

Glucans Synthesized In Situ in Experimental Salivary Pellicle Function as Specific Binding Sites for *Streptococcus mutans*

KURT M. SCHILLING† AND WILLIAM H. BOWEN*

Departments of Dental Research and Microbiology, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, New York 14642

Received 20 August 1991/Accepted 9 October 1991

Many researchers have suggested that the role of glucan-mediated interactions in the adherence of *Streptococcus mutans* is restricted to accumulation of this cariogenic bacterium following its sucrose (i.e., glucan)-independent binding to saliva-coated tooth surfaces. However, the presence of enzymatically active glucosyltransferase in salivary pellicle suggests that glucans could also promote the initial adherence of *S. mutans* to the teeth. In the present study, the commonly used hydroxyapatite adherence assay was modified to include the incorporation of glucosyltransferase and the synthesis of glucans in situ on saliva-coated hydroxyapatite beads. Several laboratory strains and clinical isolates of *S. mutans* were examined for their ability to adhere to experimental pellicles, either with or without the prior formation of glucans in situ. Results showed that most strains of *S. mutans* bound stereospecifically to glucans synthesized in pellicle. Inhibition studies with various polysaccharides and fungal dextranase indicated that α 1,6-linked glucose residues were of primary importance in the glucan binding observed. Scanning electron microscopic analysis showed direct binding of *S. mutans* to hydroxyapatite surface-associated polysaccharide and revealed no evidence of trapping or cell-to-cell binding. *S. mutans* strains also attached to host-derived structures in experimental pellicles, and the data suggest that the bacterial adhesins which recognize salivary binding sites were distinct from glucan-binding adhesins. Furthermore, glucans formed in experimental pellicles appeared to mask the host-derived components. These results support the concept that glucans synthesized in salivary pellicle can promote the selective adherence of the cariogenic streptococci which colonize human teeth.

The synthesis of extracellular glucans from dietary sucrose contributes to the virulence of *Streptococcus mutans*, *S. sobrinus*, and *S. cricetus*, the three species of mutans streptococci most commonly associated with the etiology of dental caries in humans (6, 9, 17). Results from several studies have shown that glucan synthesis catalyzed by bacterial glucosyltransferase (GTF) can enhance the pathogenic potential of dental plaque by promoting the accumulation of large numbers of cariogenic streptococci on the teeth of humans and experimental animals (12, 23, 47). Although glucan production is not required by *S. mutans* or *S. sobrinus* for initial adherence to saliva-coated surfaces in vitro (5, 13, 44) or for colonization of the teeth of humans or laboratory animals in vivo (48, 49), evidence from several studies suggests that glucan-dependent adherence and accumulation by cariogenic streptococci are critical processes in the development of pathogenic dental plaque, especially on smooth surfaces of the teeth (17, 22, 47).

Many strains of *S. mutans* and *S. sobrinus* react with glucans and are agglutinated by minute amounts of high-molecular-weight dextrans (9, 15, 32). Glucan binding appears to be unique to the mutans group of oral streptococci, and it is associated with the expression of multiple glucan-binding adhesins by these bacteria (31, 39). *S. mutans* and *S. sobrinus* produce multiple GTF isoenzymes which display a high affinity for glucans (32, 33, 39). Although the GTFs are secreted enzymes, under certain conditions they can become strongly associated with streptococcal cell surfaces and can apparently mediate glucan-induced agglutination (18). In

addition to GTFs, *S. mutans* and *S. sobrinus* are known to produce non-enzymatic glucan-binding proteins (GBPs). For example, Russell (39) isolated a 74.0-kDa GBP from *S. mutans* 3209 which was devoid of detectable GTF activity. Subsequently, a gene coding for a nonenzymatic GBP was isolated from *S. mutans* 3209 (40), and a similar gene has been identified in *S. mutans* GS-5 (37). Similarly, McCabe and Hamelik (31) have reported the isolation of multiple, nonenzymatic GBPs from *S. sobrinus* 6715. Results from studies by Mooser and Wong (33) suggest that at least some of the *S. sobrinus* GBPs are proteolytic breakdown products of GTFs.

Many researchers have suggested that glucan binding functions primarily in the sucrose-induced accretion of cariogenic streptococci, following their sucrose (i.e., glucan)-independent adherence onto saliva-coated surfaces of the teeth (5, 44). However, evidence indicates that glucan binding could also play a role in the initial adherence of *S. mutans* and *S. sobrinus* to the teeth. For example, results from several studies have demonstrated that enzymatically active GTF is present in the soluble fraction of whole human saliva and is incorporated into the initial salivary pellicle which forms on the teeth (38, 41). In addition, data from in vitro studies indicate that GTF incorporated into salivary pellicle expresses enhanced enzymatic activity and that a large proportion of the glucans formed in situ is retained in the pellicle (43). As constituents of pellicle, GTF and product glucans could directly influence the initial adherence of cariogenic streptococci to tooth surfaces by providing selective binding sites recognized by GBPs.

Most model systems designed to mimic bacterial adherence to saliva-coated tooth surfaces have not included the potential effects of enzymatically active GTF in pellicle. Instead, many oral bacterial attachment assays have used

* Corresponding author.

† Present address: Unilever Research U.S. Inc., 45 River Rd., Edgewater, NJ 07020.

TABLE 1. Bacterial strains used in adherence studies

<i>S. mutans</i> strain	Serotype ^a
GS-5	c
C10449	c
MT8148	c
Ingbritt	c
Ingbritt 1600	c
Ingbritt R	c
V100	e
OMZ175	f
GL-1 through GL-8 ^b	c
NG8	c

^a Confirmed by using serotype-specific fluorescent antisera.

^b *S. mutans* GL-1 through GL-8 are clinical isolates obtained from naval recruits (provided by I. L. Sklair, Great Lakes, Ill.).

experimental pellicles derived from glandular saliva or "heat-inactivated" whole saliva, both of which lack GTF activity (4, 5, 44). Furthermore, methods designed to study the role of glucan-mediated interactions have usually involved measurement of streptococcal attachment in the presence of sucrose and therefore have not distinguished between bacterial adherence and accumulation (5, 22, 44).

It has been reported (14) that strains of *S. sobrinus*, but not strains of *S. mutans*, bind specifically to glucans on apatitic surfaces; conversely, *S. mutans* strains, but not *S. sobrinus* strains, were found to bind to salivary components in experimental pellicles. The suggestion was made that the two species of cariogenic streptococci have distinct binding sites on the teeth (14). However, because *S. mutans* produces multiple GBPs, the reported inability of *S. mutans* strains to bind to glucans on apatite surfaces is surprising and may reflect differences among laboratory strains.

In the present study, the commonly used saliva-coated hydroxyapatite (HA) adherence assay was modified to allow synthesis of glucans in situ in salivary pellicle prior to bacterial binding. Adherence experiments were conducted with *S. mutans* GS-5, a strain which does not bind appreciably to host-derived components of pellicle (35). In addition, several other laboratory strains and clinical isolates of *S. mutans* were examined for their ability to adhere to experimental pellicles, with and without the prior formation of glucans in situ. Inhibition studies utilizing various glucans and fungal dextranase were also carried out to determine the anomeric structures which are of primary importance in the glucan binding. Furthermore, scanning electron microscopy (SEM) and separation using colloidal silica were used to examine the orientation and state of aggregation of the streptococci bound to the experimental pellicles.

(Preliminary data from this study were reported in the proceedings of the Eleventh International Conference on Oral Biology, 5-7 September 1988, Hong Kong [42].)

MATERIALS AND METHODS

Bacterial strains and cultivation. The strains of *S. mutans* used in these studies are listed in Table 1. Streptococcal strains were maintained on a solid (1.7% agar) or a liquid medium containing 2.5% tryptone, 1.5% yeast extract, 0.5% K₂HPO₄, and 0.1% MgSO₄, supplemented with 1.0% fructose (TYF). We used fructose because of concern that glucose may be contaminated by dextran. Alternatively, the streptococci were grown in a "semidefined" medium (SDM) containing 1.5% Casamino Acids (Difco Laboratories), 0.13% NH₄SO₄, 0.44% KH₂PO₄, 0.68% K₂HPO₄, 0.04%

MgSO₄, 1.0% BME vitamin mixture (GIBCO Laboratories), and 1.0% fructose. Strains were stored at 70°C in either TYF or SDM supplemented with 10.0% glycerol.

Prior to storage, most *S. mutans* strains were grown on glass rods in the presence of 3.4% sucrose to permit adherent growth of the bacteria. The glass rods harboring attached bacteria were transferred four or five times into fresh sucrose-containing medium to build up adherent plaques, and then the bacteria were scraped from the glass rods and cultured on TYF plates. The bacteria were then grown in TYF or SDM broth and stored at -70°C. Preparation of the streptococcal strains in this manner generally enhanced their ability to adhere to experimental pellicles, compared with the same strains which had been passaged several times in the laboratory prior to storage. The serotypes of all streptococcal strains were confirmed by direct immunofluorescence, using fluorescein-conjugated serotype-specific antisera.

