INFECTION AND IMMUNITY, Oct. 1991, p. 3492–3497
0019-9567/91/103492-06$02.00/0

Vol. 59, No. 10

Interaction of a Salivary Mucin-Secretory Immunoglobulin A Complex with Mucosal Pathogens

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Received 21 February 1991/Accepted 19 July 1991

This study examined the interaction of a human salivary low-molecular-weight mucin (MG2) with *Staphylococcus aureus* and *Pseudomonas aeruginosa* by using both solution-phase and solid-phase assays. In solution phase, MG2 in human submandibular-sublingual saliva (HSMSL) bound to the bacterial surface; however, the highly purified mucin isoforms (MG2a and MG2b) did not. Mucin binding appeared to be dependent on heterotypic complexing between MG2 and secretory immunoglobulin A (IgA), although other salivary molecules may also be involved. In contrast, in a solid-phase assay in which HSMSL, MG2-containing fractions with secretory IgA, and purified MG2 were immobilized onto a solid surface, there was minimal adherence of *S. aureus*. The collective results suggest that mucin binding to *S. aureus* and *P. aeruginosa* may be predicated on the formation of an MG2-secretory IgA complex. Such interactions may facilitate microbial clearance from the oral cavity and play an important role in preventing colonization of the oral cavity and the respiratory tract by potential pathogens.

Pseudomonas aeruginosa and *Staphylococcus aureus* are common mucosal inhabitants which can serve as opportunistic pathogens (12, 28, 36). Bacterial colonization of the oral cavity in conjunction with the aspiration of saliva appears to act as a source or reservoir of these respiratory pathogens, which may initiate pulmonary infections (14, 15, 21, 33, 39, 40). Both *S. aureus* and *P. aeruginosa* can adhere to salivary or respiratory mucins in vitro (28, 36). These mucins likely play a role in protecting the underlying epithelial cells and facilitate clearance of pathogenic bacteria. However, under certain disease conditions (e.g., cystic fibrosis), bacterial adhesion to host cells followed by colonization and infection is favored (35). Salivary mucins can bind to and agglutinate various species of oral bacteria (9, 18, 19) and may modulate microbial colonization through specific interactions with bacterial surface adhesins (9, 18, 20, 24, 25). In addition, salivary mucins may aggregate potential respiratory pathogens such as *P. aeruginosa* to enhance their clearance from the oral cavity (16).

Human submandibular-sublingual saliva (HSMSL) contains both high- and low-molecular-weight mucins (MG1 and MG2, respectively) (22, 26, 27). MG2 (120 to 140 kDa) comprises a single polypeptide chain with O- and N-linked oligosaccharides which account for approximately 70% of the mucin's weight (26). The major di- and trisaccharides of MG2, Galβ1,3GalNAc and NeuAcα2,3Galβ1,3GalNAc, interact with oral bacteria containing galactose or sialic acid-binding adhesins, respectively (18, 19, 25). Recent studies have found that MG2 can exist as two isoforms, MG2a and MG2b, which differ in their fucose and sialic acid contents (27). In the present study, we examined the interaction of HSMSL and MG2 isoforms with *P. aeruginosa* and *S. aureus* by using both solution- and solid-phase assays. Our findings indicate that MG2 complexing with secretory immunoglobulin A (sIgA) may be an important determinant for MG2 interaction with these mucosal pathogens.

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MATERIALS AND METHODS

Materials. The following materials were obtained from the indicated sources: Trypticase soy broth, Todd-Hewitt broth, MacConkey no. 2 and Staphylococcus no. 110 prepared-medium agar plates (BBL Microbiology Systems, Cockeysville, Md.); yeast extract (Difco Laboratories, Detroit, Mich.); chloramine T, bovine serum albumin (BSA; fraction V), sodium cyanoborohydride, dithiothreitol, *N*-2-hydroxyethylpiperazine-*N'*-2 ethanesulfonic acid (HEPES), fibronectin from human plasma, rabbit anti-human salivary amylase, rabbit anti-human sIgA (α-chain specific), and Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.); affinity-purified goat anti-rabbit or anti-mouse IgG–horseradish peroxidase conjugate, 4-chloro-1-naphthol (horseradish peroxidase color development reagent), acrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persulfate, silver stain kit, and prestained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) low-range molecular protein standards consisting of phosphorylase *b*, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme (Bio-Rad Laboratories, Richmond, Calif.); SpectraPor 3 dialysis membrane (molecular weight cutoff, 3.5 kDa; Spectrum Medical Industries, Los Angeles, Calif.); Sephadex, Sephacryl, and Sepharose gels (Pharmacia Fine Chemicals, Piscataway, N.J.); Tween 20 (Fisher Scientific, Fair Lawn, N.J.); *N*-succinimidyl-3-(4-hydroxyphenyl)propionate (Pierce Chemical Co., Rockford, Ill.); $Na^{125}I$ (14.3 mCi/μg; Amersham Corp., Arlington Heights, Ill.); iodoacetamide (Eastman Kodak Co., Rochester, N.Y.); sIgA (Calbiochem Corp., San Diego, Calif.); Immobilon P membrane (Millipore Corp., Bedford, Mass.); polypropylene test tubes (17 by 100 mm; Laboratory Products Sales, Rochester, N.Y.); polyvinyl Mic-2000 96-well microtiter plates (Dynatech Laboratories, Alexandria, Va.); and Mighty Tall electrophoresis and Semiphor TE 70 transfer units (Hoefer Scientific Instruments, San Francisco, Calif.). We have previously described mouse monoclonal anti-MG1 (5) and rabbit polyclonal anti-MG2 (4) antibodies.

