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Comparative Hydrophobicities of Oral Bacteria and Their Adherence to Salivary Pellicles

R. J. GIBBONS* AND I. ETHERDEN

Forsyth Dental Center and Harvard School of Dental Medicine, Boston, Massachusetts 02115

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Oral bacteria were found to differ in their surface hydrophobicities as determined by their ability to adsorb to hexadecane. Strains of *Actinomyces viscosus*, *A. naeslundii*, *Streptococcus sanguis*, *S. mitis*, and *Bacteroides gingivalis* proved highly hydrophobic. Strains of *B. intermedius*, *S. salivarius*, *S. mutans*, and *B. melaninogenicus* were less hydrophobic, whereas strains of *Actinobacillus actinomycetemcomitans* were hydrophilic. An overall correlation was noted between the adsorption of bacteria to hexadecane and their numbers which attached to experimental salivary pellicles formed on hydroxyapatite surfaces. This suggests that hydrophobic bonding plays an important role in this process. Pellicles prepared from saliva which had been extracted with chloroform-methanol to remove lipids adsorbed comparable numbers of *S. sanguis* and *A. viscosus* and increased numbers of *S. mutans*. Analyses of adsorption isotherms indicated that pellicles formed from lipid-depleted saliva contained increased numbers of binding sites for the *S. mutans* strains studied, and this likely accounts for their enhanced adsorption. Absorption of saliva with 10% octyl or phenyl Sepharose reduced the protein content of saliva by almost half, but the numbers of bacteria which attached to pellicles prepared from such absorbed saliva were similar to or higher than those which attached to control pellicles. These observations suggest that saliva does not contain unique highly hydrophobic salivary macromolecules which serve as essential pellicle receptors for the bacteria studied. The data obtained are consistent with the view that hydrophobic bonding together with interactions between complementary molecules are involved in bacterial attachment to salivary pellicles on teeth.

Bacteria attach to the teeth and oral mucosal surfaces of humans in a highly selective manner and attachment is thought to be the first step leading to colonization (4, 9). Teeth are covered by a membranous film, termed the acquired pellicle, which is formed by the selective adsorption of salivary components onto the apatitic mineral of enamel (4, 9). The initial attachment of bacteria to teeth is therefore thought to involve interactions between surface components of the organism and immobilized salivary components comprising the enamel pellicle.

Several recent studies have suggested that the hydrophobic properties of bacteria may be an important factor in their adherence to host tissues (11, 16, 19, 22). In the case of the oral cavity, it has been recently reported that a high percentage of streptococci (14) and of aerobically cultured bacteria derived from samples of human dental plaque exhibit hydrophobic properties as judged from their ability to adhere to hexadecane (25). Also, Nesbitt and co-workers (12) demonstrated that strains of *Streptococcus sanguis* have a high affinity for hydrocarbons, and they suggested that its adherence to tooth surfaces may depend in part on the formation of hydrophobic bonds between surface components of the streptococci and adsorbed salivary proteins comprising the pellicle (12, 13). The present investigation compared the adherence of selected strains of oral bacteria to hexadecane, a hydrophobic hydrocarbon, and to experimental salivary pellicles. In addition, the influence of salivary lipids and other hydrophobic macromolecules on bacterial attachment to experimental pellicles was studied.

MATERIALS AND METHODS

Cultures and cultural conditions. All bacterial strains were obtained from stocks stored in 50% glycerol at −20°C from the culture collection of the Forsyth Dental Center, Boston, Mass. During the period of assay, *Streptococcus* sp. and *Actinomyces* sp. strains were maintained by weekly transfer on tryptic soy agar plates containing 5% sheep blood (Scott Laboratories, Fiskeville, R.I.) and in tubes of Todd-Hewitt broth (Difco Diagnostics, Madison, Wis.) and Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), respectively. Strains of *Bacteroides* and

Actinobacillus species were transferred on Trypticase soy blood agar plates supplemented with 0.5 mg of sodium succinate and 0.5 μg of menadione per ml. The *Bacteroides* sp. strains were also maintained in Todd-Hewitt broth supplemented with 0.5 μg of menadione and 5 μg of hemin per ml, whereas the *Actinobacillus* sp. strains were propagated in mycoplasma base broth (BBL) supplemented with 0.5% glucose, 5 μg of hemin per ml, and 2 mg of filter-sterilized $NaHCO_3$ per ml. All cultures were incubated at 35°C in Brewer jars containing 80% N_2–10% H_2–10% CO_2.

