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Adherence of Mutans Streptococci to Other Oral Bacteria

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Adherence of mutans streptococci to strains of *Actinomyces viscosus*, *Streptococcus sanguis*, and *Streptococcus mitis* immobilized on a nitrocellulose membrane was measured. Strains of *Streptococcus mutans*, *S. sobrinus*, and *S. rattus* bound in a lactose-independent manner to a variety of the actinomyces and streptococci. Most of these reactions could proceed in the presence of whole saliva although adherence of *S. rattus* BHT to the streptococci was inhibited by salivary molecules. In contrast, adherence of *S. mutans* 10449 and KPSK2 to *A. viscosus*, *S. sanguis*, and *S. mitis* was enhanced by salivary molecules. *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 binding to *A. viscosus* NC3 and *S. sanguis* G9B exhibited saturation kinetics. Adherence to *A. viscosus* NC3 was of a higher avidity than adherence to *S. sanguis* G9B. Attachment of *S. mutans* KPSK2 to *S. sanguis* G9B and of *S. mutans* OMZ 176 to *A. viscosus* NC3 and *S. sanguis* G9B was inhibited by heat treatment of the mutans streptococci. Attachment of *S. mutans* KPSK2 to *A. viscosus* NC3 and of *S. rattus* FA-1 to *A. viscosus* NC3 and *S. sanguis* G9B was unaffected by heat. These observations suggest that the mutans streptococci can adhere to a variety of early plaque bacteria by several distinct mechanisms. Such interactions may be important in the colonization of tooth surfaces by the mutans streptococci.

The mutans streptococci are considered important pathogens in dental caries. In the oral cavity the mutans streptococci are found almost exclusively on the teeth; however, the mechanisms by which they initially colonize tooth surfaces are poorly understood. It is generally believed that initial attachment entails a dextran-independent, specific interaction between bacterial cell wall proteins and salivary glycoproteins in the tooth pellicle. Subsequently, if sucrose is present, dextran-mediated interactions allow further accumulation (11, 28). A number of potential salivary adhesins have been identified in the mutans streptococci. These include the antigen B-related molecules (10, 13, 23, 24, 26) and a 74-kilodalton surface protein (1). It is well established that these molecules can bind to salivary components (22, 25); however, definitive evidence that they function as adhesins in vivo has yet to be obtained. Indeed, adhesion of the mutans streptococci to saliva-coated hydroxyapatite, an in vitro model of the tooth surface, is of relatively low affinity and can exhibit nonsaturation binding kinetics (2, 7, 29). Thus, initial attachment of the mutans streptococci to tooth surfaces may not depend on complementary interactions between streptococcal and salivary molecules.

An alternative to the hypothesis that the mutans streptococci attach to the tooth pellicle is that the organisms adhere to the plaque already established on the tooth surface. The concept of interspecies binding as an important colonization mechanism was first proposed by Bladen et al. (3) and Gibbons and Nygaard (12). Following these reports a wide variety of interbacterial binding interactions was demonstrated (16, 17, 21). Studies of the mutans streptococci, however, have revealed a very limited range of interspecies binding. In a comprehensive survey of strains representing all eight serotypes of the mutans streptococci, Crowley et al. (8) observed significant binding only between strains of *Streptococcus cricetus* (serotype a) and certain strains of *Actinomyces naeslundii* and *A. odontolyticus*. Similarly, other studies have found that the mutans streptococci bind either very weakly or not at all to common plaque bacteria (5, 9, 12). These investigations have relied on the production of interspecies coaggregates as an indication of bacterial binding. However, the formation of coaggregates is not an inevitable corollary of interbacterial binding; therefore, coaggregation assays have the potential to produce false-negative results. In this study, an assay to measure binding directly between bacteria was developed. This assay was used to demonstrate interspecies adherence of strains of the mutans streptococci to *Streptococcus sanguis*, *S. mitis*, and *Actinomyces viscosus*, major constituents of early bacterial plaque.

