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Porphyromonas gingivalis Proteinases as Virulence Determinants in Progression of Periodontal Diseases[1]

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***Porphyromonas gingivalis*, one of the major causative agents of periodontal diseases, produces large amounts of arginine- and lysine-specific cysteine proteinases in cell-associated and secretory forms, which are now referred to as Arg-gingipain (Rgp) and Lys-gingipain (Kgp), respectively. A number of studies have revealed that these proteinases are closely associated with the periodontopathogenesis of this bacterium: destruction of periodontal connective tissues, disruption of host defense mechanisms, and development and maintenance of inflammation in periodontal pockets. With respect to the physiology of the bacterium, Rgp and Kgp are indispensable for it to obtain nutrients from the environment, since it cannot utilize saccharides as carbon/energy sources for growth and totally depends on peptides and amino acids that are provided from environmental proteins by Rgp and Kgp. Furthermore, proteolytic activities of Rgp and Kgp contribute to processing/maturation of various cell-surface proteins of *P. gingivalis*, such as *fimA* fimbrilin (a subunit of major fimbriae), 75-kDa protein (a subunit of minor fimbriae), hemagglutinins, and the hemoglobin receptor protein, which are important for the bacterium to colonize and proliferate in the gingival crevice and to invade the periodontium. These findings strongly indicate critical roles of Rgp and Kgp in the virulence of *P. gingivalis*.**

Key words: Arg-gingipain, cysteine proteinases, Lys-gingipain, periodontal diseases, *Porphyromonas gingivalis*.

Periodontitis is chronic inflammation in the periodontium caused by bacterial infection. Formation of a periodontal pocket (deep and morbid gingival crevice), destruction of supporting connective tissue, and loss of alveolar bone take place in progression of this disease. Serological analysis of blood samples of patients with periodontitis and cultural and molecular technological analyses of microorganisms in the periodontal pockets have demonstrated that *Porphyromonas gingivalis* (*P. gingivalis*), a Gram-negative anaerobic bacterium, is one of the most etiologically important agents associated with periodontal diseases (*1*). Furthermore, experimental animal infection models using primate and rodent have proved the periodontopathogenesis of *P. gingivalis* (*2*).

A number of virulence factors that are produced by *P. gingivalis* have been intensively characterized (*3*). Among them, arginine- and lysine-specific cysteine proteinases, which specifically cleave synthetic and natural substrates after arginine and lysine residues, respectively, have received considerable attention due to their strong ability to degrade a broad range of host proteins (*4–12*). These proteinases have been historically referred to as trypsin-like enzymes, but now are called Arg-gingipain (Rgp) and Lys-gingipain (Kgp). Molecular genetical analyses have recently revealed that Rgp and Kgp have a variety of functions in addition to the degradation of host proteins. The present review summarizes recent progress in biochemical and molecular genetical studies on Rgp and Kgp.

Enzymatic properties of Arg-gingipain (Rgp) and Lys-gingipain (Kgp)

Rgp and Kgp isolated from the culture supernatant of *P. gingivalis* exhibited single protein bands on SDS–polyacrylamide gel electrophoresis with apparent molecular masses of 44 and 51 kDa, respectively (*13*, *14*). These masses are consistent with the sizes of the nondenatured Rgp and Kgp estimated by gel filtration, indicating that they function in a monomeric form. However, it is noteworthy that Rgp and Kgp occasionally make a protein complex with adhesin(s) derived from the carboxy-terminus of the initial translation products, as described afterwards (*15*, *16*).

Biochemical and enzymological analyses revealed several interesting features of Rgp and Kgp (*13*, *14*). Rgp and Kgp have specific cleavage sites: Rgp cleaves the carboxy-terminal peptide bond of arginine residues in peptides, whereas Kgp cleaves the carboxy-terminal side of lysine residues. Rgp and Kgp show their maximal proteolytic activity with a thiol-reducing agent at neutral to slightly alkaline pH. Although they belong to the cysteine proteinase family, they possess some of the properties of serine proteinases

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and metalloendopeptidases. Chloromethylketones of tosyl-L-lysine (TLCK) and tosyl-L-phenylalanine (TPCK) strongly inhibit Rgp and Kgp, but general serine proteinase inhibitors such as diisopropylfluorophosphate (DFP) and phenylmethylsulfonyl fluoride (PMSF) do not. Host proteinase inhibitors such as cystatins, serpins, and tissue inhibitors of metalloproteinases (TIMPs) have no effect on either enzyme, suggesting that Rgp and Kgp resist these host defense systems and can exhibit their proteolytic activities in the periodontal environment. Metal chelators such as EDTA, EGTA, and phosphoramidon are powerful inhibitors of Rgp, but not of Kgp. Rgp is markedly inhibited by leupeptin, antipain, and chymostatin, whereas Kgp is only moderately sensitive to these inhibitors. In contrast, Kgp is markedly inhibited by iodoacetamide, whereas Rgp is not sensitive to this compound.

