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Adherence of *Porphyromonas (Bacteroides) gingivalis* to *Streptococcus sanguis* In Vitro

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Intergeneric bacterial adherence is responsible for the complexity of the microbiota in human dental plaque and is believed to enable some extraneous bacteria to initially colonize the human oral cavity. Some current evidence indicates that *Streptococcus sanguis*, an early colonizer of teeth, enhances subsequent colonization by *Porphyromonas (Bacteroides) gingivalis*, a bacterium associated with advanced adult periodontitis. In this study, selected strains of *P. gingivalis* and *S. sanguis* were tested for their adherence activities in vitro. A differential filtration assay was devised in which one member of the test pair was radiolabeled. Heterogeneous aggregates that formed in mixed suspensions were collected on polycarbonate filters (8-μm pore size) and were washed free of individual bacteria and small homologous clumps. *P. gingivalis* 381, W50, JKG7, and 33277 adhered to *S. sanguis* G9B, M5, Challis 6, and 38. *P. gingivalis* A7A1-28 did not adhere well to *S. sanguis* under these conditions. More precise measurements of intergeneric adherence were obtained with an alternative assay with radiolabeled *P. gingivalis* and an artificial dental plaque composed of *S. sanguis* coupled to cyanogen bromide-activated agarose beads. CNBr-agarose was selected as the supporting matrix for the plaque because it was uniformly and permanently coated with *S. sanguis* and because *P. gingivalis* had negligible adherence activity for streptococcus-free beads. *P. gingivalis* W50 grown to the early stationary phase adhered to *S. sanguis*-coated beads in higher numbers than either midlogarithmic- or late-stationary-phase cells. Intergeneric adherence was not inhibited or reversed by the presence of lactose or other monosaccharides or disaccharides. Pretreatment of either bacterium with trypsin or proteinase K reduced subsequent adherence by 86 to 100%. Neuraminidase treatment of *P. gingivalis* caused 98% reduction of adherence, whereas similar treatment of *S. sanguis* caused only a 2% loss. Preincubation of *P. gingivalis* at 60°C for 30 min decreased subsequent adherence to *S. sanguis*-coated beads by 94%. Adherence was reduced by 96% when bacteria were assayed while suspended in human whole saliva or when pretreated with saliva and subsequently assayed in buffer. The concentration of whole human saliva required to inhibit 50% adherence in this assay was 23 μg per ml (1:200 dilution). Suspension of the bacteria in normal rabbit serum resulted in 94% inhibition of adherence. These data indicate that saliva and serum may be important host defense factors for controlling *Porphyromonas-Streptococcus* adherence.

The phenomenon of intergeneric bacterial adherence is believed to contribute to the formation and maturation of human dental plaque (4, 5, 10, 27–29). Several genera of oral bacteria appear to join this complex microbiota and persist by adhering to other bacteria already attached to tooth surfaces. The adherence mechanisms appear to involve complementary molecules on the opposing cell surfaces, some of which are lectin-carbohydrate in nature (3, 11, 15). To date, several agglutinating pairs of bacteria have been identified and studied (2, 9, 10, 20, 31).

Porphyromonas (Bacteroides) gingivalis (23) has received recent attention because of its occurrence in mature dental plaque and its prominent association with advanced adult periodontitis (24, 25, 30). The mechanisms by which these bacteria colonize tooth surfaces and the adjacent periodontal tissues have not been characterized. Early studies by Slots and Gibbons (24) showed that the introduction of an extraneous strain of *P. gingivalis* into the oral cavity of human volunteers resulted in rapid colonization of dental plaque but not of clean tooth enamel. Microscopic examination of bacterial suspensions mixed in vitro indicated that *P. gingivalis* adhered to several *Streptococcus* and *Actinomyces* species that are known early colonizers of tooth surfaces. A subsequent study by Kolenbrander et al. (13) with a flocculation assay found that selected strains of *P. gingivalis* did not form macroscopic agglutinates with selected strains of gram-positive plaque-forming bacteria in vitro. More recently, adherence of *P. gingivalis* with *Actinomyces viscosus* was detected in vitro by using radiolabeled bacteria in a hydroxyapatite bead assay (17, 22). The latter observation indicates that more sensitive assays are required to detect and quantitate bacterial adherence that results in formation of predominantly microscopic agglutinates.

