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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

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The influence of culture media on various properties of *Streptococcus mutans* was investigated. Strains of *S. mutans* (serotypes *c*, *d*, *f*, and *g*) were grown in a complex medium (Todd-Hewitt broth [THB]) or a synthetic medium (SYN). The SYN cells, in contrast to THB cells, did not bind extracellular glucosyltransferase and did not produce in vitro adherence. Both types of cells possessed constitutive levels of glucosyltransferase. B13 cells grown in SYN plus invertase-treated glucose possessed the same level of constitutive enzyme as THB cells. In contrast to THB cells, the SYN cells of seven serotype strains did not agglutinate upon the addition of high-molecular-weight dextran/glucan. Significant quantities of lower-molecular-weight (2×10^4 or 7×10^4) dextran and B13 glucan were bound by SYN cells. SYN cells agglutinated weakly in anti-glucan serum (titers, 0 to 16), whereas THB cells possessed titers of 32 to 256. Evidence for the existence of a second binding site in agglutination which does not possess a glucan-like polymer has been obtained. B13 cells grown in invertase-treated THB agglutinated to the same degree as normal THB cells. The nature of this site is unknown. SYN cells possess the type-specific polysaccharide antigen. B13 cells did not bind from THB a glycoprotein which reacts with antisera to the A, B, or T blood group antigens or which allows agglutination upon the addition of dextran. The results demonstrate that *S. mutans* grown in a chemically defined medium possesses markedly different biochemical and biological activities than cells grown in a complex organic medium.

Numerous investigations, summarized in recent reviews (9, 39), have established an important role for *Streptococcus mutans* in the colonization of tooth surfaces in humans and animals and in the development of dental plaque. Various biochemical and immunological characteristics of this organism, such as the in vitro adherence to hard surfaces, the synthesis and localization of glucosyltransferase (GTF), the effect of glucan and GTF antibodies on in vitro adherence, glucan- or dextran-induced cell agglutination, the synthesis and binding of glucans, and the antigenic type-specific polysaccharides of the cell wall, have been extensively investigated (6, 7, 11, 22, 31, 42-44).

Various conditions of nutrition and growth are known to affect the morphological and biochemical properties of streptococci and other bacteria (5, 41). These properties include the composition, thickening, and synthesis of cell wall (5, 18, 40), acid production (53), dextran, levan, and intracellular polysaccharide syntheses (10, 50, 53), and enzyme formation (36).

Complex culture media have been employed in these studies on *S. mutans*. Recently, chemically defined media which support growth of various cariogenic streptococci have been developed (2, 3, 26, 51, 52, 54). Certain strains grown in a synthetic medium were found to possess significantly lower levels of cell-associated GTF activity (20), whereas the reverse was true when a complex medium was used. The cell-free GTF prepared from the former medium exhibited a specific activity 17 times greater than that from the complex medium (38). The distribution of cell-bound and cell-free GTF was also found to be different when cells were grown in a complex or a defined medium (20, 25, 31, 43, 45). Similar observations have been reported for *Streptococcus bovis* (1), *Streptococcus salivarius* (55), and *Streptococcus sanguis* (4).

In view of the function of the surface of the *S. mutans* cells in (i) the binding of GTF and the subsequent in situ synthesis of glucan (6, 12, 24,

35, 44), (ii) the binding of added glucan or dextran (7, 22, 45, 56), and (iii) antibody binding to surface polysaccharide antigenic polymers (28, 43), it was considered of interest to determine these characteristics in cells grown in a synthetic medium. Such studies may contribute to our understanding of the nature and function of these binding site(s).

MATERIALS AND METHODS

Growth of the organisms. *S. mutans* strains HS6 (serotype *a*), FA1 (*b*), Ingbritt (*c*), B13 (*d*), MT703 (*e*), MT557 (*f*), AHT (*g*), and OMZ65 (*g*) were used in this study. Strain AHT, formerly classified as type *a*, has recently been found to be type (*g*) (S. Hamada, N. Masuda, and S. Kotani, Arch. Oral Biol., in press). Cells were grown in Todd-Hewitt broth (THB) supplemented with 1.8% glucose and salts (17) or a synthetic medium (SYN) modified from that described by Terleckyj et al. (51, 52). In SYN all L-amino acids were present at one-tenth the amount of that described by these workers. At transfer 5, strains AHT, B13, MT703, and OMZ65 were found to produce culture turbidities in this medium equivalent to those in THB. In addition, the B13 extracellular GTF activity was 2.6 times that in the medium with the full quantity of amino acids. Cultures were incubated at 37°C for 18 h, harvested by centrifugation, washed three times with 0.85% saline at 4°C, and then stored in 0.02% sodium azide solution, lyophilized, or heat-treated (34).