GTF preparation. The GTF used to make experimental pellicles for the in vitro bacterial adherence studies was prepared from *S. mutans* GS-5 culture fluid. Bacteria from a starter culture were inoculated into dialysis tubing (molecular weight cutoff = 12,000) containing 150.0 ml of SDM, immersed in 2 liters of the same medium. The streptococci were grown in the dialysis tubing to concentrate secreted exoproteins. Following overnight growth at 37°C with stirring, the cells were removed by centrifugation at 12,000 × g and the culture supernatant (usually pH 5.0 to 5.5) was adjusted to pH 6.5 by addition of NaOH. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) and the preservative NaN₃ were added to the culture supernatant fluid to give final concentrations of 1.0 and 3.1 mmol, respectively. This concentrated culture supernatant was subjected to diafiltration against a buffer containing 50.0 mmol of KCl, 1.0 mmol of CaCl₂, 1.0 mmol of potassium phosphate, and 0.1 mmol of MgCl₂ (pH 6.5) (referred to as buffered KCl) over a 30,000-molecular-weight cutoff ultrafiltration membrane (Amicon YM30) in a stirred-cell ultrafiltration device (Amicon model 8400). The *S. mutans* GS5 GTF preparations used for these experiments polymerized between 0.02 and 0.05 μmol of glucose per ml per min, as determined by the radioactive GTF assay described below.

In vitro bacterial adherence assay. In the present study, a model for bacterial adsorption to HA similar to that described by Clark et al. (4) was modified to include the incorporation of GTF in the experimental pellicles formed on HA beads. A diagrammatic summary of the modified bacterial adherence assay is shown in Fig. 1.

To radiolabel bacteria, streptococci were grown in SDM containing 10 mCi of [³H]thymidine (Amersham Corp.) per ml. After harvesting by centrifugation, the labeled streptococci were dechained by brief sonication (Braun-Sonic 1510; three to four 30-s pulses at 400 W), washed, and resuspended to the appropriate cell concentration in buffered KCl. Bacterial concentrations were measured by optical density, using a calibration curve for optical density at 540 nm versus bacterial numbers as quantitated in a Petroff-Hausser chamber.

For experimental pellicles, 40-mg samples of HA beads (surface area, 0.63 cm²/mg; BDH Chemicals Ltd.) were initially suspended in buffered KCl. The HA beads were treated for 45 min at 37°C with buffer (HA), parotid saliva (PS/HA), or 2.0 mg of bovine serum albumin (BSA/HA) per ml. PS was collected from a 30-year-old male donor by using a modified Lashley cup (27). Secretion of PS was stimulated with sucrose-free lemon candy. Freshly collected saliva was

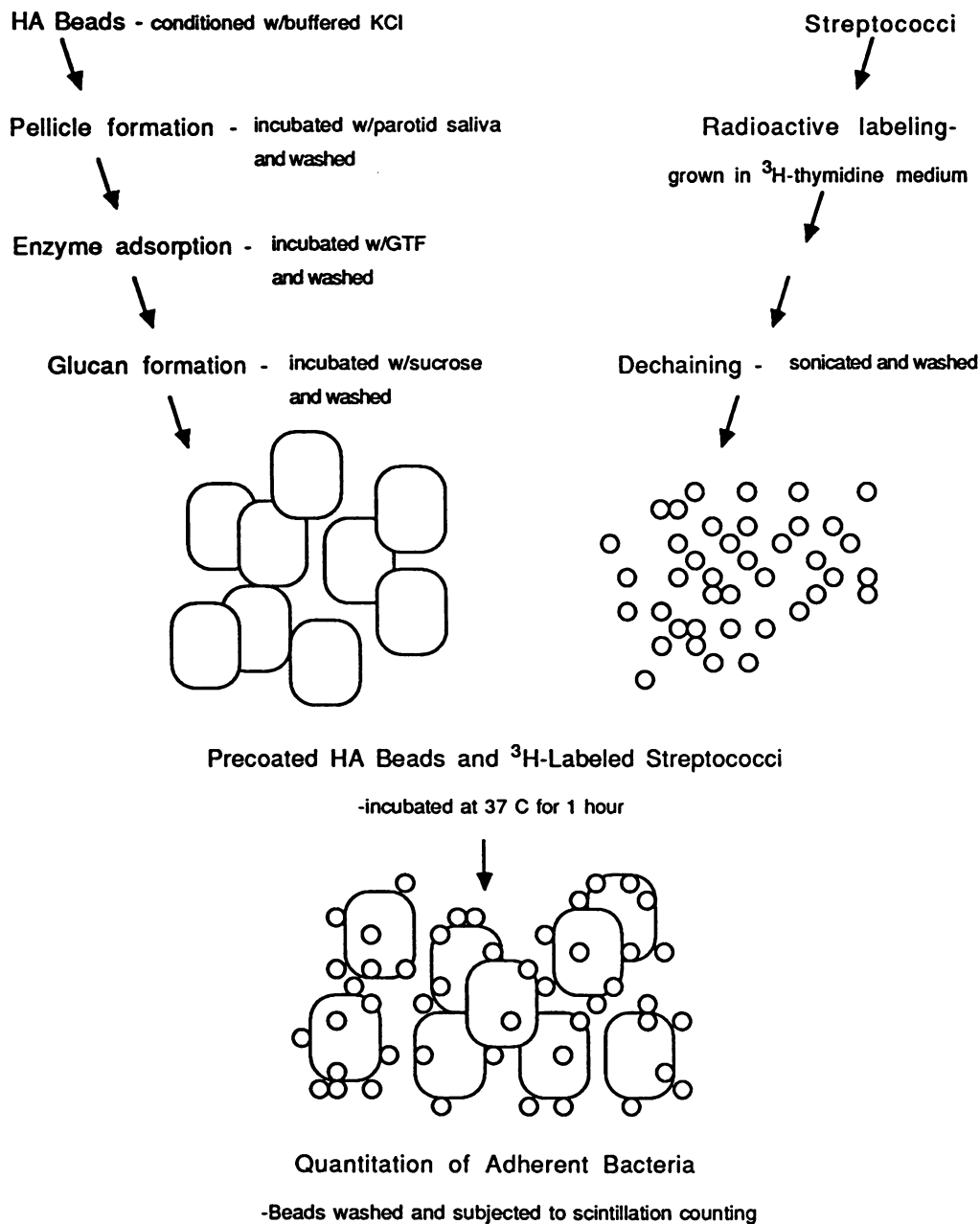


FIG. 1. Modified bacterial adherence assay.

used in all experiments. BSA-coated HA served as a control surface; studies have shown that treatment of HA beads with BSA drastically reduces adherence of oral streptococci to beads (14).

Following the initial treatment, the HA beads were washed and treated for 60 min at 37°C with GTF (prepared as described above) to permit incorporation of the enzyme into the experimental pellicles; control samples were treated with buffered KCl. Next, the HA beads were washed and incubated for 120 min at 37°C with 1.0 ml of 100.0 mmol of sucrose in buffered KCl to permit in situ glucan synthesis by the adsorbed GTF; control samples were incubated with buffered KCl. Following removal of excess sucrose and unbound glucans by washing with buffered KCl, the HA

beads were immediately used in the adherence assay described below.

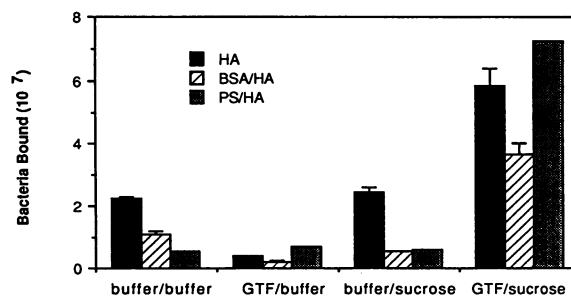
The amount of in situ formed glucans incorporated into the experimental pellicles prepared for the adherence assay was quantitated by incubating duplicate HA samples, precoated with PS and GTF, with 100.0 mmol of [^{14}C]glucosylsucrose (1,000 cpm/ μmol of sucrose; New England Nuclear) for 120.0 min at 37°C. After the samples were washed with buffered KCl, incorporation of radioactive glucose into adherent polymer was quantitated by direct scintillation counting of the HA beads. The average amount of glucan which remained attached to the HA beads was determined for each experiment and ranged from 0.3 to 2.0 μmol of incorporated [^{14}C]glucose per sample.

To quantitate bacterial adherence to experimental pellicles, prepared HA beads were combined in polypropylene microcentrifuge tubes with 1.0 ml of [^3H]thymidine-labeled streptococci, suspended at the appropriate concentration in buffered KCl, and incubated for 1.0 h at 37°C with gentle inversion on a hematology mixer (Thermolyne). The HA beads were then washed with buffered KCl, rinsed into vials by using scintillation fluid, and counted directly to determine the number of adherent bacteria.

Effects of passage of HA beads through colloidal silica following *S. mutans* adherence. To determine whether unbound glucan-induced streptococcal aggregates formed during the adherence assay, the method was modified to include passage of the HA beads harboring adherent streptococci through the colloidal silica preparation, Percoll (Pharmacia). *S. mutans* GS-5 (10^9 cells per ml), either suspended in buffered KCl or aggregated by prior incubation in 0.5 mg of dextran T500 (500,000 molecular weight; Pharmacia) per ml for 15 min at 22°C, was incubated with HA beads coated with PS plus in situ formed glucans [(PS+glucan)/HA] as per the normal adherence assay. To separate HA beads from bacterial cells and cell aggregates, the beads were rinsed into miniscintillation vials containing 4.0 ml of 100% Percoll. After the beads settled, the Percoll was aspirated and the beads were rinsed and scintillation counted to determine adherent bacteria.

