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ACTIVITY OF BRANCHED DEXTRANS IN THE ACCEPTOR REACTION OF A GLUCOSYLTRANSFERASE (GTF-I) FROM *Streptococcus mutans* OMZ176*

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ABSTRACT

The ability of several native and chemically synthesized, branched dextrans to stimulate the activity of an α-D-glucosyltransferase (GTF-I) of *Streptococcus mutans* has been compared. The enzyme catalysed the transfer of glucosyl residues from sucrose with the formation of water-insoluble (1→3)-α-D-glucan. The rate of this reaction was greatly increased in the presence of dextran, and the extent of stimulation was negatively correlated with the degree of branching of the added dextran. The results refute the concept that growth of water-insoluble glucan occurs from the multiple, non-reducing termini of dextran acceptors.

INTRODUCTION

One of the models proposed for α-D-glucan produced by *S. mutans* described a structure containing sequences of (1→6)-linked α-D-glucose residues together with sequences of (1→3)-linked residues[1]. This was consistent with the production by *S. mutans* OMZ176 of two distinct α-D-glucosyltransferases, GTF-S and GTF-I, which catalyze the synthesis, from sucrose, of the (1→6)- and (1→3)-linked chains, respectively[2]. The two types of chain were shown attached to one another through branch linkages, the premise being that each enzyme produced a glucan that could function as an acceptor for the other enzyme. In support of this concept, it was shown that low concentrations of either GTF-S itself, or the highly branched, soluble dextrans synthesized by GTF-S, stimulated GTF-I to produce greatly increased amounts of water-insoluble glucan.

The model, as depicted, gave little insight into the precise location on the dextran chains where the (1→3)-linked glucan chains could be attached. Two possibilities may be considered: (*a*) transfer to the (1→3)-linked glucosyl side-chains could occur, or (*b*) a glucose residue on the main chain, in between branch points, could act as an acceptor. Our preliminary studies revealed that the rate of incorporation of D-[^{14}C]glucose from [^{14}C]sucrose into water-insoluble glucan as

*Dedicated to Professor L. F. Leloir on the occasion of his 80th birthday.

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catalysed by GTF-I could be increased substantially by the addition of a chemically synthesized, linear (1→6)-α-D-glucan[3]. A comparison between dextrans indicated that the ability of the linear dextran (mol. wt. 3.2×10^4) to stimulate glucan synthesis by GTF-I was approximately the same as that of *Leuconostoc mesenteroides* B-512 dextran (mol. wt. 1.8×10^4), despite the fact that B-512 dextran, with ~5% of (1→3) branch linkages, contained ten times more non-reducing terminals than the linear dextran. This result indicated that *sequences* of (1→6)-linked D-glucose residues, rather than end groups, could be the structural feature, common to both dextrans, that is necessary for stimulating the activity of GTF-I. This view was reinforced by the finding that the soluble dextran produced by *L. mesenteroides* B-1355, which contains no sequences of (1→6)-linked residues, had virtually no effect on the rate of synthesis of water-insoluble glucan by GTF-I. This dextran has a highly ramified structure, with 11% of branch linkages, and the chains contain alternating (1→6)- and (1→3)-linked α-D-glucose residues[4].

In the present work, we wished to confirm whether it is the sequences of unbranched, α-D-(1→6)-linked D-glucosyl residues, or the non-reducing terminals in dextrans, that constitute an acceptor region for growing (1→3)-glucan chains. Additions to linear regions would involve, as a first step, the synthesis of a branch linkage. It is already known that *S. mutans* OMZ176 GTF-I, when incubated with sucrose in the presence of isomaltose oligosaccharide-acceptors, catalyzes the introduction, by means of a (1→3) branch linkage, of a D-glucosyl side-chain to the D-glucose residue penultimate to the reducing terminal[5]. Isomaltotetraose, -pentaose, and -hexaose were poor acceptors, but branching activity of the enzyme increased greatly when the d.p. of the acceptor was 7 or above. Furthermore, glucosyltransferases responsible for the synthesis of water-insoluble glucan were prepared from *S. mutans* strains K1-R (ref. 6) and 6715-15 (ref. 7), and were shown to introduce (1→3)-branch linkages into B-512 dextran fractions. The modified dextrans then became more efficient[6] acceptors for GTF-I, and the conclusion was that the *new* side-chains were elongated by the addition of (1→3)-linked glucose residues[7].