All cultures grown in SYN were originally inoculated from a THB culture (18 h). Five daily transfers into fresh SYN were performed before harvesting the cells in order to avoid carry-over from THB.

To remove any contaminating sucrose from the glucose used in SYN and THB, 62 g of glucose and 10 mg of yeast invertase (62,000 U; grade X; Sigma Chemical Co.) were dissolved in 200 ml of water and incubated for 2 h at 55°C. The solution was then dialyzed against water, the dialysate was concentrated under a vacuum, and the glucose was determined (27). A similar treatment was carried out on 15 g of dry THB powder (Difco). Water (400 ml) and 5 mg of invertase were added, and the mixture was incubated at 55°C for 2 h and autoclaved. Invertase-treated glucose and the salts mixture (S. Hamada and H. D. Slade, Arch. Oral Biol., in press) were sterilized by filtration and combined with invertase-treated THB to give the complete culture medium. Three transfers in these media were performed before the cells were used for GTF binding or glucan/dextran agglutination.

Assay for cell-associated GTF activity. Lyophilized cells of *S. mutans* (2 mg) suspended in 0.9 ml of 0.05 M potassium phosphate buffer (KPB; pH 6.8) were incubated with 12.5 μmol of sucrose ([*U*-^{14}C]-glucose; 0.32 μCi/μmol) at 37°C for 18 h. The cells were collected by centrifugation, washed three times with KPB, suspended in 10 ml of scintillation fluid (3a70B; Research Products International Corp.), and counted in a Beckman liquid scintillation counter (model LS-100; Beckman Instruments, Inc.). The glucan present in the supernatant was precipitated with 2 ml of 95% ethanol, incubated at 4°C for 2 h, filtered onto glass fiber disks, and counted (28). The activity of the enzyme bound was expressed as the total micromoles of [^{14}C]glucose incorporated per milligram of cells per minute in the cell-associated plus cell-free glucan.

Effect of NaF on glucan synthesis. The following mixture was used to determine the effect of NaF on the synthesis of glucan from sucrose: 0.5 ml of NaF (0.4%) was mixed with 0.4 ml of cell suspension (2.0 mg of cells) and held for 5 min at room temperature; 0.1 ml of [^{14}C]sucrose was added, and the solution was mixed and incubated for 18 h. The cell-associated and cell-free glucans synthesized were determined as described above.

Binding of GTF enzyme to THB and SYN cells of *S. mutans*. Binding assays were performed in mixtures containing 0.4 ml of cells (5 mg/ml of suspension), increasing amounts of GTF, and KPB to a final volume of 2 ml. After incubation at 37°C for 30 min, the cells were centrifuged, washed three times with buffer, and assayed for cell-associated GTF activity as described above. The GTF used was obtained from concentrated (Amicon PM-10 ultrafilter) culture fluid of SYN B13 (serotype *d*) cells. One unit of GTF activity is defined as the amount of enzyme required to incorporate 1.0 μmol of [^{14}C]glucose from sucrose into total polysaccharide per min at 37°C.

Preparation of *S. mutans* soluble glucans. Overnight cultures of *S. mutans* strains HS6 (serotype *a*), FA1 (*b*), GS5 (*c*), B13 (*d*), and MT703 (*e*) grown in THB supplemented with 1.8% glucose and salts at 37°C were centrifuged. The supernatant was filtered, and sucrose was added to a final concentration of 2.5%. The pH of the mixture was adjusted to 7.0, and the mixture was incubated at 37°C for another 18 h. The mixture was centrifuged, and an equal volume of cold 95% ethanol was added to the supernatant; the mixture was then incubated at 5°C for an additional 2 h. After centrifugation, the precipitated material was dissolved in water and centrifuged, and the supernatant containing the soluble polysaccharide was lyophilized.

