Involvement of a Lysine-specific Cysteine Proteinase in Hemoglobin Adsorption and Heme Accumulation by Porphyromonas gingivalis*

Kuniaki Okamoto‡‡, Koji Nakayama†, Tomoko Kadowaki‡‡, Naoko Abe‡, Dinath B. Ratnayake†, and Kenji Yamamoto‡‡‡

From the Departments of *Pharmacology and †Microbiology, Kyushu University Faculty of Dentistry, Higashi-ku, Fukuoka 812-8582, Japan

The oral anaerobic bacterium Porphyromonas gingivalis, a major pathogen of advanced adult periodontitis, produces a novel class of cysteine proteinases in both cell-associated and secretory forms. A lysine-specific cysteine proteinase (Lys-gingipain, KGP), as well as an arginine-specific cysteine proteinase (Arg-gingipain), is a major trypsin-like proteinase of the organism. Recent studies indicate that the secreted KGP is implicated in the destruction of periodontal tissue and the disruption of host defense mechanisms. In this study, we have constructed a KGP-deficient mutant to determine whether the cell-associated KGP is important for pathophysiology of the organism. Although the mutant retained the strong ability to disrupt the bactericidal activity of polymorphonuclear leukocytes, its hemagglutination activity was reduced to about one-half that observed with the wild-type strain. More important, the mutant did not form black-pigmented colonies on blood agar plates, indicating the defect of hemoglobin adsorption and heme accumulation of the organism, and in the ability to degrade fibrinogen. These results suggest the possible involvement of KGP in the hemoglobin binding and heme accumulation of the organism and in the bleeding tendency in periodontal pockets.

The proteinases of the Gram-negative, black-pigmented anaerobe Porphyromonas gingivalis are believed to be involved in a wide range of pathologies of progressive periodontal disease (reviewed in Refs. 1 and 2). Recently, the trypsin-like activity associated with the organism is found to be attributable to either Arg-X- or Lys-X-specific cysteine proteinase (where X is an unknown amino acid residue) (3). These two enzymes have now been termed Arg-gingipain (gingipain-R, RGP) and Lys-gingipain (gingipain-K, KGP) on the basis of their peptide cleavage specificity after arginine and lysine residues, respectively. Catalytic and structural studies have revealed that these enzymes are a novel class of the cysteine proteinase family (1).

Recently, it has been suggested that the proteolytic activities of RGP and KGP are involved in the pathogenesis of progressive periodontal disease through the following mechanisms: (i) directly degrading structural proteins of the periodontal tissues (4–12), (ii) disrupting host defense mechanisms (7, 13–19), (iii) activating or stimulating the expression of hemagglutinins (20), (iv) processing and translocating adhesion molecules (21), and (v) inducing or stimulating inflammation through the production of chemical mediators (10, 22, 23). Previous studies of RGP-deficient mutants constructed by use of suicide plasmid systems revealed that RGP plays a major role in the disruption of polymorphonuclear leukocyte (PMN) functions and the hemagglutination and fimbriation by the organism (20, 21). However, little information is available about to what extent KGP contributes to the entire virulence of P. gingivalis. To gain some insight into this question, it is necessary to undertake the molecular genetic approach.

So far, three genes encoding Lys-X-specific cysteine proteinases have been cloned and sequenced, but all of the genes seem to be essentially equivalent to one another (24–26). Southern hybridization analyses have also suggested that a single KGP-encoding gene exists on the chromosome of P. gingivalis (24). The nucleotide sequence of the kkg gene of P. gingivalis strain and the deduced amino acid sequence have suggested that the precursor of KGP comprises at least four domains: the signal peptide, the amino-terminal propeptide, the catalytic proteinase domain, and the carboxyl-terminal hemagglutinin domain. In the present study, we have constructed a KGP-deficient mutant via disruption of the kkg gene by use of suicide plasmid systems to analyze the function of KGP in the organism. The results provide evidence suggesting that KGP is associated with hemagglutination, hemoglobin binding and heme accumulation by the organism, and the bleeding tendency in periodontal pockets and that it is not directly implicated in the production of virulence factors responsible for suppression of the bactericidal activity of PMNs.

