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Isolation and Characterization of *Actinomyces viscosus* Mutants Defective in Binding Salivary Proline-Rich Proteins

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Recent studies have provided evidence for human salivary proline-rich proteins (PRPs) serving as potential receptors in the acquired pellicle for *Actinomyces viscosus* type 1 fimbriae. We report here the isolation of mutants derived from *A. viscosus* T14V-J1 which are defective in binding to PRPs partially purified from parotid gland saliva. Mutagenesis with ethyl methanesulfonate preceded enrichment for cells nonreactive with PRPs by successive adsorptions with PRP-treated latex beads. Screening was accomplished by random selection of 250 isolated colonies from each of four enrichment cycles and reaction with PRP-treated latex beads in microtiter plates. Two mutants of independent origin were examined for adherence to hydroxyapatite treated with either PRPs, proline-rich glycoproteins, deglycosylated proline-rich glycoproteins, or whole saliva. Additional surface properties that were examined included agglutination with polyclonal antisera to type 1 and type 2 fimbriae, agglutination by a monoclonal antibody to type 1 fimbriae that inhibits adherence of the parent strain to saliva-treated hydroxyapatite, the ability to bind monoclonal antibody to the type 1 fimbrial subunit, and lactose-reversible coaggregation with *Streptococcus sanguis* 34. Both mutants exhibited reduced binding to hydroxyapatite treated with whole saliva or salivary protein preparations but were still capable of reaction with antiserum to type 1 and type 2 fimbriae. In addition, these mutants possessed the ability to bind monoclonal antibody to the type 1 fimbrial subunit in amounts comparable to the amount bound by the parent strain but were not agglutinated by the adherence-inhibiting monoclonal antibody. When considered with previously published data, these results suggest that an adhesive molecule is probably associated with type 1 fimbriae and allows for the interaction of *A. viscosus* with constituents in the salivary pellicle.

Actinomyces viscosus is a primary colonizer of human tooth surfaces and consequently is of central importance in dental plaque development. *A. viscosus* may be a significant factor in gingivitis (26) and periodontitis (19, 40). One of the two antigenically and functionally distinct fimbrial types present on the surface of this bacterium, designated type 1, has been strongly implicated as having an essential role in the attachment of this organism to saliva-treated surfaces (10, 11). Unlike the type 2 fimbriae of this organism, for which investigations have revealed a conventional lectin-carbohydrate-like interaction between both these surface structures and receptors of certain streptococci (4) and neuraminidase-treated erythrocytes (12), detailed studies at the molecular level of type 1 fimbrial interaction with specific salivary receptors are few.

Gibbons et al. (14, 15) have implicated acidic proline-rich proteins (PRPs) derived from parotid or submandibular saliva as promoting the attachment of *A. viscosus* to apatitic surfaces. In addition, recent findings from our laboratory (22), obtained by utilizing colloidal gold-labeled PRPs as probes in conjunction with fimbria-deficient mutants of *A. viscosus* (7), lend further support to the proposed functional role of PRP binding to type 1 fimbriae. Clark et al. (9) have confirmed and extended these observations by using polystyrene as well as hydroxyapatite (HA) surfaces to examine the effect of various salivary proteins, including proline-rich glycoproteins (PRG), on the adsorptive behavior of *A. viscosus* T14V-J1 and fimbria-deficient mutants of this strain and have shown that rabbit polyclonal fimbria-specific anti-type 1 immunoglobulin G and Fab fragments inhibit adsorption of type 1-positive actinomyces to HA treated with PRPs and PRG. Recently, however, Cisar et al. (3) have reported that a panel of monoclonal antibodies to the type 1 fimbriae, which also react with the cloned subunit, failed to inhibit attachment of *A. viscosus* T14V to saliva-treated HA (SHA) when tested in the adherence assay. One possibility to account for these results would provide for a fimbria-associated molecule that is essential for adherence but is genetically and antigenically distinct from the fimbrial subunit.

