were irrigated for 42 h with 10:4:3 ethyl acetate–pyridine–water. Guide strips were scanned in a Baird-Atomic Radiochromatogram Scanner, Model RSC-363, to detect zones of radioactivity. After scanning, the components of the hydrolyzates were detected by silver nitrate–sodium hydroxide spray-reagent (see Fig. 3).

RESULTS

D-[^{14}C]Glucose incorporated from sucrose into insoluble dextran by the dextransucrase activity of the washed, insoluble-dextran cell-complex was not solubilized by amyloglucosidase or dextranase, under conditions in which glycogen was degraded completely to D-glucose by each enzyme. When the dextransucrase reaction was conducted in the presence of soluble clinical dextran, however, a dextransucrase component of the complex was solubilized and most of the ^{14}C-glucosyl label

was incorporated into a soluble polysaccharide fraction (see ref. 14) which was readily degraded by the two hydrolases. The soluble enzyme-component had a specific activity of 1.5 IU/mg protein and was free of levansucrase and dextranase. It contained a low invertase activity that hydrolysed sucrose in the dextran-synthesizing digests to give glucose amounting to 5% of the total reducing sugars released. The dextransucrase activity was eluted in fractions at, or near to, the void volume of a Sepharose 4B gel filtration column (not shown), indicating that it was associated with a high molecular weight, polydisperse aggregate. A higher proportion of activity was eluted in the fractions immediately following the exclusion limit on a column saturated and eluted with a soluble clinical-dextran solution (1 mg/ml), but significant dissociation of the aggregate did not occur.

The soluble enzyme-preparation, which contained clinical dextran (100 μg/ml), initially synthesized a small amount of soluble polysaccharide from sucrose, but then rapidly synthesized water-insoluble dextran and was inactivated (see ref. 14). Additional clinical dextran (1 mg/ml, Table I) or limit dextran (not shown) in the reaction mixture apparently stimulated dextran synthesis, but 8% hydrolyzed dextran (1 mg/ml) and methyl α-D-glucoside (1 mg/ml) did not stimulate or inhibit dextran synthesis, as measured by the incorporation of ^{14}C-glucosyl label from sucrose into polysaccharide (Table I). Panose, glycogen, amylose, laminaran, and nigeran did not stimulate nor inhibit polysaccharide synthesis under these conditions (1 mg/ml). Higher concentrations of methyl α-D-glucoside (19–150 mg/ml) apparently inhibited dextran synthesis, as measured by the ^{14}C-glucosyl incorporation assay, but an

TABLE I

THE REACTION OF DEXTRANSUCRASE IN THE PRESENCE OF CLINICAL DEXTRAN, HYDROLYZED CLINICAL DEXTRAN AND METHYL α-D-GLUCOSIDE[a]

Acceptor added	*Dextran synthesis (μg/ml)*	*Fructose release (μg/ml)*
None	90	80
Clinical dextran (1 mg/ml)	160	—
2%-Hydrolyzed dextran (1 mg/ml)	120	—
4%-Hydrolyzed dextran (1 mg/ml)	100	—
8%-Hydrolyzed dextran (1 mg/ml)	90	—
Methyl α-D-glucoside (1 mg/ml)	90	80
Methyl α-D-glucoside (19 mg/ml)	60	120
Methyl α-D-glucoside (38 mg/ml)	40	170
Methyl α-D-glucoside (150 mg/ml)	10	200

[a]Soluble dextransucrase (0.02 IU) was incubated for 60 min at 37° in solutions (1 ml) containing [^{14}C]sucrose ([U-^{14}C]glucosyl, 6.7 mg, 0.25 μCi), 50mM sodium phosphate buffer, pH 6.8, and the amount of acceptor indicated. All mixtures also contained clinical dextran (10 μg/ml) added with the enzyme solution. Dextran synthesis was determined from the ^{14}C-incorporation into ethanol-insoluble polysaccharide, and release of fructose was determined as described in Materials and Methods.

increased release of reducing sugars (fructose) showed that the transglucosylation reaction was in fact stimulated (Table I). The apparent K_m value for methyl α-D-glucoside in this reaction was about 10^{-1}M.