EXPERIMENTAL PROCEDURES

Media and Conditions for Cell Growth—P. gingivalis cells were grown anaerobically (10% CO₂, 10% H₂SO₄, 80% N₂) in enriched BHI broth (containing, per liter, 37 g of brain heart infusion (Difco), 5 g of yeast extract (Difco), 1 g of cysteine, 5 mg of hemin, and 1 mg of vitamin K₁) and on enriched BHI agar (containing, per liter, 15 g of agar (Nakarai, Tokyo, Japan), 37 g of brain heart infusion, 5 g of yeast extract, 1 g of cysteine, 5 mg of hemin, and 1 mg of vitamin K₁). L broth (containing, per liter, 10 g of tryptone (Difco), 5 g of yeast extract, and 5 g of sodium chloride) was used for growing Escherichia coli cells. For selection or maintenance of the antibiotic-resistant strains, antibiotics were added to the medium at the following concentrations: ampicillin, 200 μg/ml; erythromycin, 10 μg/ml.

DNA Manipulation—Chromosomal DNA was isolated from P. gingivalis cells by the guanidine isothiocyanate method (27) with the Iso-Quick DNA extraction kit (MicroProbe, Garden Grove, CA) for Southern blot analyses.
**Plasmid Construction**—Suicide plasmids constructed in this study are depicted in Fig. 1. An ~3.5-kbp BamHI fragment of plasmid pNKD (24), containing the gene of whole KGP proteinase domain, was ligated to pUC118, which was digested with EcoRI, filled in by the Klenow enzyme, digested with BamHI, and treated with alkaline phosphatase. The resulting plasmid was then digested with EcoRI and self-ligated to make a deletion within the kgp gene, giving rise to plasmid pNKV-2. A BamHI chromosomal fragment (3.3 kbp) of pNKV-2 was then ligated to pKD283 DNA (28) that had been linearized with BamHI and treated with alkaline phosphatase, resulting in plasmid pNKD.

**DNA Probes and Southern Blot Hybridization**—Two synthetic oligonucleotides, 5'-GCTAGTGCTGCTCCGGTCTTCTTGG-3' (Probe I) and 5'-GGCAGAGCTACTATTGGAGTGTCGG-3' (Probe II), were obtained from Greiner Japan (Tokyo, Japan) and Kurabo (Osaka, Japan), respectively, and were labeled with fluorescein-dUTP (Amersham Pharmacia Biotech, Little Chalfont, UK). Chromosomal DNA of wild-type and KGP-deficient strains was digested with HindIII and subjected to a 0.8% agarose gel for Southern blot analysis. Digested genome DNA was transferred to a nitrocellulose membrane (Schleicher & Schuell) essentially according to Southern (29). Hybridization with probes I and II and transferred to a nitrocellulose membrane (Schleicher & Schuell) essentially according to Southern (29). Hybridization with probes I and II and detection of signals was done by using the ECL 3'-oligolabeling (Amersham) and the SuperSignal™Nucleic Acid (Pierce), respectively.

Electrotransformation of *P. gingivalis* with pNKD Plasmid DNA— *P. gingivalis* cells were anaerobically grown to 6 × 10^9/ml in 37 °C in enriched BHI broth. The cells were then harvested by centrifugation, washed with the electroporation solution (300 mM sucrose), and resuspended in 0.1 volume of the same solution. 10 μl of plasmid DNA solution (300 μg of DNA/ml in TE buffer) were added to 0.4 ml of the cell suspension. The volume of the DNA-containing cell suspension was poured into a cuvette for electroporation (Pulsar™ cuvette with 0.2-cm electrode gap, Bio-Rad). Electroporation was performed at 2.0 kV with an electroporation apparatus (Gene Pulsar™, Bio-Rad). These procedures were carried out at 4 °C. The cell suspension was immediately mixed with 10 ml of prewarmed enriched BHI broth and incubated anaerobically at 37 °C for 15 min. The absorbance of the supernatant was measured at an optimal density (650 nm) of 0.44. The bacterial suspensions were centrifuged at 10,000 g for 30 min and resuspended in 10 mM sodium phosphate buffer (pH 7.0) containing 0.05% Brij 35. After dialysis against the same buffer at 4 °C overnight, insoluble materials were removed by centrifugation at 25,000 × g for 30 min. 20 μg of human fibrinogen were incubated with the respective dialysates (1 μg) for 4 h at 37 °C, and then electrophoresis was performed on SDS gels. The gel was stained with Coomassie Brilliant Blue R-250. Fifty points of each band were measured and averaged by Microcomputer Imaging Device.

