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Purification and Antigenicity of a Novel Glucan-Binding Protein of *Streptococcus mutans*

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A novel glucan-binding protein (GBP) having an apparent molecular mass of 59 kDa (GBP_{59}) has been purified from *Streptococcus mutans* SJ by a combination of affinity chromatography on α-1,6-linked glucan, gel filtration chromatography, and ion-exchange chromatography. GBP_{59} was distinct from the quantitatively predominant *S. mutans* GBP (GBP_{74}) on the basis of size, elution position in a salt gradient, and antigenicity. Rat antisera to purified GBP_{59} and GBP_{74} did not cross-react. GBP_{59} is apparently immunogenic in humans, since immunoglobulin A (IgA) antibody in 20 of 24 adult parotid saliva samples was shown to react with GBP_{59} in an enzyme-linked immunosorbent assay. The glucan-binding activity of GBP_{59} was confirmed by anti-GBP_{59} immunogold labelling of Sephadex G-50 that had been preincubated with *S. mutans* culture supernatant. GBP_{59} could be detected in culture supernatants of all laboratory strains of *S. mutans* (e.g., Ingbritt), as well as all strains of *S. mutans* that had been recently isolated from young children. GBP_{59} was often the only component in protease inhibitor-containing 4-h *S. mutans* culture supernatants that reacted with human parotid salivary IgA antibody in Western blot (immunoblot) analyses. These studies suggest that GBP_{59} is a structurally and antigenically distinct *S. mutans* GBP that can elicit significant levels of salivary IgA antibody in humans.

Extracellular polysaccharides have been implicated in bacterial adherence and pathogenesis in several biological systems (14, 31). In the oral cavity, the ability of mutans group streptococcal organisms to colonize and accumulate on the tooth surface has been associated, in part, with the extracellular synthesis of glucans from sucrose (3, 8, 11, 32). The glucan-mediated accumulation of mutans group streptococci in dental plaque appears to be enhanced by an interaction with cell-associated glucan-binding proteins (GBPs) (18). The resulting accumulation of the aciduric mutans group streptococci can lead to the secretion of metabolic acids that have the potential to dissolve the tooth structure.

Several oral streptococcal proteins can bind glucans. For example, glucosyltransferases (GTFs) not only catalyze the synthesis of glucans but also bind these polysaccharides, apparently via repeating sequences in the C-terminal third of the molecule (1, 6, 7, 10, 19, 23). Other GBPs are synthesized by mutans streptococci that do not have GTF enzymatic activity. The biochemical and genetic characteristics and amino acid sequence of a *Streptococcus mutans* GBP secreted as a 74-kDa protein have been described by Russell and coworkers (2, 4, 20). Landale and McCabe (15) have described a *Streptococcus sobrinus* GBP that is a homodimer of 7,500-molecular-weight subunits. Wu-Yuan and Gill (36) have described 87- and 81-kDa GBPs synthesized by *S. sobrinus* B13. Each of these mutans streptococcal GBPs displays affinity for glucans rich in α-1,6-glucosyl linkages (5, 15).

In this report, we describe an additional extracellular product of *S. mutans* that binds glucans. This GBP has an apparent molecular mass of 59 kDa (GBP_{59}) and appears to be antigenically distinct from the major *S. mutans* GBP (GBP_{74}). Of further interest is the observation that GBP_{59} appears to be significantly more immunogenic in humans than other GBPs.

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MATERIALS AND METHODS

Bacteria. *S. mutans* SJ, used for the preparation of GBPs, was initially grown anaerobically (10% CO_2, 90% N_2) overnight in a chemically defined medium as previously described (27). The final cultivation in sealed 16-liter vessels was accomplished in 8 to 12 h at 37°C. Four recent *S. mutans* isolates (ST192, LH200, RH201, and DI190) from 3- to 7-year-old children were also cultivated in the chemically defined medium. Culture supernatants from these recent isolates were obtained by centrifugation at 27,000 × *g* after 4 h of anaerobic growth. The final pH was 6.9 to 7.2. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was then added to a concentration of 1 mM. The culture supernatants were filtered on Durapore GVWP filters (Millipore Corp., Bedford, Mass.), dialyzed at 4°C against dilute Tris-HCl (pH 6.8)–0.01 mM PMSF, and then concentrated (50-fold) in a Spedi-Vac system (Savant Instruments Inc., Farmingdale, N.Y.) prior to use in Western blot (immunoblot) analyses.

Antigens. GBPs were purified from *S. mutans* SJ by a combination of affinity chromatography on Sephadex G-150 (Pharmacia Fine Chemicals, Piscataway, N.J.), gel filtration chromatography on Superose 6 (FPLC; Pharmacia), and ion-exchange chromatography on Mono-Q HR 5/5 (Pharmacia). Bacteria were cultivated in sucrose-free defined medium as described above. GBPs were removed from the neutralized unconcentrated culture medium by 4 h of incubation with Sephadex G-150 (predominantly an α1,6-linked glucan). Bound proteins were removed by exposure to 3 M guanidine HCl. GTFs were separated from other GBPs by FPLC with Superose 6 in 6 M guanidine HCl. *S. mutans* GTFs prepared in this manner synthesize 95% of their glucan products in the water-soluble form (29). Non-GTF GBP pools of *S. mutans* were further separated by ion-exchange chromatography on Mono-Q HR 5/5 (Pharmacia) as described in Results.