Adherence to hexadecane. The ability of the strains to adhere to hexadecane was used as a measure of their relative surface hydrophobicity as described by Rosenberg et al. (18, 19). Bacteria were harvested from 48-h cultures grown in appropriately supplemented Todd-Hewitt broth. The organisms were washed twice and suspended in PUM buffer (22.2 g of $K_2HPO_4 \cdot 3H_2O$, 7.26 g of KH_2PO_4, 1.8 g of urea, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter, pH 7.1) (18). The suspensions were adjusted to an optical density of 0.85 at 550 nm (1-cm light path), using PUM buffer as a blank in a Gilford spectrophotometer. Duplicate samples (3.0 ml) of the bacterial suspensions were placed in Pyrex tubes (18 by 150 mm) and 400 μl of hexadecane was added. Control suspensions were prepared without hexadecane. The suspensions were equilibrated in a water bath at 30°C. Each tube was then mixed on a Vortex mixer for two 30-s periods with 5 s in between and permitted to stand until the phases separated. The lower aqueous phase was carefully removed, and its optical density was determined at 550 nm. The values were expressed as the percentage of bacteria that remained in the aqueous phase compared with control suspensions which were not incubated with hexadecane but which were otherwise treated similarly. It was found that small deviations from the procedure outlined affected the values obtained.

Bacterial attachment to experimental salivary pellicles. Bacterial attachment to experimental salivary pellicles was determined as previously described (2, 6). The bacteria were labeled by growing them in the presence of 5 to 10 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) per ml; their specific activities ranged from 1,000 to 4,000 cpm/10^6 organisms. Reaction mixtures (125 μl) in microtitration plates consisted of 5 mg of saliva-treated hydroxyapatite (HA) beads (S-HA) and the [^{3}H]thymidine-labeled bacteria in 0.05 M KCl containing 1 mM $CaCl_2$, 1 mM PO_4 (pH 6.0), and 0.1 mM $MgCl_2$ (buffered KCl). Estimates of the numbers of binding sites and the affinities for bacterial attachment to the S-HA surfaces were determined with the Langmuirian model previously described (2, 6, 7). The bacterial concentrations used for the isotherms ranged from 1×10^7 to 20×10^7 per ml.

Saliva collection. Samples of whole unstimulated saliva from adult donors were collected in containers chilled in ice. The saliva was heated at 60°C to retard enzymatic activity and clarified by centrifugation at 10,000 × *g* for 10 min (5). Samples were stored at −20°C before use. The ability of the clarified saliva to agglutinate washed bacterial cells was determined as previously described (8).

Chloroform-methanol extraction of saliva. The ability of bacteria to attach to experimental pellicles prepared from whole saliva or saliva which had been depleted of lipids was determined. Samples (10 ml) of clarified whole human saliva were lyophilized. A portion of the lyophilized material was then extracted with 3 ml of chloroform-methanol (2:1) for 45 min by continuous mixing at room temperature (20, 21). The mixtures were centrifuged and the residue was reextracted two additional times with 3-ml samples of chloroform-methanol. The residue was then dried with N_2 and dissolved in water. Control samples of saliva were similarly lyophilized and dissolved in water for comparison. Samples of the whole saliva and the lipid-extracted saliva were used to prepare salivary pellicles on HA beads as described above, and the number of bacteria which attached to the two types of pellicles was determined.

Preparation of octyl and phenyl Sepharose-absorbed saliva. Bacterial adsorption to pellicles prepared from saliva which had been absorbed with hydrophobic adsorbents was also determined. Samples of octyl and phenyl Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) were washed with water, equilibrated with buffered KCl for 30 min, and suspended in buffered KCl to make a 10% (vol/vol) suspension. Samples (2.0 ml) of saliva were incubated with 10, 20, or 40% (vol/vol) packed phenyl or octyl Sepharose beads derived from the standardized suspensions for 1 h at room temperature with continuous inversion. The mixtures were centrifuged, and the supernatant liquors were used for preparing experimental salivary pellicles on HA beads. Samples of the salivas were analyzed for protein (1) and subjected to polyacrylamide gel electrophoresis, using procedures previously described (3).

