INFECTION AND IMMUNITY, Feb. 1979, p. 446-452
0019-9567/79/02-0446/07$02.00/0

Vol. 23, No. 2

Preparation of Glucosyltransferase from *Streptococcus mutans* by Elution from Water-Insoluble Polysaccharide with a Dissociating Solvent

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Received for publication 27 October 1978

Glucosyltransferase (EC 2.4.1.5) was obtained by dissociation from water-insoluble polysaccharide in the presence of 6 M guanidine-hydrochloride. Water-insoluble polysaccharide was synthesized by cell-free culture supernatants from *Streptococcus mutans* strain 6715. Gel filtration of the glucosyltransferase on a column of 8% agarose in phosphate buffer, followed by filtration on a column of 4% cross-linked agarose in 6 M guanidine-hydrochloride, gave a 23-fold enrichment of the enzyme. The enriched glucosyltransferase preparation contained 22% carbohydrate and eluted at a position corresponding to a molecular weight of 422,000. Polyacrylamide gel (5%) electrophoresis of this preparation revealed two regions which stained for protein, formed water-insoluble polysaccharide in the presence of sucrose, and precipitated with antisera directed to crude glucosyltransferase preparations. The guanidine-eluted enzyme could be primed by 5×10^{-5} M dextran T10 (molecular weight, 10,000). High-molecular-weight glucan and a possible glucan-binding protein were also obtained after the final gel filtration step (4% cross-linked agarose) in addition to glucosyltransferase.

The ability of cariogenic *Streptococcus mutans* to participate in the formation of dental plaques has been related to the presence of extracellular glucosyltransferase (GTF; EC 2.4.1.5) enzymes which synthesize both water-soluble and water-insoluble glucose polymers (glucans) from sucrose (9). The formation of insoluble glucan has been assigned special significance not only because this form of the polysaccharide may facilitate colonization and accumulation of *S. mutans* on tooth surfaces but also because it creates a barrier for diffusion of acids in plaque (12, 27). Methods of GTF purification have therefore been sought to provide enzyme to study the colonization processes of *S. mutans* and to provide antigen for experiments designed to interfere immunologically with the pathogenesis of cariogenic streptococci. Immunization of rodents with GTF-containing antigen preparations has been shown to reduce disease caused by infection with the same (11, 28) or different (24) strains of *S. mutans* from which the antigen was derived. Although GTF is the most probable protective antigen in these experiments, other antigens are present in some of the preparations used for immunization. The ability to obtain GTF free of other antigens in a yield sufficient for immunization experiments will help to resolve the role of GTF as a protective antigen. We have investigated an approach to GTF preparation which exploits the fact that a significant proportion of enzyme remains bound to the glucan product after incubation with sucrose. In this report we describe this approach in which enzyme bound to water-insoluble glucan synthesized by *S. mutans* 6715 is released by exposure to the dissociating solvent 6 M guanidine-hydrochloride (GuHCl).

MATERIALS AND METHODS

Bacteria and growth conditions. *S. mutans* strain 6715 was originally obtained from J. van Houte. This strain is resistant to streptomycin at concentrations of 2,000 μg/ml. *S. mutans* organisms were grown anaerobically (10% CO_2, 90% N_2) for 16 to 24 h at 37°C in 5 liters of chemically defined medium (25). Cells were then removed by centrifugation at 13,700 × *g* for 15 min at 4°C.

6 M GuHCl elution of enzyme from WIP. The *S. mutans* culture supernatant is brought to pH 6.3 with 5 N NaOH. Water-insoluble polysaccharide (WIP) is then prepared by incubation of the GTF-containing culture supernatant with 10% sucrose for 48 h at 37°C. Approximately 50 g of WIP (wet weight) and 10 g of water-soluble polysaccharide (wet weight) per liter of original culture supernatant are formed under these conditions, and greater than 97% of the total polysaccharide synthesized is glucan (21). Bacterial growth is inhibited with 0.02% sodium azide. The WIP is obtained by centrifugation at 13,700 × *g* for 15 min at 4°C. The pellet is washed twice with cold distilled water and twice with 0.01 M sodium phos-

phate, pH 6.8 (PB), to which 0.02% sodium azide is added. The washed WIP is then agitated for 1 h at room temperature in a volume of 6 M GuHCl (grade 1, water soluble; Sigma Chemical Co., St. Louis, Mo.) which is twice the weight of the polysaccharide. The enzyme-containing supernatant is collected by centrifugation at 13,700 × *g* at 4°C for 15 min. Guanidine is removed by dialysis against PB in 0.02% sodium azide, and the retentate is concentrated by negative pressure.

