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Cell Surface Proteins of Oral Streptococci

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Whole cells of representative strains of oral streptococci (*Streptococcus sanguis*, *S. mitis*, and *S. salivarius*) were radiolabeled by the lactoperoxidase method of radioiodination. The labeled polypeptides obtained by extraction of whole cells with boiling sodium dodecyl sulfate were analyzed by polyacrylamide gel electrophoresis and autoradiography. Of the total radioactivity, ca. 70% was released by treating whole cells with trypsin, suggesting that the labeling was confined to proteins located on the cell surface. Most *S. sanguis* strains studied gave a characteristic banding pattern consisting of a high-molecular-weight (120,000 [120K] to 63K) group of six proteins. Three low-molecular-weight (12K, 16K, and 18K) proteins were also detected in many strains.

Streptococcus sanguis is among the first organisms to attach to tooth surfaces, apparently via an interaction with salivary components which form a pellicle on the tooth surface (8, 17). In mature dental plaque, large numbers of *S. sanguis* are still present, and these organisms are often found in close association with other organisms characteristic of mature plaque (17, 22). The interactions of *S. sanguis* with the salivary pellicle and other bacteria have been mimicked by in vitro assays in an attempt to define the streptococcal surface molecules involved in these processes (1, 9, 18, 24, 38). A cell wall protein or glycoprotein (26, 37) may mediate saliva-dependent adherence to hydroxyapatite, and other proteins have been suggested for the adherence and coaggregation models described for *Actinomyces viscosus* (9).

Biologically active surface proteins have been detected in gram-negative bacteria by radioiodination combined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (2, 5, 7, 14, 21, 27, 33, 45). Furthermore, analysis of these surface proteins by SDS-PAGE has been suggested as a taxonomic tool (44, 48) for many species, including oral streptococci such as *S. mutans* (11, 19, 39, 40). In this study, we have identified a discrete number of surface proteins (polypeptides) in many strains of *S. sanguis*. These protein profiles have been compared with those of other oral streptococci and appear to serve as an additional taxonomic tool for these organisms. The identification of these proteins might eventually be useful in determining the biological properties of these surface components.

MATERIALS AND METHODS

Bacterial strains. The strains used in these studies, their sources, and appropriate references describing their physiological and serological characteristics are listed in Table 1. Stock cultures were kept lyophilized and frozen at −70°C; the latter were used to inoculate starter cultures (1).

Media and growth conditions. The FMC medium of Terleckyj et al. (47), supplemented with 0.019 M Na_2CO_3 and 100 μg each of L-asparagine and L-glutamine (designated as FMCCAG) per ml, was used throughout this study. Starter cultures were inoculated into FMCCAG, incubated overnight at 37°C, harvested (16,320 × *g*, 15 min, 4°C), washed thrice with PBS (0.05 M sodium phosphate buffer [pH 7.2], 0.15 M NaCl), and suspended in PBS to a turbidity corresponding to 1,000 Klett units (2×10^{10} cells per ml; Klett-Summerson colorimeter with a number 47 filter).

Radioiodination. The lactoperoxidase method (28) for radioiodination of cell surface proteins, as described by Bjorck and Kronvall (3), was used. Each reaction mixture contained 200 μl of the bacterial suspension, 10 μl of $Na^{125}I$ (100 μCi), 90 μl of PBS, and 5 μl of a lactoperoxidase solution (2 μg/ml in PBS). H_2O_2 (0.15%, 5 μl in PBS) was added to initiate the reaction; another 5 μl was added after 5 min. Iodination was carried out for 10 min at ambient temperature (19 to 21°C) and stopped by the addition of 1 ml of ice-cold PBS; the cells were harvested by centrifugation (13,750 × *g*, 5 min) and washed five times with cold PBS. The specific activity of the radioiodinated cells ranged between 1 and 2 μCi/mg of cell dry weight. The radioiodinated samples were counted with an Intertechnique (Dover, N.J.) CG 4000 gamma counter.

