INFECTION AND IMMUNITY, Sept. 1991, p. 2948–2954
0019-9567/91/092948-07$02.00/0

Vol. 59, No. 9

Delineation of a Segment of Adsorbed Salivary Acidic Proline-Rich Proteins Which Promotes Adhesion of *Streptococcus gordonii* to Apatitic Surfaces

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Cells of several strains of *Streptococcus gordonii* attached in much higher numbers to experimental pellicles formed from samples of submandibular or parotid saliva on hydroxyapatite (HA) beads than to buffer controls. The nature of the salivary components responsible were investigated by preparing experimental pellicles from chromatographic fractions of submandibular saliva obtained from Trisacryl GF 2000M columns. Adhesion of *S. gordonii* Blackburn was promoted by two groups of fractions. The adhesion-promoting activity in the first group of fractions was associated with the family of acidic proline-rich proteins (PRPs), while that of the second group is as yet unidentified. Experimental pellicles prepared by treating HA with 2 μg of pure 150-amino-acid-residue PRPs (PRP-1, PRP-2, and PIF-s) promoted adhesion of *S. gordonii* Blackburn cells to an extent comparable to that obtained with unfractionated saliva. However, pellicles prepared from a 106-residue PRP (PRP-3) were significantly less effective, and those prepared from the amino-terminal tryptic peptide (residues 1 to 30) of the PRP and the salivary phosphoprotein statherin were completely ineffective in promoting adhesion. Although adhesion of several strains of *S. gordonii* was promoted by adsorbed PRP-1, the adhesion of several strains of *Streptococcus sanguis* or *Streptococcus oralis* was either not affected or only weakly enhanced by this protein. *S. gordonii* cells bound avidly to PRPs adsorbed onto HA beads, but the streptococci did not appear to bind PRPs in solution, since concentrations of PRP as high as 200 μg/ml did not inhibit binding of bacterial cells to pellicles prepared from pure PRP. *S. gordonii* cells also attached well to PRP or a synthetic decapeptide representing residues 142 to 150 of the PRP when the peptide was linked to agarose beads. Studies with a series of synthetic decapeptides indicated that the minimal segment of PRP which promoted high levels of *S. gordonii* adhesion was the carboxy-terminal dipeptide Pro-Gln (residues 149 and 150).

Bacteria which attach to and accumulate on the teeth are responsible for initiating dental caries and various types of periodontal diseases (7, 15, 23, 35). This recognition has stimulated studies concerning the mechanisms by which bacteria attach to the surfaces of teeth. The dental enamel is covered by a thin membranous film termed the acquired pellicle. This film is generally less than 1 μm thick and is formed by the selective adsorption of components in oral fluids to the surface of the dental mineral. Components of saliva, including mucins, proline-rich proteins (PRPs), immunoglobulin A, lysozyme, etc. (6, 7, 16, 29, 30), and of crevicular fluid, such as albumin (29, 30), have been detected in natural pellicles. In addition, bacterial components such as glucans and glucosyltransferases may also be present (30, 31). The initial attachment of bacteria to the tooth surface is thought to involve stereochemically specific interactions between proteinaceous adhesins on the bacterial surface and macromolecular constituents making up the acquired pellicle (5, 7, 8, 12).

Recent studies of salivary components which can function as receptors on apatitic surfaces for bacterial adhesins have indicated that adsorbed salivary acidic PRPs can promote the adhesion of several *Actinomyces* species (11), as well as strains of *Bacteroides gingivalis*, *Bacteroides loescheii*, and certain other prominent oral bacteria (12). During the course of these studies, we observed that some strains of *Streptococcus gordonii* also bind avidly to PRPs adsorbed on hydroxyapatite (HA) surfaces. The present report describes some of the characteristics of this binding and delineates a segment of the PRPs which promotes binding of *S. gordonii* cells.

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MATERIALS AND METHODS

Cultures and cultural conditions. *S. gordonii* (formerly *Streptococcus sanguis*) Blackburn and ATCC 10558 and *S. sanguis* 804 and *Streptococcus oralis* (*Streptococcus mitis*) 75 were obtained from A. Coykendall, University of Connecticut. Other strains of *S. gordonii* and *S. sanguis* ATCC 10556 were obtained from M. Kilian, Royal Dental College, Arhus, Denmark. *S. sanguis* 35 and 29 were obtained from W. Liljemark, University of Minnesota.

All strains were stored in 50% glycerol at −20°C to avoid repeated subculture. For experimental purposes, inocula from the glycerol stocks were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures were incubated anaerobically in Brewer jars filled with 80% N_2, 10% H_2, and 10% CO_2 at 35°C. Early-stationary-phase cells were used in all experiments.

