INFECTION AND IMMUNITY, Nov. 1988, p. 2990–2993
0019-9567/88/112990-04$02.00/0

Vol. 56, No. 11

# NOTES

# Adsorbed Salivary Proline-Rich Protein 1 and Statherin: Receptors for Type 1 Fimbriae of *Actinomyces viscosus* T14V-J1 on Apatitic Surfaces

R. J. GIBBONS,[1]* D. I. HAY,[1] J. O. CISAR,[2] AND W. B. CLARK[3]

*Forsyth Dental Center, Boston, Massachusetts 02115[1]; National Institute of Dental Research, Bethesda, Maryland 20892[2]; and University of Florida, Gainesville, Florida 32610[3]*

Received 16 May 1988/Accepted 8 August 1988

**Use of specific fimbria-defective mutants derived from *Actinomyces viscosus* T14 has shown that salivary acidic proline-rich protein 1 and statherin serve as receptors for type 1 fimbriae which mediate attachment of the organism to experimental pellicles on apatitic surfaces.**

*Actinomyces viscosus* is prominent in human supra- and subgingival dental plaques (8, 10). This filamentous bacterium preferentially colonizes the teeth, and it appears to actually require the presence of teeth in order to colonize the mouth, since it is usually not detected in the oral cavities of predentate infants (8). In adults, elevated proportions of *A. viscosus* have been associated with gingivitis (18, 25) and root surface decay (9).

Typical strains of *A. viscosus* possess two types of fimbriae, which can be distinguished antigenically and functionally. Type 2 fimbriae are associated with a galactose- and *N*-acetylgalactosamine-specific lectin that mediates attachment to receptors on certain bacteria and mammalian cells (2, 3, 21). On the other hand, type 1 fimbriae mediate attachment of *A. viscosus* cells to salivary pellicles formed on apatitic surfaces similar to those of teeth (4, 5, 6, 26). Interactions involving type 1 fimbriae are not inhibited by galactosides or by other sugars studied.

In contrast to *A. viscosus*, typical strains of *Actinomyces naeslundii* frequently contain only type 2 fimbriae (3) and they attach relatively poorly to salivary pellicles on mineral surfaces (5, 7). Recent studies have shown that attachment of *A. viscosus* cells to apatitic surfaces is greatly promoted by adsorbed salivary proline-rich proteins (PRP) and by the protein statherin; other components of saliva are ineffective (13). Adsorption of *A. viscosus* cells to experimental pellicles prepared from pure PRP-1 is not inhibited by lactose, which suggests that type 1 rather than type 2 fimbriae are responsible. Furthermore, adsorbed PRP molecules do not promote the attachment of *A. naeslundii* cells which possess only type 2 fimbriae (13). Recently, mutants of *A. viscosus* which are defective in the production of either type 1 or type 2 fimbriae have been obtained (4). The purpose of the present investigation was to compare the adhesion of these specific fimbria-defective mutants to experimental pellicles formed from pure salivary PRP or statherin.

Parental *A. viscosus* T14V-J1 ($1^+2^+$) possesses type 1 and type 2 fimbriae. The isolation of fimbria-defective mutants from this strain has been described (4). Mutant 5519 ($1^+2^-$) is defective in the synthesis of type 2 fimbriae, while mutant 5951 ($1^-2^+$) is defective in the synthesis of type 1 fimbriae. Mutant 147 ($1^-2^-$) is defective in both types of fimbriae (4). The organisms were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10 μCi of [$^3$H]thymidine (New England Nuclear Corp., Boston, Mass.) per ml at 35°C in Brewer jars filled with 80% $N_2$, 10% $H_2$, and 10% $CO_2$ as previously described (11). Cells from overnight cultures were washed three times and suspended in 0.05 M KCl containing 1 mM phosphate (pH 6.0), 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ (buffered KCl) supplemented with 5 mg of bovine serum albumin (BSA) per ml (12).

