INFECTION AND IMMUNITY, May 1991, p. 1661–1666
0019-9567/91/051661-06$02.00/0

Vol. 59, No. 5

Aggregation of Group A Streptococci by Human Saliva and Effect of Saliva on Streptococcal Adherence to Host Cells

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Received 19 October 1990/Accepted 7 February 1991

The aggregation of group A streptococci by whole, stimulated human saliva (WHS) and the effect of saliva on streptococcal adherence to host cells was investigated. WHS samples from 11 individuals were found to aggregate both M^+ and M^- group A streptococci to various degrees. The aggregating activity was sensitive to heat, EDTA, EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid], sodium dodecyl sulfate, and lipoteichoic acid. None of the simple sugars tested, mercaptoethanol, albumin, or nonionic detergents had any effect on aggregation. The aggregating activity of EDTA-treated saliva was restored by 0.1 mM Ca^{2+} and 1.0 mM Mn^{2+} but not by up to 5 mM Mg^{2+}. Only streptococci from the stationary phase were aggregated. Hyaluronidase treatment of streptococci from the exponential phase of growth restored their ability to be aggregated, suggesting that the hyaluronic acid capsule interferes with agglutination. Adsorption of WHS by one strain of *Streptococcus pyogenes* removed aggregating activity for other strains of *S. pyogenes* and *Streptococcus sanguis* but not agglutinins for *Escherichia coli*, suggesting that the agglutinin is specific for certain gram-positive bacteria. Molecular sieve chromatography of WHS and identification of streptococcus-binding components of saliva suggest that either a glycoprotein of ~360 kDa or a mucin of saliva of >1,000 kDa mediates aggregation of streptococci. WHS also inhibited adherence of *S. pyogenes* to buccal epithelial cells.

Substances in saliva can interact both with mucosal-cell surfaces and with invading bacteria, and these interactions may have a profound influence on the fate of bacteria entering the oral cavity. For example, fibronectin can bind to both bacteria (3, 5, 23) and epithelial cells (25). Fibronectin bound to epithelial-cell surfaces can promote the adherence of certain gram-positive bacteria and inhibit the adherence of certain gram-negative bacteria (1, 7, 23–25). Saliva also contains glycoproteins that can stimulate adherence of *Escherichia coli* to host cells in one case (7) and block adherence in another (2). In addition, whole saliva can induce aggregation of a variety of oral bacteria, and this is thought to facilitate the removal of these bacteria from the oral cavity (11). Saliva may also promote intergeneric bacterial aggregation (13).

Thus, saliva may influence the fate of a bacterium through one of the following mechanisms: (i) promotion of clearance of the bacteria by aggregation either directly or via another oral microbe; (ii) promotion of adherence to mucosal surfaces by acting as a bridge between the bacteria and host surfaces; (iii) promotion of adherence of one species of bacterium to another species of bacterium that has colonized the surface; (iv) blocking of adherence by coating the surface of the bacteria (steric hindrance) or by direct binding to the adhesin. Therefore, studies on the interaction of saliva with bacteria are necessary in order to obtain a clearer understanding of how bacteria attach to the host's surfaces in the complex milieu of the oral cavity. Because there is a paucity of information on interactions between group A streptococci and saliva, we initiated studies on streptococcus-saliva interactions. Herein, we report that whole human saliva (WHS) contains substances that can aggregate group A streptococci and can alter streptococcal attachment to host cells.

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MATERIALS AND METHODS

Reagents. Sugars and chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless specified otherwise. Lipoteichoic acid (LTA) was extracted from M type 5 *Streptococcus pyogenes* and purified as previously described (6).