Bacterial strains and culture conditions. *S. aureus* isolates 15163, 15194, and 15334 from sputum samples from male

pneumonia patients were kindly provided by J. Mylotte, Veterans Administration Hospital, Buffalo, N.Y. *P. aeruginosa* R1 and 1244 (piliated nonmucoid sputum isolates) and M35 (a nonpiliated mucoid isolate) from cystic fibrosis patients were kindly provided by R. Ramphal, University of Florida, Gainesville, and R. L. Boyd, University of Texas, San Antonio. *P. aeruginosa* and *S. aureus* isolates were maintained as frozen cultures at −70°C in Trypticase soy broth and Todd-Hewitt broth, respectively, supplemented with 5% yeast extract and 10% glycerol. For routine use, *P. aeruginosa* and *S. aureus* isolates were subcultured on MacConkey no. 2 and Staphylococcus no. 110 prepared-medium agar plates, respectively, under limited oxygen tension. Isolated colonies of *P. aeruginosa* and *S. aureus* were grown in Trypticase soy broth and Todd-Hewitt broth, respectively, each supplemented with 5% yeast. After static incubation for 8 to 16 h at 37°C under limited oxygen tension, bacteria were inoculated (1% [vol/vol]) into larger batch cultures. Unless otherwise noted, bacteria were grown statically at 37°C under limited oxygen tension for approximately 6 h to mid-log phase. They were harvested by centrifugation at 1,900 × *g* and washed three times in 0.01 M sodium phosphate with 0.154 M NaCl, pH 7.2 (PBS). Bacteria were suspended to an A_{660} of 1 in PBS. These suspensions correspond to 1×10^9 *S. aureus* and 5×10^8 *P. aeruginosa* per ml.

Modification of HSMSL. HSMSL was collected and processed as previously described (29). Reductive methylation (RM) of HSMSL was performed by employing the conditions described earlier (13, 27). Briefly, samples were solubilized (10 mg/ml) in 0.1 M HEPES containing 6 M guanidine-HCl, pH 7.5, and incubated with formaldehyde (50 M excess with respect to lysine) in the presence of 100 mM sodium cyanoborohydride for 24 h at 37°C. RM-HSMSL was dialyzed against cold distilled water with a SpectraPor 3 dialysis membrane and lyophilized. For reduction and alkylation (RA), HSMSL (5 to 10 mg/ml) was dissolved in 0.1 M Tris HCl with 6 M guanidine-HCl, pH 8.5, and reduced with dithiothreitol (150 times the SH content of the sample) for 24 h at room temperature. Reduced samples were alkylated with iodoacetamide (twofold molar excess relative to dithiothreitol) for 1 h at room temperature. Both procedures were performed in the dark in a nitrogen atmosphere. RA-HSMSL was dialyzed against cold distilled water with a SpectraPor 3 dialysis membrane and lyophilized.

Preparation of mucin fractions. As outlined in Fig. 1, MG2a, MG2b, and crude mucin fractions (pool A and pool A2) were prepared from the lyophilized HSMSL as recently described (27). Mucins which were both RM and RA were designated RM/RA-MG2a and RM/RA-MG2b.

Preparation of ^{125}I-labeled samples. HSMSL (reconstituted pools A through D [1.0:0.5:1.0:0.5 [wt/wt]; Fig. 1), RM/RA-MG2a and RM/RA-MG2b were iodinated by the chloramine T method (10). Samples (50 to 100 μg) in 100 μl of 250 mM sodium phosphate buffer, pH 7.2, were treated with 10 μl each of $Na^{125}I$ (1 mCi) and chloramine T (10 μg in phosphate buffer). After 90 s, the reaction was terminated by the addition of 100 μl of a mixture of sodium metabisulfite and potassium iodide (2 mg each per ml of water). This mixture was desalted by gel filtration on a column (1 by 10 cm) of Sephadex G-25 with PBS as an eluant. Aliquots (5 μl) of column fractions (1 ml) were monitored for radioactivity with a Beckman 5500 gamma counter. Materials eluting at the void volume were pooled and diluted with PBS to 4×10^7 cpm for HSMSL and 2×10^7 cpm for RM/RA-MG2a and RM/RA-MG2b per 0.5 ml.

