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Antigenic Characterization of Fimbria Preparations from *Streptococcus mutans* Isolates from Caries-Free and Caries-Susceptible Subjects

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The adhesion of pathogenic bacteria to the host surface is an essential step in the development of numerous infections, including dental caries. Attachment of *Streptococcus mutans*, the main etiological agent of human dental caries, to the tooth surface may be mediated by glucan synthesized by glucosyltransferase (GTF) and by cell surface proteins, such as P1, which bind to salivary receptors. Fimbriae on the surfaces of many microorganisms are known to function in bacterial adhesion. Previous studies in this laboratory have initially characterized the fibrillar surface of *S. mutans*. The purpose of this investigation was the comparison of the antigenic properties of fimbria preparations of *S. mutans* isolates from five caries-resistant (CR) and six caries-susceptible (CS) subjects. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of *S. mutans* fimbrial preparations revealed five major protein bands at 200, 175, 157, 86, and 66 kDa in preparations from CR and CS subjects. Immunoblot analysis indicated the presence of the same major bands recognized by anti-*S. mutans* fimbria antisera. Furthermore, the 175- and 157-kDa bands were recognized by antibodies to P1 and GTF, respectively. Immunoblot analysis with antisera to the fimbria preparation, to P1, or to GTF indicated that the levels of fimbria-reactive components and P1 and GTF antigens were higher in *S. mutans* fimbria preparations from CS subjects than in those from CR individuals. For example, four of six fimbria preparations from CS patients had demonstrable P1, and all had GTF. In contrast, only two of five CR fimbrial preparations exhibited P1 and GTF. Enzyme-linked immunosorbent assay demonstrated similar results for levels of GTF antigen in the fimbrial preparations from CR and CS subjects. The results suggest that differences between the compositions of *S. mutans* fimbriae in CR and CS individuals may play an important role in the virulence of this microorganism in dental caries.

Streptococcus mutans is considered the main etiological agent of dental caries in humans and experimental animals (32, 37). An early step in the pathogenesis of any microorganism is its adherence to and colonization of host tissue. Attachment of the microorganism to the tooth surface may be mediated by glucan synthesized by glucosyltransferase (GTF) (13, 19, 47) and by other cell surface proteins, such as surface antigen I/II (18, 44, 49) (also known as P1 [2, 4, 5, 12]), protein B (45), PAC (40), MSL-S (8), and SR (1), which bind to salivary receptors.

Fimbriae have been considered as candidates for mediating microbial attachment. These structures, also called fibrils, are long hairlike extracellular appendages (33) which have been identified on several gram-negative microorganisms but have been demonstrated on only a few gram-positive bacteria, including *Actinomyces naeslundii* (9), *Streptococcus sanguis* (39), and *Streptococcus parasanguis* (41). These organelles are responsible for the adherence of bacteria to a variety of eukaryotic cell surfaces and facilitate bacterial colonization of host tissues (36). Previous studies in this laboratory have characterized fimbriae from a laboratory strain of *S. mutans* (11).

Bacteria have developed complex and varied mechanisms to present to eukaryotic receptors adhesins which promote attachment and colonization of mucosal surfaces and, in many cases, the subsequent invasion of these tissues (21, 23). Fimbriae have attracted considerable interest as potential vaccines on the premise that antifimbrial antibodies may prevent effective colonization (50). Because adsorption is a prerequisite for bacterial colonization of tooth surfaces, it may be possible to prevent colonization of oral bacteria by immunization with a vaccine consisting of purified fimbrial adhesins (7, 10). The purpose of this study was the isolation of enriched fimbria preparations from *S. mutans* isolates from five caries-resistant (CR) and six caries-susceptible (CS) subjects and the comparison of the antigenic properties of the fimbria preparations.

MATERIALS AND METHODS

Clinical evaluation of CR and CS subjects. Volunteers were recruited from research laboratory staff and patients at Indiana University School of Dentistry and Riley Hospital for Children Dental Clinic, Indiana University, Indianapolis. These studies were carried out with informed consent and were approved by the Institutional Review Board of Indiana University-Purdue University at Indianapolis. Unstimulated whole saliva was collected, and subjects were screened for the number of decayed, missing, and filled surfaces (DMFS) as described earlier (15). Volunteers who were free of carious lesions were designated CR subjects. Patients who had a DMFS score greater than 5 and/or had three or more active unrestored lesions were designated CS individuals.

