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Molecular Basis for the Different Sucrose-Dependent Adherence Properties of *Streptococcus mutans* and *Streptococcus sanguis*

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The enzymatic and adherence properties of *Streptococcus mutans* GS5 and *S. sanguis* ST3, both isolated from human carious lesions, have been compared. During growth in sucrose media, *S. mutans* GS5 adheres to smooth surfaces approximately three times more effectively than does *S. sanguis* ST3. However, strain ST3 does not display sucrose-dependent adherence under nongrowth conditions, whereas strain GS5 displays significant adherence. Although both organisms synthesize both water-soluble and -insoluble glucans, the glucosyltransferases from *S. mutans* GS5 synthesize much more adherent glucan molecules than do the comparable enzymes from *S. sanguis* ST3. Both cell types bind exogenous glucosyltransferases synthesized by strain ST3 equally well, whereas cells of strain GS5 bind the comparable enzyme fraction that it synthesizes to a greater degree than do cells of *S. sanguis* ST3. However, in contrast to the results with cells of *S. mutans* GS5, the absorption of the glucosyltransferase activity synthesized by *S. mutans* GS5 to the surface of *S. sanguis* ST3 results in low levels of subsequent sucrose-dependent adherence. These results are discussed in terms of the molecular basis for the sucrose-dependent adherence of the oral streptococci to smooth surfaces.

A variety of experimental approaches have substantiated the important role of *Streptococcus mutans* in the development of dental caries in humans and experimental animals (10). Nevertheless, it is possible that a variety of oral microorganisms found in dental plaque may be involved in the actual demineralization of tooth enamel. One of the predominant species of streptococci isolated from dental plaque has been shown to be *S. sanguis* (1). Members of this group are quite similar to *S. mutans* in a number of properties, including the elaboration of glucosyltransferase (GTF) (EC 2.4.1.5) enzymes capable of synthesizing both water-soluble and -insoluble glucan polymers from dietary sucrose (2). The ability of *S. mutans* to colonize smooth surfaces and initiate plaque formation has been shown to be primarily dependent on the synthesis of adherent insoluble glucan molecules (9). Although various strains of *S. sanguis* can also synthesize significant quantities of insoluble glucan molecules, these organisms do not induce smooth-surface caries formation when inoculated into experimental animals, as occurs with most strains of *S. mutans* tested (10). Chemical analysis of the glucan products synthesized by these two species of oral streptococci suggests that this difference may be related to the formation of different types of insoluble glucan molecules by the two microorganisms (3, 11). Furthermore, recent results from this laboratory (14) have suggested that the synthesis of adherent insoluble glucans by *S. mutans* is not in itself sufficient for sucrose-dependent adherence to smooth surfaces in vitro. In this regard, the sucrose-polymerizing activities and adherence properties of *S. sanguis* ST3, isolated from a human carious lesion, have been investigated and compared with those of cariogenic *S. mutans* GS5 (serovar *c*). These results are discussed in terms of the molecular basis for the adherence of oral streptococci to smooth surfaces.

MATERIALS AND METHODS

Organisms. Cariogenic *S. mutans* GS5 (7) was originally obtained from R. J. Gibbons (Forsyth Dental Center, Boston, Mass.) in 1971 and has been maintained as previously described (16). *S. sanguis* ST3, provided by S. Hamada (Osaka University Dental School, Osaka, Japan), was isolated from a carious lesion of a Japanese child in 1975 and was transferred biweekly into Todd-Hewitt (Difco) broth (THB). The classification of strain ST3 as a member of the *S. sanguis* group was made on the basis of its

colonial morphology and fermentation patterns (S. Hamada, personal communication). In addition, unlike all strains of *S. mutans* examined in this laboratory, antibodies directed against the GTF activities of *S. mutans* GS5 (21) do not inhibit the corresponding enzyme activities of *S. sanguis* ST3.

Preparation of cells and enzyme fractions. Cells were grown in THB supplemented with the appropriate carbon source for the indicated time periods at 37°C. After harvesting, the cells were washed three times with 0.9% NaCl and resuspended to 1/10 the original volume in 0.9% NaCl–0.02% NaN_3 (SCA). These cell suspensions were utilized as described below for the determination of enzyme activities and adherence properties. The extracellular enzyme preparations used in these experiments were either dialyzed supernatant fluids from the growth cultures or crude enzyme preparations (CEP) used as previously described (19).