In contrast to these results, the extent of stimulation of activity of *S. mutans* 6715 glucosyltransferase by two B-512 dextran fractions (mol. wt. 9,400 and 193,000) was used to support a mechanism of water-insoluble glucan growth from the multiple, non-reducing terminals of the dextran acceptors[8]. We have therefore examined a selection of dextrans, having more non-reducing end-groups than B-512 dextran, to test whether they were more, or less, efficient as acceptors for GTF-I. The degree of branching of the dextrans ranged from 5 to 42%. Thus, their degree of linearity, *n*, defined as the average number of unsubstituted glucose residues in between branch points, varied from 21 down to 0.4.

METHODS AND MATERIALS

Isolation of enzyme. — *Streptococcus mutans* GTF-I was separated from batch-culture filtrates of strain OMZ176 that had been grown[9] overnight at 37°

under anaerobic conditions in complex medium supplemented with 2% of D-glucose, with the pH controlled at 6.0. The bacteria were removed by centrifuging at 4000*g* for 10 min at 4°, and the cell-free filtrate (8 L) was concentrated to 160 mL in a hollow-fibre concentrator fitted with an H1P10 cartridge (Amicon Corporation). The volume was further decreased to 65 mL in a stirred cell (Amicon Model 402) fitted with a PM10 Diaflo ultrafiltration membrane. The retentate was dialysed against sodium citrate buffer (pH 6.5, 50mM), and then ammonium sulphate was added to give 50% saturation. The precipitated protein was dissolved in potassium phosphate buffer (pH 6.5, 10mM) and dialysed overnight against the same buffer. A portion (18 mL) was applied to a column (42 × 3 cm) of hydroxylapatite (Bio-Gel HTP) prepared in the same buffer. The column was eluted with a linear gradient of potassium phosphate buffer (pH 6.5), and GTF-I was eluted at a concentration of 0.4M. Fractions containing GTF-I activity were combined, and dialysed against sodium citrate buffer (pH 6.5, 50mM).

Determination of GTF-I activity. — GTF-I assays were performed by determining the incorporation of label from [U-^{14}C]sucrose into polysaccharide. The standard mixture (0.25 mL), which contained [U-^{14}C]sucrose (2.5 mg, 18 mCi/mol), sodium citrate buffer (25mM, pH 6.0), dextran T70 (25 μg), and GTF-I (usually 20–30 mU), was incubated for 30 min at 35°, and then ethanol (2 vol) was added to stop the reaction and precipitate the polysaccharide. After one h at 4°, the glucan was sedimented by centrifuging, and washed twice with methanol. The precipitate was resuspended in methanol (1 mL) and filtered through a Whatman 3 MM paper disc (25 mm) held in a Millipore assembly. After washing with methanol (20 mL), the paper was transferred into a scintillation vial and dried for 30 min at 50°. A toluene cocktail (8 mL) was added, and the radioactivity was counted in a liquid-scintillation counter (Beckman Model LS 9000). One unit (U) is defined as the amount of GTF-I that will incorporate 1 μmol of D-glucose per min into dextran under the foregoing conditions.

Effect of branched dextrans on the activity of GTF-I. — A series of dextrans replaced dextran T70 in the mixture just described for GTF-I assays. All of the tests described in this paper refer to the effect of various conditions on GTF-I activity, and all are based on the standard mixture (0.25 mL). The amount of sucrose (1%) remained constant, and the concentration of GTF-I remained in the range 80–120 mU/mL, but the incubation time, and the concentrations of dextran and sodium citrate buffer, were varied. Reaction times were proportional to the concentration of added dextran[6].