In a further attempt to examine the molecular nature of the binding of type 1 fimbriae of *A. viscosus* to PRPs, we have developed a method for the isolation of mutants of *A. viscosus* T14V-J1 which possess type 1 fimbriae and yet are deficient in their ability to bind to pellicles of partially purified acidic PRPs. The results provide additional evidence for the hypothesis that *A. viscosus* possesses an adhesin that functions in association with type 1 fimbriae to mediate cell adsorption on salivary pellicles, but the adhesin may be genetically distinct from the fimbriae.

MATERIALS AND METHODS

Bacterial strains. *A. viscosus* T14V-J1, a streptomycin-resistant isolate of strain T14V, was originally isolated as previously described (39). This strain, which possesses both type 1 and type 2 fimbriae, was obtained from the culture collection at the University of Florida Periodontal Disease Research Center (College of Dentistry, University of Florida, Gainesville). Fimbria-deficient mutant strain 147, lacking both type 1 and type 2 fimbriae, and strain 5951, lacking only type 1 fimbriae, were derived from *A. viscosus* T14V-J1 as recently described (7) and were kindly provided by J. O.

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Cisar, National Institute of Dental Research. *Streptococcus sanguis* 34 was also provided by J. O. Cisar. All strains were maintained as multiple frozen stocks in tryptic soy broth (TSB) (BBL, Cockeysville, Md.) containing 20% glycerol at −80°C. Unless otherwise indicated, all strains were grown aerobically in TSB overnight at 37°C. Defibrinated sheep blood-supplemented agar (Difco Laboratories, Detroit, Mich.) was used as the solid medium throughout this study. Antibiotic resistance was confirmed on Columbia CNA agar (BBL) containing 100 μg of streptomycin per ml.

Preparation of saliva and purification of saliva-derived molecules. Salivary acidic PRPs were partially purified as described recently (21). PRG and deglycosylated PRG (dPRG) were also prepared by procedures described previously (24, 25, 28) and were generously provided by M. J. Levine, State University of New York, Buffalo. PRP-1 was kindly supplied by D. I. Hay, Forsyth, Boston, Mass. Whole saliva was collected in chilled containers and clarified by centrifugation at 12,000 × *g* for 10 min prior to treatment of the HA beads.

Latex bead preparation. Preparation of latex beads for enrichment was performed as described by Clark et al. (9). Briefly, latex beads (particle diameter, 15.7 μm) (Sigma Chemical Co., St. Louis, Mo.) were diluted 1:50 in the carbonate-bicarbonate buffer (pH 9.6) of Voller and Bidwell (36), washed three times, and suspended to an optical density of 1.0 (A_{650}) in the same buffer. The beads were divided into four aliquots and pelleted, and the supernatants were removed. Ten micrograms of PRPs (1 mg/ml) in the same buffer was added to each tube of beads and gently mixed for 2 h at room temperature prior to overnight storage at 4°C. Following one wash of the beads in adsorption buffer (50 mM KCl, 1 mM K_2PO_4, 1 mM $CaCl_2$, 0.1 mM $MgCl_2$), blocking buffer (adsorption buffer with 2 mg of bovine serum albumin [BSA] per ml) (Sigma) was added for 15 min to block unoccupied sites on the beads. Each aliquot of the bead suspension was transferred to separate 1.5-ml microcentrifuge tubes. The bead suspension was pelleted, and the supernatant was removed prior to addition of the bacterial suspension.

Ethyl methanesulfonate mutagenesis. An overnight culture of *A. viscosus* T14V-J1 was diluted 1:20 into fresh TSB and allowed to grow to mid-log phase. Ethyl methanesulfonate (Sigma) was added to 0.15 M, and the culture was vortexed briefly and incubated further for 1 h at 37°C. This time of exposure was long enough to allow for 10% survival. Cells were recovered by centrifugation and washed twice in phosphate-buffered saline (PBS), pH 7.0. The pellet was resuspended to 20 times the original volume in fresh TSB and grown overnight at 37°C to allow for expression of the mutation. Ten milliliters of the culture was removed, centrifuged, and washed twice in adsorption buffer. The cells were finally suspended to 5×10^8 to 5×10^9 CFU/ml in the same buffer prior to addition to the first tube of PRP-treated latex beads (PRP-LB).

