Active Sites of Salivary Proline-Rich Protein for Binding to *Porphyromonas gingivalis* Fimbriae

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Porphyromonas gingivalis fimbriae specifically bind salivary acidic proline-rich protein 1 (PRP1) through protein-protein interactions. The binding domains of fimbrillin (a subunit of fimbriae) for PRP1 were analyzed previously (A. Amano, A. Sharma, J.-Y. Lee, H. T. Sojar, P. A. Raj, and R. J. Genco, *Infect. Immun.* 64:1631–1637, 1996). In this study, we investigated the sites of binding of the PRP1 molecules to the fimbriae. PRP1 (amino acid residues 1 to 150) was proteolysed to three fragments (residues 1 to 74 [fragment 1–74], 75 to 129, and 130 to 150). ¹²⁵I-labeled fimbriae clearly bound fragments 75–129 and 130–150, immobilized on a polyvinylidene difluoride membrane; both fragments also inhibited whole-cell binding to PRP1-coated hydroxyapatite (HAP) beads by 50 and 83%, respectively. However, the N-terminal fragment failed to show any effect. Analogous peptides corresponding to residues 75 to 89, 90 to 106, 107 to 120, 121 to 129, and 130 to 150 of PRP1 were synthesized. The fimbriae significantly bound peptide 130–150, immobilized on 96-well plates, and the peptide also inhibited binding of ¹²⁵I-labeled fimbriae to PRP1-coated HAP beads by almost 100%. Peptides 75–89, 90–106, and 121–129, immobilized on plates, showed considerable ability to bind fimbriae. For further analysis of active sites in residues 130 to 150, synthetic peptides corresponding to residues 130 to 137, 138 to 145, and 146 to 150 were prepared. Peptide 138–145 (GRPQGPPQ) inhibited fimbrial binding to PRP1-coated HAP beads by 97%. This amino acid sequence was shared in the alignment of residues 75 to 89, 90 to 106, and 107 to 120. Six synthetic peptides were prepared by serial deletions of individual residues from the N and C termini of peptide GRPQGPPQ. Peptide PQGPPQ was as inhibitory as peptide GRPQGPPQ. Further deletions of the dipeptide Pro-Gln from the N and C termini of peptide PQGPPQ resulted in significant loss of the inhibitory effect. These results strongly suggest that PQGPPQ is the minimal active segment for binding to *P. gingivalis* fimbriae and that the moiety of the Pro-Gln dipeptide plays a critical role in expressing binding ability.

The significant pathogenicity of *Porphyromonas gingivalis* has been well documented for several forms of periodontal disease (6, 21). Bacterial adherence to host tissues promotes colonization by the organism as a first step in the development of infections. The organism expresses a number of potential virulence factors which have been implicated in colonization (17, 21). Among these, fimbriae play a major role in bacterial colonization by adherence to oral surfaces including dental and mucous pellicles and to plaque-forming bacteria (17). *P. gingivalis* can bind to saliva-coated surfaces through protein-protein interactions between fimbriae and salivary components on the surfaces (4, 14). The fimbriae may also act as an adhesin to promote the interaction of the anaerobe with gram-positive early colonizers of the teeth through specific protein interactions. A fimbria-deficient mutant of *P. gingivalis* had a significant loss of ability to bind both saliva-coated hydroxyapatite (HAP) beads (15) and gram-positive bacteria (1). We previously reported that salivary acidic proline-rich proteins (PRPs) and statherin strongly promote attachment of *P. gingivalis* fimbriae to apatitic surfaces and that PRP is more effective than statherin (4). These interactions were not inhibited by charged ions, amino acids, or carbohydrates such as monosaccharide, disaccharide, hexosamine, or sialic acid, but they were inhibited by synthetic fimbrillin peptides (3, 14). These results sug-

gested that PRPs and statherin possess specific binding domains for interaction with fimbriae.