RESULTS

Bacterial adherence to hexadecane and to experimental salivary pellicles. More than 85% of the cells of the *Actinomyces viscosus*, *A. naeslundii*, *S. sanguis*, *S. mitis*, and *Bacteroides gingivalis* strains studied adsorbed to hexadecane under the conditions used (Table 1); this indicates that their cell surfaces possess strongly hydrophobic properties. As a group, these strains also adsorbed in high numbers to experimental pellicles when tested at low (10^7 per ml) and at high (10^9 per ml) concentrations (Table 1). *B. intermedius* 581, *S. salivarius* strains 9GS2 and SS2, *S. mutans* JBP, MT3, and BHT, and *B. melaninogenicus* D23 and 287 adsorbed in somewhat lower proportions to hexadecane, and these organisms also tended to attach in lower numbers to S-HA (Table 1). Two strains of *Actinobacillus actinomycetemcomitans* studied attached poorly to hexadecane, and they adsorbed in only low numbers to S-HA.

Bacterial attachment to experimental pellicles prepared from lipid-depleted saliva. Increased numbers of *S. mutans* strains JBP and BHT attached to pellicles prepared from chloroform-methanol-extracted saliva (Table 2). This increased bacterial attachment was noted when both high and low streptococcal concentrations were used. In contrast, *S. sanguis* FC-1 and C5

TABLE 1. Comparison of bacterial adsorption to hexadecane and to S-HA

Organism	% Bacteria remaining in aqueous phase after hexadecane partitioning	No. of bacteria ± SE (× 10^5) adsorbed to S-HA when assayed at:	
		10^7/ml	10^9/ml
Strongly hydrophobic strains			
A. viscosus LY7	9 ± 0.4	7.0 ± 0.1	195 ± 4
A. viscosus CK8	8 ± 0.4	9.8 ± 0.8	182 ± 7
A. viscosus T14	11 ± 3	6.3 ± 0.1	126 ± 3
A. naeslundii 12104	14 ± 1	4.5 ± 0.1	128 ± 3
S. sanguis C5	6 ± 0.3	6.2 ± 0.1	79 ± 7
S. sanguis FC-1	12 ± 1	5.3 ± 0.8	181 ± 1
S. mitis RE-7	15 ± 4	6.3 ± 0.3	122 ± 5
B. gingivalis 381	8 ± 2	8.5 ± 0.4	498 ± 39
Moderately hydrophobic strains			
B. intermedius 581	29 ± 5	4.2 ± 0.4	176 ± 7
S. salivarius SS2	20 ± 3	0.3 ± 0.2	3 ± 0.1
S. salivarius 9GS2-R	46 ± 1	0.6 ± 0.1	5 ± 0.1
S. mutans JBP	34 ± 2	1.4 ± 0.1	62 ± 4
S. mutans MT3	27 ± 1	1.2 ± 0.1	55 ± 4
S. mutans BHT	36 ± 2	1.1 ± 0.1	38 ± 3
B. melaninogenicus D23	46 ± 2	1.2 ± 0.2	23 ± 1
B. melaninogenicus 287	61 ± 5	1.4 ± 0.1	42 ± 3
Hydrophilic strains			
A. actinomycetemcomitans 29253	100 ± 1	0.7 ± 0.1	25 ± 2
A. actinomycetemcomitans Y4	84 ± 4	ND[a]	31 ± 3

[a] ND, Not determined.

and *A. viscosus* LY7 cells adsorbed comparably to pellicles prepared from control or from lipid-depleted saliva (Table 2).

The lipid-depleted saliva was found to agglutinate washed cells of *S. mutans* JBP, *S. sanguis* FC-1, and *A. viscosus* LY7 in comparable titers as untreated whole saliva. Also, when the chloroform-methanol extracts were dried under N_2 and suspended in buffered KCl by sonic oscillation, the emulsions did not cause bacterial aggregation.

Analyses of adsorption isotherms indicated that the pellicles formed from lipid-depleted saliva contained increased numbers of binding

TABLE 2. Bacterial attachment to HA beads treated with whole saliva or chloroform-methanol-extracted saliva

Organism	Bacteria (× 10^6) available in reaction mixture	No. of bacteria ± SE (× 10^6) attached to 5 mg of HA treated with:	
		Whole saliva	$CHCl_3$-MeOH-extracted saliva
S. mutans JBP	2.5	0.17 ± 0.05	0.25 ± 0.03
	20.0	1.11 ± 0.04	1.84 ± 0.08[a]
S. mutans BHT	2.5	0.06 ± 0.01	0.10 ± 0.01[a]
	20.0	0.39 ± 0.03	0.77 ± 0.02[a]
S. sanguis FC-1	2.5	1.09 ± 0.01	1.22 ± 0.03
	20.0	8.0 ± 0.51	6.85 ± 0.57
S. sanguis C5	2.5	1.72 ± 0.05	1.79 ± 0.01
	20.0	9.55 ± 2.3	9.75 ± 0.01
A. viscosus LY7	2.5	1.81 ± 0.02	1.73 ± 0.06
	20.0	10.20 ± 0.05	11.20 ± 0.02

[a] $P < 0.05$ different from whole-saliva control.