Gel filtration. (i) 8% agarose. Dialyzed and concentrated 6 M GuHCl eluates (80 ml) were gel filtered at 4°C on a column (5 by 90 cm) containing 8% agarose (Bio-Rad Laboratories, Richmond, Calif.) previously equilibrated in PB plus 0.02% sodium azide. Fractions (8 ml each) were collected at a flow rate of 50 ml/h. The column effluent was monitored at 280 nm. Selected fractions were also assayed for enzymatic activity after incubation with sucrose by measuring the release of total reducing sugars (Somogyi procedure [26]) and glucose (glucose oxidase procedure, Worthington Biochemicals Corp., Freehold, N.J.). Protein content in individual pools was estimated by the Lowry method (16), whereas carbohydrate was determined by the technique of Dubois et al. (4).

(ii) Cross-linked 4% agarose. The GTF pool obtained by gel filtration on 8% agarose was concentrated by negative pressure. Sufficient solid GuHCl was added to the pool to achieve a concentration of 6 M. After stirring for 1 h, the GTF pool was gel filtered on a column (2.6 by 90 cm) of Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) which had been equilibrated with 6 M GuHCl. Little agarose-derived carbohydrate is released in dissociating solvents because of covalent cross-links created between individual agarose chains. Fractions (4 ml each) were collected at a flow rate of 12 ml/h. The column effluent was monitored at 280 nm. Individual fractions were dialyzed against PB, after which they were analyzed for carbohydrate (4), protein (16), and enzymatic activity (26). This column was also used for estimation of molecular weight. Samples (1 ml each) containing 30 to 60 mg were equilibrated in 6 M GuHCl and applied at a flow rate of 12 ml/h. The components used to standardize the column were lysozyme (Mann Research Lab., New York, N.Y.), bovine serum albumin (Sigma); bovine immunoglobulin G (Sigma), and dextran T500 (Pharmacia). The molecular weights of these materials were 14,400, 67,000, 160,000, and 500,000, respectively.