Dextran- or glucan-induced agglutination of *S. mutans* cells. Dextran- or glucan-induced agglutination was carried out in glass tubes (12 by 75 mm) containing 0.2 ml of cells (1 mg, dry weight) and an appropriate amount of dextran T2000 (molecular weight, 2×10^6) or glucan in 1.6 ml of KPB. A stock solution of cells (50 mg in 10 ml) was sonically treated for 15 s to break up the clumps before use. After incubation at 37°C for 1 h, the degree of cell agglutination in each tube was examined microscopically at 10× magnification and scored on a visual basis as 0 (no agglutination) to 4+ (large clumps). Agglutination was also studied by using a spectrophotometric procedure. The same reaction mixture contained in a glass tube (10 by 75 mm) was used as described above. Cell suspensions before the addition of dextran were measured for their optical density at 550 nm. An appropriate amount of dextran T2000 was added, and the mixture was incubated at 37°C for the desired amount of time. At the end of each incubation period tubes were withdrawn from the water bath, mechanically mixed for 20 s, and allowed to stand at room temperature for 10 min, and the optical density was measured at 550 nm. Control tubes consisting of cell suspensions were included in each case. The extent of agglutination

induced by dextran T2000 was expressed as percent reduction in optical density compared with that of the original cell suspension.

Adherence assay. Assays for cell adherence due to cell-associated GTF were carried out as previously described (13, 34).

Binding of labeled dextrans to *S. mutans* cells. ^{14}C- and ^{3}H-labeled dextrans T20 and T70 (molecular weights, 2×10^4 and 7×10^4, respectively) were used for binding experiments. The reaction mixture contained 1.6×10^9 cells, labeled dextran (100 μg of ^{3}H-labeled dextran T70 or 50 μg of ^{14}C-labeled dextran T20) in 0.6 ml of KPB. After incubation at 37°C for 1 h, cells were filtered onto glass fiber disks, washed three times with KPB, and counted.

Agglutination of *S. mutans* cells by blood group typing antisera. *S. mutans* serotypes *a* to *g* grown in either THB or SYN were tested for their ability to be agglutinated by blood typing antisera. This was carried out in titration plates, with each well containing 0.8 mg of cells in 0.15 ml of KPB–0.85% saline and 10 μl of serially diluted anti-blood type sera (anti-A, -B, and -AB). Cell agglutination was read after incubation at 37°C for 1 h and at 4°C overnight. The highest dilution of antiserum that showed visible agglutination was recorded as the titer.

Serological procedures. Antisera against whole cells of various *S. mutans* strains grown in THB or SYN were prepared as described previously (27). Formalin-treated sucrose-grown cells of *S. mutans* 10449 were used as immunogens for the preparation of anti-glucan serum. The antigens of all seven strains of *S. mutans* were extracted at 121°C from whole cells grown in SYN, and polyglycerol phosphate antigen was removed from the extract on a diethylaminoethyl-Sephadex column (14).

Agglutination of whole cells by antiserum was determined by mixing 5 μl of cell suspension (1 mg/ml in 0.85% NaCl) and 5 μl of serum in a capillary tube (catalog no. 2953-C13; A. H. Thomas Co.) and incubating at 36°C for 1 h, followed by incubation at 4°C overnight. Readings were taken at the end of each incubation period.

Materials. Dextran T2000 (molecular weight, 2×10^6) was purchased from Pharmacia Fine Chemicals. *carboxyl*-^{14}C-labeled dextran T20 (molecular weight, 2×10^4; 2.16 mCi/g), *methoxyl*-^{3}H-labeled dextran T70 (molecular weight, 7×10^4; 33.6 mCi/g), and sucrose ([*U*-^{14}C]glucose; 275 mCi/mmol) were purchased from New England Nuclear Corp. Anti-A, -B, and -AB blood grouping sera were obtained from Ortho Diagnostics, Inc.

Dextran is a threadlike structure which possesses 95% α-1,6 glucose linkages; the remainder are α-1,3. A total of 40% of the branches contain one glucose unit, 45% contain two units, and the remaining 15% contain three or more units. The branching is considered to be uniform regardless of molecular size (Pharmacia Fine Chemicals, personal communication). There is no evidence to indicate that the ^{14}C-labeled dextrans are less active in agglutination than the unlabeled polymers.

