Fibril-Mediated Adherence of *Actinomyces viscosus* to Saliva-Treated Hydroxyapatite

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Fibril-mediated adherence of *Actinomyces viscosus* strain T14V cells to saliva-treated hydroxyapatite was studied. Fibrils were purified by ammonium sulfate precipitation and differential centrifugation from the crude supernatant of whole cells that were sheared by one passage through a French pressure cell. Purified fibrils and crude supernatant inhibited strain T14V adherence to saliva-treated hydroxyapatite to similar extents. However, anti-strain T14V serum and antifibril specific antibody completely abolished strain T14V adherence. The blocking immunoglobulin could be adsorbed from anti-T14V serum by strain T14V whole cells, by purified fibrils, and, to a lesser extent, by cell walls. It was concluded that fibrils mediate adherence of strain T14V cells to saliva-treated hydroxyapatite. In addition, fibril preparations were shown to contain more than 95% protein and to be antigenically homogeneous by immunodiffusion and Laurell rocket immunoelectrophoresis. Purified fibril preparations showed serological identity with the virulence-associated 1 antigen of Lancefield-extracted T14V cells, whereas crude supernatants contained both virulence-associated 1 and virulence-associated 2 antigens, as shown by rocket immunoelectrophoresis.

The ability of bacterial cells to interact with host tissue in a specific manner is thought to be the primary event by which most indigenous and pathogenic microorganisms initiate colonization of a host (22). Little is known about the surface structures possessed by various bacteria, which apparently must interact with host tissue components to enable the microorganisms to become firmly attached and thereby resist elimination by host defense mechanisms (21). For example, fibrils, fimbriae, and pili have been implicated in the adsorption of many gram-negative (4, 6, 15, 16, 27, 42, 44, 46, 47) and gram-positive (26, 34, 35, 54) microorganisms to host tissue. Pili have been shown to mediate adherence of gonococci to tissue culture cells (49), to erythrocytes (6), and to sperm cells (28) and of *Escherichia coli* to intestinal epithelial cells (27, 42) and kidney cells (47).

Actinomyces viscosus, a microorganism implicated in the etiology of gingivitis (29, 30, 38, 50) and periodontitis (29-31), may require specialized cell surface structures to promote adsorption and subsequent colonization on teeth, epithelial cells, and other bacteria in plaque. Fibrils have been implicated in the coaggregation of *A. viscosus* (7, 41) or *A. naeslundii* (7, 17) with oral streptococci and in the adherence of *A. naeslundii* to epithelial cells (19). Girard and Jacius (23) proposed that *Actinomyces* adsorption was mediated by the fibrils which they observed in electron photomicrographs. Brecher et al. have

shown differences in virulence of *A. viscosus* strains T14V and T14AV in monoinfected germ-free rats and conventional rats (3). Furthermore, these authors have shown that the prerequisite for virulence of strain T14V is its ability to attach to and colonize the teeth and gingival crevice of rodents, resulting in bone loss characteristic of periodontal disease (3). In this model, the failure of strain T14AV to result in alveolar bone loss was attributed to its colonization pattern. Strain T14AV colonizes pits and fissures, but lacks the ability to colonize the gingival crevice region of teeth and therefore cannot induce periodontal disease in these laboratory animals (3). *A. viscosus* strain T14V possesses a virulence-associated antigen(s) identified by immunoelectrophoresis (24, 45) or by immunodiffusion (8, 9), which is not detectable in the avirulent variant *A. viscosus* strain T14AV. In addition, morphological and ultrastructural studies of strain T14V and T14AV have shown a characteristic fibril structure on the cell surface of strain T14V that is undetectable on the avirulent strain (3, 45). Cisar and co-workers have identified these fibrils isolated from cell walls as a virulence-associated antigen (8, 9). We have shown recently that the adherence of *A. viscosus* strain T14V to saliva-treated hydroxyapatite (HA) is abolished by heat or proteolytic enzymes and that nonadherent cells do not possess surface fibrils (53). However, an a priori role of fibrils in the adherence of strain

T14V cells to saliva-treated HA surfaces has not been demonstrated.