Enzyme assays. GTF activity was determined by incorporation of [^{14}C]glucose from sucrose-[*U*-^{14}C]glucose (specific activity, 275 mCi/mmol; New England Nuclear Corp., Wilmington, Mass.) into ethanol-insoluble polysaccharide as previously described (23). Fructosyltransferase (EC 2.4.1.10) activity was determined in a similar fashion by using sucrose-[1-^{3}H,N]fructose (specific activity, 2 Ci/mmol; New England Nuclear). The standard incubation (2 h at 37°C) mixture contained 0.1 ml of enzyme solution and 0.018 μg of [^{14}C]-glucose-labeled sucrose (or 0.0080 μg of [^{3}H]fructose-labeled sucrose) in 0.2 ml of PB (5). The stimulation of GTF activity by dextran T10 (Pharmacia) was studied by the addition of buffer containing 5, 10, 50, 100, 500, and 1,000 μg/ml concentrations of dextran T10 (molecular weight = 10,000). The proportions of water-insoluble and water-soluble glucan synthesized by enzyme fractions were determined by the method of Linzer and Slade (15). After incubation, the reaction was first stopped by addition of 0.9 ml of cold PB. The mixture was then filtered by vacuum through 2.5-cm glass fiber filter disks (Gelman Type A/E, Gelman Instrument Co., Ann Arbor, Mich.) to retain water-insoluble glucan. The disks were then washed twice with 1.5 ml of cold PB. To permit measurement of total glucan synthesis, the reaction was first stopped by addition of 0.9 ml of cold 95% ethanol and allowed to stand for 15 min at 4°C. After mixing (Vortex-Genie), the mixtures were filtered on the glass fiber filter disks, and the disks were washed with 2 volumes of cold 95% ethanol. The filters were air-dried, placed in 10 ml of Ready-Solv solution IV (Beckman Instruments, Inc., Palo Alto, Calif.), and counted in a liquid scintillation spectrometer (Model LS-100C, Beckman). GTF activity was also determined by measuring the amount of fructose released after incubation with 0.125 M sucrose for 2 h at 37°C. Total reducing sugars were measured by the Somogyi technique (26). Glucose release was measured by the glucose oxidase assay (Worthington Biochemicals Corp.). Using these assays, 1 unit (U) of GTF activity is defined as the amount of enzyme required to incorporate 1.0 μmol of glucose from sucrose into glucan per min at 37°C.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed by the method of Davis (3) without the use of sample or stacking gels. Samples were mixed with 50% glycerol and layered directly onto the 5% separating gels. Proteins were subjected to electrophoresis on 100-mm gels for approximately 1 h at 4 mA/gel. Protein in the gels was stained with Coomassie brilliant blue R (Sigma). GTF activity in gels was detected by incubating washed gels in an assay mixture containing [*U*-^{14}C]sucrose (5 mCi/mmol) by the method of Chludzinski et al. (1). After incubation for 3 h at 37°C, gels were washed with two changes of distilled water, then sliced in 2-mm sections. Gel slices were dissolved with 0.2 ml of 30% hydrogen peroxide overnight, followed by the addition of 4 ml of Ready-Solv solution IV (Beckman) and counted in a liquid scintillation spectrometer (Beckman). WIP synthesis was determined by incubation of duplicate gels in 0.125 M sucrose in PB overnight at 37°C. White bands of polysaccharide appearing in the gels indicated regions of enzymatic activity. Antigenic activity in gels was detected by a modified immunodiffusion technique. After electrophoresis, the gel was sliced in 2-mm widths. The gel slices were placed in preformed 5-mm wells of immunodiffusion slides (3 ml of 1.2% agarose per slide), then macerated and suspended in a small amount of PB. The macerated slices were then reacted against a central 5-mm well filled with a representative hamster antiserum containing antibody activity against GTF of *S. mutans* 6715. This antiserum was obtained by retroorbital bleeding 10 days after four weekly injections in the salivary gland region of the enzyme-containing 8% agarose void volume pool (described above) in complete Freund adjuvant (ca. 0.05 U/injection) (28).

RESULTS

Time course of GTF elution. Initially it was found that GTF activity could be eluted from WIP synthesized by *S. mutans*. A time course experiment was performed to determine the effect of different exposure intervals of WIP from *S. mutans* 6715 to 6 M GuHCl on the release of enzymatic activity, protein, and carbohydrate. Samples (10 g each) of WIP were exposed to 20 ml of 6 M GuHCl for 0.5, 1, 3, 5, 8, 20, or 60 h. The GTF activity released is presented in Fig. 1. Between 1 and 20 h, both the GTF activity and the protein released increased by about 1.5-fold. However, during the same time interval, the dissociative effects of the guanidine on glucan structure caused the amount of carbohydrate released to increase by 3.8-fold, thus changing the carbohydrate/protein ratio from 0.5 to 1.5. Therefore, to minimize the glucan contribution to the enzyme preparation, the 1-h incubation interval was selected to obtain enzyme for further purification.

Enrichment of GTF. A representative preparation of GTF from *S. mutans* 6715 is shown in Table 1. Elution of WIP with 6 M GuHCl for 1 h yielded nearly half of the GTF activity which existed in the original culture supernatant. During dialysis of the eluate, a precipitate formed which contained 16% of the enzymatic activity. Concentration of the eluate by negative pressure further resulted in a 15% loss of activity. The guanidine-eluted enzyme preparation was then gel filtered on 8% agarose. All of the enzyme activity eluted near the void volume of the column, as measured by release of total reducing sugars (absorbance at 540 nm) (Fig. 2). This represented an 82% recovery of the enzymatic activity applied to the column. Carbohydrate and protein assay of the opalescent void volume pool 1 (fractions 28 to 38) indicated that 90% of this pool was carbohydrate. Since this soluble carbohydrate was not in a dissociating medium, the coelution of the enzyme with the carbohydrate, presumably glucan, could result from reassociation of these components. Therefore, to eliminate these potential noncovalent interactions, the enzyme-containing void volume pool was equilibrated with 6 M GuHCl and gel filtered on a column of 4% cross-linked agarose which was also equilibrated with 6 M GuHCl

