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Binding Sites of Salivary Statherin for *Porphyromonas gingivalis* Recombinant Fimbrillin

ATSUO AMANO,[1] KOSUKE KATAOKA,[1] PERIATHAMBY A. RAJ,[2] ROBERT J. GENCO,[2,3] AND SATOSHI SHIZUKUISHI[1]*

Department of Preventive Dentistry, Osaka University Faculty of Dentistry, Suita, Osaka 565, Japan,[1] and Department of Oral Biology, School of Dental Medicine,[2] and Department of Microbiology, School of Medicine and Biomedical Science,[3] State University of New York at Buffalo, Buffalo, New York 14214

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We investigated the binding sites of salivary statherin involved in the interaction with *Porphyromonas gingivalis* recombinant fimbrillin (r-Fim). Synthetic peptides representing statherin analogs were used to localize the binding domains of statherin. Peptide F4 (residues 29 to 43) significantly bound to r-Fim and inhibited r-Fim binding to statherin-coated hydroxyapatite beads. Successive peptides in which pairs of amino acid residues were deleted starting at the N terminus of peptide F4 were synthesized. Peptide N1 without Leu-29–Tyr-30 had significantly reduced direct binding and inhibition ability. The deletions of residues 31 to 40 had little effect on interaction with r-Fim. The tripeptide N6 representing Tyr-41–Thr-42–Phe-43 retained significant binding to r-Fim. Another set of peptides was synthesized by deleting individual amino acid residues from the C and N termini of peptide F4 to identify functional residues among the five putative functional residues 29, 30, and 41 to 43. Peptide C1 missing Phe-43 lost over 50% of its binding ability. Binding ability was gradually reduced with deletions from the peptides. Peptide C5 (amino acids 31 to 40) weakly affected direct binding and inhibition. Collectively, the results of this study suggests that Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43 are important binding regions that mediate the binding of statherin to *P. gingivalis* fimbrillin.

Porphyromonas gingivalis is a gram-negative black-pigmented anaerobe considered a putative periodontopathogen associated with several forms of periodontal disease (26). *P. gingivalis* is primarily isolated from the subgingival flora of patients with periodontitis. It is also isolated from tonsils, tongue, gingiva, and buccal mucous membranes in patients with periodontitis (4, 5). The adherence of *P. gingivalis* to supragingival surfaces is necessary for the initial step of periodontal infection. Salivary proteins adsorb to teeth, mucous membranes, and oral bacteria. Although the extent to which early colonization of *P. gingivalis* depends on salivary components is unclear, those salivary components to which *P. gingivalis* binds strongly likely play a critical role in mediating the adherence to saliva-coated oral surfaces.

Fimbriae are thought to be important in mediating the adherence of this bacterium to host tissues such as epithelial cells, fibroblasts, and salivary proteins (15). The *P. gingivalis* mutant with an inactivated *fimA* gene cannot bind to human gingival fibroblasts, epithelial cells (9), and whole-saliva-coated hydroxyapatite (HAP) beads (14). We showed that fimbriae are required for the adherence of *P. gingivalis* cells to whole-saliva-coated HAP beads (13). Inhibition studies with various reagents suggested that fimbriae can bind to salivary proteins probably through protein-protein interactions and that fimbrillin has specific binding domains for salivary proteins (13). Recombinant fimbrillin protein (r-Fim) of *P. gingivalis* has been generated in *Escherichia coli*, and it is chemically, antigenically, and serologically identical to the native fimbrillin (19, 21, 24). r-Fim bound to whole-saliva-coated HAP to a greater extent than native fimbriae and was thus used to identify salivary components that mediate the binding of *P. gingivalis* through its fimbriae (2). Salivary statherin and proline-rich proteins (PRP) had the highest binding ability for r-Fim among the nine major salivary components from human submandibular-sublingual saliva which were tested (2). These two salivary molecules may promote the adherence of *P. gingivalis* to saliva-coated oral surfaces through specific interactions. For a better understanding of the first step of oral *P. gingivalis* infection and to design inhibitors of these interactions which may interfere with colonization, these interactions must be analyzed. The localization of active binding domains of *P. gingivalis* r-Fim to statherin and PRP1 has been examined (1). r-Fim has binding sites for statherin and PRP1 located in residues 126 to 146 and the C-terminal region from amino acids 266 to 337.

