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Purification and Characterization of a Primer-Independent Glucosyltransferase from *Streptococcus mutans* 6715-13 Mutant 27

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Affinity chromatography on Sephadex G-50 and subsequent ion-exchange chromatography on Trisacryl-M–DEAE were used to purify the glucosyltransferase (GTF) enzymes produced by mutant 27 of *Streptococcus mutans* 6715-13. Complete separation of three types of GTF, including a primer-independent GTF capable of synthesizing a slightly branched, water-soluble glucan (GTF-S), was obtained. The characteristics of this primer-independent GTF-S were compared with those of the normally occurring primer-dependent GTF-S. The K_m for sucrose was easily obtained for each enzyme (10^{-2} M), but the K_m for dextran could only be determined for the primer-dependent GTF-S (5×10^{-7} M for clinical dextran of molecular weight 60,000 to 90,000). The primer-independent GTF-S did not respond catalytically to the presence of either clinical dextran or the highly branched, water-soluble glucan produced by primer-dependent GTF-S, although it was capable of binding these polysaccharides at a noncatalytic site and of responding to the low-molecular-weight acceptor 1-*O*-methyl-α-D-glucopyranoside. The water-soluble glucan product of primer-independent GTF-S was a superior priming glucan for primer-dependent GTF enzymes as compared with the glucan product of primer-dependent GTF-S. The presence of primer-independent GTF-S in reaction mixtures stimulated glucan synthesis by primer-dependent GTF-S and by GTF synthesizing water-insoluble glucan by at least 10-fold, whereas the presence of similar amounts of primer-dependent GTF-S had no effect on synthesis by GTF synthesizing water-insoluble glucan. Primer-independent GTF-S appears to be a potent source of priming glucan for the primer-dependent GTF enzymes. Its possession of a noncatalytic binding site for glucan, the first observed for the GTF of *S. mutans*, suggests that it may also serve as a glucan receptor on the *S. mutans* cell surface.

The synthesis of extracellular, water-insoluble glucans from sucrose is necessary for the formation of dental plaque by *Streptococcus mutans* (7). It is generally understood that this polymerization is catalyzed by two types of extracellular glucosyltransferase (GTF): one synthesizing a water-soluble product from sucrose (GTF-S) and another synthesizing a water-insoluble product from sucrose (GTF-I). These two types of GTF, when combined, synthesize a complex, highly branched, adherent, water-insoluble glucan (7, 23, and 27). Glucan synthesis by the GTF-I of *S. mutans* 6715 requires the presence of a water-soluble primer glucan, a requirement which can be fulfilled by the product of GTF-S (27). The GTF-S produced by wild-type *S. mutans* 6715, however, itself requires a primer glucan (21) and thus is not a likely source of primer glucan.

Multiple forms of GTF-S recently were reported from this laboratory (12) and by Mukasa and co-workers (21, 22, 25, 26). Two types of GTF-S were observed; one required a primer dextran and synthesized a highly branched, water-soluble product, and the other had no primer requirement and synthesized a water-soluble product that was only slightly branched. Other reports of primer-independent GTF-S (e.g., 9, 29) gave no indication that two distinct GTF-S enzymes might be produced by *S. mutans*. The observations of multiple GTF-S enzymes cited above, however, clearly indicated that two very different GTF-S enzymes were coproduced by strain 6715 (12, 22). Tsumori et al. (26) demonstrated that these two distinct GTF-S enzymes were produced in *S. mutans* serotypes a, d, and g but not in other serotypes.

The roles of the two GTF-S enzymes in the process of complex glucan synthesis and plaque formation are not understood. Although it has been suggested that the soluble glucan produced by primer-independent GTF-S serves as the required primer for the other GTF enzymes (22), supporting evidence has not been published. This report describes a purification procedure which allows complete separation of the three types of GTF. The characteristics of the two purified GTF-S enzymes are compared, and the relative abilities of each to provide primer glucan for other GTF enzymes are assessed. Preliminary results of comparisons of the GTF-S enzymes have been presented elsewhere (12).

MATERIALS AND METHODS

Mutant 27 of *S. mutans* 6715-13 was obtained from M. L. Freedman (University of Connecticut Health Center, Farmington, Conn.). This noncariogenic mutant produces large quantities of primer-independent GTF-S, synthesizes excessive amounts of water-soluble glucan, and does not form adherent deposits (3, 4, 12). Cells were grown anaerobically at 37°C in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) -yeast extract medium (13) supplemented with cysteine hydrochloride (10 ml of freshly prepared sterile cysteine hydrochloride [5 mg/ml] per liter of medium). After overnight growth, the cells were pelleted by centrifugation (23,000 × *g*), and the cell-free supernatant was neutralized with NaOH, adjusted to 50% saturation with solid $(NH_4)_2SO_4$, and stirred overnight at 4°C. The resulting precipitate was harvested by centrifugation (23,000 × *g*) and dissolved in 0.02% NaN_3 to obtain a crude extracellular protein preparation.