The assay techniques described in this report were designed to measure the ability of *P. gingivalis* to adhere to *Streptococcus sanguis* under a variety of environmental conditions. In the solid-phase assay, streptococci are covalently coupled to agarose beads; the resulting biofilm is used as an adherence substrate for radiolabeled porphyromonads in the presence and absence of saliva and serum. The streptococcus-coated beads with adherent *P. gingivalis* are separated from planktonic bacteria by differential filtration. Because *P. gingivalis* has negligible binding affinity for untreated beads, essentially all of the radioactivity detected

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TABLE 1. Adherence of ^{3}H-labeled *S. sanguis*[a] to *P. gingivalis* as measured by a filtration assay

P. gingivalis strain	Source	Adherence[b] to *S. sanguis* strain:			
		G9B	M5	Challis 6	38
381	ATCC 2561	16 ± 6	28 ± 7	25 ± 11	26 ± 2
A7A1-28	ATCC 53977	1 ± 0.5	1 ± 0.7	6 ± 2	3 ± 0.4
W50	ATCC 53978	30 ± 8	8 ± 3	14 ± 8	28 ± 5
33277	ATCC 33277	19 ± 5	27 ± 3	42 ± 16	ND
JKG7	W. Loesche	23 ± 14	22 ± 3	26 ± 6	ND

[a] Kilian et al. (8) recently proposed that *S. sanguis* should be divided into *S. sanguis* (type strain 10556, serotype 1) and a new species, *S. gordonii* (type strain 10558, serotype 2). Accordingly, strains M5 and Challis 6 may be renamed *S. gordonii*. *S. sanguis* G9B and 38 will remain unchanged.

[b] Adherence indices ± standard deviations are calculated as the average quotient of heterologous and homologous adherence. Each value represents five to eight assay mixtures. ND, Not determined.

on the beads is a direct consequence of intergeneric adherence.

MATERIALS AND METHODS

Bacteria and culture conditions. *S. sanguis* strains (Table 1) were generously provided by Burton Rosan, University of Pennsylvania, Philadelphia. *P. gingivalis* (23) strains were obtained from the culture collection of the Oral Biology Department, State University of New York at Buffalo.

Streptococci were grown at 37°C in chemically defined medium (26) to the early-stationary phase of growth as determined by turbidity measurements at 600 nm. *P. gingivalis* was grown in 3% tryptic soy broth supplemented with 0.5% yeast extract (Difco Laboratories, Detroit, Mich.), 5 μg of hemin per ml, and 1 μg of vitamin K (Sigma Chemical Co., St. Louis, Mo.) per ml. The cultures were incubated at 34°C in an atmosphere of 5% CO_2, 10% H_2, and 85% N_2. Growth was monitored turbidimetrically, and bacteria were harvested by centrifugation at selected growth phases. Both bacterial populations were washed twice with phosphate-buffered saline (0.01 M sodium phosphate [pH 7.2] containing 0.15 M sodium chloride).

For experiments with radiolabeled *S. sanguis*, bacteria were grown in medium containing [^{3}H]adenine (ICN Biomedicals, Costa Mesa, Calif.) at 2.5 μCi/ml. *P. gingivalis* was radiolabeled by growing it in medium containing [*methyl*-^{3}H]thymidine (ICN) at 2.5 μCi/ml. Specific radioactivitiy (counts per minute per 10^8 cells) was approximately 25,000 for *P. gingivalis* and 80,000 for *S. sanguis*.

Adherence assays. Selected strains of *P. gingivalis* and *S. sanguis* were screened for adherence activity by using a differential filtration assay. Bacteria were washed twice with assay buffer (0.01 M sodium phosphate [pH 7.2], 50 mM potassium chloride, 0.1 mM magnesium chloride, 0.1 mM calcium chloride) and suspended to optical densities of 1.0 and 0.5 for *P. gingivalis* and *S. sanguis*, respectively. *S. sanguis* chains were dispersed with low-intensity ultrasonication in a Biosonic III (Bronwill Scientific, Rochester, N.Y.). For the adherence assays, 1 ml of radiolabeled *S. sanguis* (4×10^8 cells) suspension was combined with 0.5 ml of the *P. gingivalis* (8×10^8 cells) suspension and mixed at 22°C for 1 h. Bacterial agglutinates were separated from free bacteria by filtration on polycarbonate membranes with 8-μm pores (Nucleopore Corp.) After individual bacteria were washed through the filter with 20 ml of buffer (four aliquots), the radioactivity remaining on the filters was counted by liquid scintillation spectrometry. As a control for spontaneous self-agglutination, the two bacteria were incubated separately for 60 min and combined immediately before filtration. Interbacterial adherence is expressed as an index which is the quotient of the filter radioactivity obtained with the test mixture and that obtained with the control mixture.

