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Streptococcus sanguis Surface Antigens and Their Interactions with Saliva

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Saliva-binding molecules of *Streptococcus sanguis* and their receptors were investigated. Streptococcal cell surfaces were extracted with a barbital buffer and examined immunochemically. Strains G9B and Blackburn, which adhere specifically to saliva-coated hydroxyapatite via immunologically related adhesins, possess 80-, 62-, and 52-kilodalton (kDa), and 52-, 42-, and 29-kDa polypeptides, respectively, which correlate with adhesion to saliva-coated hydroxyapatite. Nonadherent strains Adh$^-$ and M-5 lack these antigens. In an immunoblot overlay, the putative adhesins bound to a 73-kDa receptor present in submandibular saliva but not in parotid saliva. G9B also contains a 160-kDa surface protein which bound to an unidentified receptor in both submandibular and parotid saliva samples. Blackburn barbital-extracted components bound to 78- and 70-kDa receptors in parotid saliva. These bacterial-salivary interactions may be important in the regulation of oral ecology.

All bacteria that pass through the oral cavity come into contact with saliva. Saliva molecules can mediate a number of reactions with bacteria, such as bactericidal and inhibitory effects (22), provision of growth substrates (11), aggregation (23), and enhancement or inhibition of adherence to oral surfaces (9, 13). Interactions with saliva are thus critical in determining the fate of bacteria in the mouth.

Streptococcus sanguis is one of the first organisms to colonize tooth surfaces (8) and subsequently constitutes a large (up to 70%) proportion of supragingival plaque (37). The important role that *S. sanguis* plays in the initiation and maturation of dental plaque is partially due to its interactions with saliva molecules. However, the mechanisms of these interactions are poorly understood.

S. sanguis appears to adhere to the salivary pellicle deposited on tooth surfaces by a complex process. Lectin-like (28, 29), electrostatic (12), and hydrophobic (30) binding, alone or in combination, may be involved, possibly with two or more salivary receptors of different affinities (10). Hydrophobic proteins (3) and sialic acid-containing glycoproteins (4, 25, 38) may function as salivary receptors. The bacterial adhesins have yet to be fully defined but are thought to be associated with surface fimbrial structures (15, 27).

The other major activity that results from salivary interactions with *S. sanguis* is aggregation, which may be partially responsible for bacterial clearance from the mouth (33). A number of salivary molecules appear capable of mediating bacterial aggregation. However, attention has been focused on a group of high-molecular-weight glycoproteins, some of which exhibit blood group activity and possess functional sialic acid residues (17, 20, 24, 36). The nature of the bacterial receptors is largely unknown, although activity has been associated with surface proteins (26, 31) and lipoteichoic acid (18).

The purpose of this study was to identify molecules involved in *S. sanguis*-saliva interactions and assess the extent to which they correlate with adhesion and aggregation. An immunoblot overlay technique was used to demonstrate reactions between immunochemically defined *S. sanguis* surface proteins and components of submandibular and parotid saliva.

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MATERIALS AND METHODS

Bacteria and culture conditions. *S. sanguis* G9B and M-5 are reference strains originally isolated from dental plaque. Strain Blackburn (NCTC 10231) was obtained from R. M. Cole, National Institutes of Health, Bethesda, Md. Strain Adh$^-$ is a poorly adhering (see below) spontaneous mutant of G9B isolated from a chemostat culture (manuscript in preparation). After initial isolation, the strains were maintained at −70°C and subcultured only infrequently. The strains were characterized physiologically by the criteria of Carlsson (7) and Facklam (16) and fit the Facklam biotype I category. On the basis of the data of Rosan (32), strains G9B and Adh$^-$ are serotype 1, whereas M-5 and Blackburn are serotype 2. All of these strains have approximately the same saliva-mediated aggregating activity (35).

Cells were grown overnight at 37°C in Trypticase peptone broth (BBL Microbiology Systems, Div. Becton Dickinson and Co., Cockeysville, Md.) supplemented with 5 g of yeast extract (BBL) and 0.5% glucose as a carbon source.

Saliva. Stimulated parotid saliva and submandibular saliva samples were used. Parotid saliva was collected in Curby cups from 15 individuals and pooled. Submandibular saliva was collected from one individual, previously found to have high adherence-promoting activity, by a plastic mouthpiece collector custom fit for the subject (5). The salivas were clarified by centrifugation (44,000 × *g*, 15 min) and either aliquoted and stored at −20°C or freeze-dried and stored at +4°C.