Statherin, which is a characteristic component of human saliva, is a unique acidic and carbohydrate-free phosphoprotein (10). It inhibits the primary and secondary precipitation of calcium salts (10) and is tightly adsorbed to enamel surfaces (11). It has been reported that the polar N-terminal six residues are important for the inhibitory effect on calcium precipitation (10) and that the N-terminal five residues and Glu-26 are involved in the binding to hydroxyapatite (18). Statherin, only when it is preadsorbed to a solid surface such as HAP, promotes bacterial adhesion by organisms such as *P. gingivalis* (2), *Actinomyces viscosus* (6, 7), *A. naeslundii* (23), and *Fusobacterium nucleatum* (25) to HAP surfaces. Statherin may undergo a conformational change when adsorbed from the solution to HAP surfaces (7, 10). As a result, hidden receptors called cryptitopes are exposed and then available for binding to bacterial adhesins (7, 10). It has been surmised from a conformational study that the C-terminal portion, which, unlike the N-terminal portion, is flexible enough to provide cryptitopes used for anchoring the molecule to the surface, promotes bacterial adherence (18). However, the nature of the domains of statherin involved in bacterial adherence remain unclear.

* Corresponding author. Mailing address: Department of Preventive Dentistry, Osaka University Faculty of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565, Japan. Phone: 81(6) 879-2921. Fax: 81(6) 879-2925.

TABLE 1. Amino acid sequence of statherin and its synthetic peptides

Peptide (residues)	Amino acid sequence
	10 20 30 40
Statherin (1–43)	DSSEEKFLRRIGRFGYGYGPYQPVPEQPLYPQPYQPQYQQYTF
F1 (1–15)	DSSEEKFLRRIGRFG
F2 (5–15)	EKFLRRIGRFG
F3 (15–29)	GYGYGPYQPVPEQPL
F4 (29–43)	LYPQPYQPQYQQYTF
F5 (19–43)	GPYQPVPEQPLYPQPYQPQYQQYTF

Here, we investigated the binding sites of statherin to *P. gingivalis* r-Fim.

MATERIALS AND METHODS

Bacterial culture conditions. *P. gingivalis* 381 was grown and radiolabeled with [^{3}H]thymidine as previously described (2). The labeled cells were centrifuged, washed three times with 50 mM KCl containing 1 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$, pH 6.0 (KCl buffer) (3), and processed for *P. gingivalis* binding assays as described previously (2).

Purification of r-Fim and statherin. r-Fim (molecular mass, 41 kDa) expressed in *E. coli* was purified and iodinated as previously described (1, 2). The specific activity of iodinated protein was 9.8 mCi/μmol. Statherin was purified from human submandibular-sublingual saliva collected from a 33-year-old female donor as reported previously (17).

Preparation of synthetic statherin peptides. Synthetic peptides (F1 to F5 [Table 1]) were prepared as described previously (16). The remaining peptides (N1 to N6 and C1 to C5 [see Fig. 3 and 4]) were commercially synthesized and purified at the Sequence and Peptide Synthesis Facility of Hokkaido System Science Co. (Sapporo, Hokkaido, Japan). The amino acid sequences and mass values of products were confirmed with a 477A/120 gas-phase automatic sequencer (Applied Biosystems, Foster City, Calif.) and a fast atom bombardment mass spectrometer using the JMS-HX100/JMA-3100 data system (JEOL Ltd., Tokyo, Japan), respectively.

Dot blot assay. A polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, Calif.) was activated with 100% methanol and equilibrated with KCl buffer. Statherin and synthetic statherin peptides were dissolved in KCl buffer and loaded into wells of a Bio-Dot apparatus (Bio-Rad) by mild aspiration. The membrane with absorbed peptides was coated with 2% lipid-free bovine serum albumin (BSA; A-7030; Sigma Chemical Co., St. Louis, Mo.) and incubated with 3 ml of ^{125}I-r-Fim (20 pmol/ml) for 3 h at room temperature. Thereafter, the membrane was washed with KCl buffer containing 100 mM NaCl and subjected to autoradiography as described previously (2). For assay, a set of two membranes with absorbed peptides was prepared. One membrane was used for a radioactive experiment, and the other was incubated with KCl buffer and washed in the same manner as the radioactive duplicate to measure the amount of peptide remaining on the membrane, using a Hitachi 835 S amino acid analyzer (Hitachi Ltd., Tokyo, Japan).

