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What is This?
The Activity of Glucosyltransferase Adsorbed Onto Saliva-coated Hydroxyapatite

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This study aimed to determine physical and kinetic properties of glucosyltransferase (GTF) adsorbed onto hydroxyapatite (HA) surfaces. For development of a solid-phase enzyme assay, 4.0-mg samples of washed HA powder were exposed to centrifuged whole saliva (WSHA) or buffer, and subsequently exposed to a GTF solution. The activities of GTF adsorbed to HA and that remaining in solution were measured. WSHA was more effective in adsorbing GTF than was naked HA. Enzyme activity on the surface of WSHA was enhanced; more activity was detected on WSHA than was apparently removed from solution. A similar effect was observed when GTF was adsorbed to naked HA from a mixture with lysozyme or saliva; however, no enhancement was seen when GTF was adsorbed from a mixture with albumin. Compared with GTF in solution, adsorbed GTF displayed activity over a much wider range of pH values. Temperature-activity profiles indicated that GTF adsorbed to surfaces had a lower temperature optimum (40°C) than did soluble enzyme (45°C), and that the bound enzyme was more resistant to adverse effects of heat at elevated temperatures. The majority of glucan made by GTF adsorbed to parotid saliva-coated HA remained attached to the surface. The activity of lysozyme adsorbed to HA was reduced by adsorption of GTF to the same surface and was almost completely abolished by formation of glucans by the adsorbed GTF. These results suggest that soluble bacterial enzymes found in saliva can be incorporated into pellicle, interact with host-derived molecules on the surfaces of teeth, express enzymatic activity, and potentially influence the biological properties of pellicle.


Introduction.

Results from several recent studies have shown that soluble, cell-free, bacterial products, including glucosyltransferase (GTF) and fructosyltransferase (FTF), are present in human whole saliva and are incorporated into acquired enamel pellicle at the earliest stages of its formation (McCabe and Donkersloot, 1977; Rölla et al., 1983a, 1983b; Scheie and Rölla, 1984, 1986). Furthermore, GTF and FTF in saliva and pellicle are enzymatically active, as shown by their ability to synthesize glucans and fructans from sucrose. The GTF-catalyzed synthesis of water-insoluble glucans from dietary sucrose is known to contribute to the pathogenic potential of Streptococcus mutans and Streptococcus sobrinus (De Stoppelaar et al., 1971; Hamada and Slade, 1980; Larrimore et al., 1983; Tanzer et al., 1985). Although glucan production is not required for initial attachment of S. mutans to surfaces, evidence from several studies suggests that sucrose-dependent adherence and accumulation of cariogenic streptococci are critical processes in the development of pathogenic dental plaque, especially on smooth surfaces (Gibbons, 1984; Hamada and Slade, 1980; Rölla et al., 1983b, 1983c). It has been postulated that adherent interactions between insoluble glucans formed on tooth surfaces and those formed on bacterial surfaces play a critical role in sucrose-enhanced bacterial colonization (Rölla et al., 1983b, 1983c). The formation and retention of glucans in pellicle, prior to or concomitant with bacterial colonization, could have a profound influence on the biology of pellicle and the development of dental plaque. Although GTF adsorbed to saliva-coated surfaces represents a form of the enzyme likely to be germane to what occurs in vivo, a detailed examination of the biological and enzymatic properties of immobilized GTF has not been reported. Evidence from several studies has shown that the kinetic properties of an enzyme immobilized on a solid surface can differ significantly from those observed for the same enzyme in solution (Hofstee, 1973; Laidler and Bunting, 1980; Woodward, 1985). It was noted in a study by Rölla et al. (1983b) that, following exposure of hydroxyapatite to cell-free, human whole saliva containing detectable levels of GTF, more glucan-forming activity was detected adsorbed onto the HA than was removed from saliva. This observation is in contrast to a loss of enzyme activity reported by Parniak et al. (1983) following adsorption of a GTF preparation from Streptococcus sanguis onto naked hydroxyapatite. Results from these studies suggest that interactions between GTF and salivary components on saliva-coated surfaces are worthy of further exploration.