S. sobrinus GTF-I, used as a positive control for the enzyme assay, was prepared by affinity chromatography (Sephadex G-150), gel filtration chromatography (Superose 6), and ion-exchange chromatography (Mono-Q HR 5/5) as previously described (16, 17, 28, 29). *S. sobrinus* GBP_{87} was prepared in a

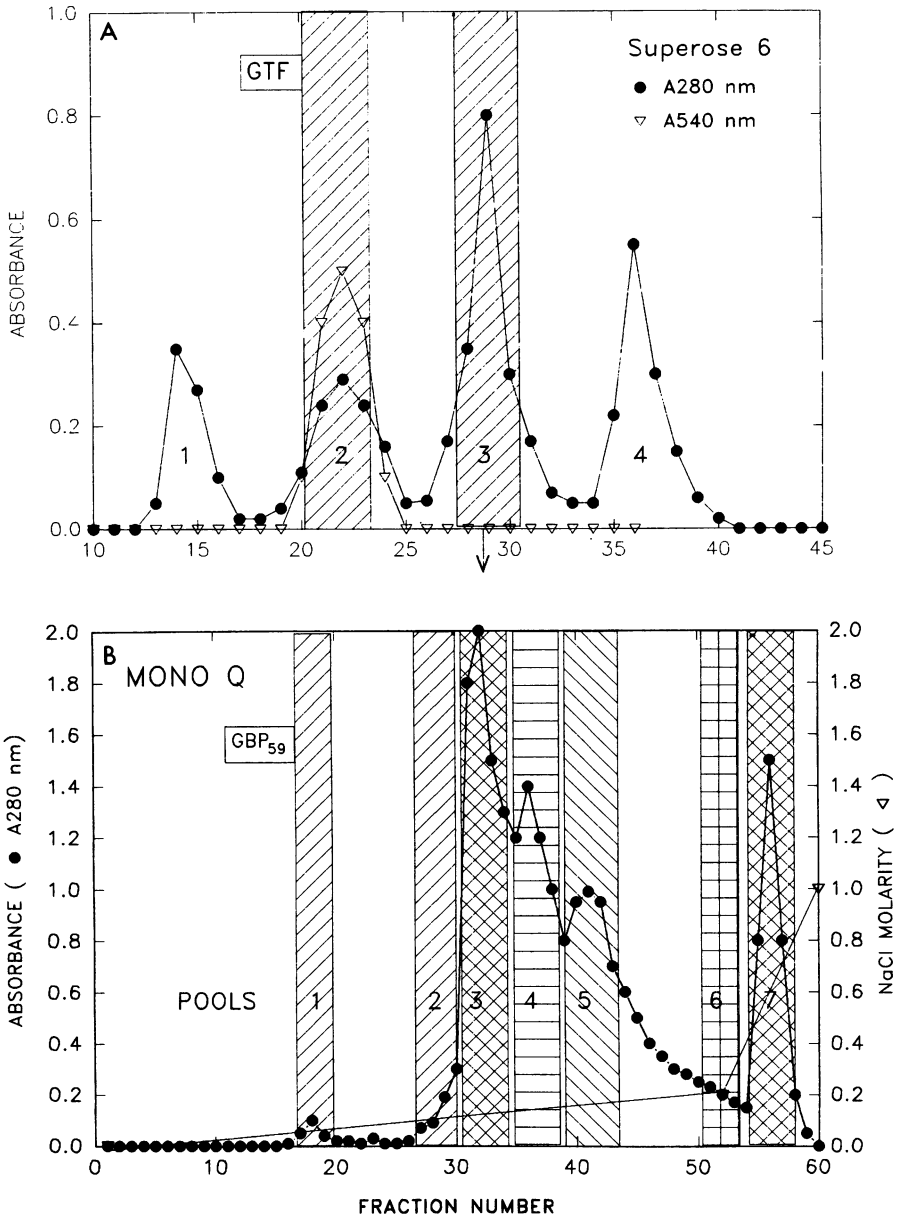

FIG. 1. Purification of GBPs of *S. mutans* SJ. (A) A_{280} elution profile of GBPs eluted from Sephadex G-150 by 3 M guanidine HCl and separated with Superose 6 in 6 M guanidine HCl. GTF enzymatic activity (release of total reducing sugars) of fractions eluting in pool 2 is illustrated by the open triangles, which indicate the A_{540} after the Somogyi assay. (B) Elution profile of GBPs in Superose 6 pool 3 after ion-exchange chromatography on a Mono-Q HR 5/5 column. The NaCl gradient used to elute the GBPs is indicated by the line connecting the open triangles. (C) Rechromatography of Mono-Q pool 3.

manner similar to that described above for *S. mutans* GBPs. *S. sobrinus* GBP_{87} eluted at 0.015 M NaCl in a 0 to 1 M NaCl gradient imposed on a 0.02 M bis-Tris–6 M urea–HCl (pH 6.5) buffer system.