MATERIALS AND METHODS

Bacteria and culture conditions. *S. mutans* GS-5, 10449, KPSK2 (serotype c), and LM-7 (serotype e), *Streptococcus sobrinus* OMZ 176 (serotype d) and 6715 (serotype g), and *Streptococcus rattus* BHT and FA-1 (serotype b) were from the culture collection maintained at the School of Dental Medicine, University of Pennsylvania. *S. sanguis* G9B, CC5A, M5, and Challis and *S. mitis* 10557 are oral isolates also from the School of Dental Medicine, University of Pennsylvania. *A. viscosus* NC3, EG4, CW171, and JK46 are oral isolates provided by B. J. Moncla, University of Washington. Stock cultures were maintained at −70°C in Trypticase peptone broth (BBL Microbiology Systems, Cockeysville, Md.) containing 15% glycerol. Cells were grown overnight under anaerobic conditions (85% N_2, 10% H_2, 5% CO_2) at 37°C in Trypticase peptone broth supplemented with 5 g of yeast extract (Difco Laboratories, Detroit, Mich.) per liter and 0.5% glucose. Numbers of bacteria were determined in a Klett-Summerson photometer previously calibrated for each species by microscopic counting of cells in a Petroff-Hausser chamber. To metabolically label bacteria, 10 μCi of [^{32}P]- or [^{3}H]thymidine (Amersham Corp. Arlington Heights, Ill.) per ml was added to the medium. Resulting specific activities varied between strains and from day to day, ranging between 8×10^{-5} and 2×10^{-4} cpm per cell.

Binding assay. An interbacterial binding assay was developed to measure the adherence of mutans streptococci (test

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organisms) to *S. sanguis*, *S. mitis*, and *A. viscosus* (base organisms) immobilized on a solid support. *S. sanguis*, *S. mitis*, and *A. viscosus* strains were harvested and washed twice in buffered KCl (5 mM KCl, 2 mM K_2PO_4, 1 mM $CaCl_2$ [pH 6.0]) by centrifugation (10,000 × *g*, 10 min) and then suspended in buffered KCl. Samples (50 μl) of the cell suspensions were added to individual wells of a dot blot apparatus (Bio-Rad Laboratories, Richmond, Calif.) containing a nitrocellulose membrane (0.45-μm pore size; Schleicher & Schuell Co., Keene, N.H.), and a vacuum was applied. The nitrocellulose with dots of immobilized bacteria (base organism blot) was removed and washed three times (15 min each) in buffered KCl containing 0.1% Tween 20 (KCl-Tween) to remove loosely bound organisms. In preliminary experiments to determine the numbers of base organisms that attach to the nitrocellulose and are thus available for binding to the test organisms, radiolabeled base organisms were employed. Subsequently, when examining binding of mutans streptococci to the base organisms, unlabeled *S. sanguis*, *S. mitis*, and *A. viscosus* cells were used. The base organism blot can be used immediately in the binding assay or stored for up to a week at 4°C in a humid chamber without loss of binding ability.

Radiolabeled mutans streptococci were harvested, washed twice in buffered KCl by centrifugation (10,000 × *g*, 10 min), and then suspended in KCl-Tween (3 ml). The bacteria were added to base organism blots and rotated for 2.5 h at 37°C. Unbound cells were decanted, and the blot was washed four times (15 min each) in KCl-Tween. The experimental areas of the nitrocellulose were excised and examined by scintillation spectroscopy (ScintiVerse II cocktail; Fisher Scientific Co., Kent, Wash.). Numbers of test organisms bound to the base were calculated from the amount of radioactivity (counts per minute) associated with the nitrocellulose. Background nonspecific binding of test organisms was assessed by excising equivalent areas of naked nitrocellulose, which were in contact with the test organisms during the assay, and measuring the numbers of bacteria attached.

