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Enzymatic Modification of Bacterial Receptors on Saliva-Treated Hydroxyapatite Surfaces

R. J. GIBBONS[1,2]* AND I. ETHERDEN[1]

Forsyth Dental Center[1] and Harvard School of Dental Medicine,[2] Boston, Massachusetts 02115

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Certain properties of experimental pellicles formed by the adsorption of salivary components on hydroxyapatite surfaces change over time. To determine whether enzymes likely to be present in the oral environment could induce such changes, pellicles were treated with saliva which had been incubated for 18 h at 35°C to promote the elaboration of microbial enzymes. This treatment markedly reduced the numbers of *Streptococcus mutans* MT3 and JBP and *S. sanguis* FC-1 and C5 cells which attached, but it had little or no effect on the attachment of *S. mitis* RE7, *Actinomyces viscosus* LY7 and CK-8, *Bacteroides gingivalis* 381, or *B. melaninogenicus* subsp. *intermedius* 581. Heating the incubated saliva at 60°C for 30 min partially reduced its pellicle-modifying activity, whereas heating at 80°C for 30 min or 100°C for 15 min completely eliminated such activity. This indicated that the saliva contained heat-labile substances, presumably enzymes, which could affect the pellicle receptors involved in the attachment of *S. mutans* and *S. sanguis*. Treatment of saliva-treated hydroxyapatite with commerically obtained enzyme preparations also affected bacterial attachment. Thus, treatment with galactose oxidase reduced the numbers of the *S. mutans* strains which attached, whereas treatment with neuraminidase reduced the adsorption of *S. sanguis* FC-1 but not that of *S. sanguis* C5. Treatment with β-glucosidase preparations derived from almonds significantly reduced the attachment of all of the streptococcal strains studied, but, when subjected to isoelectric fractionation, the adherence-inhibiting activity did not correlate directly with β-glucosidase activity. Treatment of the pellicles with trypsin or eight other glycosidases did not affect streptococcal attachment. Exposure of the enzymatically modified pellicles to fresh saliva did not restore the streptococcal receptors. Collectively, the data suggest that some bacterial receptors in the pellicle coating of teeth can be modified by enzymes likely to be present in the oral environment, and these interactions may affect oral bacterial ecology.

Dental caries and periodontal diseases are caused by bacterial deposits, called "dental plaques," which accumulate on various surfaces of the teeth (11, 12, 22). This has prompted studies of the mechanisms by which bacteria attach to the teeth and other surfaces of the mouth. Teeth are covered by a membranous film, termed the "acquired pellicle," which is thought to be formed by the selective adsorption of salivary components to the hydroxyapatite (HA) mineral of enamel (8, 12). The attachment of bacteria to the tooth surface therefore involves interactions between surface components of the microorganism and the salivary constituents comprising the pellicle. In the case of several oral bacterial species, this interaction may involve lectin-like components of the organism which bind to saccharide receptors in the salivary glycoproteins making up the pellicle (8, 12).

The properties of experimental salivary pellicles formed on HA surfaces appear to change over time. For example, fewer *Streptococcus mutans* cells attach to pellicles formed for 24 h than to pellicles formed for 1 h (2). Similarly, the permselective properties of pellicles change over a 1-week period (24, 25), and the solubility of pellicles is thought to decrease over time (13, 17). These changes may be caused by the continued adsorption of specific salivary components, partial degradation of the adsorbed salivary macromolecules by enzymatic activities in the oral environment, or intermolecular interactions which may occur between the closely packed molecules adsorbed on the HA surface. As the adsorption of proteins to HA is thought to reach equilibrium in less than 1 h, the latter possibilities appear most likely.

Glycosidases and proteases potentially capable of degrading salivary components are elabo-

rated by a variety of oral bacteria (4, 7, 18, 19). In addition, lysosomal enzymes derived from leukocytes in the gingival crevice may also be present in oral fluids (5, 23). Such bacterial or host-derived enzymes could alter receptors for bacteria in the pellicle and thereby affect bacterial attachment and colonization on the teeth. The present investigation determined the effect of pretreating experimental salivary pellicles with various enzyme preparations on the subsequent attachment of selected oral bacteria.