A solid-phase assay was devised for quantitative measurement of intergeneric bacterial adherence and to permit evaluation of the modulating effects of saliva and serum. *S. sanguis* was suspended in 0.1 M sodium bicarbonate–0.15 M NaCl (pH 7.2) to an optical density of 2 at 600 nm. Hydrated cyanogen bromide-activated Sepharose 4B beads (1.5-ml packed volume) were added to 7.0 ml of the *S. sanguis* suspension and mixed for 10 min at 22°C by tumbling in a sealed vessel. Uniform coating of the beads with streptococci was facilitated by centrifuging the mixture at $2,000 \times g$ for 10 min. The sediment was suspended by gentle vortexing, and the centrifugation was repeated three times. The streptococcus-coated beads (strep-beads) were collected on a 25-mm polycarbonate filter membrane (8-μm pores) and washed four times with 5 ml of assay buffer. As a final wash, the strep-beads were suspended in 12 ml of assay buffer, allowed to settle for 1 h at 22°C, and then suspended in 3 ml. Preliminary experiments with radiolabeled streptococci showed that the bacteria were irreversibly bound to the beads. In the adherence assay, 0.5 ml of ^{3}H-labeled *P. gingivalis* suspension was mixed with 0.05 ml of strep-beads suspension or with 0.05 ml of the suspension of CNBr-agarose beads (negative control) for 1 h at 22°C. Preliminary experiments showed that blocking the activated CNBr beads with 0.5 M glycine did not change the already low binding of *P. gingivalis*. Thus, a blocking agent was not used in subsequent experiments. The assay mixtures were filtered on a 25-mm polycarbonate membrane (8-μm pores) to remove planktonic ^{3}H-labeled *P. gingivalis*. The filters and beads were washed four times with 5 ml of phosphate-buffered saline before radioactivity was counted by liquid scintillation spectrometry.

Collection and processing of saliva and serum. Unstimulated human whole saliva from a healthy, 28-year-old male (blood group A+) was collected on ice in tubes containing 2 ml of enzyme inhibitor solution (0.1 M Tris hydrochloride [pH 7.5], 2% disodium EDTA, 10% *n*-propanol, 2 mM phenylmethylsulfonyl fluoride). The 30 ml of saliva was clarified by centrifugation at $10,000 \times g$ for 30 min. Blood was obtained from six New Zealand White rabbits, and the sera were pooled. Serum complement was inactivated by heating at 60°C for 30 min. Protein concentration was determined by the method of Hartree (6) with bovine serum albumin as the standard.

Treatment of bacteria. The effects of saliva and serum on interbacterial adherence were evaluated in competitive inhibition and blocking experiments. For competitive inhibition, *P. gingivalis* and strep-beads were each suspended in undiluted serum or saliva for 30 min at 20°C and then combined for the assay. In the blocking experiment, the bacteria were separated from the host secretions by centrifugation after the 30-min incubation. After unbound host components were washed away with three changes of assay buffer, the bacteria were combined and assayed for adherence activities.

Bacteria were pretreated with selected enzymes to determine the chemical nature of the adhesins. Bacteria were suspended to an optical density at 600 nm of 1.0 in assay buffer containing 1 mg of the indicated enzymes per ml. Trypsin type XIII (Sigma) and proteinase K (protease XI;