Enrichment and screening. Enrichment proceeded by the addition of 1 ml of the cell suspension described above to a tube of PRP-LB. Sterility was maintained throughout the entire procedure. The tube was allowed to rotate at 4°C for 1 h, and the bead-cell suspension was pelleted for 15 s in a microcentrifuge. The beads were discarded following removal of the supernatant to a fresh tube of PRP-LB, and the enrichment cycle was repeated three times. Prior to each addition of the cell suspension to a fresh tube containing the latex bead preparation, 100 μl of the supernatant was removed, diluted in PBS, and plated on blood agar. All plates were incubated aerobically at 37°C for 72 h.

Following incubation of the plates, 250 colonies were randomly selected from each of the four enrichment passes, together with 250 preenrichment colonies, with sterile toothpicks. Each colony was separately mixed with 30 μl of adsorption buffer in the individual wells of round-bottom microtiter test plates (Dynatech Laboratories, Inc., Alexandria, Va.) until the colony was thoroughly suspended. Fifteen microliters of this suspension was removed to the corresponding well of a master plate containing an equal volume of 30% glycerol. Following transfer of all colonies, each master plate was immediately stored at −80°C. The latex bead preparation described above for enrichment was adjusted to 1.0 μg of PRPs per ml for screening, and 100 μl was added to each well of the test plate. The entire plate was allowed to mix vigorously on a rotary shaker for 2 min. The following scale was used to score for the presence of aggregates: 0, no aggregation; 1, aggregation visible by microscope only; 2, definite aggregation visible by eye; 3, well-defined aggregates with some turbidity; 4, large aggregates with a clear supernatant and no remaining turbidity. The presumptive isolates were purified twice on blood agar following their removal from the corresponding master plate.

Adsorption of Adh$^-$ mutants to HA. The adsorption of [^{3}H]thymidine-labeled cells to SHA and HA treated with various salivary protein preparations was performed as described elsewhere (8, 9).

Agglutination of Adh$^-$ mutants by polyclonal and monoclonal antibody to type 1 fimbriae and by polyclonal antibody to type 2 fimbriae. Bacterial agglutination assays were performed as previously described (2). Monospecific rabbit antisera to *A. viscosus* T14V type 1 and type 2 fimbriae were prepared according to procedures described elsewhere (5, 10). A monoclonal antibody, 8B, to the subunit of type 1 fimbriae has been recently described (3). The monoclonal antibody-secreting line, 86-49E, has been described (33). Briefly, bacteria were harvested, washed twice in coaggregation buffer (20 mM Tris-HCl [pH 7.8], 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 150 mM NaCl), and suspended to 10^9 cells per ml in the same buffer. A 25-μl portion of the cell suspension was added to the wells of polyvinyl V-bottom microtiter plates (Dynatech) to which 25 μl of appropriately diluted antibody to either type 1 or type 2 fimbriae within a concentration range of 0.01 to 100 μg/ml had been added. Buffer controls were included for each experiment. The plate was mixed for 30 s on a rotary shaker, incubated at room temperature for 2 h, and then stored overnight at 4°C. Scoring for aggregation was determined as described in Table 2, footnote *b*.

Coaggregation assay. The coaggregation assay was performed as described previously (6). Cells were grown overnight in medium containing 5 g of yeast extract (Difco), 5 g of tryptone (Difco), 5 g of K_2HPO_4, 0.5 ml of Tween 80, and 1 g of glucose per liter for actinomyces or 5 g of glucose per liter for *S. sanguis* 34. The cells were washed twice in coaggregation buffer and suspended to an A_{600} of 1.5 (220 Klett units). Equal volumes (0.2 ml) of *S. sanguis* 34 and the strain to be tested were mixed in test tubes (10 by 75 mm), vortexed for 15 s, and incubated at room temperature for 15 min or overnight at 4°C. Scoring for coaggregates was determined as described in Table 2, footnote *b*. Controls for self-aggregation were similarly scored. All assays were performed in duplicate. Lactose (up to 0.1 M) (Sigma) was added to all reactions positive for coaggregation. Following

the addition of lactose, the tubes were shaken gently for 15 s and scored as described above.