^{3}H-labeled *S. sanguis* ST3 suspensions were prepared by inoculating 150 ml of 0.5% Casamino Acids (Difco)–salts (6)–1% glucose medium containing 1.5 ml of [^{3}H]thymidine (1.0 mCi/ml) with a washed suspension of *S. sanguis* ST3. After growth in a GasPak (BBL) anaerobic system for 18 h at 37°C, the labeled cells were collected on membrane filters (0.45 μm, Millipore Corp.), washed with SCA, and resuspended in 10 ml of SCA.

Enzyme assays. Soluble and insoluble glucan syntheses by the GTF preparations were measured as previously described (19). Fructosyltransferase (EC 2.4.1.10) (FTF) activity was assayed as described earlier (20). Adherent glucan synthesis was determined by incubating the standard GTF reaction mixture (substituting SCA for water) in 22-mm glass scintillation vials for 18 h at 37°C. The supernatant fluids were then carefully aspirated from the vials, and the adherent glucan layers on the bottom of the vials were gently washed three times with 2.0 ml of 0.9% NaCl. After drying, the vials were counted as previously described (18).

Adherence. The adherence of nongrowing cell suspensions to glass surfaces was determined as described previously (18). The adherence measured represents the net result of both the attachment of the cells to smooth surfaces and the attachment of the cells to each other. Adherence during growth was measured by inoculating 5-ml cultures of THB–1% glucose or sucrose with the appropriate organism (0.1-ml inoculum). The cultures were then incubated at a 30° angle for 18 h at 37°C. The culture fluids containing the nonadherent cells were carefully decanted and diluted in 0.5 N NaOH, and the absorbance was determined at 540 nm. The adherent cells were gently washed three times with 0.9% NaCl and suspended in 5.0 ml of 0.5 N NaOH, and the turbidities were measured at 540 nm.

The adherence of ^{3}H-labeled *S. sanguis* was determined by mixing ^{3}H-labeled *S. sanguis* (3.3×10^8 colony-forming units [CFU], 2,135 cpm/10^8 CFU), 0.10 M potassium phosphate buffer (pH 6.0), 2% sucrose, and SCA in a total volume of 0.5 ml directly in scintillation vials. After incubation for 18 h at 37°C, the supernatant fluids were aspirated and the adherent cell layers were washed three times with 2.0 ml of 0.9% NaCl. The vials were then directly counted after drying.

Cellular interaction with glucans. Visible agglutination of cells in the presence of exogenous sucrose or glucans was determined as previously described (8), except that the reactions were followed for up to 1 h. The adherence of cell suspensions to preformed glucan layers was carried out as described earlier (17). The glucan layers were synthesized by using the CEP from culture fluids of *S. mutans* GS5. [^{14}C]glucan binding to cells was measured by incubating the appropriate concentration of [^{14}C]glucan (0.34 μCi/mg), cells, and SCA in a total volume of 1.0 ml at 37°C for 30 min. After further incubation at 7°C for 18 h, the cells were collected on glass fiber filters (Whatman), washed three times with 2.0 ml of 0.9% NaCl, and washed once with 3.0 ml of methanol, and the dried filters were counted as previously described.

Enzyme binding. Live or heat-killed cells (100°C for 30 min) were incubated in a reaction mixture containing various levels of the CEP from either *S. sanguis* ST3 or *S. mutans* GS5 together with SCA in a total volume of 2.0 ml. After incubation at 37°C for 30 min, the mixtures were further incubated for 18 h at 7°C. The cell suspensions were centrifuged at 13,000 × *g* for 10 min, washed with 5.0 ml of 0.9% NaCl, and finally suspended in 2.0 ml of SCA. These cell suspensions were then used to determine cell-associated enzyme activities and adherence properties.

Materials. [*U*-^{14}C]sucrose-glucose (212 mCi/mmol), [*U*-^{3}H]sucrose-fructose (4.75 mCi/μmol), and [*methyl*-^{3}H]thymidine (20 Ci/mmol) were obtained from New England Nuclear Corp. Soluble [^{14}C]glucan (0.34 μCi/mg) was prepared as previously described (19). Dextran T10 and T2000 were products of Pharmacia Fine Chemicals.