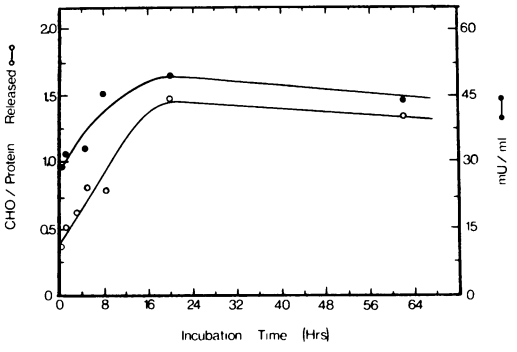

FIG. 1. *Time course of enzymatic activity, carbohydrate, and protein released from WIP. Volumes (10 ml each) of centrifuged WIP from S. mutans 6715 were incubated with 20 ml of 6 M GuHCl for the time indicated. Symbols: ○, ratio of carbohydrate (4) to protein (16) released in the supernatant after centrifugation; ●, milliunits of GTF activity (26) released per milliliter of supernatant.*

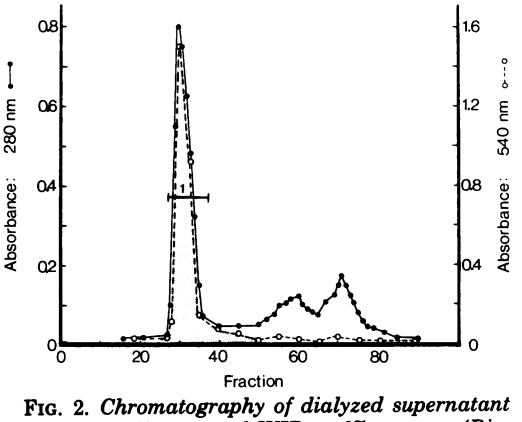

FIG. 2. *Chromatography of dialyzed supernatant of 6 M guanidine-treated WIP on 8% agarose (Bio-Gel A1.5m agarose). Fractions (8 ml each) were collected from a column (5 by 90 cm) at a flow rate of 50 ml/h. Sodium phosphate (0.01 M, pH 6.8) was the eluting buffer. Symbols: ●, absorbance at 280 nm; ○, absorbance at 540 nm of the Somogyi assay for release of total reducing sugars after incubation of 0.1-ml portions of the fractions with 0.125 M sucrose. The elution position of the enzymatic activity is indicated by the shaded region.*

TABLE 1. *Enrichment of GTFs from S. mutans 6715*

Fraction	Vol (ml)	Total protein (mg)	Total activity (U)	Sp act (U/mg of protein)	Purification (fold)	Yield (%)
Culture supernatant	5,000	300	156	0.52	1	100
6 M GuHCl eluate	380	22	68	3.08	6	43
8% Agarose	166	5.8	36	6.14	12	23
4% Agarose (CL)	55	1.3	15	11.85	23	10

(Fig. 3). The void volume peak (pool A, fractions 35 to 47) contained 255 μg of carbohydrate per μg of protein. This material reacted in gel diffusion with antibody directed against cell-associated glucan of *S. mutans* strain 6715 (13). All of the enzyme material was found to elute in the second peak (pool B, fractions 55 to 70) which contained 10% of the activity in the original culture supernatant, representing a 23-fold overall enrichment. Glucose represented greater than 97% of the labeled sugar in ethanol-insoluble polysaccharide synthesized by this fraction from sucrose, using the sucrose-[*U*-^{14}C]glucose and sucrose-[1-^{3}H,N]fructose incorporation assays. Water-insoluble glucan comprised 44% of the total glucan synthesized by this fraction, using the glass fiber filter disk assay (15). The ratio of protein to carbohydrate in the peak (pool B) was 3.8:1, suggesting either that the GTF is a glycoprotein or that some glucan still remained bound. The molecular weight of this fraction was estimated to be 422,000 by comparison with the elution position of molecular weight standards chromatographed on the same column. A third peak (pool C, fractions 71 to 79) of protein eluted just after the enzyme activity. This peak contained 5 μg of carbohydrate per μg of protein.