Effect of saliva on interspecies binding. Whole paraffin-stimulated saliva was collected from several healthy volunteers, pooled, clarified by centrifugation (10,000 × *g*, 10 min), and stored at −20°C. Base organism blots were incubated with saliva for 1 h at 37°C and then washed three times (15 min each) in buffered KCl. Washed mutans streptococci were suspended in saliva at a concentration of 2×10^8 cells per ml of saliva and incubated at 37°C for 1 h. The cells were harvested, suspended in KCl-Tween, and sonicated briefly to disperse saliva-induced aggregates. Microscopic examination revealed that this mild sonication did not cause breakup of streptococcal chains. The saliva-treated mutans streptococci were then tested for adherence to the saliva-treated base organisms and for nonspecific binding to the nitrocellulose as described above.

Effect of lactose on interspecies binding. To examine potential inhibition of binding by lactose, test and base organisms were preincubated in buffered KCl containing lactose at a final concentration of 0.1 M for 1 h at 37°C. Lactose (0.1 M final concentration) was also included in the KCl-Tween during the binding assay.

Heat treatment of mutans streptococci. Mutans streptococci were suspended in buffered KCl at a concentration of 4×10^9 cells per ml and heated at 85°C for 45 min. Control cells were held on ice for 45 min. The bacteria were diluted to 4×10^8 cells per ml in KCl-Tween and tested for binding as described above. Heat treatment did not affect the specific activity of the cells.

Coaggregation. Mutans streptococci were tested for coaggregation with *S. sanguis*, *S. mitis*, and *A. viscosus* essentially as described by Cisar et al. (5). Bacteria were suspended to 2×10^9 cells per ml in buffered KCl, and equal volumes (400 μl) of two bacterial suspensions were mixed together. Controls containing 200 μl of each suspension alone made up to 400 μl with KCl were included. After 1 and 24 h at room temperature, coaggregation was scored on a 0 to +4 scale, where 0 represented no coaggregation and +4 represented maximal coaggregation (5).

RESULTS

Development of the base organism blot. The ability of *S. sanguis*, *S. mitis*, and *A. viscosus* to adhere to the nitrocellulose support over a range of input cell concentrations was determined. The maximum number of these base organisms that would attach to the nitrocellulose was $2.0 \times 10^8 \pm 0.38 \times 10^8$ (standard deviation; $n = 6$). This value was achieved at an input concentration of 1×10^9 cells in 50 μl for *S. sanguis* and *S. mitis* and 6×10^8 cells in 50 μl for *A. viscosus*. A comparison of the numbers of base organisms bound after the blot was washed three times in KCl-Tween with the results after washing six times with a 2.5-h incubation at 37°C between washes three and four revealed no significant differences. These latter conditions more closely mimic the events that occur during an interbacterial binding assay. Treating the base organism blot with saliva also did not cause any loss of bacteria. Thus, under the conditions of the assay, binding of *S. sanguis*, *S. mitis*, and *A. viscosus* to the nitrocellulose base is essentially irreversible. In all subsequent experiments base organism blots were prepared to contain 2×10^8 cells.

As a positive control for interbacterial binding, strains of *A. viscosus* and *S. sanguis* that coaggregated were tested in the blot assay. At an input cell concentration of 4×10^8 cells per ml, 18 to 25% of *A. viscosus* NC3 and CW171 bound to *S. sanguis* G9B and CC5A.