SDS-PAGE. For SDS-PAGE polyacrylamide slab gels (17.5% each) with a 5% stacking gel were run in a Tris-glycine-sodium dodecyl sulfate (SDS) buffer (4, 23) in a Bio-Rad model 220 (Bio-Rad Laboratories, Rockville Center, N.Y.) slab gel apparatus. The 17.5% polyacrylamide gels used in this study were capable of resolving the major proteins of interest. Preliminary studies indicated that a 10% separating gel could resolve some of the higher-molecular-weight proteins (greater than 120,000 [120K]) but many of the lower-molecular-weight proteins of some strains were lost because of their migration with the buffer front. Whole-cell extracts were prepared by boiling cells for 20 min in a buffer containing 0.08 M Tris-hydrochloride (pH 6.8), 10% glycerol, 10% β-mercaptoethanol, and 2% SDS (SDS sample buffer); the extracts were clarified by centrifugation at 13,750 × *g* for 5 min. The soluble proteins were loaded onto the stacking gel and electrophoresed at a current of 30 mA per gel at room temperature until the tracking dye was within 1 cm from the gel bottom. Tracking dye (0.001% bromphenol blue in sample buffer) was run in separate wells at the periphery of the slabs. After electrophoresis, the gels were stained for protein with 0.025% Coomassie brilliant blue in 25% isopropanol–10% acetic acid overnight, fixed with 0.0025% Coomassie brilliant blue in 10% isopropanol–10% acetic acid for 2 h, and destained in 10% acetic acid (16). The stained gels were dried under vacuum and autoradiographed at −70°C with Kodak XAR-5 X-ray film and Du Pont

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TABLE 1. Streptococcus strains used

Species and strain	Serotype
S. sanguis[a]	
G9B	1
CC5A	1
32A	1
38	1
72 × 36	1
Challis-6	2
Wicky	2
ATCC 10558	2
M5	2
903	
S. mitis[b]	
ATCC 10557	
ATCC 6249	
S. salivarius[b]	
9652	
CM6	
71 × 45	
S. mutans[c]	
HS6	a
BHT	b
FA1	b
Ingbritt	c
GS-5	c
KPSK-2	c
PK-1	c
OMZ176	d
LM7	e
6715	g

[a] Data from reference 36.
[b] Data from reference 1.
[c] Classification of Brathall (6) and Hamada and Slade (20).

Cronex Lightning-Plus intensifying screens (46). The proteins that were used for standards and their molecular weights were β-galactosidase (120K), phosphorylase *a* (94K), bovine serum albumin (68K), ovalbumin (43K), chymotrypsinogen (25.7K), cytochrome *c* (11.7K), and insulin (6.5K).

Chemicals. Radioactive iodine ($Na^{125}I$) was purchased from Amersham Corp., Arlington Heights, Ill. Reagents for acrylamide gels were from Bio-Rad Laboratories.

RESULTS

Comparison of the total and radioiodinated proteins of *S. sanguis* G9B. Our initial studies were performed with this strain because it is being used to determine the molecular basis of saliva-mediated adherence and aggregation (1, 37, 38). The ^{125}I-labeled proteins are compared with the stained preparation of the same extract in Fig. 1. These studies indicated that only a discrete number of polypeptides (11 to 14) were iodinated compared with the total number of proteins present. Many of the iodinated proteins are not seen in the stained preparation (Fig. 1) because their concentration was small compared with that of the total protein found in the cell.

Effects of trypsin on radioiodinated proteins. After iodination, the washed cells of strain G9B were suspended in PBS, trypsin was added to yield a final concentration of 150 μg/ml, and the reaction mixtures were incubated for 1 and 2 h at 37°C. Controls containing cells plus buffer were run simultaneously. After incubation the cells were chilled, harvested, washed three times with cold PBS, solubilized in the SDS sample buffer, and electrophoresed. The supernatant fluid of trypsin-treated cells was counted, solubilized in SDS sample buffer, and also electrophoresed. Of the total radioactivity, ca. 75% was solubilized within 2 h of incubation of the cells with trypsin. Only low-molecular-weight (less than 12K) proteins remained on the trypsin-treated cells (Fig. 2). The loss of these cellular proteins after trypsin treatment supports the assumption that iodinated proteins are located on the surface of this organism. The autoradiographs of *S. sanguis* (Fig. 1 and 2) were typical of those for most strains. They contained high-molecular-weight proteins ranging from 110K through 63K. Intermediate-size proteins (49K through 27K) either were present in lower concentrations or did not iodinate well. This may account for the variability that we detected in these proteins among the strains of *S. sanguis* (Fig. 3). The low-molecular-weight proteins (12K, 16K, and 18K) appeared to iodinate well. Although the loss of proteins associated with the trypsin treatment might suggest digestion of many of these components, autoradiographic analysis of the supernatant after trypsin treatment showed the presence of the 12-kilodalton (kd) 36-kd, 63-kd, and 93-kd peptides (data not shown). Examination of these supernatants by crossed-rocket immunoelectrophoresis before and after trypsin treatment also indicated a low susceptibility to trypsin digestion (Rosan, et al., Program Abstr. Int. Assoc. Dent. Res. abstr. no. 200, 1983).

