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In Vitro Inhibition of Adherence of *Streptococcus mutans* Strains by Nonadherent Mutants of *S. mutans* 6715

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Four nonadherent mutants from *Streptococcus mutans* 6715 mutant UAB66 (serotype g) with similar phenotypes were shown to inhibit the adherence of adherence-proficient *S. mutans* serotypes c and g strains. One mutant, UAB108, was shown to inhibit adherence by wild-type strains representing serotypes a, d, and e as well. This inhibition of adherence was seen with pairs of strains grown in partially defined (PD) medium supplemented with 1% sucrose in both microtiter plates and glass tubes. The inhibiting factor was present in culture supernatant fluids of inhibiting strains grown in PD medium plus 1% sucrose and was heat stable. Ethanol precipitation of culture supernatant fluids of these strains yielded a water-soluble polymer which effectively inhibited the adherence of UAB66. This polymer, isolated from UAB108, was also shown to inhibit the adherence of UAB66 at lower concentrations than that needed to inhibit adherence with dextran T10. Partially purified glucosyltransferase, isolated from the culture supernatant fluids of glucose-grown UAB108, produced a water-soluble glucan which was shown to inhibit the adherence of UAB66 as well. The methods developed permit rapid screening for strains or mutants of strains or both that inhibit adherence or plaque formation or both by wild-type strains of *S. mutans*

Dental caries, resulting from infection by *Streptococcus mutans* in the presence of dietary sucrose, are generally considered to be due to the interaction of several factors. These factors include adherence of the bacteria to the tooth surface mediated by proteins (32) and water-insoluble glucans (WIG; 3, 9, 10, 12, 19, 21, 25, 26, 35), aggregation in a plaque matrix (6, 8), and acid production with subsequent enamel demineralization (15, 18). In an effort to better understand the interaction of these factors, we previously isolated and characterized a number of mutants of *S. mutans* which are defective in one or more of these virulence characteristics. We divided these mutants into at least 13 phenotypic groups based on phenotypic traits (27). Mixed cultivation in sucrose-containing medium of two nonadhering mutants of differing phenotypic traits (UAB95 and UAB230) allowed complementation for adherence, and these mutants complemented each other for caries production in gnotobiotic rats as well (24).

One unexpected observation from in vitro complementation studies was that several mutants, namely UAB108, UAB113, UAB124, and UAB244, inhibited the adherence of both mutant and wild-type control strains (24). These mutants fall into the same phenotypic group (27). Although there are slight differences in their colony morphologies on mitis salivarius (MS) agar, all of these mutant strains produce colonies that are submerged in a clear sticky fluid. All are devoid of dextranase activity and are adherence defective.

In this study, we more closely examine this phenomenon of inhibition of adherence by UAB108. Results indicate that an ethanol-precipitable polymer present in the supernatant fluid of UAB108 cultures grown in sucrose-containing medium effectively inhibits adherence of wild-type UAB62 (serotype c) and UAB66 (serotype g). In addition, a water-soluble glucan (WSG) synthesized from sucrose by glucosyltransferase (GTF) isolated from glucose-grown UAB108 was also shown to inhibit the adherence of its parent UAB66. The companion paper (34) describes more fully the properties of this inhibitory glucan and its mode of action.

MATERIALS AND METHODS

Bacterial strains and culture media. The adherence-proficient parental strains and their adherence-deficient mutants used in this study are listed in Table 1. The isolation of these mutants and methods to describe and quantitate the adherence attributes and to measure dextranase activities were previously given (27). UAB108, UAB113, UAB124, and UAB244 are devoid of dextranase activity. Additional strains representing the serotypes a through f and wild-type isolates used from our collection are as follows: (i) serotype a, AHT and HS6; (ii) serotype b, BHT; (iii) serotype c UA159, UA174, MT8148, V318, GS5, UA101, and UA130; (iv) serotype d, OMZ176; (v) serotype e, LM7; (vi) serotype f, OMZ175; and (vii) *S. mutans* 3098791 possible serotype c/e and *S. sanguis* Challis. All strains were maintained at −70°C in 1% peptone (Difco Laboratories, Detroit, Mich.) and 5% glycerol and at 4°C on brain heart infusion (BHI) agar (Difco) slants. Slants were transferred monthly.