Adherence of mutans streptococci to *S. sanguis*, *S. mitis*, and *A. viscosus*. The mutans streptococci demonstrated a variety of binding patterns with the base organisms (Table 1). *S. mutans* GS-5 (serotype c) adhered to *A. viscosus* NC3, EG4, and CW171 but not to strain JK46 or to any of the streptococcal strains. *S. mutans* 10449 (serotype c) bound to *A. viscosus* NC3 and CW171 as well as to *S. sanguis* G9B and *S. mitis* 10557. *S. mutans* KPSK2 (serotype c) showed a binding pattern similar to that of 10449, except that KPSK2 did not adhere to *S. mitis* 10557. *S. mutans* LM-7 (serotype e) attached to all of the actinomyces tested but not to any of the streptococci. *S. sobrinus* OMZ 176 (serotype d) bound to all of the actinomyces and streptococci, whereas *S. sobrinus* 6715 (serotype g) bound to all of the streptococci but to only one strain of *A. viscosus*. *S. rattus* BHT and FA-1 (serotype b) bound to all of the base organisms. The ratio of bound mutans streptococci cells to base organism cells varied between 1:15 and 1:5 for the *S. mutans* and *S. sobrinus* strains and between 1:8 and 1:1.3 for the *S. rattus* strains.

Interactions between the mutans streptococci and *S. sanguis*, *S. mitis*, and *A. viscosus* were also investigated in the coaggregation assay. In contrast to the results obtained in the blot assay, none of the mutans streptococci coaggregated with any of the streptococci or actinomyces with the exception of *S. sobrinus* 6715, which coaggregated weakly (+1) with *A. viscosus* NC3, EG4, and JK46.

Effect of saliva on the adherence of mutans streptococci to *S. sanguis*, *S. mitis*, and *A. viscosus*. To examine the influence of

TABLE 1. Adherence of mutans streptococci[a] to *A. viscosus*, *S. sanguis*, and *S. mitis*

Strain of mutans streptococci	Mean ± SD (n = 6) no. (10^7) of mutans streptococci bound to:								
	A. viscous				*S. sanguis*				*S. mitis* 10557
	NC3	EG4	CW171	JK46	G9B	CC5A	M5	Challis	
S. mutans GS-5	2.7 ± 0.61	4.6 ± 0.40	1.5 ± 0.31	—[b]	—	—	—	—	—
S. mutans 10449	2.3 ± 0.75	—	2.1 ± 0.41	—	1.3 ± 0.45	—	—	—	2.7 ± 0.64
S. mutans KPSK2	4.1 ± 0.32	—	2.5 ± 0.47	—	2.1 ± 0.38	—	—	—	—
S. mutans LM-7	3.1 ± 0.36	1.3 ± 0.45	2.9 ± 0.61	3.0 ± 0.49	—	—	—	—	—
S. sobrinus OMZ 176	3.9 ± 0.40	3.0 ± 0.28	4.4 ± 0.17	2.9 ± 0.36	2.8 ± 0.31	3.2 ± 0.33	1.7 ± 0.28	1.3 ± 0.38	3.0 ± 0.25
S. sobrinus 6715	5.3 ± 0.38	—	—	—	1.7 ± 0.15	1.2 ± 0.23	3.5 ± 0.24	4.5 ± 0.36	3.9 ± 0.61
S. rattus BHT	15.1 ± 1.3	7.6 ± 0.74	12.2 ± 0.51	10.3 ± 0.26	5.6 ± 0.34	9.6 ± 0.27	7.9 ± 0.43	2.5 ± 0.37	8.5 ± 0.28
S. rattus FA-1	13.4 ± 0.87	8.2 ± 0.17	11.8 ± 0.99	10.9 ± 0.63	6.5 ± 0.14	14.3 ± 0.51	7.4 ± 0.45	5.9 ± 0.20	13.7 ± 0.64

[a] Input cell concentration: 4 × 10^8 cells per ml.
[b] —, Counts not significantly above background values.

saliva on binding, radiolabeled mutans streptococci and base organism blots were incubated with saliva before the binding assay. Pretreatment with saliva could either enhance, inhibit, or have no effect on the various adherence reactions (Table 2). Binding of *S. mutans* GS-5 and LM-7, *S. sobrinus* OMZ 176 and 6715, and *S. rattus* FA-1 was unaffected by saliva. *S. rattus* BHT adherence to *S. sanguis* and *S. mitis* was prevented by saliva, whereas adherence to *A. viscosus* was unchanged. In contrast, adherence of *S. mutans* 10449 and KPSK2 to all of the base organisms was markedly enhanced by saliva.