The preliminary separation of glucan-binding proteins from other proteins in the crude extracellular protein preparation was achieved by affinity chromatography (15) on Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, N.J.). These isolated proteins were further fractionated by

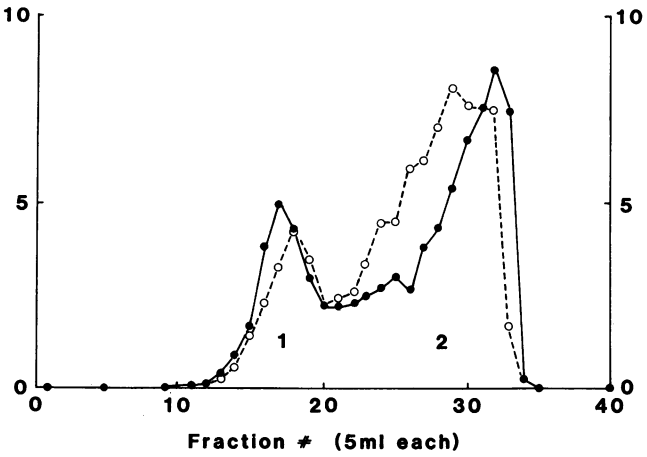

FIG. 1. Affinity chromatography elution profile. A crude extracellular protein preparation (400 ml) was applied to a bed (2.6 by 35 cm) of Sephadex G-50 equilibrated with 0.02% NaN_3. Exhaustive washing with 0.02% NaN_3 (300 ml) was followed by elution with 6.0 M guanidine hydrochloride. Only the elution profile is shown. Symbols: ●, milligrams of protein per fraction; ○, IU of GTF per fraction. 1 and 2 represent peaks.

ion-exchange chromatography on a bed (2.6 by 8 cm) of Trisacryl-M–DEAE (LKB Instruments, Inc., Rockville, Md.) equilibrated with sodium acetate buffer (0.01 M; pH 5.5). The charged ion-exchange columns were eluted with gradients of NaCl (0 to 0.25 M) in the same buffer. Column effluents were monitored at 280 nm, and 5-ml fractions were collected. Carbohydrate contents of fractions were estimated by the phenol-sulfuric acid method (2), and protein contents were determined with Coomassie brilliant blue G250 reagent (20). GTF activity was detected by assaying the incorporation of isotope from [U-^{14}C]sucrose (ICN Pharmaceuticals Inc., Irvine, Calif.) into alcohol-insoluble material (13) during 15 min of incubation at 37°C. The routine reaction mixture for this purpose contained 0.05 ml of enzyme source, 0.025 ml of [U-^{14}C]sucrose (1.67 μCi; 33.33 mg of purified sucrose in 1 ml of H_2O), and 0.025 ml of sodium phosphate buffer (0.5 M; pH 6.0) or the same buffer containing clinical dextran (0.5 mg/ml; molecular weight, 60,000 to 90,000; ICN). Studies of the priming effectiveness of glucans synthesized by the GTF-S enzymes were done with the same reaction mixture by substituting GTF-S glucans (0.125 mg/ml) for clinical dextran. Assessments of the priming effectiveness of the GTF-S enzymes required a slight modification of the reaction mixture to accommodate assays of various enzyme combinations. Enzyme solutions were adjusted to provide 5×10^{-3} IU/0.025 ml. The total volume of the reaction mixture was increased to 0.125 ml to allow the combination of up to three enzymes (0.025 ml each). Portions (0.025 ml) of H_2O were substituted for each omitted enzyme. Other conditions were as described for the routine assay. Glucans synthesized by GTF-S or GTF-I under these conditions were not precipitated by centrifugation (37,000 × *g*) or retained by membrane filters (20-μm pores).

Fructosyltransferase was assayed in routine reaction mixtures with [U-^{14}C]fructose-sucrose. Assays to determine the effects of pH upon GTF activity were done with 0.25 M sodium acetate buffer or 0.25 M sodium phosphate buffer. Sucrose solutions used in assays of GTF and fructosyltransferase were purified by ultrafiltration through a Diaflo PM10 membrane (Amicon Corp., Lexington, Mass.) to remove contaminating dextran. One IU of GTF was arbitrarily defined as the amount of enzyme catalyzing the incorporation of 1 μmol of glucose from sucrose into glucan (alcohol-insoluble material) per min under the conditions described above and in the presence of clinical dextran. Dextranase and invertase were assayed as previously described (8, 17). Branching-enzyme activity was detected by assaying the incorporation of isotope from nonreducing end-labeled [^{14}C]isomaltosaccharides into acceptor dextran (10).