Bacterial strains and growth conditions. *Streptococcus sanguis* Challis (originally obtained from R. Doyle, University of Louisville, Louisville, Ky.) and group A streptococcal strains of M type 5, M type 1, T1av (an M^- variant of M type 1 group A streptococci), and an M type 28 strain in which the M-protein gene was deleted (originally obtained from V. Fischetti, Rockefeller University, New York, N.Y.) were stored at −80°C in Todd-Hewitt broth containing 20% normal rabbit serum. The stock cultures were subcultured in Todd-Hewitt broth at 1:500 and incubated at 37°C for 18 h. Type 1 fimbriated *E. coli* CSH50 was grown in brain heart infusion broth for 18 h. The bacteria were washed in 0.15 M NaCl–0.10 M KH_2PO_4–0.1% NaN_3 (pH 7.0) (PBS) and suspended in PBS to the desired concentration.

Collection of saliva. Approximately 35 ml of Parafilm-stimulated WHS was collected from each individual into chilled tubes. The saliva samples were clarified by centrifugation at 20,000 × *g* for 15 min and stored at −80°C until needed.

Adsorption of saliva with bacteria. Five milliliters of saliva was adsorbed three times with pellets from 5 ml of the bacterial suspension (optical density at 530 nm, 1.5) and tested for aggregating activity. To determine what components of saliva were bound by *S. pyogenes*, saliva adsorbed with group A streptococci as indicated above was electrophoresed into a 5 to 15% gradient polyacrylamide gel with a 4% stacking gel under reducing and nonreducing conditions. Duplicate gels were stained with Coomassie brilliant blue R-250 or with periodic acid-Schiff (PAS) reagent (27).

Aggregation assays. Aggregation assays with clarified WHS from each donor were done with 400 μl of WHS, 50 μl

of bacteria, and 50 μl of 0.5% Tween 20. Controls contained PBS instead of saliva. The mixtures were rotated for 30 min at ambient temperature in 1.5-ml polypropylene tubes and centrifuged at 300 × *g* for 5 min, and 150 μl was removed from the supernatant and placed in microtiter wells (NUNC, Roskilde, Denmark). The A_{410} was measured with a microtiter plate reader (Dynatech Laboratories, Inc., Alexandria, Va.). The bacterial concentrations were adjusted such that an absorbance of approximately 1.0 was obtained in PBS controls. The percentage of aggregation was calculated according to the following formula: [1 − (test absorbance/PBS control absorbance)] × 100.

Assays for inhibitors of aggregation were done with 400 μl of saliva, 50 μl of bacteria, and 50 μl of test substance. Controls consisted of 400 μl of PBS instead of saliva. Samples were then treated as described above. The percentage of inhibition of aggregation was calculated by the following formula: (test absorbance/PBS control absorbance) × 100.

The aggregating activities of fractions from Sepharose 6B chromatography were determined by adding 400 μl of each fraction to a solution containing 50 μl of M type 5 group A streptococci, 25 μl of 1% Tween 20, and 25 μl of 10 mM $CaCl_2$. The mixtures were then treated as described above.

Determination of effect of growth phase on aggregation of streptococci by saliva. M type 5 *S. pyogenes* was grown in Todd-Hewitt broth at 37°C. The optical density at 530 nm was measured at intervals, and at various times, portions of the bacteria were removed, washed in PBS, and assessed for their abilities to be aggregated by saliva as described above. Streptococci from the different growth phases were treated with 1 mg of hyaluronidase (Sigma) per ml in PBS for 30 min at 37°C, washed two times in PBS, and then assessed for aggregation by saliva.

Adherence assays. The effect of WHS on the adherence of streptococci to human buccal epithelial cells immobilized on microtiter wells was determined by an enzyme-linked immunosorbent assay (ELISA) as previously described (19). Briefly, 50 μl of streptococci was added to 50 μl of WHS or 50 μl of PBS in microtiter wells containing immobilized buccal epithelial cells. In some cases, the bacteria were pretreated with saliva for 30 min at 37°C and washed once before being added to the microtiter wells. Buccal epithelial cells were pretreated with saliva by adding 100 μl of saliva to the appropriate wells, incubating them at 37°C for 30 min, and washing them once with PBS before adding streptococci. After the streptococci were added, the plates were rotated horizontally for 30 min at room temperature. The wells were washed four times with PBS and heat fixed at 60°C for 15 min. The adherence of streptococci was measured by an ELISA utilizing rabbit anti-M type 5 group A streptococci as described previously (5, 19). Controls for nonspecific binding (negative controls) consisted of wells treated in an identical manner except that they contained no epithelial cells. The percentage of adherence was calculated according to the following formula: [(A_{410} of test well − A_{410} of negative control)/(A_{410} of PBS control − A_{410} of negative control)] × 100.