Measurement of Luminol-dependent CL Response—CL response of PMNs was measured according to the method described previously (7). Hemagglutination Assay—Overnight cultures of *P. gingivalis* strains were centrifuged, washed twice with PBS, and resuspended in PBS at an optimal density of 660 nm of 0.44. The bacterial suspensions were then diluted in a 2-fold series with PBS. A 100-μl aliquot each of the dilutions was mixed with an equal volume of sheep erythrocyte suspension (2.5% in PBS) and incubated in a round-bottom microtiter plate at room temperature for 3 h.

**RESULTS**

Construction of a KGP-deficient Mutant and Southern Blot Analyses—A KGP-deficient mutant was constructed via gene disruption by use of a suicide plasmid containing a part of the *kgp* gene. The suicide plasmid pNKD contained an amino-

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**Fig. 1. Plasmid construction.** Amp, ampicillin resistance; Em, erythromycin resistance; GUS, β-glucuronidase gene; ori, replication origin functioning in *E. coli*; flI (~1G, flII (~ intergenic region; lacZ, β-galactosidase gene; ColE1 ori, replication origin. The black region is a partial *kgp* gene (signal sequence, amino-terminal prodomain, proteinase domain, and a part of carboxyl-terminal prodomain). Restriction sites: BamHI, H, HindIII, RI, EcoRI, RV, EcoRV.
Roles of Lys-gingipain in P. gingivalis

FIG. 2. Chromosomal structures around the kgp regions of the Em' transformants (A) and Southern blot analysis of their chromosomal DNA (B). A. DNA regions complementary to the kgp-specific oligonucleotides that were used as DNA probes for Southern blot analysis were indicated under the physical map of the chromosomal DNA of the wild-type strain. Four possible structures arise from plasmid integration by a single cross-over (a and b) and gene conversion (c and d). The white boxes indicate the EcoRI segment of the kgp gene. Arrowsheads show a deletion of the segment. Restriction sites: B, BanHI; H, HinIII; RI, EcoRI; RV, EcoRV. Em', erythromycin resistance. B, chromosomal DNAs of the wild-type strain and KGP-mutants were digested with HindIII and subjected to a 0.8% agarose gel for Southern blot analysis. Probe I, 5'-GCTAGGTGCTGGCCCGTCTTCCTTGG-3'; and probe II, 5'-GCCAGGACTACTATTGGAGTGTCGG-3'. Lanes 1, the wild-type ATCC33277; lanes 2, KDM16; lanes 3, KDM35.

diagram

FIG. 3. Immunoblot analyses of cell extracts and culture supernatants of the kgp mutant with antibodies recognizing RGP and KGP. Both cell extracts (lanes 1–3) and culture supernatants (lanes 4–6) from ATCC33277, KDM16, and KDM35 (each 20 µg of protein) were subjected to SDS-polyacrylamide gel electrophoresis on 10% gels. Proteins separated on the gels were transferred to nitrocellulose membranes and immunostained with antibodies recognizing both RGP and KGP. Lanes 1 and 4, ATCC33277; lanes 2 and 5, KDM16; lanes 3 and 6, KDM35. The 51-kDa protein of the ATCC33277 that is lacking in KDM35 is indicated by closed circles.

Whereas that of d-type transformants has one HindIII fragment (1 kbp) hybridizing to probe I and no DNA fragment hybridizing to probe II. The chromosomal DNA of the wild-type parent has one HindIII fragment (1.2 kbp) hybridizing to probes I and II. Southern analysis using probes I and II revealed that KDM16 and KDM35 were of types c and d, respectively (Fig. 2B).

Western Blot Analysis with Antibodies Recognizing Both KGP and RGP and the KGP Activity—To determine whether the transformant KDM35 was devoid of KGP protein, immunoblot analysis of the mutant was performed by use of antibodies reacting with both KGP and RGP (Fig. 3). The culture supernatant of ATCC33277 (wild-type parent) showed four clear protein bands with apparent molecular masses of 51, 44, 40, and 32 kDa and a smearing band of 70–100 kDa. There was no significant difference in electrophoretic profiles between KDM16 and ATCC33277, although the 40- and 32-kDa proteins were markedly decreased in KDM16. The 32-kDa protein was also detected in KDM35. However, both the cell extract and culture supernatant of KDM35 was devoid of the 51-kDa band. The 40-kDa protein could be detected by immunoblotting of a large amount of the supernatant proteins of KDM35, whereas the 51-kDa protein was not observed under the same conditions (data not shown). Therefore, the 51-kDa protein appeared to be KGP. On the other hand, the proteins of 44, 40, 32, and 70–100 kDa seemed to be derived from the kgp gene products, although its expression level was slightly different between the wild-type parent and its mutants, which was consistent with a previous study (20).