RESULTS

Sucrose-dependent adherence during growth. *S. mutans* GS5 displayed marked adherence to glass surfaces in vitro when grown in THB supplemented with sucrose (Table 1), as is typical of organisms of this species (25). When sucrose was replaced by glucose, adher-

TABLE 1. *Adherence of S. mutans GS5 and S. sanguis ST3 during growth*[a]

Organism	Carbon source	Adherence[b] (%)
S. mutans GS5	Glucose (1%)	29
S. mutans GS5	Sucrose (1%)	76
S. sanguis ST3	Glucose (1%)	7
S. sanguis ST3	Sucrose (1%)	24

[a] Each organism was inoculated into THB medium (5 ml) with the indicated carbon source and incubated at 37°C for 18 h. Adherent and nonadherent cells were quantitated as described in the text.

[b] $\{A_{540}$ (adherent cells)/[A_{540} (adherent cells) + A_{540} (nonadherent cells)]$\} \times 100$, where A_{540} is the absorbance at 540 nm.

ence was reduced but was still pronounced. This latter observation is probably the result of the presence of contaminant sucrose in the growth medium (18). *S. sanguis* ST3 also displayed significant sucrose-dependent adherence during growth. However, the adherence observed was about one-third that of *S. mutans* GS5. As with *S. mutans* GS5, the adherence of strain ST3 was also reduced when sucrose was replaced by glucose. The adherence of certain strains of *S. sanguis* to smooth surfaces during growth in sucrose broth has also been observed previously (22). The growth rates of both organisms were nearly identical in THB-glucose medium, and the final yield of cells of both strains was increased approximately 32% when glucose was replaced by sucrose.

Sucrose-dependent adherence of nongrowing cell suspensions. Washed cell suspensions of *S. mutans* GS5 grown in THB-glucose medium adhere very strongly to glass in the presence of sucrose (Fig. 1). In contrast, no detectable adherence could be measured after the incubation of *S. sanguis* ST3 with sucrose. In the presence of sucrose, these latter cells formed a gel-like layer on glass surfaces, which was readily removed during the washing procedure employed in the assays.

Interaction of cell suspensions with glucans. The ability of *S. mutans* to agglutinate in the presence of a variety of glucan polymers is one of the prominent characteristics of this group of organisms (8). As reported previously for other strains of *S. mutans* (8), strain GS5 agglutinates readily in the presence of dextran T2000 or after incubation with sucrose, indicating an interaction of the cells with glucan polymers (Table 2). In contrast, no visible agglutination was observed when *S. sanguis* ST3 was incubated with dextran T2000. However, as with *S. mutans* GS5, the incubation of *S. sanguis* ST3 cells with sucrose resulted in a visible agglutination of the cells.

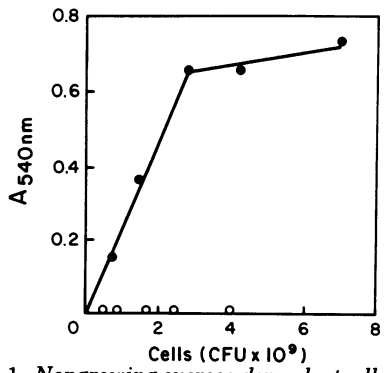

FIG. 1. *Nongrowing sucrose-dependent adherence of S. mutans GS5 and S. sanguis ST3. Various levels of the oral streptococci were incubated in the presence of sucrose, and the resultant adherence was quantitated as previously described (18). Symbols: ●, S. mutans GS5 (1.4 × 10¹⁰ CFU/ml); ○, S. sanguis ST3 (8.1 × 10⁹ CFU/ml). A_{540nm}, Absorbance at 540 nm.*

TABLE 2. *Interaction of S. mutans GS5 and S. sanguis ST3 with glucans*

Cells	Adherence to preformed glucan layers[a] (%)	Dextran-induced agglutination[b]	Sucrose-induced agglutination[b]
S. mutans GS5	7.6	+	+
S. sanguis ST3	3.9	−	+

[a] Preformed glucan layers synthesized by the CEP of *S. mutans* GS5 were prepared (17), and either *S. mutans* GS5 (2.7 × 10⁹ CFU) or *S. sanguis* ST3 (2.0 × 10⁹ CFU) cells were added. After incubation for 18 h at 37°C, the adherent cells were washed and quantitated as previously described. Adherence is presented as the percentage of added cells complexed to the glucan layers.

[b] Agglutination in the presence of dextran T2000 or sucrose (1%) was carried out as described in the text.