Electrophoresis of native proteins was done by the discontinuous polyacrylamide gel rod method of Davis (1). The sodium dodecyl sulfate technique of Weber and Osborn (28) was used to estimate molecular size. Sodium dodecyl sulfate gels were cast in a slab format (0.05 by 12.5 by 14 cm). Isoelectric focusing was carried out in 6% polyacrylamide gel slabs (0.05 by 14 by 16 cm) containing 5% pH 3 to 10 ampholytes (Accurate Chemicals, Garden City, N.Y.). Gels were stained for protein with Coomassie brilliant blue R250. GTF activity was detected by incubating unfixed gels overnight at room temperature in sodium phosphate buffer (0.05 M; pH 6.0) containing 1% sucrose and 0.1% clinical dextran. Zones of glucan synthesis were stained in washed gels by the periodic acid-Schiff procedure as described by Zacharius et al. (30).

The extent of branching in the product of primer-independent GTF-S was estimated by an indirect method. ^{14}C-labeled glucans synthesized by purified GTF-S were digested with *Penicillium* sp. endodextranase (25 IU in sodium phosphate buffer [0.05 M; pH 6.0]; grade I; Sigma Chemical Co., St. Louis, Mo.). Hydrolysis by endodextranase was monitored by estimating the reducing sugar contents of reaction mixtures (18). Incubation for 24 h at 37°C was followed by chromatography on a calibrated column (10) of Bio-Gel P2-400 (1.6 by 90 cm) equilibrated with 0.02% NaN_3. Radioactivity in each fraction (2 ml) was determined by liquid scintillation chromatography of aliquots (0.2 ml) dissolved in a scintillation cocktail containing Scintisol (Isolab, Inc., Akron, Ohio). The proportions of endodextranase limit products so obtained were used to estimate the extent of branching of glucan synthesized by primer-independent GTF-S. Isolated products larger than isomaltose (see Fig. 6) were again digested with endodextranase to assure that they were limit products.

The nomenclature used here to identify the GTF enzymes will be as described previously (12). GTF zones resolved by polyacrylamide gel electrophoresis (1) were identified as GTF-S or GTF-I according to the nature of their products and then numbered according to their relative mobilities in 7% C polyacrylamide gels, beginning with the most rapidly migrating zone. Primer-dependent GTF-S was designated GTF-S1,S2 (two zones of activity usually are resolved), and primer-independent GTF-S was designated GTF-S4.

RESULTS

Purification. Affinity chromatography yielded two peaks containing protein and GTF activity (Fig. 1 and Table 1). Electrophoretic analyses (data not shown) indicated that GTF-S1,S2 was present only in peak 1 (fractions 13 to 21) from this column, whereas GTF-S4 was present only in peak 2 (fractions 22 to 34). GTF-I isozymes were present in both peaks. Neither invertase activity nor fructosyltransferase activity was detected in the protein peaks eluted from the Sephadex G-50 affinity chromatography column.

Ion-exchange chromatography of the pooled fractions

from each peak yielded the profiles shown in Fig. 2. Most GTF-S4, free of other proteins, passed through the column unimpeded when the Sephadex G-50 peak 2 pool was applied. It was collected in the voided fractions (Table 1, DEAE column 2, void; not shown in Fig. 2). A smaller portion of GTF-S4 bound to the column and was eluted shortly after application of the gradient (Fig. 2B, peak 1, fractions 2 to 40; Table 1, DEAE column 2, peak 1). Only the GTF-S4 collected in the voided fractions was used in the experiments described here. GTF-S1,S2 was eluted from the column charged with the Sephadex G-50 peak 1 pool at 0.2 M NaCl (Fig. 2A, peak 3, fractions 225 to 240; Table 1, DEAE column 1, peak 3). GTF-I was eluted from both columns in a single peak at 0.9 M NaCl (Fig. 2A and B, peak 2, fractions 140 to 160; Table 1, DEAE columns 1 and 2, peak 2). Each peak of GTF activity was pooled and dialyzed against 0.02% NaN_3. The proteins and GTF activities present in the pooled and dialyzed GTF peaks were analyzed by polyacrylamide gel electrophoresis (Fig. 3). No cross-contamination of preparations was detected. Assays of GTF peak fractions, pooled GTF peaks 1, 2, and 3, and pooled void protein fractions of DEAE column 2 (GTF-S4; Table 1, DEAE column 2, void) for carbohydrate content were negative. Dextranase was detected in fractions 200 to 220 immediately preceding the GTF-S1,S2 peak from the Trisacryl-M–DEAE column charged with the Sephadex G-50 peak 1 pool (Fig. 2A, peak 3) but was absent from this peak.