The present report describes the purification and partial characterization of surface fibrils from strain T14V whole cells. We used anti-T14V serum and antifibril specific antibody to reveal that fibrils mediate the adherence of strain T14V to saliva-treated HA. The fibrils were found to be composed of protein and showed antigenic identity with the virulence-associated antigen 1 described by Powell et al. (45).

MATERIALS AND METHODS

Cultures and culture conditions. *A. viscosus* strain T14V was kindly provided by B. F. Hammond, University of Pennsylvania, Philadelphia. All cultures were stored as lyophilized stocks or as multiple frozen stocks at -80°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 20% glycerol. Tritium-labeled cells of *A. viscosus* strain T14V were prepared fresh from the frozen stocks for each experiment by growing the organisms in tryptic soy broth with dextrose (Difco) containing $10\ \mu\text{Ci}$ of [^3H]thymidine per ml (Schwarz/Mann, Orangeburg, N.Y.). Organisms from 16-h cultures were harvested by centrifugation at $1,300 \times g$ for 10 min, washed twice, and suspended in 0.05 M buffered KCl, pH 7.3 (1 mM potassium phosphate, 1 mM CaCl_2 , 0.1 mM MgCl_2), as previously described (10, 53).

Preparation of fibrils. A modification of the method described by Buchanan (5) for the purification of gonococcal pili was used to isolate surface fibrils from *A. viscosus* strain T14V cells. In preliminary experiments, strain T14V cell cultures were sheared for 5 min in a blender, and the fibrils were isolated by using a series of steps, including differential centrifugation and ammonium sulfate precipitation, as described below. However, subsequent trials demonstrated that large quantities of cells could be sheared more quickly by using a French pressure cell; therefore, this method was used in these studies. Bacterial cells from 9 liters of tryptic soy broth cultures (wet weight, 100 g) were washed in 0.05 M potassium phosphate buffer (pH 7.2) and resuspended in the same buffer to form a thick paste. The cell paste was passed once through a French pressure cell (American Instrument Co., Silver Spring, Md.) at $10,000\ \text{lb/in}^2$. One passage resulted in less than 2% cell disruption, as measured by release of material absorbing at 260 nm. The product was centrifuged at $48,300 \times g$ for 20 min to remove whole cells and cell walls. The crude supernatant was centrifuged for 24 h at $160,000 \times g$. The pellet was suspended in 20 ml of 0.1 M tris(hydroxymethyl)aminomethane (pH 7.5) and sonicated at full power for 1 min with a microultrasonic cell disrupter (Kontes Corp., Vineland, N.Y.). After centrifugation for 10 min at $23,700 \times g$, the supernatant was mixed with an equal volume of 20% ammonium sulfate and left at 4°C for 24 h. This solution was subsequently centrifuged for 15 min at $30,900 \times g$. The pellet was suspended in 10 ml of 10% ammonium sulfate and blended in a Vortex mixer for 1 min. After centrifugation for 15 min at $30,900 \times g$, the pellet was sus-

pended in 10 ml of filtered distilled water, sonicated for 1 min at full power, mixed with an equal volume of 20% ammonium sulfate, and again left at 4°C for 24 h. The centrifugation process through the suspension in 10% ammonium sulfate was repeated. The resulting pellet was suspended in filtered distilled water, dialyzed against four changes of distilled water, and lyophilized (15 mg of purified fibrils). Each purification step was monitored by electron microscopy and Laue rocket immunoelectrophoresis, as described below.

Preparation of cell walls. Cell walls were provided by David Brown, University of Florida. *A. viscosus* strain T14V washed cells were broken in a Braun model MSK cell homogenizer (Bronwill Scientific Inc., Rochester, N.Y.), and walls were isolated and purified by the method of Bleiweis et al. (2).

Antisera. Antisera to *A. viscosus* T14V were provided by David Brown and John Fitzgerald, University of Florida. Hyperimmune sera were prepared in adult New Zealand rabbits by a series of intravenous injections of *A. viscosus* T14V cells prepared as described below. Cells were grown to late exponential phase in tryptic soy broth without dextrose (Difco) supplemented with 0.1% yeast extract (Difco) and 1% glucose, harvested, and washed with saline. The washed cells were suspended at 20 mg (wet weight) per ml in saline and placed in a boiling water bath for 15 min as described by Powell et al. (45).