The interaction of *S. mutans* with glucan molecules can also be demonstrated after incubation of the cells with preformed glucan layers on glass surfaces (17). Both *S. mutans* GS5 and *S. sanguis* ST3 adhere to glucan layers, with the former cells adhering to a greater degree than the latter cells. The adherence of *S. mutans* GS5 to preformed layers in these experiments (Table 2) is much less than the 93% reported previously (17) for these same cells. Recent results indicate that strain GS5 has become altered in its ability to interact with glucans after continuous subculture (14).

A direct measurement of the interaction of the two cell types with soluble glucan molecules was also carried out with [^{14}C]glucan prepared as previously described (19). *S. sanguis* ST3 bound significant amounts of [^{14}C]glucan, whereas *S. mutans* GS5 was much less active in binding the soluble polymer (Fig. 2). The low level of glucan binding exhibited by strain GS5 is apparently also due to a decrease in glucan interaction displayed by these cells after continuous subculture. The ability of *S. sanguis* ST3 to bind [^{14}C]glucan is comparable to that measured for the original cultures of *S. mutans* GS5 grown from lyophilized stored samples and not maintained for long periods by continuous subculture (14).

Effects of *S. mutans* GS5 on the sucrose-dependent adherence of *S. sanguis* ST3. Despite the relatively low levels of sucrose-dependent adherence observed for *S. sanguis* (Fig. 1), these organisms are one of the predominant species of streptococci found in smooth-

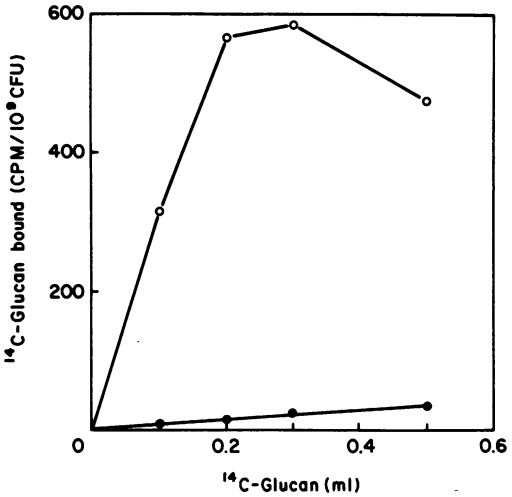

FIG. 2. *Binding of [^{14}C]glucan by S. mutans GS5 and S. sanguis ST3. Cell suspensions of the streptococci were incubated with the indicated levels of [^{14}C]glucan (22,000 cpm/ml), and the binding of the radioactive glucan was measured as described in the text. Symbols: ●, S. mutans GS5 (1.65 × 10^9 CFU); ○, S. sanguis ST3 (1.49 × 10^9 CFU).*

surface dental plaque (1). It was of interest, therefore, to determine whether the adherence of *S. sanguis* ST3 to smooth surfaces could be augmented by the presence of *S. mutans* and sucrose. The addition of sucrose did not significantly increase the adherence of ^{3}H-labeled *S. sanguis* to glass surfaces (Table 3), as was previously observed (Fig. 1). However, in the presence of viable cells of *S. mutans* GS5, there was a significant (threefold) increase in the adherence of the ST3 cells. Thus, the sucrose-dependent adherence of *S. mutans* GS5 to smooth surfaces might also trap cells of *S. sanguis* ST3 into the adherent layer. In addition, *S. sanguis* cells might also adhere to *S. mutans*-glucan layers subsequent to their formation, as suggested by the results of Table 2.

GTF activities of *S. mutans* GS5 and *S. sanguis* ST3. One possible explanation for the inability of cell suspensions of *S. sanguis* ST3 to adhere to smooth surfaces (Fig. 1) is that these cells might synthesize primarily extracellular, as opposed to cell-associated, GTF activity. To evaluate this possibility, the distribution of GTF activity was determined in cultures of strain ST3 and compared with that of *S. mutans* GS5 (Table 4). A comparison of the two cell cultures revealed that strain ST3 possessed much higher cell-associated GTF activity than did *S. mutans* GS5. The magnitude of this difference varied from experiment to experiment, but the cell-associated activity from strain ST3 was generally much greater than that of strain GS5. It was also of interest to examine the nature of the glucan products synthesized by the two species of oral streptococci. The extracellular GTF enzymes of both cell types synthesize large amounts of both soluble and insoluble glucans (Table 5). However, the structures of the glucan products synthesized by the two organisms appear to be quite different since *S.*