Kinetic comparison of the GTF-S enzymes. Levels of glucan-synthesizing activity and sucrose-independent, dextran-branching activity over a range of pHs are shown in Fig. 4. Branching activity was not detected in the GTF-S4 preparation but was readily detected in the GTF-S1,S2 preparation, in which its maximum, at pH 4.5 to 5.0, corresponded to the more acidic maximum of glucan-synthesizing activity.

Preliminary studies suggested that the rate of glucan synthesis by GTF-S4 was not affected by the presence of primer dextran. Subsequent kinetic studies of the GTF-S enzymes indicated that their K_m values for sucrose were similar (10^{-2} M). GTF-S1,S2 had a K_m value of 5×10^{-7} for dextran, whereas no K_m value for dextran could be determined for GTF-S4. Rather, the activity of GTF-S4 was completely unaffected by the presence of dextran over a concentration range of 10^{-7} to 2.5×10^{-4} M (Fig. 5), an indication that the GTF acceptor reaction usually associated with the presence of exogenous dextrans (16) was not occurring. Increasing concentrations of the GTF acceptor 1-*O*-methyl-α-D-glucopyranoside (0.25 to 1.25 M) were included in GTF-S4 reaction mixtures to determine whether the inhibition of glucan synthesis would occur as expected (16). This acceptor markedly inhibited glucan synthesis by GTF-S4, causing 53% inhibition at 0.25 M and 90% inhibition at 1.25 M.

Isoelectric focusing of the GTF-S enzymes yielded pI values similar to those obtained by other investigators (22). GTF-S1,S2 focused in two zones, at pI 3.9 and pI 4.1, whereas GTF-S4 focused in a series of zones, at pI values ranging from 6.77 to 8.0, with major zones at pI 6.77 to 7.0. Zones of GTF-I focused at pI 4.6 to 5.0 and 5.4 to 5.9. Molecular weight estimates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were as follows: 165,000 and 175,000 for GTF-S1,S2; 150,000 and 160,000 for GTF-S4; and 157,000, 165,000, and 175,000 for GTF-I.

Glucans synthesized by GTF-S1,S2 and GTF-S4. The endodextranase resistance of the [^{14}C]glucan synthesized by GTF-S1,S2 was reflected by the absence of an increase in reducing sugar residues during treatment with endodextranase and by the elution of almost all of the treated glucan in the void volume of the P2-400 column (Fig. 6). The [^{14}C]glucan synthesized by GTF-S4, in contrast, was completely degraded to glucose, isomaltose, tetrasaccharide, and pentasaccharide (G, G2, G4, and G5, respectively) and minor amounts of larger oligosaccharides (Fig. 6). The G and G2 peaks were confirmed to be glucose and isomaltose, respectively, by paper chromatography (10). Products G4 and G5 were resistant to further endodextranase action, thus indicating they were branched limit oligosaccharides. They probably were analogous to the branched oligosaccharides derived from the hydrolysis of *Leuconostoc mesenteroides* B512 dextran by *Penicillium* endodextranase (23). The proportions of these branched oligosaccharides and of isomaltose and glucose suggested that the glucan product of GTF-S4 contained 93% 1,6-α-D-linked residues and 7% 1,3,6-linked branch residues. This indirect estimate of linkages was in agreement with the direct linkage analyses reported for the products of a similar primer-independent GTF-S isolated from wild-type *S. mutans* 6715 (22).

TABLE 1. Purification of GTF enzymes from *S. mutans* 6715-13 mutant 27

Step	Total protein		Activity			Purification (fold)
	mg	%	IU	%	IU/mg	
1. Supernatant	1,240	100.0	980	100.0	0.8	1.0
2. 50% Ammonium sulfate	512	41.3	957	97.6	1.9	2.4
3. Sephadex G-50[a] (four columns)						
Peak 1	46	3.7	80	8.2	1.7	2.1
Peak 2	228	18.4	236	24.1	1.0	1.3
4a. DEAE column 1[a]						
Peak 2	27.4	2.2	14.8	1.5	0.5	0.6
Peak 3	1.5	0.1	4.7	0.5	3.1	3.9
4b. DEAE column 2[a]						
Void	9.0	0.7	113	11.5	12.6	15.8
Peak 1	7.3	0.6	37.4	3.8	5.12	6.4
Peak 2	32.5	2.6	20.5	2.1	0.6	0.8

[a] Peaks from four Sephadex G-50 columns were pooled for appilcation to the DEAE columns. Pooled peak 1 was applied to DEAE column 1, and pooled peak 2 was applied to DEAE column 2.