Carbohydrates. — Dextran fractions derived from the hydrolysis of native dextran B-512(F) were purchased from Pharmacia (South Seas). The molecular weights of these dextrans (T2000, T500, T70, T40, and T10) are shown in Table I. Native, bacterial dextrans[10] isolated from cultures of *Leuconostoc mesenteroides* strains NRRL B-512(F), B-742 (fraction S), B-1142, and B-1191, and from *Streptococcus viridans* NRRL B1351, were kindly provided by Drs. Allene Jeanes and M. E. Slodki.

TABLE I

EFFECT OF MOLECULAR WEIGHT OF THE DEXTRAN ACCEPTOR ON THE AVERAGE RATE OF α-(1→3)-D-GLUCAN SYNTHESIS

Dextran acceptor	*Molecular weight*		*Rate of α-(1→3)-D-glucan synthesis Cpm/min of incubation*
	$\bar{M}_n$	$\bar{M}_w$	
Native	1.7×10^6	50×10^6	367
T2000		2×10^6	366
T500	1.9×10^5	4.7×10^5	385
T70	3.7×10^4	6.8×10^4	393
T40	2.8×10^4	4.1×10^4	384
T10	6.2×10^3	1.0×10^4	298
None			21

TABLE II

LINKAGE ANALYSIS OF SOLUBLE DEXTRANS TESTED AS ACCEPTORS FOR GTF-I

Dextran	*Non-reducing terminals (%)*	*(1→6)-Linked residues (%)*	*(1→3)-Linked residues (%)*	*(1→3)-Branch points (%)*
B-512(F)[a,b]	4.2	91.2		4.6
T70[b]	4.6	91.6		3.8
B-1351[c]	5.8	83.3		10.5
B-1191[d]	21.8	59.6		17.2
B-1142[d]	33.3	35.0	1.3	30.4
B-742(S)[e]	38	25		28
V39[f]	10	80		10
V17	25	50		25
V37	33	34		33
V32	42	16		42
OMZ176[g]	31	40	7	22
AHT[g]	32	29	8	31

[a]The B fractions are native dextrans from NRRL strains. [b,c,d]Methylation analysis from refs. 15, 16, and 17, respectively. [e]This dextran also contains 8% of (1→4)-branch points (personal communication from Dr. M. E. Slodki). [f]The chemically synthesized V dextrans were analysed by ^{1}H-n.m.r. spectroscopy[12]. [g]Glucans from *S. mutans* strains were analysed by Professor S. Svensson.

A family of stereoregular (1→6)-α-D-glucans, V17, V32, V37, and V39, having randomly distributed, (1→3)-linked glucosyl side-chains, were synthesized chemically by a polymerization method[11] that was modified[12] for copolymerization and α-glycosidation. The sequence-distribution of branch linkages in the synthetic dextrans was calculated from reactivity ratios of the initial monomers, based on random copolymerization. The linkage analysis of the dextrans is shown in Table II.

Soluble-dextran fractions from *Streptococcus mutans* strains AHT and OMZ176 were prepared by incubating sucrose with cell-free filtrates obtained from growth in the chemostat at a low dilution-rate (D 0.05 h^{-1}) in glucose-limited medium. Under this growth condition, glucosyltransferase activity was high[13], and

was mainly[14] GTF-S. The minor amount of GTF-I (<10% of the total GTF) catalyzed the synthesis of a low proportion of (1→3)-linked sequences, leading to a mixed-linkage glucan.

A soluble carboxymethyl derivative of *Aspergillus niger* (1→3)-α-D-glucan was kindly provided by Dr. H. J. Phaff. [U-^{14}C]Sucrose (20.5 mCi/mmol) was purchased from ICN Tracer Labs.