Adhesion assay. Bacterial adhesion to saliva-coated hydroxyapatite (SHA), an in vitro model of the tooth surface, and inhibition of adhesion by antibody were measured by the methods of Rosan et al. (34).

Barbital extraction of bacteria. Cells were harvested and washed three times in H_2O by centrifugation at 10,000 × *g* for 10 min at 4°C. The pellet was suspended at 5 mg/ml in cold barbital buffer (2 mM sodium barbital, pH 8.6) and vortexed for 30 min at 4°C. The cells were harvested by centrifugation (10,000 × *g*, 10 min, 4°C), and the supernatant was collected. The pellet was suspended at 25 mg/ml and reextracted. The supernatants were combined, and the remaining cells were removed by passage through a cellulose acetate filter (0.2-μm pore size). The extract was dialyzed against H_2O at 4°C and lyophilized.

Production of antisera. All antisera were raised in female New Zealand White rabbits. Antisera to formalinized whole cells of strains G9B, Adh$^-$, M-5, and Blackburn were produced as described previously (32). These antisera have been used extensively as reference reagents both in our laboratory and in other laboratories.

Salivary immunogen was prepared by suspending 200 μg of lyophilized saliva in 0.5 ml of physiological saline and mixing this with an equal volume of Freund complete adjuvant (Sigma Chemical Co., St. Louis, Mo.). Two rabbits were inoculated intramuscularly with 0.5 ml of the mixture. This procedure was repeated four times with Freund incomplete adjuvant over a period of 2 months. The rabbits were boosted by subcutaneous immunization and bled 7 and 14 days later.

Monospecific antisera were raised to streptococcal proteins in barbital extract (containing 100 μg of protein), separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and stained with Coomassie blue (see below). The bands of interest were excised and macerated in physiological saline. The mixture was added to an equal volume (approximately 0.5 ml) of adjuvant and rabbits were immunized on the schedule used for saliva.

SDS-PAGE. Vertical slab gels of 10% (wt/vol) polyacrylamide (Bio-Rad Laboratories, Richmond, Calif.) with a 10-mm 3.6% (wt/vol) polyacrylamide stacking gel were run in a Mighty Small II gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) with the buffer system of Laemmli (19). Samples in 0.05 M Tris hydrochloride (pH 8.6) containing 2% (wt/vol) SDS (Bio-Rad), 10% (vol/vol) glycerol (Fisher Scientific Co., Pittsburgh, Pa.), 5% (vol/vol) 2-mercaptoethanol (Sigma) and 0.002% bromophenol blue were heated at 100°C for 3 min just before application to the gel. Electrophoresis was at 30 mA per gel until the bromophenol blue reached the bottom of the gel. Proteins were stained with 0.25% (wt/vol) Coomassie brilliant blue R (BioRad) in methanol-acetic acid-water (4.5:1:4.5 [vol/vol]) for 2 h, destained in the same solvent, and swollen to approximately their original size in 7% (vol/vol) acetic acid. Gels were calibrated with Sigma high- and low-molecular-weight standards.

Immunoblots and overlay immunoblots. Proteins resolved by SDS-PAGE were transferred to nitrocellulose paper (0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) by electrophoretic blotting (39). Transfer was at 115 mA for 20 h in 20 mM Tris buffer containing 1.125% (wt/vol) glycine and 20% (vol/vol) methanol. The nitrocellulose was cut into strips and soaked for 30 min in blocking buffer (phosphate-buffered saline containing 0.1% Tween 20). The transfers were treated with antiserum diluted in blocking buffer for 1 h at room temperature, and then washed three times (10 min each time) with blocking buffer. Antigen-antibody binding was localized by incubation for 30 min with a 1-in-500 dilution of anti-rabbit immunoglobulin G peroxidase conjugate (Southern Biotech Associates, Birmingham, Ala.). After being washed twice with blocking buffer and rinsed with phosphate-buffered saline, enzyme reactions were developed in 0.1 M Tris hydrochloride (pH 7.6) containing 0.05% (wt/vol) 3,3'-diaminobenzidene tetrahydrochloride (Sigma) and 0.01% (vol/vol) hydrogen peroxide. Controls with normal rabbit serum were included in all experiments.