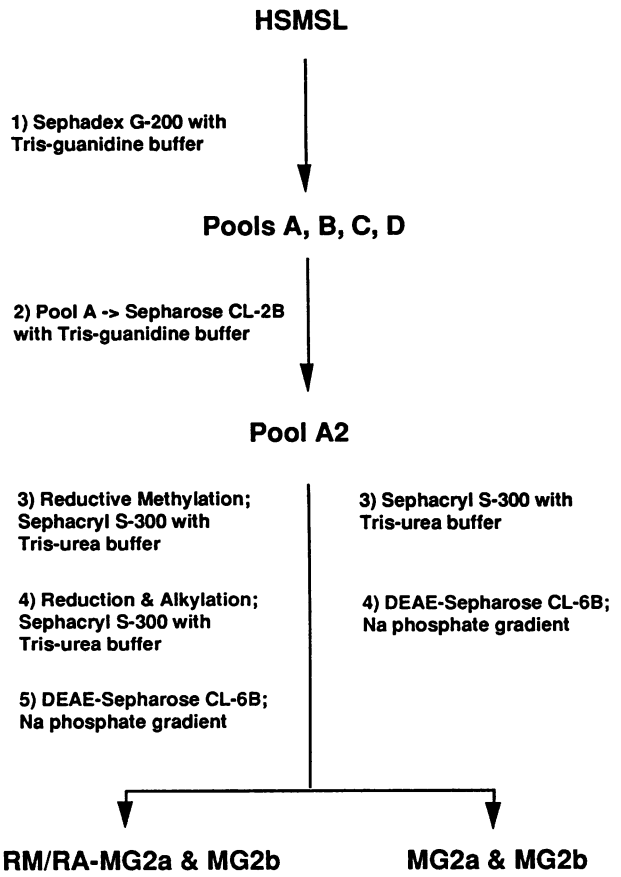

FIG. 1. Preparation of low-molecular-weight salivary mucin isoforms.

Initial attempts to metabolically label bacteria with [*methyl*-^{3}H]thymidine resulted in low specific activities. Consequently, *S. aureus* isolate 15334 was exogeneously labeled with ^{125}I (10). Bacteria were grown to early stationary phase, harvested, and washed in PBS. About 5×10^{10} cells were suspended in 2 ml of PBS with 1 mCi of $Na^{125}I$, after which chloramine T (50 μg in 50 μl of PBS) was added. After 90 s, the reaction was terminated by the addition of 500 μl of a mixture of sodium metabisulfite and potassium iodide (2 mg each per ml of water). The mixture was diluted with 6 ml of PBS and centrifuged at 1,900 × *g* for 5 min, and the cells were washed three times with 6 ml of PBS. A specific activity of ~1 cpm/10^2 bacteria was obtained.

Electrophoretic methods. SDS-PAGE was performed according to the method of Laemmli (17). Samples were solubilized in 0.064 M Tris buffer, pH 6.8, containing 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. Following electrophoresis, gels were stained for 1 h with 0.25% Coomassie brilliant blue in methanol-acetic acid-water (4/1/5 [vol/vol/vol]), destained in methanol-acetic acid-water (1/1/8 [vol/vol/vol]), placed in 10% acetic acid for 24 h, and then stained with periodic acid-Schiff reagent (PAS) (7). Alternatively, gels were silver stained following the manufacturer's (BioRad) instructions. Iodinated materials were visualized by autoradiography performed on dried gels at −70°C for 24 to 48 h with X-Omat AR film by using an intensifying screen.

Western (immunoblot) transfer was performed according to the methods of Burnett (2) and Towbin et al. (32). Immobilon P-bound antigens were incubated for 30 min in

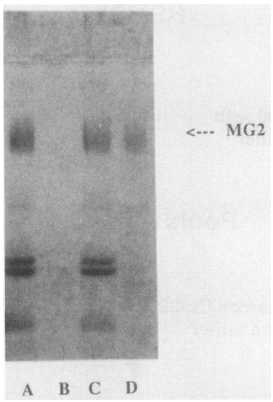

FIG. 2. SDS–7.5% PAGE of HSMSL components which bind to *S. aureus* isolate 15334. Gels were stained with Coomassie brilliant blue and counterstained with PAS. Lanes: A, HSMSL alone; B, extract of *S. aureus* incubated with PBS; C, HSMSL after depletion by *S. aureus*; D, HSMSL components which bind *S. aureus*. Similar results were obtained with *P. aeruginosa* 1244.

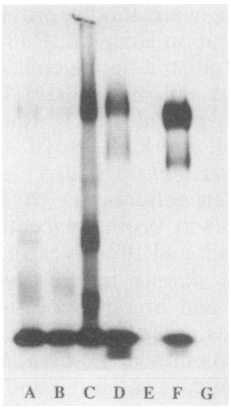

FIG. 3. SDS–7.5% PAGE and autoradiography of ^{125}I-HSMSL components which bind to *S. aureus* isolate 15334. For each experiment, 500 μl of ^{125}I-HSMSL (4×10^7 cpm) or ^{125}I-MG2 isoform (2×10^7 cpm) was added to bacteria. Lanes: A, ^{125}I-HSMSL (20/500 μl applied to the gel); B, ^{125}I-HSMSL after depletion by *S. aureus* (20/500 μl applied to the gel); C, ^{125}I-HSMSL components which bind to *S. aureus*. The bacterial pellet was washed with 5 ml of PBS and then extracted overnight at room temperature with 100 μl of SDS-PAGE sample-solubilizing buffer. Approximately 20/100 μl were applied to the gel. Lanes: D, ^{125}I-RM/RA-MG2a after depletion by *S. aureus*; E, extract from *S. aureus* incubated with RM/RA-MG2a; F, ^{125}I-RM/RA-MG2b after depletion by *S. aureus*; G, bacterial extract from *S. aureus* incubated with RM/RA-MG2b. Similar results were obtained with *P. aeruginosa* 1244.

blocking buffer (0.01 M Tris HCl–0.154 M NaCl, pH 7.2 [TBS], containing 5% BSA) and then incubated with the appropriate dilution of the rabbit or mouse antiserum in blocking buffer for 30 to 60 min. After being washed in blocking buffer, membrane-bound antigens were incubated with horseradish peroxidase-conjugated, affinity-purified goat anti-rabbit IgG or goat anti-mouse IgG for 1 h, washed three times, and visualized by horseradish peroxidase color developing reagent.

