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INHIBITION OF *Streptococcus mutans* 6715 GLUCOSYLTRANSFERASES BY SUCROSE ANALOGS MODIFIED AT POSITIONS 6 AND 6'*

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ABSTRACT

Sucrose derivatives modified at position 6 (6-deoxysucrose, 6-thiosucrose, 6,6'-dithiodisucrose, and 6,6'-dideoxy-6,6'-difluorosucrose) were tested as inhibitors of the two *Streptococcus mutans* 6715 glucosyltransferases. 6-Deoxysucrose was the best inhibitor studied, competitively inhibiting the soluble-D-glucan forming enzyme (GTF-S) and the insoluble-D-glucan forming enzyme (GTF-I) with K_i values one order of magnitude lower than the sucrose K_m values. 6-Thiosucrose was also a competitive inhibitor for both enzymes. 6,6'-Dithiodisucrose and 6,6'-dideoxy-6,6'-difluorosucrose only inhibited GTF-I; 6,6'-dithiodisucrose gave mixed inhibition and 6,6'-dideoxy-6,6'-difluorosucrose gave uncompetitive inhibition. 6-Thiosucrose was a substrate for both enzymes to produce acceptor products when acceptors were present. GTF-I synthesized *de novo* a water-insoluble, (1→3)-6-thio-α-D-glucan from 6-thiosucrose.

INTRODUCTION

Various strains of oral *Streptococci* produce glucosyltransferases which utilize sucrose as a glucosyl donor in the production of soluble and insoluble D-glucans[1]. The importance of these enzymes and the D-glucans they produce in the development of dental caries has made them the subject of many studies[2–4]. We have been particularly interested in studies on inhibitors and alternate substrates for these enzymes because of the information on the catalytic site obtained from these studies and also the possibility of the development of anti-caries agents[5].

Inhibitors for these enzymes come in many forms, such as: lipoteichoic acids, which are competitive inhibitors of both the insoluble- and the soluble-glucan-forming glucosyltransferases, GTF-I and GTS-S, respectively[6]; mutastein, a protein isolated from *Aspergillus terreus*, which inhibits[7] GTF-I; ribocitrin, an oligosaccharide consisting of three D-ribose residues and citric acid, which acts as a noncompetitive

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inhibitor of dextransucrase[8,9]; and xanthene dyes, including Methylene Blue, Rose Bengal, and Eosin Yellowish, which appear to be competitive inhibitors[10,11]. More conventional inhibitors of these enzymes include nojirimycin, 1-deoxynojirimycin, acarbose[12,13], and ascorbic acid[14]. Several sucrose analogs have also been tried, including 6,6′-dideoxysucrose[15], which was reported not to be an inhibitor; 6,6′-diamino-6,6′-dideoxysucrose; and 6-amino-6-deoxysucrose[16].

Various sugars have been investigated as possible glycosyl donors for these enzymes. Early work showed that partially methylated sucrose would not act as a donor[17]. Since then, several glucosyl donors have been reported, including α-D-glucopyranosyl fluoride[15,18], α-D-glucopyranosyl α-L-sorbofuranoside[19], 4-*O*-β-D-galactopyranosyl-β-D-fructofuranosyl α-D-glucopyranoside[20], various glucooligosaccharides[21], and *p*-nitrophenyl α-D-glucopyranoside[22]. All of these compounds transfer a D-glucosyl unit to the enzyme. Because α-D-glucosyl fluoride is a substitute for sucrose as a D-glucosyl donor, several other α-glycosyl fluorides have been tested as donors, including 2-deoxy-D-*arabino*-hexopyranosyl fluoride, α-D-allopyranosyl fluoride, and 6-deoxy-α-D-glucopyranosyl fluoride. None of these compounds acted as a glycosyl donor[15]. A more extensive study of glycosyl fluorides, including the α- and β-fluorides of several sugars, showed that only α-D-glucopyranosyl fluoride was able to donate a glycosyl unit to the glucosyltransferase, and that most of the α anomers were competitive inhibitors, whereas the β anomers and free sugars were noncompetitive inhibitors[23].

Recently, we have synthesized 6,6′-dideoxy-6,6′-difluorosucrose[24], 6-deoxysucrose, and 6-thiosucrose[25] to test as possible inhibitors, or glycosyl donors, or both for the glucosyltransferases. We report herein kinetic studies on these sucrose analogs with both GTF-I and GTF-S from *Streptococcus mutans* 6715, and the formation of (1→3)-6-thio-α-D-glucan by the reaction of GTF-I with 6-thiosucrose.