Pathological properties of Rgp and Kgp

Both Rgp and Kgp have the ability to degrade a number of host proteins, and these degradative activities are thought to be closely associated with their pathological significance (Fig. 1). Both Rgp and Kgp can degrade collagens (type I and IV), major components of periodontal connective tissue (*4–7*, *13*, *14*). Extracellular matrix proteins such as fibronectin and laminin are also susceptible to these enzymes (*8*, *10*, *17*). These *in vitro* findings suggest the possibility that Rgp and Kgp contribute directly to destruction of host tissue *in vivo*.

Moreover, Rgp and Kgp have the ability to disrupt the host defense mechanisms. They degrade and inactivate immunoglobulins such as IgG, IgA, and secretory IgA (*13*, *14*), and cytokines such as TNFα and interleukin 6 (*18*, *19*). Rgp and Kgp effectively inhibit the generation of radical oxygen species from activated polymorphonuclear leukocytes, indicating that these proteinases can impair cellular components of the defense mechanisms (*13*, *14*). Since Rgp and Kgp are hardly inactivated by host proteinase inhibitors (*13*, *14*), they are highly likely to damage host defense mechanisms.

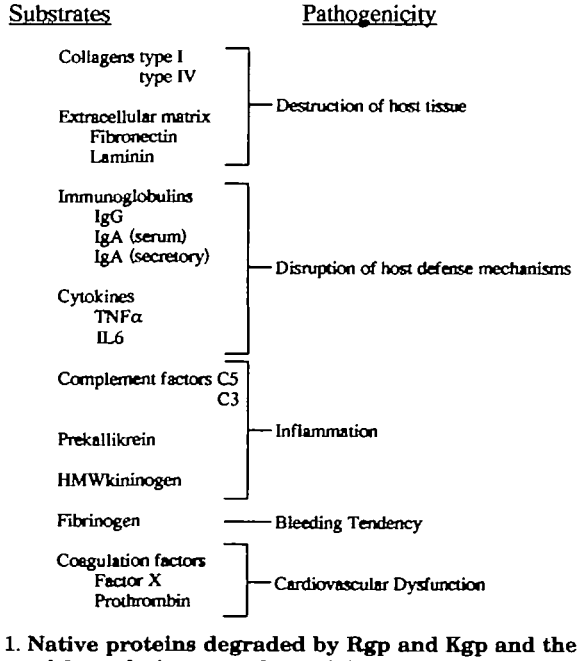

Fig. 1. **Native proteins degraded by Rgp and Kgp and the realtion of degradation to pathogenicity.**

Rgp and Kgp contribute to development and maintenance of inflammation in the periodontium through activation of the kallikrein-kinin cascade (*20*), dysregulation of the complement cascade (*21*), and dysregulation of the coagulation cascade (*22*). Rgp functions as a potent vascular permeability enhancer through the production of plasma kallikrein and the subsequent release of bradykinin (*20*). Rgp can produce a chemotactic factor by cleaving the complement C5 (*21*). The marked infiltration of leukocytes into periodontal lesions may be caused and/or accelerated by the chemotactic factor produced by Rgp. On the other hand, Rgp and Kgp suppress the bactericidal activity of leukocytes (*13*, *14*) and degrade C3 and C3-derived opsonins (*21*), which may allow *P. gingivalis* cells to evade attacks by phagocytes. Kgp has the activity to digest fibrinogen (*17*, *23*). This activity of Kgp may be related to the bleeding tendency of periodontal lesions. Rgp can cleave and activate one of the coagulation factors (factor X), suggesting its potential role in cardiovascular complications (*22*). These abilities of Rgp and Kgp indicate that they play important roles in the virulence of *P. gingivalis*.

Soluble Rgp and Kgp purified from the culture supernatant of *P. gingivalis* have been used for characterization of the pathological properties of Rgp and Kgp. However, molecular biological and biochemical analyses have revealed that Rgp and Kgp exist in various forms: soluble, membrane-associated, and protein-complex forms (*15*, *16*). The membrane-associated and protein-complex forms may have different pathological properties from the soluble forms.