Solution-phase assay for mucin-bacterium interactions. Suspensions of bacteria (0.5×10^9 to 1.0×10^9 cells) were placed in polypropylene tubes (17 by 100 mm) coated with 0.05% Tween 20 in TBS, centrifuged at $1,900 \times g$ for 10 min at 5°C, and washed with 5 ml of PBS. Then 1.0 ml of freshly collected HSMSL, 0.5 ml of reconstituted salivary samples (1 to 5 mg/ml in PBS), or 0.5 ml of sIgA (1 mg/ml in PBS) was added to the bacteria. The mixtures were incubated for 30 min at 37°C and then centrifuged at $1,900 \times g$ for 10 min at 5°C. An aliquot of the supernatant was incubated with 4× SDS-PAGE sample-solubilizing buffer at room temperature overnight and then examined by SDS-PAGE. The bacterial pellet was washed with 5 ml of PBS and then extracted overnight at room temperature with 100 μl of SDS-PAGE sample-solubilizing buffer. Following centrifugation at $11,000 \times g$ for 2 min, the bacterial extract was examined by SDS-PAGE. For iodinated samples, 0.5 ml of HSMSL (4×10^7 cpm) or MG2 isoform (2×10^7 cpm) was added to the bacteria. Controls consisted of 0.5 ml of PBS added to bacteria and 0.5 ml of HSMSL added to tubes without bacteria.

Solid-phase assay for mucin-bacterium interactions. Approximately 0.1 ml of reconstituted salivary samples in PBS (1 to 6 mg/ml), BSA (10 mg/ml), or fibronectin (0.1 mg/ml) or PBS alone was added to a 96-well polyvinyl microtiter plate and incubated at 37°C for 18 h. Unadsorbed samples were aspirated, and the wells were washed three times with 0.05% Tween 20 in PBS and incubated for 2 h at 37°C with 0.05% Tween 20 in PBS to block unbound sites. Control experiments using ^{125}I-RM/RA-MG2a or ^{125}I-RM/RA-MG2b indicated that ~15 to 25% of these materials were immobilized onto the surface of the wells. The plates were washed three times with PBS and incubated with a 0.1 ml of ^{125}I-labeled bacteria ($\sim 10^7$ cells) for 1 h at 37°C. Unbound bacteria were aspirated, and the wells were washed three times with PBS. Wells containing adherent ^{125}I-bacteria were cut, and bound radioactivity was determined with a Beckman 5500 gamma counter. The numbers (means ± standard deviations) of adherent bacteria were calculated, and differences were tested by using a one-way analysis of variance program from the Stat View 512$^+$ Macintosh-compatible computer program (Brainpower Inc., Calabasas, Calif.).

RESULTS

Preliminary characterization of saliva-bacterium interactions. A solution-phase assay was employed to identify salivary components which bind to *S. aureus* isolates 15163, 15194, and 15334 as well as to *P. aeruginosa* R1, 1244, and M35. Bacteria exposed to either lyophilized or freshly collected HSMSL were extracted with 2% SDS and then compared by SDS-PAGE with intact HSMSL and 2% SDS extracts of bacteria exposed to PBS alone. Only a few salivary components bound to the bacteria tested (Fig. 2, lane D). In contrast, components of similar size were not found in SDS extracts of bacteria incubated with PBS alone (Fig. 2, lane B). No components could be visualized from extracts of HSMSL added to tubes without bacteria. Similar results were obtained when *S. aureus* was heated at 65°C for 30 min prior to incubation with HSMSL. The major component bound to all bacteria was a PAS-staining glycoprotein of ~125 kDa which was identified by Western transfer with a monospecific antibody as MG2. Western transfer analysis of the bacterial extracts with other specific antibodies detected sIgA but not amylase or MG1. Similar findings were obtained with ^{125}I-HSMSL, which demonstrated MG2 binding as well as binding of other salivary components (Fig. 3, lanes A through C). Since *S. aureus* isolates 15163, 15194, and 15334 and *P. aeruginosa* R1, 1244, and M35 gave identical