Molecular sieve chromatography of saliva. Approximately 10 ml of WHS was dialyzed against PBS at 4°C, applied to a column (2.6 by 95 cm) of Sepharose 6B equilibrated with PBS, and eluted with a flow rate of 0.5 ml/min. Fractions (10 ml each) were collected, and the A_{280} and aggregating activity of each fraction were determined. In addition, each fraction was assayed for sugar content by the method of Devine et al. (6a). Fractions 20 and 21, 22 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, and 81 to 100 were pooled, dialyzed against distilled water by utilizing tubing retaining molecules of ≥10 kDa, and lyophilized. The lyophilized material from each pool was redissolved in 2 ml of PBS.

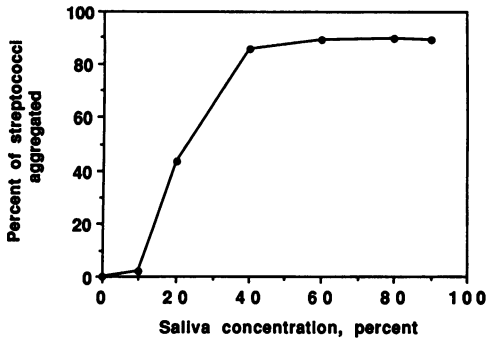

FIG. 1. Concentration-dependent aggregation of group A streptococci by stimulated WHS. Dilutions of saliva were tested for the ability to aggregate M type 5 group A streptococci as described in Materials and Methods.

Detection of IgA antibodies in saliva fractions from Sepharose 6B chromatography. The redissolved material from each pool (see above) was diluted 1:5 in 0.05 M sodium bicarbonate, pH 9.5. One hundred microliters of each dilution was added to microtiter wells and incubated at 37°C for 2 h. The wells were washed in 0.05 M Tris-HCl–0.15 NaCl (pH 7.5) (Tris-saline) containing 0.05% Tween 20. Immunoglobulin A (IgA) in the wells was detected with peroxidase-labeled rabbit antibodies to the alpha chain of human IgA diluted 1:1,000 in Tris-saline containing 0.05% Tween 20. IgG and IgM were detected with peroxidase-labeled rabbit antibodies specific for the H chain of human IgG and IgM.

RESULTS

Aggregation of group A streptococci by saliva. The assay used to measure aggregation allows quantitation of aggregated bacteria by comparing the absorbance of bacteria in PBS to that of bacteria in saliva after a low-speed centrifugation. The assay employed can rule out any contribution due to autoaggregation, since a low-speed spin removes the autoaggregated bacteria from the supernatant in PBS controls. Tween-20 was added to the aggregation mixtures to eliminate the problem of nonspecific binding of bacteria to the walls of the reaction tube.

Initial experiments indicated that saliva can aggregate M type 5 *S. pyogenes* in a dose-related fashion (Fig. 1), a finding in agreement with the observations of Komiyama and Gibbons (10). The percentage of saliva required to obtain maximal aggregation varied (40 to 90%) from one week to the next. Saliva samples from 11 individuals were tested for the ability to aggregate M^+ and M^- strains of group A streptococci (Table 1). There was no apparent correlation between the absence or presence of M protein and aggregation, which suggests that M protein is not a major factor in aggregation. None of the saliva samples strongly aggregated all of the strains tested, and at least one strain of streptococci was strongly aggregated by all of the saliva samples.