GTF-S4 as a source of substrate for primer-dependent GTF-S and GTF-I. Soluble glucans were prepared with GTF-S4 or GTF-S1,S2 in reaction mixtures containing unlabeled sucrose and lacking primer dextran, purified by precipitation with 66% ethanol, and dissolved in 0.02% NaN_3. Equal amounts (0.125 mg/ml) of each glucan were added, singly or in combination, to reaction mixtures containing [U-^{14}C]sucrose and GTF-S or GTF-I (5×10^{-3} IU, determined in the presence of primer dextran). The amount of glucan synthesized by GTF-S1,S2, GTF-S4, or GTF-I was determined after 15 min of incubation. Neither of the soluble glucans affected the synthesis of glucan by GTF-S4, but both enhanced synthesis by GTF-S1,S2 and GTF-I, with the glucan synthesized by GTF-S4 being superior to that synthesized by GTF-S1,S2 (Fig. 7). Incubation of each GTF enzyme (5×10^{-3} IU), singly or in various combinations, in reaction mixtures containing [U-^{14}C]sucrose without primer

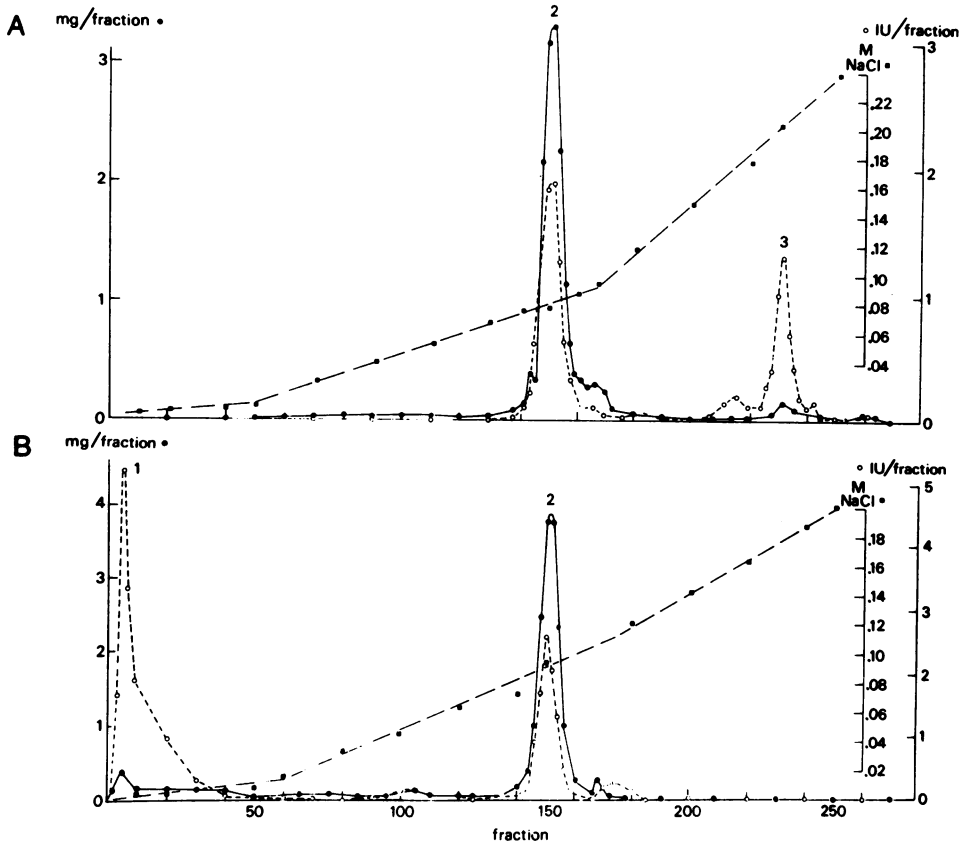

FIG. 2. Ion-exchange chromatography of affinity-purified proteins on Trisacryl-M–DEAE. Affinity chromatography peaks 1 and 2 (Fig. 1, fractions 13 to 21 and 22 to 34, respectively) were applied separately to the ion-exchange column. Voided fractions are not shown. (A) Ion-exchange chromatography of affinity chromatography peak 1; (B) chromatography of peak 2. Symbols: ●, milligrams of protein per fraction; ○, IU of GTF per fraction; ■, NaCl concentration (molar).

indicated that the presence of GTF-S4 greatly enhanced total glucan synthesis by the primer-dependent GTF enzymes (Fig. 8). The presence of GTF-S1,S2, in contrast, only slightly enhanced total glucan synthesis by primer-dependent GTF-I.