Acidic PRPs, major components of human saliva, have been reported to act as salivary receptors for several plaque-forming bacteria, such as *Eikenella corrodens*, *Prevotella loescheii*, *Actinomyces viscosus*, *Actinomyces naeslundii*, *Actinomyces israelii*, *Streptococcus mutans*, *Streptococcus gordonii*, *Streptococcus sanguis*, *Streptococcus uberis*, and *P. gingivalis* (7–10). Acidic PRPs have been thought to be able to promote bacterial adherence through unique hidden receptors called cryptitopes. It has been proposed that the PRP1 molecule undergoes a conformational change when adsorbed to HAP surfaces (8–10); as a result, the cryptitopes are exposed and are responsible for binding to bacterial adhesins. There are ten variations in the commonly occurring acidic PRPs derived from two loci and posttranslational modification (10–12). Among these variants, PRP1, PRP2, and PIF-slow are 150-residue proteins which differ only in residues 4 and 50, while PRP3, PRP4, and PIF-fast are 106-residue proteins which are identical to the first 106 residues of the larger proteins. Since PRP3 and PRP4 were relatively less effective in promoting *P. gingivalis* cell attachment to apatitic surfaces, the cryptitope(s) responsible for the organism has been suggested to reside in the C-terminal 44 residues of the larger PRP (10). However, the nature of the PRP domains interacting with *P. gingivalis* remains unclear.

The functional domains of fimbrillin (a subunit of fimbriae) for binding to PRP1 and statherin have been specified (3), and the active binding sites of statherin for fimbrillin have been localized (2). In continuation of these previous studies, the

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specific domain of the PRP1 molecule that interacts with *P. gingivalis* fimbriae was investigated in this study.

MATERIALS AND METHODS

Bacterial culture conditions. *P. gingivalis* ATCC 33277 was grown and radio-labeled with [^3H]thymidine as described previously (4). The cells were centrifuged, washed 3 times with a 50 mM KCl solution containing 1 mM KH_2PO_4 , 1 mM CaCl_2 , and 0.1 mM MgCl_2 (pH 6.0) (KCl buffer) (4), and prepared for the *P. gingivalis* binding assays as described previously (4).

Purification of fimbriae. Fimbriae were purified from *P. gingivalis* ATCC 33277 by the method of Yoshimura et al. (20) and iodinated as described previously (4). The specific activity of the iodinated protein was 1.8 mCi/ μmol of fimbriin (a subunit of fimbriae).

Preparation and proteolysis of PRP1. PRP1 was purified from human submandibular-sublingual saliva collected from three male donors by the method described by Ramasubbu et al. (18), as in our previous studies (3, 4). Purified PRP1 (1 mg) was dialyzed overnight at 4°C against 4 M urea containing 0.1% sodium dodecyl sulfate (SDS) and finally dialyzed against 10 mM Tris hydrochloride (pH 9.5). The dialysate was subjected to proteolysis with lysendopeptidase (*Achromobacter* protease I; Wako Pure Chemicals, Tokyo, Japan) for 6 h at 30°C at an enzyme-to-substrate molar ratio of 1:200. The resulting peptide fragments were separated by high-performance liquid chromatography with a C_4 reverse-phase column as described previously (5). The amino acid sequencing and amino acid analysis of these fragments were performed with a 477A/120 gas-phase automatic sequencer (Applied Biosystems, Foster City, Calif.) and a Hitachi 835 S amino acid analyzer (Hitachi Ltd., Tokyo, Japan), respectively, as previously described (5).

Dot blot assay. The dot blot assay was performed as described previously (2). PRP1 and the fragments digested by proteolysis were immobilized on a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, Calif.) with a Bio-Dot apparatus (Bio-Rad Laboratories). 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 -labeled fimbriae (20 pmol/ml) for 3 h at room temperature. Thereafter, the membrane was washed with KCl buffer containing 100 mM NaCl followed by autoradiography.

Preparation of peptides analogous to PRP1. Analogous peptides corresponding to the amino acid sequence of PRP1 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 and by fast atom bombardment mass spectrometry with the JMS-HX100/JMA-3100 data system (JEOL Ltd., Tokyo, Japan), respectively.