Inhibition of r-Fim and whole-cell binding to statherin. The binding of ^{125}I-r-Fim and ^{3}H-*P. gingivalis* cells to statherin-coated HAP was assayed as described previously (2). HAP beads (2 mg) in a tube containing 100 μl of statherin (100 μg/ml) were incubated overnight at room temperature. One hundred microliters of ^{125}I-r-Fim (5 nmol/ml) or ^{3}H-whole cells (2.5×10^8/ml) was added to tubes containing statherin-coated HAP beads, and the tubes were incubated at room temperature for 1 h. The binding level was calculated by subtracting the nonspecific binding obtained by a preincubation of the HAP beads with 500 μl nonlabeled r-Fim (50 nmol/ml) at room temperature for 1 h. All assays were performed in triplicate on three separate occasions. Variance is represented as the mean ± standard deviation, and differences were compared by using Student's *t* test.

Assays of binding of r-Fim to statherin and peptides. The direct binding of r-Fim to smaller synthetic statherin peptides was evaluated by using 96-well enzyme-linked immunosorbent assay (ELISA) plates (flat bottom, amino plate type A; Sumitomo Bakelite Co., Ltd., Tokyo, Japan). The plate was processed to provide covalent bonds between the well surface and adsorbed peptides as specified in the manufacturer's manual. Briefly, the wells were activated with 100 μl of 2% glutaraldehyde in phosphate-buffered saline (PBS; pH 7.4) and then washed twice with PBS. One hundred microliters of peptide (2 μmol/ml) in PBS was added to each well and then the plate was incubated at 4°C overnight. According to the manufacturer's data (unpublished observations), when the concentration of peptide solution is sufficiently saturated, one functional group formed on a well binds one peptide molecule. An equal amount of peptide should have been fixed to each well (about 80 pmol, as calculated by the company). The wells were washed three times with PBS containing 0.1% Tween 20 (PBST) and coated with 100 μl of 1% lipid-free BSA in PBST at room temperature for 1 h. Following three washes with PBST, the wells were incubated with 100 μl of r-Fim (5 nmol/ml) in PBST at room temperature for 3 h. The wells were washed three times with PBST and then coated with 1% lipid-free BSA. Anti-r-Fim rabbit serum diluted 1:5,000 (100 μl) (19) was added to the wells, which were placed at 4°C overnight. The antibodies that reacted with r-Fim were detected by using a 1:1,000 dilution of alkaline phosphatase-conjugated anti rabbit immunoglobulin G (heavy plus light chain; Zymed Laboratories, Inc., San Francisco, Calif.) at room temperature for 3 h. The enzyme reaction proceeded in diethanolamine buffer (pH 9.8), using *p*-nitrophenyl phosphate (Sigma) as a substrate. The reaction was terminated with 0.1 N NaOH, and the color intensity was measured at 405 nm. Lipid-free BSA (2 mM) was used as a control to determine the nonspecific binding level. Variance is represented as the mean ± standard deviation, and differences were compared by using Student's *t* test.

Analytical methods. The protein content of samples was determined by means of a Bio-Rad protein assay kit, using BSA as a standard as specified in the manufacturer's manual. The content of peptide was measured by means of dry weight or amino acid analysis.

RESULTS

Region of statherin exhibiting binding to *P. gingivalis* fimbrillin. Five synthetic peptides (F1 to F5) were used to identify the segment of statherin involved in binding to r-Fim (Fig. 1). Various amounts of peptide absorbed on the membranes were removed during the experimental process. The amounts of the five peptides bound to the membrane were therefore optimized. The control membrane was washed with KCl buffer exactly as done for the experimental site. The dots were then excised, and the amount of peptide was determined by amino acid analysis. As a result, different amounts of each peptide were loaded onto the membrane so that 0.35 nmol of each peptide remained bound. In brief, 0.4 nmol of statherin, 0.45 nmol of peptides F1 and F2, 0.55 nmol of peptides F3 and F5, and 0.8 nmol of peptide F4 were loaded onto the membrane by mild aspiration. As shown in Fig. 1, a dot of peptide F4 appears as clear as statherin in terms of the interaction with ^{125}I-r-Fim.