The nature of this aggregation was investigated by determining if various treatments or chemicals could block saliva-induced aggregation (Table 2). Treatment of saliva at 95°C for 10 min totally abolished the aggregating activity, whereas heating at 56°C for 10 min had no effect on aggregation.

TABLE 1. Aggregation of group A streptococci by saliva from different individuals

Saliva sample	% Aggregation of group A streptococci by WHS			
	M type 1	M type 5	T1Av	M⁻
1	50	97	27	71
2	31	44	68	10
3	50	83	61	38
4	49	88	61	52
5	6	92	41	17
6	17	24	53	13
7	70	97	79	43
8	73	86	78	50
9	70	93	90	50
10	51	87	77	39
11	ND[a]	49	88	58

[a] ND, Not done.

EDTA, EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], sodium dodecyl sulfate (SDS), and LTA also inhibited aggregation of streptococci. None of the simple sugars tested, mercaptoethanol, or nonionic detergents had any significant effect. Periodate treatment of saliva had only a minor effect (21% inhibition) on aggregation. The lack of any effect with mercaptoethanol suggests either that the agglutinin contains no disulfide-linked subunits or that these subunits are active in the reduced state.

Sepharose 6B chromatography of saliva indicated that only those fractions eluting in the void volume had any significant aggregating activity (fractions 20 and 21, Fig. 2), suggesting the possibility that a high-molecular-weight mucin(s) is the main component in saliva mediating streptococcal aggregation. Coommassie blue staining of SDS gels of the Sepharose 6B fractions indicated that fractions 20 and 21 contained two major bands of ~360 and 60 kDa in the reduced state (Fig. 3). The 60-kDa band was not apparent in the nonreduced state, whereas the mobility of the 360-kDa band remained unchanged in the nonreduced state. Fractions 20 and 21 exhibited low A_{280}, and their sugar/protein ratio was five times higher than that of other fractions. Such ratios are indicative of mucins. Fractions 20 and 21 were found to contain an apparent high-molecular-weight (>1,000-kDa), PAS-positive material that entered the SDS stacking gel only under reducing conditions, suggesting that these fractions contain mucins.

IgA does not appear to be involved in the aggregation of streptococci. This is suggested by the fact that the majority of IgA was found in fractions 31 to 40, which had little or no aggregating activity (Fig. 2).

Effect of cations on aggregation of streptococci by saliva. The influence of calcium, manganese, and magnesium on aggregation was also investigated (data not shown). As little as 0.1 mM calcium and 1 mM manganese restored the aggregating activity of EDTA-treated saliva, whereas magnesium had no effect. In control experiments, calcium and manganese aggregated streptococci in the absence of saliva at 3 and 1.5 mM, respectively; these concentrations are above those needed to restore the aggregating activity of EDTA-treated saliva. Interestingly, manganese was more effective than calcium in promoting aggregation of strepto-

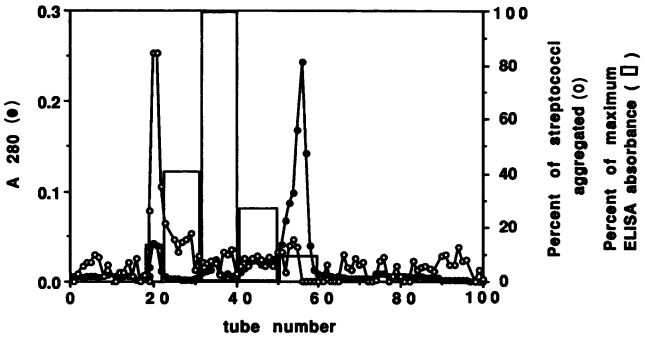

FIG. 2. Molecular sieve chromatography of WHS. Approximately 10 ml of clarified WHS was applied to a Sepharose 6B column. Fractions (10 ml each) were collected, and the A_{280} and the aggregating activity of each fraction were determined as described in Materials and Methods. Columns represent the relative amount of IgA detected in the fractions indicated (expressed as a percentage of maximum ELISA readings; A_{410} of 1.2).