MATERIALS AND METHODS

Cultures and culture conditions. The bacterial strains used were obtained from the culture collection of the Forsyth Dental Center. Streptococci were maintained in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and on mitis salivarius plates (Difco Laboratories, Detroit. Mich.). The *Actinomyces* strains were grown in Trypticase soy broth (BBL) and on Trypticase soy agar plates (BBL). *Bacteroides gingivalis* 381 and *B. melaninogenicus* subsp. *intermedius* 581 were grown in Todd-Hewitt broth supplemented with 5 μg of hemin and 0.2 μg of menadione per ml. All cultures were incubated at 35°C in Brewer Anaerobic Jars (Becton, Dickinson & Co., Rutherford, N.J.) containing 80% N_2, 10% H_2, and 10% CO_2.

[^{3}H]thymidine-labeled bacteria were prepared by growing the organisms in their respective broths supplemented with from 2 to 10 μCi of [^{3}H]thymidine per ml (New England Nuclear Corp., Boston, Mass.) Cells from overnight cultures were harvested by centrifugation, 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).

Bacterial adherence to saliva-treated HA. Bacterial attachment to experimental salivary pellicles was determined by previously described techniques (1). Briefly, samples of whole unstimulated saliva were collected from adult donors in containers chilled in ice. The saliva was heated at 60°C unless noted otherwise, clarified by centrifugation at 10,000 × *g* for 10 min, and stored at −20°C before use. Samples (5 mg) of spheroidal HA beads (BDH, Poole, England) which had been equilibrated with buffered KCl were incubated with 0.125 ml of clarified whole saliva at room temperature with continuous inversion for 60 min to form experimental pellicles. The saliva-treated HA (S-HA) beads were then washed twice with buffered KCl to remove unadsorbed saliva. The ability of bacteria to attach to the S-HA was determined by incubating the beads with 1.25×10^7 ^{3}H-labeled bacteria suspended in buffered KCl (0.125 ml). The mixtures were continuously inverted at room temperature for 60 min, and then the beads were washed three times with buffered KCl to remove unattached organisms. The number of bacteria attached to the S-HA beads was determined by scintillation counting (1).

The effect of treating the experimental pellicles with various enzymes was determined by incubating twice-washed samples of S-HA beads with 0.05 ml of either active or heat-inactivated (100°C for 15 min) enzyme for 60 min. The beads were then washed twice with buffered KCl, and the ability of [^{3}H]thymidine-labeled bacteria to attach was determined. Neuraminidase (type 1X), β-glucosidase (almond), β-amylase, and β-*N*-acetylglucosaminidase were dissolved in 0.05 M acetate buffer at pH 5, whereas α-mannosidase was studied in 0.05 M acetate buffer, pH 4.5. The following enzymes were used in 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer at the following pHs: galactose oxidase, 6; α-fucosidase and α-galactosidase, 6.5; β-glucuronidase, α-amylase, and α-glucosidase, 7; β-galactosidase (*Escherichia coli*), 7.5; and hyaluronidase (bovine testicular) and trypsin (2× crystallized), 8. All enzymes were obtained from Sigma Chemical Co., St. Louis, Mo., and were tested at a concentration of 2 U/ml, except for α-fucosidase and α-galactosidase, which were studied at concentrations of 0.1 and 0.4 U/ml, respectively.

Incubated saliva was also used as a source of enzymes likely to be present in the oral environment. Preparations were obtained by incubating samples of whole unstimulated saliva at 35°C for 18 h. The saliva was then clarified by centrifugation and stored at −20°C before use. In some experiments, pellicles were formed on HA with incubated saliva. Bacterial attachment to such pellicles was compared with attachment to pellicles formed from samples of the same batch of saliva which had been heated at 60°C for 30 min.