Effects of exogenously added polysaccharides on *S. mutans* adherence to experimental pellicles. Radioactively labeled *S. mutans* GS-5 cells were mixed with the various polysaccharides at the concentrations indicated (see Table 4) for 15.0 min at room temperature. The bacterium polysaccharide mixtures were then added to HA beads coated with experimental pellicles in the standard adherence assay. The soluble polysaccharides tested were a 9,000-kDa dextran (>90% poly- α 1,6-glucose; Sigma Chemical Co.), dextran T500 (average molecular weight, 500,000) (>90% α 1,6-glucose; Pharmacia), inulin (poly- β 2,1-fructose; Sigma), and levan (poly- β 2,6-fructose; Sigma). Polysaccharides which were insoluble in aqueous buffer were sonicated into suspension in buffered KCl prior to mixing with labeled bacteria. The insoluble polysaccharides tested included starch (poly- α 1,4-glucose), nigeran (poly- α 1,3; α 1,4-glucose) (Sigma), and glucan synthesized from sucrose by GTF from a growth culture supernatant of *S. sobrinus* 6715 (mutan). The mutan was isolated by methanol precipitation, and experiments showed it to be refractile to hydrolysis with 35.7 U of α 1,6-specific dextranase (*Penicillium* sp.; Sigma) per ml at pH 6.0 for 3.0 h at 37°C, as determined by using glucose oxidase (45) and dinitrosalicylic acid-reducing sugar assays (29). Similar mutan preparations have been reported by others to be composed of mostly α 1,3-linked glucosyl units, the remainder of the glucose residues being α 1,6 linked (16).

Treatment of experimental pellicles with fungal dextranase. Dextran was selectively removed from experimental pellicles by enzymatic treatment. This was accomplished by incubating the HA beads, coated with PS or PS plus in situ formed glucan, with 70.0 U of dextranase (1,6-D-glucan 6-glucanhydrolase) (*Penicillium* sp.; Sigma) per ml in buffered KCl for 1.0 h at 37°C. In control samples, the pellicle-coated HA beads were treated with (i) heat-inactivated dextranase (70°C for 1.0 h); (ii) dextranase plus the protease inhibitors PMSF (1.0 mmol) and soybean trypsin inhibitor (0.1 mg/ml); or (iii) PMSF and soybean trypsin inhibitor alone. Following dextranase treatment, the HA beads were washed with buffered KCl and were incubated with radioactively labeled streptococci in the standard adherence assay.



HA Treatment Prior to Addition of *S. mutans* GS-5

FIG. 2. Adherence of *S. mutans* GS-5 to glucans synthesized in situ in experimental pellicles. HA beads coated with buffer (HA), 2.0 mg of BSA/HA per ml, or PS/HA were exposed to either *S. mutans* GTF or buffer, followed by 100 mmol of either sucrose or buffer. Values represent mean of three samples (\pm standard deviation).

SEM. SEM was used to examine the structure of experimental pellicles formed on HA beads and to directly observe the binding of *S. mutans* to HA beads. For SEM analysis, the experimental pellicles, PS/HA, and (PS+glucan)/HA, were prepared as described above. To fix the pellicles for SEM, the samples were incubated overnight in 1.0% phosphate-buffered glutaraldehyde (pH 6.8). Duplicate PS/HA and (PS+glucan)/HA samples were incubated with *S. mutans* cells in the standard bacterial adherence assay and then were fixed overnight in the 1.0% glutaraldehyde. Samples were dehydrated by successive treatments with ethanol (50, 75, 90, and 99%), critical-point dried (Pousinis, Samdri PVT), and sputtered with gold-palladium to a thickness of approximately 120 Å (12 nm). The prepared samples were viewed with a Jeol 820 scanning electron microscope.

RESULTS

Adherence of *S. mutans* GS-5 to glucans formed in situ in experimental pellicles. Bacterial adherence experiments were conducted with *S. mutans* GS-5, a well-studied serotype c laboratory strain. Pellicles were formed by treatment of HA beads with PS, BSA, or buffer (HA). As seen in Fig. 2, *S. mutans* GS-5 cells bound in much higher numbers to naked HA beads than to BSA/HA or PS/HA. Furthermore, comparison of *S. mutans* GS-5 binding to PS/HA to that observed for BSA/HA failed to reveal any saliva-specific binding. To determine the ability of *S. mutans* to adhere to glucans formed in pellicle, samples of PS/HA, BSA/HA, and HA were exposed to GTF prepared from *S. mutans* GS-5. The samples were then incubated with sucrose to permit in situ glucan synthesis by the adsorbed GTF. Results clearly showed that the presence of glucans formed on HA, PS/HA, and BSA/HA surfaces promoted a large increase in the binding of the bacteria compared with the adherence observed to the same pellicles treated with buffer alone (Fig. 2). Treatment of the HA, PS/HA, or BSA/HA with either GTF alone or sucrose alone resulted in little change in the observed streptococcal binding, indicating that glucans provided the additional bacterial binding sites in the experimental pellicles (Fig. 2).

Populations of mutans streptococci found in salivas from humans range from 10^3 to 10^8 cells per ml (50). Therefore, adherence of *S. mutans* GS-5 to experimental pellicles was determined over a large range of cell concentrations. Experiments were conducted as before, comparing the binding of

TABLE 2. Effects of cell concentration on adherence of *S. mutans* GS-5 to experimental pellicles

Bacteria added (per ml)	Bacteria (SD) bound to exptl pellicles ^a	
	PS/HA	(PS+glucan)/HA
2.00×10^6	$1.11 (0.63) \times 10^5$	$1.30 (0.07) \times 10^6$
5.00×10^6	$1.60 (0.29) \times 10^5$	$3.92 (0.43) \times 10^6$
1.00×10^7	$2.05 (0.28) \times 10^5$	$6.42 (0.52) \times 10^6$
5.00×10^7	$1.01 (0.31) \times 10^6$	$3.21 (0.27) \times 10^7$
1.00×10^8	$1.94 (0.26) \times 10^6$	$6.98 (0.20) \times 10^7$
5.00×10^8	$6.45 (1.35) \times 10^6$	$1.87 (0.05) \times 10^8$
1.00×10^9	$1.14 (0.14) \times 10^7$	$3.30 (0.09) \times 10^8$
2.00×10^9	$2.26 (0.19) \times 10^7$	$5.79 (0.34) \times 10^8$

^a Mean values ($n = 3$) from a single experiment are given and are representative of at least three experiments.

S. mutans GS-5 to HA coated with PS and preformed glucans with that observed for HA coated with PS alone. As shown in Table 2, *in situ* formed glucans functioned as binding sites for *S. mutans* GS-5 at cell concentrations ranging from 2×10^6 to 2×10^9 cells per ml. The presence of glucans in experimental pellicles increased streptococcal binding by approximately 8-fold at the lowest cell concentration and up to 55-fold at the highest cell concentration compared with that observed for PS/HA.

Lack of aggregate formation during binding of *S. mutans* GS-5 to (PS+glucan)/HA. Results from studies by Drake et al. (9) and others (20, 51) have shown that high-molecular-weight dextrans induce agglutination of *S. mutans* cells. Therefore, it is conceivable that desorption of *in situ* formed glucans from (PS+glucan)/HA could have resulted in aggregation of the bacteria, causing them to pellet with the HA beads and to be counted as bound to the experimental pellicle. To determine whether unbound, glucan-induced streptococcal aggregates formed during the adherence assay, the method was modified to include passage of the HA beads, harboring adherent bacteria, through the colloidal silica preparation Percoll (Pharmacia). Percoll is used by many researchers to separate particles or cells by density or size or both. The HA beads quickly sink to the bottom of 100% Percoll, and control experiments showed that radiolabeled *S. mutans* cells, in diffuse suspension or agglutinated by 500-kDa α 1,6-rich dextran, were retarded by the Percoll (data not shown). Results from experiments using this technique indicated that >90% of the bacteria which were detected in the standard assay as adherent to (PS+glucan)/HA were still attached to the HA beads even after the beads were passed through Percoll (Table 3). In contrast,

TABLE 3. *S. mutans* cells bound to HA after passage through Percoll (Pharmacia)

Bacterial suspending medium ^a	Percoll separation	Bacteria bound to (PS+glucan)/HA (10^8) ^b
Buffer	—	1.82 (0.09)
Buffer	+	1.71 (0.39)
Dextran T500, 0.5 mg/ml	—	1.53 (0.06)
Dextran T500, 0.5 mg/ml	+	0.75 (0.07)

^a *S. mutans* cells were added to HA beads either suspended in buffer or aggregated by prior incubation with 500,000-molecular-weight dextran.

^b The data, mean values ($n = 3$) of the number of bacteria remaining bound to HA beads after passage through Percoll, are from a single experiment and are representative of at least two experiments; standard deviations are given in parentheses.

when bacteria which had been agglutinated by the addition of high-molecular-weight dextrans were incubated with the precoated HA beads, approximately 50% of the bacteria which were counted as adherent in the standard adherence assay were removed by passage of the HA beads through Percoll (Table 3). These results strongly suggest that the observed adherence of *S. mutans* GS-5 to experimental pellicles containing glucans performed *in situ* was not a result of the loose association of cellular aggregates with the surfaces of HA beads.