RESULTS

Although the final product of the action of GTF-I with sucrose is a water-insoluble (1→3)-α-D-glucan, it cannot be assumed that *all* of the product is insoluble at early stages in the reaction when dextran is present. It is well known that when dextran is greatly in excess, the total glucan product of GTF-I activity is soluble when the extent of reaction is low. In the present study, where the ability of different dextrans to stimulate GTF-I activity was to be compared, it was necessary to determine the glucan product after short reaction-times. If the proportion of water-insoluble glucan in the total glucan product varied with dextran concentration and with the extent of reaction, then the conversion of sucrose into water-insoluble glucan would not give a true indication of GTF-I activity. To investigate this point, two series of nine mixtures (0.25 mL) were prepared, each series containing [^{14}C]sucrose (2.5 mg), dextran T70 (either 0.1, 0.2, or 0.4 mg/mL), GTF-I (20 mU), and sodium citrate buffer (25mM, pH 6.0). After incubation at 35° for 30, 60, and 120 min, the first set was treated with ethanol (0.5 mL), and the total glucan product was counted as described in Methods. Tubes of the second set were boiled to stop the reaction, and the water-insoluble fraction of the glucans was collected by centrifuging, washed once with water (1 mL) and once with methanol.

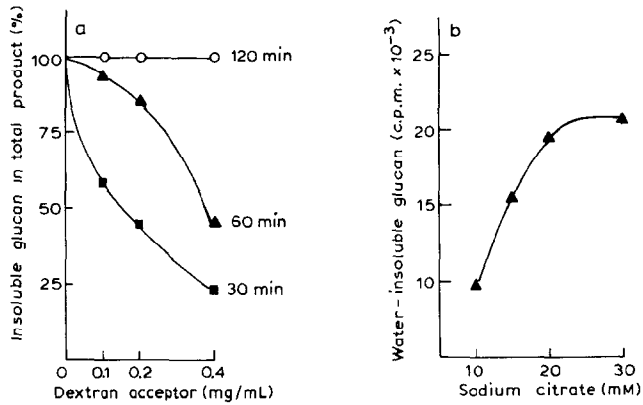

Fig. 1. (a) Effect of the concentration of dextran T70 on the proportion of water-insoluble glucan in the total product synthesized from sucrose by GTF-I. Mixtures (0.25 mL) containing [U-^{14}C]sucrose, dextran acceptor, GTF-I (20 mU), and sodium citrate buffer (30mM) were incubated at 35° for periods up to 2 h. (b) Effect of the concentration of sodium citrate buffer (pH 6.0) on the synthesis of water-insoluble glucan. Mixtures (0.25 mL) containing [U-^{14}C]sucrose and dextran T70 acceptor (50 μg) were incubated for 60 min with GTF-I (20 mU) and buffer.

The glucans were suspended in methanol and collected by filtration on paper discs by the procedure already described for 66% ethanol-insoluble product (total glucan). The results (Fig. 1a) showed that in those mixtures containing the highest concentration of dextran, a higher proportion of the glucan product remained soluble after 30 and 60 min of reaction. Only after incubation for 2 h did the product become completely water-insoluble. These results proved that, even when the dextran concentration in assay mixtures was kept low, the full extent of stimulation of GTF-I activity could only be determined by precipitating the soluble glucan product.

Dextran-stimulated GTF-I activity, as determined from the radioactivity of the water-insoluble glucan product, was also dependent on the concentration of sodium citrate buffer. When standard mixtures (0.25 mL) containing [U-^{14}C]-sucrose, dextran T70 (0.2 mg/mL), and GTF-I (20 mU) were buffered with increasing concentrations of sodium citrate buffer, a 2-fold increase in synthesis of water-insoluble glucan occurred when the buffer concentration was raised from 10 to 25mM (Fig. 1b). The dependence of glucan solubility on salt concentration emphasised the need to precipitate the soluble glucan product. Accordingly, in all further assays of GTF-I activity, the *total* product, namely soluble glucan together with water-insoluble glucan, was determined as described in Methods.

