INFECTION AND IMMUNITY, July 1986, p. 173–178
0019-9567/86/070173-06$02.00/0

Vol. 53, No. 1

Calcium-Dependent Salivary Agglutinin with Reactivity to Various Oral Bacterial Species

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Received 3 September 1985/Accepted 9 April 1986

Human parotid agglutinins from three individuals were isolated by adsorption to and desorption from strains of *Streptococcus mutans* belonging to serotypes a, b, c, d, and e and strains of *Lactobacillus casei*, *Actinomyces viscosus*, and *Streptococcus sanguis*. The desorption was achieved by suspending centrifuged saliva-coated microorganisms in 10 mM phosphate buffer (pH 6.8) containing 0.154 M sodium chloride. After another centrifugation, agglutinin activity was recovered in the supernatants. The *L. casei* strain was not agglutinated by any of the agglutinin extracts or by saliva, but all the other strains were agglutinated to a variable extent. However, all strains, including the nonagglutinating *L. casei* strain, adsorbed and desorbed agglutinins active for other strains. The agglutinin extracts from *S. mutans* serotype *c*, *S. sanguis*, and *A. viscosus* were purified and characterized by electrophoretic and immunological techniques. The purified preparations were positively stained for protein and carbohydrate, and the molecular weights were estimated to be 440,000. All agglutinin extracts needed calcium in the range of 0.1 to 0.5 mM to be active, and for a single strain, all agglutinins gave the same degree of agglutination, indicating that the isolated agglutinins may be of the same molecular species, a hypothesis that was also confirmed by the preliminary characterization of the purified agglutinins. This type of agglutinin, which seems to exert its activity among various bacterial species, could be important in mediating bacterial coaggregation and thus may add to the effect of specific agglutinins in the clearance of bacteria from the human mouth.

Salivary agglutinins are considered to participate in the bacterial clearance of the human mouth (14) by reacting with bacterial surface structures to block their adherence. Such agglutinin-coated bacteria cannot compete for similar salivary structures in the saliva-derived film lining oral surfaces. Agglutinated or agglutinin-coated microorganisms stay in the fluid saliva and are swallowed. Among the agglutinins, high-molecular-weight glycoproteins have been shown to be of importance (7, 11, 16, 19). The involvement of reactive groups such as sialic acid (22, 23) and different hexoses (12, 19, 26) in glycoproteins agglutinating different bacterial strains has indicated a specificity in bacterium-agglutinin reactions. However, most of these studies have been concerned with agglutinating activity in whole-saliva samples. In whole saliva, which is a mixture of different glandular secretions and crevicular fluid, agglutinins originating from a certain secretion may complex with other agglutinins or secretory glycoproteins (1, 8, 24) and thus more easily evade identification. By selecting a salivary secretion from a single type of gland, difficulties in isolation and preparation of agglutinins can be limited.

In the present study a method described by Rundegren and Ericson (27) for isolating agglutinins in parotid saliva was used. This method is based on the adsorption of agglutinins to microorganisms followed by desorption of agglutinins in phosphate buffer. The method makes it possible to test the agglutinin activity of salivary extracts isolated via an affinity reaction with a single bacterial strain. The purpose of this study was to compare the characteristics of parotid agglutinins isolated from several common oral bacterial species by determining the pattern of activity for each agglutinin with all the bacterial species.

MATERIALS AND METHODS

Saliva. Unstimulated parotid saliva samples were collected from three healthy male donors 1 h after breakfast. The saliva samples, which were used freshly, were collected in ice-chilled tubes by means of Lashley cups and diluted with an equal volume of 10 mM potassium phosphate buffer (pH 6.8) in 0.154 M sodium chloride (PBS). For the purification of agglutinins, parotid saliva samples from one person (subject 1) were collected separately in batches which were kept frozen at –80°C before use. Three batches of saliva, one for each of the three agglutinins purified, were collected.