Acquired enamel pellicle is thought to influence plaque development and carries progression through a number of mechanisms. Pellicle provides molecules which function as binding sites for the selective adherence of micro-organisms (Levine et al., 1985). Furthermore, several host-derived salivary enzymes which may influence the success of colonizing bacteria, including lysozyme, salivary peroxidase, and amylase, have been demonstrated to be active in experimental pellicles (Germine and Tellefson, 1986; Pruitt and Adamson, 1977). Finally, studies by Zähringer et al. (1976) and others indicate that pellicle forms a permselective barrier which influences enamel demineralization-remineralization processes. The presence of GTF and product glucans on tooth surfaces could modify these important biological properties of pellicle.

The present study aimed to compare the properties of GTF adsorbed onto hydroxyapatite surfaces with those of the enzyme in solution. The glucan-forming activity was measured with GTF in solution or adsorbed to hydroxyapatite treated with saliva, lysozyme, albumin, or buffer. The optimum pH and temperature for activity of GTF in solution were compared with those observed for the adsorbed enzyme. In addition, the adhesion of glucans synthesized by GTF adsorbed onto hydroxyapatite was determined. Finally, lysozyme adsorbed to hydroxyapatite was used as a model system to examine the effects of GTF and product glucans on antibacterial enzymes bound to surfaces.

Materials and methods.

Glucosyltransferase preparation.—Streptococcus sobrinus 6715-15 cells were grown in a medium containing 2.5% tryp-
tone, 1.5% yeast extract, 0.3% glucose, and 1.0% sorbitol, which was ultrafiltered through a 5.0-kd molecular weight cut-off (MWCO) membrane (YMS, Amicon, Danvers, MA). Bacteria from a starter culture were inoculated into dialysis tubing (MWCO = 12.0 kd) containing 150.0 mL of medium, placed in 2 liters of the same medium. Cells were grown in dialysis tubing to concentrate products secreted by the bacteria. Following overnight growth at 37°C with stirring, the cells were removed by centrifugation at 12,000 g, and the protease inhibitor, phenylmethylsulfonyl fluoride (PMSF) (1.0 mmol/L), and the preservative, NaN3 (0.02%), were added to the culture supernatant fluid.

Culture supernatant fluid (CSF) in this form, or CSF which had been dialyzed (MWCO = 12.0 kd) extensively against a buffer containing 1.0 mmol/L imidazole HCl, 50.0 mmol/L KCl, and 0.02% NaN3 (pH = 6.5) were used as sources of glucosyltransferase (GTF) in these investigations.

Saliva.—Whole saliva (WS) from one donor with low levels of S. mutans (data not shown) and negligible salivary GTF was collected over ice and clarified by centrifugation (17,000 g, 20 min). Parotid saliva (PS) was collected from the same donor by means of a modified Lashley cup (Lashley, 1916). Chewing of paraffin wax film (Parafilm "M", American Can Co., Greenwich, CT) was used to stimulate whole saliva production, and secretion of parotid saliva was stimulated by the painting of the tongue with citric acid (2.0%). Sodium azide (0.02%) was added to both secretions as a preservative.

Preparation of GTF adsorbed onto hydroxyapatite.—Four mg samples of hydroxyapatite powder (approximate surface area = 50 m2/g) (Biogel HPT, BioRad Laboratories, Richmond, CA) were placed in 1.5-mL polypropylene microfuge tubes and washed with a solution containing 50.0 mmol/L KCl, 1.0 mmol/L CaCl2, 1.0 mmol/L potassium phosphate (0.35 mmol/L K2HPO4 plus 0.65 mmol/L KH2PO4), and 0.1 mmol/L MgCl2 (pH = 6.5) (buffered KCl). Following low-speed centrifugation (18 g) and decanting, the hydroxyapatite (HA) was exposed to 1.0 mL buffered KCl, whole saliva, parotid saliva, 2.0 mg/mL bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO), or 2.0 mg/mL hen egg white lysozyme (HEWL) (Sigma) and incubated with gentle rocking (Speci-mix, Thermolyne Corp., Dubuque, IA) for 20 minutes at 37°C. The treated HA samples were then washed three times with buffered KCl and exposed to the GTF preparation (diluted 1:1 with buffered KCl) for 10 min at 37°C with gentle rocking. In experiments exploring the ability of GTF to adsorb onto HA from solutions containing other proteins, the GTF preparation was diluted with equal amounts of saliva, 2.0 mg/mL albumin, 2.0 mg/mL lysozyme, or buffered KCl and exposed to washed HA samples. Supernatants from the HA - GTF incubations were retained and assayed for remaining GTF activity by means of the liquid-phase radioactive glucan assay described below.

