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Adherence of Oral Streptococci to Salivary Glycoproteins

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We used an overlay method to study the ability of human salivary glycoproteins to serve as receptors for several strains of streptococci that colonize the oral cavity. Parotid and submandibular-sublingual salivas were collected as ductal secretions, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. The resulting blots were overlaid with [^{35}S]methionine-labeled bacteria, and salivary components to which the bacteria bound were detected by autoradiography. Potential glycoprotein receptors were identified for 8 of the 16 strains tested. In three cases (*Streptococcus sanguis* 72-40 and 804 and *Streptococcus sobrinus* OMZ176), highly specific interactions with a single salivary component were detected. Removal of sialic acid residues from the low-molecular-weight salivary mucin prevented adherence of one of these strains (*S. sanguis* 72-40), suggesting that this saccharide either mediates binding or is a critical component of the receptor site. In the remaining five strains (*Streptococcus gordonii* G9B and 10558, *S. sanguis* 10556, and *Streptococcus oralis* 10557 and 72-41), interactions with multiple salivary components, including the low-molecular-weight salivary mucin, highly glycosylated proline-rich glycoproteins, and α-amylase, were detected. These results suggest that some oral streptococci can bind specifically to certain of the salivary glycoproteins. The interactions identified may play an important role in governing bacterial adherence and clearance within the oral cavity.

One of the factors influencing the adherence of streptococci to oral surfaces is the availability of salivary receptors for these organisms (15, 53). These receptors can influence bacterial adhesion in several ways. In solution, they can induce the aggregation of oral bacteria (11–13, 28, 39, 40). Such aggregation can promote adhesion of bacteria to oral tissues and/or other bacteria (49), but it can also facilitate removal of oral bacteria by swallowing or flushing (19). Adsorbed to oral surfaces, salivary receptors are more likely to promote bacterial adhesion. For example, the adherence of streptococci, particularly *Streptococcus sanguis* and *Streptococcus mitis*, to the salivary components that coat the tooth surface is thought to be one of the first steps in the formation of dental plaque (20).

Bacterial adhesion is often the result of specific interactions between the carbohydrate portions of receptor glycoproteins and protein complexes termed adhesins on bacterial cell surfaces (45, 59). An example is the interaction between the sialic acid residues of human salivary agglutinin and an *S. sanguis* protein, SSP-5 (9). A variety of indirect techniques, including the inhibition of agglutination by simple saccharides and the production of monoclonal antibodies against receptors, have been used to determine the structural requirements of these interactions (5, 26). These methods can provide information concerning receptor specificity but usually cannot identify specific salivary components as receptors for particular bacteria. More direct studies have been hampered by the requirement that these salivary components be purified before interactions can be demonstrated.

An extremely useful technique that eliminates the requirement for purified receptor molecules involves overlaying glycolipids separated by thin-layer chromatography with radiolabeled bacteria (7, 8, 24, 27, 31, 33, 52). This approach leaves the bacterial cell surface intact, thus taking into consideration that adhesins may be multimeric protein complexes. In a previous study, we showed that the glycolipid overlay technique can be modified to detect glycoprotein receptors. Glycoproteins transferred to nitrocellulose were overlaid with radiolabeled bacteria, and specific receptors for *Staphylococcus aureus* and *Fusobacterium nucleatum* were identified (47). Subsequent studies showed that *F. nucleatum* adhered to either a low-molecular-weight or a high-molecular-weight human glycosylated proline-rich glycoprotein (PRG) via biantennary oligosaccharides containing three fucose residue substitutions but lacking sialic acid. Interestingly, one antenna of the major oligosaccharide contained two fucose substitutions and the other was not fucosylated, suggesting that this unusual structure played an important role in the highly specific bacterium-receptor interactions observed (21).