TABLE 2. Effect of various treatments and substances on the aggregation of M type 5 group A streptococci

Treatment or substance[a]	Concn	% Inhibition of aggregation of saliva from:	
		Donor 1	Donor 2
95°C, 10 min		100	100
56°C, 10 min		6	15
Periodate, 180 min	0.2 M	21	ND[b]
EDTA	0.02 M	100	97
EGTA	0.02 M	100	ND
LTA	1 mg/ml	100	59
SDS	0.1%	30	76
Tween 20	0.1%	3	7
Triton X-100	0.1%	2	6
Galactose	0.1%	1	2
Fucose	0.1%	0	1
N-Acetylneuraminic acid	0.1%	1	ND
N-Acetylglucosamine	0.1%	4	7
N-Acetylgalactosamine	0.1%	2	6
Mercaptoethanol	0.1%	1	6

[a] Periodate, EDTA, and EGTA were removed by dialysis against PBS prior to testing for effects on aggregation. In addition to the sugars listed, the following sugars had no inhibitory effect on the aggregation of streptococci: glucose, mannose, xylose, arabinose, arabinogalactan, glucoroniclactone, lactose, and fructose.

[b] ND, Not done.

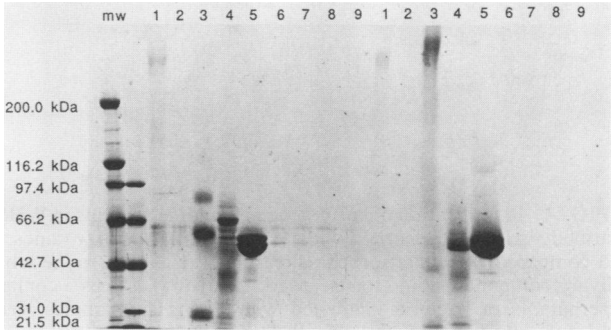

FIG. 3. A 5 to 15% gradient SDS-polyacrylamide gel of saliva fractions from Sepharose 6B chromatography. Lanes 1 to 9 correspond to fractions 20 and 21, 22 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, and 81 to 100, respectively. The first set of lanes 1 to 9 is reduced, and the second set of lanes 1 to 9 is nonreduced.

TABLE 3. Adsorption of salivary agglutinins by various bacteria

Species adsorbing saliva	Aggregation (% of control[a])					
	M⁻	T1av	M type 1	M type 5	*S. sanguis*	*E. coli*
Streptococcus strain						
M⁻	3	5	3	2	30	ND[b]
T1av	11	0	0	7	24	ND
M type 1	2	4	1	8	22	ND
M type 5	2	5	5	0	10	85
S. sanguis	0	0	0	5	0	ND
E. coli	ND	ND	ND	89	ND	0

[a] Controls consisted of aggregation by unadsorbed saliva.
[b] ND, Not done.

cocci in the absence of saliva, whereas calcium was more effective than manganese in the presence of saliva. This suggests either that a component in saliva cross-links streptococci via calcium salt bridges or that the agglutinin needs calcium to maintain a structure necessary for aggregation.

Adsorption of salivary agglutinins by various bacteria. Adsorption of saliva with one strain of group A streptococci removed aggregating activity for other strains of group A streptococci and 70 to 90% of the aggregating activity for *S. sanguis* (Table 3). In contrast, adsorption of saliva with *E. coli* did not remove aggregating activity for *S. pyogenes*. The data suggest that *S. pyogenes* and *S. sanguis* react with the same agglutinin but that this agglutinin does not react with *E. coli*.