Effect of lactose on the adherence of mutans streptococci to *S. sanguis*, *S. mitis*, and *A. viscosus*. Preincubation with and the continuous presence of lactose had no effect on the binding of the mutans streptococci to any of the base organisms.

Binding curves of *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 adherence to *A. viscosus* NC3 and *S. sanguis* G9B. *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 were selected for further study, and adherence to *A. viscosus* NC3 and *S. sanguis* G9B was measured over a range of cell input concentrations (Fig. 1 and 2). Attachment of *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 to both base organisms was proportional to the input number up to an input cell concentration of about 6 × 10^8 to 8 × 10^8 cells per ml, where saturation was reached. At saturation, adherence of *S. rattus* FA-1 to both base organisms was greater than that of *S. sobrinus* OMZ 176, which in turn was greater than that of *S. mutans* KPSK2.

Effect of heat on the adherence of *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 to *A. viscosus* NC3 and *S. sanguis* G9B. Heat treatment of *S. mutans* KPSK2 reduced adherence to *S. sanguis* G9B by about 55% but had no

TABLE 2. Effect of saliva on the adherence of mutans streptococci[a] to *A. viscosus*, *S. sanguis*, and *S. mitis*

Strain of mutans streptococci	Pretreatment with saliva[b]	Mean ± SD (n = 3) no. (10^7) of mutans streptococci bound to:								
		A. viscosus				*S. sanguis*				*S. mitis* 10557
		NC3	EG4	CW171	JK46	G9B	CC5A	M5	Challis	
GS-5	−	3.3 ± 0.47	3.9 ± 0.38	1.8 ± 0.18	—[c]	—	—	—	—	—
	+	2.8 ± 0.35	4.2 ± 0.63	1.1 ± 0.27	—	—	—	—	—	—
10449	−	3.2 ± 0.37	—	1.4 ± 0.54	—	1.7 ± 0.42	—	—	—	2.9 ± 0.58
	+	11.0 ± 0.06	6.6 ± 0.71	12.1 ± 1.3	1.5 ± 0.38	5.9 ± 0.69	1.3 ± 0.30	2.4 ± 0.56	2.3 ± 0.34	6.2 ± 0.36
KPSK2	−	3.7 ± 0.49	—	2.8 ± 0.85	—	1.6 ± 0.70	—	—	—	—
	+	16.8 ± 1.9	2.4 ± 0.12	15.5 ± 2.5	6.2 ± 0.64	5.7 ± 0.58	1.1 ± 0.34	4.9 ± 0.29	14.6 ± 0.60	12.5 ± 0.95
LM-7	−	4.0 ± 0.61	1.7 ± 0.17	2.3 ± 0.29	2.5 ± 0.45	—	—	—	—	—
	+	2.9 ± 0.53	1.4 ± 0.24	1.8 ± 0.16	3.1 ± 0.77	—	—	—	—	—
OMZ176	−	4.8 ± 0.27	3.3 ± 0.13	4.9 ± 0.37	3.4 ± 0.41	3.4 ± 0.32	3.2 ± 0.62	1.6 ± 0.59	1.1 ± 0.40	3.5 ± 0.63
	+	4.1 ± 0.55	3.6 ± 0.24	4.3 ± 0.31	3.9 ± 0.25	3.7 ± 0.19	2.5 ± 0.46	1.3 ± 0.21	1.7 ± 0.38	2.8 ± 0.43
6715	−	5.9 ± 0.42	—	—	—	2.3 ± 0.39	1.8 ± 0.17	3.4 ± 0.39	5.1 ± 0.40	4.5 ± 0.56
	+	6.2 ± 0.57	—	—	—	2.3 ± 0.45	1.6 ± 0.36	3.8 ± 0.26	4.8 ± 0.33	4.0 ± 0.38
BHT	−	13.1 ± 1.6	8.5 ± 0.76	12.3 ± 1.3	11.4 ± 0.68	6.0 ± 0.61	10.6 ± 1.0	7.1 ± 0.20	2.2 ± 0.57	7.8 ± 0.73
	+	15.8 ± 1.2	7.9 ± 0.84	12.2 ± 0.61	11.8 ± 0.93	—	—	—	—	—
FA-1	−	12.5 ± 1.0	6.9 ± 0.74	13.1 ± 0.67	9.8 ± 0.59	6.7 ± 0.55	13.3 ± 0.84	8.2 ± 0.75	5.8 ± 0.16	12.4 ± 0.70
	+	12.1 ± 0.94	7.5 ± 0.65	12.7 ± 0.81	9.0 ± 0.66	5.9 ± 0.76	13.7 ± 0.95	8.8 ± 0.23	5.6 ± 0.19	11.9 ± 0.53