Strains were cultured standing at 37°C in BHI broth (Difco) or partially defined (PD) medium (27) containing either 1% sucrose (PD scr) or 2% glucose. Colony morphology characteristics were examined on MS agar (Difco) after 48 h of anaerobic incubation (GasPak anaerobe system with a CO_2-H_2 atmosphere; BBL Microbiology Systems, Cockeysville, Md.) at 37°C (27).

Inhibition of adherence by growing cells. Inhibition of adherence of wild-type strains representing serotypes a through f, parent strains, and adherence-proficient mutants by adherence-deficient mutants was examined by crystal

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TABLE 1. Bacterial strains

Strain	Source (serotype)	Phenotype[a]	Colony morphology[b]
UAB62	PS14 (c)	Str^r Rif^r Adh$^+$ Dex$^+$	Rough, hard, slightly irregular, droplike, slightly adherent
UAB66	6715 (g)	Str^r Spc^r Adh$^+$ Dex$^+$	Rough, slightly soft, irregular, convex with a depressed center, adherent with part of colony submerged in agar
UAB108	UAB66	Str^r Spc^r Adh$^-$ Dex$^-$	Large, smooth, very soft, irregular, raised, contained within a clear sticky fluid
UAB113	UAB66	Str^r Spc^r Adh$^-$ Dex$^-$	Smooth, soft, round, raised, surrounded by a clear fluid
UAB122	UAB66	Str^r Spc^r Adh$^+$ Dex$^-$	Soft, round, convex with a rough dull rim and a smooth shiny center
UAB124	UAB66	Str^r Spc^r Adh$^-$ Dex$^-$	Smooth, soft, slightly irregular, convex, surrounded by a clear fluid
UAB158	UAB66	Str^r Spc^r Adh$^+$ Dex$^-$	Rough, hard, irregular, convex with depressed center
UAB241	UAB66	Str^r Spc^r Adh$^-$ Dex$^+$	Very rough, grainy, round, slight cone shape
UAB244	UAB66	Str^r Spc^r Adh$^-$ Dex$^-$	Smooth, soft, round, convex, surrounded by a clear fluid
UAB631	6715 (g)	Str^r Adh$^+$ Dex$^-$	Same as UAB66

[a] Adh$^-$, Adherence defective; Adh$^+$, adherence proficient; Dex$^-$, dextranase defective; Dex$^+$, produces dextranase, Rif^r, rifampin resistant; Spc^r, spectinomycin resistant; Str^r, streptomycin resistant (27).
[b] On MS agar after 48 h of anaerobic incubation (27).

violet staining of adhered growth in microtiter plates (24; Costar, Cambridge, Mass.) and in glass tubes as previously described (27). The brand of microtiter plates used was selected to yield optimal attachment in sucrose-containing medium by the parental strains UAB62 and UAB66 and was not necessarily optimal for attachment by other wild-type *S. mutans* strains of various serotypes. Quantitation of inhibition of adherence of UAB62 and UAB66 by UAB108 was determined by using sterile disposable borosilicate test tubes (13 by 100 mm) containing 3 ml of PD scr. These tubes were inoculated with 100 μl of 18- to 20-h BHI broth-grown cultures of UAB108, UAB62, and UAB66, separately and in pairs, and incubated standing at 37°C for 24 h. (Each of these three strains grows at the same rate and achieves the same density after overnight growth in BHI broth.) Tubes were vortexed, and the culture supernatant fluids were poured off. Adherent cells were washed twice with 3 ml of buffered saline with gelatin (BSG; 2), and these washes were pooled with the corresponding supernatant fluids. Culture supernatant and wash fluids were then centrifuged for 15 min at 10,000 rpm in an SS34 rotor of a Sorvall SS3 centrifuge to sediment the cells. The resulting pellet was suspended in 3 ml of BSG. BSG (3 ml) was also added to tubes containing adherent cells, and all fractions were sonicated with a microprobe for 30 s at 100 W (Braun-Sonic 1510; B. Braun, Melsunge, AG) to disperse clumps (24). Cell suspensions were then diluted in BSG and plated on MS agar. After 48 h of anaerobic incubation at 37°C, the relative cell numbers of strains in each fraction (based on colony morphologies) were determined.