Enzyme assays.—Liquid-phase GTF assays were performed by a method similar to that described by Robrish et al. (1972) and Germaine et al. (1974). Solutions containing GTF diluted (1:1) with buffered KCl were incubated in a substrate mixture containing 100.0 mmol/L [U-glucosyl-14C]-sucrose (New England Nuclear, Boston, MA) (0.014 μCi/reaction), 20.0 μmol/L dextran (9.0 kd) (Sigma), and 0.02% NaN3 in buffered KCl (pH = 6.5). The 0.6-mL reaction mixtures were incubated at 37°C for the times indicated, and stopped by the addition of 0.9 mL of ice-cold absolute methanol (MeOH) or by being placed in an ice bath. Radioactive glucans were precipitated in 5.0 mL MeOH over 25-mm glass fiber filters (Whatman Ltd., Maidstone, England) in a multi-sample vacuum manifold (Millipore Corp., Bedford, MA). Following three washes with 5.0 mL of MeOH, filters were dried and measured for radioactive activity in a scintillation counter (LS-1801, Beckman Instruments, Wakefield, MA).

For assay of the GTF activity adsorbed to HA, the solid-phase samples were exposed to 0.6 mL of the substrate mixture described for liquid-phase assays. After incubation at 37°C with gentle rocking for the indicated time, reactions were stopped by the addition of 0.9 mL of cold MeOH. For quantitation of total radioactive MeOH-insoluble glucan, complete reaction mixtures (including HA) were precipitated over glass fiber filters, washed, and counted for radioactivity as described above.

Effects of temperature and pH on enzyme activity.—For determination of the effects of temperature on the activity of GTF, soluble and adsorbed forms of the enzyme were prepared as described above. All samples were incubated with the radiolabeled substrate mixture in water baths at controlled temperatures ranging from 10° - 90°C (5° increments). The adsorbed and soluble GTF samples were incubated simultaneously, and the temperatures were carefully controlled. After two hours, the reactions were stopped by the addition of ice-cold MeOH, and total radioactive MeOH-insoluble glucans were quantitated as described above.

For investigation of the effects of pH on GTF activity, culture supernatant fluid, dialyzed as described above to remove most of its buffering capacity, was used as the source of enzyme. The GTF samples, either adsorbed onto HA or diluted (1:1) with buffer of the appropriate pH, were incubated with radioactive substrate mixtures containing 100.0 mmol/L [U-glucosyl-14C]-sucrose, 20.0 μmol/L dextran, 0.02% NaN3, and 25.0 mmol/L buffer of the appropriate pH, with either sodium acetate (pH 3.5 - 5.5) or sodium phosphate (pH 5.5 - 8.0). All samples were incubated at 37°C with gentle rocking for the time indicated and were assayed for total radioactive MeOH-insoluble glucans as described above. The pH values of all samples were checked at the beginning and the end of the incubation period to ensure that changes in value had not occurred.

Adherent glucans.—Following incubation with radioactive substrate, solid-phase enzyme reactions were stopped by placement in an ice bath and mixed by gentle inversion, and the supernatants decanted as the HA-glucan complex settled. The HA was washed twice with distilled H2O, and the washes and supernatants were combined and assayed for radioactive glucans as described above. The radioactive glucan associated with the solid phase was assayed separately as described above.