In this report, we present results obtained by using this overlay method to identify salivary glycoprotein receptors for 16 strains of streptococci that colonize various surfaces within the oral cavity. Subsets of the salivary glycoproteins, ranging from one to several components, were identified as receptors for half the strains tested. In one case, specific details of the adherence mechanism were elucidated by showing that terminal sialic acid residues were necessary for receptor activity. Together, these results suggest that specific salivary molecules are involved in the adherence and clearance of the strains for which binding was observed. In addition, these data are further evidence of the utility of this overlay method for detecting bacterial interactions with specific receptors that are components of the complex mixtures of proteins and glycoproteins that constitute biological fluids and tissue extracts.

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MATERIALS AND METHODS

Materials. Bovine serum albumin fraction V, prestained molecular weight standards for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and neuraminidase (type III) from *Vibrio cholerae* were purchased from Sigma Chemical Co., St. Louis, Mo. Nitrocellulose membrane (0.45-μm pore size) was obtained from Bio-Rad (Richmond, Calif.). Trypticase soy broth was purchased from Becton Dickinson Microbiology Systems (Cockeysville, Md.), and yeast extract was purchased from Difco Laboratories (Detroit, Mich.). [^{35}S]methionine (1,159 Ci/mmol) was obtained from New England Nuclear (Wilmington, Del.). Hyperfilm (X-ray film) is a product of Amersham (Arlington Heights, Ill.). Affinity-purified goat anti-rabbit immunoglobulin G was obtained from Jackson Immuno Research Laboratories, Inc. (West Grove, Penn.). PNGase F (peptide *N*-glycosidase F) was purified according to the method of Tarentino et al. (55).

SDS-PAGE and transfer of proteins. Parotid and submandibular-sublingual salivas were collected separately from 10 individuals of known blood types. The samples were collected as ductal secretions into an equal volume of SDS-PAGE loading buffer, consisting of 0.1 M phosphate buffer (pH 7.0) containing 6 M urea, 1% SDS, 1% β-mercaptoethanol, 0.015% bromphenol blue. Molecular weight standards were as follows: M_r 26,600, triose-phosphate isomerase; M_r 36,500, lactic acid dehydrogenase; M_r 48,500, fumarase; M_r 58,000, pyruvate kinase; M_r 84,000, fructose-6-phosphate kinase; M_r 116,000, β-galactosidase; M_r 180,000, α_2-macroglobulin. The saliva samples (100 μg of protein per lane) were separated on 7% slab gels (32), proteins were stained with silver (43), and highly glycosylated glycoproteins were stained with periodic acid-Schiff (57). Proteins were transferred to nitrocellulose membranes according to the procedure of Towbin et al. (56) and were visualized by staining with India ink (23) or amido black (56). After blotting, gels were routinely stained as described above to estimate the efficiency of transfer. The receptor patterns of the samples from two of the subjects represented the major variations observed among the 10 saliva donors. Therefore, only the results obtained with these samples are shown in Fig. 1 through 5. Subject 1 had blood type B+, Le(a−b−), and was a secretor. Subject 2 had blood type O+, Le(a−b+), and was also a secretor.

Immunostaining of nitrocellulose transfers. To confirm the identity of salivary components, nitrocellulose transfers were incubated with antibodies that reacted with specific salivary molecules and then were immunostained. Nonspecific binding was blocked by incubating blots for 1 h in Tris-buffered saline (TBS) (0.05 M Tris-HCl–0.15 M NaCl, pH 7.4) containing 5% nonfat dried milk (Carnation). The transfers were then incubated overnight in rabbit polyclonal antisera diluted in the blocking buffer as follows: anti-human α-amylase, 1:100; anti-human glycosylated PRG, 1:20; and anti-human cystatin, 1:20. These antibodies were the kind gift of Michael Levine, State University of New York at Buffalo. A rabbit polyclonal antibody produced against a synthetic peptide, the sequence of which was deduced from the cDNA of a cloned intestinal mucin (22), reacted specifically with the low-molecular-weight salivary mucin (MG2). This antibody was used at a dilution of 1:100 and was the kind gift of James Gumm and Young Kim, University of California San Francisco. After exposure to antibody, the blots were washed (5 min each time) once in TBS, twice in TBS containing 1% Triton X-100–0.5% deoxycholic acid–0.1% SDS, and twice in TBS again. Then they were incubated for 2 h in a 1:500 dilution of affinity-purified goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase. The blots were washed as described above, and the color was developed by adding a solution of diaminobenzidine (6 mg/10 ml of TBS) and 0.03% H_2O_2. Control blots were exposed to the secondary antibody alone.