Immunoblot overlays were prepared by incubation of the nitrocellulose strips containing immobilized proteins with either saliva or barbital extracts diluted in blocking buffer. After 1 h at room temperature, the strips were washed three times (30 min each time) with blocking buffer, reacted with antibody to the overlay antigen (either saliva or barbital extract), and developed as described above.

Identical results were obtained when phosphate-buffered saline containing 10% newborn calf serum and 0.2% Triton X-100 was used as the blocking buffer. The presence of additional factors, such as divalent cations provided by newborn calf serum, does not, therefore, generate new binding sites.

Chemical analysis. Protein concentration was estimated by the method of Bradford (6), with bovine serum albumin as a standard.

RESULTS

Adhesion characteristics of bacterial strains. The SHA adherence properties of *S. sanguis* strains and the ability of homologous and heterologous antisera to inhibit this adhesion are given in Table 1. G9B, a serotype 1 strain, adhered best to SHA. Blackburn, a serotype 2 strain, did not adhere as well as G9B, but its adhesion was still threefold better than that of either Adh$^-$ or M-5. All of the strains adhered to the same extent to control uncoated hydroxyapatite (K_aN = 1). Antisera to G9B and Blackburn, but not Adh$^-$ or M-5, inhibited adhesion of both of these strains to SHA. No effect of either homologous or heterologous antisera on the adhesion of Adh$^-$ and M-5 was observed. It appears, therefore, that strains G9B and Blackburn possess specific salivary adhesins which, although functionally not identical, are immunologically related.

Barbital extracts. To identify bacterial components which might contribute to salivary binding, a barbital extraction procedure was used to remove surface components. After extraction, the cells remained viable and no nucleic acid leakage was detected by radioisotope or spectrophotometric analysis. SDS-PAGE of extracts of the four strains revealed similar patterns, with many matching bands (Fig. 1). Two of the major differences were the presence of 80- and 160-kilodalton (kDa) polypeptides in G9B but not in Adh$^-$, M-5, or Blackburn; therefore, monospecific antisera against these antigens were produced as described in Materials and Methods.

Immunochemical characterization of barbital extracts. Immunoblots were used to investigate the barbital extracts further. In general, homologous and heterologous antibodies reacted with most of the proteins in the extracts, revealing major antigens of 66, 63, and 35 kDa common to all of the strains.

Immunoblots of the G9B extract are shown in Fig. 2. Antibodies to G9B (lane 1) and Blackburn (lane 4) reacted strongly with 160-, 80-, and 52-kDa antigens in addition to

TABLE 1. Adhesion of *S. sanguis* strains to SHA and effect of antisera on adhesion

S. sanguis strain	Adhesion to SHA[a]	Inhibition of adhesion by antiserum to[b]:			
		G9B	Adh$^-$	M-5	Blackburn
G9B	38	++	−	−	+
Adh$^-$	1	−	−	−	−
M-5	1	−	−	−	−
Blackburn	3	+	−	−	++

[a] Adhesion to SHA is expressed as the product of the affinity constant and the maximum number of binding sites (K_aN) (1). Cells (1×10^9 to 6×10^9) were added to the beads.

[b] Inhibition of adhesion to SHA at a serum dilution of 1 in 1,000 was scored as follows: ++, >90%; +, 60 to 80%; −, <5%.

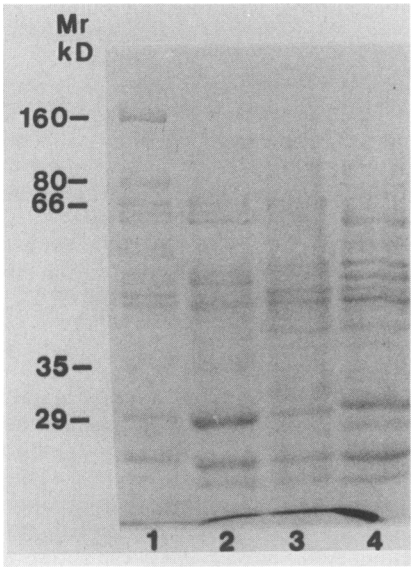

FIG. 1. Coomassie blue-stained SDS-PAGE of barbital extracts (30 μg of protein) from *S. sanguis*. Lanes: 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; 4, strain Blackburn.