Bacteria. The strains used in this study were *Actinomyces viscosus* VPI 371 (L. V. Holdeman, Virginia Polytechnic Institute and State University); *Lactobacillus casei* ATCC 7469; *Streptococcus mutans* AHT (29) serotype a, BHT (29) serotype b, TH16 (25) serotype c, OMZ176 (15) serotype d, and LM7 (13) serotype e; and *Streptococcus sanguis* TH12 (25). Lyophilized cultures were inoculated on blood agar plates (17) and incubated for 24 h at 37°C in 5% carbon dioxide in nitrogen. The following broth cultures were prepared. The *A. viscosus* strain was grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) broth, while the other strains were inoculated in a streptococcal medium (18) containing 0.4% glucose. The broth cultures were harvested at the early stationary phase and washed once with an equal volume of PBS. Stock suspensions with an optical density (OD) at 700 nm of 1.5 in PBS, as measured in a Hitachi 100-60 spectrophotometer (Hitachi, Tokyo, Japan), were prepared for each strain. Microscopic counts in cells per milliliter were 9×10^8 for *S. mutans* TH16, 2.7×10^8 for

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A. viscosus, and 4.4×10^8 for *L. casei* at an OD at 700 nm of 1.0.

Assay for agglutinin activity. The diluted saliva was tested by mixing 1 volume of 0.6 ml of saliva with 1 volume of 1.2 ml of bacterial stock suspension. Other samples were tested for agglutinin activity by suspending a pellet from 1.2 ml of bacterial stock suspension in 1.8 ml of the sample. The bacterial pellets were obtained after centrifugation at 4,000 $\times$ *g* for 20 min. Agglutination was recorded at 700 nm and 37°C by monitoring bacterial sedimentation (10). The spectrophotometric readings were quantitated according to the following mathematic expression (10) and reported as *m* values: $mt + b = \ln[(A_o - A)/A]$, where A_o is the absorbance at zero time, *A* is the absorbance at time *t* in minutes, and *b* is the intercept of the calculated line. The *m* value, which can be calculated for a set of *A* and *t* values, is related to the slope of the agglutination curve at the inflection point and is a measure of the agglutinin activity.

Desorption of salivary agglutinin-containing preparations from bacterial cells. A modification of the method described earlier (27) for the desorption of salivary agglutinins from bacteria was used. Preparation of agglutinin extracts was achieved by mixing 1 volume of diluted saliva with 2 volumes of a bacterial suspension with an OD of 1.5. A sample of 1.8 ml was immediately withdrawn from the mixture to test for agglutination. The remaining mixture was incubated at 37°C for 20 min and then centrifuged at 10,000 $\times$ *g* for 20 min at 4°C. The agglutinin-containing pellet was suspended in a volume of PBS equal to that of the supernatant. The pellet was suspended by mixing the solution on a Whirlimixer and sonicating the mixture for 5 s at amplitude 3 by using the microtip of a model W 185 sonicator (Heat Systems Ultrasonics, Plainview, N.Y.). The suspended pellet was centrifuged at 20,000 $\times$ *g* for 30 min at 4°C, and the agglutinin-containing supernatant was saved. The absorbed saliva samples and the agglutinin extracts were tested for agglutinin activity. As a control for the passive sedimentation of agglutinins, 1 volume of diluted saliva was diluted with 2 volumes of PBS and centrifuged at 10,000 $\times$ *g* for 20 min at 4°C. The tube (100 by 15 mm) content was divided from the top to the bottom in four equal fractions by careful suction.

Calcium dependence of agglutinins. All agglutinin preparations were tested with different Ca concentrations. The reaction mixtures of agglutinins and bacteria contained 0, 0.05, 0.1, 0.5, and 1.0 mM $CaCl_2$.

Agglutinin specificity. The strains were tested in two groups. One group included strains of each serotype of *S. mutans*, and the other group included the remaining strains, with the *S. mutans* serotype c strain as a reference. Within each group, an agglutinin preparation desorbed from one strain was tested for activity against all the strains. Separate saliva samples were used for the two tests, which were done twice. The absorbed saliva samples were tested for agglutinin activity and further absorbed until no residual activity could be detected for the strain used to absorb the saliva. The supernatants from these optimal absorptions were then tested for agglutinin activity against all the other strains.