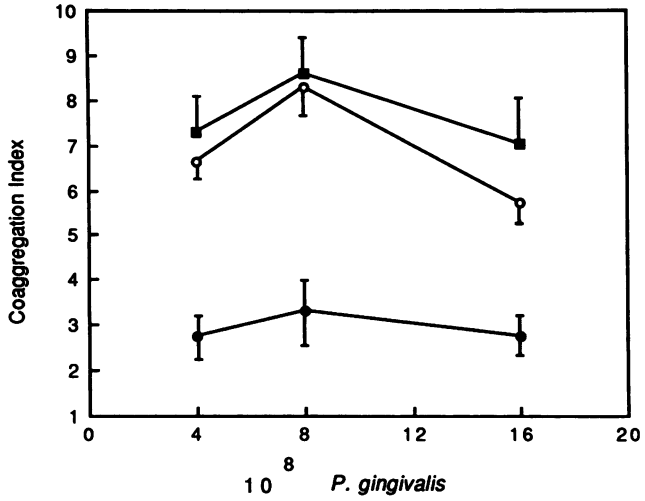

FIG. 1. Determination of the optimal cell ratio for coaggregation by *P. gingivalis* 381 and *S. sanguis* G9B. Numbers of *S. sanguis* cells per assay mixture: ●, 2×10^8; ○, 4×10^8; ■, 8×10^8.

Sigma) were used at pH 7.5, whereas neuraminidase type VI (Sigma) was used at pH 6.0. For controls, bacteria were incubated in buffer without enzyme at the indicated pHs. After 2 h at 37°C, the bacteria were harvested by centrifugation, washed three times with assay buffer, and evaluated for adherence activity by the differential filtration assay. Data are normalized to activities of the untreated controls.

Susceptibility of bacterial adhesins to denaturation with formaldehyde was also determined. Phosphate-buffered saline-washed bacteria were suspended to an optical density at 600 nm of 1.0 in phosphate-buffered saline–2% formaldehyde for 1 h at room temperature. The bacteria were then harvested by centrifugation, washed three times with coherence buffer, and assayed.

RESULTS

Screening of bacterial pairs. A differential filtration assay was used in initial experiments to detect and quantify intergeneric bacterial adherence (agglutination). Radiolabeled *S. sanguis* G9B was mixed with nonlabeled *P. gingivalis* 381 and incubated at room temperature for 60 min. The optimum numbers of bacteria from early-stationary-phase cultures were 4×10^8 *S. sanguis* and 8×10^8 *P. gingivalis* in the 1.5-ml assay mixture (Fig. 1). Visible agglutination of bacteria occurred in some of the mixed cell suspensions under these conditions; however, it was often difficult to distinguish between intergeneric agglutination and autoagglutination of *P. gingivalis* 381. Homogeneous suspensions of *P. gingivalis* 381, especially those above 10^9 cells per ml, produced visible agglutination upon standing. Adherence of *P. gingivalis* to *S. sanguis* was verified by microscopic examination of Gram-stained films. Enumeration of bacteria in small clumps indicated that 40 to 50% were streptococci.

Several strains of *P. gingivalis* were mixed with selected strains of *S. sanguis*, and the resulting intergeneric agglutination was measured by a differential filtration assay (Table 1). Agglutination occurred between all bacterial pairs, except between *P. gingivalis* A7A1-28 and *S. sanguis* G9B or M5. The agglutination indices of the active pairs ranged from 6 to 42, with the majority scoring between 20 and 30. Depending upon the bacterial pairs, the percentage of available radiolabeled cells retained by the filter was 0.2 to 1.0% for the control and 6 to 35% for test mixtures.

Adherence to strep-beads. Although the differential filtration assay served as a convenient means for quantitating intergeneric agglutination among bacteria suspended in buffered salts solutions, it was not considered appropriate when saliva or serum was included. The interaction of host proteins and glycoproteins with bacterial surfaces often results in cell agglutination, which would obscure detection of bacterially mediated adherence in this assay. Because intergeneric bacterial adherence occurs in vivo between cells bathed in saliva or coated with serum components that can leak from nearby gingival tissues, it is imperative that these host components be evaluated for their effects on interbacterial adherence in in vitro assays. Immobilization of streptococci on agarose beads avoids their agglutination by saliva and presents a plaquelike surface for *P. gingivalis* adherence. Scanning electron microscopic examination of strep-beads revealed a uniform biofilm of *S. sanguis* (Fig. 2). Small gaps in the sessile streptococci were detected occasionally on the surfaces of some beads. Calculations based upon binding of tritium-labeled *S. sanguis* cells revealed that an average of 600 streptococci were present on each 70-μm-diameter bead. Thus, the standard assay mixture contained 7.2×10^4 beads with 4.3×10^7 streptococci. Cyanogen bromide coupling of streptococci to agarose beads provided a stable and durable assay reagent. In radiolabel experiments, less than 2% of *S. sanguis* G9B cells detached from the beads during the manipulations of the adherence assay.