[a] Input cell concentration: 4 × 10^8 cells per ml. See Table 1 for species of mutans streptococci.
[b] +, Both test and base organisms were incubated with whole saliva before assay as described in Materials and Methods; −, control with no saliva.
[c] Counts not significantly above background values.

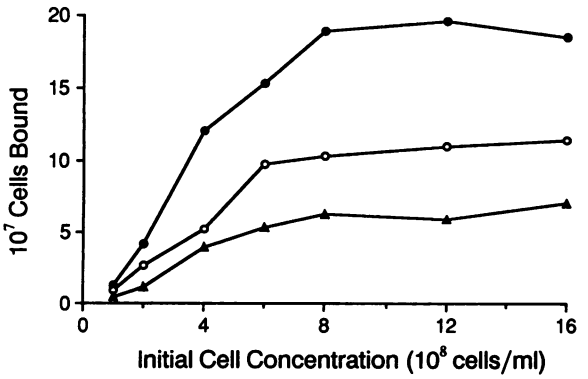

FIG. 1. Binding curves of *S. rattus* FA-1 (●), *S. sobrinus* OMZ 176 (○), and *S. mutans* KPSK2 (▲) adherence to *A. viscosus* NC3.

affect on adherence to *A. viscosus* NC3 (Table 3). Binding of *S. sobrinus* OMZ 176 to *A. viscosus* NC3 and *S. sanguis* G9B was reduced by 62 and 76%, respectively, after heat treatment. In contrast, attachment of *S. rattus* FA-1 to *A. viscosus* NC3 and *S. sanguis* G9B was not inhibited by heating.

TABLE 3. Effect of heat treatment on the adherence of mutans streptococci[a] to *A. viscosus* and *S. sanguis*

Strain of mutans streptococci	Heat treatment[b]	Mean ± SD[c] (n = 3) no. (10^7) of mutans streptococci bound to:	
		A. viscosus NC3	*S. sanguis* G9B
KPSK2	−	3.1 ± 0.49	2.2 ± 0.63
	+	3.7 ± 0.30	1.0 ± 0.17
OMZ 176	−	5.0 ± 0.33	1.7 ± 0.28
	+	1.9 ± 0.21	0.4 ± 0.05
FA-1	−	12.8 ± 0.52	5.3 ± 0.46
	+	11.6 ± 0.37	5.8 ± 0.39

[a] Input cell concentration: 4 × 10^8 cells per ml. See Table 1 for species.
[b] +, Mutans streptococci were heated to 85°C for 45 min before the assay; −, mutans streptococci were kept on ice for 45 min before the assay.