Fractionation on Biogel P-150 of the products formed by soluble dextransucrase in the presence of clinical dextran and [^{14}C]glucosyl-labelled sucrose showed that all of the [^{14}C]glucose incorporated (about 3% by weight of the dextran acceptor) was eluted with the acceptor dextran (Fig. 1); no ^{14}C-labelled material of low molecular weight was eluted other than the ^{14}C-labelled sucrose substrate. Dextran preparations, containing 6 and 25% of incorporated [^{14}C]glucose, were fractionated on Bio-Gel P-300 and Sepharose 4B columns respectively (Fig. 2a and 2b). The distribution of [^{14}C]glucosyl label in the polysaccharide peak retarded on Bio-Gel P-300 (Fig. 2a) indicates that most of the clinical dextran participating in the initial dextransucrase acceptor-reaction (6% [^{14}C]glucose incorporation) does so without undergoing a large increase in molecular weight. Synthesis of a small proportion of high-molecular-weight dextran, however, is shown by the high specific radioactivity of the excluded peak (Fig. 2a); furthermore, with the polysaccharide containing 25% of incorporated [^{14}C]glucose, elution of a discrete radioactive peak in the void volume of the Sepharose 4B column (Fig. 2b) gives evidence for the synthesis of high-molecular-weight products.

Low incorporation of [^{14}C]glucose into acceptor dextran (5% by weight) caused a marked increase in the interaction of the polysaccharide with concanavalin A (Table II). About 27% of the [^{14}C]glucose incorporated was released by amyloglucosidase during the first 3% conversion of the polysaccharide into D-glucose (not shown), but no further release of [^{14}C]glucose occurred up to 9% conversion into glucose. This contrasts with the failure of amyloglucosidase to release [^{14}C]glucose incorporated into insoluble dextran by the insoluble dextran–cell complex.

Dextran containing ^{14}C-glucose (6% by weight) incorporated from [^{14}C]-sucrose by soluble dextransucrase, was readily hydrolyzed by dextranase. Paper chromatography and autoradiography of the hydrolysis products revealed that a large proportion of the radioactivity was in a component corresponding in R_F value

TABLE II

INTERACTION WITH CONCANAVALIN A OF CLINICAL DEXTRAN BEFORE AND AFTER MODIFICATION BY DEXTRANSUCRASE ACTION (5% INCORPORATION OF [^{14}C]GLUCOSE)

Clinical dextran (mg)	*Absorbance × 100 (420 nm)*	
	Before modification	*After modification*
0.25	0.1	6
0.50	0.2	24
0.75	1.1	43
1.0	1.6	56

to isomaltose (Fig. 3, e and d). [^{14}C]Glucosyl label also was detected in two oligosaccharides (d) that were not detected in the dextranase hydrolyzate of the original clinical-dextran acceptor (f). Oligosaccharide 1 migrated between isomaltotetraose and isomaltotriose and oligosaccharide 2, a minor component that was not detected with the spray reagent, migrated between isomaltotriose and isomaltose. Treatment with dextranase of the [^{14}C]glucose-labelled polysaccharide peaks from the column of Bio-Gel P-300 (Fig. 2a) yielded radiolabelled oligosaccharides **1** and **2** from the retarded peak (Fig. 3, b), but only oligosaccharide **1** from the excluded peak (Fig. 3, a). Most of the [^{14}C]glucose-labelled polysaccharide in the retarded peak was hydrolyzed to isomaltose and oligosaccharides of low molecular weight, whereas the ^{14}C-labelled polysaccharide in the excluded peak was more resistant to dextranase treatment, a greater proportion of [^{14}C]glucose label in the hydrolyzate remaining at the origin of the chromatogram (a). Dextranase treatment of soluble, acceptor dextran containing 25% of incorporated [^{14}C]glucose resulted in the precipitation of insoluble, ^{14}C-labelled polysaccharide. The soluble fraction of this hydrolyzate contained ^{14}C-label, but its chromatographic pattern was identical to that of the hydrolyzate of unmodified clinical dextran.