Immune serum was prepared in a normal goat by a series of intramuscular and intravenous injections of strain T14V cells. Initially the goat was injected intramuscularly with 0.5 ml (10 mg) of the killed whole cell suspension mixed with 0.5 ml of complete Freund adjuvant (Difco). A second 0.5-ml (10-mg) injection of whole cells with out Freund adjuvant was given intramuscularly during week 2. At weeks 4, 6, 8, and 10, intravenous injections containing 1 mg of killed whole cells were given. Beginning with week 12 and continuing at weekly intervals, the goat was bled from the carotid artery and injected with a suspension containing 1 mg of killed whole cells.

In some experiments the goat and rabbit anti-strain T14V serum was adsorbed with strain T14V whole cells, purified fibrils, or cell walls, as described below. Strain T14V cells were grown in tryptic soy broth, harvested, washed twice, and suspended in the buffered KCl which was used in the adherence assay. Cell concentration was determined with a Spectronic 20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.), as previously described (53). A 5-ml sample of goat anti-T14V serum was adsorbed with 0.25 to 18.3 mg of fresh strain T14V cells for 60 min at 37°C . The adsorbent was removed by centrifugation at $48,300 \times g$ for 10 min. The adsorbed serum was stored at -30°C for subsequent use. To relate cell numbers to dry weights, samples of known numbers of the fresh washed whole cells used for the adsorption were lyophilized and weighed. A sample of goat anti-T14V serum was also adsorbed with varying amounts of strain T14V cell walls for 60 min at 37°C . Cell walls were removed by centrifugation at $48,300 \times g$ for 20 min. The adsorbed serum was stored at -30°C .

Goat anti-T14V serum was adsorbed with purified fibrils immobilized on Sepharose beads. Fibrils were covalently linked to CNBr-activated Sepharose beads (type 4B; Sigma Chemical Co., St. Louis, Mo.) by a

method described previously (1, 12). With this procedure approximately 75% of a 10-mg sample of fibrils attached to 5 g of beads, as measured by optical density at 280 nm. A 5-ml sample of serum was incubated with the fibril-Sepharose bead complex for 60 min at 37°C. The fibril-Sepharose bead adsorbent was removed by centrifugation at $225 \times g$ for 1 min. The adsorbed serum was stored at -30°C for subsequent use. The fibril-Sepharose bead reagent was reactivated by washing twice with 0.05 M potassium phosphate buffer, pH 2.3, and twice with 0.05 M potassium phosphate buffer, pH 7.2. Goat anti-T14V serum adsorbed with Sepharose beads without fibrils behaved like nonadsorbed anti-T14V serum in all experiments.

Rabbit anti-T14V serum was also adsorbed with the fibril-Sepharose bead reagent, as described above. Because of the high titer of the rabbit anti-T14V serum, the same 5-ml sample was adsorbed three times with the fibril-Sepharose bead reagent. After each adsorption, antifibril specific antibody was eluted from the immobilized fibrils by two 5-ml washes with 0.05 M potassium phosphate buffer, pH 2.3, and diluted immediately with 10 ml of 0.05 M potassium phosphate buffer, pH 7.2. The antifibril antibody from the three adsorptions was pooled, concentrated to 5 ml by ultrafiltration (PM-30 filter; Amicon Corp. Lexington, Mass.), and stored at -30°C.

Normal goat and rabbit sera (GIBCO Laboratories, Grand Island, N.Y.) were adsorbed similarly by all of the above-mentioned adsorbents and were used as controls.

Adherence assays. Preparation and treatment of HA beads (BDH, Poole, England) were performed by methods described previously (10). Whole paraffin-stimulated saliva from one male donor (age, 25 years) was collected, clarified, and used to treat HA, as previously described (25). Treatment of 40 mg of saliva-treated HA beads with [^3H]thymidine-labeled bacterial suspensions and subsequent scintillation counting of samples to determine numbers of cells adsorbed to HA were performed as previously described (53).