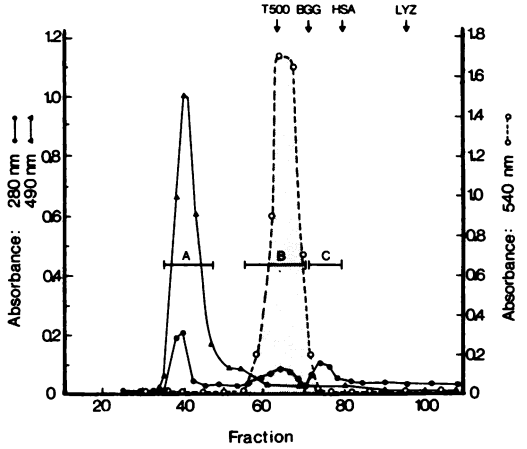

FIG. 3. *Chromatography of the GTF-containing fractions eluted from the 8% agarose column on cross-linked 4% agarose (Sepharose CL-4B). Fractions (4 ml each) were collected from the column (2.6 by 90 cm) at a flow rate of 12 ml/h; 6 M GuHCl was the eluting buffer. Symbols: ●, absorbance at 280 nm; ○, absorbance at 540 nm of Somogyi assay for release of total reducing sugars after incubation of dialyzed fractions (0.1 ml) with 0.125 M sucrose; ▲, elution position of carbohydrate, measured by phenol-H_2SO_4 method (4) as absorbance at 490 nm. The elution position of the enzymatic activity is indicated by the shaded region. The elution positions of molecular weight standards lysozyme (LYZ), human serum albumin (HSA), bovine immunoglobulin G (BGG), and dextran T500 (T500) are noted by the arrows.*

Polyacrylamide gel electrophoresis of Sepharose 4B CL pools. The purity and enzymatic activity of the three pools obtained after gel filtration on 4% CL-agarose was evaluated after electrophoresis in 5% polyacrylamide gels (Fig. 4). Pool A showed no bands staining with Coomassie brilliant blue R. Pool B contained two major protein bands, one near the origin and one which migrated 3 cm into the gel. Two or perhaps three very weakly staining bands were also observed in the gel. After electrophoresis of pool C, two bands were also observed—one at the origin, and a second band migrating approximately 5.5 cm from the origin. Duplicate gels were incubated after electrophoresis with 0.125 M sucrose overnight at 37°C to detect regions of WIP synthetic activity. No bands were observed in the pool A gel. In gels on which pool B was subjected to electrophoresis, areas of WIP enzymatic activity were noted which correponded to the two major protein bands after incubation with nonradioactive sucrose or after incubation with radioactive sucrose. In addition, water-soluble polysaccharide synthesis is suggested by the bulge in the gel (2). Only those regions corresponding to the two major protein bands in pool B formed immunoprecipitates when acrylamide slices were reacted in gel diffusion with antisera directed to pool 1 (Fig. 2) material. Incubation of gels with 0.125 M sucrose after electrophoresis of pool C material resulted in a faint band of polysaccharide corresponding to the protein-staining band seen at the origin. Although no polysaccharide was formed in the region of the protein which migrated 5.5 cm into the gel, antisera to pool 1 material (Fig. 2) formed an immunoprecipitate when reacted with gel slices taken from this region.

Dextran stimulation of GTF. The glucan synthetic activity of GTF prepared in the absence of sucrose can be primed by dextran T10 (7). Since the method of GTF preparation reported here may have resulted in bound endogenous glucan, it was of interest to determine whether exogenous dextran would prime the activity of GTF in pool B. Therefore, the amount of ethanol-insoluble glucan synthesized by pool B in the presence of various dextran concentrations was measured by using the sucrose-[*U*-^{14}C]glucose incorporation assay (Table 2). An acceleration in glucan synthesis did not occur until concentrations of more than 10^{-5} M dextran (molecular weight, 10,000) were used. The stimulating effect of dextran continued through