For adhesion studies the organisms were radiolabelled by growing them in Todd-Hewitt broth supplemented with 10 μCi of [^{3}H]thymidine (New England Nuclear, Boston, Mass.) per ml as previously described (10, 11). The streptococci were harvested by centrifugation, washed three times, and suspended in 50 mM KCl containing 1 mM KH_2PO_4, 1

mM $CaCl_2$, and 0.1 mM $MgCl_2$ at pH 6.0 (buffered KCl) and supplemented with 5 mg of human albumin (Sigma Chemical, St. Louis, Mo.) per ml as described previously (10). The suspensions were adjusted to contain between 5×10^7 and 10×10^7 streptococci per ml, based upon a standard curve relating optical density to bacterial number as determined by microscopic counts.

Collection and fractionation of saliva. Submandibular and parotid saliva samples were obtained as described previously (11). The samples were immediately dialyzed against 0.1 M $(NH_4)_2CO_3$ (pH 8.0) buffer at 4°C overnight. Chloroform (0.5%) was added to inhibit microbial growth. The dialyzed saliva was fractionated on columns of Trisacryl GF 2000M (IBF Biotechnics, Inc., Savage, Md.) at 4°C and eluted with 0.1 M $(NH_4)_2CO_3$ buffer as previously described (11). Fractions were stored frozen until used to treat HA beads for studies of bacterial adhesion.

To identify the nature of the salivary proteins responsible for promoting adhesion of *S. gordonii* cells to HA, active fractions from the Trisacryl GF 2000M columns were pooled, diluted 1 to 1 with water, and chromatographed on a column of DEAE-agarose as previously described (11). The columns were eluted with an $(NH_4)_2CO_3$ gradient (0.05 to 0.5 M), and proteins present in the fractions were examined by polyacrylamide gel electrophoresis (PAGE) as previously described (11, 12).

Preparation of a tryptic amino-terminal peptide from PRP-1. Samples of PRP-1 (8.5 mg) and trypsin (0.17 mg) (1:50 enzyme/substrate molar ratio) (TRTCPK trypsin; Worthington Diagnostics, Freehold, N.J.) were dissolved in 0.025 M (pH 8) Tris-chloride containing 1 mM $CaCl_2$ and incubated at 37°C for 2 h. The digest was chromatographed on a DEAE-agarose column and eluted with an $(NH_4)_2CO_3$ gradient (0.05 to 0.5 M). The amino-terminal tryptic peptide (residues 1 to 30), designated PRP-1(T1), was readily separated from the unretarded carboxy-terminal peptides and trypsin. This peptide is highly acidic and is considerably retarded on the column (11). Fractions containing this peptide were lyophilized, and its composition was confirmed by amino acid analysis.

Preparation of purified salivary proteins. Samples of pure PRPs and statherin were prepared from human saliva as described previously (17, 33). Their purity was assessed by several techniques, including PAGE, high-performance liquid chromatography, and sequencing (33, 34, 37).

Preparation of experimental pellicles. Experimental pellicles were prepared by exposing 5-mg samples of spheroidal HA beads (BDH Chemicals, Gallard Schlessinger Chemical Corp., Carle Place, N.Y.) to 125-μl samples of saliva or of purified proteins. The beads were washed twice with buffered KCl and treated for 30 min with 5 mg of bovine serum albumin per ml in buffered KCl to block any uncoated regions of the HA (10). The beads were then washed three times with buffered KCl and incubated with 6.25×10^6 to 12.5×10^6 [^{3}H]labelled streptococci suspended in 125 μl of buffered KCl containing 5 mg of albumin per ml. The mixtures were incubated with continuous rotation at 6 rpm at room temperature for 1 h. The beads were then washed three times with buffered KCl, and the number of streptococci which attached was determined by scintillation counting. Values were corrected for quench due to the HA beads as described previously (10, 11).

Peptide synthesis. Derivatized amino acids (tertbutyloxycarbonyl [*t*-Boc]) were of the L configuration and were purchased from Bachem Bioscience, Philadelphia, Pa. Boc-Gln was in the form of its nitrophenyl ester, and Boc-Ser was side chain protected by *O*-benzyl. Syntheses were initiated by using Boc-Gln (unprotected) or Boc-Gly esterified to a Merrifield resin (0.58 meq/g) on a Vega model 250C synthesizer controlled by an Apple IIc computer with a program based on a modification of the Merrifield method (24). The Boc-amino acid resin was suspended in and washed three times with methylene chloride, three times with ethanol, and three times with methylene chloride in the synthesizer. The resin was then washed with 50% trifluoroacetic acid containing 10% anisole for 2 min, treated with 50% trifluoroacetic acid containing 10% anisole in methylene chloride for 30 min, washed 10 times with methylene chloride, and neutralized by washing twice with 10% di-isopropyl-ethylamine (DIPEA) in methylene chloride. The second Boc-amino acid was coupled for 1 h to the deprotected amino acid resin by using a threefold molar excess of dicyclo-hexyl-carbodiimide in methylene chloride DIPEA, and hydroxybenzotriazole. Another aliquot of hydroxybenzotriazole and DIPEA was added at a twofold molar excess for an additional hour. Following coupling, the resin was washed with methylene chloride (three washes) and absolute ethanol (three washes), and an aliquot of the mixture was then tested by using the Kaiser ninhydrin procedure (19) to test for completion of coupling of the Boc-amino acid to the growing peptide chain.