ELISA. An indirect enzyme-linked immunosorbent assay (ELISA) modified from a technique described by Rennard et al. (35) was used to estimate the relative amounts of type 1 fimbriae on the surfaces of the mutants.

Flat-bottom enzyme immunoassay plates (Flow Labs, McLean, Va.) were prepared by coating the wells with 3.0 μg of purified type 1 fimbriae per ml (10) in carbonate-bicarbonate buffer, pH 9.6, overnight at room temperature. The wells were washed with PBS and then blocked with PBS containing 1% BSA and 0.05% Tween 20 (PBT). The wells were washed again with PBT prior to use.

Broth cultures of each strain to be tested were grown in TSB overnight, washed twice with PBS, and then adjusted to a concentration of 10^{10} CFU/ml in PBT. Twofold dilutions of each of the cell suspensions were made, and 50 ng of monoclonal antibody 8B to the subunit of type 1 fimbriae was added to each tube. Similarly, a standard curve was prepared with purified type 1 fimbriae within a concentration range of 3 to 0.01 μg/ml, to which 50 ng of the monoclonal antibody was added. The final volume of all reaction mixtures was 200 μl. All tubes were incubated at room temperature for 2 h, and cell-antibody or fimbria-antibody complexes were precipitated by centrifugation for 10 min at 18,000 × *g*. The supernatants containing soluble antibody were decanted, and 50 μl of this antibody was added to each of the plate wells. Positive controls consisted of fimbria-coated wells to which the antibody, in a concentration range of 0.25 to 0.002 μg/ml, was added.

The plates were allowed to incubate at room temperature for 1 h and then were washed extensively with PBT. Affinity-purified horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1,000-fold in PBT was added to each well of the plate and allowed to incubate for 1 h. Negative controls consisted of the addition of antibody conjugate to fimbria-coated wells. Following washing, bound conjugate was detected by the addition of *O*-phenylenediamine in 0.1 M citrate buffer, pH 4.5, according to the instructions of the manufacturer (Sigma). Chromophore generation was measured with the Titertek Multiskan MCC (Flow Labs) at 450 nm.

Values from the linear portion of the standard curve obtained with type 1 fimbriae were analyzed by least-squares regression analysis. The amount of type 1 fimbriae present on each of the strains was estimated, relative to the equivalent absorbance values obtained with type 1 fimbriae. The mean values calculated for each strain represent the results of at least three independent determinations.

Statistical analyses. The Duncan multiple-range test was used to determine the statistical significance between the parent strain and the mutants for the various parameters tested.

RESULTS

Isolation of Adh$^-$ mutants. Mutants nonreactive with PRP-LB were isolated following specific enrichment with these preparations. One stable mutant from the third enrichment, GB018, was isolated upon random selection of 250 isolated colonies from each of four cycles on blood agar plates. In a separate experiment, screening of an equal number of colonies yielded one isolate, GB007, also from the third enrichment cycle. Presumptive isolates from other enrichment cycles proved to be unstable. No mutants were isolated from colonies screened prior to enrichment. A score of 0 was assigned to isolates displaying no aggregation with PRP-LB, and a score of +4 was assigned to isolates yielding maximum aggregation. Isolates which failed to aggregate with PRP-LB were purified twice on blood agar plates and retested in the assay. Those isolates which repeatedly failed to aggregate PRP-LB following purification were chosen for further study. The mutants isolated in this study are of independent origin on the basis of their isolation from separate enrichment experiments.

TABLE 1. Adsorption of Adh$^-$ *A. viscosus* strains to HA treated with selected salivary proteins

Bacterial strain[a]	% Adsorption to HA (mean ± SE) treated with[b]:			
	PRP	PRG	dPRG	Whole saliva
T14V-J1	61.2 ± 2.3	26.9 ± 1.2	39.4 ± 7.7	66.2 ± 8.5
5951	0	0	0	16.6 ± 9.6
147	0	0	9.2 ± 6.9	18.2 ± 15.3
GB007	9.9 ± 2.4	7.1 ± 3.6	10.3 ± 2.3	30.9 ± 6.0
GB018	0 ± 0.9	1.9 ± 2.4	6.5 ± 5.5	27.7 ± 2.6

[a] Values for adsorption of strains T14V-J1, 5951, and 147 have been reported previously (9).