Desialylation and deglycosylation of MG2. The procedure of Prakobphol et al. (46) was used to isolate MG2. To remove sialic acid, SDS-PAGE gels of the purified protein were transferred to nitrocellulose and treated in one of two ways. In the first treatment, the blot was digested by overnight incubation at 37°C in 5 ml of phosphate-buffered saline (PBS) containing 2 U of neuraminidase. A control blot was incubated in buffer alone. Alternatively, the transfer was treated with 0.1 N HCl (70°C for 10, 30, and 60 min). The resulting blots were washed extensively in PBS to remove either enzyme or acid prior to bacterial overlay. In all cases, an identically treated nitrocellulose transfer was stained with India ink as a qualitative measure of protein loss during the desialylation and washing procedures.

Other samples of MG2 were deglycosylated by treating the lyophilized protein with anhydrous hydrofluoric acid (HF) to cleave peripheral sugars and serine- or threonine-linked *N*-acetylgalactosamine; *N*-acetylglucosamine linked to asparagine was retained. Briefly, the purified protein was methanol precipitated and completely dried in a microfuge tube. Then 1 ml of condensed HF was added, and the sample was incubated for 1 h at 0°C. The remaining HF was removed under a stream of nitrogen, and the sample was dried. The deglycosylated protein was neutralized by adding 500 ml of Tris-HCl (pH 8.0) containing 0.02% SDS and then transferred to a clean tube. The sample was methanol precipitated, dried, and resuspended in SDS-PAGE loading buffer. In other experiments, *N*-linked chains were removed by digestion with PNGase F (20 mU/mg of protein per 200 μl of 0.2 M phosphate buffer, pH 8.6). In all cases, deglycosylation was monitored by observing a reduction in electrophoretic mobility.

Preparation of labeled bacteria. The bacterial strains used in this study are listed in Table 1. Strains were stored at −70°C in Trypticase soy broth containing 0.25% yeast extract and 20% glycerol.

To metabolically label bacteria, batch cultures of the organisms were grown at 37°C to late log phase (16 to 18 h) in Trypticase soy broth containing 0.25% yeast extract and 10 μCi of [^{35}S]methionine per ml. Radiolabel incorporation was 2×10^3 to 6×10^3 bacteria/cpm. Bacteria were harvested by centrifugation and washed three times in 0.01 M phosphate buffer (pH 7.0) containing 0.154 M sodium chloride. Cells were resuspended in TBS containing 5% bovine serum albumin and briefly sonicated on ice (<10 s) to disperse aggregates. Bacteria were then adjusted to a final concentration of 5×10^8/ml by A_{620} determinations previously related to cell number by direct counting in a Petroff-Hausser chamber.

Attachment of labeled bacteria to nitrocellulose blots. Labeled bacteria were allowed to adhere to blots of electrophoretically separated salivary glycoproteins as described previously (47), after which the blots were air dried and exposed to Hyperfilm. In all cases, bacterial adherence to the molecular weight markers, which were transferred to nitrocellulose together with the salivary components, was monitored as a negative control.

TABLE 1. Streptococcal strains

Species[a]	Strain[b]	Original source
S. sanguis	10556	American Type Culture Collection
	72-40	R. Cole, National Institutes of Health
	804	J. Carlsson, University of Umea, Umea, Sweden
S. gordonii	10558	American Type Culture Collection
	G9B	B. Rosan, University of Pennsylvania
	Wickey	R. Cole, National Institutes of Health
S. mitis	903	American Type Culture Collection
S. oralis	10557	American Type Culture Collection
	72-41	R. Cole, National Institutes of Health
S. salivarius	13419	American Type Culture Collection
S. sobrinus	OMZ176	B. Guggenheim, University of Zurich, Zurich, Switzerland
	6715	P. Keyes, National Institutes of Health
S. mutans	25175	American Type Culture Collection
	Ingbritt	B. Krasse, University of Goteborg, Goteborg, Sweden
	GS-5	R. J. Gibbons, Forsyth Dental Center
	LM-7	R. J. Gibbons, Forsyth Dental Center

[a] Based on the classification scheme proposed by Kilian et al. (29).
[b] All the strains were from the bacterial culture collection of the Division of Oral Biology, Department of Stomatology, University of California San Francisco.