SEM of *S. mutans* cells bound to experimental pellicles. Although Percoll could be used to separate HA beads harboring adherent bacteria from unattached or loosely associated cellular aggregates, the possibility of cell-to-cell aggregation of the streptococci, following initial adherence to experimental pellicles, could not be explored in this assay. Furthermore, it is conceivable that some random trapping of streptococci by glucans in pellicle could have occurred. To explore this possibility, SEM was utilized to directly observe experimental pellicles, before and after the adherence of *S. mutans* cells.

Prior to SEM analysis, HA beads, prepared as described previously, were fixed in glutaraldehyde, dehydrated in ethanol, critical-point dried, and sputtered with gold-palladium. Following treatment of the HA beads with PS and GTF (no sucrose added), a macromolecular coating can be seen covering the surface of the HA (Fig. 3b) which was not seen on naked HA surfaces (Fig. 3a) ($\times 10,000$ magnification). Figure 3c, also taken at $\times 10,000$ magnification, shows the results of *in situ* glucan synthesis by GTF incorporated in the pellicle coating the beads. The glucans form an amorphous polymeric layer which appears to cover a large proportion of the HA surface. The sample shown in Fig. 3b differs from that shown in Fig. 3a only in that the HA beads were incubated with sucrose for 2.0 h prior to preparation for SEM. It is apparent from SEM analysis that *in situ* formed glucans remain attached to the apatite surface and provide new surfaces in pellicle for potential interactions with bacteria.

To visualize adherent streptococci by SEM, pellicle-coated HA beads, with and without *in situ* formed glucans, were incubated with *S. mutans* GS-5 cells as per the standard adherence assay. After rinsing the HA beads with buffer to remove unbound bacteria, the beads were fixed and prepared for SEM as described above. The photomicrograph ($\times 10,000$ magnification) in Fig. 3d shows HA beads coated with PS and GTF (no sucrose added) which had been incubated with *S. mutans* GS-5 at a concentration of 2×10^9 cells per ml. No glucans were present in the pellicle and, as can be seen, very few bacteria are visible in the field of view. However, as shown in Fig. 3e, when the same number of bacteria (2×10^9 cells per ml) was added to HA beads coated with salivary pellicle containing *in situ* formed glucans, much higher numbers of bacteria attached to the HA surfaces. The only difference between the samples shown in Fig. 3d and e was the presence of glucans in the pellicle. The difference between the number of adherent bacteria in the two samples is striking and provides additional evidence that *in situ* formed glucans promoted the adherence of *S. mutans* cells to saliva-coated HA surfaces. Higher magnification (Fig. 3f, $\times 60,000$ magnification) showed what appeared to be direct contact between *S. mutans* GS-5 and *in situ* formed glucans.

At least three observations can be noted concerning SEM analysis of the adherent streptococci. First, the relative difference, detected by SEM, between the level of adherence

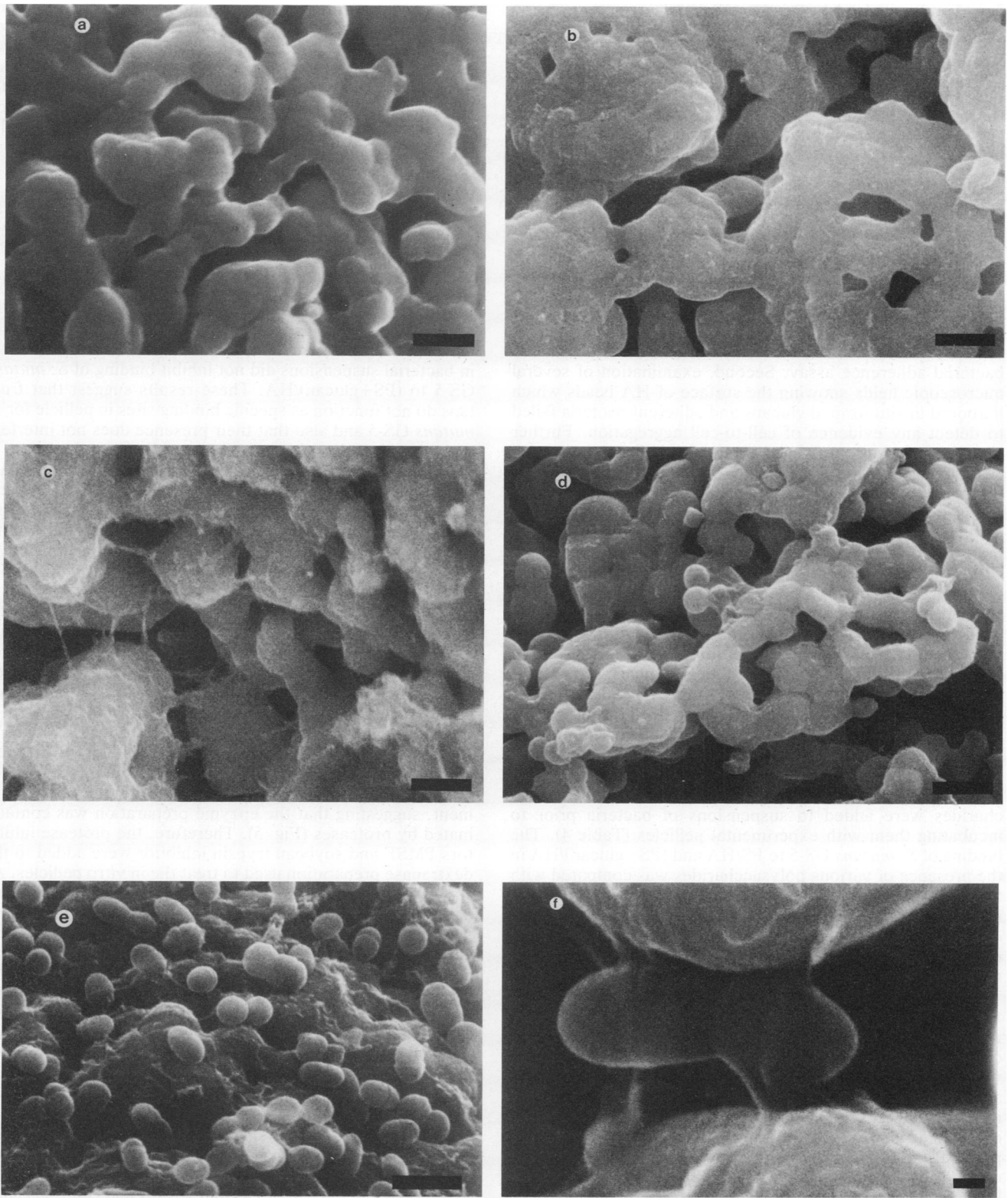


FIG. 3. SEM of *S. mutans* adherence to glucans synthesized in situ in experimental pellicle. (a) Naked HA beads. (b) HA beads coated with pellicle (PS and GTF). (c) HA beads coated with pellicle and incubated with sucrose to allow in situ formation of glucans. (d) *S. mutans* GS-5 cells bound to pellicle-coated HA beads (bacteria added at 2.0×10^9 cells per ml). (e and f) *S. mutans* GS-5 cells bound to HA beads coated with pellicle and in situ-formed glucans (bacteria added at 2.0×10^9 cells per ml). (a through e) Bar, $1.0 \mu\text{m}$. (f) Bar, $0.1 \mu\text{m}$.

TABLE 4. Effects of polysaccharides on adherence of *S. mutans* GS-5 to experimental pellicles

Polysaccharide added (final concn, 0.5 mg/ml)	% Control adherence ^a	
	PS/HA	(PS+glucan)/HA
Buffer	100.0	100.0
Dextran (α 1,6-glucose)	74.6	52.9
Starch (α 1,4-glucose)	153.1	95.5
Nigeran (α 1,3; α 1,4-glucose)	105.9	104.2
Levan (β 2,6-fructose)	127.7	116.0
Inulin (β 2,1-fructose)	120.2	103.5

^a Percent adherence compared with control samples in which bacteria were suspended in buffered KCl. Data shown are means ($n = 3$) from a single experiment.

of the streptococci to the pellicles containing glucans and the level of binding to pellicles formed from PS alone appeared to approximate the results obtained utilizing the radioactive bacterial adherence assay. Second, examination of several microscopic fields showing the surface of HA beads which harbored in situ formed glucans and adherent bacteria failed to detect any evidence of cell-to-cell aggregation. Further experiments were performed to ensure that the absence of cellular aggregates on the pellicles harboring glucans was not because of their being lost during preparation of the HA beads for SEM. Control samples which were formed by incubating (PS+glucan)/HA beads with mixtures of high-molecular-weight (2,000,000) soluble dextran and *S. mutans* GS-5 (i.e., aggregation-inducing conditions) showed evidence of adherent streptococcal aggregates which survived sample preparation for SEM (not shown). Finally, results from SEM analysis suggested that nonspecific trapping of bacteria did not play a role in the observed adherence of the *S. mutans* to in vitro pellicles containing glucans. Little, if any, glucan was detected covering the exposed surface of the streptococci in any field examined. In most instances, the bacteria were seen to bind in a monolayer on experimental pellicle surfaces.