[b] Percentage of available cells (4×10^6 cells per ml) adsorbed to 5 mg of HA treated with 10 μg of the respective protein per ml. Each mean and standard error was calculated from the results of six independent determinations. Adsorption to salivary pellicles was compared among strains by Duncan's multiple-range test.

Adsorption of Adh$^-$ mutants to HA treated with saliva preparations. Binding of strains T14V-J1, 5951, 147, and the Adh$^-$ mutants to HA treated with various saliva preparations, as well as whole paraffin-stimulated saliva, is shown in Table 1. Adsorption of the Adh$^-$ mutants GB007 and GB018 to each of the experimental pellicles composed of salivary PRPs—PRP, PRG, and dPRG—was negligible (range, 0 to 10%), and adsorption to pellicles composed of whole saliva was only moderate (range, 28 to 31%); as measured by the Duncan multiple-range test, adsorption of the mutants was statistically significantly lower than that of the parent strain to these pellicles. The parent strain, T14V-J1, adsorbed well to HA treated with whole saliva (66%) and to PRP-treated HA (61%). Adsorption of the parent strain to dPRG (39%) was lower than that to whole saliva or PRP but was lowest for PRG-treated HA (27%). The fimbria-deficient mutant (strain 147) and strain 5951, like the Adh$^-$ mutants, adsorbed poorly to all pellicles (range, 0 to 18%).

Phenotypic characterization of Adh$^-$ mutants. The properties of the mutants isolated in this study were compared with those of the parent and fimbria-deficient strains. No differences in fermentation characteristics were noted when the parent strain, 5951, and fimbria-deficient strain 147 were compared with each of the mutants (34).

The results shown in Table 2 reveal that both of the Adh$^-$ mutants were capable of being agglutinated with both polyclonal antibody to type 1 fimbriae and monoclonal antibody to the type 1 fimbrial subunit, suggesting that the fimbrial structure was not significantly altered by the mutagenic treatment. Both mutants were also capable of being agglutinated with anti-type 2 antiserum, as well as able to coaggregate with *S. sanguis* 34. Neither of these mutants, however, was capable of being agglutinated by the adherence-inhibiting monoclonal antibody, 86-49E, even when concentrations as high as 10 μg of the antibody per ml were used. It should be noted that as little as 5 μg of this adherence-inhibiting monoclonal antibody per ml will inhibit adherence of the

TABLE 2. Phenotypic properties of Adh$^-$ *A. viscosus* strains

Bacterial strain	Agglutinating concn (μg/ml) of antifimbrial antibody[a]				Coaggregation[b]
	Type 1	Type 2	8B[c]	86-49E[d]	
T14V-J1	0.08	0.15	<0.01	<0.01	3
5951	>10	0.04	>10	>10	3
147	>10	>10	>10	>10	0
GB007	0.04	<0.01	<0.01	>10	3
GB018	0.08	<0.01	<0.01	>10	2

[a] Lowest antibody concentration necessary to achieve agglutination.
[b] Coaggregation with *S. sanguis* 34, expressed on a scale of 0 for no coaggregation to 3 for maximum coaggregation. All reactions positive for coaggregation were reversed by the addition of 0.1 M lactose.
[c] Monoclonal antibody to the type 1 fimbria subunit.
[d] Adherence-inhibiting monoclonal antibody.

parent strain to SHA by 75%, whereas 100 μg of monoclonal antibody 8B per ml was unable to cause significant inhibition of adherence in this assay (3).

On the other hand, the parent strain was readily agglutinated by 86-49E, suggesting that the adhesive component remains functional. We confirmed the results of Cisar et al. (7) that both strains 5951 and 147 lack type 1 fimbriae and that strain 147 lacks type 2 fimbriae as well as the ability to coaggregate with *S. sanguis* 34. Neither of these fimbria-deficient mutants was agglutinated by the adherence-inhibiting monoclonal antibody.