Purification of agglutinins. Agglutinin-containing supernatants prepared from batches of 50 ml of saliva from the same donor and desorbed from *S. mutans TH16*, *S. sanguis* TH12, or *A. viscosus* VPI 371 were used for the isolation of agglutinins. The supernatant volumes were reduced 40 times by using a model 8200 ultrafiltration cell (Amicon Corp., Danvers, Mass.) with a PTHK 10 polysulfone filter (Millipore Corp., Bedford, Mass.) and a molecular weight cutoff at 100,000. The retained residues were chromatographed on a Bio-Gel A-5m (Bio-Rad Laboratories, Richmond, Calif.) column (bed volume, 180 ml; length, 90 cm) equilibrated with PBS at a flow rate of 12 ml/h. Fractions with agglutinin activity were concentrated eight times by volume on an Amicon ultrafiltration cell with a PTGC 10 polysulfone filter (Millipore) and a molecular weight cutoff of 10,000. The residues were analyzed for proteins and immunoglobulin A (IgA) and used in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and immunodiffusion. Samples from the various steps of purification were withdrawn for agglutinin activity assays. The TH16-purified agglutinin was tested with all strains by suspending 5 μl of purified agglutinin and 595 μl of PBS in 1.2 ml of bacterial stock suspension.

SDS-PAGE. Electrophoresis was carried out in 5% polyacrylamide rod gels (5 by 80 mm) in the presence of 0.1% SDS by using a GE 2/4 LS gel electrophoresis apparatus (Pharmacia, Uppsala, Sweden). A continuous buffer system with 0.05 M sodium phosphate (pH 7.0) was used (28). A high-molecular-weight standard (Pharmacia) was treated with 1% SDS–1% 2-mercaptoethanol at 60°C for 15 min before use. Other samples were treated under the same conditions, except that they were left unreduced. The rods were run at a constant current of 4 mA per rod for 20 h and stained with Coomassie brilliant blue R-250 (E. Merck AG, Darmstadt, Federal Republic of Germany) and periodic acid-Schiff reagent (Merck).

Preparation of antiserum. An agglutinin preparation to *S. mutans* TH16 was used to raise antibodies in rabbits. To obtain an antigen concentration high enough for a successful immunization, I omitted the gel filtration step in the purification procedure. The antigen (1 mg/ml) was suspended in an equal volume of incomplete Freund adjuvant, and 0.2-ml portions were injected subcutaneously every 14 days for 8 weeks. The first bleeding was carried out 12 days after immunization 4. Thereafter, immunization was carried out every 4 weeks, and bleedings took place 12 days after each immunization. The antiserum was absorbed with secretory IgA (Sigma Chemical Co., St. Louis, Mo.) by titration, and sodium azide was added at a final concentration of 0.1% (wt/vol). The serum was stored at 4°C.

Immunodiffusion. Ouchterlony double diffusion was carried out in 1% (wt/vol) agarose A (Pharmacia) in a buffer (pH 8.6) containing 22 mM 5.5-diethylbarbituric acid (Merck) and 73 mM Tris (Sigma). Samples of agglutinin preparations (15 μl) and antiserum (10 μl) were placed into 4-mm-diameter wells. Secretory IgA (15 μl) was added to one well as a control for the absorption of the antiserum. After 48 h of diffusion, the gels were washed, dried, and stained with Coomassie brilliant blue R-250.

Immunoblotting. Slab (100 by 140 by 1.5 mm) SDS-PAGE in 0.1% SDS of purified TH16 agglutinin, undiluted parotid saliva, and parotid saliva absorbed with strain TH16 was carried out in a 5% polyacrylamide gel by the method of Laemmli (20) by using a model 2001 electrophoresis unit (LKB, Bromma, Sweden). The absorbed saliva was obtained after the preparation of agglutinin extracts and concentrated by ultrafiltration (Millipore PTGC 10) to the same protein concentration as the undiluted parotid saliva. The samples were denatured as described above. Each sample was applied to two equal sections of the gel. After electrophoresis, the gel was cut in two; one part was stained with Kenacid blue (BDH, Poole, England), and the other was subjected to Western blotting by the method of Burnette (4). The transfer of proteins to nitrocellulose (Trans-Blot Trans-