Isoelectric fractionation of almond β-glucosidase preparations. Samples of β-glucosidase derived from almonds (Sigma) were fractionated by subjecting them to flat bed isoelectric focusing in Sephadex G-25 gels (Pharmacia Fine Chemicals, Piscataway, N.J.) with an LKB apparatus and ampholines which formed a gradient of between pH 3.5 and pH 9. After focusing, the gel was divided into 30 fractions, and the proteins present were eluted with buffered KCl. Each fraction was dialyzed against three changes of buffered KCl to remove the ampholines and then assayed for its ability to modify receptors for *S. mutans* MT3 on S-HA. The absorbance of each fraction at 280 nm was determined, and each was assayed for α- and β-galactosidases, α-mannosidase, *N*-acetylglucosaminidase, α- and β-glucosidases, and α-fucosidase with the appropriate nitrophenylated substrates (Sigma).

RESULTS

Modification of experimental pellicles by exposure to incubated saliva. Previous studies have suggested that experimental pellicles become modified over time. To determine whether these changes could be caused at least in part by enzymatic activities, we studied the effect of treating S-HA with samples of saliva which had been incubated at 35°C for 18 h to promote the elaboration of microbial enzymes. Pretreatment of S-HA with incubated saliva reduced the numbers of *S. mutans* MT3 cells which attached by almost 80% (Table 1). Heating the incubated saliva at 100°C for 15 min abolished its adherence-inhibiting effect. Pretreating the S-HA with unheated saliva or with samples heated at 60°C for 30 min also reduced the numbers of *S. mutans* MT3 cells which attached by 66 and 22%, respectively, whereas saliva heated at 80°C for 30 min or at 100°C for 15 min had no effect (Table 1). Thus, there appeared to be heat-labile

TABLE 1. Effect of treating S-HA with samples of incubated or heated saliva on subsequent attachment of *S. mutans* MT3

Treatment (1h) of S-HA	No. of *S. mutans* MT3 cells ($\times 10^6$) adsorbed per 5 mg of S-HA[a]	*S. mutans* MT3 cells attached to S-HA relative to buffer control (%)
Buffered KCl	1.8 ± 0.1	100
Incubated saliva	0.4 ± 0.1	22
Heat-inactivated incubated saliva	2.1 ± 0.2	116
Unheated saliva	0.8 ± 0.1	44
Saliva heated at 60°C for 30 min	1.4 ± 0.1	78
Saliva heated at 80°C for 30 min	1.7 ± 0.4	94
Saliva heated at 100°C for 15 min	1.7 ± 0.1	94

[a] Mean ± the standard error.

TABLE 2. Effect of treating S-HA with incubated saliva on subsequent bacterial attachment

Organism	Bacterial adsorption relative to S-HA treated with buffered KCl (%)	
	S-HA treated with incubated saliva	S-HA treated with heat-inactivated incubated saliva[a]
A. viscosus LY7	102	109
A. viscosus CK-8	100	98
S. mutans JBP	59[b]	125
S. mutans MT3	22[b]	124
S. sanguis C5	12[b]	116
S. sanguis FC-1	11[b]	76[c]
S. mitis RE7	73[d]	80[c]
B. melaninogenicus 581	90	145
B. gingivalis 381	85[c]	96

[a] Heat-inactivated at 100°C for 15 min.
[b] $P < 0.01$ different from treatment with buffered KCl (Student's t test).
[c] $P < 0.1$ different from treatment with buffered KCl (Student's t test).
[d] $P < 0.05$ different from treatment with buffered KCl (Student's t test).

substances which were presumed to be enzymes in incubated and in unheated saliva which modified the pellicle receptors for this organism.