Specificity of binding of *S. mutans* to experimental pellicles: effect of exogenously added polysaccharides. Various polysaccharides were added to suspensions of bacteria prior to incubating them with experimental pellicles (Table 4). The binding of *S. mutans* GS-5 to PS/HA and (PS+glucan)/HA in the presence of various polysaccharides was compared with that observed for bacteria suspended in buffer alone. Results indicated that low-molecular-weight (9,000) dextrans rich in α 1,6 linkages were the most effective inhibitors of binding to in situ formed glucans. Presumably the 9,000-molecular-weight dextrans interacted with glucan-binding adhesins, but were too small to cross-link the streptococcal cells. Quantitation of streptococcal adherence in the presence of high-molecular-weight (500,000) α 1,6-rich dextrans was complicated by the formation of large aggregates of bacteria which were difficult to separate from the HA beads. However, this aggregation also suggested that adhesins on *S. mutans* mediated stereospecific interactions with α 1,6-linked glucose residues. Addition of mutan, an insoluble glucan prepared from *S. sobrinus* GTF which has been shown by others to be a highly branched insoluble heteroglucan (mostly α 1,3-glucose, some α 1,6-glucose) (16), also gave variable results because of aggregation of the bacteria. The two other glucans tested, starch (α 1,4-glucose) and nigeran (alternating α 1,3; α 1,4-glucose) did not influence binding of the streptococci to in situ formed glucans and did not induce their aggregation.

The ability of 9,000-molecular-weight dextran to inhibit binding of *S. mutans* to experimental pellicles was tested over a range of dextran concentrations (Fig. 4). The degree of inhibition of *S. mutans* adherence to (PS+glucan)/HA was directly related to the concentration of the low-molecular-weight dextran. In contrast, adherence to PS/HA was only slightly inhibited by the 9.0-kDa dextran, and the inhibition was not related to dextran concentration. These results suggest that structures composed of α 1,6-linked glucose residues provide specific binding sites for *S. mutans* GS-5 in (PS+glucan)/HA.

The *S. mutans* GS-5 GTF preparation used in these experiments also contains low levels of active fructosyl-transferase which synthesizes fructans from sucrose. To determine whether the fructosyl structures played a role in *S. mutans* adherence, exogenously added fructans were also tested for their influence on adherence of *S. mutans* GS-5 to experimental pellicles. As seen in Table 4, the addition of inulin (mostly β 2,1-fructose) or levan (mostly β 2,6-fructose) in bacterial suspensions did not inhibit binding of *S. mutans* GS-5 to (PS+glucan)/HA. These results suggest that fructans do not function as specific binding sites in pellicle for *S. mutans* GS-5 and also that their presence does not interfere with glucan-mediated interactions.

In addition to the glucans and levans, some simple sugars were also tested (0.5 mg/ml) for their effects on adherence. The disaccharides isomaltose (6-*O*- α -D-glucopyranosyl-D-glucopyranose) and nigerose (3-*O*- α -D-glucopyranosyl-D-glucopyranose) and the trisaccharide melezitose (glucopyranosyl-sucrose) did not affect adherence of *S. mutans* GS-5 to (PS+glucan)/HA or PS/HA (data not shown).

Effects of dextranase treatment of experimental pellicles. The specificity of *S. mutans* adherence to pellicles containing in situ formed glucans was explored further in studies involving treatment of experimental pellicles with fungal dextranase (α 1,6-D-glucan 6-glucan hydrolase; *Penicillium* sp.). Results showed that *S. mutans* adherence to (PS+glucan)/HA was greatly reduced by prior treatment of the experimental pellicle with dextranase (Fig. 5). Furthermore, *S. mutans* adherence to PS/HA, which is devoid of glucans, was also significantly inhibited by dextranase treatment, suggesting that the enzyme preparation was contaminated by proteases (Fig. 5). Therefore, the protease inhibitors PMSF and soybean trypsin inhibitor were added to the dextranase preparation used to treat the in vitro pellicles. As shown in Fig. 5, inclusion of the protease inhibitors during dextranase treatment of PS/HA restored binding of *S. mutans* GS-5 to control levels. In contrast, streptococcal adherence to (PS+glucan)/HA was drastically reduced by dextranase treatment of the pellicle, with or without protease inhibitors present. Binding to (PS+glucan)/HA was partially restored to control levels when the dextranase was heat treated (70°C, 1.0 h) prior to use. These results further implicate α 1,6-linked glucose residues as forming the binding sites for *S. mutans* GS-5 in (PS+glucan)/HA. The dextranase preparation used in these experiments hydrolyzed 9,000- and 500,000-molecular-weight, α 1,6-rich dextrans but failed to catalyze hydrolysis of nigeran or mutan as detected by using glucose oxidase (45) to determine glucose and dinitrosalicylic acid to determine reducing sugar (29).

Adherence of various laboratory strains and clinical isolates of *S. mutans* to experimental pellicles. Results obtained in the present study showed that *S. mutans* GS-5 bound in low numbers to experimental pellicles derived from PS. However, results from studies by others indicate that strains of *S. mutans* differ with respect to their ability to bind to host-

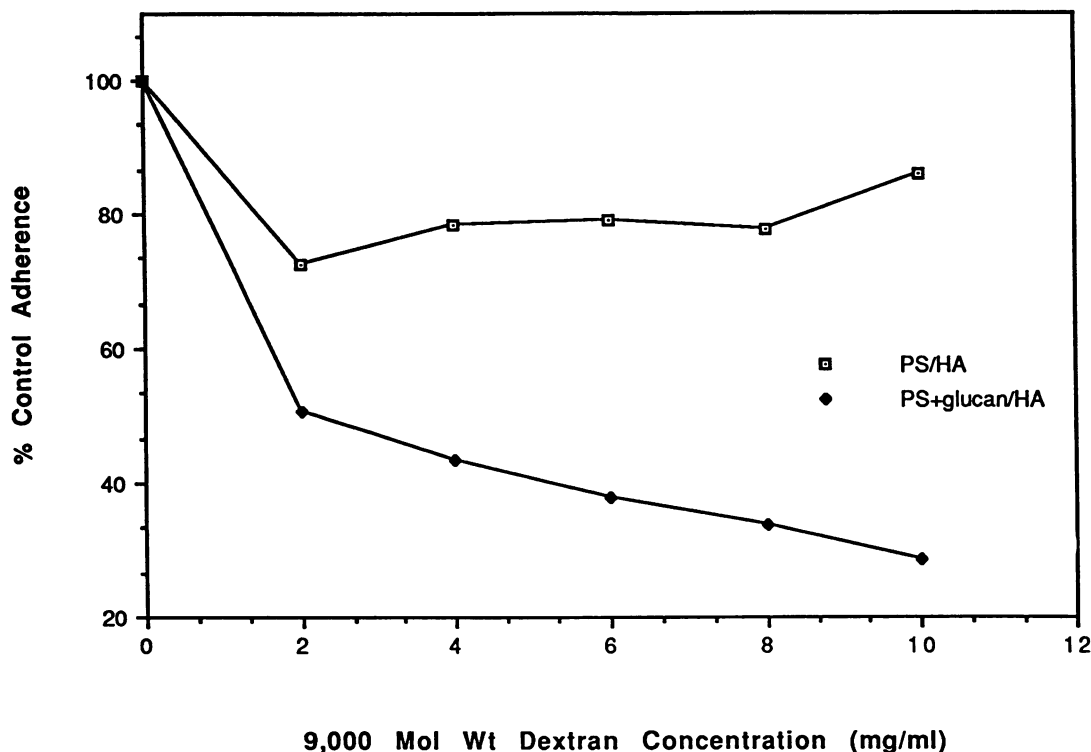


FIG. 4. Binding of *S. mutans* GS-5 to experimental pellicles in the presence of increasing concentrations of low-molecular-weight dextran. *S. mutans* GS-5 (final concentration, 2×10^9 cells per ml) was mixed for 15 min at 22°C with increasing concentrations of 9,000-molecular-weight soluble dextran (>90.0% α 1,6 linkages), after which the bacterium-polysaccharide mixtures were added to the prepared HA beads. Values represent means of three samples.

derived components in salivary pellicle (14). In addition, evidence from several studies has shown that the ability of *S. mutans* to be agglutinated by soluble dextrans also varies from strain to strain and can be lost upon repeated passage of the bacteria in vitro (15, 25). Therefore, several laboratory strains and clinical isolates of *S. mutans* were tested for the ability to attach to glucans formed in situ in pellicle.