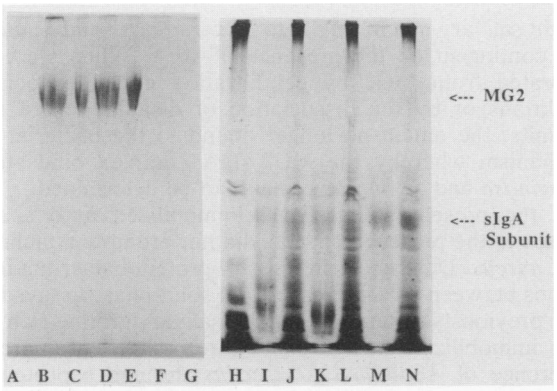

FIG. 4. SDS–7.5% PAGE of *S. aureus* isolate 15334 extracts following incubation with salivary pools A and A2. Lanes A through G were stained with PAS. Lanes H through N were silver stained. Lanes: A and H, extract of *S. aureus* isolate 15334 incubated with PBS; B and I, pool A; C and J, pool A components which bind *S. aureus*; D and K, pool A2; E and L, pool A2 components which bind *S. aureus*; F and M, sIgA; G and N, sIgA components which bind *S. aureus*.

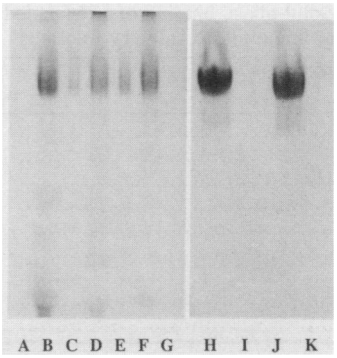

FIG. 5. SDS–7.5% PAGE. Effects of chemical treatment of HSMSL on MG2 interactions with *S. aureus* isolate 15334. Gels were stained with PAS. Lanes: A, extract of *S. aureus* incubated with PBS; B, HSMSL after depletion by *S. aureus*; C, HSMSL components which bind *S. aureus*; D, RM-HSMSL after depletion by *S. aureus*; E, RM-HSMSL components which bind *S. aureus*; F, RA-HSMSL after depletion by *S. aureus*; G, RA-HSMSL components which bind *S. aureus*; H, RM/RA-MG2b after depletion with *S. aureus*; I, extract from *S. aureus* incubated with RM/RA-MG2b; J, MG2b (no RM and RA) after depletion with *S. aureus*; K, extract from *S. aureus* incubated with MG2b (no RM and RA). Similar results were obtained with *P. aeruginosa* 1244.

results, subsequent experiments were performed with *S. aureus* isolate 15334 and *P. aeruginosa* 1244.

When purified RM/RA-MG2a and RM/RA-MG2b were incubated with *S. aureus* isolate 15334 or *P. aeruginosa* 1244, SDS-PAGE followed by PAS staining of the bacterial extracts revealed that the purified mucin isoforms did not bind to these bacteria. This finding was verified by using ^{125}I-RM/RA-MG2a and ^{125}I-RM/RA-MG2b (Fig. 3, lanes D through G). These studies were repeated with *S. aureus* isolate 15334 grown in chemically defined medium (34), and similar results were obtained, indicating that any adsorbed medium components were not responsible for binding activity.

Effects of various mucin purification steps on MG2-bacterium interactions. The finding that purified MG2 isoforms did not bind to bacteria suggested that perhaps some stage of the purification process affected the mucin's ability to bind to bacteria. Crude mucin fractions (pool A and pool A2; Fig. 1), which contain MG2, sIgA, and smaller peptides (25), were tested in the solution-phase assay. The MG2 in these fractions bound to *S. aureus* isolate 15334 and *P. aeruginosa* 1244 (Fig. 4, lanes C and E). In addition, silver staining of these bacterial extracts revealed the presence of sIgA subunits (Fig. 4, lanes J and L). These observations suggested that sIgA or smaller salivary peptides may influence mucin binding to these bacteria. Previous studies have shown that MG2 can be separated from sIgA by repeated recycling of pool A2 on gel filtration matrices under dissociating conditions or by RA, which dissociates sIgA subunits (26, 27). Smaller peptides can be dissociated from MG2 by RM (26). Consequently, the effects of sIgA and the smaller salivary peptides on MG2-bacterium interactions were examined in the solution-phase assay by using RA-HSMSL as well as RM-HSMSL. Interestingly, MG2 from RM-HSMSL bound to bacteria; however, MG2 from RA-HSMSL did not (Fig. 5, lanes E and G, respectively). In addition, MG2b preparations depleted of sIgA (RM/RA-MG2b or MG2b prepared by Sephacryl S-300–6 M urea chromatography of pool A2) did not bind to *S. aureus* or *P. aeruginosa* (Fig. 5, lanes I and K, respectively). Incubation of sIgA alone with *S. aureus* and *P. aeruginosa* resulted in binding to these bacteria (Fig. 4, lane N). Collectively, these data, which are summarized in Table 1, suggest that a complex involving MG2 and sIgA may be required for mucin binding to these bacteria. Preliminary experiments with purified MG2 and sIgA (1 mg/ml each) incubated with *S. aureus* resulted in only minimal restoration of MG2 binding (data not shown). The reason for our inability to restore MG2 binding in the presence of sIgA is currently unknown. Other cofactors such as cations might be required. Studies are under way to explore this point further.