Inhibition of adherence by culture supernatant fluids. Strains UAB62, UAB66, UAB108, UAB113, UAB122, UAB124, and UAB244 were grown for 18 to 20 h at 37°C in 50 ml of PD scr. Cells were removed by two successive centrifugations at 15,000 rpm for 15 min in an SS34 rotor of a Sorvall SS3 centrifuge, and the culture supernatant fluids were split into two 25-ml fractions, one of which was boiled for 20 min. Each fraction was diluted with 25 ml of 2× PD medium, and sucrose was added to a final concentration of 1%. The pH was adjusted to approximately 6.8 with NaOH. Three milliliters of each supernatant fluid sample was added to borosilicate glass disposable test tubes and inoculated with 100 μl of one of the adherence-proficient strains, i.e., UAB122, UAB62, or UAB66. Supernatant fluids from these strains were used as controls. After 18 to 20 h of incubation standing at 37°C, the tubes were scored for adherence on a scale of 0 to 4+ as described elsewhere (27).

Inhibition of adherence by ethanol precipitates. Culture supernatant fluids (500 ml) of adherence-inhibiting strains UAB108, UAB113, UAB124, and UAB244 grown in PD scr medium for 48 h standing at 37°C were centrifuged as described above and treated with 3 volumes of ethanol at 4°C for 1 h. The resulting precipitates were collected by centrifugation, washed three times with ethanol, and then dissolved in 250 ml of water. Samples were dialyzed against water for 24 h. The carbohydrate concentration was determined by the phenol-sulfuric acid method (5). PD medium (2×) was diluted with an equal volume of dialyzed polymer, and sucrose was added to a final concentration of 1%. To 3 ml of PD scr plus polymer, a 100-μl inoculum of an 18- to 20-h BHI broth-grown UAB66 culture was added. After overnight incubation (for 18 to 20 h) standing at 37°C, the tubes were scored for adherence as described elsewhere (27).

For quantitation of the amount of UAB108 polymer needed to inhibit adherence, the dialyzed polymer solution was lyophilized and then added to PD scr at concentrations of 4, 8, and 12 mg/ml. Controls contained either dextran T10 (Sigma Chemical Co., St. Louis, Mo.) at 10, 25, or 50 mg/ml or no additional polymer. To 3 ml of PD scr, PD scr plus polymer, and PD scr plus dextran T10 media, a 100-μl inoculum of overnight UAB122, UAB62, or UAB66 was added. After 24 h of incubation standing at 37°C, the tubes were scored for adherence as previously described (27).

Inhibition of adherence by glucan synthesized by UAB108 GTF. A partially purified GTF preparation from 1-liter of culture supernatant fluid from UAB108 grown in PD medium with 2% glucose was prepared as described previously (24) and used to synthesize WSG in vitro by suspending 20 μg of partially purified GTF per ml in phosphate-buffered saline plus 5% sucrose. This mixture was incubated for 18 h at 37°C and then mixed with 2 volumes of ethanol for 2 h at 4°C. The precipitate was removed by centrifugation, dissolved in water, and then reprecipitated with 2 volumes of ethanol. After centrifugation, the precipitate was lyophilized. PD scr (3 ml) with and without added glucan was inoculated with 100 μl of cells of UAB66 grown overnight in BHI broth and incubated standing for 18 h at 37°C. Adherence was scored as described elsewhere (27).

Colony morphology and size analyses. Cultures of UAB66 (wild type) and its adherence-inhibiting mutants were grown anaerobically in BHI broth for 24 h, diluted, and then plated onto MS agar and incubated anaerobically for 18, 24, 36, and 48 h. Colony diameters and morphologies and amounts of glucan were recorded.