Prior to the amino acid addition steps, at selected cycles, a percentage of the protected peptide resin was removed from the synthesis mixture in such a way that all the glycine-substituted analogs possessing a C-terminal Gln could be prepared in a sequential manner starting with various peptide intermediates of the original synthesis.

Cleavage and extraction of peptides from their supports. Cleavage of each of the peptide resins was performed in a Peninsula HF apparatus in the presence of the anisole (1.2 ml/g of peptide resin) and methylethylsulfide (1 ml/g) at 0°C for 1 h, after which the mixture was thoroughly dried under high vacuum. The mixture was then washed with cold anhydrous ether, extracted with alternate washes of water and glacial acetic acid, and then lyophilized.

Purification of crude synthetic product. Crude synthetic peptides were desalted and purified by gel filtration on a column of Sephadex G-25 (120 by 2.0 cm), which had been equilibrated with 0.1 M NH_4HCO_3 (pH 8.0). Column effluent was monitored by UV A_{254} and A_{206} with an LKB Uvicord III monitor.

Characterization of synthetic peptides. Samples were hydrolyzed in 5.7 N HCl for 22 h at 110°C, dried, reconstituted, and applied to an amino acid analyzer (Applied Biosystems model 420A derivatizer). Amino acids were quantified as their phenylthiocarbamyl derivatives on an Applied Biosystems model 130A high-performance liquid chromatograph. Homogeneity of the purified synthetic peptides was evaluated by NH_2-terminal sequencing (4) by preview analysis (36) using an Applied Biosystems gas-phase sequencer (model 470A).

Linking of peptides to CNBr-agarose beads. To study the adhesion-promoting activity of proteins and peptides which do not adsorb readily to HA, the molecules were linked to CNBr-activated Sepharose 6MB agarose beads (Pharmacia LKB Biotechnology, Piscataway, N.J.) according to instructions supplied by the manufacturer. Briefly, the activated beads were swollen in 0.001 M HCl for 15 min and washed with the HCl (200 ml/g of dried beads) on a sintered glass filter. The protein or synthetic peptide to be coupled to the beads was dissolved in coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl [pH 8.3]), added to the washed beads (5 ml/g of dry beads), and rotated end over end for 2 h at room tempera-

TABLE 1. Attachment of streptococcal strains to HA treated with submandibular saliva or PRP-1

Organism	Strain	Source	Mean no. (±SE) (10^5) of streptococci attached/5 mg of HA treated with:		
			Buffered KCl	Submandibular saliva	PRP-1 (50 μg/ml)
S. gordonii	Blackburn	A. Coykendall	0.69 ± 0.06	6.70 ± 0.13	9.11 ± 0.50
S. gordonii	10558	A. Coykendall	0.37 ± 0.06	0.36 ± 0.21	0.20 ± 0.09
S. gordonii	SK6	M. Kilian	0.58 ± 0.08	3.34 ± 0.58	3.62 ± 0.44
S. gordonii	SK7	M. Kilian	0.32 ± 0.02	3.76 ± 0.44	3.98 ± 0.06
S. gordonii	SK8	M. Kilian	0.31 ± 0.01	1.07 ± 0.07	2.26 ± 0.42
S. gordonii	SK9	M. Kilian	0.13 ± 0.01	3.23 ± 0.11	1.28 ± 0.02
S. sanguis	10556	M. Kilian	0.05 ± 0.02	2.54 ± 0.02	0.21 ± 0.01
S. sanguis II	35	W. Liljemark	0.25 ± 0.02	7.50 ± 0.15	0.27 ± 0.02
S. sanguis II	29	W. Liljemark	0.25 ± 0.01	3.50 ± 0.20	0.44 ± 0.06
S. sanguis	804	A. Coykendall	0.12 ± 0.04	6.70 ± 0.28	0.36 ± 0.01
S. oralis (*S. mitis*)	75	A. Coykendall	0.28 ± 0.01	4.70 ± 0.01	0.70 ± 0.01
S. oralis (*S. mitis*)	C5	Forsyth Dental Center	0.32 ± 0.08	3.90 ± 0.30	0.48 ± 0.06

ture. The beads were sedimented by low-speed centrifugation (300 × *g*) and washed twice with coupling buffer. The supernatants from these steps were recovered so that the coupling efficiency could be determined. Any remaining CNBr groups were blocked by treating the beads with glycine (0.2 M) in coupling buffer on a sintered glass filter, and the product was then washed five times alternately with acetate buffer (0.1 M sodium acetate, 0.5 M NaCl [pH 4.0]) and coupling buffer. The beads were washed off the filter with coupling buffer and stored at 4°C until use. Typically, 285 mg of dry beads were used to give 1 ml of swollen coupled beads. The amounts of different proteins or peptides coupled to the gel are given in the descriptions of the individual experiments.