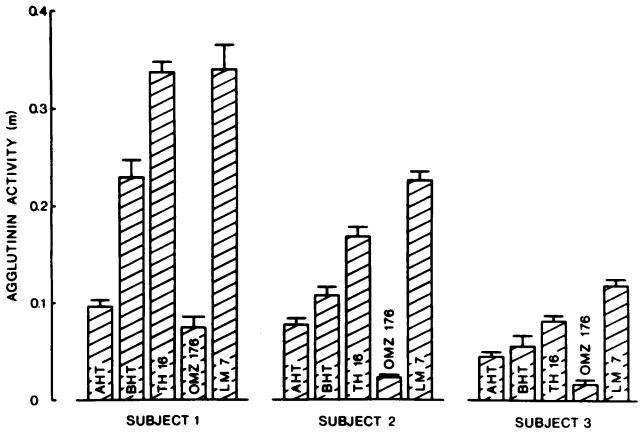

FIG. 1. Agglutinin activities of the three subjects for *S. mutans* strains. Shown for each strain is the mean ± standard deviation of agglutination induced by the five different agglutinin extracts desorbed from the *S. mutans* strains.

fer Medium; Bio-Rad) was carried out at 10 V/cm for 6 h in a Trans-Blot Cell (Bio-Rad). The nitrocellulose sheet was incubated with antiserum to strain TH16 agglutinin and then with a peroxidase-conjugated swine antibody to rabbit immunoglobulins (Dako, Glostrup, Denmark). The antigen was visualized in two steps. First, the antigen was incubated for 10 min in 0.05% (wt/vol) 3,3-diaminobenzidine tetrahydrochloride (Fluka AG, Buchs, Switzerland)–0.03% (wt/vol) cobalt chloride–0.03% (wt/vol) nickel ammonium sulfate in 50 mM phosphate buffer (pH 7.4). Second, the same solution was changed, and H_2O_2 was added at a final concentration of 5×10^{-4}% (vol/vol). The staining was stopped by rinsing with water.

Analyses. Protein was determined by the method of Bradford (2) by using the Bio-Rad protein assay and bovine gamma globulin (Bio-Rad protein standard I) as a standard. The content of IgA in purified agglutinin preparations was

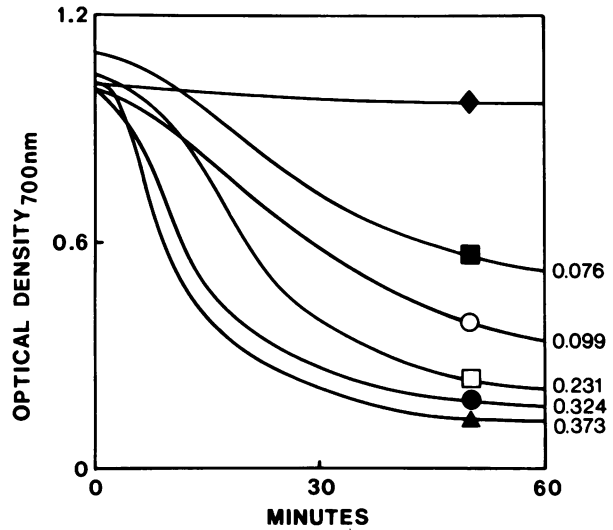

FIG. 2. Agglutination induced with the LM7 agglutinin of subject 1 and bacterial suspensions of AHT (○), BHT (□), TH16 (●), OMZ176 (■), and LM7 (▲). The corresponding *m* values are shown to the right of the curves. Spontaneous bacterial sedimentation of the TH16 bacterial suspension with PBS substituted for agglutinin is shown (◆, control).