***S. mutans* strains.** A laboratory strain of *S. mutans* (*S. mutans* TH16, serotype c) and one *S. mutans* isolate from each of five CR and six CS subjects were used in this study. *S. mutans* TH16 was originally isolated from a human caries lesion and has been shown to be cariogenic in a rat model (17a). *S. mutans* serotype c strains were detected by colony morphology and staining with serotype-specific antisera (kindly provided by Ariel Thomson, National Caries Program, National Institute of Dental Research, Bethesda, Md.) in whole-saliva samples from all volunteers as described earlier (15). No other serotypes were observed. Briefly, unstimulated whole saliva samples were diluted in sterile saline, vortexed for 30 s,

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and plated in duplicate on mitis salivarius agar plates (Difco Laboratories, Detroit, Mich.) supplemented with bacitracin (0.2 U/ml) and 15% sucrose for isolation of *S. mutans* after incubation for 3 days at 37°C in an atmosphere of 5% CO_2 in air. The one predominant *S. mutans* colony type from each of five CR and six CS subjects was isolated and frozen in aliquots at −80°C. One type of colony morphology was detected for each subject.

Preparation of fimbria-enriched fractions. Fimbria preparations from the various strains of *S. mutans* were obtained following a modification of the method used by Morris and colleagues (39). Briefly, *S. mutans* strains were grown in Todd-Hewitt broth (Difco) supplemented with 1% glucose for 18 h at 37°C in 5% CO_2, and the fimbriae were removed from the cells by a shearing technique. *S. mutans* cells from 3-liter batches of culture were harvested by centrifuging at 10,000 × *g* for 15 min at 4°C, washed once in fimbria buffer (10 mM phosphate-buffered saline, 1 mM $CaCl_2$, and 1 mM phenylmethylsulfonyl fluoride [pH 7.2]), and frozen as a pellet at −20°C overnight. Phenylmethylsulfonyl fluoride was added to inhibit endogenous proteolytic digestion of fimbrial proteins, and $CaCl_2$ was added to reduce aggregation. Frozen cells were thawed, suspended in fimbria buffer, and blended in a Waring blender for two 1-min cycles at high speed. After blending, unbroken cells and cell wall and membrane debris from each *S. mutans* strain were removed by centrifuging (10,000 × *g*, 4°C, 10 min) and the supernatant, containing the fimbriae, was retained and centrifuged at 110,000 × *g* for 2 h. The resulting fimbria pellet was resuspended in fimbria buffer and centrifuged again at 10,000 × *g* for 10 min to remove cell debris and aggregated fimbriae. The supernatant containing the fimbria preparation was divided into aliquots and frozen at −80°C until used. Protein concentrations were determined by a microprotein assay (Bio-Rad Laboratories, Hercules, Calif.).

SDS-PAGE of fimbria preparations. Reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were prepared by the method of Laemmli (27). Resolving and stacking gels of 10 and 3% acrylamide (National Diagnostics, Atlanta, Ga.), respectively, were prepared in a minigel apparatus (Mini-Protein II; Bio-Rad). Samples (0.3 mg of protein/ml) were placed in a boiling water bath for 7 min with equal volumes of SDS-PAGE sample buffer (pH 6.8) containing 0.167 M Tris-HCl, 27% glycerol, 5.3% SDS, 13.3% 2-mercaptoethanol, and 0.1% bromphenol blue, and 50 μl of each boiled sample, containing 15 μg of protein, was loaded into the wells. Samples were electrophoresed for 45 min at a constant voltage (150 V). Gels were stained for protein with Coomassie brilliant blue and silver nitrate dual staining.