Adhesion of bacteria to peptides linked to agarose beads. Samples of peptides linked to agarose beads were washed three times with buffered KCl containing 5 mg of albumin per ml and suspended to make a 10% (vol/vol) suspension. Samples (60 μl) of the suspension were added to microtitration plates, and 60 μl of washed ^{3}H-labelled streptococcal cells suspended at a concentration of 10^8/ml in buffered KCl containing albumin was added. The mixtures were rotated at 6 rpm for 90 min at room temperature. The beads were then permitted to settle, and the supernatant liquor was carefully removed. The beads were washed three times with buffered KCl and transferred to scintillation vials to determine the number of bacteria which attached.

RESULTS

Attachment of *S. gordonii* Blackburn to experimental pellicles. Cells of *S. gordonii* Blackburn attached in much higher numbers to HA beads which had been pretreated with samples of human submandibular (Table 1) or parotid saliva (data not shown) than to those which had been treated with buffered KCl. To determine the nature of the salivary components responsible for promoting adhesion of the organism, experimental pellicles were prepared from fractions of submandibular and parotid saliva obtained from Trisacryl GF 2000M columns. Attachment of *S. gordonii* Blackburn cells to the HA beads was promoted by two groups of fractions from both the submandibular and parotid saliva samples (Fig. 1A and B). Fractions 23 to 28 and 29 to 33 from submandibular saliva were pooled, and each pool was rechromatographed on a DEAE-agarose column. The adhesion-promoting components in the first group of fractions from the Trisacryl columns were found to be associated with the family of salivary PRPs (Fig. 2A) when analyzed by PAGE. The adhesion-promoting material in the second group of fractions eluted from the DEAE column just prior to the phosphoprotein statherin (Fig. 2B) but has not yet been characterized.

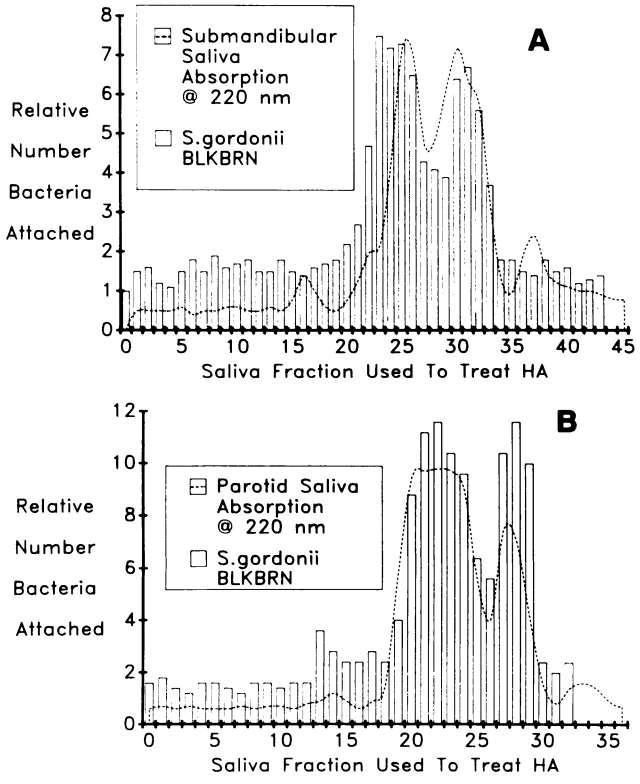

FIG. 1. Attachment of *S. gordonii* Blackburn cells to HA treated with fractions of submandibular saliva (A) or parotid saliva (B) obtained by chromatography on columns of Trisacryl GF 2000M. The A_{220} values of fractions and the number of *S. gordonii* cells which attached are indicated. Note that two groups of fractions promoted adhesion of the organism.