Immunization. Six 60-day-old Sprague-Dawley rats were injected subcutaneously at 21-day intervals with GBPs, obtained from Superose 6 chromatography and Mono-Q HR 5/5 chromatography, in complete Freund's adjuvant (first injection) and incomplete Freund's adjuvant (two subsequent injections). Antigen doses were 7 μg (*S. mutans* GBP_{74} and GBP_{59}) and 10 μg (*S. mutans* GTF [Superose 6 pool 2]). Blood was drawn for antibody analysis 42 days after the first injection. Sera were obtained by centrifugation of the clotted blood and stored frozen at −20°C until use in Western blot analyses. The immunoglobulin G (IgG) fraction of each serum was prepared by binding serum IgG to recombinant protein G (GammaBind G; Genex Corp., Gaithersburg, Md.) in 0.01 M sodium phosphate–0.15 M sodium chloride (pH 7.0), washing, and release with 1 M acetic acid. The IgG antibody preparations were then dialyzed against 0.02 M sodium phosphate–0.15 M NaCl–0.2% sodium azide buffer (pH 6.5) (PBSA) and stored frozen at −70°C.

Measurement of enzymatic activity. GTF activity eluting in the Superose 6 column fractions (10 μl) was determined by

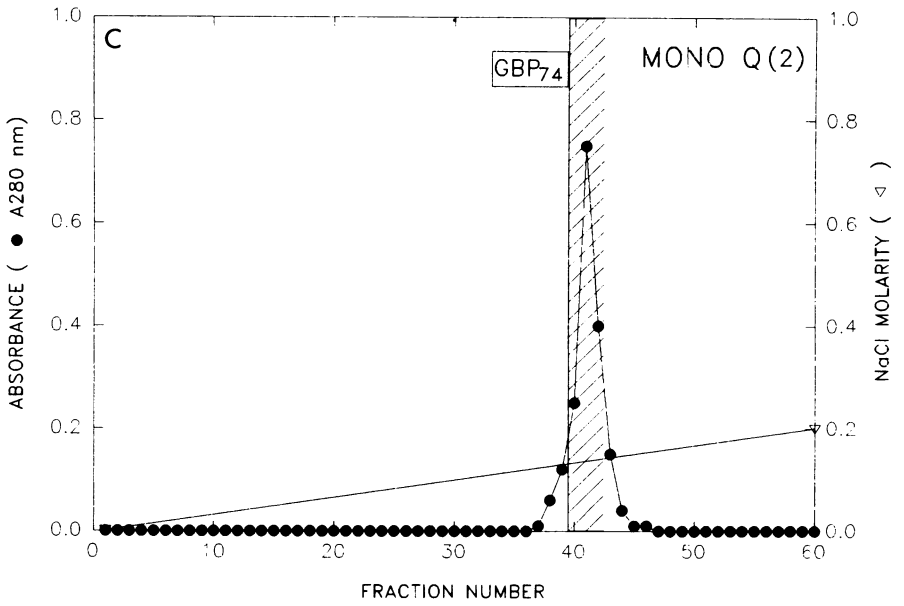

FIG. 1—*Continued.*

measuring the amount of fructose released after incubation with 0.125 M sucrose for 1 h at 37°C. Total reducing sugars were measured at A_{540} by the Somogyi technique (30). GTF activity in pooled fractions was also measured with a ^{14}C-glucosyl-labelled sucrose (specific activity, 310 mCi/mmol; New England Nuclear Corp., Burlington, Mass.) incorporation assay. In brief, approximately 0.03 μg of *S. mutans* GBP_{59}, 0.43 μg of GBP_{74}, or 0.53 of *S. sobrinus* GTF-I in 0.24 ml of PBSA was incubated with 43 nCi of glucosyl-labelled sucrose (approximately 100,000 cpm), 38 μg of dextran T10 (Pharmacia), and 1.6 mg of cold sucrose. After 17 h of incubation at 37°C and the addition of 3 mg of dextran T10 per ml as a carrier, ethanol was added to a concentration of 70%. The precipitated polysaccharide was collected by centrifugation, washed twice with 70% ethanol, and solubilized with distilled water, and the radioactivity was counted as previously described. Fructosyltransferase activity was measured in a similar manner, but with 96 nCi of ^{14}C-fructosyl-labelled sucrose (261 mCi/mmol; approximately 175,000 cpm) and 3 mg of cold sucrose. After 16 h of incubation, 1.0 mg of dextran T10 was added as a carrier. Specific activity was calculated by multiplying the fraction of radioactivity incorporated into the product by the total amount of sucrose added and dividing the result by time and then by moles of enzyme. Specific activity calculated in this manner is lower than the true catalytic constant, since saturating concentrations of sucrose may not have been used, and under these conditions the reaction may not have been linear.