Effects of adsorbed GTF on activity of adsorbed lysozyme.—Samples of 20.0 mg of HA beads (approximate surface area = 0.63 cm2/mg) (BDH Chemicals Ltd., Poole, England) were exposed to 1.0 mL of 0.1 mg/mL hen egg white lysozyme (HEWL) (Sigma) for 60 min. The HEWL-coated HA beads (HEWL-HA) were washed with buffered KCl and exposed for 30 min to a culture supernatant preparation containing GTF from Streptococcus mutans GS-5 grown in complex medium (described above). Control samples of HEWL-HA were exposed to 1.0 mL of 1.0 mg/mL BSA or buffer for 30 min. Following a second wash, the samples of HEWL-HA with associated GTF were exposed to buffered KCl or to a substrate solution containing 100.0 mmol/L sucrose and 20.0 μmol/L dextran, for 120 min at 37°C. Control samples were exposed to buffer or sucrose for the same duration. After a final wash in buffered KCl, all samples were exposed to a suspension of Micrococcus lysodeikticus cells (Sigma) (absorption at 540 nm = 0.5) and rocked gently at 37°C. The lytic activity of adsorbed lysozyme was determined by measurement of changes in absorbance at 540 nm (A540) in a spectrophotometer (DU-8B, Beckman Instruments, Wakefield, MA). Activity was expressed as the decrease in A540 over time (Table 4). Exposure
of micrococal cells to naked HA, GTF-coated HA, and BSA-coated HA did not result in a significant decrease in $A_{440}$.

**Results.**

*Activity of GTF adsorbed onto HA surfaces.*—Following exposure of soluble GTF to buffer-treated HA, the enzyme activity detected on the HA corresponded to approximately one-half of the GTF removed from solution (Table 1). In contrast, when the soluble GTF was exposed to WSHA, over twice as much enzyme was removed from solution by WSHA than was removed by naked HA. Furthermore, comparison of the level of GTF activity detected on WSHA with the amount removed from solution indicated significant enhancement of GTF activity by WSHA (Table 1). The saliva used in these studies was selected for its low GTF content, and as shown in Table 1, saliva-coated HA alone expressed negligible activity.

Glucosyltransferase could apparently be selectively adsorbed onto HA in the presence of other proteins which bind to the mineral. For instance, when GTF was adsorbed onto naked HA in the presence of saliva, rather than onto pre-formed WSHA, significant amounts of enzyme still adsorbed in an active form (Table 1). Again, more activity was detected adsorbed onto the HA than was removed from solution, indicating enhancement by salivary components adsorbed onto HA.

Proteins of different ionic nature influenced the ability of GTF to adsorb to HA. For instance, when GTF was adsorbed onto HA from a mixture of the enzyme and lysozyme (HEWL), more GTF adsorbed to the mineral than was adsorbed from a mixture of GTF and buffer, and the bound enzyme expressed the predicted glucan-synthesizing activity (Table 2). However, when adsorbed from a mixture with albumin (BSA), significantly less GTF adsorbed onto HA than was observed for GTF mixed with buffer, and the bound enzyme displayed diminished activity (Table 2).

*Effects of pH and temperature on adsorbed GTF.*—The influence of pH on the activity of adsorbed GTF differed greatly from that observed for the enzyme in solution (Fig. 1). As shown in Fig. 1a, the activity of GTF in solution exhibited a marked dependence on pH, with very low activity at acidic pH values and a maximum activity at approximately pH 6.0. However, the activity of GTF adsorbed onto HA coated with parotid saliva (PSHA) was much less influenced by the pH of the reaction mixture (Fig. 1a). In fact, GTF adsorbed onto PSHA exhibited over 85% maximal activity across the pH range 4.7 to 7.5. GTF adsorbed onto HA coated with bovine serum albumin (AHA) or uncoated HA also exhibited a broad pH optimum, with high activity expressed at acidic pH values (Fig. 1b). However, the activity of GTF bound to AHA was more affected by changes in pH than was seen with GTF adsorbed to HA or PSHA.

Differences between the properties of GTF in solution and those of the enzyme adsorbed to HA surfaces were also detected when the effects of temperature on enzymatic activity were compared. When assayed for the quantity of glucan synthesized as a function of temperature, GTF in solution exhibited greatly reduced activity at extremes of temperature, and a sharp peak of maximum activity at 45°C (Fig. 2a). In contrast, when GTF was adsorbed onto HA treated with parotid saliva, bovine serum albumin, or buffer, all three forms of adsorbed enzyme exhibited a lower optimum temperature (40°C) (Fig. 2b). Furthermore, GTF adsorbed onto these surfaces was less adversely affected by heat, expressing two to three times as much activity at elevated temperatures (50° and 55°C) as that observed for enzyme in solution.