Radiolabeled *P. gingivalis* W50 was tested for its ability to adhere to *S. sanguis* G9B-coated agarose beads. *P. gingivalis* grown to the early-stationary phase adhered in higher numbers than did *P. gingivalis* from the midlogarithmic and late-stationary growth phases (Fig. 3). Importantly, the *P. gingivalis* did not adhere well to streptococcus-free beads; there was a 25-fold differential between the strep-bead values and the control bead values. Maximum adherence of *P. gingivalis* W50 to strep-beads occurred within 2 h at room temperature (Fig. 4). At the highest value (5500 cpm), 2.2×10^7 *P. gingivalis* cells were bound to beads containing 4.3×10^7 *S. sanguis*. When whole saliva was used as the suspending fluid in the assay, adherence of *P. gingivalis* W50 was reduced 95%. The inhibitory effect of saliva was unchanged over 18 h of incubation (Fig. 4).

Effects of saliva and serum. The inhibitory activity of human whole saliva and normal rabbit serum on adherence of *P. gingivalis* to *S. sanguis* is summarized in Fig. 5. A 50% inhibition of adherence was achieved with a 1:60 dilution of saliva and with a 1:500 dilution of normal rabbit serum. The protein concentrations of these solutions were 23 and 174 μg/ml, respectively. Thus, saliva appeared to be 7.5 times more effective than serum as an adherence inhibitor.

The inhibitory effects of saliva on interbacterial adherence (Fig. 5) indicate that saliva components bind to the bacterial surfaces and mask the adhesins that mediate attachment. To determine which bacterium was adversely effected by saliva, a blocking experiment was conducted in which one member of the pair was preincubated with undiluted saliva, washed with buffer, and then assayed with the untreated partner. Results for percent adherence ± standard deviation were as follows: no saliva treatment, 100% ± 19%; saliva-coated strep-beads, 14% ± 4%; saliva-coated *P. gingivalis* W50, 35% ± 11%; saliva treatment of both species, 11% ± 1% (data normalized to adherence values obtained with untreated bacteria [7,394 ± 1,386 cpm]). These data indicate