Immunoelectron microscopy. Sephadex G-50 (in Tris-buffered saline [TBS]–bovine serum albumin [BSA] buffer [pH 6.8]) was incubated with concentrated *S. mutans* SJ culture supernatant (grown in defined medium [27]) for 5 h at 4°C. The coated Sephadex beads were washed on a Quik-Sep column (IsoLab, Akron, Ohio) with TBS-BSA. Aliquots of the coated, washed Sephadex beads were incubated for 5 h at 4°C with rat IgG antibody to *S. mutans* GTF (Superose 6 pool 2) or *S. mutans* GBP_{59} or IgG from sham-immunized rats. After the removal of unbound rat IgG by washing with TBS-BSA, 300 μl of gold-labelled goat anti-rat IgG (20-nm particles; Zymed Laboratories, South San Francisco, Calif.) was incubated with each Sephadex fraction overnight at 4°C. The gold-labelled goat anti-rat IgG had been dialyzed in Tris–0.1% BSA (pH 7.0) and was used at a 1:20 dilution of the original stock. Gold-labelled goat anti-rat IgG was also added to an *S. mutans* culture supernatant-coated Sephadex fraction that had been incubated with buffer instead of rat IgG. Each Sephadex fraction was then washed in a filter apparatus containing Whatman GF/F glass fiber filter paper.

Samples were prepared for immunoelectron microscopy by fixation with 2 ml of Karnovsky's glutaraldehyde-formaldehyde fixative in 0.1 M cacodylate buffer (pH 7.1), rinsing with cacodylate buffer, dehydration in 50% ethanol two times and in 95% ethanol three times, and air drying. Each sample was then coated with carbon and examined in a JEOL JSM-6400 scanning electron microscope for bound gold particles at magnifications ranging from 600- to 10,000-fold.

SDS-PAGE and immunoblotting. Polyacrylamide gel electrophoresis (PAGE) of proteins was performed for 1 h at 17 mA per gel with 7% polyacrylamide gels containing 0.01% sodium dodecyl sulfate (SDS) and with 4% stacking gels in an air-cooled slab gel apparatus (Mighty Small, Hoefer Scientific Instruments, San Francisco, Calif.) as previously described (26). Separated proteins were electrophoretically transferred to nitrocellulose for 1 h at 200 mA. After blocking was done, the blotted proteins were incubated with the respective rat antisera (1:200 final dilution) or human parotid saliva samples (1:30 final dilution). The initial incubation was performed with trays when blotted proteins were being analyzed against one antiserum or with a PR-150 Deca-Probe incubation manifold (Hoefer) when reactions with several antisera were being evaluated simultaneously. After incubation with rat antisera or human saliva samples, membranes were developed for IgG or IgA antibody with 1:100 dilutions of biotinylated affinity-purified goat anti-rat gamma-chain or anti-human alpha-chain reagent, respectively (Tago). Bands were visualized with streptavidin-horseradish peroxidase (Zymed), and then 0.05% 4-chloro-1-naphthol, 16.7% methanol, and 0.015% hydrogen peroxide were added (26). Banding patterns were measured

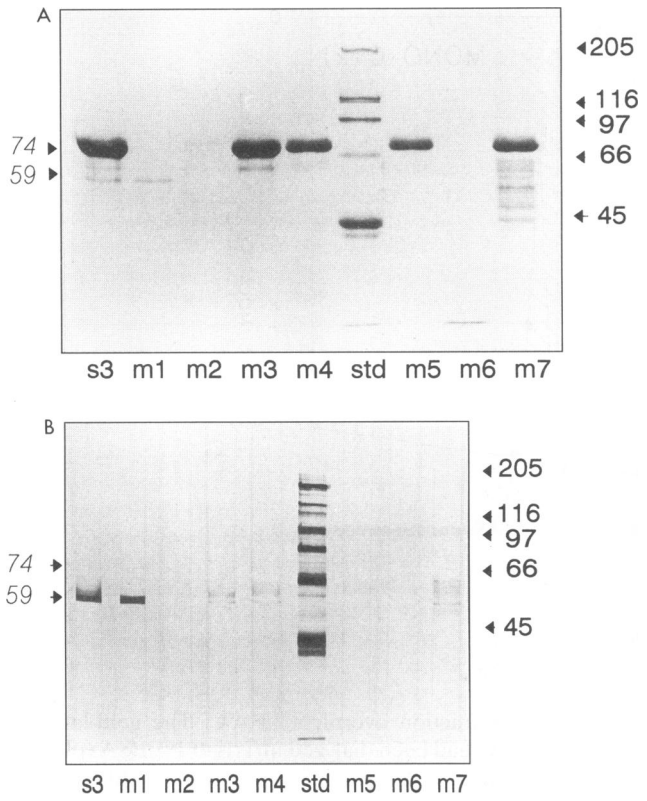

FIG. 2. Characteristics of Mono-Q pools after SDS-PAGE and Western blot analyses. (A) Coomassie blue-stained components of Superose 6 pool 3 (s3) and Mono-Q pools (m1 to m7) after SDS-PAGE with 7% gels. The molecular masses (in kilodaltons) of the standards are indicated at the right. (B) Western blot analysis of the same pools probed with human saliva (subject 1) and developed with an α-chain-specific reagent. The molecular masses (in kilodaltons) of the biotinylated standards are indicated at the right.

densitometrically (Hoefer) and analyzed by use of a GS-365W data system (Hoefer).