Samples of submandibular saliva were obtained by using a custom-made collecting device. Acid candy was used to stimulate salivary flow, and samples were immediately dialyzed against 0.1 M $NH_4HCO_3$ (pH 8.0) buffer which contained 0.5% $CHCl_3$ at 4°C overnight (13). The saliva was then fractionated on columns of Trisacryl GF 2000 (LKB, Bromma, Sweden) at 4°C and eluted with 0.1 M $NH_4HCO_3$ buffer as previously described (13). Experimental pellicles were prepared by exposing 5 mg of spheroidal hydroxyapatite (HA) beads (BDH Chemicals, Gallard-Schlesinger Chemical Corp., Carle Place, N.Y.) to 125 μl of unfractionated saliva or to each column fraction for 1 h. The beads were then washed twice with buffered KCl and treated for 30 min with buffered KCl-BSA to block any uncoated regions of the HA. After being washed, the beads were incubated with $6.25 \times 10^6$ [$^3$H]thymidine-labeled actinomyces cells suspended in 125 μl of buffered KCl-BSA. After incubation with continuous rotation for 1 h at room temperature, the beads were washed three times and the number of actinomyces cells which attached was determined by scintillation counting as previously described (11, 12).

Parental strain T14V-J1 ($1^+2^+$) and mutant 5519 ($1^+2^-$) attached in significantly higher numbers to HA treated with unfractionated submandibular saliva than did mutant 5951 ($1^-2^+$) or 147 ($1^-2^-$) (Table 1). These observations are consistent with previous studies (4–7). Treatment of HA with chromatographic fractions of submandibular saliva produced two broad peaks of adhesion-promoting activity for

* Corresponding author.

TABLE 1. Adsorption of parental and fimbria-defective mutant strains of *A. viscosus* to Experimental Pellicles on HA[a]

| Pellicle source | No. ($10^6$) (mean ± SE) of *A. viscosus* cells adsorbed per 5 mg of HA | | | |
|---|---|---|---|---|
| | Strain T14V-J1 ($1^+2^+$) | Strain 5519 ($1^+2^-$) | Strain 5951 ($1^-2^+$) | Strain 147 ($1^-2^-$) |
| Buffer | 0.63 ± 0.01 | 0.86 ± 0.01 | 0.22 ± 0.05 | 0.14 ± 0.02 |
| Submandibular saliva | 5.19 ± 0.03 | 4.64 ± 0.03 | 2.81 ± 0.23 | 1.15 ± 0.13 |
| PRP-1 (100 μg/ml) | 5.44 ± 0.19 | 4.96 ± 0.23 | 0.33 ± 0.05 | 0.45 ± 0.04 |
| PRP-1 (20 μg/ml) | 5.19 ± 0.16 | 5.02 ± 0.06 | 0.39 ± 0.07 | 0.27 ± 0.01 |
| Statherin (200 μg/ml) | 5.56 ± 0.14 | 5.18 ± 0.33 | 0.94 ± 0.08 | 0.05 ± 0.01 |
| Statherin (20 μg/ml) | 1.32 ± 0.24 | 1.37 ± 0.06 | 0.10 ± 0.04 | 0.38 ± 0.01 |

[a] Reaction mixtures (125 μl) contained 5 mg of HA beads and $6.25 \times 10^6$ $^3$H-labeled bacteria in buffered KCl-BSA.

parental strain T14V-J1 ($1^+2^+$) (data not shown) and mutant strain 5519 ($1^+2^-$) (Fig. 1). These two salivary fractions are known to include the acidic PRPs and the protein statherin, respectively (13). The adhesion of strain 5951 ($1^-2^+$) was not strongly promoted by any salivary fraction (Fig. 1).

The adhesion of parental strain T14V-J1 ($1^+2^+$) and the fimbria-defective mutants was determined by using HA beads treated with pure PRP-1 or statherin (Table 1). Samples of these proteins were prepared from human saliva as previously described (13, 22). Their purity was assessed by polyacrylamide gel electrophoresis, by high-performance liquid chromatography, and by sequencing (22–24, 27, 28). Pellicles prepared from 20-μg/ml solutions of pure PRP-1 were as effective as unfractionated submandibular saliva in promoting the adhesion of strain T14V-J1 ($1^+2^+$) and mutant 5519 ($1^+2^-$) (Table 1). Pellicles formed from high concentrations of statherin (200 μg/ml) also promoted good adhesion of these strains. In contrast, the adhesion of mutant 5951 ($1^-2^+$) or 147 ($1^-2^-$) was not promoted by either adsorbed PRP-1 or statherin (Table 1). The presence of 0.05 M lactose did not significantly inhibit adhesion of strain T14V-J1 or 5519 to any of the pellicles studied (data not shown). These observations are consistent with previous reports that have shown that the type 1 fimbriae of *A. viscosus* cells are primarily responsible for mediating attachment to unmodified salivary pellicles on HA surfaces (4–7). Our data further indicate that the type 1 fimbriae of *A. viscosus* cells interact with adsorbed PRP-1 and statherin molecules on HA surfaces.