***S. sanguis* and *S. mitis*.** Additional strains of *S. sanguis*, *S. mitis*, and *S. salivarius* and representatives of the various

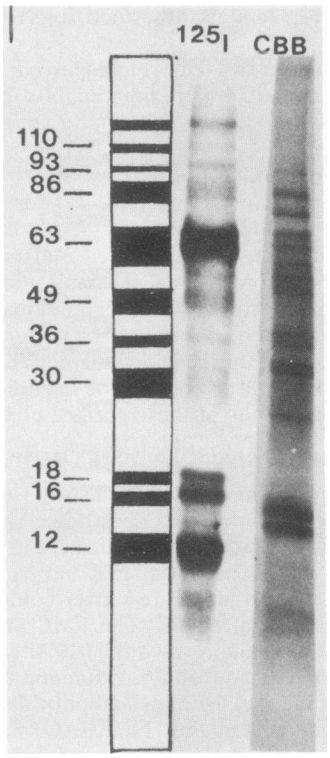

FIG. 1. Autoradiograph of SDS-PAGE–separated proteins of *S. sanguis* G9B cells labeled with ^{125}I (^{125}I) and a Coomassie-stained preparation of the same extract (CBB). Molecular weights shown to the left of the diagram are based on linear regression analysis of protein standards described in the text.

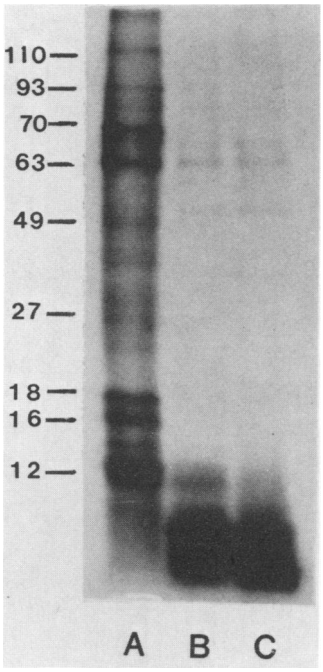

FIG. 2. Autoradiograph depicting effects of trypsin treatment on whole cells of ^{125}I-labeled *S. sanguis* G9B. Lanes: A, autoradiograph of SDS-PAGE of control cells; B, cells treated for 1 h with trypsin; C, cells treated for 2 h with trypsin.

serotypes of *S. mutans* were iodinated, and their surface protein profiles were examined by SDS-PAGE. The results for most strains of *S. sanguis* serotypes 1 and 2 (Fig. 3A and B, respectively) were similar to those shown above for strain G9B and reinforce the impression that there are two sets of proteins: a high-molecular-weight group containing 120K through 63K proteins and a low-molecular-weight group containing 18K through 12K proteins. Since the high- and low-molecular-weight groups were widely distributed, they might represent the major structural proteins of the cell surface. However, there were differences among some strains that could be significant in terms of biological function. Thus, strain Wicky (Fig. 3B), which is used extensively as a recipient (34) in transformation studies, showed consistent differences from other *S. sanguis* strains in both its high-molecular-weight and low-molecular-weight proteins. In addition, a number of intermediate-size proteins were much more evident in this strain.

The idea that biological differences among *S. sanguis* strains might be more closely associated with the intermediate- and low-molecular-weight proteins is supported by observing the autoradiographs of strains CC5A and 903. Strain CC5A is a corncob-forming strain (24), whereas strain 903 is similar antigenically but does not form corncobs with *Bacterionema matrochutii* (31). Although both strains have the high-molecular-weight proteins that are typical of *S. sanguis*, they differed significantly in their intermediate- and low-molecular-weight-range proteins in our study (Fig. 4). Indeed, in both of these strains, we began to see resemblances to the *S. mitis* protein profiles (Fig. 5) in which the intermediate- and low-molecular-weight proteins appeared to be more prominent.