KGP can specifically cleave the Lys-X peptide bond. The proteolytic activity on the synthetic substrate t-butyloxycarbonyl-Val-Leu-Lys-MCA was scarcely detectable in either the cell extract or the culture supernatant of KDM35, whereas KDM16 showed the same activity in these fractions as the parent ATCC33277 (Fig. 4A). On the other hand, the hydrolizing activity on the synthetic substrate carbobenzoxy-Phe-Arg-MCA, which represents the RGP activity, was not significantly changed in either fraction of KDM35, as well as KDM16 (Fig. 4B). Thus, KDM35 was found to be devoid of KGP at the protein and activity levels, indicating that KDM35 is a KGP-deficient mutant that possesses a deletion within the proteinase domain region of the kgp gene.

Effect on CL Response of PMNs—The culture supernatant of the wild-type strain contained potent virulence factors, which disrupt the bactericidal function of PMNs (37). Both RGP and KGP purified from the culture supernatant of P. gingivalis...
exhibited the potent suppressive activity against the CL response of PMNs stimulated by serum-activated zymosan (7, 33). Further, in agreement with the previous results (20), the culture supernatant of the RGP-null mutant (the rgpA rgpB double mutant, KDP112) was shown to almost completely lose the inhibitory effect on the CL response of PMNs, confirming that RGP is responsible for suppression of the bactericidal function of PMNs (Fig. 5A). In contrast, the culture supernatant of the KGP-deficient mutant (KDM35), like the wild-type strain and KDM16, resulted in the intense inhibition of the CL response of PMNs, suggesting that contribution of KGP to the inhibition of the bactericidal activity of PMNs by the culture supernatant of P. gingivalis is not as much as that of RGP and that KGP is not directly involved in the production of P. gingivalis factors responsible for the inhibition.

Hemagglutination—In our previous study (20), we found that the RGP-null mutant showed a greater decrease in the hemagglutinating activity observed with the wild-type strain. Because the initial translation product of KGP appeared to contain the hemagglutinin domain in the carboxyl-terminal region (24), which is significantly homologous to that of the RGP gene product (38), it is of special importance to determine whether KGP is related to hemagglutination of P. gingivalis. The RGP-null mutant almost completely lost the intense hemagglutinating activity observed with the wild-type strain. Although KDM16 had the same hemagglutinating activity as the wild-type strain, KDM35 reduced the extent of hemagglutination to one-half those of the wild-type strain and KDM16 (Fig. 5B). These results indicate a significant contribution of the cell-associated KGP to the hemagglutination of P. gingivalis.

Black Pigmentation—As shown in Fig. 6, P. gingivalis strains produce black-pigmented colonies on laked blood agar plates. It is generally accepted that the black pigments are heme, which is an absolute requirement for growth of P. gingivalis (39, 40), and that it is probably derived from erythrocytes in the natural niche for the organism. Therefore, it is particularly important for the organism to aggregate and lyse erythrocytes to survive in vivo (41, 42). To determine whether KGP is involved in the formation of black-pigmented colonies, KDM35 was grown on laked sheep blood agar. Although KDM16, like the wild-type strain, developed black-pigmented colonies, KDM35 formed less pigmented colonies (Fig. 6). Further, it is questionable whether KDM35, the RGP-null mutant, and the wild-type strain can form black-pigmented colonies when grown on enriched tryptic soy agar plates with or without 2% hemoglobin (data not shown). On the plate without hemoglobin, neither of them showed both cell growth and black pigmentation. However, in the presence of hemin in enriched tryptic soy agar plates each strain resulted in the cell growth but not black pigmentation. In the presence of hemoglobin, only KDM35 did not form black-pigmented colonies, but it retained the cell growth activity. These findings indicate that KGP seems to be involved in the hemoglobin adsorption and the heme accumulation by the organism.