EXPERIMENTAL

Enzymes. — *Streptococcus mutans* 6715 was grown on the medium described by Ciardi *et al.*[26]. After removal of cells and concentration using a Millipore Pellicon filtration system, the glucansucrases were eluted from a Bio-Gel A-15m column[27]. Two glucansucrases (GTF-S and GTF-I) were separated by ion-exchange chromatography on DEAE-cellulose[28] in 20mM sodium phosphate buffer, pH 6.8, containing 0.02% NaN_3, and a gradient of 0 to 0.2M NaCl. Further purification of the glucansucrases was accomplished by a second ion-exchange chromatography step[29] using DEAE-Bio-Gel A with the same phosphate buffer and a 0 to 0.2 M NaCl gradient. Final purification of each enzyme was accomplished by affinity chromatography[29] on Sephadex G-50.

Glucansucrase activity, determined by a radiochemical assay using [U-^{14}C]sucrose[30], is given in International Units (IU), *i.e.*, μmol of D-glucose incorporated per min into D-glucan at pH 5.2 and 37°, in the presence of 3.3 mg of

dextran T-10/mL. The specific activity of purified GTF-S was 50 ±7 IU/mg of protein, and that of GTF-I was 87 ±7 IU/mg of protein. GTF-S contained 57 μg of carbohydrate per mg of protein.

Carbohydrates. — [U-^{14}C]Sucrose was obtained from Schwarz/Mann Inc. (Div. of Mediscience, Inc., Spring Valley, NY 10977). 6-Thiosucrose, 6,6'-dithiodisucrose, and 6-deoxysucrose were prepared by the method of Binder and Robyt[25]. 6,6'-Dideoxy-6,6'-difluorosucrose was prepared by the method of Zikopoulos *et al.*[24]. Dextran T-2000 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

[U-^{14}C]6-Thiosucrose. — [U-^{14}C]Sucrose (0.23 mg, 200 μCi) was dissolved in *N,N*-dimethylformamide (1 mL). *p*-Toluenesulfonic acid (2 mg) and 2,2-dimethoxypropane (0.45 mL) were added. The mixture was kept for 45 min and then de-ionized with Rexyn R.G. 6 (OH^-) anion-exchange resin and filtered, and the filtrate concentrated *in vacuo* to 1/10. Pyridine (1 mL) and benzoyl chloride (0.15 mL) were added at 0°, and the mixture was kept overnight at 20°. Unlabeled 4,6-*O*-isopropylidenesucrose hexabenzoate[25] (100 mg) in dichloromethane (1 mL) was added, and the solution was washed twice with a saturated $NaHCO_3$ solution and once with water. The solvent was evaporated, and the resulting foam dissolved in hot ethanol (5 mL) and kept for 2 h at −20°. The crystals were filtered off and dissolved in dichloromethane (0.25 mL), and diethyl ether was added (3 mL), followed by slow addition of hexane (5 mL) with stirring to give pure crystalline 4,6-*O*-isopropylidenesucrose hexabenzoate. The synthesis of [U-^{14}C]6-thiosucrose from this point was carried out as previously reported for 6-thiosucrose[25]. The final yield of label in 6-thiosucrose was 2.3%, giving a specific activity of 0.13 mCi/mmol.

Chromatography. — T.l.c. was performed on Whatman K5F 0.25-mm silica gel plates (Whatman Inc. Clifton, NJ 07014). Chromatograms were developed by one ascent in 3:1 (v/v) acetonitrile–water, and spots detected by spraying with 20% H_2SO_4 in methanol and charring at 110° for 10 min.

Gel filtration chromatography of glycans was carried out on a Bio-Gel P-10 column (1 × 18 cm), equilibrated and eluted with formamide; 0.5-mL fractions were collected.

Enzyme-digest conditions. — All digests were conducted at 37° in 50mM sodium acetate buffer, pH 5.2, containing 0.01% NaN_3. 1,4-Dithiothreitol was added to digests containing 6-thiosucrose to prevent its oxidation to the disulfide, 6,6'-dithiodisucrose.

Preparation and characterization of reaction products. — To 100mM 6-deoxysucrose (30 μL) was added 30 μL of one of the following solutions: buffer, 100mM sucrose, or 100mM maltose. To each of these solutions was added GTF-S (0.10 IU, 30 μL) or GTF-I (0.08 IU, 15 μL). After 3 h, aliquots of the digest containing sucrose were deposited on t.l.c. plates and, after 23 h, aliquots from the other digests were deposited. Similar experiments were carried out for 6-thiosucrose, but with addition of 20mM 1,4-dithiothreitol. Digests containing 6-thiosucrose and GTF-I, with and without added maltose, were sampled over a period of 23 h and analyzed by t.l.c.

6-Thiosucrose (0.5 g in 8 mL) was treated with GTF-I (1.3 IU, 0.25 mL) in buffer containing 80mM 1,4-dithiothreitol. After five days, another 1.3 IU of GTF-I was added and, after an additional four days, the insoluble product was collected by centrifugation, washed twice with D_2O, and dissolved in pyridine (2 mL). A ^{13}C-n.m.r. spectrum of the product was obtained with a JEOL FX-90Q, Fourier-transform n.m.r. spectrometer, at 22.5 MHz, with proton decoupling, and a sample temperature of 80°. Field-frequency locking was provided by residual D_2O in the sample.