Immunoblotting of fimbria preparations. Fimbrial preparations were electrophoresed in duplicate on SDS-PAGE gels; one of each pair was dually stained with Coomassie brilliant blue and silver nitrate, and the other was electroblotted onto a nitrocellulose membrane (Bio-Rad). Proteins on the SDS-PAGE gels were transferred to a nitrocellulose membrane by the protocol of Towbin et al. (51) with a minitransblot electrophoretic transfer cell (Bio-Rad). The transfer was completed after 45 min at a constant voltage of 75 V. The blot was washed once for 10 min with washing buffer supplemented with 0.5% Tween-20 (WBT, pH 7.4) containing 0.02 M Tris and 0.3 M NaCl, followed by overnight fixation and blocking in WBT containing 0.5% glutaraldehyde. The membranes were washed three times for 10 min each in WBT and probed for P1 antigen with monoclonal antibody to P1 (lot A-10-A8C; kindly provided by Arnold S. Bleiweis, University of Florida, Gainesville), probed for *S. mutans* fimbrial antigens with rabbit antibody to enriched *S. mutans* TH16 fimbriae (11), and probed for GTF antigen with rat anti-GTF antibody (kindly provided by Daniel J. Smith, Forsyth Dental Center, Boston, Mass.) diluted 1:5,000, 1:500, and 1:5,000 in WBT, respectively. The antibodies were incubated with the membranes for 1 h at room temperature. The membranes were washed three times in WBT and incubated for 1 h with each of the following: (i) anti-mouse immunoglobulin G (IgG) (Fc specific)–alkaline phosphatase conjugate (diluted 1:1,000 in WBT; Sigma Chemical Co., St. Louis, Mo.), (ii) anti-rabbit IgG–alkaline phosphatase conjugate (1:1,000 dilution; Sigma), and (iii) anti-rat IgG (whole molecule)–alkaline phosphatase conjugate (1:30,000; Sigma). The membranes were washed three times with WBT. Antibody binding was visualized by the addition of alkaline phosphatase substrate (*p*-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate; Bio-Rad) dissolved in 100 mM Tris-HCl (pH 9.5). Color development was stopped by rinsing the nitrocellulose membranes with deionized water. Gels and blots were analyzed and compared with an UltroScan XL laser densitometer and GelScan XL software (Pharmacia LKB Biotechnology AB, Uppsala, Sweden).

ELISA for fimbrial, GTF, and P1 antigens. Fimbrial preparations from *S. mutans* isolates from CR and CS subjects were assayed for fimbrial, GTF, and P1 antigenic determinants by a modification of a previously described enzyme-linked immunosorbent assay (ELISA) (15). Polystyrene microtiter plates (EIA, Linbro; Flow Laboratories, Inc., McLean, Va.) were coated (100 μl/well) with the fimbria preparations (1 μg of protein/ml diluted in 0.1 M carbonate-bicarbonate buffer, pH 9.6) and incubated at 37°C for 3 h and overnight at 4°C. Unbound antigens were removed by washing the wells three times with saline containing 0.05% Tween 20 (Tween-saline), 200 μl of 1% bovine serum albumin (Sigma) in carbonate buffer was added to each well, and plates were incubated for 1 h at 25°C. After plates were washed, aliquots (100 μl/well) of rabbit anti-*S. mutans* TH16 fimbriae, rat anti-GTF, and monoclonal anti-P1 (each diluted 1:5,000 in Tween-saline) were added to the wells and incubated for 3 h at 37°C. The plates were washed and incubated for 3 h with 100 μl of anti-rabbit IgG (Fc-specific)–horseradish peroxidase (diluted 1:1,000; Cappel Division, Organon Teknika Corp., West Chester, Pa.) for fimbrial antigenic determinants, with anti-rat IgG (whole molecule)–horseradish peroxidase conjugate (diluted 1:35,000; Sigma) for GTF determinants, and with anti-mouse IgG (Fc-specific)–alkaline phosphatase conjugate (diluted 1:1,000; Sigma) for P1 determinants. Plates were washed, and the reaction was developed with horseradish peroxidase or alkaline phosphatase substrate solutions containing *ortho*-phenylenediamine dihydrochloride (Sigma) in citrate buffer (pH 5.0) containing H_2O_2 (100 μl/well) for the antifimbria and anti-GTF assays or with *p*-nitrophenyl phosphate (200 μl/well; Sigma) in 10% diethanolamine (Sigma) buffer (pH 9.6) for the anti-P1 assay, respectively. Color development was monitored between 10 and 30 min, and the reaction was stopped with 100 μl of 2 N H_2SO_4 or with 50 μl of 3 N NaOH, respectively. The amount of color which developed was measured at 490 or 405 nm, respectively, in the microtiter plate with a Thermomax kinetic microplate spectrophotometer (Molecular Devices, Menlo Park, Calif.).