In order to determine what components of saliva were selectively removed by group A streptococci, saliva was adsorbed with streptococci and electrophoresed into a 5 to 15% polyacrylamide gel. At least seven bands were removed by streptococci (Fig. 4B, lane 2). It is of interest that a high-molecular-weight, PAS-positive material that entered the stacking gel (Fig. 4D, lane 1) was not completely adsorbed by group A streptococci, suggesting that this substance reacts poorly with the streptococci. This band may be identical to the mucin MG1 (14), because it has an M_r of >1,000 kDa, enters the stacking gel only when reduced (suggesting disulfide-linked subunits), and is detected only with the PAS stain (suggesting a high degree of glycosylation).

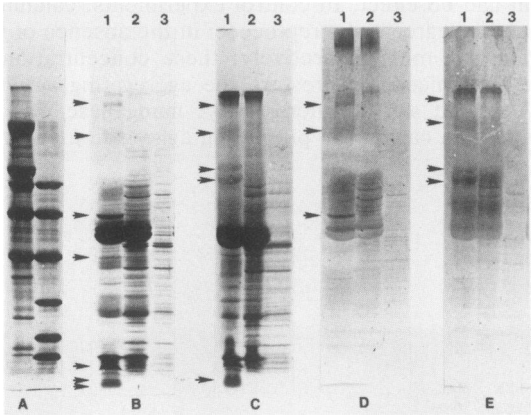

FIG. 4. Identification of streptococcus-binding proteins in WHS. Clarified WHS was adsorbed with M type 5 *S. pyogenes* (lanes 2) and compared with unadsorbed saliva (lanes 1) to determine what salivary components bind to streptococci (arrows). Lanes 3 contain supernatants of bacteria incubated with PBS. The samples were electrophoresed in a 5 to 15% gradient SDS-polyacrylamide gel under reducing (A, B, D) and nonreducing (C and E) conditions. Panels A, B, and C were stained with Coomassie brilliant blue R-250, and panels D and E were stained with PAS reagent. Panel A contains high- and low-molecular-weight standards obtained from Bio-Rad (200, 116.2, 97.4, 66.2, 42.7, 31, 21.5, and 14.7 kDa).

One band that binds to streptococci (top arrow, Fig. 4B, lane 1) is similar to the agglutinin described by Rundegren and Arnold (21, 22). This conclusion is based on the observations that it has a similar M_r of ~400 kDa, stains positive with both PAS and Coomassie, and is found in the fractions from Sepharose 6B chromatography that aggregate group A streptococci. Furthermore, the agglutinin described by Rundegren and Arnold aggregates *S. sanguis*, and *S. sanguis* and *S. pyogenes* appear to react with the same agglutinin since adsorption with one bacterium removes aggregating activity for the other (Table 3).

Of the other bands that bind to group A streptococci, only one has been identified with any certainty. The third arrow from the top in Fig. 4B, lane 1, points to albumin, which is known to bind to group A streptococci (12). Because the aggregating activity of saliva was found exclusively in the void volume from Sepharose 6B chromatography and because the low-molecular-weight, streptococcal-binding substances were not found in this fraction, it is unlikely that the low-molecular-weight components that bound to streptococci are involved in aggregation.

The possibility exists that instead of being adsorbed by the streptococci, the salivary proteins were degraded by streptococcal proteases. However, we have found that even proteins that are very sensitive to a variety of proteases, such as fibronectin, were not degraded when bound to M type 5 *S. pyogenes* (unpublished data). Thus, it is unlikely that the salivary proteins were degraded by streptococcal proteases.

Role of hyaluronic acid capsule in aggregation by saliva. The surface structures of group A streptococci vary during different phases of growth. One of the components that changes is the hyaluronic acid capsule that accumulates on the surface during the exponential phase of growth and is subsequently lost as the organism enters stationary phase (8). To determine if this variation had any affect on streptococcal aggregation by saliva, the organisms were isolated at different times in their growth curve and tested for aggregation (Fig. 5). As expected, bacteria from the stationary phase of growth were readily aggregated, but those from the exponential phase were not aggregated. To ascertain whether this failure to be aggregated was due to the presence of capsule, the organisms in exponential phase were treated with hyaluronidase and retested for their ability to be aggregated by saliva. The hyaluronidase treatment fully restored aggregating activity, which suggests that the hyaluronic acid capsule interferes with the agglutination of streptococci by saliva.