The laboratory strains used in the present study have been

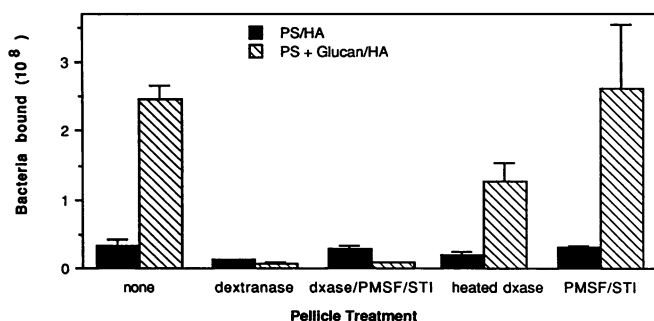


FIG. 5. Effects of treatment of experimental pellicles with fungal dextranase (dxase) on adherence of *S. mutans* GS-5. Pellicle-coated HA beads were treated with dextranase (1,6-D-glucan 6-glucanhydrolase) prior to incubation with radiolabeled *S. mutans* GS-5 (2.0×10^9 cells per ml). Control samples included those treated with either dextranase plus the protease inhibitors PMSF (1.0 mmol/liter) and soybean trypsin inhibitor (STI; 0.1 mg/ml), the protease inhibitors alone, or heat-inactivated dextranase (70°C, 60.0 min). Values represent means of three samples (\pm standard deviation).

studied by researchers for many years and have been passaged numerous times in vitro. Results showed that the ability of the laboratory strains of *S. mutans* to bind to glucans formed in situ varied greatly from strain to strain (Table 5). Five of eight laboratory strains tested bound in significantly higher numbers to (PS+glucan)/HA than observed for PS/HA; these included serotype c strains GS-5, C10449, NG-8, and Ingbritt, serotype e strain V100, and serotype f strain OMZ175. In contrast, in situ formed glucans did not promote binding of serotype c strains, Ingbritt, and MT8148 to experimental pellicles.

Laboratory strains of *S. mutans* also were highly variable

TABLE 5. Adherence of laboratory strains of *S. mutans* to experimental pellicles

<i>S. mutans</i> strain (serotype)	Bacteria (SD) bound to exptl pellicles (10^8) ^a	
	PS/HA	(PS+glucan)/HA
GS-5 (c)	0.09 (0.02)	1.10 (0.10)
C10449 (c)	0.51 (0.12)	1.88 (0.17)
MT8148 (c)	0.73 (0.07)	0.42 (0.05)
Ingbritt (c)	0.62 (0.06)	0.39 (0.05)
Ingbritt 1600 (c)	0.64 (0.08)	4.62 (0.46)
Ingbritt R (c)	1.99 (0.12)	1.92 (0.09)
V100 (e)	1.65 (0.12)	1.99 (0.08)
OMZ175 (f)	2.39 (0.14)	3.28 (0.24)

^a Bacteria were added at 2.0×10^9 cells per ml, except for V100 and OMZ175 which were added at 10^9 cells per ml. Data shown are means ($n = 3$) from a single experiment.

TABLE 6. Adherence of clinical isolates of *S. mutans* to experimental pellicles

Clinical <i>S. mutans</i> isolate ^a	Bacteria (SD) bound to exptl pellicles (10 ⁸) ^b		
	BSA/HA	PS/HA	(PS+glucan)/HA
GL-1	0.32 (0.01)	0.37 (0.02)	1.95 (0.14)
GL-2	0.32 (0.05)	0.51 (0.06)	2.28 (0.12)
GL-3	0.53 (0.03)	1.73 (0.30)	2.36 (0.07)
GL-4	0.23 (0.03)	1.28 (0.13)	1.62 (0.06)
GL-5	0.16 (0.01)	1.35 (0.16)	1.15 (0.37)
GL-6	0.24 (0.03)	1.21 (0.11)	1.79 (0.07)
GL-7	0.27 (0.01)	1.31 (0.04)	1.45 (0.02)
GL-8	0.28 (0.08)	0.99 (0.02)	0.82 (0.03)

^a Isolates were obtained from I. L. Shklair and were confirmed as serotype c by using type-specific fluorescent antisera.

^b All strains were added at 10⁹ cells per ml, except *S. mutans* GL-1, which was added at 2.0 × 10⁹ cells per ml. Data shown are means (n = 3) from a single experiment.

with respect to binding to pellicles formed from PS alone. Strains Ingbritt R, V100, and OMZ175 bound in significantly higher numbers to PS/HA than observed with the other strains; *S. mutans* GS-5 displayed the lowest binding to PS/HA. It is important to note that, among laboratory strains of *S. mutans*, the ability to bind to host-derived pellicle constituents varied independently from the ability to adhere to in situ formed glucans.

Clinical isolates of *S. mutans* were also tested for their ability to adhere to experimental pellicles (Table 6). The isolates were obtained from one source and have not been repeatedly subcultured in vitro. As shown in Table 6, most of the *S. mutans* isolates bound in highest numbers to pellicles containing glucans. However, they varied greatly in their relative ability to attach to pellicles formed from PS alone. Isolates GL-1 and GL-2 bound poorly to PS/HA and resembled *S. mutans* GS-5 in their overall adherence properties. In contrast, the remaining isolates bound in significantly higher numbers (four- to eightfold higher) to PS/HA than observed for BSA/HA.

For those *S. mutans* strains which bound in high numbers to both PS/HA and (PS+glucan)/HA, it was necessary to determine the contribution of host-derived components versus in situ formed glucans in forming the bacterial binding sites in (PS+glucan)/HA. Therefore, several of the isolates were also tested for adherence to PS/HA and (PS+glucan)/HA in the presence of soluble, low-molecular-weight dextrans, which act as specific inhibitors of binding to glucans in pellicle. As shown in Table 7, the addition of 1.0

mg of dextran (9,000 molecular-weight) per ml to suspensions of several different *S. mutans* isolates resulted in significant inhibition of their adherence to (PS+glucan)/HA, but did not affect binding to PS/HA. Furthermore, for all isolates tested, the number of bacterial cells which bound to (PS+glucan)/HA in the presence of 1.0 mg of dextran per ml was found to be much lower than the number which bound to PS/HA. This observation is of particular interest because it suggests that glucans formed in situ in salivary pellicle are able to block or mask host-derived components in (PS+glucan)/HA which function as bacterial binding sites in PS/HA. Otherwise, in the presence of low-molecular-weight dextrans, the streptococci should still have bound to (PS+glucan)/HA at levels similar to that observed for PS/HA.

DISCUSSION

Binding of mutans streptococci to glucans formed in situ in experimental pellicles. Results from the present study have demonstrated that strains of *S. mutans* can bind specifically to glucans formed in experimental salivary pellicles. These results suggest that glucan-mediated interactions are not restricted to the secondary accumulation of bacteria following adherence independent of the presence of sucrose, as implied by many researchers (5, 44), but also may facilitate the initial adherence to tooth surfaces by *S. mutans*. Furthermore, glucans could also contribute to the initial adherence of *S. mutans* to immature plaque, which usually contains other GTF-secreting bacterial species such as *S. sanguis*.

Results from SEM and separation studies with colloidal silica indicated that adherence of *S. mutans* GS-5 to pellicles containing in situ formed glucans involved binding of the streptococci to HA surface-associated polysaccharides and was not caused by cell-to-cell binding (Table 3; Fig. 3). Furthermore, no evidence of nonspecific trapping on the surfaces of the beads was revealed by SEM. These results appear to be in contrast to those obtained by Staat et al. (44), who used SEM to examine *S. sobrinus* 6715, which attached to saliva-coated HA surfaces in the presence and absence of sucrose. The *S. sobrinus* cells which attached to HA in the presence of sucrose displayed extensive glucan-mediated cell-to-cell adherence; streptococci which bound to HA in the absence of sucrose were observed to bind in discrete units. Based on these and other data, Staat et al. (44) concluded that glucan-mediated adherent interactions were important for accumulation of *S. sobrinus* on saliva-coated surfaces and were not involved in their initial adherence.

TABLE 7. Effect of low-molecular-weight dextran on adherence of clinical isolates of *S. mutans*

Clinical <i>S. mutans</i> isolate	PS/HA (bacteria bound to exptl pellicles [10 ⁸]) ^a		% Inhibition	(PS+glucan)/HA (bacteria bound to exptl pellicles [10 ⁸])		% Inhibition
	Alone	With dextran		Alone	With dextran	
GL-3	1.73 (0.30)	ND	ND	2.36 (0.07)	0.82 (0.03)	65.0
GL-4	1.28 (0.13)	ND	ND	1.62 (0.06)	0.66 (0.04)	59.1
GL-5	1.35 (0.16)	ND	ND	1.15 (0.37)	0.45 (0.05)	60.9
GL-6	1.21 (0.11)	1.17 (0.13)	3.3	1.79 (0.07)	0.22 (0.02)	72.2
GL-7	1.31 (0.04)	1.23 (0.06)	6.2	1.45 (0.02)	0.50 (0.04)	65.5
GL-8	0.99 (0.02)	1.01 (0.08)	0.0	0.82 (0.03)	0.23 (0.05)	72.0

^a Bacteria were added at 10⁹ cells per ml in either the presence or the absence of 1.0 mg of 9,000-molecular-weight dextran per ml. The effect of dextran on bacterial binding to PS/HA and to (PS+glucan)/HA is expressed as percent inhibition. Data shown are means (n = 3) from a single experiment; standard deviations are shown in parentheses. ND, not determined.

However, their model was not designed to distinguish between glucan-mediated adherence and sucrose-mediated accumulation resulting from de novo synthesis of glucans.

Data from the present study conflict with observations reported by Gibbons et al. (14). Results of their studies showed that specific strains of *S. mutans*, including *S. mutans* JPB, were not able to bind to glucans on apatite surfaces, but did interact with host-derived components in experimental pellicles. Most likely, the conflicting results are due to differences in the expression of GBPs by the streptococcal strains used in the two studies. Strain-to-strain differences in glucan binding by *S. mutans* have been observed elsewhere. For instance, Inoue et al. (19) have reported that high-molecular-weight dextrans agglutinated strains of *S. sobrinus* and *S. cricetus*, but did not agglutinate representative strains of *S. mutans*. However, results from studies by several other groups have demonstrated that many strains of *S. mutans*, including some of the same ones examined by Inoue and colleagues, could be agglutinated by minute amounts of high-molecular-weight dextrans (9, 20, 51).