Effect of molecular weight of dextran on the stimulation of GTF-I activity. — The native dextrans, the chemically synthesized dextrans, and the enzymically synthesized dextrans that are compared in this study provide a series containing between 5 and 42% of (1→3)-α-D-glucosidic branch-linkages. However, the dextrans also differ greatly in their molecular weights. Therefore, the effect of the degree of branching of each dextran on its stimulation of GTF-I could not be ascertained with this diverse series unless it could be proved that the molecular weight of a dextran had no influence on its reactivity. To test this point, use was made of a series of commercial dextrans (Pharmacia Fine Chemicals) derived from *L. mesenteroides* NRRL B-512(F) dextran by controlled, partial acid hydrolysis. Each dextran, at concentrations of 0.1 and 0.2 mg/mL, was incubated at 35° in standard mixtures (0.25 mL) containing [U-^{14}C]sucrose, GTF-I (30 mU), and sodium citrate buffer (25mM, pH 6.0), for 30 and 60 min, respectively. The product was precipitated with ethanol and counted (see Methods). The counts, divided by the time of incubation, gave the average rate of glucan synthesis catalyzed by GTF-I, expressed as the rate of incorporation of ^{14}C-glucose per min (Table I). The results indicated that variation in $\bar{M}_w$ from 41×10^3 to 50×10^6 had no significant effect on the acceptor reaction. Only the smallest dextran ($\bar{M}_w$ 10,400) showed a slightly decreased ability to stimulate GTF-I activity.

Our best estimates of $\bar{M}_w$ of the chemically synthesized, branched dextrans, based on data from their immediate precursors and a known degree of degradation for V39, are as follows: V39, 77,700; V17, 43,700; V37, 39,800; and V32, 11,300. It was concluded that the efficiency of the native and synthetic dextrans could be compared in the acceptor reaction with GTF-I, despite the variation in their molecular weights.

Acceptor efficiency of branched dextrans. — The dextrans were added at concentrations of 0.05, 0.10, and 0.15 mg per mL to mixtures (0.25 mL) containing [U-^{14}C]sucrose, GTF-I (20 mU), and sodium citrate buffer (25mM, pH 6.0). Incubation times at 35° were 15, 30, and 45 min, respectively. Synthesis of α-(1→3)-glucan was thus determined after reaction times that were proportional to the concentration of added dextran, and under these conditions the average rate of glucan synthesized was constant for a given dextran. Plots of [^{14}C]glucose incorporated into 66% ethanol-insoluble glucan against the concentration of acceptor dextran gave straight lines (Fig. 2), the slopes of which were measured to give a value for the efficiency of each dextran as an acceptor for GTF-I. Because dextran T70 gave the highest stimulation of GTF activity and was therefore the most efficient acceptor, this dextran was given an efficiency value of 90 units (see Discussion). All other dextrans were compared with T70, and an example is given in Fig. 2, where the rate of glucan synthesis in the presence of V32 dextran and T70 dextran, respectively, is shown. Comparison of the slopes indicated that the stimulation of glucan synthesis due to the acceptor reaction with V32 dextran was 19% of that with T70 dextran. Hence the efficiency value for V32 dextran was set at 17 (T70 dextran = 90).

When this procedure was applied to dextrans of known structure (Table II), a highly significant negative correlation was found between the extent of branching of a dextran and its ability to stimulate GTF-I activity expressed as efficiency values (Fig. 3). The correlation coefficient was −0.988. Extrapolation of the line of best fit indicated that a dextran would become totally unable to act as an acceptor only when the extent of branching was 50.3%. The maximum value for branching is 50%, when every glucose residue on the main chain bears a glucosyl side-chain.

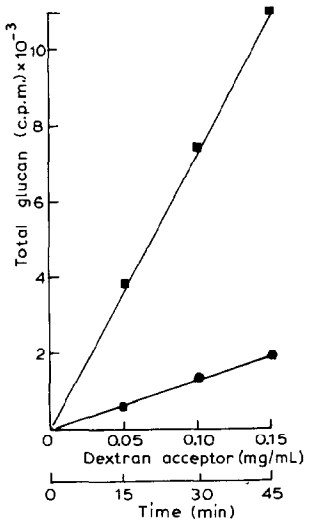

Fig. 2. Rate of glucan synthesis in mixtures (0.25 mL) containing [U-^{14}C]sucrose, sodium citrate buffer (25mM, pH 6.0), GTF-I (20 mU), and either T70 (■) or V32 (●) dextran acceptor.