RESULTS

Growth of *S. mutans* strains in SYN. All seven serotype strains of *S. mutans* grew well after transfer 5 in SYN. Growth was more dispersed and uniform than in THB. No clumping of cells was noted. The growth rate in SYN was approximately one-half that in THB (52).

Adherence of *S. mutans* THB and SYN cells. Both THB and SYN lyophilized cells were examined for their ability to adhere to the surface of glass tubes when incubated in the presence of sucrose for 18 h at 37°C. Except for strain FA1 (serotype *b*), all THB cells formed an adherent film on the glass surface, with values of the adhered cells ranging from 55 to 73% of the total cells in the culture. However, the SYN cells formed flaky clumps which only loosely attached to the glass surface and which were easily rinsed off during the washing procedure.

Binding of GTF to *S. mutans* THB and SYN cells. Cells of *S. mutans* grown in THB or SYN were examined for their ability to synthesize glucan after exposure to cell-free GTF from strain B13. All cells were exposed to the enzyme for 30 min at 37°C, washed three times with KPB, and incubated with [^{14}C]sucrose. The amount of [^{14}C]glucan incorporated into the total cell-associated and cell-free glucans was used as an expression of the activity of the bound enzyme. Figure 1 shows that each strain synthesized glucan and that the quantity of glucan increased as the quantity of enzyme added increased from 0 to 50 μl. The serotype *c*, *d*, and *f* strains showed an approximate 70% increase, whereas the serotype *g* strain showed little change. The result with the serotype *c* strain is

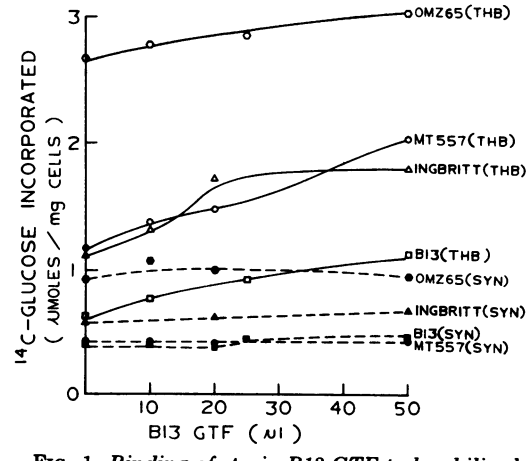

FIG. 1. *Binding of strain B13 GTF to lyophilized S. mutans cells. Both THB and SYN cells of S. mutans strains Ingbritt (serotype c), B13 (d), MT557 (f), and OMZ65 (g) were used. Cells were incubated with increasing amounts of B13 GTF (0.23 mg of protein per ml), washed, and assayed for cell-associated GTF activity as described in the text. The total glucan synthetic activity of the B13 GTF enzyme was 7.5 U/mg of protein.*

in agreement with another strain of the same serotype (24).

In contrast, SYN cells did not show an increase in glucan synthesis at the levels of enzyme added (Fig. 1). The result was the same with each of the four strains. Strain B13 cells grown in SYN containing invertase-treated glucose also did not show an increase in glucan synthesis, and the level of the constitutive enzyme, as judged by glucan synthesis, was the same. It appears that the constitutive cell-associated GTF does not require for its attachment to the cell wall a glucan-like polymer which has its origin in sucrose. Recent results, however, which show that GS5 (serotype *c*) cells grown in SYN can bind added GTF, indicate that strain differences may exist. B13 grown in SYN plus sucrose, however, are able to bind GTF and synthesize glucan (Hamada and Slade, in press).

The absence of significant activity by the intracellular invertase enzymes of *S. mutans* (23, 49) was shown by the ineffectiveness of NaF on either lyophilized THB or SYN B13, Ingbritt, or OMZ65 cells.

Agglutination of *S. mutans* SYN cells by dextran T70 or T2000, glucans, or sucrose. Previous work from our laboratory has shown that THB cells of *S. mutans* serotypes *a* to *g* varied in the extent of their agglutination in the presence of added dextran T2000 or glucans prepared from five serotype strains of *S. mutans*. Strains B13 (serotype *d*), OMZ65 (*g*), and AHT (*g*) showed the strongest agglutination (56). In the present study, no agglutination of SYN cells was induced by dextran T2000 or glucans from strains HS6 (*a*), FA1 (*b*), GS5 (*c*), B13 (*d*), and MT703 (*e*) (data not shown).