Assays of fimbrial binding to analogous peptides. The direct binding of fimbriae to synthetic peptides of PRP1 was evaluated with 96-well enzyme-linked immunosorbent assay (ELISA) plates (flat bottom amino plate type A; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) as described previously (2). Briefly, the wells were activated with 100 μl of 2% glutaraldehyde in phosphate-buffered saline (PBS) (pH 7.4). One hundred microliters of PRP1 (2 $\mu\text{mol}/\text{ml}$) or peptide solution (2 $\mu\text{mol}/\text{ml}$) in PBS was added to each well, and the plate was then incubated at 4°C overnight. The wells were washed three times with PBS containing 0.1% Tween 20 (PBST) and coated with 100 μl of 2% Block Ace (casein solution prepared from homogenized milk; Snow Brand Co. Ltd., Sapporo, Japan) in PBST at room temperature for 1 h. After being washed three times with PBST, the wells were incubated with 100 μl of fimbriae (5 nmol/ml) in PBST at room temperature for 3 h. The wells were washed three times with PBST and then coated with 2% Block Ace. Antifimbria rabbit serum diluted 1:5,000 (100 μl) (19) was added to the wells, which were stored at 4°C overnight. Antibodies that reacted with the fimbriae were detected by measuring color intensity at 405 nm as described previously (2). Lipid-free BSA (100 μl at 2 mM) was used as a control to subtract the nonspecific binding level. All assays were performed in triplicate on three separate occasions.

Inhibition by peptides of binding of fimbriae and whole cells to PRP1. The assay of binding of ^{125}I -labeled fimbriae and ^3H -labeled *P. gingivalis* cells to PRP1-coated HAP was carried out by the method described previously (2-4). Briefly, HAP beads (2 mg) in a tube were incubated with 100 μl of PRP1 solution (100 $\mu\text{g}/\text{ml}$) overnight at room temperature. One hundred microliters of peptide solution (1 $\mu\text{mol}/\text{ml}$) or PRP1 (1 $\mu\text{mol}/\text{ml}$) as an inhibitor and 100 μl of ^{125}I -labeled fimbriae (5 nmol/ml) or 200 μl of ^3H -labeled whole cells ($5 \times 10^8/\text{ml}$) were added to a tube containing PRP1-coated HAP beads and incubated at room temperature for 1 h. The specific binding level was calculated by subtracting the nonspecific binding level, which was obtained by preincubation of HAP beads with nonlabeled fimbriae (500 μl at 50 nmol/ml) at room temperature for 1 h, from the binding level with labeled fimbriae. The inhibitory rate was calculated by comparing the specific binding levels with and without inhibitors. All assays were performed in triplicate on three separate occasions.

Analytical methods. SDS-polyacrylamide gel electrophoresis was performed with a Phast System (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) on a PhastGel gradient high density gel (Pharmacia LKB) according to the manufacturer's instructions. Samples were dissolved in 10 mM Tris hydrochloride buffer (pH 8.0) with 2.5% SDS, 1 mM EDTA, and 0.01% bromophenol blue and immersed in a boiling water bath for 5 min. An electrophoresis calibration kit

FIG. 1. SDS-polyacrylamide gel electrophoresis analysis of PRP1 fragments digested with lysendopeptidase. PRP1 (residues 1 to 150) was subjected to proteolysis with lysendopeptidase (*Achromobacter* protease I). The resulting peptides were separated by high-performance liquid chromatography with a C_4 reverse-phase column. Lanes: a, high molecular mass standard; b, PRP1; c, fragment 1-74; d, fragment 75-129; e, fragment 130-150; f, low molecular mass standard.

(Pharmacia LKB) was used for molecular weight markers. The gels were stained with 0.1% Coomassie brilliant blue R-350. The protein content of the samples was determined with a Bio-Rad protein assay kit with BSA as a standard, according to the manufacturer's instructions. The peptide content was measured by dry weight or amino acid analysis.