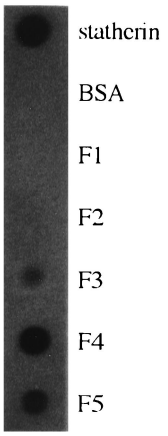

FIG. 1. Dot blot assays to determine which segment is involved in the binding to r-Fim. A polyvinylidene difluoride membrane with absorbed statherin, synthetic peptides (F1 to F5), and BSA was incubated with ^{125}I-r-Fim for 3 h at room temperature. The membrane was washed with KCl buffer containing 100 mM NaCl and then visualized by autoradiography.

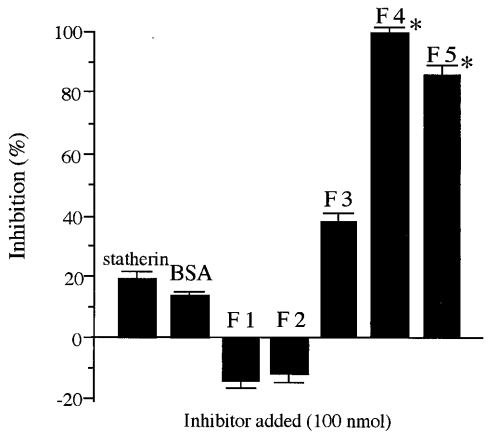

FIG. 2. Inhibition by synthetic peptides in the binding of ^{125}I-r-Fim to HAP beads coated with statherin. The binding of ^{125}I-r-Fim to statherin-coated HAP beads was assayed as described previously (2). HAP beads in a tube were incubated with 100 μl of statherin (100 μg/ml) overnight at room temperature. One hundred microliters of peptide inhibitor (100 nmol) and 100 μl of ^{125}I-r-Fim (0.5 nmol) were added to a tube containing statherin-coated HAP beads and incubated for 1 h. The binding level was calculated by subtracting the nonspecific binding level, which was obtained by the prior incubation of HAP beads with 500 μl nonlabeled r-Fim (50 nmol/ml). All assays were performed in triplicate on three separate occasions. Means were significantly different from each other. *, $P < 0.001$.

Peptide F4 had a higher affinity than peptide F5, which has 10 more amino acid residues at the N terminus. On the other hand, peptides F1 and F2 and the negative control BSA did not react. These results suggested that the C-terminal segment (residues 29 to 43) contains the binding domain for *P. gingivalis* r-Fim.

Inhibition by peptides of the binding of r-Fim statherin. We confirmed the findings from the dot blot assay, in which the direct binding of peptide was qualitatively evaluated, by means of inhibition experiments with the peptides (Fig. 2). A 200-fold excess of peptide (100 nmol) and ^{125}I-r-Fim (0.5 nmol) were simultaneously added to a tube. This concentration of peptide was sufficient to inhibit r-Fim binding to statherin-coated HAP beads to a maximal inhibition level. Peptide F4 inhibited r-Fim binding by 100%. Although peptide F5 contained the same amino acid sequence as peptide F4, its inhibition level was 83%. When the amount was doubled (200 nmol), the inhibition by peptide F5 increased only to 89% (data not shown). Peptide F3 inhibited by 40%, and the N-terminal segments (peptides F1 and 2) had no effect. Statherin in solution showed little inhibitory effect because of its cryptitic conformation as described previously (2). However, the synthetic peptides were inhibitory in solution, suggesting that the binding sites are exposed on the synthetic peptides.

Determination of the binding sites by using N-terminal truncated peptides. To determine which sites are involved in the binding, we synthesized six peptides in which amino acid residues were deleted in pairs from the N terminus of peptide F4 as shown in Fig. 3. The dot blot assay was performed to assess the binding abilities of these peptides. However, variable small amounts of short peptides such as N5 and N6 remained bound to the polyvinylidene difluoride membrane after washing with KCl buffer. Hence, the dot blot assay could not be applied to determine the binding properties of these short