The ability of *A. viscosus* T14V-J1 and its fimbria-defective mutants to bind to adsorbed PRP-1 was also studied by using polystyrene latex beads (15.7-μm diameter, LB16; Sigma Chemical Co., St. Louis, Mo.). Suspensions (0.5%) of the beads were incubated with the 50-μg/ml solutions of the protein in 0.05 M carbonate buffer at pH 9.5. For comparison, beads were also coated with BSA or asialofetuin prepared by mild acid hydrolysis from fetuin (Sigma). The beads were washed twice and suspended in buffer containing 2 mg of BSA per ml. Samples (100 μl) of the protein-coated beads were mixed with $5 \times 10^6$ bacteria in microdilution plates on a rotary shaker and then examined for agglutination.

Parental strain T14V-J1 agglutinated latex beads coated with either PRP-1 or asialofetuin, but not beads coated with BSA (Fig. 2). Mutant 5519 ($1^+2^-$) agglutinated only beads coated with PRP-1, while mutant 5951 ($1^-2^+$) agglutinated only beads coated with asialofetuin. Mutant 147 failed to agglutinate any of the beads tested (Fig. 2). These observations further substantiate that type 1 fimbriae bind to adsorbed PRP-1 molecules, whereas type 2 fimbriae do not.

PRP-1 and statherin are nonglycosylated proteins (15, 22–24, 27, 28), and the fimbriae of *A. viscosus* cells have also

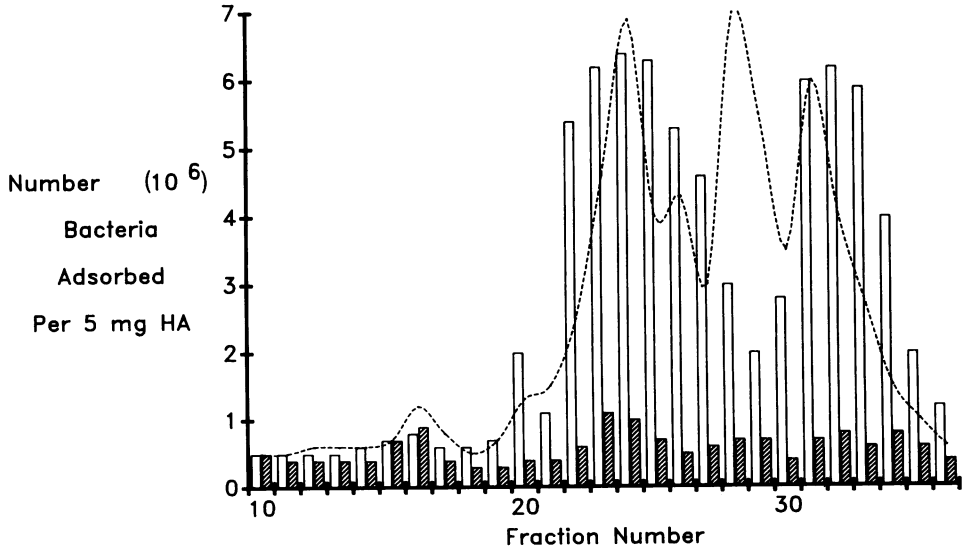


FIG. 1. Attachment of *A. viscosus* 5519 ($1^+2^-$) and 5951 ($1^-2^+$) to HA treated with submandibular saliva fractions obtained by chromatography on columns of Trisacryl GF-2000. Symbols: ----, absorption of the fractions at 220 nm; □, mean number of 5519 ($1^+2^-$) cells which attached; ▨, mean number of 5951 ($1^-2^+$) cells which attached.