Statistical analysis. The ELISA data was reduced by computing the means and standard errors of the means of the absorbances of triplicate determinations per sample. The results were analyzed by the paired *t* test, and significant differences were defined as a P value of ≤ 0.05.

RESULTS

Subject demographics. The mean age (± standard deviation) of the five CR subjects was 25.4 ± 7.8 years (ranging from 17 to 38 years) and that of the six CS subjects was 13.2 ± 16.2 years (ranging from 4 to 46 years). The CR subject group consisted of four males and one female; all were Caucasians, and they had no carious lesions or restorations. The CS group was composed of three males and three females; five were Caucasian and one was African-American, and they had a DMFS score of 12.5 ± 9.0.

Protein analysis of fimbrial preparations. Fimbrial preparations obtained from *S. mutans* isolates from CR and CS subjects were compared by SDS-PAGE to detect differences in protein composition (Fig. 1A and 2). In reducing SDS-PAGE of enriched fimbrial preparations of *S. mutans* isolates dual stained with Coomassie brilliant blue and silver stain, five extensively stained major protein bands having molecular masses of approximately 200, 175, 157, 86, and 66 kDa were observed in fimbria preparations from CR and CS subjects. Additional lower-molecular-mass minor bands, ranging from 20 to 72 kDa, and higher-molecular mass minor bands, ranging from 125 to 135 kDa, may be other fimbrial components, fragments derived from lower-molecular-weight bands, or, less likely, contaminating nonfimbrial components. No obvious differences in the number of major or minor bands were observed between fimbrial preparations from CR and CS subjects. The densities of the bands were greater in the preparations from CS subjects, particularly the 66-kDa band, which was present at higher levels in fimbria preparations from isolates from CS subjects than it was in those from CR subjects. This was demonstrated by the significantly larger area under the curve when the 66-kDa protein band was scanned with the densitometer (mean for the CS group, 0.74 ± 0.53; mean for the CR group, 0.10 ± 0.09), although this protein was present at some level in all preparations.

Immunological analysis of fimbrial preparations. To detect antigenic differences between proteins of *S. mutans*-enriched fimbrial preparations from CR and CS subjects, separated proteins on polyacrylamide gels were transferred to nitrocellulose membranes and probed with antibodies to enriched *S. mutans* TH16 fimbriae, to GTF, or to P1 (Fig. 1B and 3 to 5). Antibody to *S. mutans* fimbriae recognized a large number of antigens in the enriched *S. mutans* fimbrial preparations from CR and CS subjects (Fig. 3). In striking contrast was the significantly larger amount of immunoreactive components in the preparations from the CS group than in the fimbria samples from the CR group. One major protein (M_r, 86,000) was shown to be present in approximately equal amounts in *S. mutans* isolates from both CR and CS subjects when they were probed with