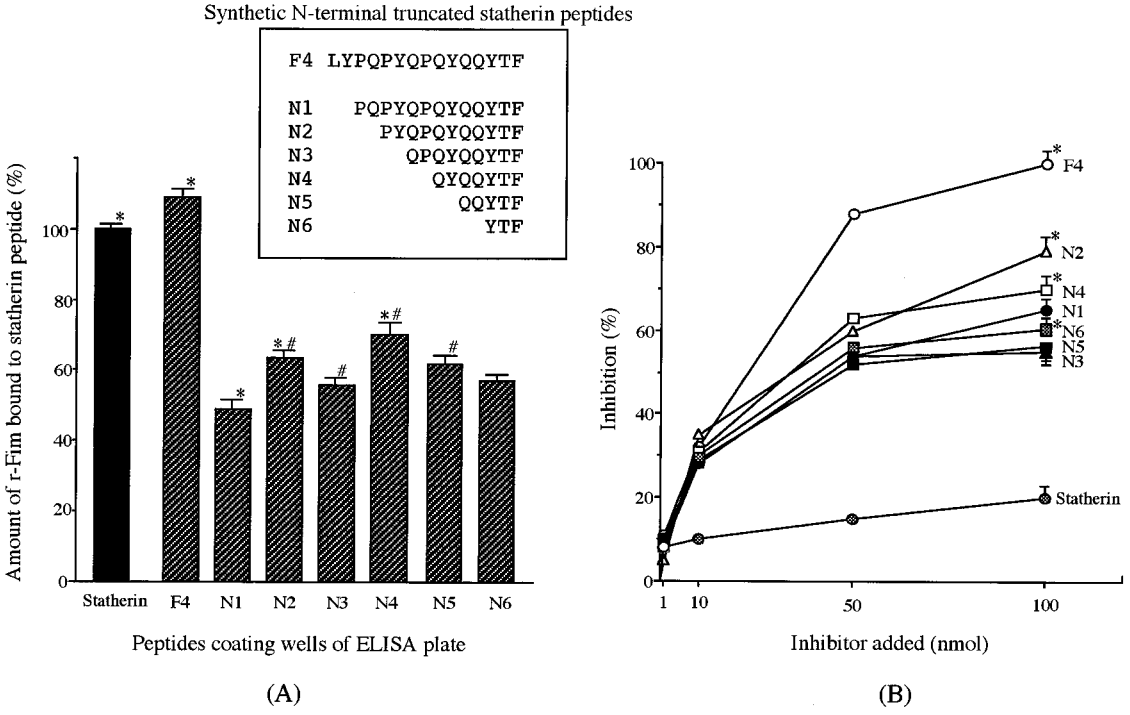

FIG. 3. Determination of the binding sites by using the N-terminal truncated peptides. (A) Direct binding of r-Fim to peptides N1 to N6 fixed on wells of ELISA plates. One hundred microliters of peptide (2 μmol/ml) in PBS was added to each well, and the plate was then incubated at 4°C overnight. The wells were coated with 1% lipid-free BSA in PBST at room temperature for 1 h. After three washes with PBST, the wells were incubated with 100 μl of r-Fim (5 nmol/ml) in PBST at room temperature for 3 h. The amount of adsorbed r-Fim was detected with anti-r-Fim rabbit serum as described in the text. Lipid-free BSA (2 mM) was used as a control to subtract the nonspecific binding level. Means were significantly different from each other. *, $P < 0.005$; #, $P < 0.05$. (B) Inhibition by peptides N1 to N6 of the binding of ^{125}I-r-Fim to HAP beads coated with statherin. One hundred microliters of peptide inhibitor (100 nmol) and 100 μl of ^{125}I-r-Fim (0.5 nmol) were added to a tube containing statherin-coated HAP beads and incubated for 1 h. The binding level was calculated as described in the legend to Fig. 2. All assays were performed in triplicate on three separate occasions. Means were significantly different from each other. *, $P < 0.001$.