Immobilized-mucin-binding assay. The adherence of ^{125}I-labeled *S. aureus* isolate 15334 to MG2 was also examined in a solid-phase assay. To establish a range over which subsequent assays could be evaluated, immobilized fibronectin, which bound $\sim 6 \times 10^6$ cells, served as a positive control, while immobilized BSA, which bound $\sim 4 \times 10^5$ cells, served as a negative control. *S. aureus* bound to all MG2-containing fractions to the same extent as BSA, which was 1 order of magnitude lower than *S. aureus* adherence to fibronectin (Table 2). We have treated *S. aureus* with iodine (cold) and chloramine T and subsequently performed solution-phase assays using MG2 fractions which contain MG2 and sIgA (e.g., pool A). Our findings indicate that iodination and chloramine T oxidation of *S. aureus* did not inhibit MG2

TABLE 1. Summary of MG2 and sIgA solution-phase interactions with *S. aureus* and *P. aeruginosa*

Salivary sample[a]	MG2 binding	sIgA present
HSMSL	+	+
Pool A	+	+
Pool A2	+	+
RM-HSMSL	+	+
RA-HSMSL	−	Subunits dissociated
RM/RA-MG2a	−	−
RM/RA-MG2b	−	−
MG2b	−	−

[a] Data were obtained from samples that were not radiolabeled. MG2b was prepared by Sephacryl S-300–6 M urea chromatography of pool A2 without RM and RA (Fig. 1).

TABLE 2. Solid-phase binding of ^{125}I-labeled *S. aureus* to immobilized salivary fractions

Immobilized protein (mg/ml)[a]	Mean no. of CFU adherent ± SD (10^5)
Uncoated	5.25 ± 2.70
BSA[b] (10)	4.14 ± 2.54
Fibronectin (0.1)	58.13 ± 23.87[c]
HSMSL (6)	8.56 ± 1.71
Pool A (3)	4.12 ± 2.26
Pool A2 (3)	4.43 ± 2.62
MG2a[d] (1)	2.86 ± 1.03
MG2b[d] (1)	5.35 ± 2.89
sIgA (1)	3.62 ± 0.87

[a] A total of 5×10^7 bacteria (5×10^5 cpm) was added to each well. Each value was obtained from assays run in triplicate.

[b] *S. aureus* bound to uncoated wells to the same extent as to BSA-coated wells.

[c] $P < 0.001$.

[d] MG2a and MG2b were prepared by Sephacryl S-300–6 M urea chromatography of pool A2 without RM and RA (Fig. 1).

binding. These results suggested that immobilized MG2, either in purified form or in the presence of sIgA, did not appreciably promote the adherence of *S. aureus*.

DISCUSSION

Mucins have a propensity for homotypic and heterotypic complexing due to their negatively charged sialic acid and sulfate residues as well as the presence of hydrophobic domains, properties which make these glycoproteins amphipathic (1). Salivary mucins have been shown to participate in noncovalent interactions with sIgA (22, 26), lysozyme (29), and lipids (30) which may result in a heterotypic complex between two or more salivary molecules. Until recently, the biological significance of such complexing has remained obscure. Indeed, it has been postulated that the complexing of mucin with protective molecules such as immunoglobulins or lysozyme may help to concentrate these components at various tissue-environmental interfaces (19). Alternatively, the complex itself may serve as a functional unit which possesses properties different from those of its individual components. Several recent investigations have begun to clarify this issue. For example, self-association or homotypic complexing of mucin monomers in aqueous solutions may be required for certain bacterial interactions. Brack and Reynolds (1) have reported that a high-molecular-weight rat salivary mucin supercomplex, consisting of >99% mucin, can agglutinate *Streptococcus mutans*. However, this agglutination activity was inhibited upon complex dissociation in high-ionic-strength buffer containing 6 M urea. Heterotypic complexing between the salivary proline-rich glycoprotein and albumin can enhance this glycoprotein's lubricating properties at an enamel-glass interface (11). Several studies have indicated that heterotypic complexing may be required in mucin-mediated microbial interactions. Ericson et al. (6) have suggested that sIgA in complex with salivary agglutinins may be more efficient at inducing aggregation of *S. mutans* than free sIgA. Magnusson and Stjernstrom (23) have shown that incubation of *Salmonella typhimurium* with sIgA renders these bacteria highly mucophilic for gastric mucin, suggesting that a synergistic relationship may exist between sIgA and mucins. The present study provides another example of the dependence of mucin-bacterium interactions on complexing of mucin with another macromolecule. Here, the binding of the low-molecular-weight salivary mucin, MG2, to *P. aeruginosa* and *S. aureus* was contingent on the presence of sIgA. When sIgA was separated from MG2 by gel filtration under dissociating conditions or by the dissociation of disulfide-linked sIgA subunits, the mucin no longer bound to the bacteria. The mechanism whereby the MG2-sIgA complex binds to *P. aeruginosa* and *S. aureus* remains to be determined.