Although strain 10557 (Fig. 5) is classified as *S. sanguis*, nearly every investigation of its properties including DNA homologies (12) indicates that it is an atypical *S. sanguis* strain (10). Indeed, there still seems to be considerable confusion in classifying the two species *S. sanguis* and *S. mitis*. It is easy to see how this confusion could occur, even by observing surface protein patterns of strains CC5A, 903, 10557, and 6249. Strain 6249 is classified as *S. mitis*. In studying the autoradiographs of these four strains compared

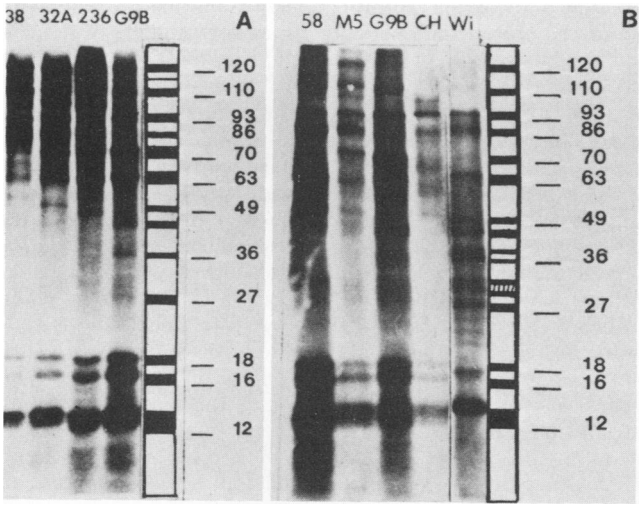

FIG. 3. Comparison of autoradiographs of SDS-PAGE proteins of ^{125}I-labeled *S. sanguis*. (A) Strains 38, 32A, 72 × 36 (236), and G9B of serotype 1; (B) strains 10558 (58), M-5, G9B (control), Challis (CH), and Wicky (Wi) of serotype 2. Molecular weights were calculated as described in the legend to Fig. 1. Cross hatching indicates bands that might represent a doublet.

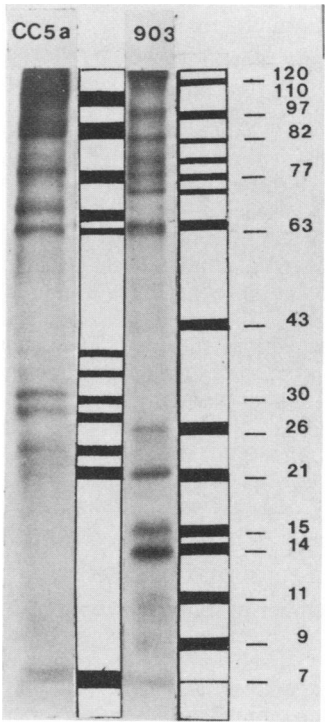

FIG. 4. Comparison of SDS-PAGE protein profiles of ^{125}I-labeled *S. sanguis* CC5A and 903.

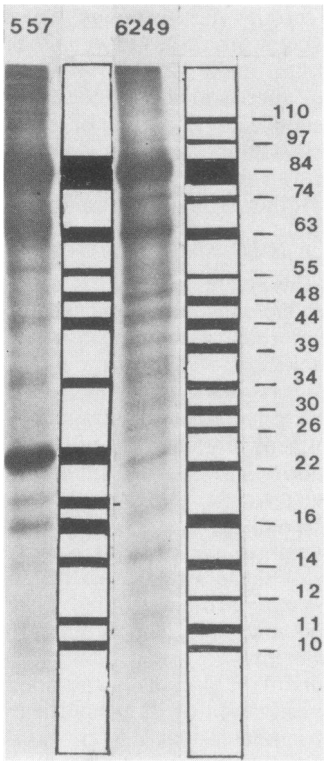

FIG. 5. Autoradiographs of SDS-PAGE protein profiles of *S. sanguis* 10557 (557) and *S. mitis* 6249.

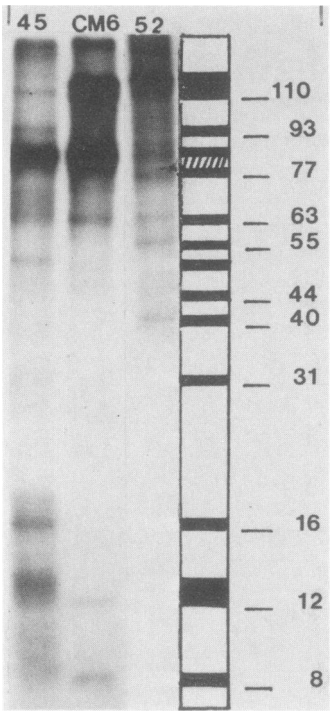

FIG. 6. Autoradiographs of SDS-PAGE protein profiles of ^{125}I-labeled *S. salivarius* 7145 (45), CM6, and 9652 (52). The cross-hatching in the diagram indicates bands that might represent a doublet.

with those of the typical *S. sanguis* strains, (Fig. 3) we were struck by two observations: first, the absence of the distinct high- and low-molecular-weight groups (particularly in strains 10557 and 6249) and second, the large number and distribution of surface proteins. At least 18 proteins appeared to be present in strain 6249. Although we have only drawn 12 proteins in strain 10557, there were almost an equal number of faint bands which were present.