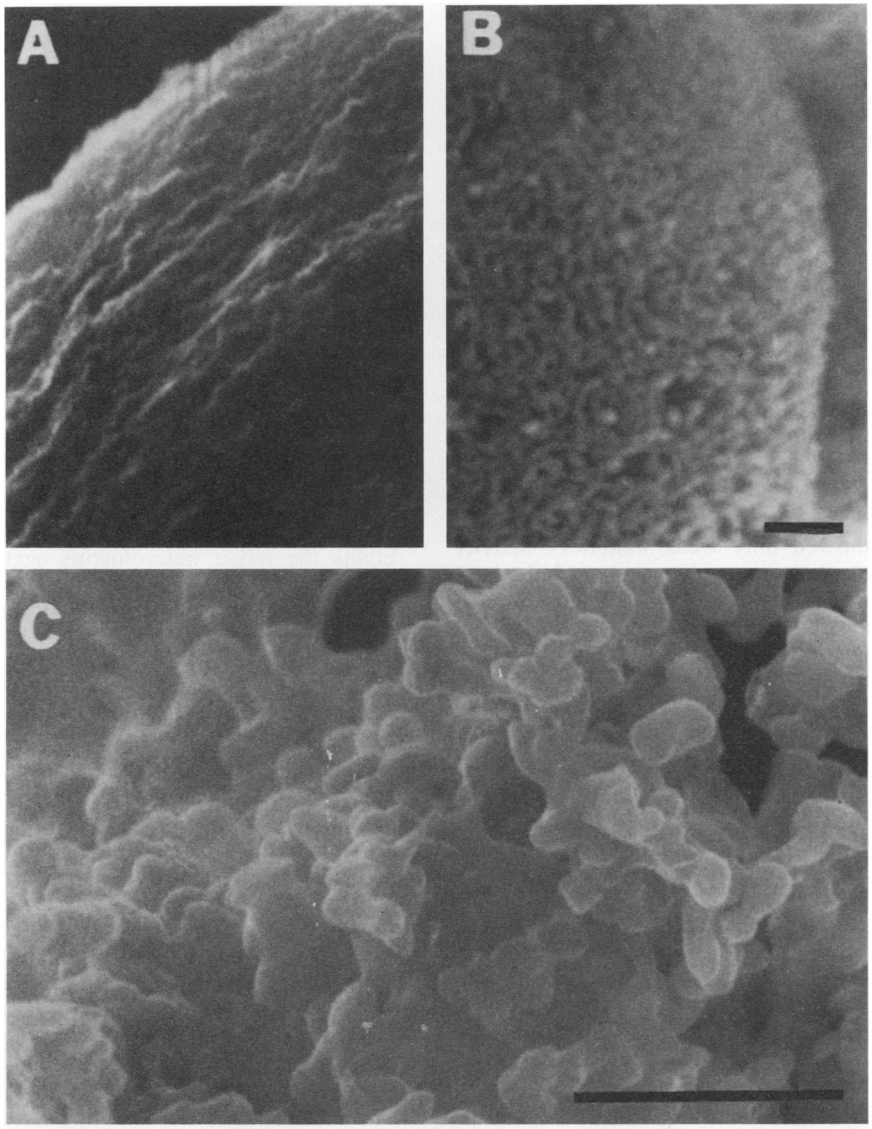

FIG. 2. Scanning electron photomicrographs of bare agarose beads (A) and agarose beads coated with *S. sanguis* G9B (B and C). Bars, 5 μm.

that saliva components bind to both cell surfaces and modulate subsequent interbacterial adherence.

Nature of adhesins. To identify the nature of cell surface components mediating interbacterial adherence, one member of the pair was pretreated with selected enzymes and tested against the untreated partner cells. Protease treatment of either bacterium resulted in >86% loss of adherence activity (Table 2). Neuraminidase caused 98% loss of activity in *P. gingivalis* but only 2% loss of *S. sanguis* activity. The effect of neuraminidase on *P. gingivalis* may be a result of nonenzymatic binding of protein to cell surfaces rather than enzymatic removal of sialic acid, since *N*-acetylneuraminic acid and *N*-acetylneuramin-lactose were only weak inhibitors of adherence (see below). The neuraminidase effects cannot be attributed to protease contamination of the enzyme preparation. In a separate experiment, a quantity of enzyme equivalent to that used to treat bacteria failed to digest radiolabeled bovine serum albumin.

Selected monosaccharides and disaccharides were tested

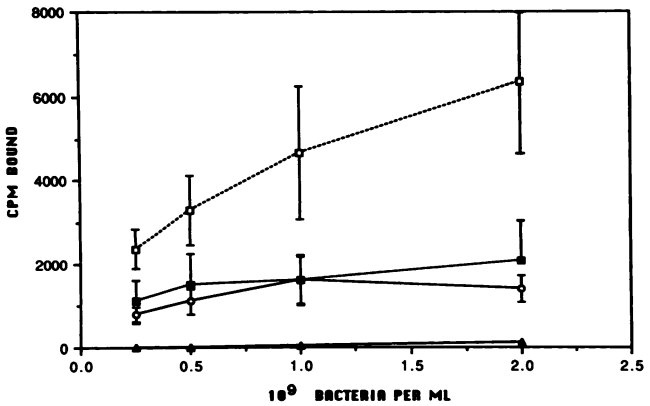

FIG. 3. Effect of growth phase on *P. gingivalis* W50 adherence to strep-beads: ○, logarithmic phase at 24 h; □, early-stationary phase at 48 h; ■, late-stationary phase at 72 h.

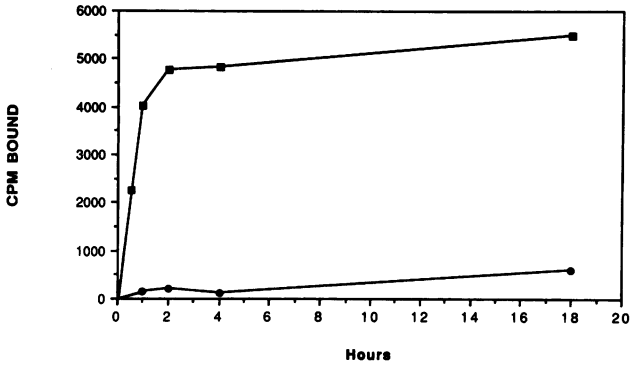

FIG. 4. Effect of saliva and time on *P. gingivalis* W50 adherence to strep-beads: ■, bacteria suspended in buffer solution; ●, bacteria suspended in human whole saliva.

for inhibitory activity in the strep-bead adherence assay. At 50 mM, all sugars showed only weak inhibitory activity (Table 3). Thus, there was no evidence of carbohydrate specificity. Pretreatment of *P. gingivalis* at 60°C for 30 min decreased adherence with untreated strep-beads by 94%, whereas viable *P. gingivalis* adhered normally to heat-treated strep-beads (108%).