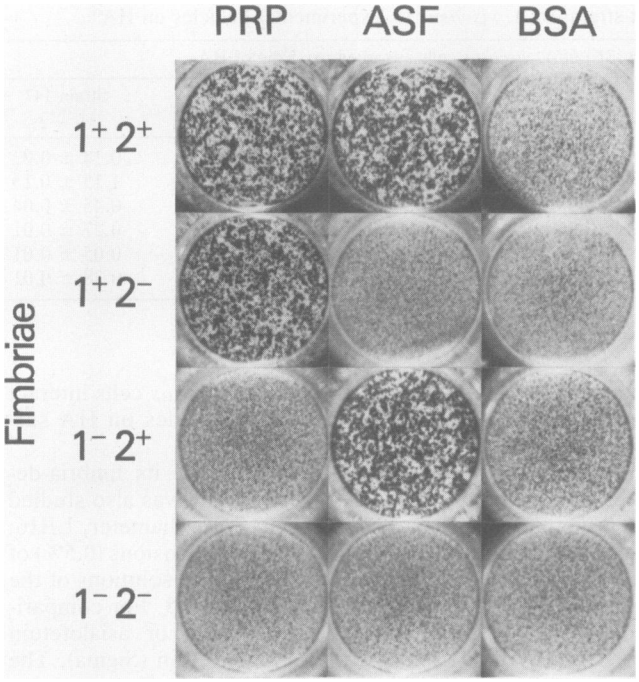


FIG. 2. Aggregation of latex beads coated with PRP-1 (PRP), asialofetuin (ASF), and BSA by cells of parental *A. viscosus* T14V-J1 ($1^+2^+$) and fimbria-defective strains 5519 ($1^+2^-$), 5951 ($1^-2^+$), and 147 ($1^-2^-$). Note that strains possessing type 1 fimbriae aggregated PRP-1-coated latex beads, while strains possessing type 2 fimbriae aggregated asialofetuin-coated latex beads.

been reported to be free of carbohydrate (6, 26). Therefore, the interaction of type 1 fimbriae with adsorbed PRP-1 or statherin molecules appears to represent a clear example of a protein-protein stereochemical interaction which mediates bacterial adhesion. The PRPs and statherin are characteristically found in saliva (16, 17), and these proteins adsorb with high affinity to apatitic minerals (14, 15, 19). Both proteins would therefore be expected to be present in the acquired pellicle which forms on teeth, and this prediction has been confirmed in the case of the PRPs (1, 15, 17). The present study has now shown that these proteins serve as major salivary receptors for the type 1 fimbrial adhesins which mediate attachment of *A. viscosus* cells to pellicles formed on HA surfaces. These findings provide a molecular explanation for the tropism which *A. viscosus* cells display for teeth.

This investigation was supported in part by Public Health Service grants DE-02847, DE-03915, DE-07009, DE-05429, and DE-00115 from the National Institute for Dental Research.