the common antigens. Antibodies to M-5 (lane 3) failed to react, and antibodies to Adh$^-$ (lane 2) reacted weakly, with the 80- and 52-kDa antigens. However, both of these antisera reacted strongly with the 160-kDa antigen. Antiserum raised to the 80-kDa polypeptide (lane 5) reacted with the immunizing antigen, the 52-kDa antigen, and a new antigen of 62 kDa (lane 5, arrow). None of the polyvalent antisera detected the 62-kDa antigen, possibly because it is obscured by the nearby 63-kDa band or because it consists mostly of hidden determinants exposed by SDS-PAGE and thus only represented in the 80-kDa antiserum raised to the SDS-treated polypeptide. Antiserum to the 160-kDa polypeptide bound only to this antigen (lane 6).

None of the antisera detected the 80-, 62-, or 52-kDa antigens in the barbital extracts of Adh$^-$ or M-5 (Fig. 3 and 4). These strains also had only small quantities of the 160-kDa antigen.

Blackburn barbital extract (Fig. 5) also possessed little of the 160-kDa antigen and lacked the 80- and 62-kDa antigens. However, the 52-kDa antigen was present, and the 80-kDa antiserum (lane 5) reacted with two additional antigens of 42 and 29 kDa not seen in G9B. Antibodies to Adh$^-$ (lane 2) but not M-5 (lane 3) bound to the 42-kDa antigen, and these antibodies also appeared to react with the 29-kDa antigen. However, this is probably an unrelated comigrating polypeptide, since the 80-kDa antiserum did not detect a 29-kDa antigen in the Adh$^-$ and M-5 barbital extracts (Fig. 3 and 4, lanes 5) and strain Blackburn antiserum reacted about twice as strongly with the antigen(s) in this region than the Adh$^-$ and M-5 antisera (Fig. 5, lanes 2 to 4).

These results indicate that the 80-, 62-, and 52-kDa antigens of G9B are immunologically related to the antigens of 52, 42, and 29 kDa in strain Blackburn. Antisera to G9B and Blackburn, which inhibited adhesion of these two strains to SHA, reacted with the 80- and 52-kDa antigens, and Blackburn antibodies also reacted with the 42- and 29-kDa antigens. Since all of these antigens were detected by the anti-80-kDa serum, for simplicity we shall refer to them as the 80-kDa antigen complex. Antisera to Adh$^-$ and M-5, which had no effect on adhesion, reacted weakly or not at all with the 80-, 52-, and 29-kDa antigens, although Adh$^-$, but not M-5, antibodies did give a strong band with the 42-kDa antigen.

Binding of streptococcal proteins to saliva. Streptococcal-salivary binding was visualized by overlay immunoblots. Both

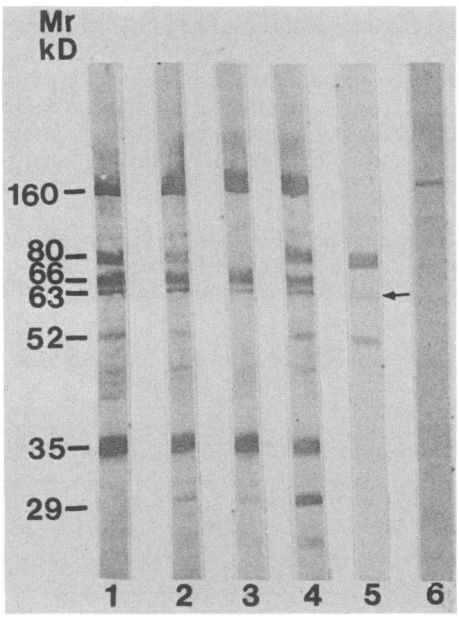

FIG. 2. Immunoblot of *S. sanguis* G9B barbital buffer extract (2 μg of protein) probed with antisera, diluted 1 in 100, raised against (lanes) 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; 4, strain Blackburn; 5, the 80-kDa antigen; and 6, the 160-kDa antigen.