Limit-dextran acceptor (prepared by exhaustive treatment of clinical dextran with amyloglucosidase) was incubated for 60 min with [^{14}C]sucrose (0.15 μCi/10 μg) and soluble dextransucrase. All of the [^{14}C]glucose (0.5 μg, 12,000 c.p.m.) incorporated into the acceptor (10 mg) was retarded on a Sepharose 4B column and was eluted in the same volume as the original limit-dextran (not shown). When the incorporated [^{14}C]glucose was chased with non-radioactive sucrose (50 mg/ml) for 15 min before separation on the Sepharose 4B column, 6% of the ^{14}C-glucosyl label appeared in a discrete polysaccharide component that was excluded from the column

TABLE III

INSOLUBILIZATION OF [^{14}C]GLUCOSYL LABELLED ACCEPTOR BY DEXTRANSUCRASE ACTION IN THE PRESENCE OF NON-RADIOACTIVE SUCROSE[a]

Dextransucrase activity (IU/ml)	*Dextran*		*[^{14}C]Glucosyl label*	
	Soluble (μg/ml)	*Insoluble (%)*	*Soluble (c.p.m./ml)*	*Insoluble (%)*
0	1,010	0	2,520	0
0.005	590	42	340	87
0.02	410	59	180	93

[a]Solutions (1 ml) containing soluble dextransucrase as indicated, sucrose (4 mg/ml), [^{14}C]glucosyl labelled limit-dextran (1 mg/ml) and 10 mM sodium phosphate were incubated at pH 6.0 for 4 h at 37°. Each solution was then centrifuged at 37,000 × *g* for 10 min, the supernatant solution was dialyzed against water and its content of soluble dextran and the associated radioactivity were determined as described in Materials and Methods. The [^{14}C]glucosyl-labelled limit-dextran used in this experiment was purified from a digest in which it had been previously prepared by incubation under similar conditions until it contained 0.4% by weight of incorporated [^{14}C]glucose.

TABLE IV

DEXTRAN SYNTHESIS BY DEXTRANSUCRASE IN THE PRESENCE OF INCREASING AMOUNTS OF SOLUBLE DEXTRAN[a]

Dextran added (mg/ml)	*Dextran synthesis (μg/ml)*	
	From fructose released	*From [^{14}C]glucose incorporated*
None	130	120
0.5	380	400
2	370	370
4	320	320
10	310	300
50	330	300

[a]Soluble dextransucrase (0.04 IU/ml) was incubated with [^{14}C]sucrose ([U-^{14}C]glucosyl) and the amounts of dextran indicated, for 60 min as in Table I. Endogenous dextran (40 μg/ml) was present in all mixtures (see Table I). The [^{14}C]glucose incorporated into dextran was determined as described in Materials and Methods. Fructose released by action of dextransucrase was determined by subtracting twice the amount of D-glucose released (D-glucose oxidase method) from the total production of reducing sugars (Nelson determination).

(not shown). A four-times higher specific radioactivity of the excluded peak, compared with that of the retarded peak, indicated that [^{14}C]glucose-labelled limit dextran was preferentially incorporated into the product of high molecular weight. Similarly, [^{14}C]glucose-labelled (0.4% by weight) limit dextran was preferentially incorporated into an insoluble dextran fraction when it was incubated with fresh dextransucrase and unlabelled sucrose (Table III).

The greatest apparent stimulation of dextran synthesis ([^{14}C]glucose incorporation) and transglucosylation (fructose release) occurred when soluble dextransucrase was incubated (1 h) with clinical dextran at a concentration of 0.5 mg/ml. Dextran concentrations of 2 mg/ml and higher caused lower stimulations (Table IV). The same result was obtained for dextran acceptor precipitated from 67% ethanol, and when 90% ethanol was used as the wash solution in the assay of [^{14}C]dextran synthesis. These observations, and the correspondence between release of fructose and ^{14}C-incorporation into dextran, establish that there is a real decrease in the stimulation of dextran synthesis at the higher concentrations of acceptor.