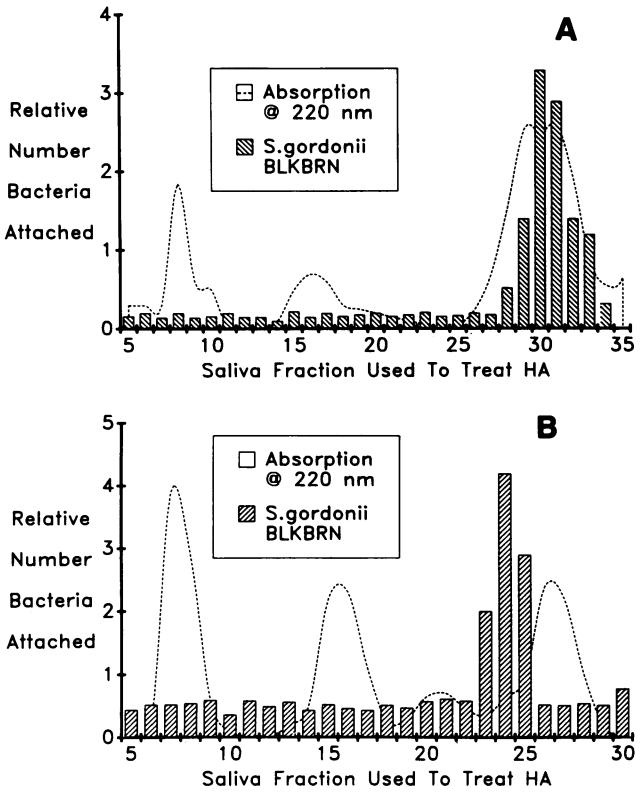

FIG. 2. Attachment of *S. gordonii* Blackburn cells to HA treated with subfractions of submandibular saliva obtained by chromatography on DEAE-agarose columns. The A_{220} values of fractions and the number of *S. gordonii* Blackburn cells which attached are indicated. The adhesion-promoting components in panel A were found to be associated with the family of PRPs when analyzed by PAGE. The adhesion-promoting component(s) in panel B eluted just prior to the phosphoprotein statherin.

Attachment of *S. gordonii* Blackburn cells to experiment pellicles prepared from pure salivary PRPs. The structural relationships of the family of acidic PRPs present in human saliva have been established (1, 17, 37). Three members of this group are 150-residue proteins, designated PRP-1, PRP-2, and PIF-s. PRP-1 and PIF-s differ only in residues 4 and 50, in which there is a transposition between asparagine and aspartic acid; PRP-2 has aspartate at both positions. Three other PRPs (PIF-f, PRP-3, and PRP-4) are 106-residue proteins which are identical to the first 106 residues of the larger proteins. They are thought to be derived from the larger proteins by a posttranslational proteolysis, and the 44-residue peptide (residues 107 to 150) anticipated from this cleavage has been identified in human saliva (18).

Experimental pellicles prepared by treating HA beads with as little as 2 μg of highly purified PRP-1 promoted a high level of *S. gordonii* attachment which was comparable to that obtained with unfractionated saliva (Fig. 3). PRP-2 and PIF-s produced comparable effects (data not shown). However, pellicles prepared from PRP-3 were significantly less effective, while those prepared from salivary statherin were completely ineffective in promoting *S. gordonii* adhesion (Fig. 3).

Adhesion of other streptococcal strains to experimental pellicles. The adhesion of additional strains of *S. gordonii* and of strains of *S. sanguis* and *S. oralis* to HA surfaces was

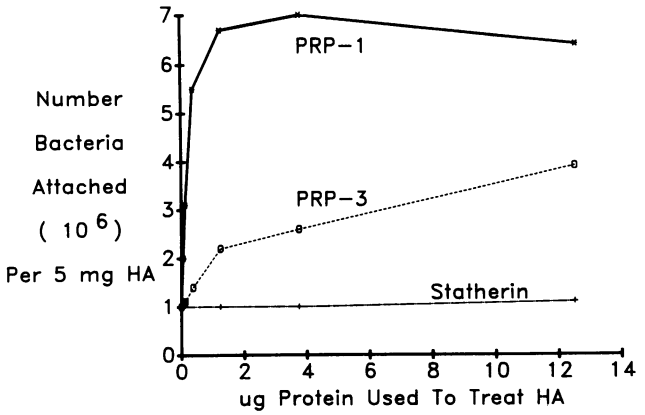

FIG. 3. Attachment of *S. gordonii* Blackburn cells to experimental pellicles formed on HA beads from pure salivary PRP-1, PRP-3, and statherin.

also found to be strongly promoted by components present in submandibular saliva (Table 1). *S. gordonii* 10558 proved to be an exception; its adhesion to HA was not promoted by treatment with submandibular saliva. With the exception of strain 10558, the adhesion of other strains of *S. gordonii* tested to experimental pellicles was also found to be strongly promoted by adsorbed PRP-1. However, the adhesion of the strains of *S. sanguis* or *S. oralis* tested to HA was either not affected or only weakly enhanced by pretreatment with highly purified PRP-1 (Table 1).