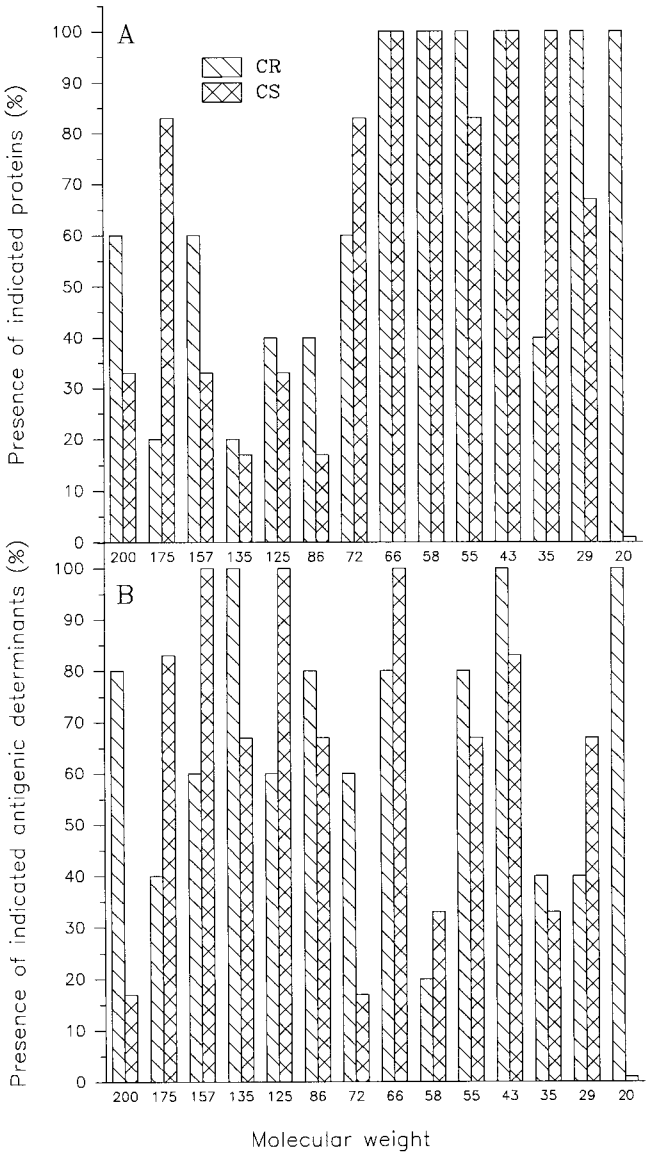

FIG. 1. Presence of proteins (A) and antigenic determinants (B) in enriched fimbrial preparations (15 μg) of *S. mutans* isolates from five CR and six CS subjects in Coomassie brilliant blue- and silver-stained reducing SDS–10% PAGE gels and in immunoblots probed with rabbit antibody to enriched *S. mutans* TH16 fimbriae, respectively. Percentages reflect number of fimbria preparations from CR or CS subjects expressing the presence of the stated protein or antigenic determinant.

anti-*S. mutans* TH16 antibody to fimbriae, while several other antigens (M_rs, 175,000, 157,000, and 66,000) were stained in more preparations from CS *S. mutans* isolates than in those from CR isolates. The 200-kDa antigen was recognized in four enriched fimbrial preparations from CR subjects but in only one fimbrial preparation from a CS subject.

The levels of immunoreactive-protein expression of GTF and P1 were significantly higher in fimbria preparations from CS subjects than in those from CR individuals. Four of six enriched *S. mutans* fimbria preparations from CS subjects had demonstrable P1 (M_r, 175,000) (Fig. 4B), and all had GTF (M_r, 157,000) (Fig. 5B). In contrast, only two of five enriched fimbrial preparations from CR subjects exhibited P1 and GTF (Fig. 4A and 5A, respectively).

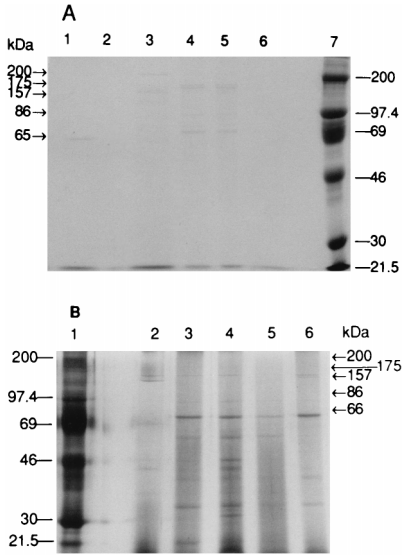

FIG. 2. Representative reducing SDS–10% PAGE analysis of fimbrial preparations (15 μg) of *S. mutans* TH16 and *S. mutans* isolates from five CR (A) and four CS (B) subjects stained with Coomassie brilliant blue and silver stain. (A) Fimbrial preparations from CR5 (lane 1), CR4 (lane 2), CR3 (lane 3), CR2 (lane 4), CR1 (lane 5), TH16 (lane 6), and molecular mass standards (lane 7). (B) Molecular weight standards (lane 1) and fimbrial preparations from TH16 (lane 2), CS1 (lane 3), CS2 (lane 4), CS3 (lane 5), and CS4 (lane 6). Molecular masses of standards and major fimbrial proteins (200, 175, 157, 86, and 66 kDa) are indicated on the sides.