RESULTS

Purification of GBP_{59}. GBPs were obtained from unconcentrated culture supernatants of defined medium-grown *S. mutans* SJ by adsorption onto Sephadex G-150 and then 3 M guanidine HCl elution. GBPs were separated from GTFs by FPLC with Superose 6 in 6 M guanidine HCl (Fig. 1A). GTF activity (measured both chemically [release of total reducing sugars] and isotopically {incorporation of [^{14}C]glucose from ^{14}C-glucosyl-labelled sucrose into labelled glucan}) was detected only in Superose 6 pool 2. PAGE of Superose 6 pool 3 revealed (Fig. 2A, lane s3) a major protein band with an estimated molecular mass of 74 kDa (GBP_{74}) and several faster-migrating minor bands, including a band migrating at a position corresponding to approximately 59 kDa (GBP_{59}). Superose 6 pool 3 was then applied to a Mono-Q HR 5/5 column containing 0.02 M bis-Tris–6 M urea–HCl (pH 6.5) and eluted in this buffer with a gradient formed from 0 to 1 M NaCl (elution rate, 1 ml/min; slope, 0.6 mM NaCl per ml) (Fig. 1B). Pools (m1 to m7) were prepared from the eluting proteins and characterized by PAGE (Fig. 2A) and by Western blotting with human parotid saliva as an antibody source (Fig. 2B). GBP_{59} (pool m1) was eluted at a position corresponding to 0.085 M NaCl (Fig. 2A). GBP_{74} (pool m3) was eluted primarily at 0.13 M NaCl (Fig. 2A), although several pools eluting later also contained significant amounts of GBP_{74}. GBP_{74} was further enriched by rechromatography of pool m3 with the same buffer system but with a shallower, 0.05 to 0.18 M, NaCl gradient (Fig. 1C). Significantly more GBP_{74} (mean purified amount, 403 μg) than GBP_{59} (mean purified amount, 20 μg) could be obtained with this affinity purification strategy.

Superose 6 pool 3 and the Mono-Q pools were then analyzed by Western blotting for components that were immunologically reactive with IgA antibody in human parotid saliva (Fig. 2B). Interestingly, a strong band was detected at the migration position of GBP_{59} in Superose 6 pool 3 (s3) and Mono-Q pool 1 (m1). No salivary IgA reactivity was detected at the migration position of GBP_{74}, despite its significantly higher concentration in pools s3 and m3.

Glucan-binding and enzymatic activities of GBP_{59}. The glucan-binding activity of GBP_{59} was indicated from its repeated detection as a minor component after affinity chromatography of *S. mutans* SJ culture supernatants with Sephadex G-150. The glucan-binding activity was confirmed by first incubating an *S. mutans* SJ culture supernatant with Sephadex G-150. The pore size of this grade of Sephadex, an α1,6-cross-linked glucan, is insufficient to permit penetration of 59-kDa globular proteins or the immunological reagents used in the assay. The presence of GBPs was immunologically probed with nonspecific rat IgG or rat IgG antibody to *S. mutans* GTF or to *S. mutans* GBP_{59}. Gold-labelled goat anti-rat IgG antibody was used as the detection system. Culture supernatant-coated Sephadex preparations exposed to rat anti-GTF or rat anti-GBP_{59} antibody contained many beads that had more than 50 gold particles per field at magnifications of ×5,000 to 10,000. In contrast, few gold particles could be detected in *S. mutans* culture supernatant-coated Sephadex preparations that had been incubated with IgG from sham-immunized rats or with buffer.

Table 1 summarizes the enzymatic activity of GBP_{59}. Little or no GTF or fructosyltransferase activity could be detected under the conditions of the assay.

Antigenicity of *S. mutans* GBPs in rats. Figure 3 illustrates the reaction of rat antiserum specific for each *S. mutans* GBP. An antiserum to *S. mutans* GBP_{74} reacted with the corresponding GBP_{74} immunogen (Fig. 3) and with an 87-kDa GBP (data not shown) that had been purified from *S. sobrinus* 6715 by similar techniques (see Materials and Methods). However, the anti-GBP_{74} serum did not show an observable reaction with *S. mutans* GBP_{59}. In contrast, rat antiserum to *S. mutans* GBP_{59} reacted with GBP_{59} but not with purified *S. mutans* GBP_{74} (Fig. 3) or with *S. sobrinus* GBP_{87} (data not shown).

The specificity of the antiserum to *S. mutans* GBP_{59} was further explored in the experiment shown in Fig. 4. Culture supernatant from *S. mutans* Ingbritt and GBPs from *S. mutans* Ingbritt and CE (i.e., protein eluted in 3 M guanidine HCl from Sephadex G-150 after incubation with the respective culture supernatants) were separated by SDS-PAGE. Coomassie blue staining revealed many proteins in the *S. mutans* culture supernatant and prominent staining of GBP_{74} in the guanidine eluates (Fig. 4A). In contrast, Western blots developed with the anti-GBP_{59} serum revealed GBP_{59} rather than the quantitatively predominant GBP_{74} (Fig. 4B). These results indicate that *S. mutans* GBP_{59} and GBP_{74} are antigenically distinct.