***S. salivarius* and *S. mutans*.** The autoradiographs of the *S. salivarius* strains showed (Fig. 6) high-molecular-weight proteins similar to those seen in *S. sanguis* (e.g., 110K, 93K, and 63K) and proteins of 16 kd and 12 kd. However the overall pattern was different, and with the exception of the 110K, 77K, and 63K bands, each strain appeared to be distinct; obviously, more strains will have to be examined. The complexity of iodinated proteins in *S. mutans* appeared to be related partially to serotype. Thus, serotypes *a* and *b* were similar, and each contained a major iodinated band at 63K (Fig. 7). Strains in serotype *c* had similar high-molecular-weight proteins, albeit differences in the degrees of iodination were apparent. Strain OMZ 176 (serotype *d*) was one of the few oral streptococci which did not contain a 63K protein, but a 73K protein was detected. Most *S. mutans* strains contained a low-molecular-weight protein (9.6K).

DISCUSSION

A discrete number of iodinated proteins have been found among the oral streptococci studied. It is probable that these proteins are localized on the surface because the lactoperoxidase used to catalyze the reaction does not penetrate the cell membranes (28) and no labeling occurred in the absence of lactoperoxidase. Furthermore, trypsin, which also does not penetrate the membrane, removes most of the surface protein. Finally, the iodinated proteins appear to represent only a small portion of the total proteins found in the cell. We concluded, therefore, that most of the iodination was confined to proteins on the cell surface.

The *S. sanguis* strains are characterized by a high-molecular-weight group of proteins (120K to 63K) and a low-molecular-weight group (18K to 12K). Since lactoperoxidase catalyzes iodination mainly at the tyrosine and histidine residues of proteins (30), it is conceivable that these heavily iodinated proteins are actually minor components of the cell surface but are rich in tyrosine and histidine residues that are accessible for labeling. The small quantity of cell surface protein is evident when unlabeled extracts from whole cells are examined in SDS-PAGE and stained with Coomassie blue (Fig. 1). On this basis, it is possible, even probable, that those proteins which did not iodinate well may also contribute significantly to the difference in biological properties among the various strains examined. The close similarity among the surface proteins of most *S. sanguis* strains would follow from the DNA-DNA hybridization studies which indicate that these strains are closely related (12). In contrast, 903 is known to be atypical (12, 31, 36); thus, it is not surprising that its protein profile is different from those of the other *S. sanguis* strains. Although the corncob-forming strain CC5A has not been studied genetically, the studies of Mouton et al. suggest a close antigenic relationship between CC5A and 903 (31). This relationship is also apparent in surface protein composition.

The *S. salivarius* strains examined (Fig. 6) are characterized by a heavily iodinated protein of 77 kd; as seen in strain 9652, this band may represent a doublet. As observed in *S. sanguis*, most surface proteins are divided between a high- and a low-molecular-weight group. Indeed, a comparison of

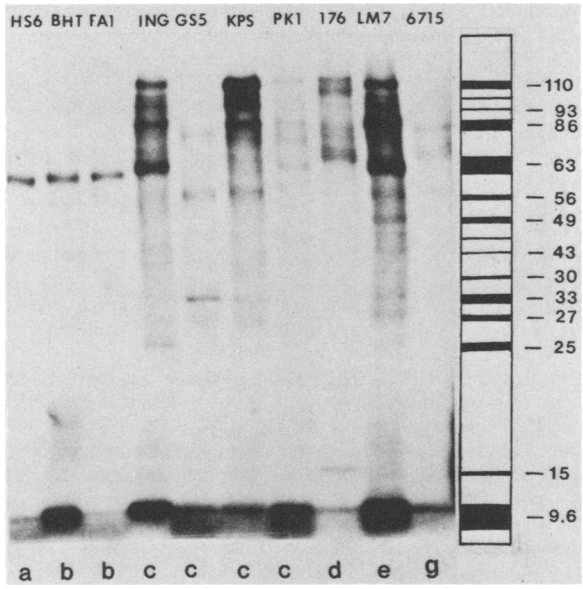

FIG. 7. Autoradiographs of SDS-PAGE profiles of ^{125}I-labeled *S. mutans*. The strain numbers are indicated at the top, and the designations are at the bottom of the figure. ING, Ingbritt; KPS, KPSK-2; 176, Omz 176. Molecular weights of the various strains are summarized in the diagram at the right.