As shown in Fig. 2, in the presence of as little as 50 μg of dextran T2000, agglutination of B13 THB cells took place within 15 min and caused a 60% decrease in optical density of the cell suspension. However, essentially no reduction in optical density of a B13 SYN cell suspension was noted under the same experimental conditions. Similar results were observed for all other strains of SYN cells.

Dextran of a lower molecular weight (dextran T70) failed to induce agglutination in either type of cells. However, like all of the THB-grown *S. mutans* cells, visible agglutination occurred readily due to cell-associated glucan synthesis when SYN cells were incubated with sucrose.

Binding of low-molecular-weight dextrans by *S. mutans* SYN cells. The ability of *S. mutans* THB cells (serotypes *a* to *g*) to bind dextrans with molecular weights of 20,000 to 70,000 has been demonstrated previously (56). In the present study, the binding of ^{14}C-labeled dextran T20 and ^{3}H-labeled dextran T70 by

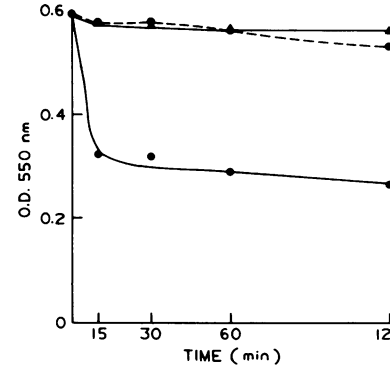

FIG. 2. *Time course of agglutination of B13 THB and SYN cells. The assay is described in the text except that 50 μg of dextran T2000 was added. Symbols: ▲——▲, B13 SYN cells plus dextran; ●- - -●, B13 THB cells only; ●——●, B13 THB cells plus dextran. Although not shown here, no decrease in optical density (O.D.) was observed in a B13 SYN cell suspension.*

these strains grown in a synthetic medium was investigated and compared with values obtained with THB cells. As shown in Table 1, although the SYN cells did not agglutinate in the presence of high-molecular-weight dextran (T2000) or glucans, the quantities of lower-molecular-weight dextran T20 or T170 bound were significant in each case. Four strains of SYN cells bound from 1.3 to 4.4 times as much dextran T20 as did THB cells. Three of these SYN strains (FA1, Ingbritt, MT557) also bound more dextran T70 than the THB cells. Table 1 shows, however, that in all cases SYN cells bound less glucan than THB cells.

Agglutination of THB or SYN cells with anti-glucan serum. Anti-glucan (anti-MT123) serum was used for the detection of glucan polymer on the cell surface of both THB and SYN cells. As shown in Table 2, all THB cells reacted positively. Strains MT703 (serotype *e*) and MT557 (serotype *f*) gave the highest titers. The SYN cells, however, were either negative or possessed a titer 1/16th that of THB cells.

Antigenic properties of *S. mutans* SYN cells. To determine whether the differences observed in the SYN cells were related to a possible loss of their serotype-specific polysaccharide antigens, Rantz-Randall extracts were prepared from all seven strains of *S. mutans* grown in SYN. All antigens gave positive reactions in the capillary precipitin test with their respective type-specific antisera.

Agglutination of *S. mutans* cells by blood group typing antisera. Complex culture media have been shown to contain components which can bind to bacterial cell surfaces and react with human blood group A, B, and AB

TABLE 1. *Binding of glucan and lower-molecular-weight dextrans by S. mutans*[a]