TABLE 3. *Effects of S. mutans GS5 on the nongrowing sucrose-dependent adherence of ^{3}H-labeled S. sanguis ST3*[a]

Cells	Sucrose (%)	Adherent cells (cpm)
^{3}H-labeled *S. sanguis* ST3	None	146
^{3}H-labeled *S. sanguis* ST3	2	171
^{3}H-labeled *S. sanguis* ST3 + *S. mutans* GS5	2	533
^{3}H-labeled *S. sanguis* ST3 + heat-killed *S. mutans* GS5	2	112

[a] ^{3}H-labeled *S. sanguis* (3.3 × 10^8 CFU, 2,135 cpm/10^8 CFU) cells were incubated with the indicated amounts of sucrose and live or heat-killed cells of *S. mutans* GS5 (1 × 10^9 CFU), and the adherence of radioactive cells was determined as described in the text.

TABLE 4. *Distribution of GTF activities in S. mutans GS5 and S. sanguis ST3 cultures*[a]

Cells	GTF (mU/10^8 CFU)	
	Cell associated	Extracellular
S. mutans GS5	0.19 (11)[b]	1.59 (89)
S. sanguis ST3	0.86 (62)	0.53 (38)

[a] Each organism was grown for 5 h in THB–1% glucose (10 ml), and the total GTF activity was determined for the washed cell suspensions and dialyzed extracellular fluids as described in the text.

[b] Numbers in parentheses indicate percentage of total culture activity.

TABLE 5. *Extracellular enzyme activities of S. mutans GS5 and S. sanguis ST3*[a]

Organism	GTF (mU/ml)		Adherent GTF[b] (cpm/mU)	FTF (mU/ml)
	Soluble	Insoluble		
S. mutans GS5	34.2	43.0	214	3.8
S. sanguis ST3	113.4	83.2	31	0

[a] The extracellular proteins of THB–1% glucose-grown cultures (200 ml) of each organism were precipitated by ammonium sulfate as previously described (19). Each dialyzed enzyme preparation was then assayed for the indicated activities as described in the text.

[b] Adherent GTF activities are expressed as the counts of ^{14}C-labeled adherent glucan synthesized in 18 h per milliunit of total GTP activity added.

sanguis ST3 synthesizes appreciable amounts of glucan molecules that are water insoluble but do not adhere strongly to glass surfaces compared with the *S. mutans* GS5 products.

In contrast to the extracellular enzymes of *S. mutans* GS5, no appreciable FTF activity could be detected in the comparable fraction from *S. sanguis* ST3. Likewise, strain GS5 possessed significant cell-associated FTF activity (0.02 mU/10^8 CFU), whereas relatively little activity could be detected for cells of strain ST3 (<0.003 mU/10^8 CFU). Previous investigations have also indicated that certain other strains of *S. sanguis* are devoid of detectable FTF activity (26).

Binding of GTF by *S. mutans* GS5 and *S. sanguis* ST3. Previous investigations (13) have suggested that the presence of the cell-associated GTF activity on *S. mutans* GS5 is the result of the binding of extracellular enzymes by the cells. Furthermore, heat-killed cells of *S. mutans* can also bind significant GTF activity (23). A comparison of enzyme binding by heat-killed cells of both *S. mutans* GS5 and *S. sanguis* ST3 indicated that strain GS5 binds its own extracellular GTF activity more efficiently than do the *S. sanguis* cells (Fig. 3A). Furthermore, the activity bound by strain GS5 catalyzes significant sucrose-dependent adherence of the cells to smooth surfaces. In contrast, relatively poor adherence of heat-killed *S. sanguis* ST3 cells occurs after the binding of the GS5 extracellular enzymes. Therefore, when both cell types bind equal levels of enzyme activity, the GS5 cells adhere much better than do the ST3 cells. Recent results that used heat-killed cells of *S. sanguis* OMZ-9 with bound *S. mutans* GTF enzymes also have indicated that relatively poor adherence occurs after incubation with sucrose (27).

Both heat-killed cell populations bound the extracellular GTF activity from *S. sanguis* ST3 equally well (Fig. 3B). Despite the binding of significant enzyme activity by the two streptococci, very little sucrose-dependent adherence could be detected for either cell type. Thus, a comparison of the adherence properties of heat-killed GS5 cells with bound enzymes from the two cell types correlates with the earlier suggestion that the GTF enzymes from *S. sanguis* ST3 do not synthesize appreciable amounts of adherent glucan molecules.