RESULTS

Proteolytic fragments of PRP1 exhibiting ability to bind to *P. gingivalis* fimbriae. PRP1 was digested to three fragments by proteolysis and each fragment was purified to homogeneity as shown in Fig. 1. Purified fragments were subjected to dot blot assay to identify regions of PRP1 involved in binding to fimbriae. The amounts of the three fragments immobilized on the membrane were optimized to be equal by amino acid analysis as described previously (reference 2 and data not shown). As shown in Fig. 2A, the intensity of the dot of the fragment corresponding to amino acid residues 130 to 150 (fragment 130-150) was observed to be as deep as that of PRP1 in terms of the interaction with ^{125}I -labeled fimbriae. Fragment 75-129 also showed a clear dot; however, fragment 1-74 showed no interaction with ^{125}I -labeled fimbriae. Fimbriae are major components that enable *P. gingivalis* to attach to PRP1-coated HAP beads (4, 14); therefore, the inhibitory effects of the fragments were examined in the binding of ^3H -labeled whole cells to PRP1 (Fig. 2B). Fragment 130-150 (100 nmol) showed the most significant inhibition activity, at 84%. Fragment 75-129 also inhibited whole-cell binding by 49%, while the inhibitory effect of fragment 1-74 was negligible. The inhibitory activities of these fragments (100 nmol) were more significant in the binding of ^{125}I -labeled fimbriae to HAP beads coated with PRP1 (data not shown). The inhibitory effects were almost 100% by fragment 130-150 and $83\% \pm 4.2\%$ (mean $\pm$ standard deviation) by fragment 75-129. The effect of fragment 1-74 was less than 5%. These results suggested that residues 75 to 150 contain functional binding domains for *P. gingivalis* fimbriae.

Determination of the binding region of PRP1 by using analogous peptides. To further localize the active binding region of inner residues 75 to 150, five synthetic analogous peptides corresponding to residues 75 to 89, 90 to 106, 107 to 120, 121 to 129, and 130 to 150 were prepared, as shown in Fig. 3. The abilities of the peptides to directly bind to fimbriae were examined (Fig. 3A). Fimbriae bound as strongly to peptide 130-150, immobilized on ELISA plates, as to PRP1. Peptides 75-89, 90-106, and 107-120 also showed considerable ability to bind to fimbriae, at 77, 78, and 86% of that of PRP1, respec-

FIG. 2. Active region of PRP1 for binding to *P. gingivalis* fimbriae. (A) Dot blot assays to determine which fragment is involved in binding to fimbriae. PRP1 fragments, BSA, and PRP1, immobilized on polyvinylidene difluoride membranes, were probed with ¹²⁵I-labeled fimbriae for 3 h at room temperature. The membrane was washed with KCl buffer containing 100 mM NaCl and then visualized by autoradiography. (B) Inhibitory effect of PRP1 fragments in the binding of ³H-labeled *P. gingivalis* cells to HAP coated with PRP1. HAP beads coated with PRP1 were incubated with 200 μ l of ³H-labeled *P. gingivalis* cells (5×10^8 cells/ml) in the presence of PRP1 (100 nmol) or each fragment (100 nmol). Nonlabeled fimbriae (500 μ l at 50 nmol/ml) was preincubated with PRP1-coated HAP beads to obtain the nonspecific binding level of the ³H-labeled cells. The inhibitory activity was calculated by subtracting nonspecific binding as background from each total binding level. All assays were performed in triplicate on three separate occasions. Data are expressed as means $\pm$ standard deviations.

tively. A single amino acid sequence (GRPQGPPQ) was shared by all active peptides, corresponding to residues 75 to 80, 92 to 97, 109 to 114, and 140 to 145. This sequence region may be involved in the interaction with fimbriae. To examine the nature of these interactions, charged amino acids (L-arginine and histidine [0.1 M]) and sodium chloride (1 M) were added to the reaction mixtures. Arginine slightly inhibited the binding of the active peptides by 15 to 20%, while the effects of histidine and sodium chloride were negligible (data not

shown). These findings suggested that all active peptides contain specific binding region(s) for fimbriae.