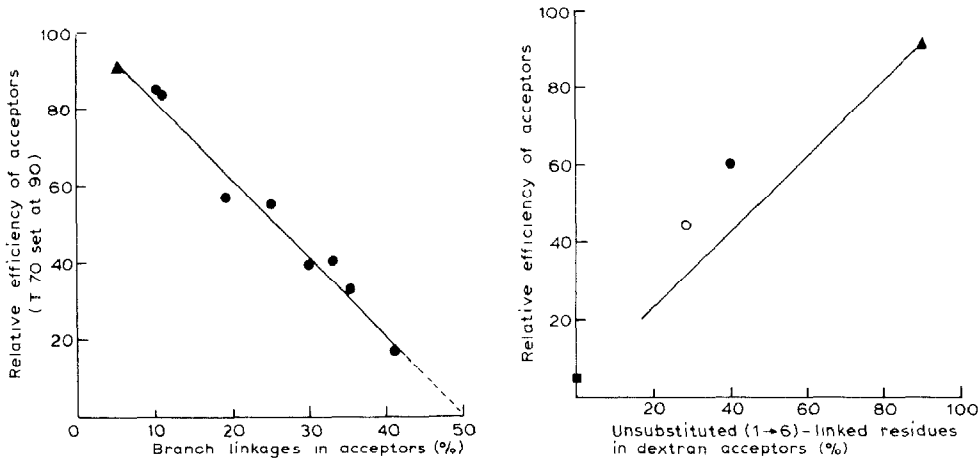

Fig. 3. Relationship between the proportion of branch linkages in dextrans and their efficiency in stimulating GTF-I to catalyze the synthesis of glucan from sucrose. Dextran T70 (▲), with 5% of (1→3)-branch linkages was given a value of 90 for its efficiency as acceptor. The efficiency of the other dextrans (●) was expressed relative to that of T70 by the procedure described for V32 dextran (see Fig. 2 and text).

Fig. 4. Comparison of the efficiency of *S. mutans* AHT soluble-glucan fraction (○), *S. mutans* OMZ176 soluble-glucan fraction (●) and (1→3)-(*O*-carboxymethyl)-α-D-glucan (■) with T70 (▲) and other dextrans as acceptors for GTF-I in standard mixtures. The solid line (correlation coefficient 0.993) relates the efficiency in the acceptor reaction to the proportion of (1→6)-linked sequences in dextrans that are free from (1→3)-linked sequences (data from Fig. 3 and Table II). The *S. mutans* glucans contain, in addition, 7–8% of (1→3)-linked sequences.

Soluble-dextran fractions from *S. mutans* strains AHT and OMZ176 were also tested as acceptors for GTF-I. Methylation analysis of these glucans confirmed their similarity to dextrans synthesized by GTF-S (31% of branching), but in addition they contained 7–8% of (1→3)-linked residues not involved in branching. The glucans were better acceptors, by a factor of up to 40% (Fig. 4), than could be expected from their content of (1→6)-linked sequences. By contrast, a soluble glucan obtained by preparing a carboxymethyl derivative of (1→3)-α-D-glucan had an efficiency value of 5, giving an average rate of glucan synthesis that was only slightly higher than the control incubated without acceptor.

DISCUSSION

The dextran-acceptor reaction was studied with a preparation of extracellular glucosyltransferase (GTF-I) obtained from a batch culture of *S. mutans* OMZ176. Under this growth condition, the production of the two major glucosyltransferases, GTF-S and GTF-I, was approximately equal[14]. The enzymes were well separated by chromatography on hydroxylapatite, GTF-S and GTF-I being eluted with 0.075 and 0.40M potassium phosphate buffer, respectively.