DISCUSSION

Bacterial colonization of clean tooth surfaces involves adherence to the salivary pellicle on the enamel surface. However, once a layer of bacteria has developed, subsequent colonization may depend on adherence to the organisms already established in the early plaque, mostly *A. viscosus*, *S. sanguis*, and *S. mitis*. Such interbacterial binding has been demonstrated between a wide variety of common plaque bacteria (17, 21), and its importance in vivo has been corroborated by electron microscopic observations of developing plaque (14) and colonization studies of gnotobiotic rats (30).

Previous investigations of adherence of the mutans streptococci to other oral bacteria have not revealed any significant interbacterial binding (5, 9, 12), with the exception of *S. cricetus*, which interacts with strains of *A. naeslundii* and *A. odontolyticus* (8). These studies employed coaggregation assays to investigate interbacterial binding, a procedure that entails mixing suspensions of the two test strains and assessing the degree of binding as a function of the amount of subsequent aggregation. This type of assay is somewhat artificial in that both species are tested in solution, whereas in vivo one of the organisms is immobilized on the tooth surface. More importantly, it relies on a phenomenon, aggregation, that is secondary to binding and may not occur if other conditions, such as the valence of the interaction, are not fulfilled. To circumvent these problems and directly measure binding between bacteria, investigators have utilized saliva-coated hydroxyapatite and agarose beads as supports for one of the test organisms (4, 18, 27). However, if the first strain does not form a continuous layer and the second strain has a high affinity for the support, binding of the second strain to exposed areas of the support surface may complicate interpretation of the results. To create a continuous layer of bacteria, Liljemark et al. (19) utilized a biological adhesive to attach organisms to tissue culture plates and enamel chips. The approach developed here, where one species is immobilized on a nitrocellulose membrane, offers a greater degree of simplicity and ease of manipulation for the study of interbacterial binding. Radiolabeling the test organism allows quantitation of the binding or, in the case of ^{32}P, visualization by autoradiography for rapid initial screening. Although it can not be stated with certainty that the base organisms form a continuous layer, the controls of naked nitrocellulose included in the assays showed no significant bacterial binding. Therefore, any exposed areas of nitrocellulose that do occur are adequately blocked by the Tween 20 in the assay buffers and do not provide attachment sites for the test organisms.

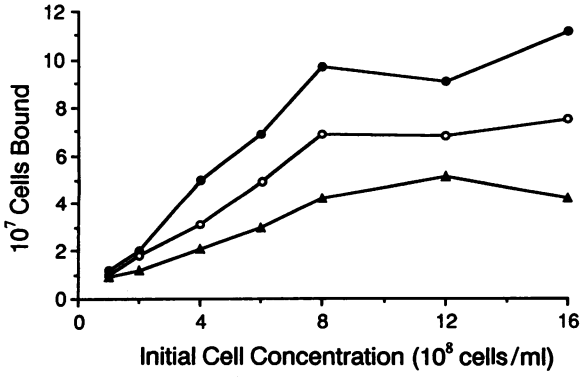

FIG. 2. Binding curves of *S. rattus* FA-1 (●), *S. sobrinus* OMZ 176 (○), and *S. mutans* KPSK2 (▲) adherence to *S. sanguis* G9B.

The utilization of a direct binding assay allowed the demonstration of a variety of adherence interactions between the mutans streptococci and *S. sanguis*, *S. mitis*, and *A. viscosus*. These interactions were not observed by coaggregation; thus, the blot assay may be a more sensitive detector of interbacterial binding than coaggregation. Alternatively, the mutans streptococci may have evolved mechanisms to prevent aggregation after initial binding to other species. The ability of bacteria to bind to each other without coaggregation may be ecologically advantageous, since the formation of coaggregates in suspension may prevent colonization by promoting clearance of the organisms from the mouth (8).