In the present study, MG2 immobilized onto a solid surface in the presence of sIgA did not promote attachment of *S. aureus*. Differences in salivary protein-bacterium interactions between solution-phase and solid-phase assays have been previously described. Human salivary proline-rich proteins immobilized on hydroxyapatite or latex promote the adherence of *Actinomyces viscosus* through a protein-to-protein interaction, but in solution phase, these proteins do not bind this microorganism (3, 8). It has been suggested that upon binding to the solid surface, the proline-rich protein undergoes a conformational change which leads to the unmasking of a receptor(s) for *A. viscosus*. Also, the binding of certain *Escherichia coli* strains to intestinal mucin can differ between solution- and solid-phase assays (37). It is interesting to note that MG2 immobilized onto glass can promote the attachment of other bacteria such as *Streptococcus sanguis* (31). It is apparent, therefore, that MG2 bound to a solid surface displays selectivity in subsequent bacterial adherence. Perhaps immobilized MG2 favors the attachment of benign microorganisms such as *S. sanguis* over the attachment of potential mucosal pathogens such as *P. aeruginosa* and *S. aureus*. In this regard, the ability of an MG2-sIgA complex to bind to potential mucosal pathogens could serve as a nonspecific host defense mechanism. Since sIgA alone binds to the bacterial surface, the functional importance of MG2 in this complex is unclear. Nevertheless, MG2-sIgA complexes may facilitate bacterial clearance from the oropharyngeal cavity by enhancing bacterial aggregation (16, 38). The ability of *P. aeruginosa* to persist and infect in the respiratory tract is correlated with the organism's ability to adhere in the oral cavity (39, 40). MG2-sIgA binding to *P. aeruginosa* and *S. aureus* in the oropharyngeal cavity and subsequent clearance may be of importance in preventing infection of the upper respiratory tract and subsequent infection of the lower respiratory tract.

ACKNOWLEDGMENTS

This study was supported in part by USPHS grants AI27401, DE00158, DE07585, and DE08240.

REFERENCES

1. **Brack, C. M., and E. C. Reynolds.** 1987. Characterization of a rat salivary sialoglycoprotein complex which agglutinates *Streptococcus mutans*. Infect. Immun. **55**:1264–1273.
2. **Burnett, W. N.** 1981. "Western blotting." Electrophoretic transfer of proteins from sodium-dodecyl sulphate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
3. **Clark, W. B., J. E. Beem, W. E. Nesbitt, J. O. Cisar, C.-C. Tseng, and M. J. Levine.** 1989. Pellicle receptors for *Actinomyces viscosus* type 1 fimbriae in vitro. Infect. Immun. **57**:3003–3008.
4. **Cohen, R. E., A. Aguirre, M. E. Neiders, M. J. Levine, P. C. Jones, M. S. Reddy, and J. G. Haar.** 1991. Immunochemistry and immunogenicity of low molecular weight human salivary mucin. Arch. Oral Biol. **36**:347–356.
5. **Cohen, R. E., A. Aguirre, M. E. Neiders, M. J. Levine, P. C. Jones, M. S. Reddy, and J. G. Haar.** 1990. Immunochemistry of

high-molecular weight human salivary mucin. Arch. Oral Biol. **35**:127–136.