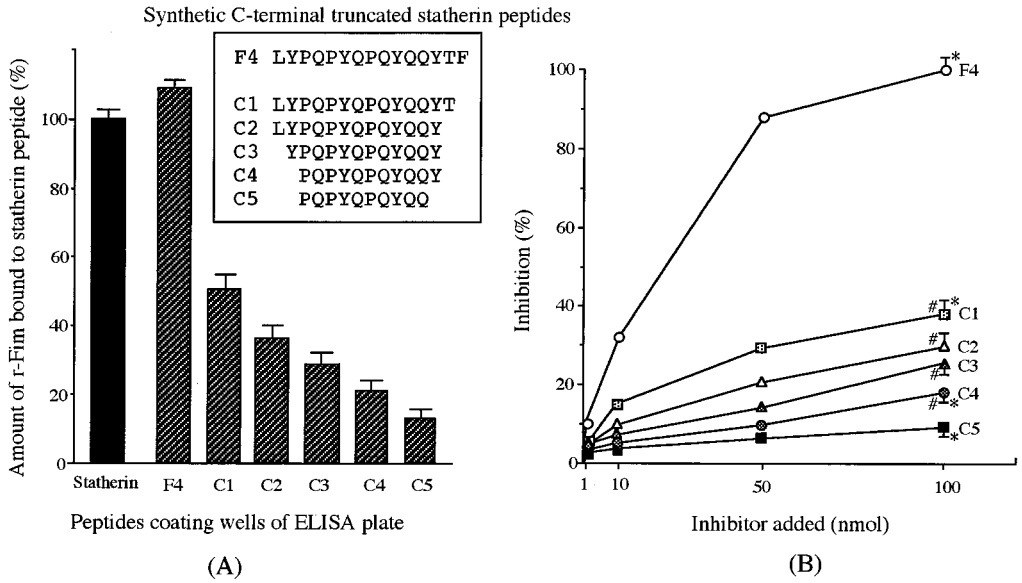

FIG. 4. Determination of the binding sites by using the C-terminal truncated peptides. (A) Direct binding of r-Fim to peptides C1 to C5 fixed on wells of ELISA plates. One hundred microliters of peptide (2 μmol/ml) in PBS was added to each well. The wells were coated with 1% lipid-free BSA in PBST and washed with PBST. The wells were incubated with 100 μl of r-Fim (5 nmol/ml) in PBST at room temperature for 3 h. The amount of adsorbed r-Fim was detected with the anti-r-Fim rabbit serum as described in the text. Lipid-free BSA (2 mM) was used as a control to subtract the nonspecific binding level. Means were significantly different from each other ($P < 0.05$). (B) Inhibition by peptides C1 to C5 of ^{125}I-r-Fim binding to HAP beads coated with statherin. One hundred microliters of peptide inhibitor (100 nmol) and 100 μl of ^{125}I-r-Fim (0.5 nmol) were added to a tube containing statherin-coated HAP beads and incubated for 1 h. The binding level was calculated as described in the legend to Fig. 2. All assays were performed in triplicate on three separate occasions. Means were significantly different from each other. *, $P < 0.001$; #, $P < 0.05$.

peptides. Therefore, direct binding in 96-well ELISA plates was used (Fig. 3A). Peptide F4 had a 10% higher binding capacity than statherin. Peptide N1 without Leu-Tyr had less than half the binding ability of peptide F4, which had the lowest capacity among peptides N1 to N6. The binding ability of peptides N2 and N4 was enhanced by about 15% by exposing the N-terminal Pro-Tyr and Gln-Tyr, respectively. The tripeptide N6 retained significant binding ability.

The abilities of the peptides to inhibit binding were similar to those in the direct binding study (Fig. 3B). The inhibitory effect of peptide N1 was significantly reduced by deleting Leu-Tyr. Peptides N2 and N4 were slightly more inhibitory than the other peptides, and the inhibition levels among peptides N3, N5, and N6 were indistinguishable. The addition of 100 nmol of peptide N6 (500 nmol/ml) inhibited binding by 60%. The results of these experiments indicated that Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43 are the binding sites of statherin for *P. gingivalis* r-Fim.

Determination of binding sites by using peptides C1 to C5. On the basis of the results in Fig. 3, peptides C1 to C5 were designed and used to identify which among the five specified residues (Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43) were functional (Fig. 4A). Peptide C1, missing Phe in the C-terminal end, lost over 50% of its binding ability. Peptides C1 to C4 showed a reduction of binding ability gradually along the deletion of residues.

A deletion of Phe-43 led to a significant loss of inhibitory effect (Fig. 4B). Peptide C2 was 10% less inhibitory than peptide C1. The truncated peptides C2 to C5 gradually lost inhibition abilities, and that of C5 was very weak.

Inhibition by peptides of the binding of *P. gingivalis* cells to statherin. The inhibitory effects of peptides were confirmed by assays of whole-cell binding to statherin-coated HAP beads (Fig. 5). Peptide F4 was 94% inhibitory at a concentration of 500 nmol/ml. Peptides N1 to N6, possessing the putative site Tyr-41–Thr-42–Phe-43, were 74 to 87% inhibitory. The inhibitory levels of peptides N2 and N4, containing Pro-33–Tyr-34 and Gln-37–Tyr-38, respectively, in the N terminus, were slightly higher than those of the other N-terminal truncated peptides. Peptide C1 and C2, which contain Leu-29–Tyr-30, inhibited by more than 60%. Peptide C3, which was missing residues 29, 30, and 41 to 43, had Tyr-30 and Tyr-41 exposed on both ends. The C3 peptide inhibited to greater extent than