Influence of PRPs in solution on attachment of *S. gordonii* cells. Prior studies have shown that *Actinomyces viscosus* cells bind strongly to PRPs adsorbed onto apatitic surfaces, but the organisms do not appear to bind to PRPs in solution nor is their adhesion inhibited by solutions of these proteins (11, 13). Similar results were obtained with *S. gordonii* Blackburn cells in the present investigation. Thus, addition of PRP-1 at a concentration as high as 200 μg/ml to the bacterial suspending fluid did not inhibit binding of *S. gordonii* cells to experimental pellicles prepared from either pure PRP-1 or from submandibular saliva (Table 2). Likewise, the addition of PRP-3 to suspensions did not affect

TABLE 2. Effect of soluble PRP on adsorption of *S. gordonii* Blackburn to experimental pellicles

Pellicle source (concn [μg/ml])	Additive (concn [μg/ml]) to bacterial suspending fluid	No. (10^5) (mean ± SE) of *S. sanguis* Blackburn adsorbed/ 5 mg of HA
Buffer	None	0.5 ± 0.2
Submandibular saliva	None	16.0 ± 1.7
	PRP-1 (200)	18.0 ± 0.8
PRP-1 (50)	None	13.0 ± 0.3
	PRP-1 (200)	24.0 ± 0.3
	PRP-1 (100)	20.0 ± 0.5
	PRP-1 (50)	18.0 ± 1.7
PRP-3 (50)	None	3.7 ± 0.3
	PRP-3 (50)	3.8 ± 0.4
Tryptic peptide PRP-1(T1) (100)	None	0.6 ± 0.3

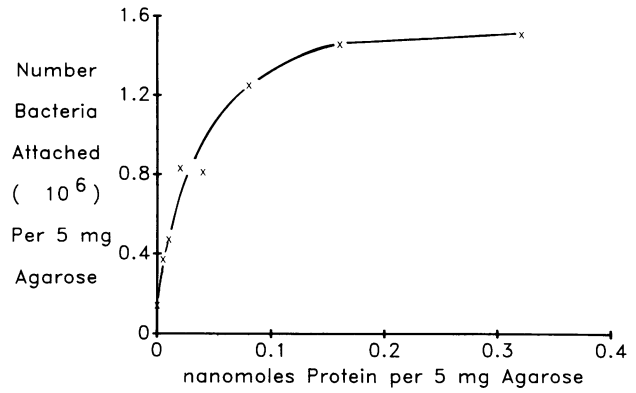

FIG. 4. Attachment of *S. gordonii* Blackburn cells to CNBr-agarose beads containing various quantities of PIF-s. Reaction mixtures (120 μl) contained 6 mg of agarose beads and 6×10^6 streptococcal cells.

adhesion of *S. gordonii* cells to HA beads containing adsorbed PRP-3 (Table 2).

Adhesion of *S. gordonii* cells to experimental pellicles prepared from the amino-terminal tryptic peptide PRP-1(T1) derived from PRP-1. Trypsin cleaves PRPs between residues 30 and 31, yielding a highly acidic peptide which is responsible for the high affinity of PRP molecules for apatitic surfaces (1, 2). However, experimental pellicles prepared by treating HA beads with solutions containing 100 μg of this peptide per ml did not promote adhesion of *S. gordonii* Blackburn cells above the level obtained with buffer-treated controls (Table 2). Thus, this segment of PRP-1 appears to lack the appropriate peptide sequences to which the adhesin of *S. gordonii* binds.

Binding of *S. gordonii* cells to peptides linked to agarose beads. Determination of the structural basis of the adhesion-promoting activity of the PRPs required study of peptides which lack an HA-binding region. Therefore, peptides were linked onto agarose beads. To determine if *S. gordonii* cells could bind to PRP linked to agarose, samples of CNBr-agarose beads were reacted with various quantities of PIF-s. Adhesion of *S. gordonii* Blackburn cells to these beads was strongly promoted in a dose-dependent manner (Fig. 4), and approximately 0.2 nmol of protein per 5 mg of agarose promoted maximal adhesion. *S. gordonii* 10558, which did not bind well to pellicles formed from submandibular saliva or pure PRP-1 (Table 1), did not bind to agarose beads containing PIF-s (data not shown).