TABLE 3. Adsorption parameters[a] for bacterial attachment to HA beads treated with whole saliva or with chloroform-methanol-extracted saliva

Organism	HA treated with:	Correlation coefficient	No. of binding sites ± SE ($\times 10^6$)	Affinity (ml/cell) ± SE ($\times 10^{-9}$)
S. mutans JBP	Whole saliva	0.96	1.32 ± 0.06	7.8 ± 0.3
	Extracted saliva	0.89	2.16 ± 0.18[b]	8.9 ± 0.1
S. mutans BHT	Whole saliva	0.90	1.32 ± 0.10	2.6 ± 0.2
	Extracted saliva	0.73	5.99 ± 1.4[b]	0.9 ± 0.2[b]
S. sanguis FC-1	Whole saliva	0.89	26.3 ± 2.0	4.7 ± 0.3
	Extracted saliva	0.99	15.9 ± 1.5[b]	10.4 ± 0.9[b]
S. sanguis C5	Whole saliva	0.99	11.5 ± 0.08	91.4 ± 2.0
	Extracted saliva	0.99	11.1 ± 0.14	100.0 ± 4.9
A. viscosus LY7	Whole saliva	0.99	12.9 ± 0.13	53.9 ± 1.1
	Extracted saliva	0.98	14.4 ± 0.36	49.1 ± 2.2

[a] Determined over the range of 1×10^7 to 20×10^7 bacteria per ml.
[b] $P < 0.05$ different from whole saliva.

sites for both *S. mutans* strains studied, and this likely accounts for their enhanced adsorption (Table 3). The affinity of *S. mutans* JBP to both types of pellicles was similar, but the affinity of strain BHT was somewhat lower to pellicles prepared from the lipid-depleted saliva. It is of interest to note that the affinity of strain BHT (serotype *b*) was significantly lower than that of *S. mutans* JBP (serotype *c*) since serotype *b* strains are infrequently isolated from humans (17).

Estimates of the numbers of binding sites and the affinities for *S. sanguis* strain FC-1 also showed statistically significant differences between pellicles prepared from whole saliva and those formed from lipid-depleted saliva, but no differences were noted for *S. sanguis* strain C5 or *A. viscosus* strain LY7 (Table 3).

Bacterial adsorption to pellicles prepared from octyl and phenyl Sepharose-absorbed saliva. Absorption with 10% (vol/vol) phenyl or octyl Sepharose reduced the protein content of saliva by almost half (Table 4), whereas absorption with 10% (vol/vol) Sepharose 4B removed only 2% of the salivary protein. This suggests that a significant proportion of salivary macromolecules contains hydrophobic regions which bind to hydrophobic adsorbents. The numbers of *A. viscosus* LY7, *S. sanguis* FC-1, and *B. gingivalis* 381 cells which attached to pellicles prepared from octyl or phenyl Sepharose-absorbed saliva were similar to those which attached to control pellicles (Table 4). However, significantly higher numbers of *B. intermedius* 581 attached to pellicles prepared from the absorbed saliva.

Absorption of saliva with increasing amounts of octyl or phenyl Sepharose removed additional protein (Table 4). Pellicles prepared from saliva absorbed with 40% (vol/vol) octyl or phenyl Sepharose adsorbed fewer cells of *S. sanguis*

TABLE 4. Effect of absorption of saliva with phenyl or octyl Sepharose on bacterial attachment to experimental pellicles

Pellicles prepared from saliva absorbed with (%, vol/vol):	Protein content (mg/ml)	No. of bacteria ± SE ($\times 10^6$) attached to 5 mg of S-HA			
		A. viscosus LY7	*S. sanguis* FC-1	*B. gingivalis* 381	*B. intermedius* 581
Unabsorbed control	0.47	8.9 ± 0.2	2.3 ± 0.1	6.7 ± 0.2	1.3 ± 0.2
Phenyl Sepharose, 10	0.23	8.5 ± 0.1	2.6 ± 0.6	8.1 ± 0.6	4.4 ± 0.1[a]
Phenyl Sepharose, 20	0.18	8.3 ± 0.3	1.7 ± 0.1[a]	7.9 ± 0.1	4.2 ± 0.2[a]
Phenyl Sepharose, 40	0.13	7.7 ± 0.2[a]	0.9 ± 0.1[a]	7.7 ± 0.2	4.0 ± 0.2[a]
Octyl Sepharose, 10	0.25	8.0 ± 0.2	2.4 ± 0.5	7.5 ± 0.1	3.3 ± 0.8[a]
Octyl Sepharose, 20	0.17	7.6 ± 0.3	1.4 ± 0.1[a]	7.8 ± 0.3	3.4 ± 0.1[a]
Ocytl Sepharose, 40	0.12	7.7 ± 0.1[a]	1.0 ± 0.1[a]	7.3 ± 0.1	4.2 ± 0.2[a]