Lyophilized preparations of purified fibrils and crude supernatant obtained by shearing whole cells in the French pressure cell were used in competitive inhibition assays with strain T14V cells and saliva-treated HA beads. Labeled strain T14V cells were suspended in buffered KCl at a concentration of 8×10^7 cells per ml. Equal volumes of buffered KCl containing various concentrations of fibrils or crude supernatant were used to dilute the labeled cells to the standard working concentration of 4×10^7 cells per ml. This mixture was assayed for adherence to saliva-treated HA. Bovine serum albumin (Sigma) and dextran (molecular weight, 18,400; Sigma) were used as controls to examine nonspecific competitive inhibition. Additional controls consisted of strain T14V cells diluted with buffered KCl alone.

Adherence assays with various antibody preparations included in the cell-HA reaction mixtures were performed. Strain T14V cells suspended at 8×10^7 cells per ml were diluted to the standard cell concentration (4×10^7 cells per ml) with an equal volume of buffered KCl containing a 1:4 dilution of one of the antibody preparations or normal serum and immediately used in the adherence assay. Microscopic exam-

inations of the reaction mixtures revealed that significant clumping did not occur at the cell concentrations and serum dilutions used. A 1:4 serum dilution was used in all antibody experiments unless indicated otherwise. Adherence data are expressed as percent adherence of control cells which were incubated with normal serum.

Immunoelectrophoresis. The antigens present in the crude supernatant and fibrils were detected by Laurell rocket immunoelectrophoresis (37), utilizing the Osserman modification (33, 43) as described previously (45). To increase their solubility, the purified fibrils (20 mg/ml) were suspended in 0.5% Triton X-100 (Sigma). In addition, 1.0% Triton X-100 was included in the agarose.

One well contained a chemical extract of strain T14V whole cells prepared by a modification of the method of Lancefield and Perlmann (36), as described previously (45).

Electron microscopy. One drop of the purified fibril preparation was placed on carbon-stabilized Parlodion film. Excess material was removed, and 1 drop of 1% uranyl acetate in water was added. Excess stain was immediately removed, and the grids were examined at 60 kV in a Zeiss EM9 S-2 electron microscope.

Chemical analysis. Protein contents were estimated by the method of Lowry et al. (39), using bovine serum albumin (Sigma) as a standard. Carbohydrate, as measured by reducing sugars, was estimated by the phenol-sulfuric acid method (14), using dextran (Sigma) as a standard. Amino acid, *N*-acetyl muramic acid, and hexosamine compositions of the purified fibrils were determined with model JLC-5AH amino acid analyzer (JOEL USA, Inc., Cranford, N.J.). The 2-mg fibril samples were hydrolyzed in a mixture containing 2 ml of 6 N HCl, 10 μl of phenol, and 5 μl of mercaptoethanol at 110°C for 22 h in nitrogen-flushed, sealed vials. After lyophilization, the samples were suspended in 4 ml of distilled water, filtered through glass wool, and analyzed.

RESULTS

Competitive inhibition of adherence. Purified fibrils or crude supernatant was included in the bacterial cell-HA suspension to examine direct inhibition of strain T14V adherence to saliva-treated HA (Fig. 1). Increasing the amount of purified fibrils or crude supernatant inhibited adherence of whole cells up to a maximum of 30%. To assess the influence of nonspecific inhibition on adherence, bovine serum albumin and dextran were included in bacterial cell-HA suspensions in quantities similar to those tested for fibrils and crude supernatant. At the highest concentration of bovine serum albumin or dextran examined (2 mg/ml), inhibition was approximately 10%.

Adherence inhibition with antisera. Goat anti-T14V serum inhibited adherence of strain T14V to saliva-treated HA (Fig. 2). Adherence decreased as the concentration of antiserum in the bacterial cell-HA suspension increased. At

FIG. 1. Competitive inhibition of *A. viscosus* T14V adherence to saliva-treated HA with crude supernatant (●), purified fibrils (■), bovine serum albumin (○), and dextran (□).