Preliminary studies showed that, for small peptides, peptide length was a significant factor in adhesion-promoting activity. Therefore, a series of decapeptides representing residues 141 to 150 of PIF-s were synthesized and linked at a concentration of 12.5 nmol/5 mg of CNBr-agarose. The sequences and analytical composition of these peptides are given in Table 3. A peptide representing residues 141 to 150 proved highly effective in promoting adhesion of *S. gordonii* Blackburn cells, compared with controls (Table 4). This peptide also promoted adhesion of two additional strains of *S. gordonii* (strains SK8 and SK9). However, a glycine-extended decapeptide containing a single Gln (residue 150) in the terminal position was ineffective in promoting adhesion of *S. gordonii*. Decapeptides containing residues 149 and 150 (Pro-Gln) and lower-numbered residues exhibited a significant level of receptivity for all *S. gordonii* strains tested when linked to agarose beads (Table 4). Thus, the terminal dipeptide (Pro-Gln) appears to be the minimal segment of PRPs which is central to the adhesion of *S. gordonii*. A glycine-extended peptide representing residues 141 to 149 and which terminated in Gly rather than Gln exhibited weak to moderate receptivity for the *S. gordonii* strains tested. This indicates that the terminal Gln residue contributes significantly to the binding. That this peptide representing residues 141 to 149 displayed some activity suggests that either the penultimate Pro residue also plays a significant role or that the internal Pro-Gln residues in this peptide contributed to the activity displayed.

DISCUSSION

In the present study, we observed that human saliva contains at least two groups of components which bind to apatitic surfaces and promote the adhesion of strains of *S. gordonii*. Adhesion-promoting components migrating in the first group of fractions eluting from Trisacryl columns were associated with the family of acidic PRPs, and treatment of HA with microgram quantities of the 150-residue PRPs promoted a level of streptococcal binding comparable to that of unfractionated saliva. The nature of the component(s) present in the second group of fractions eluting from Trisacryl columns has not yet been definitively identified and is being investigated. In this regard, it should be noted that streptococci now considered to be *S. gordonii* have been recently shown to possess an amylase receptor and to bind salivary amylase from solution (3, 32).

Several other oral bacteria have also been observed to bind to adsorbed PRPs on apatitic surfaces (11, 12). The best-characterized species to date is *A. viscosus*, which possesses an adhesin associated with type 1 fimbriae which binds to adsorbed PRPs (14). The adhesin of *S. gordonii* cells

TABLE 3. Amino acid composition of glycine-substituted analogs of PRPs[a]

Residue(s) of PRPs represented	Sequence	Composition (no. of residues/peptide)			
		Ser	Glu	Pro	Gly
150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLN		1.00		9.21
149–150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-PRO-GLN		1.00	0.98	8.12
148–150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-SER-PRO-GLN	0.93	1.00	0.99	7.05
146–150	GLY-GLY-GLY-GLY-GLY-GLY-GLN-SER-PRO-GLN	0.94	2.00	1.01	6.06
145–150	GLY-GLY-GLY-GLY-GLN-GLY-GLN-SER-PRO-GLN	0.92	3.00	0.98	5.02
144–150	GLY-GLY-GLY-PRO-GLN-GLY-GLN-SER-PRO-GLN	0.93	3.00	1.97	4.04
141–150	GLN-GLY-PRO-PRO-GLN-GLY-GLN-SER-PRO-GLN	0.92	4.00	2.98	2.02
141–149	GLN-GLY-PRO-PRO-GLN-GLY-GLN-SER-PRO-GLY	0.91	3.00	2.97	3.03

[a] Each of the purified synthetic peptides was subjected to automated sequence analysis, and the maximal observed preview was 1.3%.

TABLE 4. Attachment of *S. gordonii* strains to glycine-substituted synthetic peptides representing segments of PRPs linked to agarose beads

Residue(s) of PRPs represented	Peptide[a] linked to agarose beads	% Attachment relative to peptide 141–150		
		S. gordonii Blackburn	S. gordonii SK8	S. gordonii SK9
None	Control (no peptide)	0	0	0
150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLN	2	0	0
149–150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-GLY-PRO-GLN	88	66	63
148–150	GLY-GLY-GLY-GLY-GLY-GLY-GLY-SER-PRO-GLN	90	53	93
146–150	GLY-GLY-GLY-GLY-GLY-GLY-GLN-SER-PRO-GLN	61	66	93
145–150	GLY-GLY-GLY-GLY-GLN-GLY-GLN-SER-PRO-GLN	85	50	67
144–150	GLY-GLY-GLY-PRO-GLN-GLY-GLN-SER-PRO-GLN	88	87	133
141–150	GLN-GLY-PRO-PRO-GLN-GLY-GLN-SER-PRO-GLN	100	100	100
141–149	GLN-GLY-PRO-PRO-GLN-GLY-GLN-SER-PRO-GLY	65	29	37

[a] 12.5 nmol of peptide per 5 mg of CNBr-agarose.