The use of viable cells in the binding experiments yielded similar results (data not shown). Viable cells of both streptococci apparently bound extracellular GTF activity to a somewhat lesser degree than did the comparable heat-killed cells. The extracellular enzymes

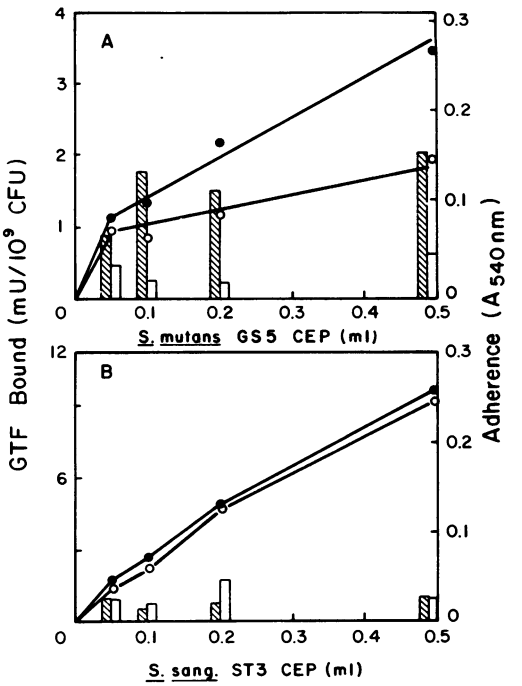

FIG. 3. *Binding of CEP to heat-killed cells of S. mutans GS5 and S. sanguis ST3 and subsequent sucrose-dependent adherence. The binding of the CEP preparations to the cells and the subsequent adherence were measured as described in the text. (A) Binding of S. mutans GS5 CEP. The indicated levels of CEP (49.9 mU/ml) were incubated with the cells and treated as described in the text. Symbols: ●, S. mutans GS5 (2.5 × 10^9 CFU); ○, S. sanguis ST3 (2.2 × 10^9 CFU); shaded bars, adherence of GS5 cells; open bars, adherence of ST3 cells. (B) Binding of S. sanguis ST3 CEP. The appropriate levels of CEP (67.9 mU/ml) were incubated with the cells and treated as described in the text. The symbols are the same as for (A). A_{540nm}, Absorbance at 540 nm.*

from *S. mutans* GS5 bound to the viable GS5 cells to a greater extent than to the ST3 cells, whereas the enzymes from *S. sanguis* ST3 bound equally well to viable cells of both cell types. These results with viable cells yield only apparent binding capabilities since it is not possible to accurately discriminate between the relative contributions of the endogenous cell-associated activity and the added activity in the binding measurements. Like heat-killed cells, live cells of *S. sanguis* ST3 that bound the GTF activity from *S. mutans* GS5 did not adhere well to smooth surfaces in the presence of sucrose.

DISCUSSION

Although *S. sanguis* is found in relatively

high concentrations on tooth surfaces (1), there does not appear to be a direct correlation between the presence of this organism and the incidence of caries (4). In contrast, several studies have suggested a direct correlation between the presence of *S. mutans* on tooth surfaces and the incidence of caries (4, 12), and various strains of this group uniformly induce carious lesions in experimental animals (10). These differences between the two oral streptococci appear to result from the inability of *S. sanguis* colonies on tooth surfaces to synthesize sufficient acid concentrations to produce enamel demineralization (10). These organisms, once attached to enamel surfaces, may not form large enough bacterial plaques by themselves for sufficient acid production due to: (i) possible nutrient-limited growth rates (28), (ii) limitations in the structural properties of the *S. sanguis* plaque matrix, or (iii) their inability to synthesize intracellular polysaccharides (29).