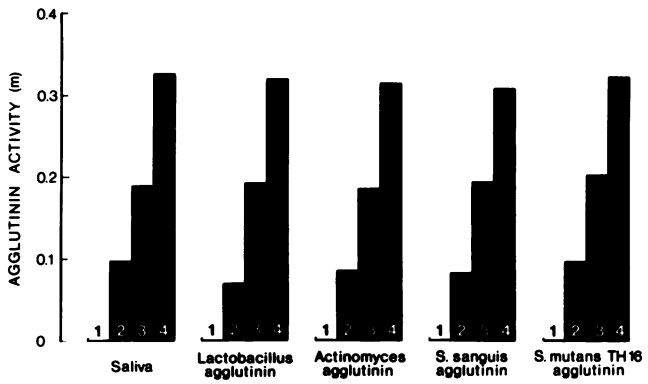

FIG. 3. Agglutinin activities of subject 1 for *L. casei* (column 1), *A. viscosus* (column 2), *S. sanguis* (column 3), and *S. mutans* TH16 (column 4). Agglutination was induced by parotid saliva and parotid agglutinin extracts desorbed from the strains.

determined by an enzyme-linked immunosorbent assay (3) with stabilized human serum (Behringwerke AG, Marburg, Federal Republic of Germany) as a standard.

RESULTS

All strains treated with parotid saliva released salivary extracts with agglutinating activity. Centrifugation at 10,000 × *g* of parotid saliva alone showed that the agglutinin activity was evenly distributed in the tube. The four 2.5-ml fractions had *m* values for *S. mutans* TH16 of 0.327, 0.321, 0.325, and 0.327 counted from the top of the tube. Thus, the agglutinins actually bind to the bacterial cells to get sedimented. The presence of calcium was necessary for the agglutinins to be active. The optimal Ca concentrations needed for agglutination to occur were between 0.1 and 0.5 mM. A concentration of 0.5 mM Ca was thus used throughout the experiments.

For each subject the agglutination rates for the *S. mutans* strains differed, but for each strain there was a low variation in agglutination rates mediated by the different agglutinin extracts desorbed from the various *S. mutans* strains (Fig. 1). Although the overall activity differed among the subjects, each subject had the same relative agglutination pattern. Parotid saliva had agglutinin activities similar to those of the agglutinin extracts. Subject 3 had the lowest agglutinin titer, but he also had a higher secretion rate, 0.19 ml/min, than did the other subjects, who had secretion rates of 0.13 ml/min (subject 2) and 0.12 ml/min (subject 1).

Agglutination curves mediated by the LM7 agglutinin extract of subject 1 are shown in Fig. 2 as an example of the basis for the calculation of the corresponding *m* values, which are indicated immediately to the right of the curves. The curves can be compared with the control curve, which represents the sedimentation of bacterial suspensions in which agglutinins are substituted with PBS. The bacterial suspensions with PBS substituted for agglutinins showed a maximum decrease in OD of 6% after 60 min. The *m* values are calculated for experimental curves which are subtracted with the OD change of control curves.

Agglutination mediated by the agglutinin extracts of subject 1 for the other bacterial species is shown in Fig. 3. Each agglutinin preparation yielded virtually the same agglutinin activity for each strain as did the agglutinin preparations *S. mutans* (Fig. 1). Subjects 2 and 3 had the same agglutination pattern as did subject 1 but with lower activities. The lowest

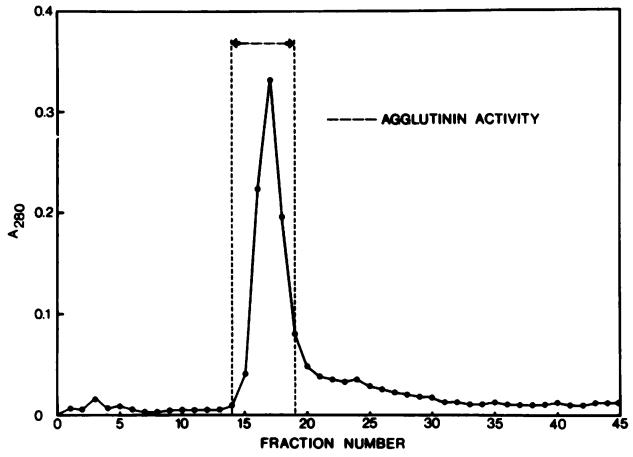

FIG. 4. Bio-Gel A chromatogram of the *S. mutans* TH16 agglutinin. Fractions of 4 ml were collected at a flow rate of 12 ml/h. The fractions containing agglutinin activity are indicated with a dotted line.

activity was recorded for subject 3 with *S. mutans* strains. *L. casei* was not agglutinated by any of the agglutinins, whereas the other strains were agglutinated to a variable degree (Fig. 3).