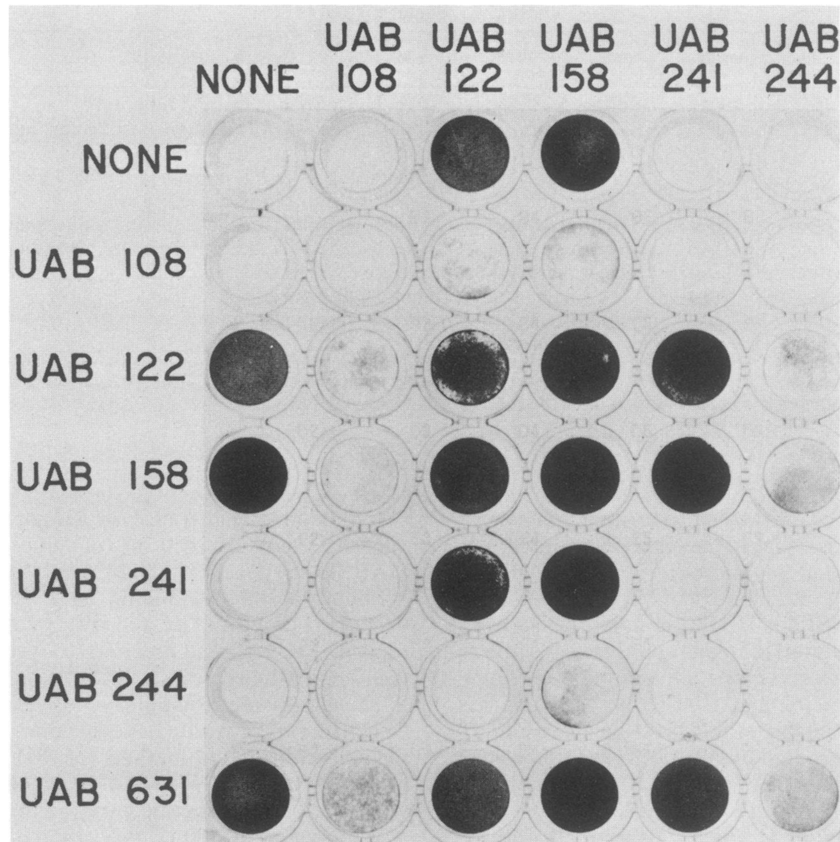

FIG. 1. Microtiter wells of adherent and nonadherent strains grown individually and in pairs in PD scr, washed to remove nonadhering cells, and stained with 1% crystal violet solution.

RESULTS

Inhibition of adherence by growing cells. Analysis of microtiter plate wells inoculated with all pairwise combinations of nonadherent and adherent strains showed both complementation for adherence and also various degrees of inhibition of adherence (24). The adherence of UAB122, UAB158, and UAB631 is inhibited by growth in PD scr with either UAB108 or UAB244 (Fig. 1). Furthermore, adherence of the wild-type UAB62 and UAB66 strains was inhibited by these strains and by UAB113 and UAB124 as well (data not shown). Similar results were obtained for the same strains with glass tubes. Examples of the inhibition of the adherence of UAB62 and UAB66 by UAB108 are shown in Fig. 2. Quantitative measures of the decrease in numbers of adhered cells with wild-type strains UAB62 and UAB66 inhibited by UAB108 are summarized in Table 2. Adherence of UAB62 was reduced approximately 48%, whereas that for UAB66 was reduced approximately 76%. Of further interest was the fact that although the total adherent cell mass was reduced in both cases, UAB108 was not detected in significant numbers in the adherent mass when it was grown with UAB62, but accounted for approximately 60% of the total CFU present in the adherent cell mass when it was grown with UAB66.

A number of wild-type strains representing various serotypes were grown with UAB108 in microtiter plates containing PD scr to determine if their adherence could also be inhibited by this mutant. The results are shown in Fig. 3. Unfortunately, strains AHT, BHT, and OMZ175 did not adhere to the microtiter wells. Neither did *Streptococcus sanguis*, which was as expected. However, of the remaining adherent wild-type strains, all were at least partially inhibited by UAB108.

Colony morphology and glucan production. The four adherence-defective mutants (UAB108, UAB113, UAB124, and UAB244) that were capable of inhibiting adherence by other *S. mutans* strains all had similar colony morphologies on MS agar (Table 1). All colonies were surrounded by a clear fluid, subsequently shown to be a WSG. UAB108 was most unusual in having colonies that were six to eight times the diameter of colonies of its parent UAB66 after 36 h of

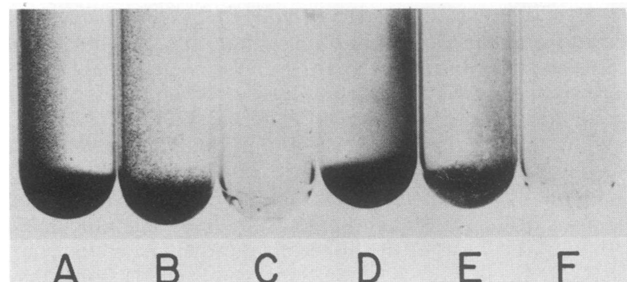

FIG. 2. Adherence of (a) UAB62, (b) UAB62 and UAB108, (c) UAB108, (d) UAB66, (e) UAB66 and UAB108, and (f) UAB108 grown in glass tubes containing PD scr medium.