During growth in the presence of sucrose (Table 1), *S. sanguis* ST3 displays about one-third of the adherence capability of *S. mutans* GS5. However, in contrast to strain GS5, washed cell suspensions of glucose-grown cells of *S. sanguis* ST3 incubated with sucrose do not display any detectable adherence to smooth surfaces (Fig. 1). These results suggest that most of the adherence of strain ST3 observed during growth in the presence of sucrose may represent adherence of the cells to the adherent glucan layers synthesized by the extracellular GTF activity produced by the cells during growth. Adherence of the cells to preformed glucan layers (Table 2) as well as the synthesis of low levels of adherent glucan by the extracellular enzymes of strain ST3 (Table 5) could be demonstrated. Although these two properties of strain ST3 are less pronounced than those of *S. mutans* GS5, they may be sufficient to account for the cellular adherence demonstrated during growth in vitro. The fact that sucrose-dependent adherence of *S. sanguis* to tooth surfaces in vivo does not appear to be significant (5) may indicate that the interaction of *S. sanguis* with glucan layers on tooth surfaces may be minimal in the human oral cavity, as has been suggested recently for *S. mutans* (30).

Previous investigations concerning the comparative structures of the glucans synthesized by the extracellular GTF enzymes of *S. mutans* and *S. sanguis* (3, 11) indicated that the products differed in their relative proportions of α-1,3- and α-1,6-linked glucose units. Although the structural requirements for adherence of glucan molecules are not precisely known, these results have been interpreted as indicating that *S. sanguis* synthesizes lower concentrations of adherent glucans than do strains of *S. mutans*. A direct comparison of adherent glucan synthesis by *S. mutans* GS5 and *S. sanguis* ST3 (Table 5) indicated that the former synthesizes approximately seven times as much adherent glucan molecules as does the latter. Therefore, assuming that the extracellular activities also reflect the properties of the cell-associated activities (18), the primary basis for the widely divergent sucrose-dependent adherence properties observed for the two oral streptococci (Fig. 1) appears to be a result of the differential synthesis of adherent glucan molecules. The relatively weak adherence of the glucan products synthesized by the enzymes of *S. sanguis* ST3 is also indicated by the inability of these enzymes bound to heat-killed cells of either oral streptococci to catalyze significant adherence (Fig. 3B). In contrast, heat-killed GS5 cells with bound homologous GTF enzymes demonstrated significant sucrose-dependent adherence to smooth surfaces (Fig. 3A). The weak sucrose-dependent adherence of *S. sanguis* ST3 may also result from the inability of these cells to bind GTF enzymes that synthesize adherent glucan or from the defective binding of glucan molecules involved in the adherence process. These latter two possibilities are suggested by the observation that heat-killed cells of *S. sanguis* that bound appreciable amounts of the GS5 enzymes displayed relatively weak adherence. These results suggest that the synthesis of adherent polymer molecules by the cells per se may not be the sole requirement for maximum sucrose-dependent adherence to smooth surfaces. A similar conclusion has also been reached from an investigation of a nonadherent variant of *S. mutans* GS5 (14). The differences observed in the relative adherence properties of the heat-killed cells (Fig. 3A) are not the result of the inability of the ST3 cells to agglutinate since heat-inactivated cells of both strains with bound GTF from strain GS5 display visible aggregation in the presence of sucrose. However, the nature of the postulated additional adherence requirement is unknown, and more detailed investigations into the adherence properties of *S. sanguis* ST3 will be required to substantiate this suggestion.

Although the binding of GTF activity by various strains of *S. mutans* can be readily demonstrated (14, 23, 27), the nature of the binding sites responsible for such interactions is still unknown. The heat-stable receptors on the cell surfaces of the oral streptococci might interact with either the protein or glucan components of the GTF complex. In addition, the GTF com-

plex might interact with glucan or other polysaccharide components on the cell surface (24). Therefore, it is not yet clear why heat-killed cells of *S. mutans* GS5 bind significantly more GTF activity from strain GS5 than do cells of *S. sanguis* ST3, whereas both cell types bind the enzymes from strain ST3 equally well.

The ability of *S. sanguis* ST3 to adhere to preformed glucan layers contrasts with previous results with *S. sanguis* 10556 (17). Furthermore, glucan-mediated agglutination of *S. sanguis* has not been previously reported although certain strains of this group agglutinate in the presence of saliva (15). As with other strains of *S. sanguis* (8), strain ST3 does not agglutinate in the presence of dextran T2000, but does aggregate when incubated with sucrose. These variable results suggest that various strains of *S. sanguis* may exhibit differential specificities on interacting with glucan molecules or, conversely, that the strains may become altered to different degrees in glucan recognition after continuous laboratory subculture, as has been recently suggested for *S. mutans* GS5 (14). These possibilities again emphasize that some caution should be exercised in drawing conclusions from the comparative properties of oral streptococci.

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