Binding of type 1 subunit monoclonal antibody to mutants. The ability of the parent strain, T14V-J1, and the Adh$^-$ mutants to bind monoclonal antibody to the subunit of type 1 fimbriae was examined to estimate the amount of this fimbrial type present on these strains. The equivalent amount of antibody bound by each of these strains was calculated relative to the amount of antibody bound by purified type 1 fimbriae. The parent strain, T14V-J1, was estimated to have 1.37 μg of type 1 fimbriae per 10^9 cells; strains GB007 and GB018 were estimated to possess 0.76 and 0.90 μg of type 1 fimbriae per 10^9 cells, respectively. The amounts for the parent and mutant strains were not significantly different when compared by the Duncan multiple-range test. No fimbriae were detected on fimbria-deficient strains 5951 and 147 by this assay.

DISCUSSION

Successful colonization of the oral cavity often requires that a bacterium attach directly to host soft or hard tissue surfaces or to other organisms with the potential to interact with specific host components (17). To begin to understand the molecular basis for colonization, it is necessary to identify the molecules present on the surface of organisms that allow for attachment to host-derived molecules. Further, those organisms capable of intergeneric coaggregation may have an additional means of establishing themselves in particular oral microenvironments (17).

Although evidence from numerous studies has suggested that type 1 fimbriae are essential in the adherence of *A. viscosus* to SHA (7, 10, 11), the nature of the receptor-binding domain(s) of the fimbriae has not been elucidated. In the present study, we have employed mutant analysis in an initial effort to characterize the adhesins associated with these fimbriae that permit the interaction of this bacterium with adsorbed salivary acidic PRPs. Utilizing this approach, we were able to select for mutants that lacked the ability to bind to PRPs. The isolation of such mutants of *A. viscosus* T14V-J1 that no longer bind to PRP-treated surfaces but retain fimbriae and reactivity both with a monoclonal antibody against the type 1 fimbrial subunit and an adherence-inhibiting monoclonal antibody supports the hypothesis that the functional activity of *A. viscosus* type 1 fimbriae may depend on a minor and as-yet-unidentified protein, as has been suggested for adherence of *Escherichia coli* to epithelial cells (30, 31). Considering that information concerning the genetics of *A. viscosus* is limited, this is a significant observation. Weiss and coworkers (37, 38) have also lacked a suitable means of genetic analysis in their studies of the adhesins of *Bacteroides loeschii* that mediate coaggregation of this organism with oral bacteria.

Previous studies in our laboratory have made use of latex beads treated with purified PRPs and other salivary proteins to examine the specificity of attachment of *A. viscosus* type 1 fimbriae to salivary pellicle receptors (9, 15). In the present study, we have modified this procedure to allow for the selection of nonadhering mutants by successive adsorptions of wild-type cells with PRP-LB from a population of cells exposed to the chemical mutagen ethyl methanesulfonate. This strategy proved to be effective when equal numbers of colonies from each enrichment stage were examined. Two stable mutants, GB007 and GB018, were selected from the third enrichment cycle in separate experiments. Enrichment schemes similar to the one reported here have been used to isolate mutants of actinomyces and streptococcal strains which are defective in their ability to coaggregate with the corresponding partner by making use of the respective wild-type partner to remove predominantly nonmutant cells from the suspension (20). Similarly, Weiss and colleagues (37) have used this method to isolate coaggregation-defective mutants of *B. loeschii*. Murchison et al. (32) have isolated *Streptococcus mutans* mutants defective in adherence and aggregation properties by using glass as the adsorptive surface. In addition, Fives-Taylor and Thompson (13) were able to isolate *S. sanguis* mutants defective in adherence to SHA.