[a] $P < 0.05$ different from pellicles prepared from unabsorbed saliva.

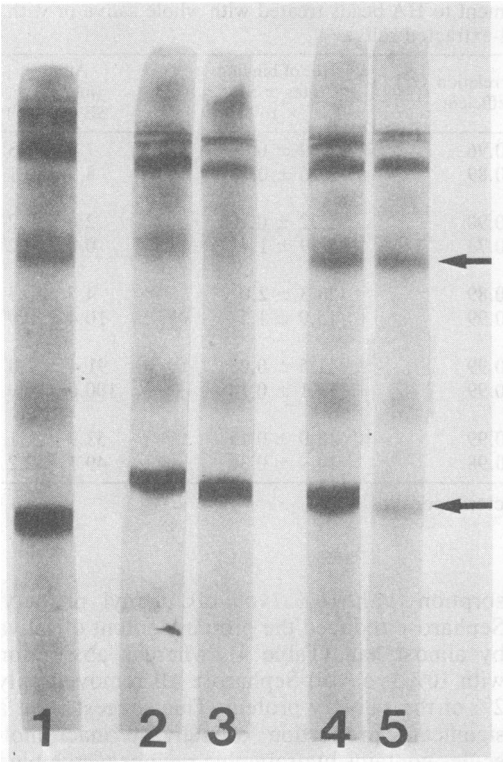

FIG. 1. Polyacrylamide gel electrophoretic analysis of 100-μl samples of saliva before and after absorption with phenyl or octyl Sepharose. Lane 1, Unabsorbed saliva; lane 2, saliva absorbed with 10% (vol/vol) phenyl Sepharose; lane 3, saliva absorbed with 40% (vol/vol) phenyl Sepharose; lane 4, saliva absorbed with 10% (vol/vol) octyl Sepharose; lane 5, saliva absorbed with 40% (vol/vol) octyl Sepharose. Note that absorption with phenyl Sepharose removed components in the amylase region (upper arrow) more effectively than did absorption with octyl Sepharose, whereas the latter adsorbent removed more material in the albumin region (lower arrow).

FC-1 and *A. viscosus* LY7. However, such pellicles still adsorbed comparable numbers of *B. gingivalis* 381 cells as control pellicles (Table 4).

Polyacrylamide gel electrophoretic analyses indicated that absorption with 10% (vol/vol) of either hydrophobic adsorbent reduced the concentration of some fast-migrating, lightly staining components to undetectable levels, but most of the major salivary constitutents were still evident (Fig. 1). Samples which had been absorbed with 40% (vol/vol) of the adsorbents appeared to have reduced concentrations of most components. Some selectivity was evident between the two adsorbents. Adsorption with 40% (vol/vol) octyl Sepharose removed almost all of a strongly staining component which migrated in the albumin region (15), whereas absorption with 40% (vol/vol) phenyl Sepharose had much less of an effect (Fig. 1). In contrast, the latter adsorbent removed almost all of a slower-migtrating component in the region of the gel where salivary amylases are found (23).

DISCUSSION

Adherence to hexadecane has been used in several recent investigations as a measure of the hydrophobic surface properties of bacteria (12, 18, 19, 25). In preliminary experiments, we noted that the extent of bacterial attachment to hexadecane was influenced by a variety of experimental variables. The type and size of test tube, the volume of bacterial cell suspension, the quantity of hexadecane, the composition of the buffer used, the temperature, and the rate and duration of mixing all influenced the values obtained. Because various investigators have used different experimental modifications of the basic procedure described by Rosenberg et al. (18), the values of hydrophobicity obtained in different studies may not be directly comparable. However, the values for the hydrophobic properties of different strains within a given investigation should be relative to each other.