Time–progress curves for acceptor reactions catalyzed by a five-fold increased enzyme activity revealed that the initial rate of reaction in the presence of 10 mg of clinical dextran was substantially lower than that in the presence of 5 mg of acceptor which, in turn, was lower than the initial rate with 1 mg of acceptor. As the reaction progressed, however, the slower reactions increased in rate and, eventually, greater amounts of dextran were synthesized at the higher concentrations of acceptor (Fig. 4). The apparent inhibition of the initial reactions by acceptor was even more marked when limit dextran was used as an acceptor in reactions catalyzed by small concen-

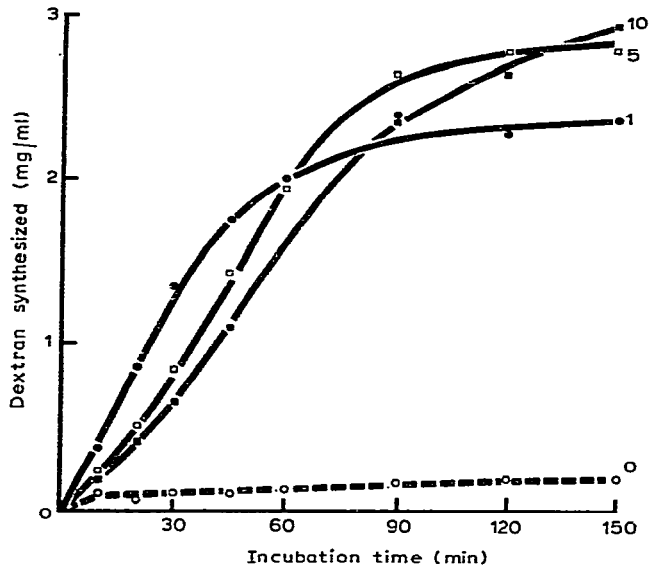

Fig. 4. Effect of clinical-dextran concentration on the rate and extent of dextran synthesis by soluble dextransucrase. Enzyme (0.2 IU) was incubated with different amounts of clinical dextran in mixtures (1 ml) containing [^{14}C]sucrose and buffer solution as in Table I. All mixtures also contained clinical dextran (20 μg/ml) added with the enzyme solution. Samples (50 μl) were removed at the times indicated and dextran synthesis was determined from the [^{14}C]glucose incorporated into ethanol-insoluble polysaccharide. Acceptor dextran added (mg/ml): ○, none; ●, 1; □ 5; ■, 10.

trations of enzyme (0.02 IU/ml). The initial reaction-rates were considerably lower than one would expect from the decreased enzyme activity but, again, they were inversely related to the concentration of acceptor (Fig. 5a). When the product formed during 120 min from 10 mg of limit-dextran acceptor was included at the same concentration in a fresh reaction-mixture, however, the initial curve for reaction progress was similar to that of dextransucrase acting in the presence of 1 mg of the limit-dextran acceptor (Fig. 5b). When synthesis was measured after reaction times directly proportional to the amounts of clinical dextran in each digest, it was directly proportional to the time of reaction (Fig. 6b). That is to say, the average rate of dextran synthesis (μg/min/ml of digest) in each mixture was approximately equal under these conditions (Fig. 6a).

An ammonium sulfate fraction of dextransucrase from *S. mutans* BHT synthesized a 75% water-soluble dextran and was not stimulated by exogenous clinical dextran, whereas a similar enzyme fraction from *S. mutans* K1-R synthesized water-insoluble dextran and was stimulated by clinical dextran and by the soluble dextran synthesized with BHT dextransucrase (Table V). Synthesis of dextran by strain K1-R enzyme was not significantly increased, however, when BHT enzyme was allowed to synthesize soluble dextran in the same reaction mixture. Under these conditions, dextran synthesis was about equal to the sum of the synthesis observed for each enzyme preparation acting independently (Table V).