*Retention of glucans produced by adsorbed GTF.*—A large proportion of radioactive glucans, synthesized by GTF adsorbed to HA surfaces, remained bound to the mineral surface. For instance, approximately 50% of the glucans formed by GTF adsorbed to naked HA remained attached to the mineral surface (Table 3). Following synthesis by GTF adsorbed onto WSHA, approximately 40% of the glucan synthesized was detected adsorbed to the WSHA. When parotid saliva was used to treat the HA, over 60% of the glucans made by the adsorbed GTF remained attached to the solid phase.

*Effect of adsorbed GTF on the activity of adsorbed lysozyme.*—Lysozyme adsorbed onto HA (HEWL-HA) retained the ability to lyse *Micrococcus lysodeikticus* cell walls, measured as decreasing optical density at 540 nm (Table 4). When GTF was adsorbed onto the HEWL-HA before exposure to *M. lysodeikticus* cells, a decrease in the lytic activity of the adsorbed lysozyme was observed (Table 4). Furthermore, when the samples of HEWL-HA with adsorbed GTF were incubated with sucrose for 120 min to allow for glucan formation, cell lysis by the bound lysozyme was reduced to less than 20% of that seen with buffer-treated HEWL-HA. In contrast, when HEWL-HA samples were treated with 1.0 mg/mL BSA, the lytic activity of the adsorbed lysozyme was observed to increase (Table 4). Incubation of *M. lysodeikticus* cells with HA, GTF-coated HA, or BSA-coated HA did not result in significant decreases in optical density (data not shown).

**Discussion.**

The formation of adherent glucans by cell-associated glucosyltransferase (GTF) of cariogenic streptococci has been implicated in the pathogenesis of dental caries by several investigators (De Stoppelaar et al., 1971; Hamada and Slade, 1980; Larrimore et al., 1983; Tanzer et al., 1985). However, recent studies demonstrating active, cell-free GTF in saliva and in the initial pellicle which forms on teeth have added a new dimension to the role of the enzyme in caries development.

**TABLE 1**

<table>
<thead>
<tr>
<th>HA Sample*</th>
<th>GTF Added</th>
<th>GTF in Supernatant</th>
<th>GTF adsorbed onto HA‡</th>
<th>GTF detected on HA§</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTF + Buffer + HA</td>
<td>39.85</td>
<td>36.29</td>
<td>3.56</td>
<td>1.76</td>
</tr>
<tr>
<td>GTF + Buffer + WSHA</td>
<td>39.85</td>
<td>31.36</td>
<td>8.49</td>
<td>13.11</td>
</tr>
<tr>
<td>GTF + WS + HA</td>
<td>39.85</td>
<td>35.29</td>
<td>4.56</td>
<td>8.91</td>
</tr>
<tr>
<td>WS + HA</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* Solutions of GTF, mixed (1:1) with bufferd KCl or with centrifuged whole saliva, were exposed to naked HA or whole saliva-coated HA (WSHA) for 10 min at 37°C. Samples were centrifuged (18 g) and the supernatants saved and assayed for activity.

- The activity of liquid- and solid-phase GTF was determined by incubation in a reaction mixture containing 100.0 mmol/L U-glucosyl-14C-sucrose, 20.0 μmol/L dextran (9000 mω) in bufferd KCl (pH = 6.5) at 37°C. Activity is expressed in arbitrary units equal to μmol of radioactive glucose incorporated into MeOH-insoluble polymer per 4 hr. For all solid-phase, n = 4; for liquid, n = 2.

‡ Calculated by subtracting the amount of GTF activity remaining in the supernatant, following exposure to HA or WSHA, from the GTF added.

§ Represents the enzymatic activity, determined experimentally, adsorbed onto HA or WSHA.

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Fig. 1 — Effects of pH on activity of GTF in solution and adsorbed onto HA surfaces. Samples were incubated for two hr at 37°C with radioactive substrate, buffered with either 25.0 mmol/L sodium acetate (pH = 3.5-5.5) or 25.0 mmol/L sodium phosphate (pH = 5.5-8.0). pH values represent final pH of reaction mixtures. Fig. 1a (top): Percent maximum enzyme activity (as a function of pH) for GTF in solution or adsorbed onto HA pre-treated with parotid saliva (PASHA). Fig. 1b: Percent maximum activity for GTF adsorbed onto HA pre-treated with 2.0% (w/v) bovine serum albumin (AHA) or buffer (HA).