The agglutinin extracts of subject 1, who had the most marked differences in agglutinin activity among the strains, were analyzed for protein content. The protein concentration for every agglutinin extract was between 5 and 6 μg/ml. The amount of protein in the corresponding supernatant from PBS-treated bacteria was consistently found to be below 0.5 μg/ml, and these supernatants were all devoid of agglutinin activity.

Saliva samples absorbed with the different bacterial strains and tested with the various strains all produced curves with the same appearance as that of the PBS control curve in Fig. 2. No agglutinin activity (*m* value, 0) could thus be found in any of the supernatants for any of the strains.

The filtrates from the 100,000-molecular-weight ultrafiltration of agglutinin extracts had no agglutinin activity for the strains from which the extracts were desorbed, although the filtrates were concentrated 500 times by volume on the 10,000-molecular-weight exclusion filter. The residues applied to the Bio-Gel agarose column contained 1.3 mg (*S. mutans*), 1.2 mg (*S. sanguis*), and 1.0 mg (*A. viscosus*) of protein. The chromatograms were similar, with a major A_{280} peak at the void volume, which coincided with all of the agglutinin activity found in the fractions (Fig. 4). The active fractions were pooled and further concentrated.

The concentrated agglutinin preparations produced somewhat trailing but homogenous bands of similar molecular weights, with an extrapolated M_r of 440,000 in SDS-PAGE after staining with Coomassie brilliant blue (Fig. 5). The agglutinin preparations stained with periodic acid-Schiff stain appeared to be identical to those stained with Coomassie brilliant blue. Immunoblotting produced a clear stain for 2 μg of TH16 agglutinin at the site for the agglutinin in the SDS gel (Fig. 6). A faint blotted band with the same mobility as the agglutinin band was detected in the parotid saliva sample containing 100 μg of protein. This band had a somewhat higher mobility than that of the closest main band in the Kenacid blue-stained gel of parotid saliva. The absorbed saliva produced a scarcely visible strain at the site for the agglutinin. The cross-reactive low-molecular-weight components detected in the saliva samples were not detected in the agglutinin sample.

The IgA contents (protein [wt/wt]) were 0.9% for the *S. mutans* TH16 agglutinin, 1.5% for the *S. sanguis* agglutinin, and 1.6% for the *A. viscosus* agglutinin.

The polyspecific agglutination pattern of the purified TH16 agglutinin was similar to those of saliva (Table 1) and the agglutinin extracts (Fig. 1 and 3). Immunodiffusion revealed identity precipitation lines among the three purified agglutinin preparations (Fig. 7). Secretory IgA produced no precipitation line at all.

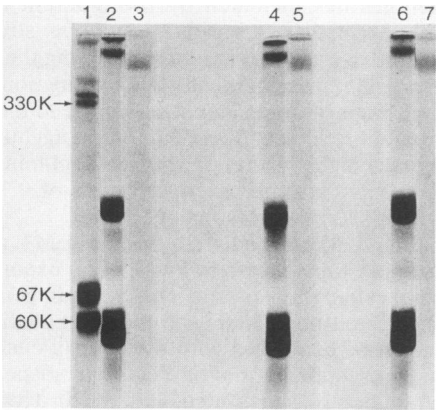

FIG. 5. SDS-PAGE of parotid saliva samples and purified agglutinins desorbed from bacterial cells treated with the parotid saliva samples. The parotid saliva samples corresponding to an agglutinin are shown immediately to the left of the agglutinin. Lanes: 1, high-molecular-weight standards (hog thyroglobulin subunit [330,000 {330K}], bovine serum albumin [67K], and bovine catalase subunit [60K]); 2, parotid saliva; 3, *S. mutans* TH16 agglutinin; 4, parotid saliva; 5, *S. sanguis* agglutinin; 6, parotid saliva; 7, *A. viscosus* agglutinin. The amounts of protein applied were as follows: high-molecular-weight standards 62 μg; parotid saliva, 100 μg; and agglutinin preparations, 20 μg.