Strain	Molecules of dextran bound per cell[b] ($\times 10^{-3}$)					
	THB cells			SYN cells		
	^{14}C-labeled dextran T20[b]	^{3}H-labeled dextran T70[b]	^{14}C-labeled glucan	^{14}C-labeled dextran T20	^{3}H-labeled dextran T70	^{14}C-labeled glucan
FA1 (*b*)	7.65 ± 0.32[c]	5.00 ± 1.282	0.39 ± 0.03	14.89 ± 1.87	13.71 ± 2.50	0.29 ± 0.07
Ingbritt (*c*)	6.61 ± 0.39	5.05 ± 1.35	0.37 ± 0.04	8.82 ± 1.16	7.35 ± 1.52	0.24 ± 0.05
B13 (*d*)	2.27 ± 0.06	2.76 ± 0.60	0.39 ± 0.04	3.64 ± 0.32	1.42 ± 0.22	0.11 ± 0.01
MT703 (*e*)	9.39 ± 0.63	4.77 ± 0.90	0.38 ± 0.02	5.98 ± 0.62	3.70 ± 0.33	0.15 ± 0.01
MT557 (*f*)	1.82 ± 0.18	1.58 ± 0.45	0.33 ± 0.01	8.03 ± 0.66	6.99 ± 0.67	0.22 ± 0.01
OMZ65 (*g*)	12.77 ± 1.66	5.81 ± 0.16	0.54 ± 0.05	7.01 ± 0.75	4.95 ± 0.98	0.22 ± 0.01
AHT (*g*)	6.11 ± 1.52	3.69 ± 1.20	0.55 ± 0.04	3.18 ± 0.57	1.69 ± 0.10	0.10 ± 0.01

[a] Assays for dextran binding were carried out as described in the text. The quantities of labeled dextran available per cell were 1.55×10^6 molecules of dextran T20 and 8.9×10^5 molecules of dextran T70.
[b] Mean values are taken from Hamada and Slade, in press.
[c] Values represent mean value ± standard deviation obtained from five determinations.

TABLE 2. *Agglutination of THB or SYN S. mutans cells by anti-glucan serum*

Strain	Agglutination titer[a]	
	THB cells	SYN cells
HS6 (*a*)	64	4
FA1 (*b*)	32	4
Ingbritt (*c*)	128	–[b]
B13 (*d*)	128	–
MT703 (*e*)	256	16
MT557 (*f*)	256	16
OMZ65 (*g*)	32	–

[a] Titers represent the highest dilution giving visual agglutination.
[b] –, Negative reaction.

TABLE 3. *Agglutination of S. mutans THB and SYN cells by blood group antisera A, B, and AB*

Strain	Agglutination titer[a]					
	THB cells with antiserum:			SYN cells with antiserum:		
	A	B	AB	A	B	AB
HS6 (*a*)	4	4	16	0	0	0
FA1 (*b*)	2	4	2	0	0	0
B13 (*d*)	8	16	32	0	0	0
MT703 (*e*)	16	0	8	0	0	0
MT557 (*f*)	64	32	0	0	0	0
6715 (*g*)	16	64	32	0	0	0
AHT (*g*)	128	512	512	0	0	0

[a] Agglutination was carried out in titration plates as described in the text. The highest dilution of antiserum that showed positive agglutination was recorded as the agglutination titer.

antisera (29, 46, 48). The AB antiserum is specific for the blood group T antigen (46). THB possesses the type A blood group substance (8). The blood group antigens are glycoprotein (47). In view of the possible function of a glycoprotein in the binding of glucan and subsequent agglutination of *S. mutans* (56), it was of interest to determine the reaction of the THB and SYN cells to blood group A, B, and T antisera and the presence of the T antigen in SYN and THB cells. In the present study, except for the lack of agglutination of MT703 (serotype *e*) cells against anti-B serum and MT557 (serotype *f*) cells against anti-T serum, the remaining five serotype strains of *S. mutans* grown in THB were agglutinated when incubated with blood typing sera A, B, and T (Table 3). Strain FA1, however, was weak in each case. MT557 (serotype *f*), AHT (*g*), and OMZ65 (*g*) were the most reactive.

SYN B13 cells did not agglutinate in A, B, or T antiserum, in agreement with the results of other workers (8). These cells were found to lack the A, B, and T antigens, and B13 THB cells also did not contain the T antigen (G. Springer, personal communication). Table 3 shows, however, that B13 THB cells agglutinated with T antiserum. This result is probably due to the presence of an antibody in AB antiserum other than the T antibody. Thus, B13 cells and AB erythrocytes share a common antigen. It appears that commercial AB antiserum is not suitable to demonstrate the presence of T antigen in *S. mutans* cells. SYN cells did not absorb any material from THB in 1 h at 37°C which reacted with A, B, or AB antiserum or which enabled them to agglutinate with dextran T2000.