DISCUSSION

The GTF enzymes produced by *S. mutans* 6715-13 mutant 27 are readily isolated and separated by affinity chromatography on Sephadex G-50 and subsequent ion-exchange chromatography on Trisacryl-M–DEAE (Fig. 1, 2, and 3; Table 1). Fructosyltransferase and invertase are eliminated in the affinity chromatography step by virtue of their inability to bind dextran. Endodextranase usually is separated from GTF during affinity chromatography, since endodextranase invariably elutes in peak 1 from the Sephadex G-50 column and, for most strains of *S. mutans*, the bulk of GTF elutes in peak 2 (11, 12). Much of the GTF from mutant 27, however, elutes in peak 1 as well as in peak 2 (Fig. 1), resulting in a peak 1 pool of GTF which is contaminated with endodextranase. Ion-exchange chromatography on Trisacryl-M–DEAE effectively separates endodextranase from GTF in Sephadex G-50 peak 1, and its absence is routinely assured by direct assays of enzyme preparations, Endodextranase was absent from the Trisacryl-M–DEAE enzyme preparations obtained here.

The growth of *S. mutans* under conditions preventing the synthesis of glucan results in the production of GTF enzymes which require primer and are stimulated by its presence (5, 19). The growth conditions and the methods of GTF isolation described here routinely yield a primer-independent GTF enzyme (GTF-S4) and two primer-dependent GTF enzymes (GTF-S1,S2 and GTF-I) from mutant 27. Carbohydrates are not present in these GTF preparations, indicating the absence of endogenous primer. GTF-S4 differs markedly from the other GTF enzymes of *S. mutans*. It does not possess the sucrose-independent branching activity (10) found in GTF-S1,S2 (Fig. 4), it lacks the requirement for primer glucan characteristic of other *S. mutans* GTF enzymes (12, 22), and its rate of glucan synthesis is neither enhanced nor diminished by the presence of primer glucan (Fig. 5). The total absence of a catalytic response to primer dextran has not been reported for other purified GTF enzymes. Although there have been other observations of primer-independent GTF-S (e.g., 9, 22, 29), the complete absence of any response to the presence of primer is unusual. This lack of response to primer glucan contrasts with the marked inhibition of GTF-S4 glucan synthesis by the low-molecular-weight acceptor 1-*O*-methyl-α-D-glucopyranoside. GTF-S4, therefore, responds catalytically to the presence of a small acceptor molecule but not to the presence of glucan. The lack of a response to various glucans, including its own product (Fig. 5 and 7), cannot be attributed to the presence of an endogenous primer glucan in preparations of the enzyme, since carbohydrates are invariably absent. Neither can it be due to the inability of GTF-S4 to bind to glucan, since it binds Sephadex G-50 (Fig. 1) and

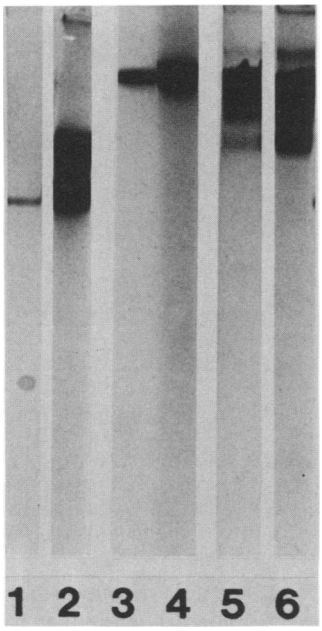

FIG. 3. Polyacrylamide gel electrophoresis of GTF pools from ion-exchange chromatography columns (Fig. 2; Table 1). Lanes 1 and 2, GTF-S1,S2 in peak 3 (Fig. 2A); lanes 3 and 4, GTF-S4 in the excluded protein pool of DEAE column 2 (Table 1, DEAE column 2, void); lanes 5 and 6, GTF-I in peak 2 (Fig. 2A and B). Lanes 1, 3, and 5 were stained for protein with Coomassie brilliant blue R250, and lanes 2, 4, and 6 were incubated overnight in 1% sucrose–0.1% clinical dextran in sodium phosphate buffer (0.05 M; pH 6.0) and then subjected to the periodic acid-Schiff procedure (30) to stain regions of glucan synthesis.

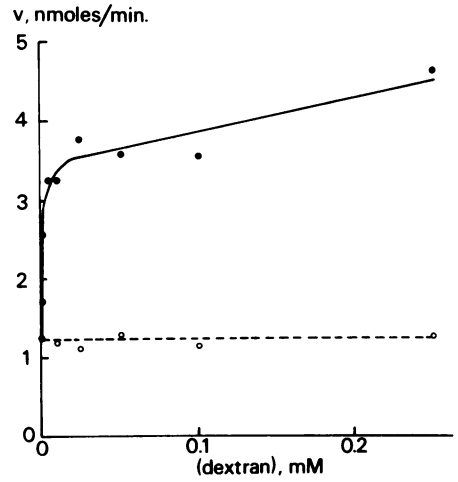

FIG. 5. Michaelis-Menten plot of the effect of dextran concentration on the rates of glucan synthesis by GTF-S1,S2 (●) and GTF-S4 (○). v, Velocity of glucan synthesis expressed as international units.

has been found to bind dextran and various *S. mutans* water-soluble glucans in affinity electrophoresis (24) studies (unpublished results). The enzyme appears, rather, to lack a catalytic site for exogenous 1,6-α-D-glucans but to possess a noncatalytic site which avidly binds these polysaccharides. This is the first observation of a GTF possessing a glucan-binding site with no apparent catalytic function.