An insoluble product from the reaction of GTF-I with 6-thiosucrose was produced by the following method and analyzed by gel filtration. GTF-I (0.52 IU, 0.1 mL) was added to 24mM 1,4-dithiothreitol in buffer (0.25 mL) containing 17 μmol of [U-^{14}C]6-thiosucrose (1.8×10^6 c.p.m.). After 24 h, the insoluble product was collected by centrifugation, dissolved in formamide (0.2 mL), and applied to a Bio-Gel P-10 column equilibrated with formamide. Fractions were assayed for ^{14}C, in a toluene cocktail, by heterogeneous scintillation-counting of aliquots dried onto Whatman 3MM filter-paper squares.

Kinetics of insoluble-product formation from 6-thiosucrose by GTF-I. — To GTF-I (80 μL, 1.7 IU/mL) was added 60mM 1,4-dithiothreitol (80 μL) containing T-2000 dextran (6 mg/mL). The mixture was incubated for 5 min, at which time 50mM 6-thiosucrose (80 μL) was added. Aliquots (25 μL) taken at various times were deposited onto 1 × 1-cm squares of Whatman 3MM filter paper. The papers were washed with methanol (5 ×) to remove methanol-soluble label, followed by liquid scintillation counting[30] to determine formation of the methanol-insoluble product. A similar experiment was performed with 50mM [U-^{14}C]sucrose to monitor (1→3)-D-glucan formation; in this experiment, the GTF-I concentration was one-third that used with 6-thiosucrose.

Inhibitor kinetics. — To test the ability of the modified sucrose compound to inhibit glucan formation by GTF-I and GTF-S, a series of 0.15-mL enzyme digests were prepared, all containing [U-^{14}C]sucrose (3.33–16.7mM), and dextran T-2000 (2 mg/mL). One of four inhibitors was present in each digest, at the specified concentrations: 0.20–1.5mM 6-deoxysucrose, 2–15mM 6-thiosucrose (these digests also contained 10mM 1,4-dithiothreitol, 6.7–21.9mM 6,6′-dideoxy-6,6′-difluorosucrose, or 6.7–21.9mM 6,6′-dithiodisucrose. In addition, the water-insoluble product obtained by GTF-I reaction with 6-thiosucrose was tested as an inhibitor of GTF-I reaction with sucrose; the insoluble-product concentration was 0.077–0.191 mg/mL, and these digests contained 10mM 1,4-dithiothreitol.

The inhibition digests contained 7 mIU of GTF-S or 5 mIU of GTF-I. Incorporation of ^{14}C into methanol-insoluble product was determined by scintillation counting, as described earlier[30]. The concentrations of 1,4-dithiothreitol used in the kinetic analysis had no significant effect on either the K_m or V_{max} of these enzymes.