Since the binding region of each active peptide might be cryptic, the inhibitory effects of the peptides in solution were examined in the binding of ¹²⁵I-labeled fimbriae to PRP1-coated HAP beads, as shown in Fig. 4. Peptide 130–150 (100 nmol) inhibited fimbrial binding to PRP1 by almost 100%, while the inhibitory effects of the remaining peptides were negligible, indicating that the cryptotope(s) of these peptides is hidden in solution as in the PRP1 molecule.

Determination of an active binding region in residues 130 to 150 of PRP1. For further analysis of the binding region in peptide 130–150, which was effective both when adsorbed to the surface and in solution, three peptides were designed in correspondence to the shared common sequence (residues 138 to 145) in the active peptides and the remaining alignments on both sides in residues 130 to 150. The synthetic peptides were as follows: peptides 130–137 (PQGPPQ), 138–145 (GRPQGPPQ), and 146–150 (GQSPQ). Preliminary studies showed that, for small peptides such as GQSPQ, peptide length was a significant factor in linking the peptide with functional groups on ELISA plates (data not shown). Hence, the direct binding assay could not be performed for these peptides, so the inhibitory effects of the peptides in solution were assayed to determine the active region. As shown in Fig. 5, peptide 138–145 was significantly inhibitory at a level almost identical to that of peptide 130–150. The effects of peptides 130–137 and 146–150 were also found to be inhibitory; however, the levels were significantly less than that of peptide 138–145.

Determination of the minimal active segment of the common sequence. It was clear that the sequence GRPQGPPQ contains the site involved in the interaction with *P. gingivalis* fimbriae, as shown in Fig. 5. To determine the minimal active segment, we synthesized six peptides in which amino acid res-

FIG. 3. Determination of the binding domain of PRP1 by using analogous peptides. Direct binding of fimbriae to peptides 75–89, 90–106, 107–120, 121–129, and 130–150, immobilized on wells of ELISA plates. One hundred microliters of each peptide (2 μ mol/ml) in PBS was added to each well, and the plate was then incubated at 4°C overnight. The wells were coated with 1% Block Ace in PBST at room temperature for 1 h. After being washed three times with PBST, the wells were incubated with 100 μ l of fimbriae (5 nmol/ml) in PBST at room temperature for 3 h. The amount of adsorbed fimbriae was detected with anti-fimbria rabbit serum as described in the text. Lipid-free BSA (2 mM) was used as a control to obtain the nonspecific binding level. The binding levels of the fimbriae were calculated by subtracting nonspecific binding from each binding value. All assays were performed in triplicate on three separate occasions. Data are expressed as means $\pm$ standard deviations.

FIG. 4. Inhibition by peptides in the binding of ^{125}I -labeled fimbriae to HAP beads coated with PRP1. One hundred microliters of each peptide (100 nmol) and 100 μl of ^{125}I -labeled fimbriae (0.5 nmol) were added to a tube containing PRP1-coated HAP beads and incubated for 1 h. Nonlabeled fimbriae (500 μl at 50 nmol/ml) were preincubated with PRP1-coated HAP beads to obtain the nonspecific binding level of the ^{125}I -labeled fimbriae. The inhibitory activity was calculated by subtracting nonspecific binding as background from each total binding level. All assays were performed in triplicate on three separate occasions. Data are expressed as means $\pm$ standard deviations.

idues were deleted from the N terminus of peptide 138–145. As shown in Fig. 6A, serial deletions of the Gly and Arg residues showed no effect. By the deletions of the Pro and then the Gln residues, however, the inhibitory effects of the peptides was dramatically reduced. It seemed likely that PQGPPQ was the minimal active segment. To examine the significance of the C-terminal residues, another set of peptides was prepared by the serial deletion of residues from the C terminus of peptide PQGPPQ, as shown in Fig. 6B. The loss of the Gln residue led to a 35% reduction of the inhibitory effect, and further deletion