Inhibition of streptococcal adherence to buccal cells by saliva. The effects of saliva on the attachment of streptococci to buccal cells were determined by an ELISA for adherence (5, 19). Assays employing differential centrifugation to separate nonadherent bacteria from cells containing adherent bacteria cannot be used with saliva because saliva agglutinates streptococci, resulting in comigration of bacteria with the epithelial cells. This highlights one of the advantages of using immobilized epithelial cells, since nonadherent bacteria are simply removed by aspiration whether aggregated or not. When the ELISA was used, saliva was found to inhibit

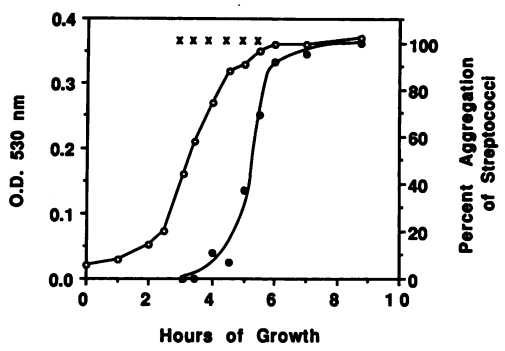

FIG. 5. Role of hyaluronic acid capsule in aggregation of M type 5 group A streptococci by WHS. *S. pyogenes* was grown in Todd-Hewitt broth at 37°C and growth was monitored by determining the optical density (O.D.) at 530 nm (○). At the indicated times, portions were removed, washed in PBS, and tested for aggregation by saliva (●) or were tested for aggregation after treatment with hyaluronidase (×).

adherence by approximately 70% (adherence was 31% ± 7%, mean ± standard deviation). Apparently the inhibitory component has to be present in the reaction mixture, since pretreatment of the epithelial cells and streptococci with saliva failed to block adherence (71% ± 24% and 113% ± 31%, respectively [mean ± standard deviation]). Adherence in the PBS control was 100% ± 12%.

DISCUSSION

Saliva contains a number of substances that can aggregate various species of oral bacteria and modulate their adhesion to surfaces in the oral cavity (1–3, 7, 10, 11, 14, 20). However, very little is known concerning the role of saliva in the attachment of group A streptococci to host cells. In this investigation, we found that stimulated WHS can induce aggregation of group A streptococci and reduce attachment of streptococci to host cells.

There was variation in the degree of aggregation of different strains of streptococci by saliva from a single donor and variation in the aggregation of a single strain by different donors. Differences in the aggregation of strains of *S. sanguis* by saliva have also been noted (11). The basis for the variability in the aggregation of different strains by saliva from a single individual is not known, but a likely explanation is that the bacteria vary in the expression of surface components. Strains of group A streptococci express different amounts of LTA and M protein on their surfaces (17), and other components probably vary as well. One surface component of streptococci that was found to be inversely related to aggregation by saliva was the hyaluronic acid capsule. Organisms from the exponential phase of growth, in which the capsule is optimally expressed, were not aggregated by saliva. Treatment of these organisms with hyaluronidase restored the ability to be aggregated by saliva, indicating that the capsule interferes with aggregation.

The variation in aggregation of a single strain by stimulated WHS from different donors is probably due to differences in concentrations of the agglutinin(s) present in saliva. One factor that could contribute to this variation is the rate of secretion, which can affect protein concentrations and the relative contributions made to WHS from the parotid and submandibular-sublingual glands (16). Maximal stimulation can increase the contribution of parotid saliva to WHS from 33 to 66%. Other factors that are known to cause variations in the composition of saliva are diurnal rhythm, age, and sex (16). Enzymatic alterations of salivary components by host and bacterial enzymes may also contribute to the variability between saliva samples.