TABLE 2. Percentage of CFU adhering and nonadhering when grown in the presence or absence of UAB108

Strain	% Adhering[a]	% Non-adhering[b]	% Decrease[c]
UAB62	52	48	
UAB66	99	0	
UAB108	0	99	
UAB62 + UAB108	23[d]	77	
UAB62	27	73	48
UAB108	<0.01	100	
UAB66 + UAB108	14[e]	86	
UAB66	24	76	76
UAB108	11	89	

[a] Values represent the percentage of CFU of any given strain found adhering to the glass surface after washing, based on the total number of CFU of that strain in each culture.

[b] Values represent the percentage of CFU of any given strain found in supernatant fluids and washings, based on the total number of CFU of that strain in each culture.

[c] Decrease in adherence when grown with UAB108 versus when grown alone.

[d] Total adherent cell mass for UAB62 grown with UAB108 was assayed for the presence of each strain. UAB62 accounted for >99.9% of the CFU present, whereas UAB108 (<0.01%) was not detected.

[e] Total adherent cell mass for UAB66 grown with UAB108 was assayed for the presence of each strain. UAB66 accounted for 40% of the CFU present, whereas UAB108 made up the remaining 60%.

anaerobic incubation. The glucan exuded by UAB108 expanded the aparent colony size another two- to threefold. After 48 h of incubation, the glucan from neighboring colonies on MS agar with 100 or more colonies had coalesced.

Inhibition of adherence by culture supernatant fluids. The ability of culture supernatant fluids of UAB108, UAB124, and UAB244 to inhibit adherence of UAB122, UAB62, UAB66 was either the same or only slightly less than that observed with growing cells (Table 3). This inhibition was not destroyed by boiling. Culture supernatant fluids (both boiled and untreated) of UAB113 were shown to inhibit the adherence of UAB122 and UAB62 as well, but these fluids were ineffective in inhibiting the adherence of UAB66. Generally, the degree of inhibition of adherence of UAB62 and UAB122 grown in the presence of mutants or mutant culture supernatant fluids was greater than that seen for UAB66 grown under either condition. The most effective inhibition was seen with mixed cultivation and supernatant fluid of UAB108 (Table 3). Examples of the inhibition of adherence by UAB108 supernatant fluid are seen in Fig. 4.

Inhibition of adherence by ethanol precipitates. Culture supernatant fluids of inhibiting strains were precipitated with ethanol, and the amount of water-soluble carbohydrate present was determined. UAB108 produced three times as much water-soluble carbohydrate as the other inhibiting strains examined, and this level of polymer was most effective in inhibiting the adherence of parent strain UAB66 (Table 4).

The ability of UAB108 polymer to inhibit the adherence of UAB66 was compared with that of dextran T10. Results demonstrated that two to four times the level of dextran T10 was needed to elicit the same level of inhibition as that seen with UAB108 polymer (Table 5).

Inhibition of adherence by glucan from UAB108. A WSG synthesized in vitro with a partially purified GTF preparation from UAB108 grown in PD medium with 2% glucose was added in increasing amounts to PD scr-grown cultures inoculated with UAB66. The results show that this glucan effectively inhibited the adherence of UAB66. The amount