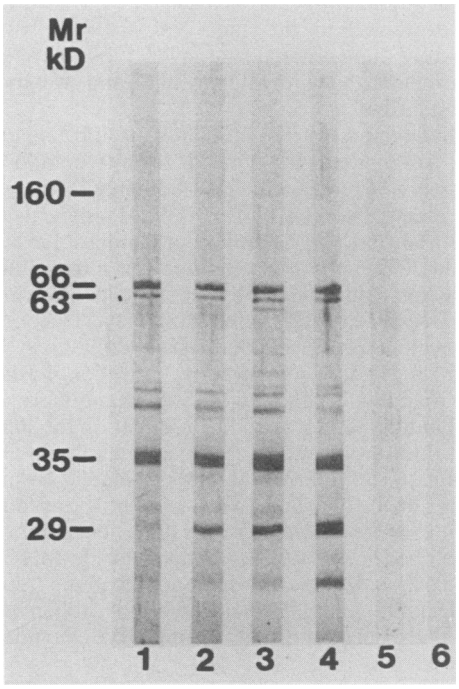

FIG. 3. Immunoblot of *S. sanguis* Adh$^-$ barbital buffer extract (2 μg of protein) probed with antisera, diluted 1 in 100, raised against (lanes) 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; 4, strain Blackburn; 5, the 80-kDa antigen; and 6, the 160-kDa antigen.

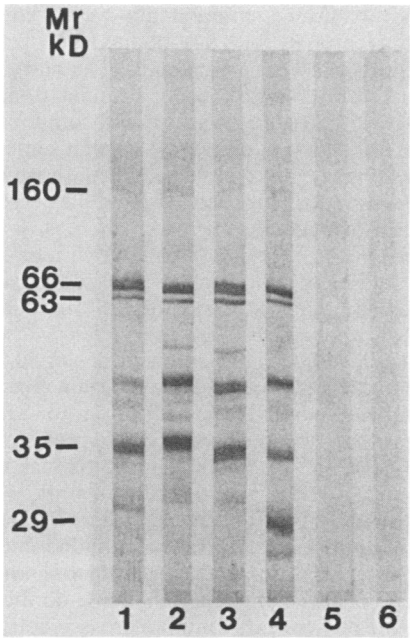

FIG. 4. Immunoblot of *S. sanguis* M-5 barbital buffer extract (2 μg of protein) probed with antisera, diluted 1 in 100, raised against (lanes) 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; 4, strain Blackburn; 5, the 80-kDa antigen; and 6, the 160-kDa antigen.

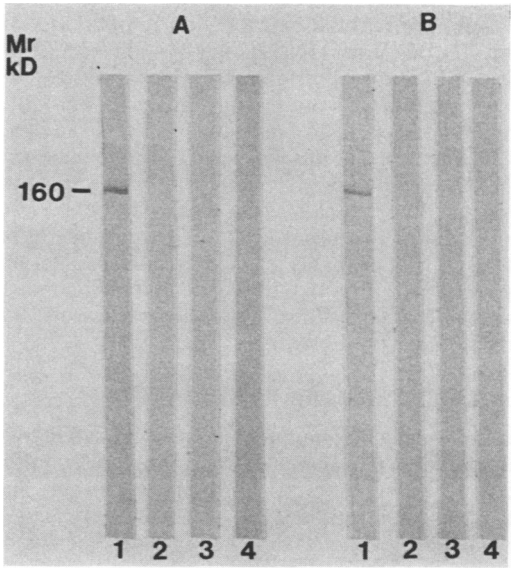

FIG. 6. Binding of saliva to barbital extracts from *S. sanguis*. (A) Barbital extracts (2 μg of protein) from (lanes) 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; and 4, strain Blackburn were separated by SDS-PAGE, immunoblotted, and overlaid with a 1-in-3 dilution of submandibular saliva. (B) The same extracts as in panel A overlaid with a 1-in-3 dilution of parotid saliva. Salivary binding was detected with homologous antibodies to saliva diluted 1 in 100.

submandibular and parotid saliva components bound to the 160-kDa polypeptide in SDS-treated G9B barbital extract (Fig. 6). However, the receptor for the 160-kDa antigen could not be detected in electrophoresed saliva.

When submandibular salivary molecules were immobilized on nitrocellulose (Fig. 7), native (non-SDS-treated) barbital-extracted antigens from G9B (lane 1) and Blackburn (lane 5) bound to a 73-kDa protein. This reaction was also detected by the 80-kDa antisera (lanes 2 and 6) and thus appeared to be mediated by the 80-kDa antigen complex. No interactions between Adh$^-$ or M-5 barbital extract and submandibular saliva were observed.