DISCUSSION

The data presented in this study indicate that *P. gingivalis* adheres readily to the surfaces of *S. sanguis* in vitro. When bacteria are suspended in buffered salts solution, this phenomenon does not always result in the formation of macroscopically visible agglutinates (flocculation); instead, fine granular or microscopic clumps occur that can be detected and quantitated by a differential filtration assay. Radiolabeled *P. gingivalis* cells also adhere to an artificial plaque composed of *S. sanguis* covalently coupled to the surface of agarose beads. This intergeneric bacterial adherence occurs at a diminished level (5 to 10%) when the bacteria are bathed in or precoated with human whole saliva, indicating that *P. gingivalis* is still capable of colonizing dental plaque by this mechanism under in vivo conditions. Thus, these observations are consistent with those of both Slots and Gibbons (24) and Kolenbrander et al. (13).

The assays described in this report are capable of detecting and quantitating the formation of microscopic bacterial agglutinates with a high degree of resolution. Differential filtration on polycarbonate membranes with 8-μm pores removes free bacteria and small homologous clumps and retains the larger heterologous agglutinates. Filtration of bacterial suspensions immediately after combining the two populations provides a convenient and accurate control for phenomena that may complicate the interpretation of test data such as direct binding of bacteria to polycarbonate filters, clogging of filter pores by agglutinates, and mechanical trapping of one bacterial partner in large autoagglutinates of the second bacterium. *P. gingivalis* 381 frequently demonstrated macroscopic autoagglutination when suspended in buffer solution at concentrations of 2×10^9 cells per ml and greater. The high sensitivity of the radioactivity-based assay permits the numbers of bacteria in the incubation mixture to be lowered to minimize autoagglutination. Although this assay was rapid and convenient for initial screening for interbacterial adherence activity of numerous bacterial pairs suspended in buffered salts solutions, it proved to be less appropriate for studies directed at characterizing the modulating effects of host components such as saliva and serum. These host secretions contain immunoglobulins and other constituents that can bind to oral bacteria and cause agglutination (1, 7, 16, 19, 21). Preliminary experiments with radiolabeled streptococci indicated that inclusion of saliva and serum in assay mixtures caused a significant (20%) increase in the numbers of bacteria retained by the filter membrane, although agglutination of these

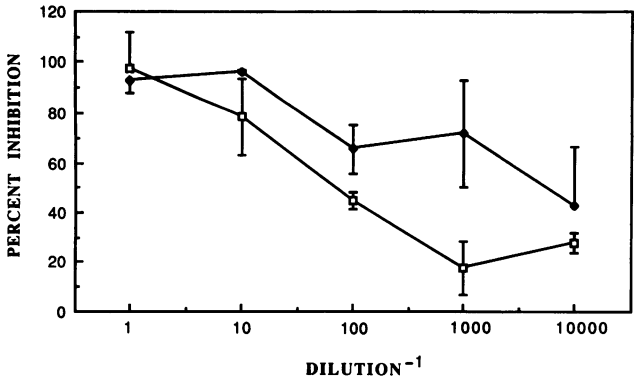

FIG. 5. Inhibition of *P. gingivalis* W50 adherence to strep-beads by selected concentrations of normal rabbit serum (●) and human whole saliva (□).

TABLE 2. Effect of enzymes on intergeneric bacterial adherence

Enzyme (1 mg/ml)	Treated bacterium	% Adherence[a]
Trypsin	*P. gingivalis* W50	13.6 ± 10.8
	S. sanguis G9B	2.0 ± 2.0
Proteinase K	*P. gingivalis* W50	3.4 ± 4.8
	S. sanguis G9B	1.0 ± 3.4
Neuraminidase	*P. gingivalis* W50	2.4 ± 3.4
	S. sanguis G9B	98.4 ± 19.7

[a] Data represent an average indexes ± standard deviations calculated from triplicate determinations.