FIG. 5. Determination of the active binding domain in residues 130 to 150 of PRP1. Synthetic peptides 130–137, 138–145, and 146–150 were used as inhibitors in the binding of ^{125}I -labeled fimbriae to PRP1-coated HAP beads. One hundred microliters of each peptide (100 nmol) and 100 μl of ^{125}I -labeled fimbriae (0.5 nmol) were simultaneously added to the tube. The experimental procedure was as described in the legend to Fig. 4. All assays were performed in triplicate on three separate occasions. Data are expressed as means $\pm$ standard deviations.

FIG. 6. Determination of active sites in residues 138 to 145 of PRP1. (A) Synthetic peptides in which amino acid residues were deleted from the N terminus of peptide 138–145 were used as inhibitors in the binding of ^{125}I -labeled fimbriae to PRP1-coated HAP beads. One hundred microliters of each peptide (100 nmol) and 100 μl of ^{125}I -labeled fimbriae (0.5 nmol) were simultaneously added to the tube. The experimental procedure was as described in the legend to Fig. 4. (B) Synthetic peptides in which amino acid residues were deleted from the C terminus of peptide 140–145 were used as inhibitors in the binding of ^{125}I -labeled fimbriae to PRP1-coated HAP beads. The experimental procedure was as described for panel A. All assays were performed in triplicate on three separate occasions. Data are expressed as means $\pm$ standard deviations.

of the Pro residue resulted in a complete loss of activity. These findings strongly suggested that PQGPPQ is the minimal active segment for binding to *P. gingivalis* fimbriae and that the moiety of the Pro-Gln dipeptide plays a critical role in binding ability.

DISCUSSION

PRP1 has been reported to promote the adhesion of several plaque-forming bacteria, and the domains of PRP1 for interactions with certain bacteria are thought to be cryptic (7–10).

Although the nature of the cryptitope(s), such as exact binding domains, remains unknown, there has been only one reported study that describes binding sites of PRP1 for a bacterium. Gibbons et al. (7) demonstrated that the cryptic segment of PRP1 which promotes *S. gordonii* adhesion resides in the Pro-Gln sequence located in the C-terminal end of PRP1. In the present study, we investigated the interaction of PRP1 with *P. gingivalis* and identified the minimal active binding site of PRP1 for *P. gingivalis* fimbriae by using a series of synthetic peptides. Amino acid residues 140 to 145 (PQGPPQ) of PRP1 have been shown to be the minimal active site for binding to *P. gingivalis* fimbriae. Since this segment resides in four regions (residues 75 to 80, 92 to 97, 109 to 114, and 140 to 145) of the PRP1 molecule, these four regions would seem to provide structural binding sites for the fimbriae. This is consistent with the findings that *P. gingivalis* fimbriae bound to immobilized peptides 75–89, 90–106, 107–120, and 130–150, which all contain the PQGPPQ segment. However, peptide 130–150 had a significant inhibitory effect on the binding of the fimbriae to PRP1-coated HAP, whereas peptides 75–89, 90–106, and 107–120 showed slight inhibitory effects when they were used as inhibitors in solution. In the inhibition studies with these peptides, a 200-fold excess of peptide (100 nmol) relative to ¹²⁵I-labeled fimbriae (0.5 nmol) was simultaneously added to a reaction mixture. These concentrations should have been sufficient to show a maximal level of activity if the peptides were inhibitory. There is little evidence available to explain these discrepancies. One possible explanation is that active sites in the analogous peptides are as cryptic as in the whole molecule. Although it is unclear whether the binding site composed of PQGPPQ acts as a ligand or receptor, the binding activity would be associated with a certain tertiary structure to form a ligandary male or receptory female. Since amino acid residues such as Pro and Gln interfere with formation of α -helix and β -sheet structure, the PQGPPQ segment should form an unordered tertiary structure. Such a structure may easily undergo conformational transformation associated with that of the whole molecule. The PQGPPQ segment might even be cryptic in peptides depending on the conformation of the molecule. Peptides 75–89, 90–106, and 107–120 contain a QGGH segment next to the PQGPPQ segment. The QGGH sequence may be a factor that interferes with the binding of fimbriae to the binding site in solution by overlapping the site. When these three peptides are adsorbed on surfaces, the PQGPPQ segment may be exposed and fimbriae may bind to them. However, the PQGPPQ segment of peptide 130–150, which does not contain the QGGH segment, might be exposed even in solution and be able to inhibit fimbrial binding to PRP1. To confirm this hypothesis, new peptides synthesized by adding amino acid residues such as QGGH next to the PQGPPQ segment may be useful for further study.