## LITERATURE CITED

1. **Bennick, A., G. Chau, R. Goodlin, S. Abrams, D. Tustian, and G. Madapallimattam.** 1983. The role of human salivary acidic proline-rich proteins in the formation of acquired dental pellicle *in vivo* and their fate after adsorption to the human enamel surface. Arch. Oral Biol. **28**:19–27.
2. **Cisar, J. O.** 1986. Fimbrial lectins of the oral actinomyces, p. 183–196. *In* D. Mirelman (ed.), Microbial lectins and agglutinins: properties and biological activity. John Wiley & Sons, Inc., New York.
3. **Cisar, J. O., V. A. David, S. H. Curl, and A. E. Vatter.** 1984. Exclusive presence of lactose-sensitive fimbriae on a typical strain (WVU45) of *Actinomyces naeslundii*. Infect. Immun. **46**: 453–458.
4. **Cisar, J. O., A. E. Vatter, W. B. Clark, S. H. Curl, S. Hurst-Calderone, and A. L. Sandberg.** 1988. Mutants of *Actinomyces viscosus* T14V lacking type 1, type 2, or both types of fimbriae. Infect. Immun. **56**:2984–2989.
5. **Clark, W. B.** 1985. The *Actinomyces* fimbriae and adherence to hydroxyapatite, p. 103–108. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
6. **Clark, W. B., T. T. Wheeler, and J. O. Cisar.** 1984. Specific inhibition of adsorption of *Actinomyces viscosus* T14V to saliva-treated hydroxyapatite by antibody against type 1 fimbriae. Infect. Immun. **43**:497–501.
7. **Clark, W. B., T. T. Wheeler, D. D. Lane, and J. O. Cisar.** 1986. *Actinomyces* adsorption mediated by type-1 fimbriae. J. Dent. Res. **65**:1166–1168.
8. **Ellen, R. P.** 1976. Establishment and distribution of *Actinomyces viscosus* and *Actinomyces naeslundii* in the human oral cavity. Infect. Immun. **14**:1119–1124.
9. **Ellen, R. P., D. W. Banting, and E. D. Fillery.** 1985. Longitudinal microbiological investigation of a hospitalized population of older adults with a high root surface caries risk. J. Dent. Res. **64**: 1377–1381.
10. **Ellen, R. P., D. N. Segel, and D. A. Grove.** 1978. Relative proportions of *Actinomyces viscosus* and *Actinomyces naeslundii* in dental plaques collected from single sites. J. Dent. Res. **57**: 550.
11. **Gibbons, R. J., and I. Etherden.** 1982. Enzymatic modification of bacterial receptors on saliva-treated hydroxyapatite surfaces. Infect. Immun. **36**:52–58.
12. **Gibbons, R. J., and I. Etherden.** 1985. Albumin as a blocking agent in studies of streptococcal adsorption to experimental salivary pellicles. Infect. Immun. **50**:592–594.
13. **Gibbons, R. J., and D. I. Hay.** 1988. Human salivary acidic proline-rich proteins and statherin promote the attachment of *Actinomyces viscosus* LY7 to apatitic surfaces. Infect. Immun. **56**:439–445.
14. **Hay, D. I.** 1973. The interaction of human parotid salivary proteins with hydroxyapatite. Arch. Oral Biol. **20**:1517–1529.
15. **Hay, D. I.** 1983. Human glandular salivary proteins, p. 319–335. *In* E. P. Lazzari (ed.), CRC handbook of experimental aspects of oral biochemistry. CRC Press, Inc., Boca Raton, Fla.
16. **Hay, D. I., D. J. Smith, S. K. Schluckebier, and E. C. Moreno.** 1984. Human salivary statherin. Relationship between concentration and inhibition of calcium phosphate precipitation in parotid saliva. J. Dent. Res. **63**:857–863.
17. **Kousvelari, E. E., R. S. Baratz, B. Burke, and F. G. Oppenheim.** 1980. Immunochemical identification and determination of proline-rich proteins in salivary secretions, enamel pellicle and glandular tissue specimens. J. Dent. Res. **59**:1430–1438.
18. **Loesche, W. J., and S. A. Syed.** 1978. Bacteriology of human experimental gingivitis, effect of plaque and gingivitis score. Infect. Immun. **21**:830–839.
19. **Moreno, E. C., M. Kresak, and D. I. Hay.** 1982. Adsorption thermodynamics of acidic proline-rich salivary proteins onto calcium apatites. J. Biol. Chem. **257**:2981–2989.
20. **Revis, G. J., A. E. Vatter, A. J. Crowle, and J. O. Cisar.** 1982. Antibodies against the Ag2 fimbriae of *Actinomyces viscosus* T14V inhibit lactose-sensitive bacterial adherence. Infect. Immun. **36**:1217–1222.
21. **Sandberg, A. L., L. L. Mudrick, J. O. Cisar, M. J. Brennan, S. E. Mergenhagen, and A. E. Vatter.** 1986. Type 2 fimbrial lectin-mediated phagocytosis of oral *Actinomyces* spp. by polymorphonuclear leukocytes. Infect. Immun. **54**:472–476.
22. **Schlesinger, D. H., and D. I. Hay.** 1977. Complete covalent structure of statherin, a tyrosine-rich acidic peptide which inhibits calcium phosphate precipitation from human parotid saliva. J. Biol. Chem. **252**:1689–1695.
23. **Schlesinger, D. H., and D. I. Hay.** 1979. Complete primary structure of a proline-rich phosphoprotein (PRP-4), a potent inhibitor of calcium phosphate precipitation in human parotid saliva, p. 133–135. *In* G. Gross and J. Meienhofer, (ed.),

Peptides: structure and biological function; Proceedings of the 6th American Peptide Symposium. Pierce Chemical Co., Rockford, Ill.

24. **Schlesinger, D. H., and D. I. Hay.** 1986. Complete covalent structure of a proline-rich phosphoprotein (PRP-2), an inhibitor of calcium phosphate crystal growth from human parotid saliva. Int. J. Peptide Protein Res. **27**:373–379.
25. **Syed, S. A., and W. J. Loesche.** 1978. Bacteriology of human experimental gingivitis: effect of plaque age. Infect. Immun. **21**: 821–829.
26. **Wheeler, T. T., and W. B. Clark.** 1980. Fibril-mediated adherence of *Actinomyces viscosus* to saliva-treated hydroxyapatite. Infect. Immun. **28**:577–584.
27. **Wong, R. S., T. Hoffmann, and A. Bennick.** 1979. The complete primary structure of a proline-rich phosphoprotein from human saliva. J. Biol. Chem. **254**:4800–4808.
28. **Wong, R. S., and A. Bennick.** 1980. The primary structure of a salivary calcium-binding proline-rich phosphoprotein (protein C), a possible precursor of a related salivary protein A. J. Biol. Chem. **255**:5943–5948.