RESULTS

The action of GTF-I and GTF-S on two different modified sucrose molecules,

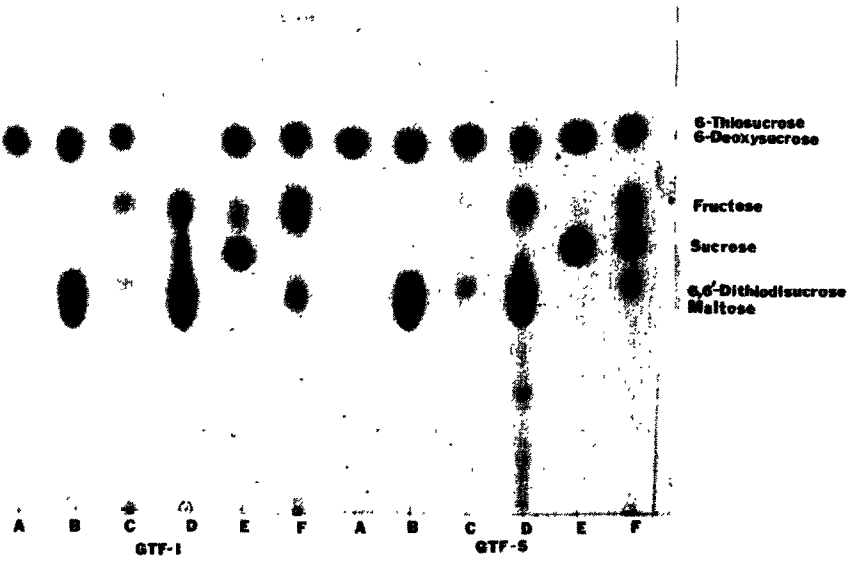

Fig. 1. T.l.c. of the action of glucosyltransferases in the presence of the following sugars: (A) 6-deoxysucrose, (B) 6-deoxysucrose and maltose, (C) 6-thiosucrose, (D) 6-thiosucrose and maltose, (E) 6-deoxysucrose and sucrose, and (F) 6-thiosucrose and sucrose. Left half of the plate shows the action of *Streptococcus mutans* 6715 GTF-I; right half of the plate shows the action of *Streptococcus mutans* 6715 GTF-S. Each mixture (3 μL) was deposited at 3 h for those digests that contained sucrose and at 23 h for the others. The plate was developed with one ascent in 3:1 (v/v) acetonitrile–water. When aliquots of the digests containing 6-thiosucrose were dried on the t.l.c. plate, air oxidation of this compound gave rise to 6,6′-dithiodisucrose, which comigrated with maltose.

6-deoxysucrose and 6-thiosucrose, was monitored by t.l.c. (see Fig. 1) under one of the following conditions: in the presence of maltose, which is an acceptor[31]; in the presence of sucrose; or in the presence of buffer only. 6-Deoxysucrose was not active with either enzyme under any of the digest conditions, and almost totally inhibited glucosyltransferase action on sucrose. The 6-thioglucosyl group of 6-thiosucrose was transferred to maltose, by both GTF-I and GTF-S , to give a homologous series of acceptor products with the release of fructose. GTF-I produced an acceptor product having a higher mobility than maltose, which is probably *O*-(6-thio-α-D-glucosyl)-(1→3)-maltose. We postulate that the first acceptor product of GTF-S in the presence of maltose is *O*-(6-thio-α-D-glucosyl)-(1→6′)-maltose, which may have the same mobility on t.l.c. as maltose[21,31]. in the absence of maltose, only GTF-I was able to utilize 6-thiosucrose, to release fructose and form a product that remained on t.l.c. at the origin. 6-Thiosucrose did not totally inhibit the utilization of sucrose by either enzyme, GTF-I being able to digest all of the sucrose.

In the presence of an acceptor, GTF-I was able to act on 6-thiosucrose rapidly (see Fig. 1). Therefore, time courses of this reaction in the presence and absence of maltose were investigated by t.l.c. (see Fig. 2). In the presence of maltose, all of the 6-thiosucrose was consumed within 9 h, whereas in the absence of maltose,

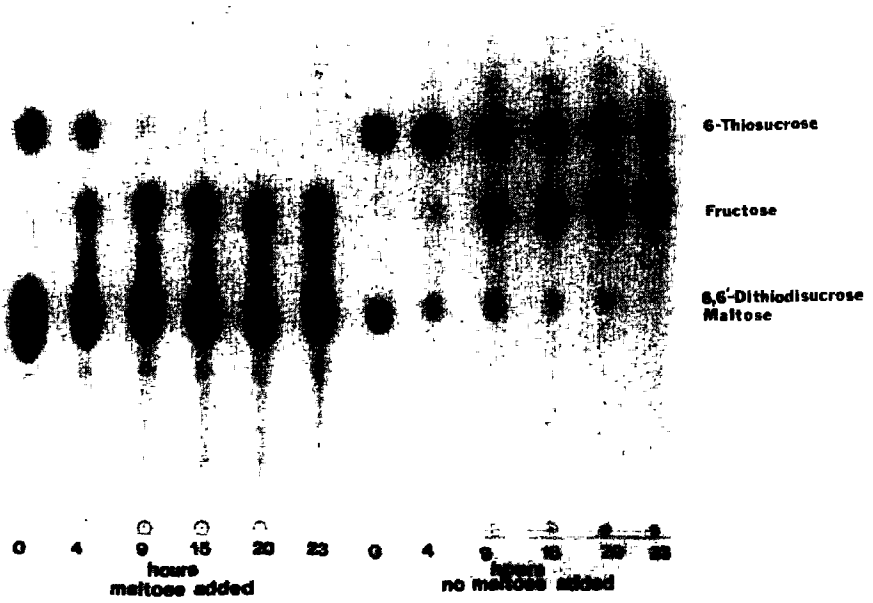

Fig. 2. T.l.c. of the products from the action of *Streptococcus mutans* 6715 GTF-I on 6-thiosucrose: (a) left half of the plate, in the presence of maltose, and (b) right half of the plate, in the absence of any added acceptor. Time points were taken at 0, 4, 9, 15, 20, and 23 h. An aliquot (3 μL) of each mixture was chromatographed for one ascent in 3:1 (v/v) acetonitrile–water. The spot in the no-maltose tracks having the same R_F as maltose is the disulfide of 6-thiosucrose (see Fig. 1).

some 6-thiosucrose was still present after 23 h of reaction, and an insoluble product was present in the reaction mixture. In addition, the insoluble product formed by GTF-I action on 6-thiosucrose failed to appear in the presence of maltose.