Hemoglobin Adsorption and Hemoglobin Receptor Protein Production—More recent work in our laboratory has resulted in the identification and purification of a prominent 19-kDa protein that was significantly expressed in P. gingivalis when grown on blood agar plate (32). This protein was found to be the hemoglobin receptor (HbR) protein (34) that was intragenically obtained with the W50 strain of P. gingivalis (45) and that hagA genes of P. gingivalis (24, 43, 44). Also, it was interesting to note that HbR protein was not expressed in nonpigmented mutants (BE1 and BR1) that were isolated from the W50 strain of P. gingivalis (45) and that the ability of these mutants to bind hemoglobin was markedly decreased (34). Therefore, to determine whether the decreased pigmentation of KDM35 is attributable to a defect of hemoglobin adsorption, we examined the mutant for the ability to bind hemoglobin. The results indicate that KDM35 binds hemoglobin to a lesser extent than the kgpA sibling strain KDM16 (Fig. 7A). Immunoblot analysis with anti-HbR antiserum revealed
that the cell extract of KDM35 grown on the laked blood agar for 3 days produced no HbR protein, whereas that of KDM16 produced a single protein band with an apparent molecular mass of 19 kDa (Fig. 7B). However, after 7 days of incubation KDM35 produced a small amount of the HbR protein, and after 17 days it produced as much as that of KDM16.

**Degradation of Fibrinogen**—Fibrinogen is a 300-kDa protein that consists of three pairs of polypeptide chains (designated Aα, Bβ, and γ) covalently linked by disulfide bonds. Thrombin is known to convert fibrinogen to fibrin monomers, leading to the forming of a fibrin gel. Because of the bleeding tendency in periodontal pockets and the observation that *P. gingivalis* gipains cleave fibrinogen (46–48), we examined whether KGP contributes to blocking blood coagulation in the periodontal pockets. For this, the culture supernatants freshly harvested from KDM35, KDP112, and the wild-type strain were incubated with human fibrinogen at 37 °C for 4 h (Fig. 8A). All of the chains were extensively degraded by the culture supernatants of the wild-type strain and the RGP-null mutant, although the former was more effective than the latter. However, the culture supernatant of the KGP-deficient mutant KDM35 was less effective in proteolysis of these chains, especially Bβ and γ chains. Densitometric scanning revealed that the degradation by the culture supernatant of KDM35 was reduced to about one-half that of the wild-type strain for the Aα and Bβ chains and one-fourth that of the wild-type strain for the γ chain (Fig. 8B).

**DISCUSSION**

In our previous study (20), we constructed RGP-deficient mutants from *P. gingivalis* ATCC33277 and provided evidence suggesting that RGP plays critical roles in inhibition of the bactericidal activity of PMNs and the hemagglutination by the organism. In this study, we have constructed the KGP-deficient mutant, designated KDM35, from *P. gingivalis* ATCC33277 by integration of the Em r suicide plasmid pNKD containing a DNA fragment of the gene to clarify the KGP function in the organism. The cell extract and culture medium of KDM35
showed no Lys-X-specific cysteine proteinase activity but still retained the same Arg-X-specific cysteine proteinase activity as that of the wild-type strain in both fractions, indicating that only the KGP proteinase gene is disrupted.

We have previously shown that the initial translation product of KGP, like RGP, contains the hemagglutinin-related sequence in the carboxyl-terminal domain (24) and that the RGP-null mutant KDP112 is almost devoid of the hemagglutinating activity and loses the inhibitory effect on the bactericidal activity of PMNs (20). More recently, we have also found that the purified KGP significantly inhibits the bactericidal activity of PMNs (37). Therefore, in this study we determined to what extent KGP contributes to the disruption of host defense mechanisms and the hemagglutinating activity by P. gingivalis by biochemical analysis of the KGP-deficient mutant KDM35. Morphologically, KDM35 possessed a similar number of characteristic kinky fimbriae on the cell surface of the wild-type strain. Taken together, the precursors of fimbriin, a major component of fimbriae, and a 75-kDa cell surface protein have been shown to undergo normal processing in KDM35. These results suggest that KGP is not significantly involved in processing and translocation of the cell surface proteins. The culture supernatant of this mutant also had the same suppressive activity on the CL response of PMNs as that of the wild-type strain, indicating that KGP has little contribution to production of virulence factor(s) responsible for disruption of the bactericidal activity of PMNs. This is consistent with the observation that the culture supernatant of RGP-null mutant almost completely lost the inhibitory effect of the culture supernatant of P. gingivalis on the CL response of PMNs (20). In contrast, KDM35 showed a significant decrease in the hemagglutinating activity, suggesting that KGP significantly contributes to the generation of hemagglutinins from the initial translation products of hemagglutinin-related genes, such as rgp1, kgp, and hagA, B, C (49), in P. gingivalis strains. However, because the RGP-null mutant is almost devoid of the hemagglutinating activity, KGP seems likely to make a relatively small contribution to the production of hemagglutinins, as compared with RGP.