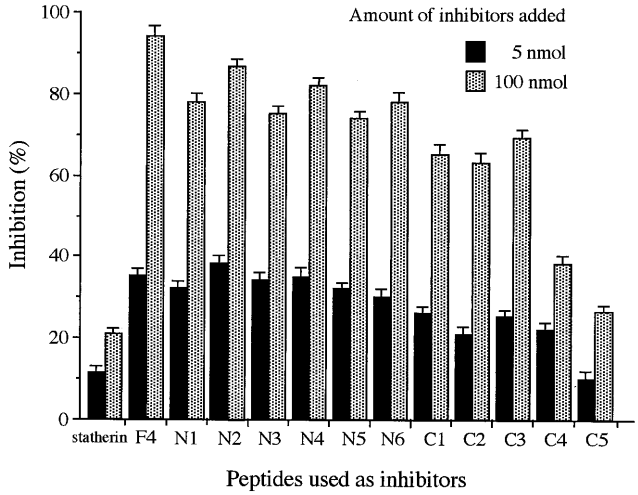

FIG. 5. Inhibition by synthetic peptides of ^{3}H-whole-cell binding to HAP beads coated with statherin. The binding of ^{3}H-whole cells to statherin-coated HAP beads was assayed as described previously (2). One hundred microliters of peptide (100 nmol) inhibitor and 100 μl of ^{3}H-whole cells (2.5×10^{8}/ml) were added to a tube containing statherin-coated HAP beads and incubated for 1 h. The binding level was calculated as described in the text. All assays were performed in triplicate on three separate occasions.

peptides C1 and C2. The inhibition levels of peptides C4 and C5 were reduced after deletion of the residues containing the putative binding sites. The inhibition effect by C5 was very weak.

The results of these experiments suggest that Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43 are elements of the binding sites that mediate interactions with *P. gingivalis* whole cells as well as fimbrillin.

DISCUSSION

We demonstrated that peptide F4 (residues 29 to 43) contains the binding sites for r-Fim by means of direct binding and inhibition studies. Intact statherin in solution showed little inhibitory effect in the binding of r-Fim to statherin-coated HAP beads, likely because of hidden cryptitopes as described previously (2). However, peptide F4 in solution seemed not to have a folded conformation to conceal the binding sites, probably because it consisted of only 15 residues. Peptide F5 (residues 19 to 43) containing the same amino acid sequence (residues 29 to 43) as peptide F4 but failed to reach the same level of inhibition as peptide F4 even at double the concentration. Hence, residues 19 to 28 in peptide F5 likely do not constitute a region that mediates binding. Peptide F3 showed weak abilities in both direct binding and inhibition. These could be due to the effect of Leu-29 and Tyr-16, -18, and -21, which are amino acids composing the binding sites proposed in this study. The N-terminal segment (residues 1 to 15) is rich in polar amino acids such as Asp, Glu, Arg, Lys, and phosphorylated Ser. The promotion of inhibition by peptides F1 and F2 in the inhibition experiment would be through nonspecific ionic interaction.

In the experiments using the N-terminal truncated peptides, two regions, Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43, exhibited strong activity and hence may act as binding sites for r-Fim. The results obtained in assays using peptides N2 and N4 suggested that Pro-33–Tyr-34 and Gln-37–Tyr-38 also contribute as functional binding sites. However, Phe-33–Tyr-34 and Gln-37–Tyr-38 were effective only when these residues were exposed at the N-terminal end. Therefore, it is questionable that these segments, which are aligned inside intact statherin, play a role in binding to *P. gingivalis* r-Fim.

In the experiments using peptides C1 to C5, Phe at the C terminus may be a critical residue. However, the sequential deletion of five specified residues led each time to a reduction in both direct binding and inhibition. Hence, it is likely that they are residues involved in the specific binding to r-Fim. Peptide C5 (residues 31 to 40) probably contains no functional binding site. Collectively, the data in this study suggest that the binding sites of statherin for r-Fim are composed of Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43.

Leu-29 and Phe-43 are highly hydrophobic because of their alkyl and phenyl radicals, respectively, and Phe-43 in the C-terminal end is negatively charged. Tyr-30 and Thr-41 have a hydroxyl group that can be negatively charged or provide a hydrogen bond. Although further studies are necessary to analyze the exact mechanism of receptor-adhesin interaction, the binding by the sites composing Leu-29–Tyr-30 and Tyr-41–Thr-42–Phe-43 might depend on a combination of hydrophobic and ionic interaction and hydrogen bonding.