In the ^{13}C-n.m.r. spectrum of the water-insoluble product formed by action of GTF-I on 6-thiosucrose (see Fig. 3), five resonances (A–E) corresponded well to the resonances of C-1–5 of an (1→3)-α-D-glucan[32], even though the solvent used for this n.m.r. spectrum was pyridine, and the thiol group has some effect on ring-carbon chemical shifts. Peak F is the resonance of C-6, linked to S. The minor resonances are due to traces of 6-thiosucrose. The product was soluble in *N,N*-dimethylformamide, dimethyl sulfoxide, and formamide, whereas mutan, the (1→3)-α-D-glucan produced by GTF-I action on sucrose, is not.

The Bio-Gel P-10 elution profile of the ^{14}C-labeled, insoluble product of GTF-I action on 6-thiosucrose is shown in Fig. 4. The polysaccharide formed in the digest (peaks A and B) has a broadly distributed mol. wt. Peak A has a mol. wt. of at least 20 000, peak B has an intermediate mol. wt., and peak C, at the included volume, is due to unreacted [U-^{14}C]6-thiosucrose. To minimize formation of cross-linked disulfides, no attempt was made to remove unreacted 6-thiosucrose before gel filtration.

Since 6-thiosucrose is a substrate for polymerization by GTF-I, we attempted to follow the kinetics of insoluble-product formation from sucrose and from 6-

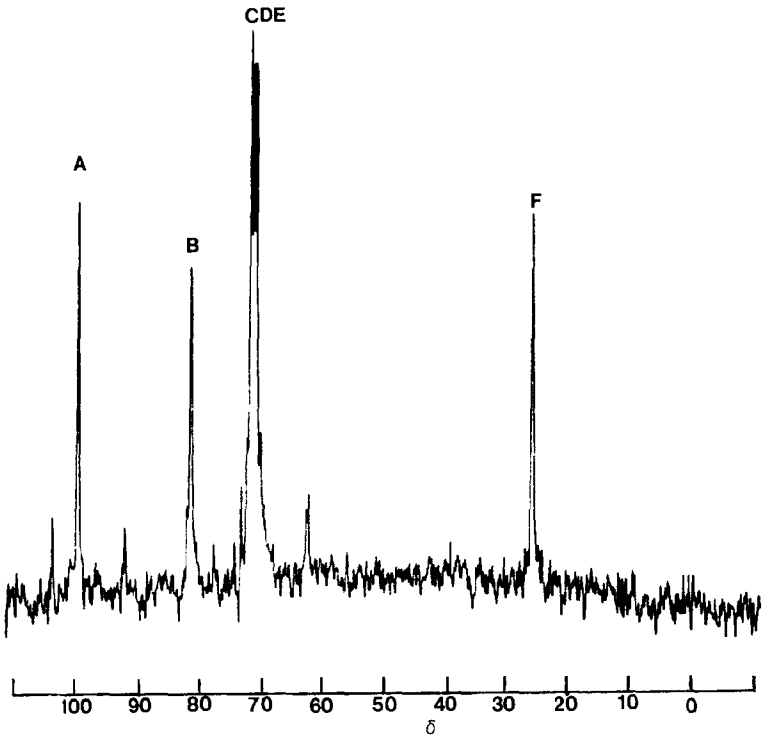

Fig. 3. ^{13}C-N.m.r. spectrum of the insoluble-glycan product of *Streptococcus mutans* 6715 GTF-I reaction with 6-thiosucrose. Resonances (from the signal of tetramethylsilane) are as follows: δ (A) 100.2, (B) 81.8, (C) 72.1, (D) 71.6, (E) 71.2, and (F) 25.7. Minor resonances were due to 6-thiosucrose. The sample was dissolved in pyridine, which served as an internal standard; sample concentration was 37 mg/mL and the temperature was 80°.

thiosucrose under identical conditions, except that the GTF-I concentration in the sucrose digest was one-third that in the 6-thiosucrose digest (see Fig. 5). The non-linear, initial velocities observed for 6-thiosucrose prevented initial-rate kinetic analysis.

To obtain the kinetic parameters for the various modified sucrose molecules reacting with GTF-I and GTF-S, we tested them as inhibitors of glucan formation from sucrose. Figure 6 is a set of Michaelis–Menten plots for GTF-I in the presence of the following inhibitors: 6-thiosucrose, 6-deoxysucrose, 6,6′-dithiosucrose, and 6,6′-dideoxy-6,6′-difluorosucrose. 6-Thiosucrose and 6-deoxysucrose both were competitive inhibitors, whereas 6,6′-dithiodisucrose gave mixed inhibition, and 6,6′-dideoxy-6,6′-difluorosucrose gave uncompetitive inhibition. Figure 7a shows the inhibition of GTF-S by 6-thiosucrose, and Fig. 7b, inhibition by 6-deoxysucrose. 6,6′-Dithiodisucrose and 6,6′-dideoxy-6,6′-difluorosucrose did not appear to inhibit glucan formation by GTF-S, but did affect GTF-I. The insoluble product from GTF-I action on 6-thiosucrose did not inhibit glucan formation by GTF-I.