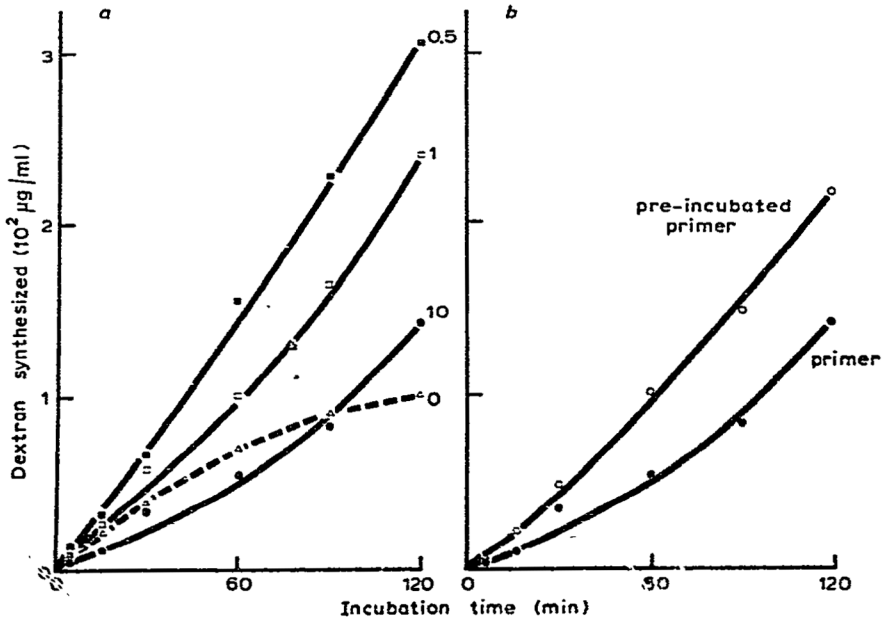

Fig. 5. Effect of limit-dextran concentration on the initial rate of synthesis of dextran by soluble dextransucrase. Reaction mixtures (1 ml) and procedures were as described in Fig. 4 except that only 0.02 IU of enzyme was used and clinical dextran was replaced by limit dextran as the exogenous acceptor. All mixtures also contained clinical dextran (150 µg/ml) added with the enzyme solution. (*a*) Synthesis in the presence of different concentrations of limit dextran (mg/ml): △, none; ■, 0.5; □, 1.0; ●, 10. (*b*) Synthesis before (●) and after (○) limit dextran (10 mg/ml) was modified in a dextransucrose reaction-mixture. Limit dextran (10 mg/ml) was incubated for 120 min in a dextransucrase reaction-mixture as in (*a*). The modified acceptor was recovered by precipitation with 67% ethanol and included at a concentration of 10 mg/ml in an identical reaction-mixture.

TABLE V

EFFECT OF DEXTRAN ACCEPTOR ON DEXTRAN SYNTHESIS BY DEXTRANSUCRASES FROM *S. mutans* STRAINS BHT AND K1-R[a]

Reaction time (h)	*Dextran synthesis (µg/ml)*					
	BHT enzyme	*K1-R enzyme*	*BHT + K1-R enzymes*	*BHT enzyme + clinical dextran*	*K1-R enzyme + clinical dextran*	*K1-R enzyme + BHT dextran*
0.25	51	13	150			
0.5	100	26	165	112	310	450
1.0	149	44	198	145	1030	730
2.0	259	67	293	243	1510	1,000

[a]Solutions (0.4 ml) containing "crude" dextransucrase preparations (0.1 ml) were incubated with or without clinical dextran or BHT dextran (250 µg/ml) in the presence of [^{14}C]sucrose, as described in Table I. Dextran synthesis was determined from the [^{14}C]glucose incorporated into ethanol-insoluble polysaccharide. The BHT dextran used was prepared in a digest containing BHT enzyme and non-radioactive sucrose, and was purified by precipitation with ethanol.