Barbital extracts from G9B, Adh$^-$, and M-5 did not react with any SDS-treated parotid saliva antigens. However,

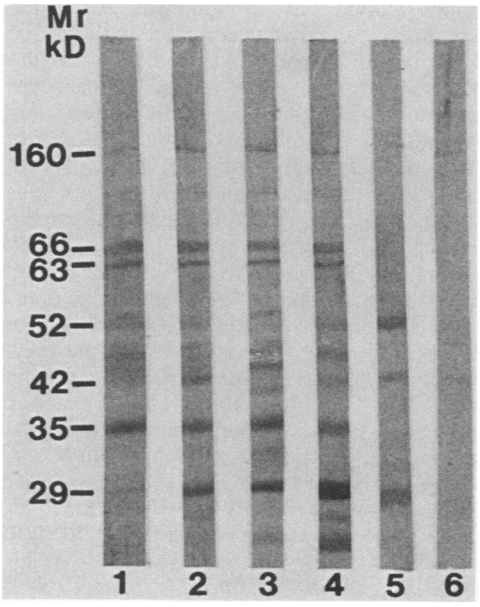

FIG. 5. Immunoblot of *S. sanguis* Blackburn barbital buffer extract (2 μg of protein) probed with antisera, diluted 1 in 100, raised against (lanes) 1, strain G9B; 2, strain Adh$^-$; 3, strain M-5; 4, strain Blackburn; 5, the 80-kDa antigen; and 6, the 160-kDa antigen.

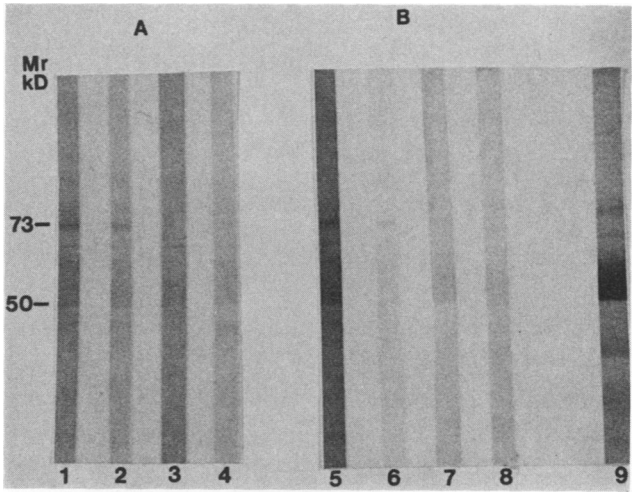

FIG. 7. Binding of *S. sanguis* antigens to submandibular saliva. Submandibular saliva (40 μg) was separated by SDS-PAGE, immunoblotted, and overlaid with barbital extract (500 μg of protein) from *S. sanguis* G9B (A) and Blackburn (B). Barbital extract-saliva binding was localized by probing with antisera raised against (lanes) 1, strain G9B; 2 and 6, the 80-kDa antigen; 3 and 7, the 160-kDa antigen; and 5, strain Adh$^-$. Lanes 4 and 8 were controls without barbital extract and showed nonspecific antibody binding. Lane 9 was a Coomassie blue stain of submandibular saliva after SDS-PAGE.

strain Blackburn barbital extract (Fig. 8) bound to antigens of 78 and 70 kDa (lane 1) which did not stain with Coomassie blue (lane 5). Since this interaction was not detected by either 80- or 160-kDa antiserum (lanes 2 and 3), these antigens do not appear to be involved in this binding.

All of the bacterial antibodies used to probe these overlays gave a diffuse band of 50 to 55 kDa with both submandibular and parotid saliva samples and a weak band of 65 kDa with submandibular saliva, regardless of the presence of barbital extract. This was probably caused by nonspecific antibody binding, since it also occurred when conjugated second antibody was reacted with saliva in the absence of anti-saliva antibodies (data not shown).

DISCUSSION

S. sanguis surface components react with saliva by poorly defined mechanisms. To understand these interactions better, we used well-characterized *S. sanguis* strains and antisera to probe the streptococcal surfaces immunochemically and investigate the bacterial and salivary molecules responsible for binding.

All of the strains studied showed similar saliva-mediated aggregation. Strains G9B and Blackburn demonstrated specific, antibody-inhibitable adhesion to SHA, whereas Adh^- and M-5 bound poorly, and binding was not inhibited by homologous or heterologous antisera. Since antibody to G9B and Blackburn, but not Adh^- or M-5, inhibited adhesion of G9B and Blackburn to SHA, a widely used in vitro model of the salivary pellicle, the salivary adhesins of these two strains are probably antigenically related. The effect of antibody on saliva-mediated aggregation was not studied because of the high agglutinating activity of all of the antisera.