Similar effects were noted with other bacterial strains. Pretreatment of S-HA with incubated saliva significantly reduced the numbers of *S. mutans* JBP and *S. sanguis* C5 and FC-1 cells that adsorbed, as compared with control S-HA, which had been treated with either buffered KCl or with heat-inactivated saliva (Table 2). However, the incubated saliva had little or no effect on the attachment of *S. mitis* RE7, *A. viscosus* LY7 and CK-8, *B. melaninogenicus* 581, or *B. gingivalis* 381. Thus, the heat-labile components in incubated saliva selectively affected the attachment of different bacterial species; this indicates that different salivary receptors are involved in their attachment to S-HA.

Essentially similar results were obtained in experiments which determined the numbers of bacteria which attached to experimental pellicles formed from heated or from incubated saliva. Many fewer *S. mutans* JBP and MT3 and *S. sanguis* C5 and FC-1 cells adsorbed to pellicles formed from incubated saliva compared with pellicles formed from 60°C-heated saliva (Table 3). However, similar numbers of *S. mitis*, *A. viscosus*, *B. melaninogenicus*, and *B. gingivalis* cells attached to the two types of pellicles.

Effect of treating S-HA with commercial enzyme preparations on subsequent bacterial adsorption. The ability of incubated saliva to modify S-HA and reduce the attachment of *S. mutans* and *S. sanguis* cells suggested that there likely are enzymes in oral fluids which can destroy or modify the pellicle receptors on S-HA surfaces for these organisms. Consequently, the effect of

TABLE 3. Bacterial adsorption to experimental pellicles formed from saliva heated at 60°C for 30 min or from saliva incubated at 35°C for 18 h

Organism	No. of bacteria adsorbed ($\times 10^6$, ±SE) per 5 mg of HA treated with:		Attachment to pellicles formed from incubated saliva compared with those formed from heated saliva (%)
	Saliva heated for 30 min at 60°C	Saliva incubated for 18 h at 35°C	
A. viscosus LY7	5.7 ± 0.6	5.4 ± 0.3	95
A. viscosus CK-8	4.5 ± 1.0	4.3 ± 0.4	96
S. mutans JBP	0.8 ± 0.1	0.3 ± 0.1	38
S. mutans MT3	1.7 ± 0.2	0.9 ± 0.1	53
S. sanguis C5	1.9 ± 0.2	0.3 ± 0.1	16
S. sanguis FC-1	2.5 ± 0.3	0.4 ± 0.1	16
S. mitis RE7	1.5 ± 0.2	1.2 ± 0.1	80
B. melaninogenicus 581	2.9 ± 0.3	3.2 ± 0.3	110
B. gingivalis 381	7.4 ± 0.7	6.0 ± 0.6	81

TABLE 4. Effect of treating S-HA with commercial enzyme preparations on subsequent bacterial adsorption

S-HA pretreatment[a]	Bacterial adsorption compared with adsorption to heat-inactivated enzyme control (%)				
	S. mutans JPB	*S. mutans* MT3	*S. sanguis* C5	*S. sanguis* FC-1	*S. mitis* 26
β-glucosidase	39[b]	24[c]	72[b]	63[c]	26[b]
Galactose oxidase	59[b]	64[d]	100	88[d]	100
Neuraminidase	99	96	88	11[c]	70
Trypsin	117	103	94	90	126

[a] Active solutions of α-mannosidase, α-galactosidase, α-glucosidase, α-amylase, α-fucosidase, β-glucuronidase, β-galactosidase, and hyaluronidase had no effect compared with heat-inactivated enzyme controls.
[b] $P < 0.05$ different from heat-inactivated control (Student's t test).
[c] $P < 0.01$ different from heat-inactivated control (Student's t test).
[d] $P < 0.1$ different from heat-inactivated control (Student's t test).

pretreating S-HA with several commercially obtained enzyme preparations was studied. Pretreatment of S-HA with β-glucosidase preparations derived from almonds significantly reduced the attachment of all of the streptococci studied (Table 4). Treatment of S-HA with galactose oxidase reduced the adsorption of *S. mutans* JBP and MT3, but it had less or no effect upon attachment of the *S. sanguis* and *S. mitis* strains studied (Table 4). Pretreatment of S-HA with neuraminidase markedly reduced the adsorption of *S. sanguis* FC-1, but it had little or no effect on *S. sanguis* C5 or the other streptococci studied. Trypsin and the other glycoside hydrolases tested did not affect the attachment of any of the strains studied compared with controls of heat-inactivated enzyme preparation (Table 4).