(Rölla et al., 1983a, 1983b; Scheie and Rölla, 1986). The formation of polysaccharides by GTF in pellicle may represent a crucial step in sucrose-enhanced plaque development. In addition, the presence of GTF and its product glucans could have profound effects on the biological properties of pellicle.

Results from the present study suggest that interactions with host-derived salivary components may enhance the pathogenic effects of GTF in pellicle by several mechanisms. First, salivary components appear to promote the uptake of GTF secreted by Streptococcus sobrinus 6715 onto hydroxyapatite (HA) (Table 1). Such interactions may improve the ability of GTF to bind to saliva-coated tooth surfaces in vivo. Furthermore, interactions between GTF and salivary constituents bound to HA surfaces enhance the glucan-synthesizing activity of the insolubilized enzyme (Table 1). The mechanisms underlying the observed enhancement of GTF activity remain to be explored.

**TABLE 2**

GTF ADSORPTION FROM SOLUTIONS OF PROTEINS

<table>
<thead>
<tr>
<th>HA Sample*</th>
<th>GTF Added†</th>
<th>GTF in Supernatant</th>
<th>GTF adsorbed onto HA</th>
<th>GTF detected on HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTF + Buffer + HA</td>
<td>52.83</td>
<td>42.40</td>
<td>10.43</td>
<td>3.71</td>
</tr>
<tr>
<td>GTF + HEWL + HA</td>
<td>52.83</td>
<td>35.84</td>
<td>16.99</td>
<td>17.64</td>
</tr>
<tr>
<td>GTF + BSA + HA</td>
<td>52.83</td>
<td>48.99</td>
<td>3.84</td>
<td>2.25</td>
</tr>
</tbody>
</table>

* Solutions of GTF were mixed (1:1) with buffered KCl, 2.0 mg/mL hen egg white lysozyme (HEWL), or 2.0 mg/mL bovine serum albumin (BSA) and exposed to HA for 10 min at 37°C.
† GTF activities in liquid and solid phases determined as described in Table 1, except that units represent μmol of radioactive glucose incorporated into glucan per 6 hr. All samples were run in duplicate.
Table 3

<table>
<thead>
<tr>
<th>HA Sample</th>
<th>% Glucan in Supernatant†</th>
<th>% Glucan Retained on HA‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTF + HA</td>
<td>52.6</td>
<td>47.4</td>
</tr>
<tr>
<td>GTF + WSHA</td>
<td>60.9</td>
<td>39.1</td>
</tr>
<tr>
<td>GTF + PSHA</td>
<td>32.5</td>
<td>67.5</td>
</tr>
</tbody>
</table>

* Solution of GTF was exposed to HA pre-treated with buffered KCl, centrifuged whole saliva, or parotid saliva for 10 min at 37°C.
† Following incubation of solid-phase GTF with the radioactive substrate mixture described in Table 1, supernatant and two H2O washes were combined and assayed for radioactive MeOH-insoluble glucans.
‡ Radioactive polymer attached to the solid phase was counted separately. All samples were run in duplicate.

Table 4

<table>
<thead>
<tr>
<th>HA Sample Preparation*</th>
<th>Exposure 1</th>
<th>Exposure 2</th>
<th>Lysozyme Activity†</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEWL</td>
<td>Buffer</td>
<td>Buffer</td>
<td>8.30</td>
<td>100.00</td>
</tr>
<tr>
<td>HEWL</td>
<td>Buffer</td>
<td>GTF</td>
<td>4.25</td>
<td>51.20</td>
</tr>
<tr>
<td>HEWL</td>
<td>GTF</td>
<td>Sucrose</td>
<td>1.30</td>
<td>15.66</td>
</tr>
<tr>
<td>HEWL</td>
<td>GTF</td>
<td>Buffer</td>
<td>11.75</td>
<td>141.57</td>
</tr>
</tbody>
</table>