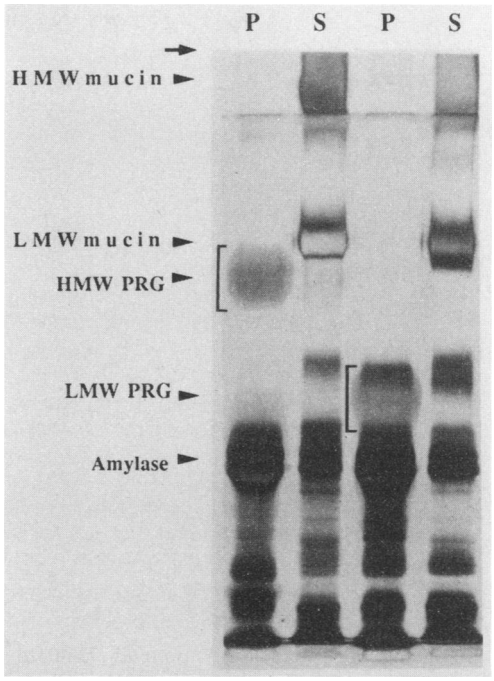

FIG. 1. SDS-PAGE of parotid (P) and submandibular-sublingual (S) salivas collected from subject 1 (lanes 1 and 2) and subject 2 (lanes 3 and 4). The components were separated on 7% polyacrylamide gels and visualized by staining with silver. Nitrocellulose blots of these samples were used in all the adherence assays. HMW, high molecular weight; LMW, low molecular weight; PRG, highly glycosylated PRG; →, origin.

RESULTS

Samples of the parotid and submandibular-sublingual salivas that were used for this study were electrophoretically separated, and the protein components were demonstrated by silver staining (Fig. 1). For a review of the structure of salivary proteins, see reference 34. Prominent components of the parotid salivas included either a high- or a low-molecular-weight glycosylated PRG. Recent evidence suggests that PRGs can vary substantially in molecular weight among individuals (2, 21). The amino acid sequence of the low-molecular-weight PRG (Fig. 1, lane 3) has been deduced from the nucleotide sequence (38), and some of the structural aspects of the *N*-linked oligosaccharides have been determined (48). We found that in certain individuals, the low-molecular-weight glycoprotein is replaced by a high-molecular-weight PRG (Fig. 1, lane 1). This glycoprotein, which cross-reacts with an antibody against the low-molecular-weight PRG (unpublished observations), has a peptide core whose sequence is related to that of the lower-molecular-weight protein and to the product encoded by the PRB-3 gene (21, 37). Like the low-molecular-weight PRG, this glycoprotein also mediates adherence of *F. nucleatum* (21, 47).

Other salivary components were identified by immunostaining blots of identical gels with specific antisera. This allowed us to unequivocally identify α-amylase, present in parotid saliva, as well as cystatin and the low-molecular-weight salivary mucin (MG2), components of submandibular-sublingual saliva. The high-molecular-weight mucin (MG1) was identified by its periodic acid-Schiff reactivity. Finally, it was also clear from these studies that some of the salivary glycoproteins migrated with anomalously high molecular weights in the Laemmli SDS-PAGE system we used. For example, the low-molecular-weight PRG (M_r, 36,900) had a slower electrophoretic mobility than α-amylase (M_r, 50,000 to 60,000). Presumably this was due, at least in part, to the high carbohydrate content of the former glycoprotein relative to that of the latter.