Inability of heated saliva to restore modified pellicle receptors for *S. mutans* MT3. Because unheated saliva and certain commercial enzyme preparations appeared to alter pellicle receptors for *S. mutans* MT3, it was of interest to determine whether modified pellicles could be "restored" by a subsequent exposure to saliva. However, when S-HA which had been modified by unheated saliva or by almond β-glucosidase preparations was secondarily exposed to heated or unheated saliva, no additional adsorption of *S. mutans* MT3 cells occurred (Tables 5 and 6). This suggests that salivary molecules containing *S. mutans* receptors do not readily absorb to enzymatically modified pellicles on S-HA surfaces.

Fractionation of β-glucosidase preparation. Because the commercially obtained β-glucosidase preparation reduced the adherence of all streptococcal strains studied when used to pretreat S-HA, it was studied further. Such enzyme preparations have been reported to contain several contaminating glycoside hydrolases (20), and consequently it was of interest to determine whether the adherence-inhibiting activity of the preparation was associated with β-glucosidase activity. Unfractionated preparations of the almond β-glucosidase were found to contain low levels of α- and β-galactosidase, α-mannosidase, and *N*-acetylglucosaminidase in addition to β-glucosidase, but α-fucosidase and α-glucosidase activities were not detected. When the preparations were subjected to isoelectric focusing, major peaks of β-glucosidase activity were present at approximately pH 3.5, 4.5, and 6.3, respectively (Fig. 1A). A peak of α-galactosidase also focused at pH 3.5, whereas peaks of β-galactosidase were detected at pH 3.6, 4.3, and 6.2, respectively. α-Mannosidase was present at pH 3.7, and a small amount of *N*-acetylglucosaminidase activity also focused at pH 3.9 (Fig. 1B).

Each fraction was assayed for its ability to affect the adsorption of *S. mutans* MT3 when used to pretreat S-HA. A band of adherence-inhibiting activity was present which focused between pH 3.6 and 4.2 (Fig. 1). This roughly correlated with the bulk of the glycoside hydrolase activity detected (Fig. 1). However, the most active fractions (fractions 12 to 14) did not directly correlate with the major peaks of β-glucosidase activity, nor did they correlate directly with the activities of the other enzymes monitored. In addition, the β-glucosidase fraction which focused at pH 6.2 was not highly

TABLE 5. Inability of saliva to restore pellicle receptors for *S. mutans* MT3 after treatment with unheated saliva

First treatment (1 h) of S-HA	Second treatment (1 h) of S-HA	No. of *S. mutans* MT3 cells (× 10^6) adsorbed per 5 mg of S-HA[a]
Buffered KCl	Buffered KCl	2.8 ± 0.1
Heated (80°C) saliva	Buffered KCl	2.7 ± 0.3
Unheated saliva	Buffered KCl	1.8 ± 0.3
Unheated saliva	Unheated saliva	1.5 ± 0.4
Unheated saliva	Heated (60°C) saliva	1.0 ± 0.2
Unheated saliva	Heated (80°C) saliva	1.7 ± 0.3

[a] Mean ± the standard error.