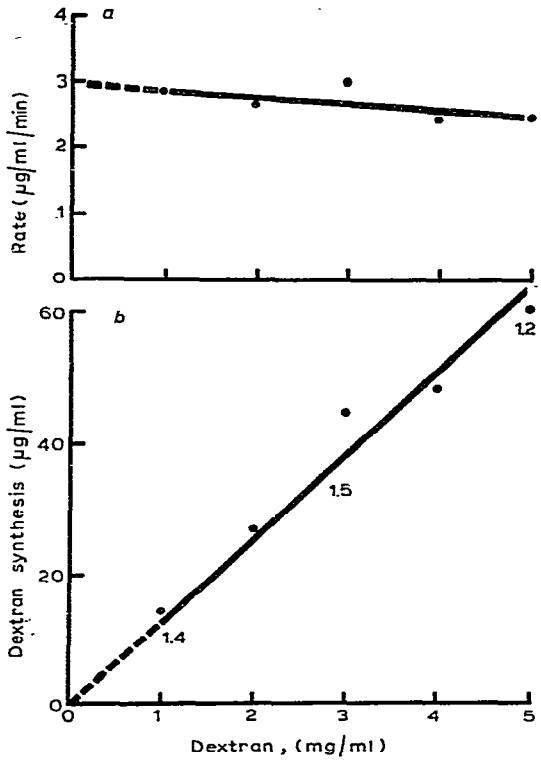

Fig. 6. Dextran synthesis and average synthetic rate when incubation times are proportional to the concentration of acceptor dextran. Reaction mixtures (5 ml) contained enzyme (0.1 IU), clinical dextran at concentrations of 1–5 mg/ml, and [U-^{14}C]sucrose and buffer as described in Table I. Incubation periods at 37° were directly related to the concentration of acceptor dextran (1 mg/ml incubated for 5 min, 2 mg/ml for 10 min, and so on). (*a*) Average rate of dextran synthesis; (*b*) dextran synthesized as a function of acceptor concentration and incubation time. Numerals below graphed line indicate the percent of incorporated [^{14}C]glucose in acceptor present at 1, 3, and 5 mg/ml after incubation for 5, 15, and 25 min, respectively.

DISCUSSION

Solubilization of the dextransucrase activity (reversibly bound) associated with the insoluble dextran–cell complex provides a convenient and specific method for preparing an enzyme fraction suitable for the study of the dextran-acceptor reaction. The solubilized activity utilizes clinical dextran as an acceptor and, in doing so, forms soluble dextran as the initial product of its reaction with sucrose[14]. The specific activity of the soluble dextransucrase preparation is comparable to those of enzyme fractions prepared by procedures involving ion-exchange chromatography and isoelectric focusing[24,25], and its state of aggregation resembles those of other preparations[26–28] in which co-purification of levansucrase and dextransucrase activities suggested the existence of a specific enzyme-complex.

The absence of levansucrase from the soluble dextransucrase preparation does

not preclude the presence of several dextransucrases. Thus, radioactive isomaltose present in the hydrolyzate of the modified dextran acceptor (Fig. 3) confirms the synthesis of (1 → 6) linkages, whereas dextranase-resistant—and water-insoluble—products are consistent with the formation of (1 → 3) linkages, for which supportive evidence is provided by preliminary methylation analyses. Enzymes synthesizing water-soluble, (1 → 6)-linked-α-D-glucans[27] or water-insoluble, (1 → 3)-linked-α-D-glucans[29] have been isolated from crude streptococcal enzyme preparations, but it remains to be confirmed if the soluble dextransucrase preparation contains activities corresponding to these.

The lack of acceptor activity of polysaccharides other than dextran, and the inability of small saccharides to compete with a low concentration of clinical dextran (Table I), indicate that the requirement for exogenous acceptor is specific for the macromolecular structure of dextran. As the interaction of dextrans with concanavalin A is related to their degree of branching or content of (1 → 2) linkages[7,30], the absence of (1 → 2) linkages in the modified dextran, as indicated by preliminary methylation analyses, suggests that increased branching is responsible for the higher reactivity of the modified dextran with concanavalin A (Table II). The small change of molecular weight that accompanies the modification (Figs. 1 and 2a) suggests that a large proportion of the additional branching is accomplished by the transfer of single D-glucosyl residues or short oligosaccharide chains to the acceptor dextran.