Reaction of GBP_{59} with human salivary IgA antibody. Twenty-four adult parotid saliva samples were tested for the presence of IgA antibody to GBP_{59} with enzyme-linked immunosorbent assay (ELISA) plates coated with purified GBP_{59}

TABLE 1. Assay for GTF and fructosyltransferase activities in GBPs

Sample	GTF activity[a]		Sample	Fructosyltransferase activity[b]	
	^{14}C-glucan cpm (mean ± SD)	Sp act		^{14}C-fructan cpm (mean ± SD)	Sp act
No enzyme	137 ± 13		No enzyme	55 ± 22	
GTF-I	4,839 ± 203	1.117	*S. mutans* CS	27,009 ± 325	
GBP_{59}[c]	136 ± 1	<0.038	GBP_{59}[c]	96 ± 2	0.07
GBP_{74}[d]	152 ± 35	<0.007	GBP_{74}[d]	36 ± 5	<0.002

[a] *S. sobrinus* 6715 GTF-I (0.53 μg) was used as the positive control for GTF activity. Data indicate the formation of total water-soluble and insoluble glucan from ^{14}C-glucosyl-labelled sucrose. Specific activity is expressed as moles of hexose incorporated per second per mole of enzyme.

[b] *S. mutans* SJ culture supernatant (CS) was used as the positive control for fructosyltransferase activity at a concentration (500×) roughly approximating the level of enrichment of the GBPs. Data indicate the total amount of fructan formed from ^{14}C-fructosyl-labelled sucrose. Specific activity is reported as described in footnote *a*.

[c] 0.03 μg.

[d] 0.43 μg.

(Fig. 5). Twenty of the 24 (83%) subjects had detectable antibody to GBP_{59}. Three of the 24 saliva samples (2 positive and 1 negative) were used to probe for GBP_{59} in culture supernatants of *S. mutans* that had been recently isolated from young children. These salivary IgA antibody preparations reacted with at least 17 components in overnight (16-h) cultures of *S. mutans* SJ (Fig. 6, first lane in each blot), including components that migrated to the positions of GTF, GBP_{74}, and GBP_{59}. Steps that were taken to minimize the possibility that GBP_{59} was a product of enzymatic degradation of larger GBPs or GTF included the addition of a protease inhibitor, PMSF, immediately after growth and the use of culture supernatants collected after only 4 h of bacterial growth (Fig. 6). A component migrating to the position of GBP_{59} was detected in all the *S. mutans* culture supernatants by immunoblotting with the two ELISA-positive saliva samples and was often the only component detectable by human salivary IgA antibody in these 4-h cultures. In separate Western blot experiments, the presence of GBP_{59} was verified in all five 4-h culture supernatants with rat anti-GBP_{59} serum; GBP_{74} was detected in three of the five culture supernatants. Salivary IgA antibodies of subjects 2 and 3 also detected a high-molecular-weight component that migrated to a position

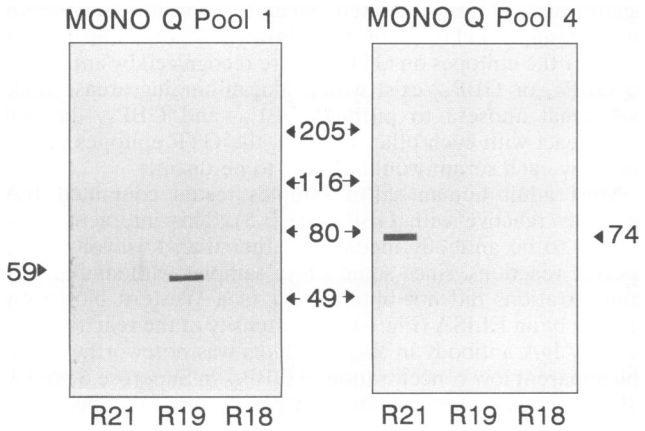

FIG. 3. Western blots of GBP pools developed with rat antisera to purified *S. mutans* GBPs. Mono-Q pool 1 (left blot) and Mono-Q pool 4 (right blot) were electrophoresed on 7% SDS-PAGE gels, transblotted, incubated with serum from a sham-injected rat (R18), serum from a rat injected with GBP_{59} (R19), or serum from a rat injected with GBP_{74} (R21), and then developed for the detection of rat IgG. Sera were tested at a dilution of 1:200. Molecular masses (in kilodaltons) of prestained standards are indicated between the blots.

associated with GTF in the 4-h culture supernatant of *S. mutans* DI190.