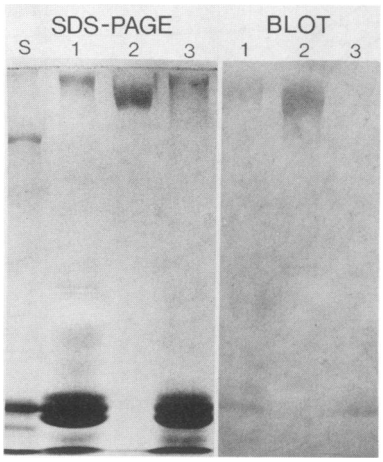

FIG. 6. SDS-PAGE and immunoblotting of parotid saliva (lane 1), strain TH16 agglutinin (lane 2), and absorbed saliva (lane 3). Proteins were transferred from an SDS gel to which 100 μg of protein (for parotid saliva and absorbed saliva) and 2 μg of protein (for TH16 agglutinin) had been applied. The amount of high-molecular-weight standard (lane S) applied was 10 μg.

TABLE 1. Agglutinin activity in mixtures (1.8 ml) of purified TH16 agglutinin (1 μg), parotid saliva (470 μg protein), and some oral strains[a]

Sample (from subject 1)	Agglutinin activity (*m* value) for:							
	S. mutans					*S. sanguis* TH12	*L. casei* ATCC 7469	*A. viscosus* VPI 371
	AHT	BHT	TH16	OMZ176	LM7			
TH16 agglutinin	0.155	0.289	0.374	0.070	0.404	0.216	0	0.199
Saliva	0.076	0.215	0.289	0.062	0.373	0.190	0	0.097

[a] Reaction mixtures contained 10^9 strain TH16 cells.

DISCUSSION

In the present study it was shown that parotid saliva agglutinins desorbed from one bacterial strain could mediate agglutination of strains representing several bacterial species, thus indicating the general activity of these agglutinins. The agglutinins were isolated by selective adsorption to microorganisms. This method has the advantage of being gentle and provides a high degree of purification in only one step (11).

The three individuals had different agglutinin activities in their saliva samples and agglutinin extracts. This is, to some extent, probably due to the different secretion rates recorded, since a high secretion rate results in a lower protein concentration in the saliva (5). The various activities might also reflect actual differences in the individual protein compositions of the saliva samples.

All strains, including the nonagglutinating *L. casei* strain, adsorbed and desorbed agglutinins active for other strains. *L. casei* may occupy salivary reactive sites responsible for agglutination. Thus, no free sites may be left over for cross-linking of agglutinin-coated cells.

In the two sets of agglutination experiments the different agglutinin extracts exhibited practically the same activities, a result that was also confirmed by the very small differences in amounts of salivary protein that could be desorbed from the saliva-coated bacteria in the experiments with subject 1. The crude and purified agglutinin preparations to strain TH16 had the same agglutination patterns for the various strains, indicating that only negligible amounts of nonagglutinin saliva components may have interfered with the agglutination mediated by the crude extract.

The agglutinins seem to be adsorbed to the cells in a general manner. Obviously, adsorption is dependent on an active calcium-stabilized configuration of the agglutinins, as suggested by the calcium dependence of the agglutinins.