GTF-S1,S2 was designated "sucrose:1,6-α-D-glucan 3-α- and 6-α-glucosyltransferase" (22, 25); that is, it was suggested to be bifunctional, capable of synthesizing from sucrose both 1,6-α-D and 1,3-α-D linkages (the latter forming branch points on 1,6-linked sequences). The presence of sucrose-independent branching activity in the GTF-S1,S2 preparation (Fig. 4), however, indicates that branch points on the product of this GTF are formed through the intercession of the endogenous branching activity, which readily functions in the absence of polymerization (10). The suggestion that the polymerization activity of GTF-S1,S2 is bifunctional is not an accurate assessment in view of the consistent presence of sucrose-independent branching activity in preparations of this enzyme (10; Fig. 4).

The dependence of GTF-I glucan synthesis rates upon

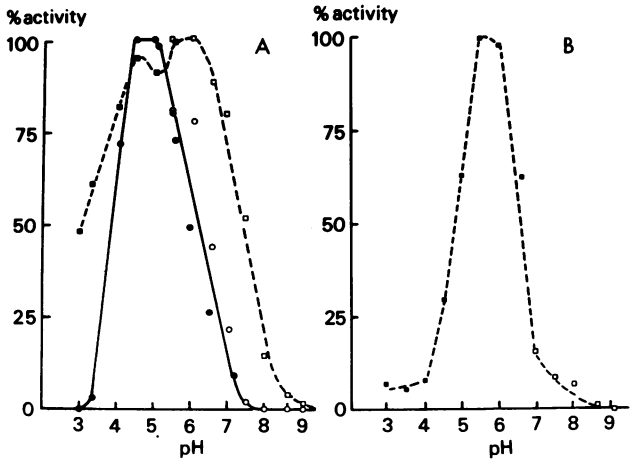

FIG. 4. Effects of pH on glucan synthesis and sucrose-independent dextran branching by GTF-S1,S2 (A) and GTF-S4 (B). Symbols: ● and ○, branching activity; ■ and □, GTF activity. Buffers used were 0.25 M sodium acetate (solid symbols) and 0.25 M sodium phosphate (open symbols).

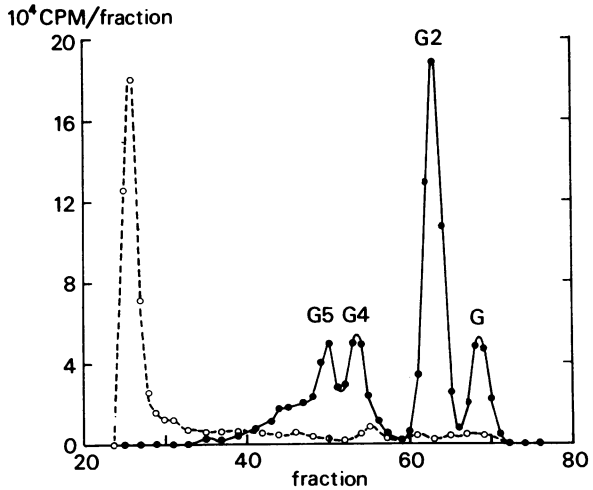

FIG. 6. Gel filtration on Bio-Gel P2-400 of the endodextranase products of water-soluble glucans synthesized in the absence of primers by GTF-S1,S2 (○) and GTF-S4 (●).

GTF-S activity was described by Walker (27), but the GTF-S used was not a primer-independent enzyme. GTF-S4 appears to be capable of providing a primer glucan well suited to the requirements of the primer-dependent GTF enzymes (Fig. 7 and 8). The observation of the superiority of the GTF-S4 product over the GTF-S1,S2 product in this regard (Fig. 7) and of GTF-S4 itself over GTF-S1,S2 (Fig. 8) suggests that the linear structure of the GTF-S4 glucan better defines a GTF primer than does the highly branched structure of the GTF-S1,S2 glucan. The resistance of the latter glucan to endodextranase action indicates that it possesses few accessible linear 1,6-α-D-linked sequences, as might be expected from the high level of branching in this glucan (21). Although increased branching of a glucan primer (or acceptor) has been demonstrated to enhance its function as a primer (16), extreme branching would impede any primer function which depends upon the presentation of an array of linear 1,6-α-D-linked sequences. The expected enhancement of the glucan synthesis rate by about 20-fold in reaction mixtures containing all three GTF enzymes was not observed (Fig. 8), suggesting that the overall rate of glucan synthesis is limited by the rate of primer glucan synthesis by GTF-S4.