Peptides 130–137 and 146–150 also inhibited the binding of *P. gingivalis* fimbriae to PRP1-coated HAP. Although these peptides do not contain the PQGPPQ segment, the sequences show high homologies to PQGPPQ. There might be another fimbria-binding site of PRP1. However, the inhibitory effect of these peptides was much less than that of the PQGPPQ segment. Therefore, the PQGPPQ segment may be a major fimbria-binding site, while other binding sites may play accessory roles.

The first 30 amino acid residues in the N terminus of PRP1 contain 13 of the 15 negatively charged amino acids in the protein, and these amino acids have been reported to provide the high affinity of PRP1 to apatitic surfaces (12, 13). Our results showed that fragment 1–74, which contains the charged residues, did not bind to the fimbriae. This could be evidence

that the interaction between fimbriae and PRP1 is not mediated by negatively charged amino acids. The result of the inhibition assay using NaCl and charged amino acids such as L-arginine and L-histidine as inhibitors may support the fact that *P. gingivalis* fimbriae bind to PRP1 through specific interaction. Judging by the amino acid residues in the active site, this specific interaction would seem to be mediated by the hydrophobicity of Pro and the hydrogen bond provided by Gln.

We searched a protein database (BLASTP-Swissprot/UPD; European Bioinformatics Institute, University of Geneva) for other human proteins with amino acid sequences homologous to the active site of PRP1. In the human PRP family, all PRPs contain several regions of PQGPPQ, and basic PRPs such as IB1 and IB6 possess active sequences such as PQGPPQ, PQGPP, and PQGPPPQ, which are also effective inhibitors (Fig. 5 and 6B). In our previous study (4), the basic PRP showed little affinity for *P. gingivalis* cells and fimbrillin. However, the protein used was the basic proline-rich peptide P-B, which was without homology to the active sequences (18). Salivary proline-rich glycoprotein also contains active sequences such as PQGPPQ, PQGPP, and PQGPPPQ. These results suggest that many salivary proline-rich proteins and glycoproteins may possess specific affinities for *P. gingivalis* fimbriae. Furthermore, the homology research revealed that human collagen alpha (I and IV) contains multiple regions of PQGPP. Fimbriated *P. gingivalis* strains have been reported to show high abilities to bind collagen types I and IV (16). This binding might be mediated by the interaction between fimbriae and the PQGPP regions in collagen.

We reported that *P. gingivalis* recombinant fimbrillin has distinct binding domains for PRP1 and statherin and proposed that the LY and TYF sequences of statherin play important roles in the interaction with *P. gingivalis* fimbriae (2). As described above, *S. gordonii* recognizes PQ residues located at the C-terminal end of PRP1, and the PQ sequence of the internal residues of PRP1 does not promote adhesion (7). However, *P. gingivalis* fimbriae recognize inner segments of statherin such as LY sequences (2). In this study, *P. gingivalis* recognized the inner segment of PRP1. These results may be helpful in understanding the diversity of adherence by many species of oral organisms.

In this study, the binding site of PRP1 for *P. gingivalis* fimbriae was determined to be the PQGPPQ segment. To understand the binding mechanism in detail, further studies, including generation of recombinant PRP1 variants, are now in progress.

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