Most of the adherence reactions, with the exception of *S. rattus* BHT binding to *S. sanguis* and *S. mitis*, could proceed in the presence of saliva, indicating probable in vivo significance. Indeed, adherence of strains 10449 and KPSK2 (*S. mutans* serotype c) to all of the actinomyces and streptococci tested was enhanced by saliva. It is unclear at present whether adherence under these circumstances is mediated

by salivary molecules adsorbed to both test and base organisms or by adsorbed salivary molecules on one strain interacting with bacterial molecules on the other or whether saliva modulates the environment to enhance bacteria-bacteria binding. In contrast, adherence by *S. mutans* GS-5, also a serotype c strain, was unaffected by salivary molecules. It is interesting to note in this context that GS-5 has been found to express a defective form of the salivary binding protein PAc which lacks the component necessary for anchorage in the cell wall (23). Possible involvement of the antigen B-related molecules in these adherence reactions, however, would appear to be limited to the serotype c strains, since adherence of strains of serotypes e (*S. mutans*) and d and g (*S. sobrinus*), which are thought to produce wall-associated, antigen B-related molecules, was not enhanced by saliva.

Many previously described interspecies binding interactions between oral streptococci and actinomyces are mediated by lectinlike molecules with a specificity for galactosyl-containing receptors and thus can be inhibited by galactosides such as lactose (5, 17, 21). The reactions described here, however, are lactose independent and so would not appear to involve related mechanisms. Since the type 2 fimbriae are considered responsible for lactose-sensitive binding of *A. viscosus* (6), the participation of these components in the interactions is unlikely. Similarly, Crowley et al. (8) found that coaggregation of *S. cricetus* (serotype a) with certain actinomyces was not lactose reversible. Further work is required to determine whether the interactions have other sugar specificities.

Adherence of *S. mutans* KPSK2, *S. sobrinus* OMZ 176, and *S. rattus* FA-1 to *A. viscosus* NC3 and *S. sanguis* G9B was examined in more detail. For all of the test organisms, more cells adhered to *A. viscosus* than to *S. sanguis*. It would appear, therefore, that the mutans streptococci bind with a higher avidity to the actinomyces than to the streptococci. The binding curves for these organisms displayed saturation kinetics, suggesting that the binding is mediated by a limited number of complementary molecules on the bacterial surfaces and that the mutans streptococci do not bind to each other. Adherence of *S. sobrinus* OMZ 176 to *A. viscosus* NC3 and *S. sanguis* G9B and of *S. mutans* KPSK2 to *S. sanguis* G9B was inhibited by heat treatment, denoting the possible involvement of surface proteins of these mutans streptococci in attachment. However, adherence of *S. mutans* KPSK2 to *A. viscosus* NC3 and of *S. rattus* FA-1 to *A. viscosus* NC3 and *S. sanguis* G9B was unaffected by heat treatment, which would tend to preclude the involvement of surface proteins on the mutans streptococci but not the possibility of a role for surface proteins of *A. viscosus* and *S. sanguis*. The variable response of the mutans streptococci to heat treatment, along with the differences in base organism specificity and affinity displayed by most of the strains of mutans streptococci, implies that a number of distinct binding mechanisms are possessed by different strains. The precise nature and identity of the adhesins and receptors that mediate these binding reactions require further study.

S. mutans and *S. sobrinus* are the most common mutans streptococci isolated from the oral cavity (20). *S. rattus* is not isolated frequently from the oral flora, although it has been found in high proportions in various African populations (20). However, mitis salivarius-bacitracin agar, a medium widely used for the isolation of the mutans streptococci, inhibits the growth of *S. rattus* (15). Therefore the true incidence of *S. rattus* in the human oral cavity may be higher than currently indicated. Successful colonization of tooth surfaces depends on the outcome of an intricate series of interactions with host and other bacterial factors. The results presented here demonstrate that adherence of *S. mutans*, *S. sobrinus*, and *S. rattus* to various initial colonizers of plaque may be one important consideration in the establishment of these species in the mouth. Furthermore, the composition of the early plaque may play a role in determining which species of the mutans streptococci are able to attach to the tooth surface.

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