ELISA for fimbrial, GTF, and P1 antigens. ELISA of *S. mutans* fimbria preparations from CR and CS subjects confirmed the significantly higher ($P \leq 0.05$) levels of reactivity with anti-GTF in fimbria preparations from isolates from CS

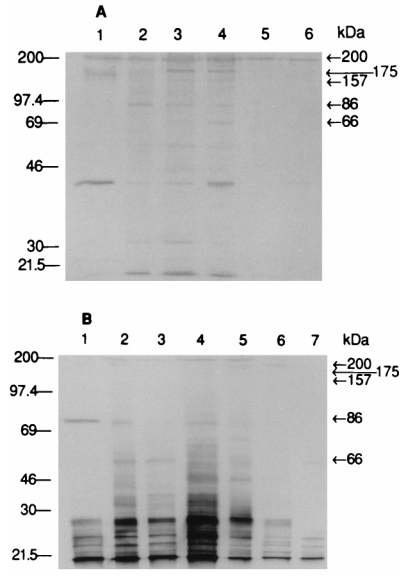

FIG. 3. Representative immunoblot analysis of fimbrial preparations (15 μg) of *S. mutans* TH16 and *S. mutans* isolates from five CR (A) and six CS (B) subjects probed with rabbit antibody to enriched *S. mutans* TH16 fimbriae. (A) Fimbrial preparations from TH16 (lane 1), CR subject 1 (CR1) (lane 2), CR2 (lane 3), CR3 (lane 4), CR4 (lane 5), and CR5 (lane 6). (B) Fimbrial preparations from TH16 (lane 1), CS1 (lane 2), CS2 (lane 3), CS3 (lane 4), CS4 (lane 5), CS5 (lane 6), and CS6 (lane 7). Molecular masses of standards and major fimbrial proteins (200, 175, 157, 86, and 66 kDa) are indicated on the sides.

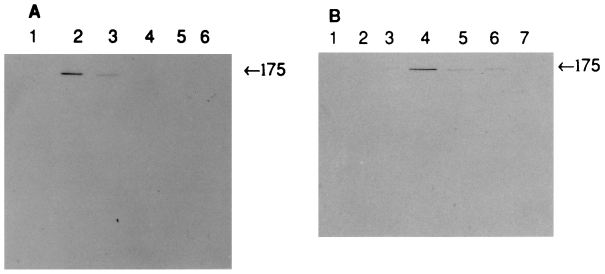

FIG. 4. Representative immunoblot analysis of fimbrial preparations (15 μg) of *S. mutans* TH16 and *S. mutans* isolates from five CR (A) and six CS (B) subjects probed with monoclonal anti-P1 antibody. (A) Fimbrial preparations from TH16 (lane 1), CR1 (lane 2), CR2 (lane 3), CR3 (lane 4), CR4 (lane 5), and CR5 (lane 6). (B) Fimbrial preparations from CS6 (lane 1), CS5 (lane 2), CS4 (lane 3), CS3 (lane 4), CS2 (lane 5), CS1 (lane 6), and TH16 (lane 7). The approximate molecular mass of P1 (175 kDa) is indicated on the side.

subjects than in those from preparations from CR subjects (Table 1) observed by immunoblotting (Fig. 5). Although fimbria preparations from CS subjects had higher levels of P1 antigen and fimbrial components than those from CR individuals, no statistically significant differences were detected between reactivities with anti-P1 and anti-fimbria antibodies to fimbria preparations from CR and CS subjects.

DISCUSSION

The specific binding of adhesion-mediating molecules (adhesins) to their complementary receptors on various host tissues is crucial to the initiation and establishment of bacterial infections. It now appears that in a number of bacterial species the adhesins are parts of complex structures protruding from the bacterial surface, which are referred to as fimbriae or fibrils (36). In addition, a considerable amount of data suggests that fimbriae mediate adherence of bacteria to host tissue surfaces