* 20 mg of HA beads were exposed to 0.1 mg/mL hen egg white lysozyme (HEWL) for 60 min (Exposure 1), washed, and exposed to either buffered KCl, GTF, or 1.0 mg/mL bovine serum albumin (BSA) for 30 min (Exposure 2). All samples were washed and incubated with either buffered KCl or a solution containing 100.0 mmol/L sucrose and 20.0 mmol/L dextran (9000 mw) for 120 min at 37°C (Exposure 3).
† Adsorbed lysozyme activity was determined by incubation of HEWL-coated HA samples with a suspension of Micrococcus lysodeikticus cells (A570 = 0.5). Following incubation for 30 min at 37°C, the OD540 was determined and the activity expressed in arbitrary units equal to -0.02 A540 per 30 min. All samples were run in duplicate. Exposure of micrococcal cells to HA treated only with buffer, GTF, GTF + sucrose, or BSA did not cause significant changes in A540.

but could involve aggregation of the enzyme by salivary components adsorbed to the HA. Indeed, several studies have shown that the activity of GTF secreted by cariogenic streptococci can be enhanced by the addition of neutral salts (i.e., ammonium sulfate, polyethylene glycol) which are presumed to aggregate the enzyme in solution (Asm et al., 1986; Kuramitsu and Wondrack, 1983; Newman et al., 1980; Mukasa et al., 1979). In these studies, it was also demonstrated that the glucans made by GTF under aggregating conditions were more water-insoluble than the products made by the same enzyme in non-aggregating conditions. Therefore, any in vivo interactions which aggregate GTF could be biologically relevant because insoluble glucans are generally considered to be more important than are soluble glucans in the etiology of dental caries (Hamada and Slade, 1980). Future studies to determine the structure and solubility of glucans made by GTF adsorbed to saliva-coated HA surfaces should improve our understanding of the biological activity of pelvic-associated GTF.

The nature of the interactions between GTF and adsorbed salivary components needs further elucidation. That ionic interactions may occur is suggested by the observed effects on the binding and activity of GTF exhibited by the anionic protein, albumin, and lysozyme, which is cationic (Table 2). However, results from studies by Rölla et al. (1984) showed that GTF (secreted by S. sobrinus) could bind to anionic, cationic, hydrophobic, and dextran chromatographic media in an enzymatically active form, able to synthesize glucans from sucrose. Clearly, a variety of interactions could mediate adsorption of GTF onto saliva-coated tooth surfaces in a form which expresses enhanced enzymatic activity.

The specific components of salivary which interact with GTF on surfaces have not yet been identified, although one of them could be lysozyme. Many salivary macromolecules found in pellicle and plaque have been shown to enhance the activity of GTF in solution. These include host-derived components such as secretory IgA (non-GTF-specific), phosphoproteins, blood group-reactive substance, and phospholipids, as well as products of bacterial origin, such as ribonucleic acid, putrescine, and cadaverine (Ciardi et al., 1978; Fukui et al., 1974; Rölla et al., 1983c; Schachtele et al., 1978). However, the influence of these substances on the activity of GTF adsorbed to saliva-coated surfaces has not been explored. Moreover, it is quite possible that salivary molecules which do not interact with GTF in solution are capable of doing so when adsorbed to apatite surfaces. For instance, in recent studies by Gibbons (1986), salivary proline-rich proteins, which failed to interact with Actinomyces viscosus cells in solution, were able to act as binding sites for A. viscosus when adsorbed onto HA surfaces. Studies utilizing purified salivary components adsorbed onto HA should facilitate the identification of those molecules which interact with GTF on surfaces.

Glucosyltransferase in pellicle and plaque is probably exposed to continually changing local environmental conditions, especially changes in pH. The pH in plaque on teeth rises and falls as a result of bacterial metabolism, and GTF on these surfaces may be exposed to pH values below 4.0 or higher than neutrality. The ability of GTF, adsorbed to saliva-coated HA, to synthesize glucans over a wide range of pH values (Fig. 1) suggests that GTF bound to saliva-coated surfaces can continue to contribute to the bulk of plaque, even during acidogenic metabolism of sucrose by plaque bacteria. Extracellular glucans made during an acidogenic sucrose challenge may serve as reserve polysaccharides, utilized by plaque bacteria when dietary carbohydrates are unavailable. In addition, results from several studies suggest that glucans may inhibit diffusion of metabolic acids and other ions from plaque, thus increasing the time of exposure of tooth surfaces to acidic conditions (Hojo et al., 1976; Melson et al., 1979), although studies by Dibdin et al. (1983) failed to show this effect.