the protein profiles of the oral streptococci indicates the consistent presence of 110K and 63K proteins. These proteins may represent one of the common protein antigens reported recently by Schöller et al. (42) for several species of oral gram-positive bacteria.

Other investigators have electrophoretically examined the total cellular proteins of *S. mutans*. These studies have shown that strains within the same serotype and genotype have similar protein profiles (11, 19, 39–41, 44, 48). At least 40 to 60 proteins were seen in these preparations: our whole-cell extracts also contained large numbers of proteins in contrast to the smaller number of surface proteins seen in the iodinated preparation. Although the function of the surface proteins cannot be described with certainty, these functions could be related to differences in the ecological distribution of the proteins. The 110K protein observed in the serotype *c* strains may represent either the Russell B antigen (41) or a cell-bound form of glucosyltransferase which has been shown to have a similar molecular weight (20). This B antigen, along with glucosyltransferase, may be involved in adhesion of *S. mutans* to hydroxyapatite (15, 41). A similar protein has been localized to the cell surface by using extracts of radioiodinated cells (49). Ultrastructural studies also indicate the surface location of this protein (29).

As stated earlier, iodination depends not only on the concentration and chemical composition of the protein but also on the accessibility of the proteins to the reagent used in the procedure. The autoradiographs of the SDS-PAGE–separated proteins, as well as the extent of radioiodination for a given strain, were highly reproducible. This was observed for the iodination of replicate samples of both a single batch of cells on the same day and different batches of cells labeled on different days. The presence of polysaccharide and other surface components could affect both labeling and solubilization in SDS; these components have also been shown to affect sensitivity to muralytic enzymes (43). A strain of *Lactobacillus casei*, known to be heavily encapsulated, iodinated very poorly under the conditions used in this study (unpublished data). Indeed, in determining the optimal conditions for iodination and SDS-PAGE of *S. sanguis* G9B, we observed that some iodinated material did not penetrate the gel. Analysis of this material indicated that it was a complex of peptidoglycan, protein, and surface polysaccharides (unpublished data). Reusch has recently utilized the fact the SDS does not cleave peptidoglycan and some covalently bound polymers to purify *S. sanguis* sacculi by gel electrophoresis (35). The sacculi were unable to penetrate the gel and thus were concentrated upon the stacking gel.

An earlier report by Larsen et al. (25) used the electrophoretic patterns of cell wall proteins to classify group A streptococci. Common surface proteins were also found among groups A, C, and G streptococci by Bjorck and Kronvall (3); 8 to 10 common cell wall proteins were observed in group B streptococcal cell walls. These proteins ranged in size from 30 to 60 kd (D. P. Krieg and S. J. Mattingly, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, D47, p. 55). Although useful taxonomically, the function of these proteins has not been described. A 31-kd surface protein has also been reported in group B streptococci (13), and three proteins, of unknown function, have been found in *S. mutans* (32).

A cell surface protein appears to mediate adhesion of *S. sanguis* G9B to saliva-coated tooth surfaces in vitro (1, 37). Heating whole cells to 95°C or proteolytic treatment of whole cells results in a dose-dependent decrease in adhesion accompanied by the loss of proteins of 86, 92, and 120 kd from the surface (37). Preliminary evidence suggested that some of these proteins appear in the supernatant and may therefore be related (B. Appelbaum and B. Rosan, J. Dent. Res., abstr. no. 1124, 1981) to the adherence-blocking activity observed in supernatants of disrupted cells. Liljemark and Bloomquist (26) have also described an 86-kd adhesin obtained from *S. sanguis* cell walls by sonication. Although the functions of the proteins described in this report are not yet understood, identification of the surface proteins of the oral streptococci will be useful as a taxonomic tool. In addition, it is probable that one or more of these proteins, alone or in concert with other surface macromolecules, will have important biological function in host-parasite interactions including adhesion to host tissues.

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