A noteworthy and unforeseen property of KDM35 was the reduced black pigmentation. The characteristic black colonies of P. gingivalis on blood agar is thought to be caused by accumulation of heme. The ability to utilize heme and heme-containing compounds has also been found in several pathogenic microorganisms (50). Black pigmentation of colonies by heme accumulation, however, is known in limited bacterial species in the genera Porphyromonas and Prevotella. Although this property is thought to be related to virulence of P. gingivalis (51), it is not yet clear how P. gingivalis cells acquire heme from erythrocytes and other host components. Recently, Fujimura et al. (34) isolated the 19-kDa hemoglobin-binding protein from the envelope of P. gingivalis by affinity chromatography. Then we found that the 19-kDa hemoglobin-binding protein was encoded by internal domain regions (e.g., HGP15 in rgp1) of multiple genes: rgp1, kgp, and hagA (32). We proposed renaming the hemoglobin-binding protein the hemoglobin receptor (HbR) domain protein. In addition, we found that nonpigmented mutants (BE1 and BR1) isolated from P. gingivalis W50 did not express the HbR domain protein and showed deficiency in hemoglobin adsorption, which indicated a close relationship among HbR production, hemoglobin adsorption, and pigmentation of P. gingivalis. P. gingivalis W50 BE1 also showed reduced virulence in mouse infection model, a decrease in trypsin-like proteinase production, and loss of hemagglutination (45, 51). The HbR domain protein is likely to be generated by proteolytic processing of the polyproteins from rgp1, kgp, and hagA. The HbR protein has a Lys residue at the carboxyl terminus, indicating involvement of KGP in this cleavage. Immunoblot analysis with anti-HbR antiserum revealed that KDM35 growing on the laked blood agar for 3 days had no proteins immunoreacted with the antiserum, whereas the kgp sibling strain KDM16 showed an immunoreactive 19-kDa protein band. The results suggest the contribution of KGP to the production of the HbR domain protein. However, the HbR protein was produced in the cells of the KGP-deficient mutant after prolonged incubation. This slow expression of the HbR domain protein of KDM35 might account for its reduced pigmentation. Why was the 19-kDa HbR domain protein detected in KDM35 after prolonged incubation? It could be explained by the fact that the carboxyl-terminal processing of the HbR domain protein in the KDM35 might be done in a much slower mode by an unknown proteinase. The proteinase should not be specific for Lys-X, because the KGP-deficient mutant showed no lysine-specific proteinase activity. Molecular mass of the HbR protein expressed in the KGP-deficient mutant was almost the same as those of the wild-type strain and KDM16. Therefore, the cleavage site by the unknown proteinase should be very close to the carboxyl-terminal Lys residue. Another possibility is that some of the HbR domains in the rgp1 and hagA genes of strain ATCC33277 may possess a different residue such as Arg at the carboxyl terminus because the nucleotide sequences of these genes of the strain have not been determined. Furthermore, we cannot rule out the possibility of the presence of another anti-HbR cross-reactive protein with a molecular mass of 19-kDa that might be expressed after prolonged incubation on blood agar plates.

The final characterization of the KGP-deficient mutant KDM35 was the association of KGP with the fibrinogenolytic activity. Fibrinogen is a major component of the coagulation cascade. Upon cleavage by thrombin, fibrin monomers polymerize and form a meshwork that traps platelets and blood cells. Although the culture supernatants of the wild-type strain and the RGP-null mutant extensively degraded all of the chains of fibrinogen, that of the KGP-deficient mutant showed a marked decrease in their degradation (especially the γ chain). It has been demonstrated that the fibrinogen Αα chain is rapidly degraded by both Arg-X- and Lys-X-specific cysteine proteinases from P. gingivalis and that Lys-X-specific cysteine proteinase(s) is a much more potent fibrinogenolytic enzyme than Arg-specific cysteine proteinase(s) (46–48). Our data in this report are also consistent with these observations. The intense fibrinogenolytic activity of KGP appears to render fibrinogen uncleavable and may contribute to a propensity for bleeding in periodontal pockets of periodontitis patients. This may therefore represent another virulence that facilitates the bacterial survival and invasion of host tissues.

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