The ability of a modified limit-dextran to sustain a higher initial rate of the dextransucrase reaction (Fig. 5a and b) and its preferential incorporation into products excluded from Sepharose 4B (not shown) or insoluble in water (Table III), indicate that it is a more-efficient acceptor than the original limit-dextran. The initial formation of a small number of highly efficient acceptor molecules would provide focal points for rapid sequential transfers in the ensuing reaction and, hence, is consistent with the synthesis of discrete dextran fractions in the presence of acceptor dextran (Fig. 2b). A progressive increase in acceptor efficiency would also explain the synthesis of macromolecular dextran by *Leuconostoc mesenteroides* dextransucrase acting on sucrose in the presence of oligosaccharide acceptors[6].

The lower initial rates of dextransucrase action at higher concentrations (10 mg/ml) of dextran and limit-dextran acceptors (Figs. 4 and 5a) probably reflect the greater amounts of synthesis (and hence time) required to convert the acceptor molecules into specific structures that support an increased reaction rate, comparable to that attained at earlier stages in the dextransucrase reaction with lower concentrations (1 mg/ml) of acceptor (Fig. 5b). Three stages in the dextransucrase acceptor-reaction, therefore, may be recognized (Figs. 4 and 5), namely, an initial, slow reaction in which efficient acceptor is produced, a rapid reaction in which efficient acceptor is used, and the final reaction, decreasing in rate as the enzyme is irreversibly inactivated (characterized as irreversible on the basis that addition of fresh, clinical dextran does not restore the lost activity[14]).

In the absence of exogenous dextran-acceptor, soluble K1-R dextransucrase uses low concentrations of endogenous dextran to synthesize insoluble dextran

rapidly with a progressive loss of activity (Figs. 4 and 5a). Although total dextran synthesis was increased at concentrations of acceptor dextran up to 0.5 mg/ml (not shown), non-linear progress curves prevented us from assessing the contribution made by stimulation of the initial reaction rate as opposed to that made by a decrease in the rate of enzyme inactivation. Any stimulation of the initial reaction-rate by very low concentrations of dextran, similar to that observed[31] for an enzyme preparation from *Streptococcus mutans* 6715, is masked completely by the rapid inactivation of the K1-R enzyme under these conditions. The non-limiting effect of soluble acceptor, observed when dextran at concentrations greater than 0.5 mg/ml is modified to the same extent (Fig. 6), confirms that a major role of the exogenous dextran acceptor is to prevent inhibition, rather than to stimulate the reaction by a mass-action effect. The water-insoluble polysaccharide that precipitates (not shown) after dextranase hydrolysis of the (1 → 6)-linked component of modified acceptor dextran (containing 25% of [^{14}C]glucose) strongly suggests that the acceptor dextran acts primarily as a soluble matrix, on which dextransucrase synthesizes potentially water-insoluble structures. The soluble, macromolecular acceptor delays the onset of precipitation and the accompanying enzyme inactivation and, in so doing, allows an increased synthesis of insoluble polysaccharide (Fig. 4). The failure of the BHT enzyme to increase dextran synthesis significantly by the K1-R dextransucrase (Table V) emphasizes that a significant enhancement of complex dextran synthesis requires the presence of substantial amounts of preformed, soluble acceptor.

Clearly, the availability of suitable acceptor is an important determinant of the structure and amount of dextran synthesized by the dextransucrase(s) of *Streptococcus mutans* K1-R. Insoluble dextran formed from sucrose by a crude enzyme-preparation from *Streptococcus mutans* OMZ 61 comprised a (1 → 6)-linked dextran backbone having (1 → 3)-linked side-chains[32], whereas the dextran product made by enzyme from strain OMZ 176 possessed a (1 → 3)-linked backbone and (1 → 6)-linked side-chains[33]. It is tempting to suggest that the mechanism of synthesis of the first of these structures is similar to that of the insoluble dextran formed by the soluble K1-R dextransucrase enzyme acting on the (1 → 6)-linked clinical-dextran acceptor. An understanding of synthesis of dextrans by crude enzyme-mixtures, and of even more-complex structures produced in mixed bacterial cultures and in dental plaque, therefore, must include a knowledge, not only of the type, quantity, and order of elaboration of the dextransucrases present but, also, of their specific requirement for acceptors that are incorporated into the structure of the final dextran products.

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