TABLE 3. Effect of sugars on adherence of *P. gingivalis* to strep-beads

Sugar (50 mM)	% Inhibition[a]
D-Galactose	33 ± 2
D-Glucose	14 ± 9
D-Glucuronic acid	11 ± 1
D-Mannose	37 ± 6
L-Rhamnose	35 ± 5
N-Acetylgalactosamine	28 ± 3
N-Acetylglucosamine	32 ± 5
N-Acetylneuraminic acid	24 ± 2
N-Acetylneuramin-lactose	17 ± 4
Cellobiose	4 ± 1
Lactose	28 ± 1
Maltose	27 ± 2
Sucrose	14 ± 2

[a] Data represent average inhibition ± ranges of duplicate assays.

bacteria by serum and saliva could not be detected macroscopically.

To facilitate the distinction between host-induced bacterial agglutination and true interbacterial adherence, a solid-phase assay was devised in which viable *S. sanguis* is coupled to CNBr-agarose. The resulting surface approximates a homologous dental plaque and can be used to study coherence between *S. sanguis* and other oral bacteria. *S. sanguis* is one of the first oral bacteria to colonize the surfaces of newly cleaned teeth and can be expected to support subsequent colonization by other bacteria (27–29). CNBr-agarose was selected as the supporting matrix because the beads are coated uniformly and irreversibly with streptococci and because *P. gingivalis* has negligible adherence activity toward the unblocked CNBr-agarose beads. Thus, should any gaps occur in the experimental plaque of *S. sanguis*, *P. gingivalis* will not adhere to the exposed agarose matrix. This is an important advantage over the saliva-coated hydroxyapatite beads used by Schwarz et al. (22) for the study of *Actinomyces viscosus* and *P. gingivalis* coaggregation. Only slightly higher numbers of *P. gingivalis* adhered to *A. viscosus*-coated saliva-coated hydroxyapatite beads than to the control beads. As an alternative method, Liljemark et al. (18) used a biological adhesive to attach *S. sanguis* to plastic and bovine enamel chips. The resulting monolayer was used successfully to detect and study adherence of *S. sanguis* with *Haemophilus parainfluenzae* and *Streptococcus sobrinus*. Although the monolayer was reported to be contiguous, the ability of *H. parainfluenzae* to attach to the adhesin-treated enamel, which could be exposed by gaps in the streptococcus monolayer, was not reported.

Human whole saliva and normal rabbit serum adversely affect adherence of planktonic *P. gingivalis* to sessile *S. sanguis*. At equivalent concentrations of protein, saliva was approximately 7.5 times more active than normal rabbit serum as an inhibitor of interbacterial adherence. Experiments in which bacteria were pretreated with saliva indicate that saliva components can bind to the surfaces of either bacterium and inhibit adherence. Ellen et al. (3) also found that saliva inhibited *P. gingivalis* adherence to *A. viscosus* in vitro. These observations indicate that saliva constitutes an important host defense mechanism for controlling colonization of supragingival plaque by *P. gingivalis*. Identification and purification of the active saliva component(s) is necessary for a better understanding of this bacterial adherence mechanism and how it may contribute to development of supragingival plaque and to the pathogenesis of periodontal infections.

Several characteristics of *P. gingivalis-S. sanguis* adherence differ significantly from those of lactose-reversible adherence mechanisms reported by Cisar et al. (2) and Kolenbrander and Anderson (11). The former mechanism produces predominately microscopic agglutinates over 60 min in buffer suspension and is inhibited by human whole saliva but not by lactose. The latter mechanism, exemplified by *A. viscosus-S. sanguis*, forms macroscopic agglutinates instantly and is unaffected by the presence of saliva (14) but is inhibited by lactose (2, 15). Adherence of *P. gingivalis* to *Fusobacterium nucleatum* is also inhibited by lactose (12). Adherence among all of these bacterial pairs is inactivated by protease treatment of the bacteria, indicating that the adherence mechanisms involve either protein-protein or protein-polysaccharide interactions. The relative abilities of these two apparently distinct mechanisms of interbacterial adherence to function in vivo remains to be determined.

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