FIG. 2. Titration of adherence inhibition activity of *A. viscosus* T14V adherence to saliva-treated HA. Symbols: ●, normal goat serum; ■, goat anti-T14V serum. Undiluted serum contained 133 mg of protein per ml. Dilutions are expressed as ratios of antiserum volume to total volume.

antisera dilutions equal to or less than 3:10, adherence was completely abolished. Normal goat serum did not diminish adherence of strain T14V cells.

Adsorption of antiserum inhibition. The inhibition activity of the goat anti-T14V serum could be adsorbed completely with strain T14V cells or purified fibrils and to a lesser degree with strain T14V cell walls (Table 1). Adherence inhibition activity was diminished in specific antisera as the number of strain T14V cells used to adsorb the serum increased, and such activity was completely eliminated after adsorption with 20 mg of cells (data not shown). However, adsorption of antisera with strain T14V cell walls did not reduce inhibition to the same extent as did whole cells. Adsorption of normal serum with whole cells or fibrils had little influence on adherence.

Adherence inhibition with antifibril specific antibody. Inhibition of strain T14V adherence to saliva-treated HA with rabbit anti-T14V serum was similar to goat anti-T14V serum inhibition (Table 2). A volume of rabbit antifibril specific antibody equivalent to the volume of anti-T14V serum inhibited strain T14V adherence to saliva-treated HA. Undiluted rabbit anti-T14V serum contained 73 mg of total protein per ml, whereas undiluted rabbit antifibril specific antibody contained 3.2 mg of total protein per ml. Adsorbed or unadsorbed normal serum did not alter adherence.

Identification and composition of fibril antigen. Electron photomicrographs of negatively stained preparations of purified fibrils (Fig. 3) indicated the relative morphological homogeneity obtained with the fibril purification procedure. Rocket immunoelectrophoresis of purified fibril preparations demonstrated only one antigen reacting with rabbit anti-T14V serum (Fig. 4). The fibril-specific antigen was found in crude supernatant preparations in smaller amounts. The fibril-specific antigens in both preparations showed identity with one of the virulence-associated antigens (virulence-associated antigen 1) in the whole cell Lancefield extract described previously by Powell et al. (45). In addition to the fibril-specific antigen, the crude supernatant contained two additional antigens detected by the anti-T14V serum. One of these showed identity with the other virulence-associated antigen (virulence-associated antigen

TABLE 1. Adsorption of goat anti-T14V and normal sera

Adsorbent	% Adherence of control ^a	
	Normal serum	Anti-T14V serum
None	100 ^b	12
T14V whole cells (7.5 mg)	87	95
Fibrils (7.5 mg)	107	93
T14V Braun cell walls (7.5 mg)	ND ^c	29

^a Serum was diluted 1:4.

^b Controls with and without normal serum in cell-HA suspensions adhered similarly.

^c ND, Not done.

TABLE 2. Effect of rabbit serum on adherence

Serum ^a	% Adherence of control
Normal ^b	100
Anti-T14V	10
Antifibril specific antibody	9

^a Serum was diluted 1:4.

^b Controls with and without normal serum in cell-HA suspensions adhered similarly.

FIG. 3. Transmission electron microscopy of uranyl acetate-stained preparation of *A. viscosus* T14V purified fibrils. $\times 86,000$.

FIG. 4. Laurell rocket immunoelectrophoresis of antigen preparations from *A. viscosus* T14V. Well 1, 250 μ g of Lancefield extract; well 2, 200 μ g of purified fibrils in 0.5% Triton X-100; well 3, 200 μ g of crude supernatant. Rabbit anti-T14V whole cell serum was used at 50 μ l/ml of agarose. The reference antigen in the Osserman trough was crude supernatant. TC, Top common; VA 1 and VA 2, virulence-associated antigens 1 and 2, respectively.

2) of the whole cell Lancefield extract, as described by Powell et al. (45). A rapidly migrating antigen was also found in the crude supernatant, but it migrated off the gel. Subsequent treatment of the fibrils and crude supernatant by the methods of Lancefield and Perlmann (36) did not alter the precipitin patterns (data not shown).