The main substance(s) in saliva that aggregates group A streptococci appears to be either a 360-kDa glycoprotein or a 1,000-kDa glycoprotein that is sensitive to heat and requires 0.1 mM calcium for optimal activity. The 360-kDa glycoprotein apparently contains no disulfide-linked subunits, since its mobility remained the same under reducing and nonreducing conditions and mercaptoethanol had no effect on the aggregating activity. These properties are similar to the agglutinin described by Rundegren and Arnold (21, 22), which is also a high-molecular-weight glycoprotein of approximately 400 kDa that requires calcium for optimal activity. The slight discrepancy in molecular weight may be due to the inherent heterogeneity found in salivary glycoproteins. Rundegren suggested that this agglutinin is relatively nonspecific, since it aggregated a variety of bacterial species from the oral cavity. The present findings—that adsorption of saliva with group A streptococci removed the agglutinating activity of saliva for *S. sanguis* and, conversely, that adsorption of saliva with *S. sanguis* removed the agglutinating activity of saliva for *S. pyogenes*—support this concept. However, *E. coli* failed to remove the aggregating activity for *S. pyogenes*, suggesting that some specificity is involved.

The high-molecular-weight mucin (>1,000 kDa) may also be involved in the aggregation of streptococci, since this component is found in the fractions that contain aggregating activity. However, the fact that saliva adsorbed with streptococci still contains significant amounts of this material yet no longer aggregates streptococci makes it questionable that this compound has a role in aggregation. It should be mentioned that since stimulated WHS was used, the specific source of the agglutinin is not yet known. Further experimentation will be required to determine if the agglutinin is a salivary gland secretion or if it has some other origin.

The nature of the streptococcal surface component involved in interactions with the salivary agglutinin is not clear. The inhibition of aggregation by LTA suggests a role for this compound. Since LTA is a chelator of cations (9), it is possible that the saliva-induced aggregation of group A streptococci is mediated by calcium bridges between the negative charges of LTA and the negative charges of mucin. A similar mechanism has been proposed for the interaction between *S. sanguis* and salivary mucins (14, 20). However, as a chelator, LTA may inhibit aggregation by competing with the agglutinin for available calcium. M protein does not appear to be centrally involved, since M-negative strains were also aggregated by saliva.

Although it is possible that saliva interacts with group A streptococci by lectinlike interactions that have been described for other bacteria (15, 18), this becomes less likely when one considers that none of the sugars tested had any effect, that periodate treatment of saliva had little effect, and that heat denaturation abolished the aggregating activity of saliva.

The aggregation of bacteria by saliva is thought to facilitate the clearance of bacteria from the oral cavity and may influence the ability of the bacteria to adhere to the oral epithelium. For example, Williams and Gibbons (26) found that a salivary glycoprotein blocked the attachment of *S. sanguis* to epithelial cells and that this inhibition correlated with aggregation of the bacteria. Similarly, saliva inhibited the adherence of group A streptococci to epithelial cells

when it was present in the reaction mixture. Pretreatment of either the epithelial cells or the bacteria failed to inhibit adherence. Since previous work indicated that LTA is a primary adhesin in the attachment of *S. pyogenes* to host cells (4), these data suggest that a salivary component binds with low affinity to the streptococcal surface and interferes with the interaction between LTA and receptors on host cells.

In conclusion, the data presented indicate that stimulated WHS can both aggregate group A streptococci and block their adherence to epithelial cells. There is a large variation in the aggregating activity of saliva from different donors, and it is interesting to speculate on whether these variations are related to the susceptibility of certain individuals to oral colonization by group A streptococci. Future studies will focus on such variations to determine if there is any relationship between concentrations of agglutinin-inhibitor in saliva and streptococcal pharyngitis.

ACKNOWLEDGMENTS

We thank Jennifer Parker and Jane Hurley for excellent technical assistance.

These studies were supported in part by Veterans Affairs medical research grants and by Public Health Service grant DE07218 from the National Institutes of Health.

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