TABLE 6. Inability of saliva to restore pellicle receptors for *S. mutans* MT3 after treatment with β-glucosidase preparations

First treatment of S-HA	Second treatment of S-HA	No. of *S. mutans* MT3 cells ($\times 10^6$) adsorbed per 5 mg of S-HA[a]
Acetate buffer (pH 5)	Buffered KCl (pH 6)	1.31 ± 0.2
Heat-inactivated β-glucosidase	Buffered KCl (pH 6)	1.58 ± 0.1
Active β-glucosidase	Buffered KCl (pH 6)	0.46 ± 0.1
Active β-glucosidase	Heated saliva (60°C for 30 min)	0.29 ± 0.1
Active β-glucosidase	Heated saliva (80°C for 30 min)	0.48 ± 0.1

[a] Mean ± the standard error.

active in affecting streptococcal adherence. Thus, the adherence-inhibiting activity of the almond β-glucosidase preparation could not be associated with any of the individual enzyme activities monitored.

DISCUSSION

Previous investigators have noted that the properties of experimentally formed pellicles change over time (2, 13, 17, 24, 25). The nature of the changes which occur are not understood, but they could be caused in part by enzymatic alterations. The data obtained in the present study indicate that at least some properties of experimental pellicles can become modified by enzymes likely to be present in the oral environment. Thus, pellicle receptors involved in the attachment of strains of *S. mutans* and *S. sanguis* were destroyed or rendered unavailable following exposure to heat-labile substances presumed to be enzymes in unheated or in incubated whole saliva or to certain commercially obtained glycoside hydrolases. Saliva samples retained some receptor-modifying activity after heating at 60°C for 30 min, but little or no activity was detected when the saliva was heated at 80°C for 30 min or at 100°C for 15 min. The ability of 60°C-heated saliva to still modify or destroy the receptors involved in the attachment of *S. mutans* appears to explain why fewer cells of this organism adsorb to experimental pellicles formed for 24 h than to those formed for 1 h (2). These observations are also consistent with those of Knox (14), who observed that saliva exhibits mucolytic enzyme activity which is destroyed by heating to 80°C.

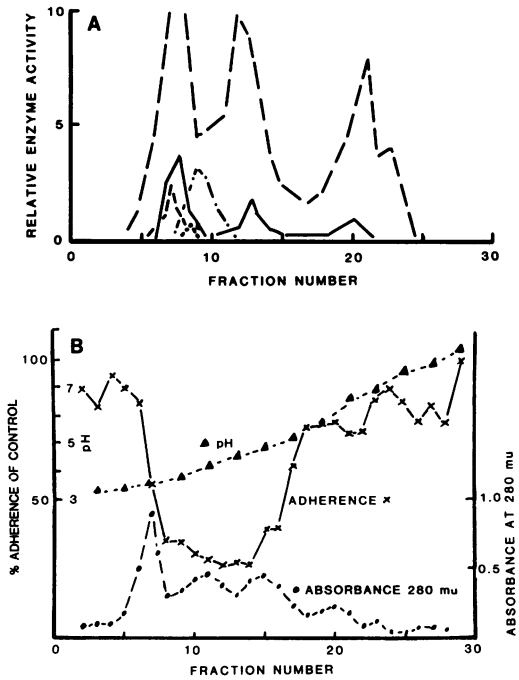

FIG. 1. Fractionation of almond β-glucosidase preparation by preparative flat bed isoelectric focusing. A. Enzyme activities: — —, β-glucosidase; ——, β-galactosidase; — - —, α-mannosidase; - - -, α-galactosidase; ....., *N*-acetylglucosaminidase. B. ▲, pH gradient established; X, adherence-inhibiting activities of fractions; ●, absorbance of fractions at 280 nm.

Most previous investigations of bacterial attachment to S-HA surfaces have used saliva heated at 60°C for 30 min to inactivate degradative enzymes. As saliva treated in this manner retains some pellicle-modifying activity, this may be a source of variation in adherence assays. We noted that similar or somewhat higher numbers of *S. mutans* cells attached to experimental pellicles formed from saliva heated at 80°C for 30 min compared with those formed from saliva heated at 60°C for 30 min (data not shown). However, we observed that this heat treatment appeared to alter several salivary macromolecules.