The present study has shown that strains of prominent oral bacteria differ in their relative hydrophobic surface properties. Such differences have also been noted by others (12, 14, 25). In view of the limited number of strains assayed, the data obtained do not permit different oral species to be ranked according to their hydrophobicities. However, in general, strains which attached well to hexadecane also attached in high numbers to experimental salivary pellicles. This correlation was noted when the organisms were assayed at low or high cell concentrations. This suggests that hydrophobic interactions play a role in the attachment of a variety of oral bacteria to salivary pellicles, and such interactions may involve high- and low-affinity binding sites (6). In some instances, differences were noted in the hydrophobicity of different strains of the same species. For example, *S. salivarius* strain 9GS2R proved less hydrophobic than strain SS2, but both strains attached in relatively low numbers to S-HA. The relatively weak adherence of *S. salivarius* strains to hexadecane and the variation noted in the hydrophobic properties of various streptococci are consistent with previous studies (12, 14, 25).

Few of the binding sites for bacteria on host tissues have been characterized. Recently, globoside glycolipids have been suggested to serve as a class of epithelial cell receptors for strains of *Escherichia coli* involved in urinary tract infections (9, 10, 19). Since human saliva has been shown to contain neutral and acidic glyco-

lipids, triglycerides, and free fatty acids (21, 22), it was of interest to determine whether these compounds served as hydrophobic binding sites in experimental salivary pellicles for the bacteria studied. Therefore, we compared the ability of *S. mutans*, *S. sanguis*, and *A. viscosus* strains to adsorb to pellicles prepared from clarified whole human saliva and from the same saliva which had been depleted of lipids by chloroform-methanol extraction. Two strains of *S. mutans* studied attached in significantly higher numbers to pellicles prepared from chloroform-methanol-extracted saliva as compared with pellicles formed from untreated whole saliva. Adsorption isotherms suggested that this increased binding was likely due to the presence of higher numbers of binding sites in the lipid-depleted pellicles. The strains of *S. sanguis* and *A. viscosus* studied adsorbed comparably to both types of pellicles, even though the extraction procedure probably caused significant denaturation of salivary proteins. Thus, it appears that salivary lipids do not serve as a class of hydrophobic binding sites for the organisms studied. In fact, the enhanced adsorption of *S. mutans* to pellicles prepared from lipid-depleted saliva argues that lipids may partially occlude the sites to which these organisms attach. In this manner, salivary lipids could serve a protective function in *S. mutans*-induced dental caries.

To determine whether there were unique highly hydrophobic salivary macromolecules which could serve as receptors for hydrophobic bacteria in experimental pellicles, experiments were performed in which samples of clarified human saliva were absorbed with different concentrations of two hydrophobic adsorbents. Absorption with 10% (vol/vol) of either octyl or phenyl Sepharose reduced the protein content of saliva by approximately 50%. However, highly hydrophobic organisms such as *A. viscosus*, *S. sanguis*, and *B. gingivalis* 381 still adsorbed to pellicles prepared from such absorbed saliva comparably to control pellicles. These observations argue against the possibility that unique highly hydrophobic salivary macromolecules serve as essential receptors for the organisms studied. In fact, significantly increased numbers of *B. intermedius* 581 attached to pellicles prepared from saliva which had been absorbed with 10% of either hydrophobic adsorbent. Increasing the concentration of the two adsorbents to 40% (vol/vol) removed additional protein from saliva, and statistically fewer cells of *A. viscosus* LY7 and *S. sanguis* FC-1 attached to pellicles prepared from it. However, polyacrylamide gel electrophoretic analysis revealed that the concentration of most salivary components was reduced by this procedure. The array and diversity of the salivary constitutents removed by absorption with 40% of these adsorbents and the comparatively weak effect that this had on bacterial attachment further suggest that specific hydrophobic salivary macromolecules are not likely required for the attachment of the organisms studied.

Although there was a general correlation between the hydrophobicity of the bacterial strains studied and their adherence to experimental pellicles, it seems unlikely that hydrophobic interactions per se can account for the highly specific way in which bacteria attach to teeth and other oral tissues. For example, *S. salivarius* strains attach poorly to teeth and to experimental pellicles, but they adhere in high numbers to human oral epithelial cells. Furthermore, *S. salivarius* cells attach well to the tongues of humans, but not to those of rodents. Such observations suggest that mechanisms in addition to hydrophobic interactions are probably involved in bacterial adherence to host tissues. This view is consistent with that of Nesbitt and co-workers, who previously suggested that interactions between complementary macromolecules as well as hydrophobic bonding are likely involved in the attachment of *S. sanguis* cells to salivary pellicles (12, 13).

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