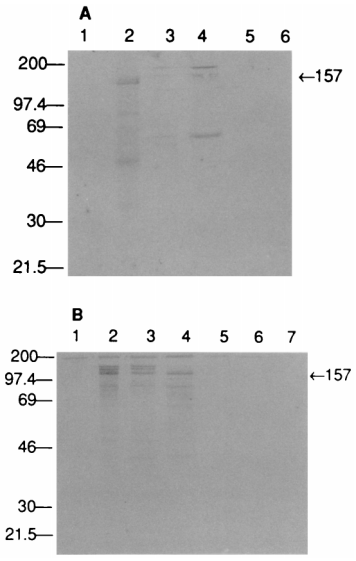

FIG. 5. Representative immunoblot analysis of fimbrial preparations (15 μg) of *S. mutans* TH16 and *S. mutans* isolates from five CR (A) and six CS (B) subjects probed with rat anti-GTF antibody. (A) Fimbrial preparations from TH16 (lane 1), CR1 (lane 2), CR2 (lane 3), CR3 (lane 4), CR4 (lane 5), and CR5 (lane 6). (B) Fimbrial preparations from TH16 (lane 1), CS1 (lane 2), CS2 (lane 3), CS3 (lane 4), CS4 (lane 5), CS5 (lane 6), and CS6 (lane 7). The approximate molecular masses of standards and GTF (157 kDa) are indicated on the sides.

TABLE 1. Reactivity of *S. mutans* fimbrial preparations from CR and CS subjects with anti-GTF, anti-P1, and antifimbria antibody in ELISA

Antibody reagent	Reactivity of preparations from[a]:	
	CR subjects (n = 5)	CS subjects (n = 6)
Anti-GTF	0.155 ± 0.049	0.213 ± 0.067[b]
Anti-P1	0.047 ± 0.004	0.120 ± 0.051
Antifimbria	1.024 ± 0.064	1.153 ± 0.070

[a] Values are the means ± standard errors of the means of the absorbance at 490 or 405 nm for triplicate determinations per sample.
[b] $P \leq 0.05$ compared with value for CR group.

(9, 21, 33, 39, 41, 50). Similarly, several putative functions have been ascribed to fimbriae in various sources. Kawata et al. (22) demonstrated that *Porphyromonas gingivalis* fimbriae stimulated bone resorption in vitro. However, immunization with highly purified *P. gingivalis* fimbrial protein protected against periodontal tissue destruction when tested in gnotobiotic-rat models. These findings suggest that antibodies to fimbriae may be protective against *P. gingivalis*-induced periodontal disease (10).

Fimbriae have been demonstrated to mediate adhesion of gram-negative (14, 23, 26, 33, 52) and gram-positive bacteria, including *S. parasanguis* (41), *S. sanguis* (39), and *A. naeslundii* (9), but such an adherence mechanism has not yet been described for *S. mutans*. This may be due, in part, to the difficulty of purifying the fimbriae; however, previous studies in this laboratory have initially characterized the fibrillar surface of a laboratory strain of *S. mutans* (11).

The results of the present study describe the antigenic characterization of fimbria preparations from *S. mutans* isolates from CR and CS subjects. All the isolates were characterized as *S. mutans* serotype c, the most prevalent serotype in the U.S. population (43). Fimbriae were easily sheared from the cell surfaces of the clinical *S. mutans* isolates, and electron-microscopic analyses revealed the appearance of short filaments (11). SDS-PAGE analysis revealed five major protein bands at 200, 175, 157, 86, and 66 kDa in *S. mutans* fimbria preparations from CR and CS subjects, with a few minor lower-molecular-weight bands. Immunoblot analysis indicated the presence of these same five major bands (200, 175, 157, 86, and 66 kDa) recognized by the anti-*S. mutans* TH16 fimbria antisera. Furthermore, the 175- and 157-kDa bands were recognized by antibodies to P1 and GTF, respectively.

The levels of expression of GTF and P1 antigens determined by immunoblotting and ELISA were higher in fimbria preparations from *S. mutans* isolates from CS subjects than in those from CR individuals. Although the P1 ELISA data does not appear to be statistically different (likely because of the low number of subjects), numerical differences are obvious. Similar to our results, proteins antigenically related to P1 were found on the surfaces of *S. mutans* serotypes c, e, and f when they were probed with monospecific antiserum (38) or with monoclonal antibodies (2, 48). Anti-P1 antisera and monoclonal antibodies have been used to detect significant quantities of P1 in the culture supernatants of some *S. mutans* strains, suggesting that in these strains P1 antigen was shed from the bacterial surface, while in other strains the protein was not released from the surface in large amounts (4, 29). Electron microscopy of immunogold-labeled bacteria has shown the P1 protein to be associated with the layer of peritrichous fibrils surrounding the cell in "retainer" strains, whereas "nonretainer" strains released P1 into the culture supernatant and did not possess a