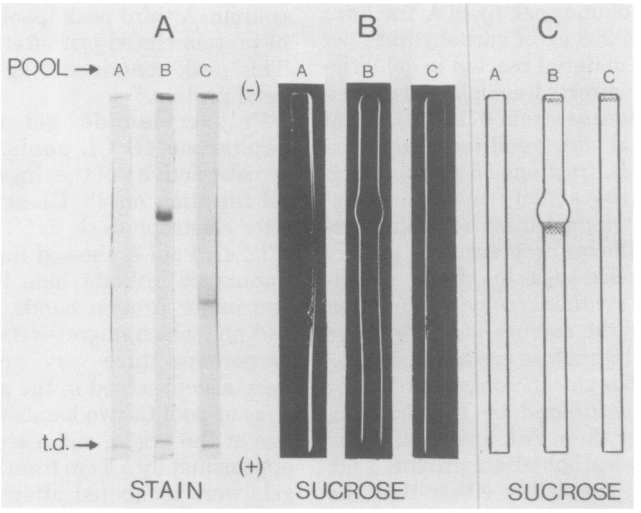

FIG. 4. *Electrophoresis of pools A, B, and C, obtained after elution on the cross-linked 4% agarose, on 5% polyacrylamide gels. Pool A protein (10 μg), pool B protein (30 μg), and pool C protein (22 μg) were applied to 100-mm gels and run at 4 mA/gel for 70 min. (A) Gels were stained with Coomassie brilliant blue R to reveal protein bands; (B) gels were incubated with 0.125 M sucrose in PB overnight at 37°C to reveal areas of WIP synthesis (white bands); (C) diagram of gels shown in B which were incubated with 0.125 M sucrose. t.d., Tracking dye position.*

TABLE 2. *Dextran stimulation of GTF activity*

Dextran T10 concn (M)	[^{14}C]glucose incorporated into glucan (nmol)[a]
0	1.18 ± 0.17
5×10^{-7}	1.33 ± 0.06
1×10^{-6}	1.57 ± 0.04
5×10^{-6}	1.30 ± 0.14
1×10^{-5}	1.55 ± 0.01
5×10^{-5}	2.00 ± 0.02
1×10^{-4}	3.50 ± 0.42

[a] Incubation mixture: 10 nmol of sucrose-[*U*-^{14}C]-glucose and 2.1 μg of enzyme in 0.2 ml of PB incubated for 2 h at 37°C. Values indicate mean of three determinations ± standard error.

the 10^{-4} M concentration, respresenting a three- to fourfold increase in GTF activity.

DISCUSSION

The dissociating solvent 6 M GuHCl has been shown to be effective in releasing GTF which is bound to WIP synthesized by *S. mutans* 6715. More than 40% of the enzymatic activity contained in the original culture supernatant can be released by 1 h of exposure of WIP to 6 M GuHCl. Enzyme yields can be increased by longer exposure to the solvent (Fig. 1) or by repeated elution of the polysaccharide. However, these procedures also increase the amount of glucan which is solubilized. Several advantages are inherent in this approach to GTF purification. Extracellular bacterial components which are not associated with the GTF are largely eliminated before purification is even begun. The use of defined media for bacterial culture also eliminates the contribution of exogenous media components. The WIP itself can be stored and used as an enzyme stock, since we have been able to prepare GTF from WIP stored at 4°C in 0.02% sodium azide for periods of 6 to 8 months without apparent detrimental effects. It is possible to obtain up to 16 U of enzyme activity per liter since between 100 and 200 mU of enzyme activity typically can be eluted from 1 g (wet weight) of packed WIP, and 30 to 80 g of polysaccharide can be synthesized from 1 liter of culture supernatant of *S. mutans* 6715. Furthermore, based on polyacrylamide gel patterns, the GTF obtained by this technique contains fewer contaminating components (Fig. 4) than are obtained by most other large-scale techniques for GTF preparation (1, 6, 10, 14, 18, 20). We have also used the GuHCl elution technique to prepare GTF from WIP synthesized by other serotypes of *S. mutans* (24).

The exposure of GTF to 6 M GuHCl does not seem to alter appreciably the enzymatic characteristics, since the specific activity of the most purified enzyme pool is at least as high as the specific activity of GTF prepared by techniques which do not include such treatment (1, 6, 18, 19). The immunological properties of GTF do not appear to be changed significantly by guanidine elution. Antisera obtained by injection of

either guanidine-eluted or chromatographically prepared GTF contain similar amounts of immunoglobulin G antibody directed to GTF (29). Both enzyme preparations also are effective as antigens which elicit similar reductions of caries (24, 28). Although the enzyme-containing 8% agarose void volume pool also contains carbohydrate, most is removed by the final step so that the enzyme which results has a carbohydrate content (22%) which is less than that reported for highly purified GTF (32 to 38%) prepared from sucrose-free culture supernatants (7). However, the activity of such an enzyme preparation, as described by Germaine et al. (7), could be primed at a 10-fold lower concentration of dextran T10 than could the guanidine-eluted enzyme. This finding may suggest that at least a portion of the carbohydrate in the guanidine-eluted enzyme preparation is residual glucan acting as endogenous primer (7). Alternatively, the enzymes responsible for WIP and water-soluble polysaccharide synthesis have been shown to differ in their ability to be stimulated by dextran (8, 14). Since the guanidine-eluted preparations contained both activities, the relationship between enzyme activity and primer dextran concentration may simply reflect this difference.