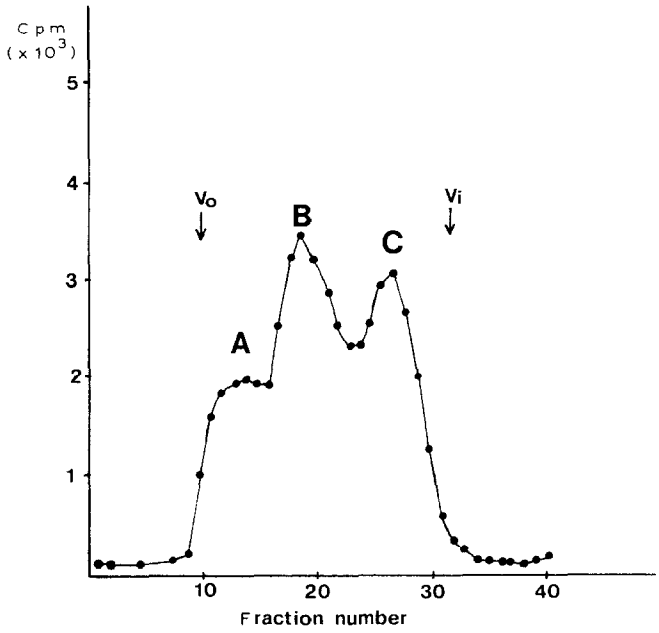

Fig. 4. Elution profile, from a Bio-Gel P-10 column (1 × 18 cm), of the insoluble product produced by *Streptococcus mutans* 6715 GTF-I reaction with [U-^{14}C]6-thiosucrose, and eluted with formamide (0 5-mL fractions collected): Peaks A and B are product, and peak C is unreacted 6-thiosucrose.

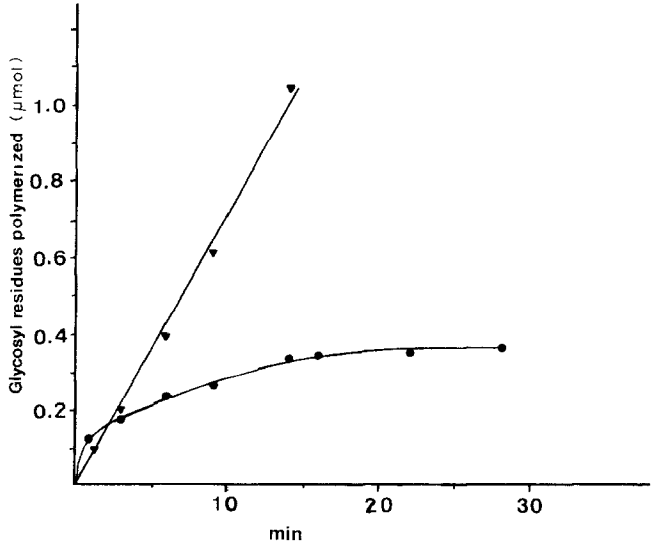

Fig. 5. Progress curve of insoluble-product formation from [U-^{14}C]sucrose (—▼—▼—▼—) or [U-^{14}C]6-thiosucrose (—●—●—●—) by the action of *Streptococcus mutans* 6715 GTF-I. Enzyme concentration in the sucrose mixture was one-third of that in the 6-thiosucrose mixture.