Immunodiffusion experiments in which crude supernatant and purified fibrils were used as

antigen sources also showed a reaction of identity between one antigen in the crude supernatant and the purified fibril antigen when developed against rabbit anti-T14V serum or rabbit antifibril specific antibody (data not shown). In addition to the fibril-specific precipitin line, three additional precipitin lines were evident when the crude supernatant and anti-T14V serum were reacted. However, only the single precipitin line was observed in the fibril-antifibril reaction or in the crude supernatant-antifibril reaction.

Chemical analysis of purified fibrils revealed preparations composed of 95.2% protein and less than 2% carbohydrate as measured by reducing sugars. Amino acid analysis (Table 3) showed that fibrils contain high quantities of aspartic acid, threonine, glutamic acid, and alanine. A small quantity (15.8%) of basic amino acids was present, whereas acidic, polar uncharged, and nonpolar amino acids were present in approximately equal quantities (25 to 30%). *N*-acetyl muramic acid and *N*-acetyl glucosamine, as well as other hexosamines, were not detected in the preparation, indicating that cell wall material did not contaminate the fibril preparation. A minimum molecular weight of 24,960 for the

TABLE 3. Amino acid composition of *A. viscosus* T14V fibrils obtained from French press shearing and lysozyme digestion

Amino acid	No. of residues per 1,000 ^a	
	French press	Lysozyme ^b
Acidic	(26) ^c	(24)
Aspartic acid	137	139
Glutamic acid	120	102
Basic	(16)	(12)
Lysine	76	95
Arginine	70	20
Histidine	12	8
Ornithine	0	ND ^d
Polar uncharged	(29)	(29)
Threonine	114	136
Glycine	86	83
Serine	74	35
Tyrosine	23	33
Cystine	0	ND
Nonpolar	(29)	(35)
Alanine	102	95
Leucine	74	75
Valine	51	62
Isoleucine	28	35
Phenylalanine	23	19
Methionine	11	1
Proline	Trace	62

^a Not corrected for losses.
^b Obtained from Cisar and Vatter (8).
^c Numbers in parentheses are percentages of total amino acids.
^d ND, Not done.

fibrils was calculated from the amino acid data. Purified fibrils subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (32) barely penetrated the gel. Therefore, it was not possible to confirm the molecular weight estimate by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

DISCUSSION

A variety of studies have shown that specific surface polymers possessed by various bacteria may interact with host tissue components (6, 13, 18, 20, 25, 27, 28, 42, 47, 49), facilitating firm attachment to a specific host tissue. For example, the adherence of *Klebsiella pneumoniae* to rat bladder epithelial cells was recently shown to be inhibited by antiserum to the whole cells and by specific antiserum to the surface pili (20). In addition, Salit and Gotschlich (47) reported that antipilus sera inhibited adherence of pilated strains of *Escherichia coli* to monkey kidney cells. In the present study the adherence of *A. viscosus* strain T14V to saliva-treated HA was shown to be mediated by surface fibrils. Adherence was completely inhibited by both goat and rabbit anti-T14V sera. Adherence inhibition activity could be adsorbed from the antisera with strain T14V whole cells and with purified fibrils immobilized on Sepharose beads. Antifibril specific antibody eluted from immobilized fibrils also inhibited the adherence of strain T14V cells to saliva-treated HA.

The adherence inhibition activity decreased significantly as anti-T14V serum was preadsorbed with increasing quantities of strain T14V cells. It is of interest that for preadsorption of the antiserum cell walls were less effective than whole cells in eliminating adherence inhibition activity. For example, when 4 mg of either whole cells or cell walls was used as adsorbent, inhibition by whole cell-adsorbed serum decreased by more than 50%, whereas inhibition by cell wall-adsorbed serum decreased less than 10%. Since whole cells have additional mass within the cells, it seems logical to expect that adherence inhibition would decrease to a greater extent if specific antisera were preadsorbed with cell walls rather than an equivalent weight of whole cells. However, this was not observed. In addition, Powell has observed recently that strain T14V cell walls neither adhere to saliva-treated HA nor possess the long surface fibrils characteristic of whole cells (J. T. Powell, Ph.D. thesis, University of Florida, Gainesville, 1979).