It is known that an overabundance of water-soluble primer results in the synthesis of predominantly water-soluble glucans by *S. mutans* GTF (16). GTF-S4 is not normally found in the extracellular fluids of *S. mutans* cultures, although preliminary studies with monoclonal antibodies indicate that it is present on the surfaces of wild-type cells (unpublished results). This enzyme is released into the culture fluid when appropriate detergents are present in the medium (22) or, under normal culture conditions, by mutants such as mutant 27 of strain 6715-13 and by mutants of strain AHT (12). The synthesis of abnormal amounts of water-soluble glucans by nonadherent mutant 27 (3, 4) suggests

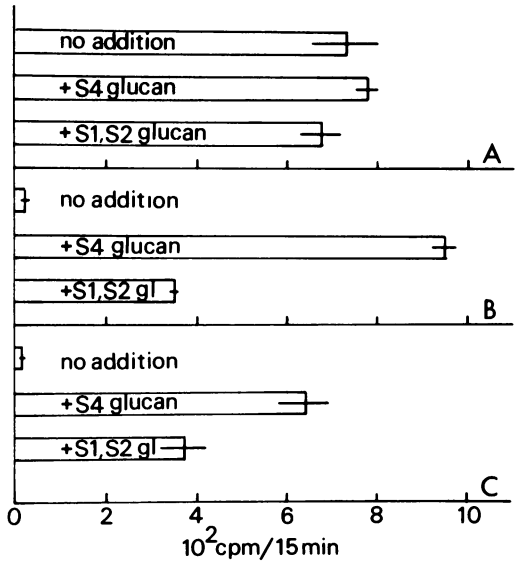

FIG. 7. Priming effectiveness of the water-soluble glucan products of GTF-S4 (+S4 glucan) and GTF-S1,S2 (+S1,S2 glucan or +S1,S2 gl) as compared with control reaction mixtures lacking a primer (no addition). Glucan synthesis by GTF-S4 (A), GTF-S1,S2 (B), and GTF-I (C) is shown. Each bar represents the mean of glucan synthesis in three replicate reaction mixtures, and standard deviations are indicated by the horizontal line at the right end of each bar.

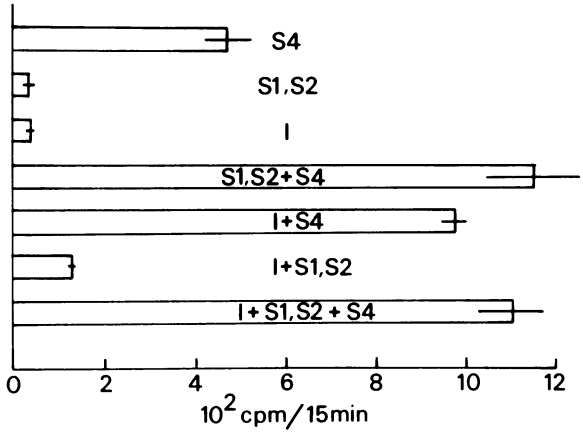

FIG. 8. Priming effectiveness of GTF-S1,S2 and GTF-S4 in the absence of exogenous primer glucan. Glucan synthesis by combinations of GTF-S1,S2 (S1,S2), GTF-S4 (S4), and GTF-I (I) is compared with glucan synthesis by individual enzymes. Data are presented as described in the legend to Fig. 7.

that its phenotype is primarily determined by the characteristic excess of GTF-S4 and the resulting overproduction of primer glucan. GTF-S4 thus appears to be a potent component of the GTF complement, capable of either aiding (Fig. 7 and 8) or disrupting (3, 4) the synthesis of water-insoluble glucans and the formation of the plaque matrix.

Gibbons and Fitzgerald (6) first suggested that the *S. mutans* cell surface glucan-binding site might reside in the active site of cell-associated GTF. Studies by Germaine and Schachtele (5) and the results of affinity chromatography (11, 12, 15) confirmed the ability of GTF to bind glucans. Other studies, however, indicated that cells depleted of GTF activity still agglutinated in the presence of glucans (14), thus suggesting that the GTF active site is not involved in glucan-mediated aggregation. An alternate candidate for the cell surface glucan-binding site is the glucan-binding protein (11), which recently was shown to possess exclusive specificity for 1,6-α-D-glucans (E. C. Landale and M. M. McCabe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, B9, p. 19). The presence of a noncatalytic glucan-binding site on GTF-S4 suggests that this enzyme could serve as a cell surface site for glucan binding and as a source of primer glucan. The possibility that other GTF enzymes possess similar glucan-binding sites is being assessed.

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