Previously, preparations of GTF which form water-insoluble glucan have generally resulted in very high-molecular-weight complexes ($\geq 1.5 \times 10^6$) (5, 14, 19, 28). In the present study, GTF activity eluted at the void volume of an 8% agarose column, suggesting a similarly high molecular weight. However, removal of glucan from the complex by gel filtration in the presence of 6 M GuHCl apparently reduced the molecular weight to 422,000 (Fig. 3). This information may lend some support to the notion that glucan is at least partly responsible for these large complexes (14, 19). The apparent molecular weight of the guanidine-eluted enzyme suggests a state of limited aggregation, possibly because of small amounts of glucan, an irreversible denaturation of the protein structure, or other effects which are responsible for the apparent 1 M NaCl-sensitive disaggregation of GTF obtained after growth of *S. mutans* in chemically defined media (22).

The nature of the components comprising the third peak (pool 3) appearing after gel filtration of the 8% agarose void volume pool is as yet unclear. However, several facts suggest that a component(s) within it may be related to the dextran-binding protein reported by McCabe and Smith (17). Presumably, this material remained bound to glucan during the initial purification step and was released from it after gel filtration in the presence of 6 M GuHCl. This component migrates nearly twice the distance of GTF in polyacrylamide gels, has no detectable transferase activity, elutes last in gel filtration, and is immunogenic in hamsters. Some of these characteristics are similar to those reported for dextran-binding protein. Experiments are now underway to characterize this pool 3 material more completely.

Thus, exposure of WIP, synthesized by *S. mutans* 6715 to 6 M GuHCl and coupled with gel filtration procedures, may be effective in providing enriched fractions of components which comprise the GTF enzyme complex (e.g., glucan, GTF, and, possibly, detran-binding protein). These components, which are recovered in relatively purified form, could then be useful in defining the events leading to *S. mutans* colonization of dental surfaces as well as the potential of these antigens to induce an immune response which will interfere with the pathogenic potential of cariogenic streptococci.

ACKNOWLEDGMENTS

This research was performed pursuant to Public Health Service contract DE-42438 and grant DE-04733 with the National Institute of Dental Research and was supported also by Public Health Service Research Career Development awards DE-70122 (to M.A.T.) and DE-00024 (to D.J.S.) from the National Institute of Dental Research.

We thank M. Dhond and W. King for expert technical assistance, C. Raymond for secretarial assistance, and T. Myoda (Dupont Research Institute) for generously supplying us with an antiserum directed to teichoic acid.

LITERATURE CITED

1. **Chludzinski, A. M., G. R. Germaine, and C. F. Schachtele.** 1974. Purification and properties of dextransucrase from *Streptococcus mutans*. J. Bacteriol. **118**:1-7.
2. **Ciardi, J. E., G. J. Hageage, Jr., and C. L. Wittenberger.** 1976. Multicomponent nature of the glucosyltransferase system of *Streptococcus mutans*. J. Dent. Res. **55**:C87-C96.
3. **Davis, B. J.** 1964. Disc electrophoresis. II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404-427.
4. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350-356.
5. **Evans, R. T., and R. J. Genco.** 1973. Inhibition of glucosyltransferase activity by antisera to known serotypes of *Streptococcus mutans*. Infect. Immun. **7**: 237-241.
6. **Fukui, K., Y. Fukui, and T. Moriyama.** 1974. Purification and properties of dextransucrase and invertase from *Streptococcus mutans* HS6. J. Bacteriol. **118**: 796-804.
7. **Germaine, G. R., A. M. Chludzinski, and C. F. Schachtele.** 1974. *Streptococcus mutans* dextransucrase: requirement for primer dextran. J. Bacteriol. **120**: 287-294.
8. **Germaine, G. R., S. K. Harlander, W-L. S. Leung, and C. F. Schachtele.** 1977. *Streptococcus mutans*

dextransucrase: functioning of primer dextran and endogenous dextranase in water-soluble and water-insoluble glucan synthesis. Infect. Immun. **16**:637–648.

9. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
10. **Guggenheim, B., and E. Newbrun.** 1969. Extracellular glucosyltransferase activity of an HS strain of *Streptococcus mutans*. Helv. Odontol. Acta **13**:84–97.
11. **Hayashi, J. A., I. L. Shklair, and A. N. Bahn.** 1972. Immunization with dextran-sucrases and glycosidic hydrolases. J. Dent. Res. **51**:436–442.
12. **Hojo, S., M. Higuchi, and S. Araya.** 1976. Glucan inhibition of diffusion in plaque. J. Dent. Res. **55**:169.
13. **Iacono, V. J., M. A. Taubman, D. J. Smith, and M. J. Levine.** 1975. Isolation and immunochemical characterization of the group-specific antigen of *Streptococcus mutans* 6715. Infect. Immun. **11**:117–128.
14. **Kuramitsu, H. K.** 1975. Characterization of extracellular glucosyltransferase activity of *Streptococcus mutans*. Infect. Immun. **13**:738–749.
15. **Linzer, R., and H. D. Slade.** 1976. Characterization of an anti-glucosyltransferase serum specific for insoluble glucan synthesis by *Streptococcus mutans*. Infect. Immun. **13**:494–500.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **McCabe, M. M., R. M. Hamelik, and E. E. Smith.** 1977. Purification of dextran binding protein from cariogenic *Streptococcus mutans*. Biochem. Biophys. Res. Commun. **78**:273–278.
18. **McCabe, M. M., and E. E. Smith.** 1977. Specific method for the purification of *Streptococcus mutans* dextransucrase. Infect. Immun. **16**:760–765.
19. **Mukasa, H., and H. D. Slade.** 1974. Mechanism of the adherence of *Streptococcus mutans* to smooth surfaces. III. Purification and properties of the enzyme complex responsible for adherence. Infect. Immun. **10**: 1135–1145.
20. **Scales, W. R., L. W. Long, and J. R. Edwards.** 1975. Purification and characterization of glucosyltransferase complex from the culture broth of *Streptococcus mutans* FA-1. Carbohydr. Res. **42**:325–338.
21. **Schachtele, C. F., G. R. Germaine, and S. K. Harlander.** 1975. Production of elevated levels of dextransucrase by a mutant of *Streptococcus mutans*. Infect. Immun. **12**:934–937.
22. **Schachtele, C. F., S. K. Harlander, and G. R. Germaine.** 1976. *Streptococcus mutans* dextransucrase: availability of disaggregated enzyme after growth in a chemically defined medium. Infect. Immun. **13**: 1522–1524.
23. **Smith, D. J., and M. A. Taubman.** 1977. Antigenic relatedness of glucosyltransferase from *Streptococcus mutans*. Infect. Immun. **15**:91–103.
24. **Smith, D. J., M. A. Taubman, and J. L. Ebersole.** 1978. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in hamsters caused by homologous and heterologous serotypes of *Streptococcus mutans*. Infect. Immun. **21**:843–851.
25. **Socransky, S. S., C. Smith, and A. Manganello.** 1973. Defined media for the cultivation of oral gram positive rods. J. Dent. Res. **52**:88.
26. **Somogyi, M.** 1945. A new reagent for the determination of sugars. J. Biol. Chem. **160**:61–73.
27. **Tanzer, J. M., M. L. Friedman, R. J. Fitzgerald, and R. H. Larson.** 1974. Diminished virulence of glucan synthesis-defective mutants of *Streptococcus mutans*. Infect. Immun. **10**:197–203.
28. **Taubman, M. A., and D. J. Smith.** 1977. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in rats and hamsters. J. Immunol. **118**:710–720.
29. **Taubman, M. A., D. J. Smith, and J. L. Ebersole.** 1978. Antibody binding of glucosyltransferase enzyme preparations from homologous and heterologous serotypes of *Streptococcus mutans*. Adv. Exp. Biol. Med. **107**:317–326.