DISCUSSION

This article describes a novel protein from *S. mutans* that appears to stimulate a significant mucosal immune response in many humans, is synthesized early in the growth phase, and demonstrates glucan-binding characteristics. Several GBPs of *S. mutans* have been described; these include GTFs that make soluble (13), insoluble (25), or both soluble and insoluble (12) glucan products and a non-GTF GBP having an apparent molecular mass of 74 kDa (20). GBP_{59} described in this article appears to be distinct from GBP_{74} or GTF for several reasons. GBP_{59} migrates in SDS-PAGE faster than GBP_{74} or GTF and is likely to be associated with one of the minor components observed by Russell during the preparation of GBP_{74} (20). GBP_{59} displays elution characteristics distinctly different from those of GBP_{74} (Fig. 1) or GTF (33) in ion-exchange chromatography. Individual antisera raised to either GBP_{59} or GBP_{74} do not cross-react (Fig. 3), and anti-GBP_{59} serum reacts only very weakly with *S. mutans* GTF isozymes (Fig. 4B). Russell et al. (22) reported that faster-migrating components seen in the GBP_{74} preparation could be prevented by the use of PMSF as a protease inhibitor. In our studies, designed to limit proteolytic degradation, GBP_{59} remained the only major antigenic component in PMSF-containing supernatants of cultures that had been incubated for short periods of time (Fig. 6). Although the three salivary IgA antibody sources used to develop the blotted culture supernatants had significant reactivity with GTF synthesized in the 16-h cultures, the 4-h cultures showed little immunological evidence of GTF synthesis. This observation would imply that immunologically reactive GBP_{59} in the 4-h cultures was not simply a degraded product of GTF. Finally, a 59-kDa component reactive with anti-GBP_{59} serum and described in the present study could be detected in an *S. mutans* strain in which the *gbp* gene had been inactivated (21a). Taken together, the data strongly suggest that GBP_{59} is not derived from GBP_{74} or GTF but that it is a genetically distinct protein.

Scholler et al. (24) have described characteristics of an extracellular protein that may be common to *Streptococcus sanguis*, *Actinomyces viscosus*, *Lactobacillus salivarius*, and several mutans streptococcal species, including *S. mutans*. These proteins could be detected in culture supernatants of these microorganisms and were found (by SDS-PAGE) to have apparent molecular masses that were similar (60 kDa) to that of GBP_{59}. However, rat antisera to purified GBP_{59} showed no reaction with *S. sanguis* culture supernatants in the 60-kDa

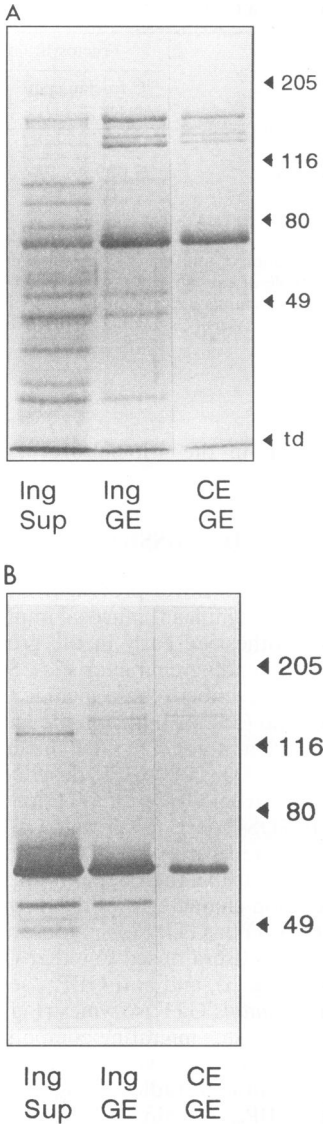

FIG. 4. SDS-PAGE (7% gels) of an *S. mutans* culture supernatant (Ing Sup) and two *S. mutans* guanidine eluates (Ing GE and CE GE) after binding to and 3 M guanidine HCl elution from Sephadex G-150. The gel in panel A was stained with Coomassie blue. td, tracking dye. The proteins in the gel were transblotted to nitrocellulose (B) and developed with 1:200-diluted serum from a rat injected with GBP_{59} from *S. mutans* (R19) as described in Materials and Methods. Guanidine eluates (GE) were tested after 50-fold concentration. The source of each guanidine eluate is indicated beneath each lane. The molecular masses (in kilodaltons) of the biotinylated standards are indicated to the right of the gel and the blot.

region of Western blots (data not shown), suggesting that GBP_{59} is not related to the extracellular proteins synthesized by these oral microorganisms.

Both GBP_{59} and GBP_{74} contain glucan-binding domains, as do mutans streptococcal GTF isozymes, which have been shown to contain several repeating sequences within the region of the molecule thought to be involved with glucan binding (1, 9, 22, 34, 35). Banas and coworkers (4) have determined the sequence of DNA coding for a GBP from *S. mutans* Ingbritt. Interestingly, the molecular mass of the processed protein was