Methylation analysis of the product synthesized by GTF-I from sucrose, in

the absence of acceptor, proved that the polysaccharide was a linear (1→3)-α-D-glucan, the mol% of 2,4-dimethyl ether and 2,4,6-trimethyl ether being 1 and 88%, respectively[1]. Hydrolysis of the polysaccharide with a specific (1→3)-α-D-endoglucanase gave 84% conversion into D-glucose[1].

Synthesis of glucan catalysed by GTF-I is greatly enhanced by low concentrations of dextran. When a series of dextrans (Pharmacia) derived from *L. mesenteroides* B-512(F) dextran was added in equal concentration (mg/mL) to the standard reaction (see Methods), the proportion of side chains and (1→6)-linked sequences in the dextran acceptors remained constant, but the molar concentration of reducing groups in the mixture containing the smallest dextran (T10, $\bar{M}_w$ 1.0×10^4) was 5000-fold higher than that containing native dextran ($\bar{M}_w$ 50×10^6). Most of the dextrans were equally effective in stimulating glucan synthesis (Table I), indicating that the reducing end-group was not involved in the acceptor reaction.

A previous comparison of the effect of two soluble glucans (Sigma Chemical Company) of different molecular weights (9,400 and 193,000) on the rate of synthesis of insoluble D-glucan by a glucosyltransferase from *S. mutans* 6715 led to the conclusion that polymer growth occurred by transfer to the non-reducing terminals[8]. By contrast, the results of the present study, with dextrans containing a wide range of (1→3)-glucosidic branch-linkages, show that it is such dextrans as T70, that have the *lowest* proportion of non-reducing terminals and the highest proportion of unbranched (1→6)-linked sequences, that are the most efficient dextrans for stimulating glucan synthesis. Dextran T70, which has ~90% of (1→6)-linked sequences, was given an efficiency value of 90. Other dextrans, with higher proportions of (1→3) branch-linkages, and therefore with more non-reducing terminals and *fewer* (1→6)-linked sequences, were progressively less efficient in the acceptor reaction (Fig. 3).

Values for the two types of branched dextrans, native and synthetic fell on the same line (Fig. 3). The synthetic dextrans thus serve as well characterized models for related, native dextrans. From their method of synthesis[11,12], the V-dextrans are a family of (1→6)-linked α-D-glucans having randomly distributed (1→3)-linked glucosyl side-chains, 95% of the branch linkages being in the α configuration. Enzymic-degradation studies revealed that native and synthetic dextrans having an equivalent content of (1→3)-branch linkages, were hydrolysed to the same extent by *Penicillium funiculosum* endodextranase. Separation of the products from *Streptococcus viridans* NRRL B-1351 dextran and V39 dextran by l.c. gave glucose, isomaltose, and the same series of branched oligosaccharides[18].

It was apparent that unbranched sequences of (1→6)-linked α-D-glucose residues are the functional regions of dextrans that promote the reaction with GTF-I. This proposal is in accord with the result that linear, synthetic (1→6)-α-D-glucan[3] was an efficient acceptor[1], despite the complete absence of side chains. The minimum length of the sequence of unsubstituted glucose residues in between branch points of an effective acceptor-dextran is not known. In the best dextran-

acceptors, for example V39 dextran (with 10% of branch linkages), B-512(F) dextran, and the Pharmacia hydrolysates (with 5% of branch linkages), >90% of the branch points are at least six (1→6)-linked residues apart[12,19]. Because of the random distribution of branch points in both native and chemically synthesized dextrans, branching may frequently occur on adjacent residues in more-highly branched dextrans. The sequence length of unbranched residues will then be higher than the average value of *n* that would be predicted by methylation analysis from the ratio of trimethyl ethers to tetramethyl ethers. A computer programme was used to calculate the number-average sequence length, diad fractions, and weight fractions of branched and unbranched sequences in the synthetic dextrans[12]. For dextran V17, which has 32% of (1→3)-branch linkages, 50% of these are linked to adjacent units on the main chain, and the number-average sequence-lengths of both branched and unbranched units are about two. Therefore, when we consider the soluble dextran synthesized by *S. mutans* OMZ176 GTF-S, which also has 32% of (1→3)-branch linkages[1], and a value of *n* close to one deduced from methylation analysis, we cannot assume that GTF-I can catalyze the transfer of a glucose unit to a residue on the main chain that is flanked on both sides by residues bearing side-chains. The ability of even the most highly branched dextrans to act as acceptors (Fig. 3) can be better explained by the probable presence of some unbranched sequences, the length of which is most likely to be two or more glucose units.