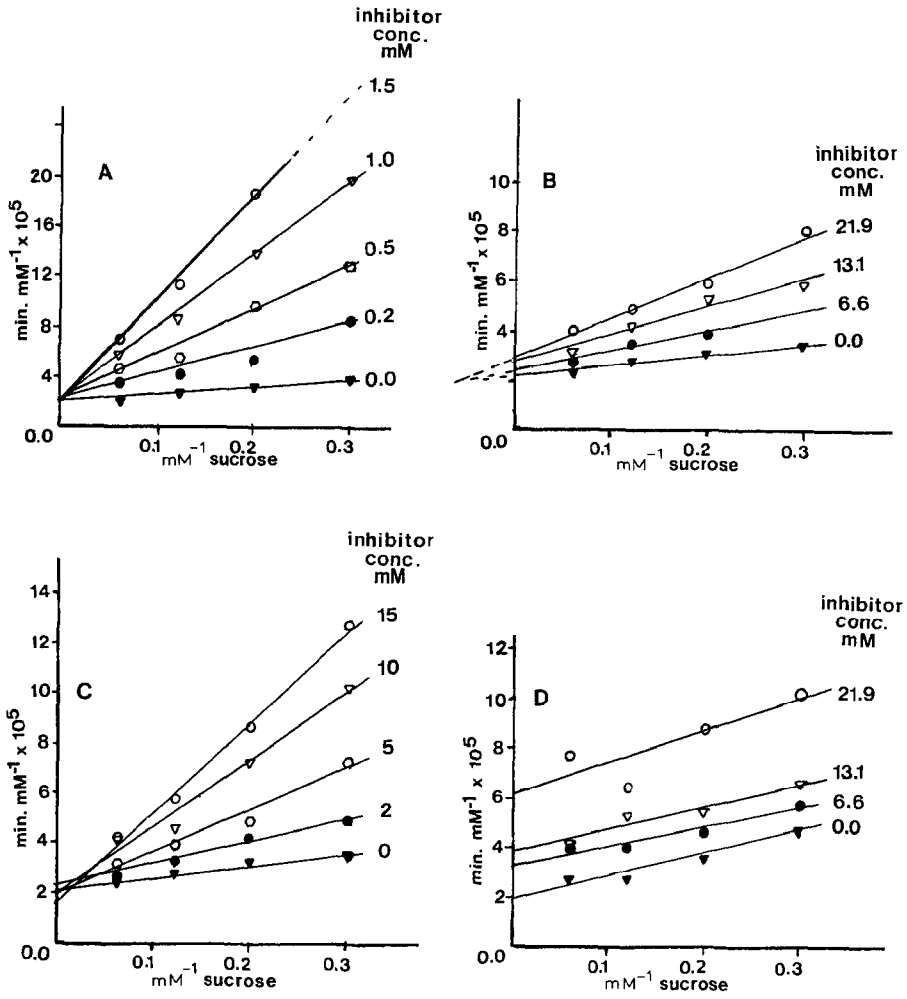

Fig. 6. Lineweaver–Burk plots of D-glucose incorporation into D-glucan by reaction of *Streptococcus mutans* 6715 GTF-I with [U-^{14}C]sucrose in the presence of one of the following inhibitors: (A) 6-deoxysucrose, (B) 6,6′-dithiodisucrose, (C) 6-thiosucrose, and (D) 6,6′-dideoxy-6,6′-difluorosucrose.

Table I gives the inhibition constants (K_i) and the type of inhibition for the various sucrose derivatives. 6-Deoxysucrose is the best inhibitor for both enzymes, followed in effectiveness by 6-thiosucrose. 6,6′-Dithiodisucrose and 6,6′-dideoxy-6,6′-difluorosucrose were inhibitors only for GTF-I.

DISCUSSION

Recently, various substrates have been reported for the glucansucrases of *Leuconostoc* and *Streptococcus*[15,18–22]; all of these substrates have an unmodified glucosyl residue, which is transferred by the enzyme. Modified glucosyl residues

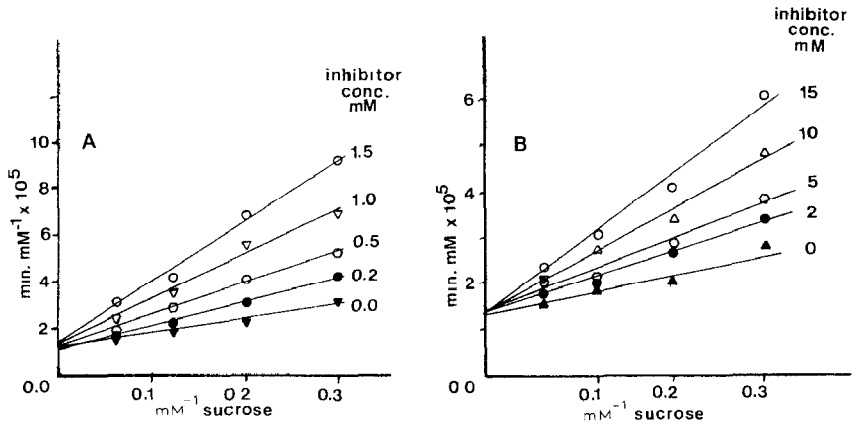

Fig. 7. Lineweaver–Burk plots of D-glucose incorporation into D-glucan by reaction of *Streptococcus mutans* 6715 GTF-S with [U-^{14}C]sucrose in the presence of one of the following inhibitors: (A) 6-deoxysucrose, and (B) 6-thiosucrose.