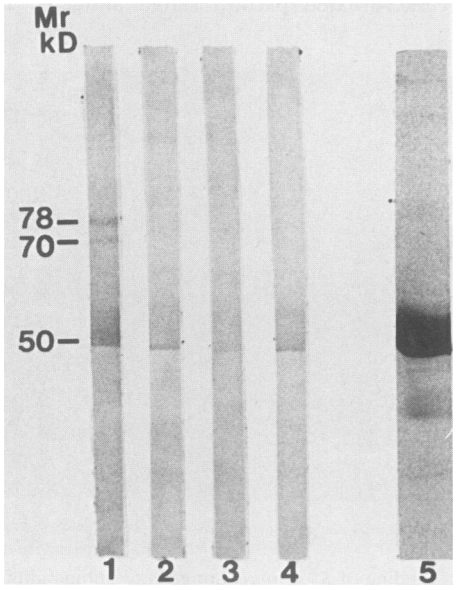

FIG. 8. Binding of *S. sanguis* antigens to parotid saliva. Parotid saliva (40 μg) was separated by SDS-PAGE, immunoblotted, and overlaid with barbital extract (500 μg of protein) from *S. sanguis* Blackburn. Barbital extract-saliva binding was localized by probing with antisera raised against (lanes) 1, strain Blackburn; 2, the 80-kDa antigen; and 3, the 160-kDa antigen. Lane 4 was a control without barbital extract and showed nonspecific antibody binding. Lane 5 was a Coomassie blue stain of parotid saliva after SDS-PAGE.

Streptococcal surface components were removed with barbital buffer, probed with antibodies, and reacted with saliva to identify interacting molecules. The barbital extracts can be expected to contain loosely associated surface molecules, along with more firmly associated components, in the process of extracellular excretion. Although some leakage of intracellular antigens might have occurred, those recognized by the antisera to formalinized whole cells were predominantly surface located.

SDS-PAGE of the extracts revealed a variety of proteins. Similar cell surface complexity has been reported by Appelbaum and Rosan (2) and Morris et al. (26). Immunochemical analyses of the barbital extracts revealed a correlation between antigenically related polypeptides of 80, 62, and 52 kDa in G9B and 52, 42, and 29 kDa in strain Blackburn (the 80-kDa antigen complex) and adhesion to SHA. These polypeptides were absent from nonadherent strains Adh^- and M-5. The G9B 80- and 52-kDa antigens reacted strongly with antibodies to G9B and Blackburn, only weakly with antibodies to Adh^-, and not at all with antibodies to M-5. The ability of antibodies to Adh^-, a nonadherent mutant of G9B, to bind to the 80- and 52-kDa antigens may be due to small amounts of these antigens remaining on the surface of Adh^- cells, the presence of common cross-reacting antigens in parent and mutant but not in M-5, or retention of the proteins in an immunologically and functionally altered form. Of the 52-, 42-, and 29-kDa antigens in Blackburn, the 52-kDa polypeptide is common with G9B, while the 42- and 29-kDa antigens seem unique to Blackburn, although they are immunologically related to G9B antigens. The structural relationships among these molecules are unclear. They might all be parts of a larger molecule, or the lower-sized polypeptides may be breakdown products of the 52- or 80-kDa (or both) proteins. Indeed, there is evidence that in G9B the 62- and 52-kDa polypeptides result from cleavage of the 80-kDa molecule by an endogenous enzyme (manuscript in preparation).