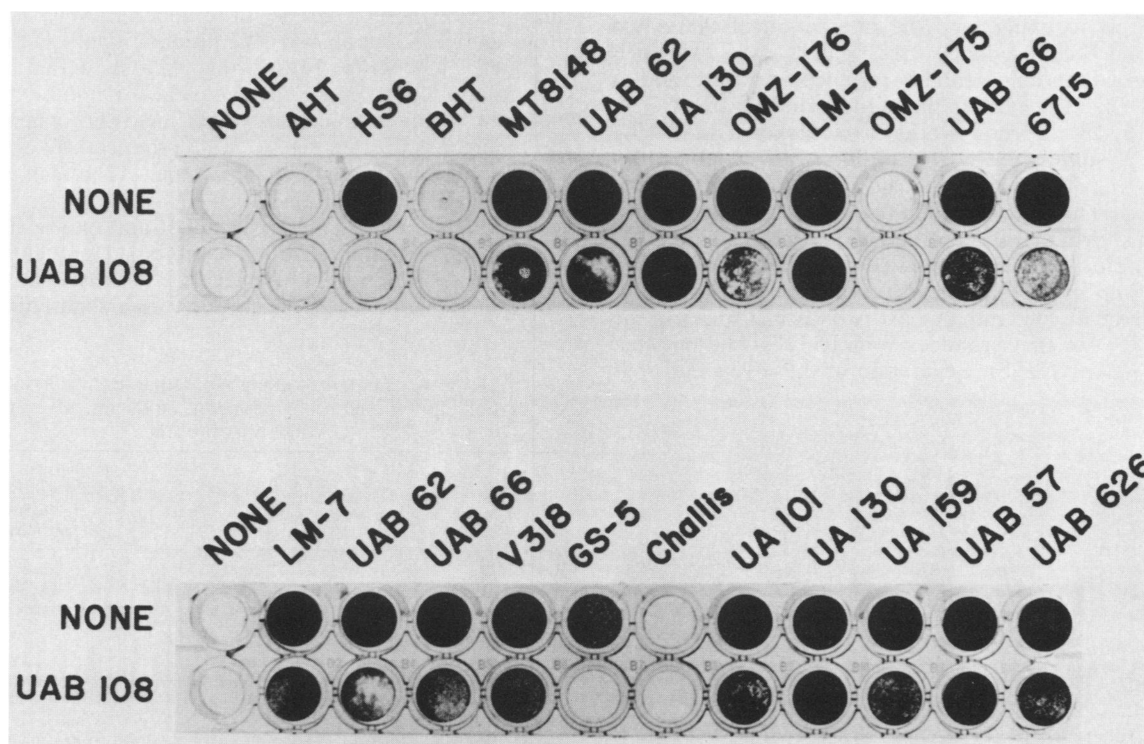

FIG. 3. Microtiter wells of strains representing serotypes a through g and *S. sanguis* Challis grown individually and in combination with UAB108 in PD scr.

TABLE 3. Adherence in the presence of growing cells or culture supernatant fluids[a]

Strain	Growing cultures in PD medium + 1% sucrose[b]			Supernatant fluids diluted with 2× PD medium					
				Unboiled			Boiled for 20 min		
	UAB122	UAB62	UAB66	UAB122	UAB62	UAB66	UAB122	UAB62	UAB66
UAB108	0	0	2+	0	1+	2+	1+	1+	2+
UAB113	1+	1+	3+	2+	2+	4+	2+	2+	4+
UAB124	1+	1+	3+	1+	2+	3+	2+	2+	3+
UAB244	1+	1+	2+	2+	2+	3+	2+	2+	3+
UAB122	4+	ND[c]	ND	4+	ND	ND	4+	ND	ND
UAB62	ND	4+	ND	ND	4+	ND	ND	4+	ND
UAB66	ND	ND	4+	ND	ND	4+	ND	ND	4+

[a] Adherence of cells was scored 0 (no adherence) to 4+ (maximum adherence) (27).
[b] PD scr mixedly infected with UAB122, UAB62, or UAB66 and each of the strains listed.
[c] ND, Not determined.

of inhibition increased as increasing amounts of glucan were added. When 0, 4.5, 9.0, 13.5, and 18.0 mg of glucan per ml were added, adherence was 4+, 3+, 2+, 1+, and 0, respectively.

DISCUSSION

The sucrose-dependent adherence of *S. mutans* via WIG is known to be an important factor in the virulence of this organism (12, 15, 21, 24, 26, 35). Studies have shown that two classes of enzymes, one which produces WSG and one which produces WIG, can be identified (1, 20, 23, 25, 31, 39). When both enzymes are present, wild-type *S. mutans* produces mostly adherent WIG, whereas only a small amount of the nonadherent WSG can be detected (1, 26). But mutants of *S. mutans* which lack the ability to produce WIG often produce increased levels of WSG, are defective in in vitro adherence, and have been shown to be less virulent than the wild-type strains in vivo (26, 35).

In an effort to understand the interaction of these two enzymes and their products, investigators have examined the effects of both commercially produced dextrans and in vitro-synthesized glucans on the production of WIG by GTF (1, 7, 8, 16, 23). Several workers have shown that WIG formation is stimulated by the addition of low-molecular-weight dextrans, indicating that these dextrans act as primers or acceptors for WIG formation (7, 14, 16). In addition, it has been shown that the addition of dextrans can influence the structure of the WIG formed (17, 36, 37).