Examples of the binding patterns we observed when blots of the salivary glycoproteins were overlaid with radiolabeled bacteria are shown in Fig. 2 through 5. In some cases (Fig. 2 to 4), binding to a single component of submandibular-sublingual and/or parotid saliva was detected. For example, *S. sanguis* 72-40 (Fig. 2) and *S. sanguis* 804 (data not shown) bound only to the low-molecular-weight mucin found in submandibular-sublingual saliva. No binding to the high-molecular-weight mucin was observed. This was not due to a failure to transfer this glycoprotein, since a high-molecular-weight component with an M_r identical to that of MG1 was detected when the blot was stained with India ink. In addition, no mucin was left in the original SDS-PAGE gel, as demonstrated by staining with periodic acid-Schiff. Even after long-term exposure of the blot to X-ray film, no other components that supported adherence of these bacteria were detected. Furthermore, no interactions between these or any other strains we tested and the protein molecular weight markers were observed (data not shown). These results suggest that *S. sanguis* 72-40 and *S. sanguis* 804 interact preferentially with the low-molecular-weight salivary mucin, which differs substantially in structure from the high-molecular-weight mucin (36).

Using MG2 purified from subject 1, we investigated the role of specific oligosaccharide residues carried by MG2 in mediating adherence of these strains. Removal of sialic acid, either by neuraminidase digestion or by mild acid treatment,

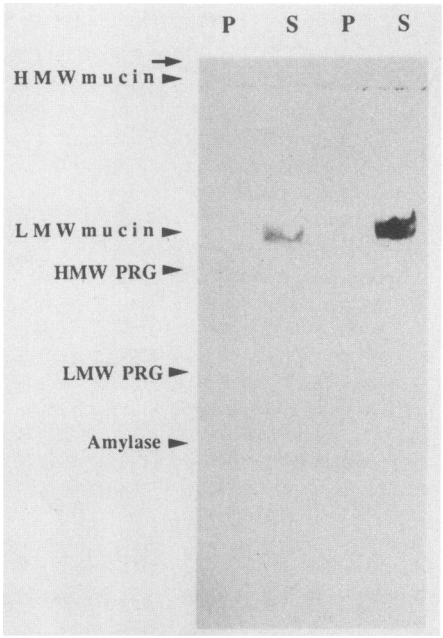

FIG. 2. Binding of ^{35}S-labeled *S. sanguis* 72-40 to the low-molecular-weight mucin. The salivary glycoproteins were separated by SDS-PAGE, transferred to nitrocellulose, and overlaid with the metabolically labeled bacterium. Adherence to purified protein was detected by autoradiography. Abbreviations are as defined in the legend to Fig. 1.

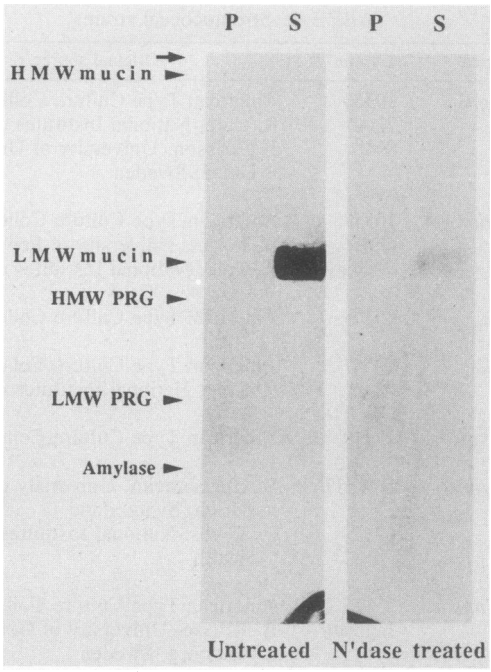

FIG. 3. Binding of ^{35}S-labeled *S. sanguis* 72-40 to purified MG2 is sialic acid dependent. Samples of purified MG2 were transferred from SDS-PAGE gels to nitrocellulose and overlaid with the metabolically labeled bacterium. Adherence to the purified protein was detected by autoradiography (Untreated). After treatment with neuraminidase (N'dase treated), adherence was substantially reduced. Abbreviations are as defined in the legend to Fig. 1.