6. **Ericson, T., D. Bratthall, and J. Rundegren.** 1979. Bacterial agglutination induced by saliva. Inhibition studies with anti-Ig antisera and serum components, p. 243–254. *In* I. Kleinberg, S. A. Ellison, and I. D. Mandel (ed.), Saliva and dental caries, special supplemental microbiology abstracts. Information Retrieval, Washington, D.C.
7. **Fairbanks, G., T. C. Steck, and D. F. H. Wallach.** 1971. Electrophoretic analysis of the major glycopeptides of the human erythrocyte membrane. Biochemistry **10**:2606–2617.
8. **Gibbons, R. J., and D. I. Hay.** 1988. Human salivary acidic proline-rich proteins and statherin promote the attachment of *Actinomyces viscosus* LY7 to apatitic surfaces. Infect. Immun. **56**:439–445.
9. **Gibbons, R. J., and V. Qureshi.** 1976. Interactions of *Streptococcus mutans* and other oral bacteria with blood group reactive substances, p. 163–182. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Microbial aspects of dental caries, supplemental microbiology abstracts, vol. 2. Information Retrieval, Washington, D.C.
10. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. **89**:114–123.
11. **Hatton, M. N., R. E. Loomis, M. J. Levine, and L. A. Tabak.** 1985. Masticatory lubrication. The role of carbohydrate in the lubricating property of a salivary glycoprotein-albumin complex. Biochem. J. **230**:817–820.
12. **Iglewski, B.** 1989. Probing *Pseudomonas aeruginosa*, an opportunistic pathogen. ASM News **55**:303–307.
13. **Jentoft, N., and D. G. Dearborn.** 1979. Labeling of proteins by reductive methylation using sodium cyanoborohydride. J. Biol. Chem. **254**:4359–4365.
14. **Johanson, W. G., Jr., D. E. Woods, and T. Chaudhuri.** 1979. Association of respiratory tract colonization with adherence of gram-negative bacilli to epithelial cells. J. Infect. Dis. **139**:667–673.
15. **Komiyama, K., B. F. Habbick, and S. K. Tumber.** 1987. Role of sialic acid in saliva-mediated aggregation of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. Infect. Immun. **55**:2364–2369.
16. **Komiyama, K., B. F. Habbick, and S. K. Tumber.** 1989. Whole, submandibular, and parotid saliva-mediated aggregation of *Pseudomonas aeruginosa* in cystic fibrosis. Infect. Immun. **57**:1299–1304.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Levine, M. J., M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li, and T. van Dyke.** 1978. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoprotein with *Streptococcus sanguis* and *Streptococcus mutans*. Infect. Immun. **19**:107–115.
19. **Levine, M. J., M. S. Reddy, L. A. Tabak, R. E. Loomis, E. J. Bergey, P. C. Jones, R. E. Cohen, M. W. Stinson, and I. Al-Hashimi.** 1987. Structural aspects of salivary glycoproteins. J. Dent. Res. **66**:436–441.
20. **Liljemark, W. F., C. G. Bloomquist, and J. C. Ofstehage.** 1979. Aggregation and adherence of *Streptococcus sanguis*: role of human salivary immunoglobulin A. Infect. Immun. **26**:1104–1110.
21. **Lindeman, R. A., M. G. Newman, A. K. Kaufman, and T. V. Le.** 1985. Oral colonization and susceptibility testing of *Pseudomonas aeruginosa* oral isolates from cystic fibrosis patients. J. Dent. Res. **64**:54–57.
22. **Loomis, R. E., A. Prakobphol, M. J. Levine, M. S. Reddy, and P. C. Jones.** 1987. Biochemical and biophysical comparison of two mucins from human submandibular-sublingual saliva. Arch. Biochem. Biophys. **258**:452–464.
23. **Magnusson, K. E., and I. Stjernstrom.** 1982. Mucosal barrier mechanisms. Interplay between secretory IgA (sIgA), IgG, and mucins on the surface properties and association of salmonellae with intestine and granulocytes. Immunology **45**:239–248.
24. **McBride, B. C., and M. T. Gisslow.** 1977. Role of sialic acid in saliva-induced aggregation of *Streptococcus sanguis*. Infect. Immun. **18**:35–40.
25. **Murray, P. A., M. J. Levine, L. A. Tabak, and M. S. Reddy.** 1982. Specificity of salivary-bacterial interactions. II. Evidence for a lectin on *Streptococcus sanguis* with specificity for a NeuAcα2,3Galβ1,3GalNAc. Biochem. Biophys. Res. Commun. **106**:390–396.
26. **Prakobphol, A., M. J. Levine, L. A. Tabak, and M. S. Reddy.** 1982. Purification of a low molecular-weight, mucin-type glycoprotein from human submandibular-sublingual saliva. Carbohydr. Res. **108**:111–122.
27. **Ramasubbu, N., M. S. Reddy, E. J. Bergey, G. Haraszthy, S. D. Soni, and M. J. Levine.** Large scale purification and characterization of the major phosphoproteins and mucins of human submandibular-sublingual saliva. Biochem. J., in press.
28. **Sanford, B. A., V. L. Thomas, and M. A. Ramsay.** 1989. Binding of staphylococci to mucus in vivo and in vitro. Infect. Immun. **57**:3735–3742.
29. **Shomers, J. P., L. A. Tabak, M. J. Levine, I. D. Mandel, and D. I. Hay.** 1982. Properties of cysteine-containing phosphoproteins from human submandibular-sublingual saliva. J. Dent. Res. **61**:397–399.
30. **Slomiany, B. L., V. L. Murty, A. Slomiany, J. Zielenski, and I. D. Mandel.** 1986. Mucus glycoprotein of human saliva: differences in the associated and covalently bound lipids with caries. Biochem. Biophys. Acta **882**:18–28.
31. **Stinson, M. W., M. J. Levine, J. M. Cavese, A. Prakobphol, P. A. Murray, L. A. Tabak, and M. S. Reddy.** 1982. Adherence of *Streptococcus sanguis* to salivary mucin bound to glass. J. Dent. Res. **61**:1390–1393.
32. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
33. **Valenti, W. M., R. G. Trudell, and D. W. Bentley.** 1978. Factors predisposing to oropharyngeal colonization with gram-negative bacilli in the aged. N. Engl. J. Med. **298**:1108–1111.
34. **van de Rijn, I., and R. E. Kessler.** 1980. Growth characteristics of group a streptococci in a new chemically defined medium. Infect. Immun. **27**:444–448.
35. **Vishwanath, S., and R. Ramphal.** 1984. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. Infect. Immun. **45**:197–202.
36. **Vishwanath, S., and R. Ramphal.** 1985. Tracheobronchial mucin receptor for *Pseudomonas aeruginosa*: predominance of amino sugars in binding sites. Infect. Immun. **48**:331–335.
37. **Wanke, C. A., S. Cronan, C. Goss, K. Chadee, and R. L. Guerrant.** 1990. Characterization of binding of *Escherichia coli* strains which are enteropathogens to small-bowel mucin. Infect. Immun. **58**:794–800.
38. **Williams, R. C., and R. J. Gibbons.** 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science **177**:697–699.
39. **Woods, D. E.** 1983. Pulmonary infection in cystic fibrosis, p. 4–15. *In* Cystic Fibrosis Foundation (ed.), Guidance, Action, Projection (GAP) Conference Proceedings. Cystic Fibrosis Foundation, Rockville, Md.
40. **Woods, D. E., J. A. Bass, W. G. Johanson, Jr., and D. C. Straus.** 1980. Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. Infect. Immun. **30**:694–699.