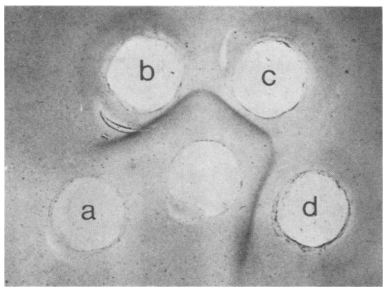

FIG. 7. Immunodiffusion of agglutinin preparations. The center well contained 10 μl of antiserum to the *S. mutans* TH16 agglutinin preparation. The other wells contained 15 μl of the following preparations: a, secretory IgA (2 μg); b, *S. mutans* TH16 agglutinin (2.4 μg); c, *S. sanguis* agglutinin (2.5 μg); and d, *A. viscosus* agglutinin (2.3 μg).

Parotid saliva contains ionized calcium in excess (21), fostering the activity of the agglutinins. Furthermore, desorption can easily be achieved in a calcium-free buffer with neutral pH and physiological ionic strength (27), indicating a low affinity for the bacteria of the agglutinins.

The chromatograms and the electrophoretic appearance of the purified agglutinins were similar to those reported earlier for strain TH16 parotid saliva agglutinin (11). In the native state, the agglutinins had a molecular weight of not less than 5×10^6, as shown by the Bio-Gel chromatograms. In SDS-PAGE, the molecular weight was estimated to be 440,000, indicating that the agglutinins exist as multiple forms of a subunit. From immunodiffusion it appears that the three agglutinins are immunochemically identical to each other but different from secretory IgA. Immunoblotting demonstrated that the agglutinins are present in parotid saliva at very low concentrations, as shown by their absence from the Kenacid blue-stained gel. The minor trace of agglutinin revealed in the absorbed saliva by the sensitive blotting technique was not enough to yield a biological agglutinin activity. As little as 1 μg of the agglutinin had a somewhat higher activity than 470 μg (protein) of parotid saliva (Table 1); this value is close to the specific activity found earlier for a purified agglutinin to strain TH16 (11).

The use of a higher salt concentration (1 M NaCl) for desorption of whole-saliva components from *S. mutans* cells results in a higher yield of many proteins, including IgA (6). The low IgA content of the present agglutinin preparations might have been due to the IgA having such a high affinity for the bacterial surfaces that only a fraction of it was desorbed by the method used. Following agglutination, coating of *S. mutans* cells with submandibular-sublingual saliva, and suspension in PBS, no agglutinin activity could be recovered in the supernatant (unpublished data), indicating the presence of a different and higher-affinity agglutinin in submandibular-sublingual saliva than in parotid saliva.

Several investigators have stressed bacterial species specificity of salivary agglutinins. These agglutinins may differ from the present one in that they are dependent on specific saccharides and on lectin-like reactions with the cells, as reported, e.g., for *S. sanguis* (22, 23) and *S. mutans* (26). In a study by Ericson and Magnusson (9), parotid saliva agglutinins to some oral strains showed a different degree of reduction in activity after adsorption to defined quantities of hydroxyapatite, this result was taken to be an indication of a specificity of the agglutinins. They found that serotype d strains of *S. mutans* were the most sensitive strains to adsorption, whereas serotype c and e strains were the most insensitive. In light of the present results, the different rates of bacterial agglutination obtained after absorption of saliva with a certain amount of hydroxyapatite might reflect differences between bacterial strains in the amount of agglutinins required for detectable agglutination to occur at all.

It was shown earlier (27) that a parotid saliva agglutinin to

strain TH16 required calcium in the order of 0.1 mM to be active. In the present study, the close concentration range of 0.1 to 0.5 mM calcium was needed to restore activity to all agglutinin preparations. This fact further supports the suggestion that the agglutinin preparations may be the same substance.

The present results indicate that, in addition to strain-specific bacterium-agglutinating substances such as secretory IgA, there exists in the human mouth a type of agglutinin which is capable of binding to and agglutinating a variety of bacterial species. Such an agglutinin could probably play an important role in mediating coaggregation of different microorganisms, thus adding to the efficiency of specific agglutinins in causing bacterial clearance.

ACKNOWLEDGMENTS

This work was supported by the Swedish Dental Society.

I thank Stig Edwardsson for valuable criticism of this manuscript and Douglas Bratthall for analyses of IgA.

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