greatly diminished the ability of this mucin to support adherence of *S. sanguis* 72-40 (Fig. 3) but did not change, as estimated by staining with India ink, the amount of protein present in the MG2 band (data not shown). Thus, sialic acid constitutes either all or a portion of the binding site on the low-molecular-weight mucin recognized by this bacterium. Additional experiments suggested that receptor activity could also be abolished by the treatment of purified MG2 with anhydrous HF, which results in the cleavage of peripheral sugars and serine- or threonine-linked *N*-acetylgalactosamine while *N*-acetylglucosamine linked to asparagine is retained. In contrast, treatment of purified MG2 with PNGase F, which removes only asparagine-linked saccharides, had no effect on the adherence of *S. sanguis* 72-40 (data not shown).

Selective interactions with salivary components other than MG2 were also observed. *Streptococcus sobrinus* OMZ176 bound specifically to a salivary component with an M_r of 45,000 (Fig. 4) in parotid saliva. The identity of this protein is unknown and is currently under investigation in our laboratory. However, it was clear that this component is not related to α-amylase, since it does not stain with anti-α-amylase antibodies. Adherence to a slightly higher-molecular-weight component present only in submandibular-sublingual saliva was also detected (Fig. 4). At the present time, the structural relationship between the two proteins that support adherence of *S. sobrinus* OMZ176 is unknown.

Streptococcus oralis 10557 (Fig. 5) and several of the other bacterial strains (*Streptococcus gordonii* G9B and 10558 and *S. oralis* 72-41) bound to multiple components in both the submandibular-sublingual and the parotid salivas. These components included MG2 and the highly glycosylated PRGs from both subjects. Interestingly, by overlaying with ^{35}S-labeled bacteria, we detected minor cross-contamination of the salivas (parotid with submandibular-sublingual and vice versa) that was not visible by silver staining. For example, autoradiography of the binding of *S. oralis* 10557 revealed trace amounts of the highly glycosylated PRG, which is found only in parotid saliva, in these particular samples of submandibular-sublingual saliva (Fig. 5). We also saw individual variation in the ability of particular salivary components from the two subjects to support binding. For example, α-amylase in the parotid saliva from subject 2 but not subject 1 promoted adherence of *S. oralis* 10557. In addition, this same bacterium bound to different, unidentified, low-molecular-weight (<40,000) components in the parotid salivas of subject 1 and subject 2. These results suggest that in this case, the structural elements that make up receptor activity are carried by more than one glycoprotein and by different glycoproteins from different individuals. This could be related to variations in glycosylation of salivary components, since these modifications are known to change with blood type and secretor status (18), as well as to the genetic polymorphisms that are typical of salivary components. More subtle variations in the binding patterns of these bacteria were also observed. For example, we consistently found that higher numbers of *S. oralis* 10557 bound to MG2 than to the glycosylated PRGs, as evidenced by the relative intensities of the two bands on the same autoradiogram. The opposite pattern was observed for binding of *S. gordonii* G9B (data not shown). This bacterium bound to the same salivary components as *S. oralis* 10557, but apparently in greater numbers to the PRGs than to MG2. The significance of this observation remains to be tested.

Table 2 summarizes the relative binding affinities we observed for the various strains tested. Binding was rated on

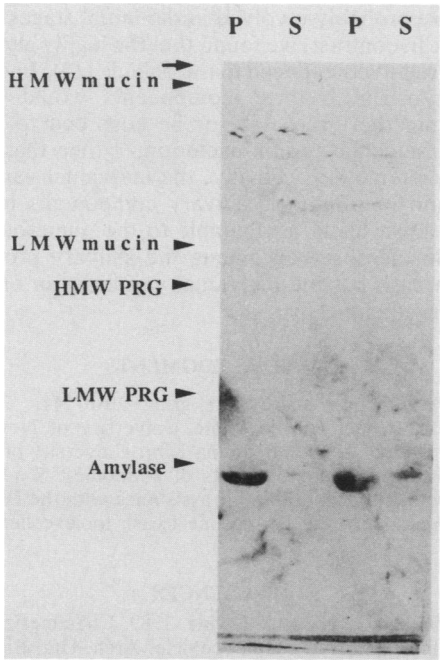

FIG. 4. Binding of ^{35}S-labeled *S. sobrinus* OMZ176 to a single component of parotid saliva (P) and a component of submandibular-sublingual (S) saliva. Abbreviations are as defined in the legend to Fig. 1.