We proposed the following hypothesis as a mechanism to understand the binding of *P. gingivalis* fimbriae to statherin (1). (i) There are three or more binding sites on fimbrillin protein, and a combination of all contributes to stable binding and retention of the fimbrillin moiety on surface bound statherin. (ii) Statherin also has several binding sites, each corresponding to a specific fimbrillin domain. (iii) Whole cells would be too large to maintain stable binding on statherin without formation of multiple binding bridges by multiple binding sites, which results in energetically stable *P. gingivalis* binding to statherin-coated surfaces. If this hypothesis is correct, the inhibition of *P. gingivalis* binding to statherin can be achieved by coverage or interference of one of the several binding sites. Our results from this study support these proposals. The peptides containing part of all of the binding sites in peptide F4 bound to r-Fim and inhibited r-Fim binding to statherin. Whole-cell binding was more clearly inhibited by incomplete peptides missing part of other residues involved in the binding (Fig. 5). Even when three residues were deleted, peptide C3, possessing Tyr-30 and Tyr-41, was effective, inhibiting 70% of the whole-cell binding. The presence of only one amino acid (Tyr-41) among the five functional residues did not lead to effective inhibition.

A synthetic peptide corresponding to residues 126 to 146 of fimbrillin (50 nmol in 200 μl of reaction mixture) inhibited ^{125}I-r-Fim binding to PRP1-, statherin-, and whole-saliva-coated HAP by 29, 32, and 34%, respectively (1). This peptide also contained the Tyr-Thr-Phe sequence at the C-terminal end. This peptide inhibited over 85% of the whole-cell binding to salivary proteins (1), and residues 126 to 146 reportedly constitute the fibronectin binding domain of fimbriae (22). Hence, the YTF sequence may play an important role in several interactions of *P. gingivalis* fimbriae with host proteins.

There is still little evidence available to explain the structural mechanism that exposes or conceals cryptitopes in statherin. Ramasubbu et al. (18) have proposed a schematic model to explain the structural characteristics of statherin, using structure prediction, circular dichroism, molecular modeling, and mechanics. According to this proposal, in the aqueous or solution phase, a dimer of statherin is formed, with the clustered N-terminal negatively charged groups completely exposed for binding to calcium nuclei. However, in solution, the potential site for adhesion to bacteria is masked by the molecular formation of the dimer. The dimer is separated into monomers by binding to enamel, using the N-terminal and poly-L-proline helices, which exposes the potential sites that are recognized by adhesive bacteria. The proposed functional sites that we found to bind the *P. gingivalis* fimbrillin reside in the middle and C-terminal portions of statherin. This schematic model can explain the molecular mechanism by which the binding site located in the C-terminal portion of soluble statherin is concealed.

PRP1 also has cryptitopes which become exposed by conformational change as the molecule adsorbs to HAP surfaces (2, 6, 7). Analysis of the binding domains of PRP1 for *P. gingivalis* fimbriae is still under way (12). It has been demonstrated that the segment of PRP1 which promotes *Streptococcus gordonii* adhesion resides in the Pro-Gln residues located in the C-terminal portion of PRP1, and the Pro-Gln sequence of the internal residues of PRP1 does not promote adhesion (8). Statherin, which failed to promote *S. gordonii* adhesion to HAP (8), also has internal Pro-Gln sequences at amino acid positions 31 to 32 and 36 to 37. These results suggested that the specific binding sites of both salivary proteins for bacterial adhesion are necessary to reside in the C-terminal end to function. Although the affinity of the five proposed sites of statherin for *P. gingivalis* r-Fim was not fully examined in this study, the C-terminal residues represented by the tripeptide YTF which retained significant binding to r-Fim might be a primary functional site. To confirm this possibility, site-directed mutagenesis to generate statherin variants is likely to yield important information when the variants are tested as components of artificial saliva.

P. gingivalis r-Fim has distinct binding domains for statherin and PRP1 (1). The domains for PRP1 might correspond to Pro-Gln in the C-terminal end as in *S. gordonii*, but the sites of statherin proposed in this study for r-Fim domains would not be recognized by *S. gordonii*. These suggestions may be helpful in understanding the selective adherence to oral surfaces by many species of oral organisms.

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