Cisar and co-workers (8, 9) isolated fibrils from strain T14V cell walls that had been digested with lysozyme. Furthermore, antibody that reacted with these isolated fibrils also reacted with intact fibrils on the outer surfaces of strain T14V

whole cells (9). The Braun procedure for obtaining cell walls removes the cell surface fibrillar material (Powell, Ph.D. thesis). However, it is possible that short fragments remain imbedded in the cell wall matrix and are available for binding antibody directed against the fibrils. Subsequent treatment of the cell walls with lysozyme apparently releases the fibril fragments isolated by Cisar and Vatter (8). Initial adsorption of a cell to a surface is thought to entail a reversible phase, whereby at equilibrium the cell remains approximately 10 nm from the surface, balanced between the repulsive effects of negatively charged surfaces and the attractive influences of Van der Waal's forces (40). Thus, it is possible that the fibril fragments that remain in the cell walls after Braun homogenization are not of sufficient length to bridge this 10-nm gap and permit walls to adhere firmly to saliva-treated HA. Considering the apparent similarity in morphology and antibody reactivity of the surface fibrils and matrix fibrils observed by Cisar et al. (8, 9) and by us, it is not surprising that the amino acid composition of the fibrils isolated by the method described here was similar to the composition obtained by lysozyme digestion of cell walls (Table 3) (8). Collectively, these data suggest that fibrils isolated by shearing whole cells may be representative surface extensions of the entire fibrils, which were isolated from enzyme-digested walls by Cisar and Vatter (8).

Fibrils were also included in bacterial cell-HA mixtures to determine whether strain T14V adherence could be competitively inhibited. Addition of fibrils or crude supernatant, which contains other cell surface components in addition to fibrils, to a suspension of strain T14V cells and saliva-treated HA decreased adherence by as much as 30%. This suggests that purified fibrils compete for the strain T14V binding sites on the saliva-treated HA, thereby blocking cellular adherence at those sites. Fibrils are also thought to mediate cell to cell aggregation of strain T14V cells (Powell, Ph.D. thesis); therefore, fibrils adsorbed to receptor sites on a saliva-treated HA surface could also act as alternate adsorption sites for strain T14V cells. Such alternate sites which mask adsorbed salivary receptor sites could promote a less effective attachment, causing adherence inhibition.

The fibrils appeared as a pure suspension, as judged by transmission electron microscopy. Antigenic homogeneity of the fibrils was shown by immunodiffusion and Laurell rocket immunoelectrophoresis. Therefore, the isolation and purification procedures employed in this study appear to yield an antigenically homogeneous protein product that represents the surface fibrils of

strain T14V. Laurell rocket immunoelectrophoresis further identified a single fibril antigen showing identity with virulence-associated antigen 1 which was previously found in Lancefield extracts of strain T14V by Powell et al. (45). A second antigen in the crude supernatant showed identity with virulence-associated antigen 2 of Powell et al. in the strain T14V Lancefield extract (45). The virulence-associated antigen 2 of Lancefield extracts was previously believed to be a result of the extraction procedure, which altered the virulence-associated antigen to form two antigenic moieties (9). However, this does not appear to be the case since crude supernatants that had not been Lancefield extracted also contained both antigens (data not shown). In addition, Lancefield extraction of fibrils did not alter the immunoelectrophoretic pattern.

Theoretically, local antibodies directed against the bacterial cell surface receptor(s) which promotes binding to the host tissue might be expected to exert some modulating influences on initial adherence and subsequent colonization. For example, cell-associated glucosyltransferase is known to enhance the accumulation of *Streptococcus mutans* in plaque on the teeth of conventional rats (11, 52). It has been shown that immunization with purified glucosyltransferase effectively reduces colonization by *S. mutans* and protects against the subsequent development of dental caries in conventional and gnotobiotic rats (51) and in hamsters (48). Thus, the isolation and identification of surface structures responsible for the adherence of *A. viscosus* strain T14V cells to saliva-treated HA surfaces and the inhibition of strain T14V adherence by antifibril specific antibody suggest that surface fibrils might be effective for inducing immunoprotection by interfering with colonization by this organism. We are currently investigating the possibility of inhibiting colonization by strain T14V in conventional animals by immunization with purified fibrils.

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