a scale of − to ++++. When there were differences in the ability of the salivary components from the two individuals to support adherence, both results are included. Binding to specific salivary components was detected in 8 of the 16 strains. For the other eight, including all the *Streptococcus mutans* strains tested, no binding was detected. In the case of *Streptococcus salivarius* 13419 this was expected, since this bacterium preferentially colonizes the tongue rather than tooth surfaces (58). The fact that different specificities were seen for different bacteria and that no binding was observed for more than half of the strains suggests the highly specific nature of those interactions detected.

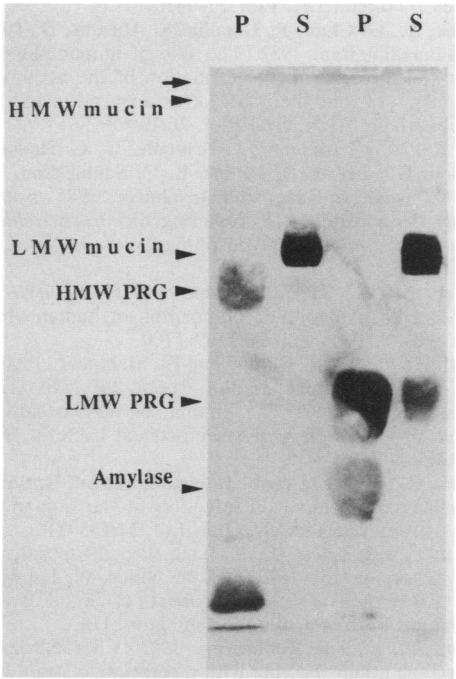

FIG. 5. Binding of ^{35}S-labeled *S. oralis* 10557 to multiple salivary components. Abbreviations are as defined in the legend to Fig. 1.

TABLE 2. Salivary receptor specificities of 16 strains of oral streptococci

Species	Strain	Binding rating[a]			
		MG2[b]	PRGs	α-Amylase	Other[c]
S. sanguis	10556	++++	++	+	+
	72-40	++	−	−	−
	804	++	−	−	−
S. gordonii	10558	−	++	−, ++	++, −
	G9B	++	++++	++	+++
	Wickey	−	−	−	−
S. mitis	903	−	−	−	−
S. oralis	10557	++++	++	−, ++	++, −
	72-41	+	+++	+++	+, +++
S. salivarius	13419	−	−	−	−
S. sobrinus	OMZ176	−	−	−	++
	6715	−	−	−	−
S. mutans	25175	−	−	−	−
	Ingbritt	−	−	−	−
	GS-5	−	−	−	−
	LM-7	−	−	−	−

[a] Binding was rated on a scale of − to ++++. Two values indicate individual differences in the ability of particular salivary components to support adherence of a bacterium. In all cases, the first result was obtained with saliva from subject 1 and the second was obtained with saliva from subject 2.
[b] Low-molecular-weight mucin.
[c] Proteins of unknown identity.

DISCUSSION

Few components of biologically relevant tissues or body fluids have been shown to serve as receptors for bacteria. To identify potential salivary receptors for oral bacteria, we used an overlay assay in which electrophoretically separated parotid and submandibular-sublingual components are immobilized on nitrocellulose. This method favors the identification of interactions with the carbohydrate portions of receptors, since oligosaccharide conformation is not altered by the electrophoretic or blotting procedures. The interaction of *F. nucleatum* with highly glycosylated PRGs is an example of a carbohydrate-based interaction detected by this method (21, 47). The identification of interactions mediated by short protein sequences is probably also possible; for example, fibroblasts attach to the tripeptide cell-binding domain of fibronectin under these conditions (25). However, adherence that depends on recognition of an extended protein conformation of the receptor or on the formation of complexes between multiple salivary components cannot be detected under the conditions we used, since the samples were reduced and denatured prior to electrophoresis.