The expression of GBPs by the mutans streptococci is influenced by a variety of factors. For instance, the culture conditions used to grow *S. mutans*, *S. sobrinus*, and *S. cricetus* have been shown to influence their dextran-binding activities (10, 51). Furthermore, repeated in vitro passage of *S. mutans* strains can diminish their ability to undergo dextran-induced agglutination. Results of studies by Gibbons and Fitzgerald (15) showed that *S. mutans* GS-5 which had been subcultured repeatedly was not agglutinated by high-molecular-weight dextrans. However, in the same study, another strain of *S. mutans* GS-5 (GS-5MR), provided by Bowen and colleagues after reisolation from a monkey, was readily agglutinated by dextran. These observations emphasize the potential problems associated with studying the adherence of laboratory strains of streptococci which may have been repeatedly passaged in vitro.

Properties of the interactions which mediate *S. mutans* binding to glucans formed in pellicle. Glucans synthesized by streptococcal GTF are generally mixed heteropolymers composed predominantly of α 1,6- and α 1,3-linked glucose residues (11, 16). However, data from the present study indicate that α 1,6-linked glucosyl structures are critical in binding of *S. mutans* GS-5 (Table 4; Fig. 4 and 5). These results are in agreement with recent observations concerning the GBPs of other species of mutans streptococci. For instance, Drake et al. (9) demonstrated that glucan-induced agglutination of *S. cricetus* AHT was inhibited by low-molecular-weight glucans rich in α 1,6 linkages, but not by glucans composed predominantly of other anomeric linkages. Furthermore, Landale and McCabe (26) utilized an affinity electrophoresis system to show that a GBP isolated from *S. sobrinus* 6715 specifically recognized ligands composed of α 1,6-linked glucose residues.

The specific adhesins which mediated the binding of *S. mutans* strains to glucans formed in pellicle remain to be identified. In the present study, *S. mutans* strains were grown under conditions which favor low levels of cell-associated GTF activity. However, in vivo, both GTF and non-GTF GBPs probably play a role (17, 18, 33, 40). The non-GTF glucan-binding adhesins produced by *S. mutans* include GBP, the product of the *gbp* gene which was first isolated by Russell and colleagues (40). Recently, Perry and Kuramitsu (37) reported the location of *gbp* in a region on the chromosome of *S. mutans* GS-5 which contains several sucrose-metabolizing genes. Antibodies raised against GBP

have been shown to inhibit sucrose-dependent accumulation of *S. mutans* Ingbritt on glass surfaces (7). However, anti-GBP and anti-GTF antisera failed to inhibit dextran-induced agglutination of *S. mutans* Ingbritt, suggesting that *S. mutans* produces non-GTF GBPs other than GBP (7).

Protein P1 (1) (also designated AgB [8], Ag I/II [1], and Pac [36]), which has been implicated in the binding of *S. mutans* to saliva-coated surfaces (1, 8, 36), has also been identified as a potential glucan-binding adhesin. Douglas and Russell have shown that antibodies specific for P1 inhibit sucrose-induced accumulation of *S. mutans* on glass surfaces (7). However, despite the indirect evidence of a role for P1 in glucan binding, results from the present study suggest otherwise. *S. mutans* GS-5 was found to bind quite well to glucans in pellicle, even though available evidence suggests that this strain produces a variant form of P1. The P1 produced by *S. mutans* GS-5 is aberrantly small (35) (i.e., 155,000 instead of the normal 190,000 molecular weight), and very little P1 is found associated with the surface of *S. mutans* GS-5. Instead, most of the protein is released into the bacterium's culture fluid (1, 21).

We have recently obtained further evidence that P1 is not involved in glucan binding by *S. mutans* (2). An isogenic P1-minus mutant of *S. mutans* NG8 (28) was found to attach to PS-coated HA in much lower numbers than its parent strain, but still bound at very high levels to pellicles containing in situ formed glucans and still successfully colonized rats fed a sucrose-rich diet (2).

Expression of multiple mechanisms of adherence by strains of *S. mutans*. Although *S. mutans* GS-5 bound poorly to pellicles derived from host-derived salivary components, several other laboratory strains and clinical isolates of *S. mutans* were found to bind at high levels to pellicles derived from PS (Tables 5 and 6). Furthermore, the adherence of many of these same strains was also promoted by the presence of in situ formed glucans. These results suggest that *S. mutans* expresses multiple adhesins with differing specificities that are able to mediate attachment of the bacteria to host-derived and bacterium-derived binding sites on tooth surfaces, an observation consistent with that reported for many other species of plaque bacteria (3, 13, 34).

Available evidence suggests that *S. mutans* may regulate expression of adhesins at the level of cell surface localization. Results of studies by McBride et al. (30) demonstrated that repeated passage of *S. mutans* in vitro resulted in the generation of hydrophilic variant strains which were unable to incorporate several extracellular proteins in their cell walls; instead, the proteins (including protein P1) were released into the surrounding medium). These strains showed decreased adherence to saliva-coated HA and reduced implantability on the teeth of human volunteers (46).

The ability of *S. mutans* to bind to glucans is also diminished by repeated passage in vitro (15, 25). However, results from the present study indicate that the expression of glucan-binding adhesins is regulated by mechanisms which are different than those controlling the expression of saliva-binding adhesins. Results showed that the ability of several *S. mutans* strains to bind to in situ formed glucans varied independently of their ability to bind to host-derived binding sites in pellicle (Tables 5 and 6).

Steric effects of glucans on host-derived components in pellicle. One unexplored but potentially significant effect of GTF and product glucans incorporated in pellicle and plaque resides in their influence on the biological activities of host-derived components adsorbed to tooth surfaces. For instance, results presented here indicate that in situ formed

glucans can mask host-derived bacterial binding sites in pellicle. This masking effect was detected with strains of *S. mutans* which are able to bind to both glucans and host-derived components (Table 7). The apparent blocking of host-derived *S. mutans* binding sites by in situ formed glucans suggests that glucans formed in pellicle could influence adherence by other species, such as *S. sanguis*, *S. mitis*, and *Actinomyces viscosus*, which bind specifically to adsorbed host-derived salivary components (3, 13, 34). It is tempting to speculate that GTF and product glucans on tooth surfaces may influence the pathogenic potential of plaque by selectively promoting binding of the mutans streptococci at the expense of other bacterial species and by protecting the adherent bacteria from some of the inimical forces which threaten their survival (43). In these ways, GTF may act as a virulence factor in a context not previously considered.

ACKNOWLEDGMENTS

We acknowledge the advice and suggestions of R. A. Burne and L. A. Tabak in various aspects of this investigation. We also acknowledge the technical assistance of Maureen Blitzer and the assistance in SEM provided by Sandra MacCormack of the Eastman Dental Center, Rochester, N.Y.

This work was supported in part by Public Health Service grant DE07907 from the National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