The results of several studies[6,7] indicate that the increased synthesis of glucan catalysed by GTF-I in the presence of acceptor dextran is due to the modification of the dextran by the formation of a (1→3) branch-linkage. Robyt and Martin[20] also assumed that the attachment of (1→3)-α-D-glucan chains to exogenous dextran-acceptor must be by (1→3) branch-linkages. Fukui *et al.* reported[7] that T10 dextran acceptor became more heavily branched after incubation with *S. mutans* 6715 GTF-I and sucrose. The product contained >93% of (1→3)-glucosidic linkages, and the (1→3)-glucan chains were attached by (1→3) branch-linkages to glucose residues on the T10 dextran chains.

Preformed, single D-glucosyl side-chains on the main chain of dextrans clearly do not confer added efficiency in the dextran-acceptor reaction of GTF-I in activity tests. Nevertheless, the synthesis by GTF-S of highly branched dextran should be advantageous *in vivo* towards the synthesis of increased amounts of water-insoluble glucan by GTF-I. First, a branched acceptor-dextran is more stable in dental plaque, being resistant to degradation by endogenous dextranases. Second, the short side-chains foster increased solubility of the growing mixed-chain glucan, and this delays the inactivation of glucosyl transferases by adsorption on to water-insoluble glucan.

S. mutans grows slowly in dental plaque, and chemostat studies[14] have indicated that at low growth-rate the proportion of GTF-S to GTF-I is approximately 10:1. Under this condition, the major product synthesized by *S. mutans* OMZ176 is a soluble glucan composed of ~90% of highly branched dextran chains

and 10% of (1→3)-α-D-glucan chains[14]. Our results show that dextrans already containing some (1→3)-linked sequences in addition to their (1→6)-linked sequences are more efficient acceptors (Fig. 4) than less-complex, synthetic dextrans with a comparable proportion of (1→6)-linked sequences. We suggest, therefore, that the reaction of GTF-I with dextran is slow until the newly-formed side chains consist of more than one and possibly three (1→6)-linked residues. Such soluble acceptors, having both (1→3)-linked chains and (1→3)-linked chains, apparently contain a structural feature that fosters the activity of GTF-I, and the reaction proceeds more rapidly with the formation of linear (1→3)-α-D-glucan chains. This is in accord with the proposals of McCabe and Smith[6], and is also consistent with the results of Fukui *et al.*[7]. It is noteworthy that a linear (1→3)-α-D-glucan, made soluble by the introduction of carboxymethyl groups, had little effect on the rate of glucan synthesis (Fig. 4). This result confirmed that long (1→3)-linked sequences, similar to those synthesized by GTF-I, are not effective as acceptors in the absence of (1→6)-linked sequences[13].

Periods when the supply of nutrients to the oral cavity is increased permit a higher growth-rate of the organisms in dental plaque. In continuous cultures of *S. mutans* strains, an increase in growth rate leads to a greatly increased productivity of GTF-I in culture filtrates[13,14]. As GTF-S predominates at low growth-rates, substantial amounts of preformed dextran acceptor may be present in the vicinity of *S. mutans* colonies in dental plaque. This acceptor most probably contains sufficient (1→3)-linked sequences as well as enough unbranched sequences of (1→6)-linked residues to stimulate the activity of GTF-I that is released by *S. mutans* at times when rapid growth can occur.

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