Preparations of GTF from culture supernatant fluids of *S. mutans* can be divided into at least two distinct forms of the enzyme, i.e., one that produces WIG (GTF-I) and one that produces WSG (GTF-S). Separation of these two enzymes has led to the observation that both are necessary for the production of adherent WIG (1, 12, 23, 25, 39). Alone, GTF-I produces a nonadherent WIG (from sucrose) which is not sensitive to dextranase. However, in conjunction with GTF-S, the enzyme preparation produces an adherent WIG which can be partially degraded by dextranase, suggesting some incorporation of WSG into the adherent WIG. The level of this glucan is higher than the combined level of glucans produced by either enzyme alone (1). Furthermore, it is apparent that the ratio of GTF-S to GTF-I is critical, and the concentration of primer dextran can either stimulate or inhibit the production of adherent WIG (1, 10, 21). Similar effects on GTF-I, either stimulation or inhibition, have been noted with added WSG synthesized in vitro from sucrose by GTF-S (1).

The inhibition of glucan production as well as inhibition of adherence, aggregation, and virulence by high-molecular-weight dextrans has also been examined (8, 9, 29). In the presence of these dextrans, little adherent WIG is formed from sucrose by combined GTF preparations, and the major product is a WSG (1, 16). Further data show that after the two fractions are separated, only GTF-I is inhibited by the addition of high-molecular-weight dextran, whereas the GTF-S is not affected (1). A WSG produced by *S. mutans* 6715 has also been shown to inhibit the synthesis of adherent WIG (1).

The phenotypic characteristics of strains like UAB108 led us to postulate that their ability to inhibit adherence might be due to the production of high levels of a WSG. On sucrose-containing (MS) agar, these strains varied slightly in colony

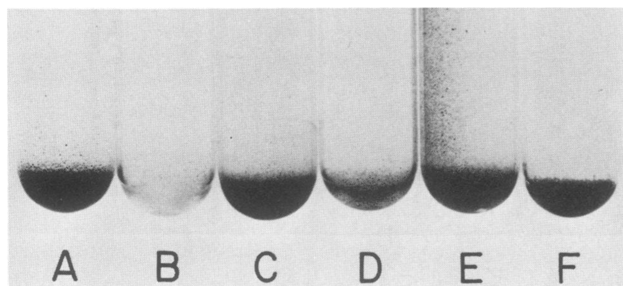

FIG. 4. Adherence of (a) UAB122 in PD scr, (b) UAB122 in UAB108 supernatant fluid, (c) UAB62 in PD scr, (d) UAB62 in UAB108 supernatant fluid, (e) UAB66 in PD scr, and (f) UAB66 in UAB108 supernatant fluid.

TABLE 4. Amount of ethanol-precipitated water-soluble carbohydrate in culture supernatant fluids and adherence of UAB66 in its presence

Strain	Carbohydrate (mg/ml)[a]	Adherence of UAB66 grown in PD medium + 1% sucrose + carbohydrate[b]
UAB108	12.8	1+
UAB113	3.5	3+
UAB124	4.1	2+
UAB244	4.1	2+
UAB66	0.5	4+

[a] Values represent the amount of carbohydrate produced by each strain per milliliter of culture.
[b] UAB66 was grown in PD scr plus carbohydrate from each strain, and adherence was scored as described elsewhere (27). The concentration of carbohydrate used was the same as that produced by each strain grown in PD scr.

TABLE 5. Adherence of strains grown with ethanol-precipitated polymer from UAB108 and dextran T10

Strains	UAB108 polymer added (mg/ml)			Dextran T10 added (mg/ml)			None
	4	8	12	10	25	50	
UAB122	0	0	0	2+	0	0	4+
UAB62	3+	1+	0	3+	2+	0	4+
UAB66	3+	2+	1+	4+	2+	1+	4+

morphology, but all produced copious amounts of a clear fluid which surrounded the colonies. It seemed probable, then, that this same extracellular exudate would be present in culture supernatant fluids of PD scr-grown cells and would therefore be available for inhibition.

In support of this hypothesis, inhibition of adherence was seen when normally adherent *S. mutans* strains were grown in pH-adjusted supernatant fluids from PD scr-grown inhibiting strains. Boiling these culture supernatant fluids did not destroy the effect, indicating that the inhibiting factor was not likely to be a protein but was most likely a heat-stable polymer (Table 3). Additionally, the supernatant fluids of UAB113 inhibited adherence of UAB122 and UAB62 only slightly and adherence of UAB66 not at all. This result suggested that although the heat-stable inhibiting factor was present in the supernatant fluids of UAB113, the two fold dilution of these fluids most likely reduced the concentration of that factor to a level insufficient to effectively inhibit the adherence of UAB66. This possibility is in accord with the findings that UAB113 produced only 27% as much WSG as did UAB108 (Table 4) and that a threefold decrease in the concentration of the UAB108 glucan also diminished the inhibition of adherence of UAB66 cells (Table 5).