Among the strains of oral streptococci studied, we detected different adherence patterns. In certain cases (*S.*

sanguis 72-40 and *S. sanguis* 804), a bacterium interacted in a very specific manner with only one salivary component. These results suggest that adherence requires recognition of a structural motif that is unique to a particular glycoprotein. For example, removal of sialic acid, a saccharide commonly found at the termini of many salivary proteins, abolished adherence of *S. sanguis* 72-40 to MG2. Significantly, other sialic acid-containing salivary glycoproteins did not support adherence of this strain. Thus, we postulate that sialic acid-containing oligosaccharides and/or the protein core to which these units are attached forms a unique recognition site that mediates adherence. In other cases (*S. gordonii* G9B and 10558, *S. sanguis* 10556, and *S. oralis* 10557 and 72-41), bacteria bound to several components. These results suggest that the epitopes necessary for adherence may be present on multiple proteins.

Some of the interactions we identified may involve protein as well as carbohydrate portions of salivary components. To distinguish between these two possibilities, we are deglycosylating the purified proteins and conjugating the reducing termini of the oligosaccharides to phosphatidyl ethanolamine dipalmitoyl. The products can then be separated by thin-layer chromatography, and potential oligosaccharide receptors can be identified by overlaying with metabolically labeled bacteria (21, 42). The structures of these neoglycolipids can also be determined by mass spectrometry (42). Structural analysis of the oligosaccharides that support binding can provide further information on the specific mechanisms governing bacterial adherence to salivary components.

Other studies have revealed interactions between oral streptococci and salivary molecules. Receptors identified include α-amylase (10, 50), high- and low-molecular-weight mucins (41, 44, 54), secretory immunoglobulin A (35), a basic salivary PRG (51), 55- and 60-kDa salivary glycoproteins (3, 4), and a 300-kDa component from submandibular-sublingual saliva (30). In that we detected binding to the mucins, α-amylase, and highly glycosylated PRGs, our results are generally consistent with many of these previous reports. Furthermore, our results show that there are strain as well as species differences in the interactions between oral streptococci and salivary components. These data are consistent with the demonstration of different salivary receptors for *S. mutans* and *S. sobrinus* (16). However, one surprising result was that no strain of *S. mutans* that we tested bound to specific salivary components. Previous work suggests that a fucose-rich, 440-kDa parotid glycoprotein (13), as well as 55- and 60-kDa salivary glycoproteins (3, 4), can aggregate cell suspensions of many oral streptococci, including *S. mutans* strains. Others have shown that an unidentified high-molecular-weight component from submandibular-sublingual saliva (>300 kDa) promoted adhesion of *S. mutans* to hydroxyapatite (30) and that *S. mutans* JBP could interact with the high-molecular-weight salivary mucins and acidic proline-rich proteins (17). These differing observations are probably due to the use of different techniques (i.e., solid-phase versus soluble assays) and bacterial strains.

Although the biological relevance of our findings remains to be determined, we can speculate on the role of the identified interactions in modulating streptococcal colonization of the oral cavity. As suggested by other investigators (49), we postulate that some salivary components promote clearance, while others facilitate adherence. MG2 (14), α-amylase (1), and acidic proline-rich proteins (6) are among the major components of the human salivary pellicle that coats the tooth surface. Bacteria that bind to these glycoproteins are probably involved in the initial stages of plaque formation. In contrast, we found that the highly glycosylated PRGs are relatively reduced in the pellicle (14). Bacteria that adhere primarily to these components would either be cleared from the oral cavity or be adsorbed to the tooth surface through bacterium-bacterium rather than pellicle-bacterium interactions. Finally, the individual variation we observed in the ability of salivary components to support binding is most likely attributable to the numerous genetic polymorphisms observed among the salivary proteins and suggests a basis for the individual variations in oral microflora observed clinically.

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