DISCUSSION

Studies by previous workers have shown that the addition of sucrose to the growth medium of *S. mutans* results in a change in the ratio of the cell-associated to cell-free forms of GTF (31, 33, 45). Glucose-supplemented cultures of *S. mutans* K1-R have low levels of cell-associated

activity (31). A more than 12% increase was obtained when these cells were grown in a sucrose-supplemented medium. The addition of 0.01% sucrose to Trypticase soy broth resulted primarily in the cell-bound form of GTF in strain 6715 (45).

Complex media may contain variable amounts of sucrose which can affect the activity and levels of cell-bound and cell-free GTF produced by *S. mutans*. A 17-fold decrease was found in the specific activity of cell-free GTF in strain 6715 grown in Trypticase soy broth compared with that from a synthetic medium (38). Strain GS5 grown in THB possessed approximately 10-fold-greater cell-bound GTF activity than that found in cells grown in a chemically defined medium (20). A more recent investigation (16) demonstrated that most of the commercially obtained THB contains trace amounts of sucrose which could promote the association of GTF with the *S. mutans* cells. Our results (Fig. 1) showing that strains of *S. mutans* THB cells (except B13) possessed higher cell-associated GTF activity than the SYN cells are in agreement with those of the latter workers. This in turn may be related to the two- to sevenfold-higher sucrose-dependent adherence displayed by the THB cells (data not shown).

The probable function of glucan and protein in the binding of GTF on the *S. mutans* cell surface has been reported (6, 24, 34, 35, 42). Extracellular GTF can bind to this site(s), synthesize cell-bound glucan from sucrose, and produce in vitro adherence (15, 34, 35, 42). The results presented in Fig. 1 show that THB cells of *S. mutans* Ingbritt (serotype *c*), B13 (*d*), MT557 (*f*), and OMZ65 (*g*) bound GTF and synthesized glucans. Increases are seen in each case. Strain MT557 (*f*) showed a twofold increase. Although not shown here, all heat-treated THB cells used in the present study bound added GTF and synthesized less than that of the lyophilized cells. These data are consistent with earlier studies (15, 24).

In contrast to the THB cells, essentially no increase in GTF-binding was observed in either lyophilized (Fig. 1) or heat-treated SYN cells (data not shown). It has been suggested that GTF might react with glucan or other polysaccharide components on the *S. mutans* surface (6, 24, 34, 35, 43). Table 2 shows that THB cells possess considerably more glucan than SYN cells. The increase in binding of GTF by THB cells (Fig. 1) may be related to the quantity of surface glucan. Recent evidence (16), in agreement with our earlier results (34, 35), suggests that cell surface glucan participates in the binding of GTF. A model based on the participation of both glucan and a heat-stable protein in the binding process has been presented (43).

In the present study a marked difference was observed between the THB and SYN cells in the agglutination by glucans or dextran T2000. The existence of binding sites on the *S. mutans* surface which are capable of binding dextran and thereby causing agglutination has been suggested (7, 11, 22, 32, 37, 39, 56). Previous work in this laboratory has indicated the presence of more than one binding site for soluble glucans on the cell surface of *S. mutans* B13 (56). Preincubation of the cells with dextran T70 did not reduce the quantity of glucan bound, and conversely the treatment of cells with glucan did not reduce the dextran T70 bound. Also, pretreatment of the cells with the lectins Con A and RCA I, and RCA II did not reduce the binding of dextran T20 or T70. The binding of glucan, however, was reduced 80%. Treatment of the cells by heat, ethylenediaminetetraacetate, sodium dodecyl sulfate, trypsin, and dextranase likewise reduced the binding of glucan but not dextran T20 or T70 (56). The effect of dextranase and trypsin indicates the binding site for glucan is a glycoprotein (56). Other studies indicate that the binding site contains a protein (6, 22, 32, 45). These results are in agreement with a model presented eariler, which pictured the binding site as a protein (42). This was later modified to include glucan (43).

We have, however, evidence that a second binding site exists. B13 cells grown in invertase-treated THB agglutinated to the same degree as those grown in normal THB. The nature of this site is unknown. It is not known whether the binding of dextran T20 and T70 and glucan by SYN cells is due in part to the secondary site. Different sites for the binding of dextran T20 and T70 and of dextran T2000 by THB cells was reported earlier (56).