The physical-chemical basis for the observed activity of surface-bound GTF over such a broad range of pH values is not known, but may involve stabilization of an active conformation of the enzyme. Changes in the physical properties of GTF following adsorption to HA surfaces are further suggested by differences in temperature-activity profiles for adsorbed and soluble forms of the enzyme (Fig. 2). The lower optimum
temperature for enzymatic activity and the increased activity at extremely elevated temperatures observed for GTF adsorbed to HA surfaces are consistent with results obtained by others, which showed that both the temperature dependence of catalytic activity (Laidler and Bunting, 1980) and resistance to heat denaturation (Shepard et al., 1983) of an enzyme can change following adsorption to surfaces. It will be of interest to test the susceptibility of surface-bound GTF to degradation by proteases and inhibition by GTF-specific antibodies, in order for us to understand better the contribution of saliva-coated surfaces to the activity, stability, and conformation of GTF in pellicle and plaque.

The presence of biologically active GTF in pellicle on teeth may influence oral microbial ecology by several mechanisms. Carioenic streptococci produce several glucan-binding proteins, including GTF, fructosyltransferase (FTF), and non-enzymatic lectin-like proteins (McCabe and Hamelik, 1978; Russell, 1979; Russell et al., 1983). Results from the present study indicate that a significant amount of glucans synthesized by GTF adsorbed to saliva-coated surfaces remain attached to the mineral surface (Table 3). Glucans synthesized and retained in pellicle on tooth surfaces could augment colonization by bacteria expressing glucan-binding proteins on their surfaces. Binding of S. mutans to surface-associated glucans has been demonstrated in studies by Gibbons and Fitzgerald (1969), utilizing teeth coated with dextrans produced by a Leuconostoc species. Furthermore, Kuramitsu (1974) showed that live or heat-killed S. mutans cells adhered to glass surfaces which were coated with glucans produced by extracellular GTF, and suggested that similar interactions may play a role on tooth surfaces in vivo.

Another unexplored but potentially significant biological effect of GTF incorporated into pellicle and plaque resides in its influence on salivary anti-bacterial enzymes, such as lysozyme and salivary peroxidase, considered to be active on oral surfaces (Germaine and Telfefson, 1986; Pruitt and Adamson, 1977). Results from the present study suggest that GTF adsorbed onto HA can interfere with the enzymatic activity of lysozyme bound to the same surface (Table 4). Inhibition of the lytic action of lysozyme was especially dramatic when the adsorbed GTF was allowed to synthesize glucans. It is tempting to speculate that GTF and product glucans on tooth surfaces may modify, in vivo, the biological activity of salivary components in pellicle which influence the success of colonizing oral micro-organisms, including molecules which serve as bacterial binding sites. By interfering with the function of host molecules in pellicle, GTF may act as a virulence factor in a context not previously considered.

In conclusion, this study presents evidence which supports the concept that the biological properties of pellicle are influenced by secreted bacterial products which are delivered to tooth surfaces in saliva and incorporated into pellicle as biologically active molecules, even perhaps in advance of bacterial colonization. Oral surfaces are colonized by large numbers of bacteria which liberate a diverse array of products into saliva. As noted by Burne et al. (1986), many of the proteins and enzymes which have been implicated as virulence factors of S. mutans and S. sobrinus are secreted exo-proteins. These include GTF, FTF, levanase, dextranase, and cell surface proteins (Burne et al., 1987; Carlsson, 1970; Guggenheim and Burckhardt, 1974; McBride et al., 1985). Furthermore, export of other unidentified proteins (McBrice et al., 1985) and lipoteichoic acids (Markham et al., 1975) by oral streptococci has also been described. Clearly, incorporation of these and other bacterial products could have profound effects on the biological activity of salivary films which form on tooth, mucosal, and even bacterial surfaces.

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