Neuraminidase treatment of experimental pellicles markedly reduced the attachment of *S. sanguis* FC-1 but not of *S. sanguis* C5 or the other streptococci studied. This indicates that the latter organisms attach to different receptors in the pellicles. These observations are consistent with earlier reports which demonstrated that some strains of *S. sanguis* attach to sialic acid residues present in salivary glycoproteins, whereas other strains of this species do not (16). It was also noted previously that neuraminidase

treatment does not affect the binding of purified salivary glycoproteins to *S. mutans* (15).

The attachment of *S. mutans* to pellicles which were treated with galactose oxidase was reduced. Earlier studies suggested that galactose residues may comprise a part of the receptors for this organism because its attachment to S-HA is inhibited by galactose and melibiose (10). Also, Levine et al. (15) noted that a purified salivary glycoprotein no longer agglutinated *S. mutans* cells after it had been treated with α-galactosidase. However, neither α- nor β-galactosidase treatment of S-HA affected the attachment of the *S. mutans* strains in the present study. This may be because the enzymes used were unable to hydrolyze the galactosyl residues present in the adsorbed salivary glycoproteins. In this regard, some types of α-fucosidases are unable to hydrolyze the terminal fucose residues of salivary mucins, whereas other α-fucosidases are active (21). Pretreatment of experimental pellicles with trypsin or several other enzymes did not affect the attachment of any of the streptococci studied.

The enzyme preparation found most active in reducing streptococcal adsorption to S-HA was β-glucosidase derived from almonds. However, such preparations contain several contaminating glycoside hydrolases (20). When the preparations were subjected to isoelectric fractionation, the pellicle-modifying activity was present in fractions associated with the bulk of the collective glycoside hydrolase activity present, but it did not correlate directly with any individual enzyme monitored. Thus, the marked adherence-inhibiting effect of the crude β-glucosidase preparation may be caused by unrecognized enzymes or the collective action of several of the glycoside hydrolases present. It is also possible that heat-labile lectins were present in the almond preparations which could bind to S-HA and mask the streptococcal receptors. Lectins capable of masking S-HA receptors for oral streptococci have been noted previously in extracts of several vegetables, fruits, and nuts (9). However, secondary exposure of β-glucosidase-modified pellicles to heated saliva would be expected to promote desorption of the bound lectins. The observation that this treatment did not promote increased streptococcal attachment (Table 6) therefore argues against the likelihood that lectins were responsible for the reduced streptococcal adherence noted. However, more definitive data are needed to adequately characterize the receptor or attachment sites for the organisms studied.

The inability of modified experimental pellicles to be readily restored by exposure to new saliva samples suggests that the altered salivary macromolecules remain on the HA surface and prevent adsorption of new salivary components. This raises the possibility that pellicles on the teeth which become naturally or artificially modified by enzymatic activities may remain depleted of bacterial receptors for appreciable periods of time. This possibility warrants additional study, because it could lead to new approaches for altering bacterial colonization of the teeth.

Several oral bacteria are known to synthesize glycoside hydrolases and proteases which may affect salivary glycoproteins (18, 19), and *S. mutans* and other oral bacteria and yeasts have been shown to degrade specific salivary macromolecules (4, 7). As saccharides are thought to comprise part of the receptors for some oral bacteria (8, 12), enzymes which alter the sugar moieties of salivary glycoproteins could destroy these receptors and thereby reduce the adherence and colonization of neighboring organisms. However, it is also possible that some enzymes might remove terminal sugars from the oligosaccharide chains of glycoproteins and expose underlying saccharide receptors. An example of the latter possibility concerns strains of *A. viscosus* and *A. naeslundii* which contain lectin-like fibrils which bind to β-galactoside-containing receptors. These organisms do not attach to such receptors on erythrocytes unless terminal sialic acid residues are first removed by neuraminidase (3, 6). Collectively, the available data suggest that modification of receptors on host tissues by microbial enzymes may represent a novel type of interaction which affects the oral bacterial ecology.

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