Confirmation of the inability to bind to adsorbed PRPs was obtained following the initial screening for presumptive mutants by latex bead agglutination and was provided by an examination of these isolates for their abilities to bind to pellicles of PRPs, PRG, dPRGs, or whole saliva that had been adsorbed onto HA. Adherence of mutants to pellicles composed solely of PRPs was significantly less than that of the parent strain, T14V-J1. Moreover, although the selection for these mutants was carried out on the basis of their reactivities with PRPs, pellicles composed of PRG, dPRGs, and whole saliva also failed to promote wild-type levels of adherence. One possible explanation for these results would be the existence of other mutations affecting domains responsible for attachment to PRG or dPRG. A more likely explanation would allow for the existence of binding sites with sequences common to both PRPs and PRG for the adhesin(s). The latter explanation is probable, given the proposed common ancestry postulated for salivary PRPs (1) and the apparent amino acid sequence homology between PRG and PRPs (9). It was previously observed that strains which lack type 1 fimbriae—strain 5951, which possesses only type 2 fimbriae, and strain 455-2, which lacks both fimbrial types—adsorbed less well to SHA than did the Adh$^-$ mutants (7).

To determine the extent to which the type 1 fimbriae of these mutants had been altered by the mutagenic treatment, it was necessary to examine their degree of fimbriation. Both

strains GB007 and GB018 bound a significant fraction of the available fimbrial subunit antibody, 8B. Further evidence for the presence of fimbriae on these mutants is shown by their ability to agglutinate with monospecific antiserum to type 1 fimbriae. When tested for other phenotypic characteristics, these mutants did not exhibit any significant difference in cell surface structure from that of the parent strain.

Recently, our laboratory produced another monoclonal antibody to the type 1 fimbriae that has the ability to inhibit the adherence of the parent strain to SHA (33). Further, this antibody was capable of agglutinating both the parent and strain 55-19, which bear type 1 fimbriae, but not strain 5951 or 147, both of which lack these fimbriae (Table 2). Neither of the Adh^- mutants was agglutinated by this monoclonal antibody, although they possess amounts of type 1 fimbriae comparable to the amount possessed by the parent. Electron microscopy together with immunogold labeling revealed that only cells possessing type 1 fimbriae appeared to be labeled with the antibody at a discrete location, whereas the monoclonal antibody to the subunit of type 1 fimbriae bound extensively along the length of the fimbriae (23). Together, this evidence further suggests that the adhesive protein(s) may represent only a minor component of the fimbriae.

Intergeneric coaggregation of *A. viscosus* with other oral bacteria has been proposed as an important mechanism for the colonization of this organism in vivo (16, 17, 29) and has been shown to be dependent on the presence of type 2 fimbriae (5). Both mutants reacted with antiserum to type 2 fimbriae and were capable of lactose-reversible coaggregation with *S. sanguis* 34.

Recent evidence from our laboratory (22), obtained by utilizing PRP-gold probes to examine the binding of these complexes to *A. viscosus* type 1 fimbriae, has suggested single-site binding on these fimbriae for the binding of salivary PRPs. In addition, evidence from other studies involving gram-negative organisms suggests that functional epitopes of fimbria-bearing bacteria may be encoded separately from those responsible for subunit synthesis or assembly. Minion et al. (30), in a study of *E. coli* type 1 fimbriae, demonstrated by deletion mutation analysis of a recombinant plasmid containing the genetic information for fimbrial synthesis and assembly that adhesive functions of these fimbriae are presumably encoded by a gene that does not participate in these functions. In a recent study, Hanson and Brinton (18) extended this observation for the mannose-sensitive type 1 pili of *E. coli* by the identification of a receptor-binding adhesin that appears to be localized at the tips of the fimbriae. In another study of the S fimbriae of *E. coli*, Moch et al. (31) were able to dissociate the fimbriae from an adhesin-enriched fraction. The purified adhesins, not the fimbriae, possessed biological activity in agglutinating human and bovine erythrocytes. London and Allen (27) have also observed that purified adhesins of *B. loeschii* had activity in agglutinating *S. sanguis* as well as neuraminidase-treated erythrocytes.

We have been able to isolate nonadherent mutants of *A. viscosus* by using the putative salivary receptor proteins as a means of selection. Future studies will make use of these mutants, in conjunction with other methodologies, to further define the immunoreactive and receptor-binding regions associated with *A. viscosus* type 1 fimbriae.

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