Further evidence that the 80-kDa antigen complex correlates with adhesion is provided by its ability to bind to submandibular saliva. This binding was established when blotted submandibular saliva was treated with G9B or Blackburn barbital extract, followed by antibodies to whole cells or to the 80-kDa protein. It did not occur, however, when saliva was reacted with blotted barbital extract, suggesting that conformational determinants are involved. Support for this concept comes from the ability of antibodies to native, but not to SDS-treated, 80-kDa antigen to block adhesion to SHA (manuscript in preparation). Similarly, Morris et al. (27) found that antibody to an *S. sanguis* native fibrillar preparation inhibited adhesion to SHA, whereas antibody to the denatured fibrillar antigen was ineffective. The 80-kDa antigens did not bind to parotid saliva. This is consistent with previous studies which demonstrated the presence of the adherence-promoting factor of saliva in submandibular saliva only (33). The molecular weight of the putative adhesin described here is in the same range as that of the adherence-blocking component obtained from *S. sanguis* by Liljemark and Bloomquist (21). However, its relationship to the fimbriae containing adhesins of >300 kDa, isolated from *S. sanguis* 12 by Morris et al. (27), and >150 kDa, found by Elder and Fives-Taylor (14) in *S. sanguis* FW213, is not clear. All of the strains examined in our study showed similar fimbriation (P. S. Handley, personal communication), suggesting that fimbriae per se do not mediate adhesion to SHA.

The salivary receptor for the 80-kDa antigen complex was

a 73-kDa protein-containing molecule. This does not seem related to the 55- and 60-kDa hydrophobic proteins in parotid saliva which Babu et al. (3) found to interfere with *S. sanguis* Challis adhesion to SHA. Unfortunately, streptococcal binding to salivary molecules of that size was not determinable in our experiments because of nonspecific antibody binding in that molecular weight region. We observed no reactions with other submandibular saliva components, such as the low-molecular-weight mucin (MG2) and proline-rich proteins, which have been implicated in *S. sanguis* adhesion (4, 38).

Bacterial-salivary interactions correlating with aggregation were not detected. However, the 160-kDa polypeptide in G9B barbital extract bound to submandibular and parotid saliva samples. Since both of these secretions contain aggregating activity for *S. sanguis* (33) and Morris et al. (26) found a putative agglutinin of 160 kDa in *S. sanguis* 12, it is possible that the 160-kDa protein mediates agglutination in G9B. Moreover, since antisera to Adh^-, M-5, and Blackburn seem to possess anti-160-kDa antibodies as potent as those found in anti-G9B serum, strains Adh^-, M-5, and Blackburn probably possess the 160-kDa protein, perhaps more tightly bound to the surface and no longer amenable to barbital buffer extraction. The salivary receptor for the 160-kDa protein was not identified. However, since it was present in both submandibular and parotid saliva samples, it is unlikely to be the high-molecular-weight (MG1), low-molecular-weight (MG2), or blood group-reactive mucins which have been implicated in aggregation (17, 20) but are not produced by the parotid gland (23). Failure to detect the 160-kDa receptor in saliva immobilized on nitrocellulose could be a consequence of denaturation during electrophoresis or electrotransfer. Alternatively, the size of the salivary molecule may be such that it does not resolve in 10% SDS-PAGE gels.

Components in barbital extract from strain Blackburn bound to molecules of 78 and 70 kDa in parotid saliva. These two components were not seen in SDS-PAGE of parotid saliva with Coomassie blue stain, implying that either they contain little protein or they are present in very small amounts in saliva. The bacterial molecules responsible for this binding were not identified, probably because they were denatured. The biological function of these interactions is unknown. However, they may reflect both the lower level of adhesion of strain Blackburn to SHA, compared with that of G9B, found here and differences in specificity for salivary pellicle between these strains reported previously (1). This is compatible with the observation that heterologous antiserum does not completely inhibit adhesion of G9B and Blackburn to SHA.

It is important to emphasize that the bacterial-salivary reactions demonstrated here are only those which can proceed in the presence of detergent (found to be essential to minimize background binding) and in which at least one of the components is resolvable by polyacrylamide gel electrophoresis and survives denaturation and transfer to nitrocellulose paper. Furthermore, since antibody was used for detection, poorly antigenic molecules may have escaped notice. Another uncertain factor is the extent to which material removed from bacteria retains its original activity and is an adequate reflection of the cell surface. Nevertheless, the sensitivity of the immunoblot overlay technique has allowed direct detection of a number of novel bacterial-salivary binding systems. These may act in concert with mechanisms proposed by other investigators to enhance adhesion or aggregation (or both), but some systems may be unique to individual strains.

The nature of the forces involved in these interactions can only be speculated upon. However, the reactions were stable in the presence of the detergent Tween 20; this tends to exclude purely hydrophobic bonding.

Further study is required to extend and confirm these interactions between *S. sanguis* and saliva and to determine more precisely their biological function and mode of action. Such increased knowledge and understanding should help elucidate the complex mechanisms which regulate the development of oral microflora.

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