which mediates adhesion to adsorbed PRPs exhibits some similarities to that of *A. viscosus*. They both bind equally well to representatives of the 150-residue PRPs (PRP-1, PRP-2, and PIF-s) adsorbed on HA surfaces. In addition, the adhesins of both organisms do not appear to interact with PRPs in solution. Thus, both adhesins evidently bind to segments of PRPs (cryptitopes) which become exposed as a result of a conformational change when the proteins adsorb to apatitic surfaces. This is very likely an important trait which has evolved to enable bacteria to attach to surface-associated molecules while avoiding interacting with soluble molecules in the secretions, which would inhibit their adhesion. However, the *S. gordonii* adhesin also displays several distinct differences from that of *A. viscosus*. The 106-residue PRP (PRP-3) was much less effective in promoting adhesion of *S. gordonii* than of *A. viscosus*. In addition, statherin at moderate concentrations was effective in promoting adhesion of *A. viscosus* cells (11), but it was completely inactive for *S. gordonii*. Mutants of *A. viscosus* defective in the synthesis of type 1 fimbriae fail to bind to either adsorbed PRPs or to salivary statherin, suggesting that the same adhesin is involved in the binding of *A. viscosus* to both phosphoproteins (14). Therefore, the adhesin of *S. gordonii* cells exhibits a clearly different pattern of binding specificities.

Treatment of HA beads with solutions containing 100 μg of the tryptic peptide PRP-1(T1) per ml did not promote *S. gordonii* adhesion. The inactivity of this amino-terminal fragment and the weak adhesion-promoting activity of the 106-residue PRPs (i.e., PRP-3) suggested that the carboxy-terminal end of the 150-residue PRPs may contain the dominant molecular segments which react with the *S. gordonii* adhesin. This was substantiated by the observation that a synthetic peptide representing residues 141 to 150 of the PRPs proved highly effective in promoting *S. gordonii* adhesion when linked to CNBr-activated agarose beads (Table 3). Data derived from a series of glycine-extended synthetic peptides indicated that the minimal segment which promoted *S. gordonii* adhesion appears to be the dipeptide representing residues 149 and 150 (Pro-Gln). The 150-residue PRPs contain 15 Pro-Gln residues, 8 of which are present in the 106-residue proteins (1, 34). Salivary statherin also contains two internal Pro-Gln residues. The inability of statherin and the feeble ability of the 106-residue PRPs to promote *S. gordonii* adhesion suggests that the adhesin of this organism does not bind effectively to all of the internal peptide residues in these proteins. However, this does not preclude the possibility that some sterically favored internal Pro-Gln dipeptides may offer binding sites.

The oral streptococci are a heterogeneous collection of species which have been difficult to definitively classify. Strains currently considered *S. gordonii* formerly were considered a subpopulation of the species *S. sanguis* (20). Strains of *S. gordonii* also possess many phenotypic similarities to strains considered to be *S. oralis* and *S. sanguis* (20). However, the classification of *S. gordonii* as a distinct species appears to be of more than academic interest since this organism exhibits a different pattern of oral colonization than those of *S. oralis* and *S. sanguis* (27, 28). Although only a limited number of streptococcal strains were studied in the present investigation, the data obtained suggest that strains of *S. gordonii* may also differ in some of their adhesive properties from other oral streptococci. For example, strains of *S. sanguis* and *S. oralis* studied did not bind effectively to adsorbed PRPs, whereas almost all strains of *S. gordonii* studied did bind. *S. gordonii* 10558 was an exception, but this organism did not bind well to pellicles prepared from submandibular saliva. Therefore, it seems likely that strain 10558 represents an adhesin-defective variant of *S. gordonii*.

Other studies have demonstrated that strains of oral streptococci may bind to different salivary molecules. For example, strains of *S. mutans* of serotype c bind mainly to a high-molecular-weight salivary mucin and to a lesser extent to PRPs and other components in submandibular saliva (9, 21), whereas strains of *S. sobrinus* do not bind well to any salivary components which adsorb to HA (10). Likewise, Liljemark et al. (22) observed that strains of *S. sanguis* and *S. mitis* are heterogeneous, and approximately 60% of strains displayed a lower adhesion to neuraminidase-treated salivary pellicles. These strains also exhibited different patterns of inhibition by galactose and *N*-acetylgalactosamine. An adhesin with a specificity for a trisaccharide consisting of sialic acid, galactose, and *N*-acetylgalactosamine has been isolated from strains of *S. sanguis* and *S. mitis* (25, 26), and the interaction of these organisms with salivary glycoproteins has been shown to be dependent on neuraminidase-sensitive receptors. However, the acidic salivary PRPs studied in the present investigation are nonglycosylated proteins, and thus the adhesin of *S. gordonii* cells which recognizes terminal Pro-Gln residues in PRPs is distinctly different from the adhesin isolated from *S. sanguis* and *S. mitis*.

ACKNOWLEDGMENTS

This investigation was supported in part by grants DE-02847, DE-03915, DE-08601, DE-01659, and DE-07009 from the National Institute for Dental Research.

We are grateful for the capable technical assistance of S. Schluckebier, A. Palmer-Kelley, and M. Ferland.

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