Inhibition of adherence by a water-soluble ethanol precipitate from culture supernatant fluids of UAB108 cells grown in PD scr medium further suggested that this factor was carbohydrate in nature (Table 5). This possibility was supported by the observation that all inhibiting strains produced extracellular water-soluble carbohydrate levels from 7 (for UAB113) to 25 (for UAB108) times that seen for the parent UAB66 (Table 4). As indicated in the accompanying manuscript (34), 97% of the dry weight of the water-soluble carbohydrate was glucose, thus justifying the conclusion that it is a glucan. Finally,WSG produced by UAB108 (glucan 108) was shown to effectively inhibit wild-type adherence and did so at a lower concentration than that needed to elicit the same inhibition of adherence with dextran T10 (see above).

The role of dextranase in the adherence of *S. mutans* is poorly understood. Several hypotheses as to its function have been offered, but there remain many unanswered questions (11, 13, 22, 30, 33, 38). It has been postulated that dextranase might play a role in the production of WIG by cleaving native WSG and thereby providing small WSG molecules that would prime the synthesis of the adherent WIG (33). Another hypothesis states that the GTF-to-dextranase ratio regulates WIG production (11). Using previously isolated dextranase-deficient (Dex^-) mutants which displayed different phenotypes, we suggested that the latter hypothesis was most likely (27). However, based on the data presented here, this suggestion may have been an oversimplification. It seems probable that the function and interaction of dextranase with GTF are much more complex. Certainly, if dextranase is needed for the cleavage of WSG for primer molecules, then mutants which lack dextranase could make high-molecular-weight WSG which would in turn inhibit the formation of WIG by other strains. It seems logical, then, that in the case of inhibition of UAB122, an adherent mutant postulated to have a partially defective (inactive) dextranase (27), the level of WSG produced by the inhibiting strain is sufficient to inhibit the formation of adherent WIG. This fact does not explain, however, the inhibition of adherence of wild-type strains.

Normally, it might be expected that the dextranase produced by the wild type would cleave the WSG produced by the mutant as well as its own WSG, and thus WIG synthesis would not be affected during mixed cultivation. But if the ratio of the dextranase enzyme to GTF worked to regulate the production of both glucans, then the absence of enzyme dextranase action in mutant strains might allow for production of relatively high levels of GTF-S by mutants, which might in turn inhibit the action of the wild-type dextranase. Alternatively, the lack of dextranase in mutants may allow for the production of structurally altered WSG molecules. Such molecules might bind wild-type dextranase in such a way as to inactivate the enzyme and thereby prevent the production of primer glucan molecules. In any event, it seems clear that a further investigation of the interaction and regulation of these enzymes is necessary.

The fact that mutants totally devoid of dextranase activity can be isolated (27, 28) suggests the presence of a single gene encoding for that enzyme, but multiple active forms of dextranase have been identified (4, 6). These forms are possibly degradative forms of the enzyme which arise from extracellular processing and proteolysis (6; J. Barrett, personal communication). Furthermore, we have shown previously that the dextranase enzyme appears to be closely associated with surface protein antigen A, since most $SpaA^-$ mutants are also devoid of dextranase activity (3). These $SpaA^-$ Dex^- mutants are totally avirulent as well. Thus, loss of dextranase activity may be due to the absence of other essential cell surface proteins. The analysis of a wide variety of Dex^- mutants isolated in this laboratory by a number of independent methods should allow us to more clearly elucidate the role of dextranase(s) in the adherence and virulence of *S. mutans* (3, 4, 27).

Inhibition of adherence of wild-type strains representing the different serotypes as well as a number of different serotype c strains by UAB108 could be clinically significant. Although representatives of several serotypes can be found, the majority of *S. mutans* strains isolated from human carious lesions in Europe and North America are found to be serotype c (3, 15). Thus, the effects of UAB108 and its glucan on the virulence of UAB66 and other strains, particularly strains of serotype c, are of prime importance and are now being examined. Preliminary results suggest that this mutant as well as glucan 108 are effective in reducing the caries caused by UAB66 in gnotobiotic rats. These data as well as the properties of glucan 108 and a possible mechanism by which it inhibits the adherence of wild-type strains are discussed further in another publication (34).

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