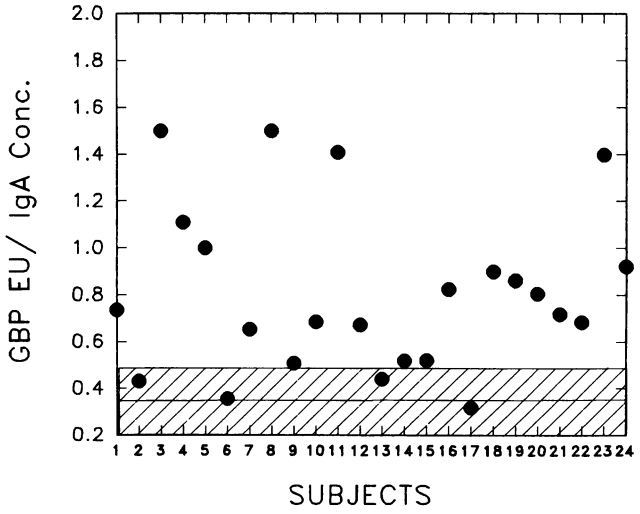

FIG. 5. Detection of human parotid salivary IgA antibody to GBP_{59} in an ELISA. Plates were coated with purified GBP_{59}. The background (shaded area) was taken as 3 standard deviations above the mean absorbance for four infant saliva samples taken before mutans streptococci were detected (4 months to 1.5 years of age). All adults were infected with mutans streptococci. Antibody activity was expressed as ELISA units (EU)/salivary IgA concentration (Conc.).

calculated to be approximately 59 kDa (4), although the product of the *gbp* clone was shown to react with antisera to GBP_{74} in Western blots. Our antiserum to GBP_{74} also recognized epitopes on *S. sobrinus* GBP_{87}, suggesting structural relationships between GBPs of different streptococcal species. In this regard, the deduced sequence of GBP_{74} contained repeated sequences that were similar to sequences in *Streptococcus downei* and *S. mutans* GTFs (4). Antisera to *S. mutans* GBP_{59} (Fig. 4) and to GBP_{74} (data not shown) reacted weakly with *S. mutans* and *S. sobrinus* GTFs in Western blot assays. Wu-Yuan and Gill (36) and Russell (21) showed that antisera to *S. mutans* GBP_{74} cross-reacted with GTFs from *S. sobrinus* and *S. downei*. However, in the present study, antiserum raised against neither GBP inhibited glucan formation by *S. sobrinus* or *S. mutans* GTF (data not shown). It remains unclear whether the epitopes on GTF that are recognized by antiserum to GBP_{74} or GBP_{59} exist within glucan-binding areas. Since polyclonal antisera to purified GBP_{59} and GBP_{74} did not cross-react with each other (Fig. 3), the GTF epitopes recognized by each serum would appear to be distinct.

Most adult human saliva samples tested contained IgA antibody reactive with GBP_{59} (Fig. 5). This interaction appeared to be antibody mediated rather than to involve nonspecific reactions, since some saliva samples with similar IgA concentrations did not bind GBP_{59} in a Western blot assay (Fig. 4) or an ELISA (Fig. 5). The intensity of the reaction with salivary IgA antibody in Western blots was noteworthy, given the apparent low concentration of GBP_{59} in Superose 6 pool 3. GBP_{59} appears to be secreted early in the growth phase of *S. mutans*, since it could be detected by human salivary IgA antibody and rat antibody to GBP_{59} in 4-h cultures of several *S. mutans* strains. In contrast, GBP_{74} showed little or no reaction in Western blot analysis with human salivary IgA antibody (Fig. 2), despite its quantitative predominance in affinity chromatography-isolated preparations of *S. mutans* GBPs. These data suggest that GBP_{59} is appreciably more immunogenic in humans than GBP_{74} and may represent an

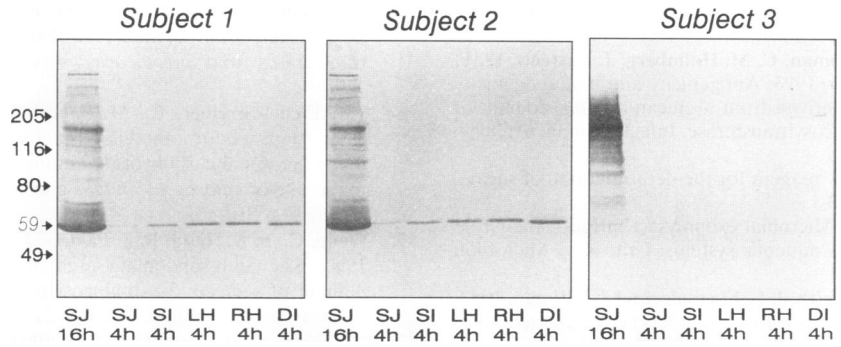

FIG. 6. Western blots of 4- and 16-h culture supernatants of five *S. mutans* isolates developed with three human parotid saliva samples. *S. mutans* ST192, LH200, RH210, and DI190 had been recently isolated from 3- to 7-year-old children. Culture supernatants were concentrated 50-fold, electrophoresed on 7% SDS-PAGE gels, transblotted, incubated with the indicated saliva sample (diluted 1:30), and developed for the detection of IgA. The source (SJ, strain SJ; SI, ST192; LH, LH200; RH, RH210; DI, DI190) and incubation time for each culture supernatant are given beneath each lane. The molecular masses (in kilodaltons) of the prestained standards are indicated to the left, together with an indication of the migration position of GBP_{59}.

important *S. mutans* antigen, since it could be detected in all *S. mutans* strains tested.

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