TABLE I

KINETIC CONSTANTS OF MODIFIED SUCROSE MOLECULES ON *Streptococcus mutans* 6715 D-GLUCOSYLTRANSFERASES[a]

Compound	*GTF-S*		*GTF-I*	
	Kinetic constant (mM)	*Type of inhibition*	*Kinetic constant (mM)*	*Type of inhibition*
Sucrose (K_m)	5.0 ±1.5		3.7 ±1.2	
6-Deoxysucrose	K_i 0.56	competitive	K_i 0.18	competitive
6-Thiosucrose	K_i 7.3	competitive	K_i 3.4	competitive
6,6′-Dithiodisucrose		not an inhibitor	K_i 8.0, K_i' 62	mixed
6,6′-Dideoxy-6,6′-difluorosucrose		not an inhibitor	K_i 10	uncompetitive

[a]Inhibition constants were determined from slope and intercept replots of Lineweaver–Burk plots.

have not been reported to be transferred[15–17,23]. We have found, however, that, of the sucrose derivatives we tested, 6-thiosucrose acts as a glycosyl donor in acceptor reactions of both glucansucrases of *Streptococcus mutans* 6715, as well as the dextransucrase of *Leuconostoc mesenteroides* B-512F (data not shown), but that only the insoluble-glucan forming enzyme (GTF-I) of *Streptococcus mutans* is able to use 6-thiosucrose for *de novo* synthesis of a modified glycan. ^{13}C-N.m.r. data (Fig. 3) suggest that this product is an α-D-(1→3)-linked 6-thioglucan.

Donation of 6-thioglucose to maltose acceptor by GTF-I is much faster than the formation of 6-thioglucan from 6-thiosucrose (Fig. 1). The acceptor products of this reaction were not identified, but by analogy with the acceptor reactions of these enzymes with sucrose and maltose[21,31] and some of our studies with GTF-I[33],

the first maltose acceptor product is probably 6″-thiopanose for GTF-S and *O*-(6-thio-α-D-glucosyl)-(1→3′)-maltose for GTF-I, and that successive 6-thioglucosyl groups are probably transferred to 3-OH of the nonreducing 6-thioglucosyl group.

The nonlinearity of the rate of polymer formation (Fig. 5) suggests product inhibition. We tested the insoluble product as an inhibitor of (1→3)-α-D-glucan formation from sucrose by GTF-I; no inhibition was observed (data not shown). One possible explanation for the nonlinear initial velocity of product formation from 6-thiosucrose is that early in the reaction 6-thioglucosyl groups are rapidly transferred to the added acceptor dextran[34,35]; later, when this acceptor is exhausted, only the slow formation of the insoluble product is observed. This slow polymerization would occur by insertion of 6-thioglycosyl residues at the reducing end of an enzyme-linked 6-thioglucan[36,37].

Among the 6-modified sucrose analogs tested as glucansucrase substrates, 6-thiosucrose is unique in its ability to donate a modified glucosyl group[15,16,23]. One possible explanation is that glycosyl transfer by the enzyme requires that the substituent at C-6 furnish a proton for a hydrogen bond to the enzyme. 6-Deoxysucrose and 6,6′-dideoxy-6,6′-difluorosucrose may fail to act as substrates for GTF-I for this reason. 6,6′-Diamino-6,6′-dideoxysucrose[16] at pH 5.2 could be a good proton donor, but its positive charge may prevent productive binding by the enzyme, thus leading to the observed uncompetitive inhibition.

Although a proton-donating group at C-6 may be required for glucosyl transfer by the enzyme, it certainly is not required for binding at the active site. Table I shows that 6-deoxysucrose is the most effective inhibitor among the compounds tested, for both GTF-I and GTF-S, and its inhibition was competitive. This is surprising, since 6,6′-dideoxysucrose has been reported not to inhibit a glucosyltransferase from *S. mutans*[15], although no data were given. The inhibition constant of 6-thiosucrose, on the other hand, is close to the Michaelis constant for sucrose, and it also acted as a competitive inhibitor.

The disulfide of 6-thiosucrose was a poor inhibitor for GTF-I and did not inhibit GTF-S at all (data not shown). The lack of inhibition of GTF-S by this compound may be due to steric hindrance of binding at the active site. The weak mixed inhibition of GTF-I may result from a slight affinity for both the sucrose-binding site and for some other binding site on the enzyme, such as an acceptor-binding site.

6,6′-Dideoxy-6,6′-difluorosucrose was proposed to be a good substrate analog for dextransucrases because fluorine has been used as a hydroxyl group analog in that it has similar electronegativity and can act as a hydrogen-bond acceptor[38]. Unfortunately, 6,6′-dideoxy-6,6′-difluorosucrose did not appear to inhibit GTF-S (data not shown), and was a weak uncompetitive inhibitor of GTF-I. Uncompetitive inhibition of a mixture of glucansucrases from *S. mutans* 6715 has also been reported for 6,6′-diamino-6,6′-dideoxysucrose, although no data were given[16].

It is apparent that the substrate requirements of *S. mutans* glucosyltransferases allow much more substitution on the fructosyl than on the glucosyl

residue of sucrose[15,18,21,22]. Substitutions on the glucosyl residue, other than the replacement of 6-OH by a thiol group, have so far resulted in the loss of the ability to act as a glucosyl donor, but not necessarily the ability to bind at the active site and inhibit the enzyme, as was the case with 6-deoxysucrose.

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