REFERENCES

1. Ayakawa, G. Y., L. W. Boushell, P. J. Crowley, G. W. Erdos, W. P. McArthur, and A. S. Bleiweis. 1987. Isolation and characterization of monoclonal antibodies specific for antigen P1, a major surface protein of mutans streptococci. *Infect. Immun.* **55**:2759-2767.
2. Bowen, W. H., K. M. Schilling, E. Giertsen, S. Pearson, S. F. Lee, A. S. Bleiweis, and D. Beeman. 1991. Role of a cell surface-associated protein in adherence and dental caries. *Infect. Immun.* **59**:4606-4609.
3. Cisar, J. D., A. L. Sandberg, and S. E. Mergenhagen. 1984. The function and distribution of different fimbriae on strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. *J. Dent. Res.* **63**:393-396.
4. Clark, W. B., L. L. Bammann, and R. J. Gibbons. 1978. Comparative estimated bacterial affinities and adsorption sites on hydroxyapatite surfaces. *Infect. Immun.* **19**:846-853.
5. Clark, W. B., and R. J. Gibbons. 1977. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. *Infect. Immun.* **18**:514-523.
6. Curtiss, R., S. A. Larrimore, R. G. Holt, J. F. Barrett, R. Barletta, H. Murchison, S. M. Michalek, and S. Saito. 1983. Analysis of *Streptococcus mutans* virulence attributes using recombinant DNA and immunological techniques, p. 95-104. *In* R. J. Doyle and J. E. Ciardi (ed.), *Glucosyltransferases, sucrose, and dental caries*. Spec. Suppl. Chemical Senses. IRL Press, Washington, D.C.
7. Douglas, C. W. I., and R. R. B. Russell. 1982. Effect of specific antisera on the adherence properties of the oral bacterium, *Streptococcus mutans*. *Arch. Oral Biol.* **27**:1039-1045.
8. Douglas, C. W. I., and R. R. B. Russell. 1984. Effect of specific antisera upon *Streptococcus mutans* adherence to saliva-coated hydroxyapatite. *FEMS Microbiol. Lett.* **25**:211-214.
9. Drake, D., K. G. Taylor, A. S. Bleiweis, and R. J. Doyle. 1988. Specificity of the glucan-binding lectin of *Streptococcus cricetus*. *Infect. Immun.* **56**:1864-1872.
10. Drake, D., K. G. Taylor, and R. J. Doyle. 1988. Expression of the glucan-binding lectin of *Streptococcus cricetus* requires manganous ion. *Infect. Immun.* **56**:2205-2207.
11. Freedman, R. J., D. Birkhed, and K. Granath. 1978. Analysis of glucans from cariogenic and mutant *Streptococcus mutans*. *Infect. Immun.* **21**:17-27.
12. Frostell, G., P. H. Keyes, and R. H. Larson. 1967. The effect of various sugars and sugar substitutes on dental caries in hamsters and rats. *J. Nutr.* **93**:65-76.
13. Gibbons, R. J. 1984. Adherent interactions that may affect microbial ecology in the mouth. *J. Dent. Res.* **63**:378-385.
14. Gibbons, R. J., L. Cohen, and D. I. Hay. 1986. Strains of *Streptococcus mutans* and *Streptococcus sobrinus* attach to different pellicle receptors. *Infect. Immun.* **52**:555-561.
15. Gibbons, R. J., and R. J. Fitzgerald. 1969. Dextran-induced agglutination of *Streptococcus mutans* and its potential role in the formation of microbial dental plaques. *J. Bacteriol.* **98**:341-346.
16. Guggenheim, B. 1970. Enzymatic hydrolysis and structure of water-insoluble glucan produced by glucosyltransferases from strains of *Streptococcus mutans*. *Helv. Odont. Acta* **5**:89-108.
17. Hamada, S., and H. D. Slade. 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* **44**:331-384.
18. Hamada, S., and M. Torii. 1978. Effect of sucrose in culture media on location of glucosyltransferase of *Streptococcus mutans* and cell adherence to glass surfaces. *Infect. Immun.* **20**:592-599.
19. Inoue, M., H. Shibata, and T. Morioka. 1982. Serotype specificity for some biochemical characteristics of *Streptococcus mutans*. *Microbios* **33**:7-14.
20. Kelstrup, J., and T. D. Funder-Nielsen. 1974. Adhesion of dextran to *Streptococcus mutans*. *J. Gen. Microbiol.* **81**:485-489.
21. Knox, K. W., L. N. Hardy, and A. J. Wicken. 1986. Comparative studies on the protein profiles and hydrophobicity of strains of *Streptococcus mutans* serotype c. *J. Gen. Microbiol.* **132**:2541-2548.
22. Koga, T., H. Asakawa, N. Okahashi, and S. Hamada. 1986. Sucrose-dependent cell adherence and cariogenicity of serotype c *Streptococcus mutans*. *J. Gen. Microbiol.* **132**:2873-2883.
23. Krasse, B. 1965. The effects of caries-inducing streptococci in hamsters fed diets with sucrose or glucose. *Arch. Oral Biol.* **10**:223-226.
24. Kuramitsu, H. K. 1974. Adherence of *Streptococcus mutans* to dextran synthesized in the presence of extracellular dextranase. *Infect. Immun.* **9**:764-765.
25. Kuramitsu, H. K. 1976. Properties of a mutant of *Streptococcus mutans* altered in glucosyltransferase activity. *Infect. Immun.* **13**:345-353.
26. Landale, E. C. and M. M. McCabe. 1987. Characterization by affinity electrophoresis of an α 1,6-glucan-binding protein from *Streptococcus sobrinus*. *Infect. Immun.* **55**:3011-3016.
27. Lashley, K. S. 1916. Reflex secretion of the parotid gland. *J. Exp. Psychol.* **1**:461-465.
28. Lee, S. F., A. Progluske-Fox, G. W. Erdos, D. A. Piacentini, G. Y. Ayakawa, P. J. Crowley, and A. S. Bleiweis. 1989. Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in the major surface protein antigen P1 (I/II). *Infect. Immun.* **57**:3306-3313.
29. Luchsinger, W. W., and R. A. Cornesky. 1962. Reducing power by the dinitrosalicylic acid method. *Anal. Biochem.* **4**:346-347.
30. McBride, B. C., E. J. Morris, and N. Ganeshkumar. 1985. Relationship of streptococcal cell surface proteins to hydrophobicity and adherence, p. 85-93. *In* S. A. Mergenhagen and B. Rosan (ed.), *Molecular basis of oral microbial adhesion*. American Society for Microbiology, Washington, D.C.
31. McCabe, M. M., and R. M. Hamelik. 1978. Multiple forms of dextran-binding proteins from *Streptococcus mutans*. *Adv. Exp. Med. Biol.* **107**:749-759.
32. McCabe, M. M., and E. E. Smith. 1975. Relationship between cell-bound dextranase and the agglutination of *Streptococcus mutans*. *Infect. Immun.* **12**:512-520.
33. Mooser, G., and C. Wong. 1988. Isolation of a glucan-binding domain of glucosyltransferase (1,6- α -glucan synthase) from *Streptococcus sobrinus*. *Infect. Immun.* **56**:880-884.
34. Morris, E. J., and B. C. McBride. 1984. Adherence of *Streptococcus sanguis* to saliva-coated hydroxyapatite: evidence for two binding sites. *Infect. Immun.* **43**:656-663.

35. Ohta, H., H. Kato, N. Okahashi, I. Takahashi, S. Hamada, and T. Koga. 1989. Characterization of a cell-surface protein antigen of hydrophillic *Streptococcus mutans* strain GS-5. *J. Gen. Microbiol.* 135:981-988.
36. Okahashi, N., C. Shahkawa, M. Yoshikawa, S. Hamada, and T. Koga. 1989. Molecular characterization of a surface protein antigen gene from serotype c *Streptococcus mutans* implicated in dental caries. *Mol. Microbiol.* 3:673-678.
37. Perry, D., and H. K. Kuramitsu. 1990. Linkage of sucrose-metabolizing genes in *Streptococcus mutans*. *Infect. Immun.* 58:3462-3464.
38. Rølla, G., J. E. Ciardi, K. Eggen, W. H. Bowen, and J. Afseth. 1983. Free glucosyl- and fructosyltransferase in human saliva and adsorption of these enzymes to teeth *in vivo*, p. 21-30. In R. J. Doyle and J. E. Ciardi, (ed.), *Glucosyltransferase, glucans, sucrose, and dental caries*. Spec. Suppl. Chemical Senses. IRL Press, Washington, D.C.
39. Russell, R. R. B. 1979. Glucan binding proteins of *Streptococcus mutans* serotype c. *J. Gen. Microbiol.* 112:197-201.
40. Russell, R. R. B., D. Coleman, and G. Dougan. 1985. Expression of a gene for glucan-binding protein from *Streptococcus mutans* in *Escherichia coli*. *J. Gen. Microbiol.* 131:295-299.
41. Scheie, A. A., K. H. Eggen, and G. Rølla. 1986. Glucosyltransferase activity in human *in vivo* formed pellicle and in whole saliva. *Scand. J. Dent. Res.* 95:212-215.
42. Schilling, K. M., M. H. Blitzer, and W. H. Bowen. 1989. Adherence of *Streptococcus mutans* to glucans formed *in situ* in salivary pellicle. *J. Dent. Res.* 68(Spec. Issue):1678-1680.
43. Schilling, K. M., and W. H. Bowen. 1988. The activity of glucosyltransferase adsorbed onto saliva-coated hydroxyapatite. *J. Dent. Res.* 67:2-8.
44. Staat, R. H., S. D. Langely, and R. J. Doyle. 1980. *Streptococcus mutans* adherence: presumptive evidence for protein-mediated attachment followed by glucan-dependent cellular accumulation. *Infect. Immun.* 27:675-681.
45. Stein, D. C., L. K. Kopec, R. B. Yasbin, and F. E. Young. 1984. Characterization of a strain of *Bacillus subtilis* DSM704 and its production of 1-deoxyinojiromycin. *Appl. Environ. Microbiol.* 48:280-284.
46. Svanborg, M., G. Westergren, and J. Olsson. 1984. Oral implantation in humans of *Streptococcus mutans* strains with different degrees of hydrophobicity. *Infect. Immun.* 43:817-821.
47. Tanzer, J. M., M. L. Freedman, and R. J. Fitzgerald. 1985. Virulence of mutants defective in glucosyltransferase, dextran-mediated aggregation, or dextranase activity, p. 204-211. In S. A. Mergenhagen and B. Rosan (ed.), *Molecular basis of oral microbial adhesion*. American Society for Microbiology, Washington, D.C.
48. van Houte, J., R. C. Burgess, and H. Onose. 1976. Oral implantation of human strains of *Streptococcus mutans* in rats fed sucrose or glucose diets. *Arch. Oral Biol.* 21:561-564.
49. van Houte, J., and S. Duchin. 1975. *Streptococcus mutans* in the mouths of children with congenital sucrose deficiency. *Arch. Oral Biol.* 20:771-773.
50. van Houte, J., and D. B. Green. 1974. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. *Infect. Immun.* 9:624-628.
51. Wu-Yuan, C. D., S. Tai, and H. D. Slade. 1979. Properties of *Streptococcus mutans* grown in a synthetic medium: binding of glucosyltransferase and *in vitro* adherence and binding of dextran/glucan and glycoprotein and agglutination. *Infect. Immun.* 23:600-608.