The agglutination of *S. mutans* does not appear to involve the type-specific polysaccharide antigens. All SYN cells possess these antigens, and each reacted strongly in the precipitin test (27) with its specific antiserum. The non-participation of these antigens as a binding site for dextran in agglutination agrees with the model reported earlier (42, 43).

Certain strains of *S. mutans* after continuous culturing in vitro in rich media gradually lose their ability to agglutinate (7, 21). We have found similar results after five daily transfers in SYN. A loss of agglutinability also occurs with *Actinomyces viscosus* (30). Among seven strains representing the six serotypes of *S. mutans* only B13 (serotype *d*), AHT (*g*), and OMZ65 (*g*) gave good agglutination with glucan and dextran T2000 after many transfers in THB (56). This in vitro loss or lack of the ability to agglutinate

may be due to synthesis of binding sites that do not possess the normal steric structure, insufficient quantity of the normal site, or no sites at all. These possibilities may also apply to the inability of SYN cells to agglutinate with T2000 dextran or glucan.

It has been suggested that in agglutination the cross-linking of cells by dextran is mediated by (i) the interaction of cell-free GTF with multiple sites on individual dextran and/or glucan molecules attached to the cell surface and (ii) dextran and/or glucan molecules of sufficient size bound to a nonenzyme dextran receptor on the cell surface (6). Our previous studies (56) indicate that the latter process plays a significant role in agglutination. The inability of dextrans T20 and T70 to produce agglutination may be due to a polymer size which is insufficient to bridge two or more *S. mutans* cells. This agrees with an earlier suggestion (7).

The participation of GTF in the binding of low-molecular-weight (1×10^4 to 7×10^4) dextran is not clear at present. Such binding has been adequately demonstrated (19, 21, 32, 45, 56). Cells grown in different culture media may possess varying affinities for dextran molecules (21, 45, 56). For example, *S. mutans* 6715 grown in a rich medium plus sucrose possessed high levels of cell-associated GTF activity and bound 17 times more dextran (2×10^4 daltons) than cells grown in the absence of sucrose (45). However, in the present investigation, the THB and SYN cells of *S. mutans* bound comparable amounts of lower-molecular-weight dextrans (^{14}C-labeled dextran T20 and ^{3}H-labeled dextran T70) (Table 1) despite their differences in cell-associated GTF levels (Fig. 1). These results indicate that the binding of lower-molecular-weight dextrans by various strains may or may not be mediated by cell-associated GTF.

The ability of THB cells to agglutinate with dextran T2000 may be due in part to the adsorption of a glycoprotein from THB. Complex media are known to contain the human glycoprotein blood group reactive components (29, 46, 48). Brain heart infusion broth and thioglycolate broth contained higher concentrations of A-reactive material than did THB or Trypticase soy broth, whereas a chemically defined medium did not contain this antigen. Several strains of *S. mutans* (serotypes *a* to *e*) and other common oral bacteria, when grown in Typticase soy broth, were agglutinated upon incubation with blood group typing serum A, B, or AB (8). Our results, show that agglutination in AB antiserum does not indicate the presence of the T antigen in the cells. Strains grown in a chemically defined medium did not react to A, B, or AB antiserum (8). Our results with SYN cells are similar and also demonstrate that SYN cells cannot synthesize and do not bind blood group reactive components from THB or a dextran-binding site necessary for agglutination. This indicates the absence of a receptor site for glycoprotein on the surface of B13 SYN cells. Since protein and glucan on the surface of *S. mutans* may act as a binding site for GTF (35), a possible glycoprotein-like substance (43), it is probable that THB-grown cells can bind the glycoprotein blood group components available in THB. This binding site for the blood group substances was not removed by washing THB cells in KPB. Table 2 shows that one or more of these antigens is present in THB cells, although a wide variation in quantity is evident.

In summary, this study indicates that certain surface properties of *S. mutans* cells relating to adherence, GTF binding, dextran/glucan binding, and agglutination undergo a change when grown in a synthetic medium as compared with those of cells grown in an organic culture medium. It is not known whether such changes occur in vivo. It is apparent, however, that the environment may greatly influence the biological and biochemical activities of *S. mutans*.

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