layer of fibrils on the cell surface (2, 4). Since P1 was identified as a major surface antigen of *S. mutans*, there has been much attention paid to its potential as a vaccine. Several groups have found surface antigen I/II (or P1) to be an effective caries vaccine in monkeys (31) and mice (20), and anti-I/II (or anti-P1) antibodies protected against colonization by *S. mutans* in humans (34, 35), monkeys (30), and rats (42). This suggests that this protein may be an effective caries vaccine (4). Decreases in the amount of P1 on the cell surface have been related to decreases in cell hydrophobicity and a corresponding loss of adherence of cells to saliva-coated hydroxyapatite (24, 28), indicating that changes on the bacterial cell surface affect the ability of *S. mutans* to colonize surfaces. Such changes may have other implications, such as allowing cells to alter their surface antigenic composition (29). This may explain the lower levels of P1 antigen in *S. mutans* fimbriae from CR subjects compared to those in preparations from CS subjects. Similar results between fimbrial preparations of isolates from CR and CS subjects were obtained with GTF.

Results from several studies have shown that glucan synthesis catalyzed by bacterial GTF can enhance the pathogenic potential of dental plaque by promoting the accumulation of large numbers of cariogenic streptococci on the teeth of humans and experimental animals (46). Although glucan production is not required by *S. mutans* for colonization of the teeth of humans or laboratory animals in vivo (53), evidence from several studies has suggested that glucan-dependent adherence and accumulation of cariogenic streptococci are critical processes in the development of dental plaque (25, 46). This may explain why *S. mutans* fimbria preparations from CS subjects had more GTF antigen than those from CR individuals.

Although it is not known exactly which proteins correspond to the 86- and 66-kDa bands, a candidate antigen for the 86-kDa band may be a fructosyltransferase, which is responsible for the formation of fructan from fructose. Results from the present study demonstrated that the 66-kDa band represents the major immunogenic band of the fimbria preparations, and although it was observed in *S. mutans* fimbria preparations from both CR and CS subjects, interestingly it was present at significantly higher levels in fimbria preparations from CS subjects, suggesting that the 66-kDa protein may be biologically important in the differences observed between *S. mutans* isolates from CR and CS subjects. Work is in progress to evaluate the role of this fimbrial protein in the virulence of *S. mutans*. The minor components stained with the anti-*S. mutans* fimbria antisera may be degraded products of the major fimbrial bands, with the possible exception of the 20-kDa protein, which was observed primarily in fimbria preparations from CR individuals, suggesting that it may also play an important biological role.

Our laboratory has previously reported that CR subjects have higher levels of salivary IgA and serum IgG antibodies to most of the *S. mutans* antigens examined than do CS individuals, suggesting that the higher levels of these antibodies may be responsible for the lower numbers of carious lesions and *S. mutans* in CR than in CS subjects (15). Previously, this laboratory demonstrated that CR individuals have significantly higher levels of salivary IgA (but not serum IgG) antibodies to an enriched fimbrillar preparation of *S. mutans* TH16 than do CS individuals, suggesting that CR subjects may be protected immunologically from dental caries by salivary IgA antibody against *S. mutans* fimbriae (11).

Challacombe et al. (6) demonstrated that approximately 60% of the naturally occurring serum antibody that bound to *S. mutans* cells was directed to P1 (or antigen I/II) and 30% was directed to GTF. Bammann and Gibbons (3) demonstrated that a significant amount of human salivary IgA antibody activity against *S. mutans* whole cells was directed to glucan and GTF determinants. Gregory et al. (16) and Gregory and Filler (17) concluded that induced salivary IgA antibodies can prevent *S. mutans* colonization. Taken together, these investigations suggest that if GTF and P1 are attached to *S. mutans* fimbriae, *S. mutans* fimbrial preparations are ideal candidates for potential vaccines on the premise that antifimbrial antibodies may prevent effective adhesion by bacteria.

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