Structural characterization of Rgp and Kgp

A variety of genes encoding arginine- or lysine-specific proteinases have been cloned from various *P. gingivalis* strains in several laboratories. The genes for arginine-specific proteinases that have been cloned and completely sequenced include *rgp-1* (Arg-gingipain 1) of HG66 (*24*), *rgpA* (argingipain A, later renamed as Arg-gingipain) of 381 (*25*), *prpRI* (protease polyprotein ArgI) of W50 (*26*), and *prtR* (protease R) of W50 (*27*). Comparison of the amino acid sequences deduced from these genes showed a high degree of similarity among them. The only significant variation is seen in *rgpA* of 381, which has a large deletion between two direct DNA repeats in the carboxy-terminal region. It is thought that all of these genes are allele in different bacterial strains, and the Rgp-encoding gene is now referred to as *rgpA* (*28*). The *prtH* gene, which was reported as an Rgp-encoding gene of W83, has been amended (*29*). The *rgpA* gene comprises four domain regions: the signal sequence, the amino-terminal propeptide, the catalytic proteinase domain, and the carboxy-terminal adhesin domain (Fig. 2A). The amino acid sequence of the catalytic proteinase domain has no significant similarity with those of known cysteine proteinases, indicating the uniqueness of this proteinase.

Genetic study with insertional gene inactivation using the cloned *rgpA* gene demonstrated that two separate loci on the chromosome of *P. gingivalis* ATCC 33277 each encoded Rgp, indicating the existence of a second gene (*rgpB*) responsible for Rgp activity (*30*). The gene *rgpB* was cloned and sequenced (*31*). *rgpB* shares similarities with *rgpA*; but the DNA region corresponding to Tyr^{654}–Val^{912} of

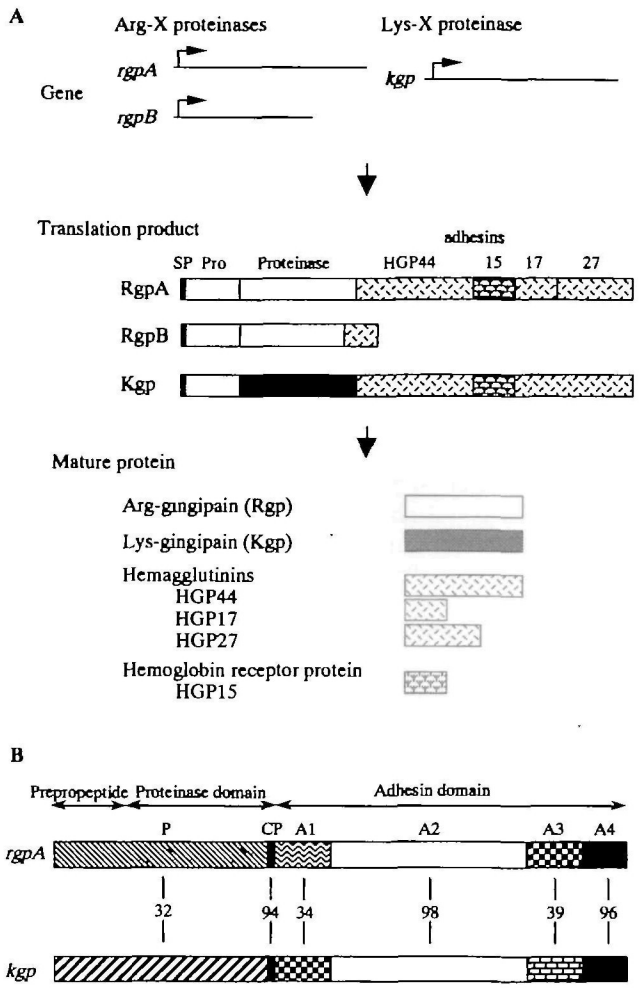

Fig. 2. **(A) Scheme of structures of the genes and encoded preproproteins of Rgp and Kgp.** The structures are based on the nucleotide sequences of *rgp* and *kgp* from the following strains: *rgpA*, HG66; *rgpB*, ATCC33277; *kgp*, 381. **(B) Similarity of *rgpA* and *kgp* genes.** Nucleotide sequences of *rgpA* from HG66 and *kgp* from 381 are divided into six regions based on the similarity in the sequences. The nucleotide number of each region is: P, 1–1974; CP, 1975–2040; A1, 2041–2595; A2, 2596–4182; A3, 4183–4734; A4, 4735–5112 in the *rgpA* gene; and P, 1–2040; CP, 2041–2106; A1, 2107–2658; A2, 2659–4239; A3, 4240–4791; A4, 4792–5169 in the *kgp* gene. The numbers in the figure represent the percentages of identity in the regions between *rgpA* and *kgp* nucleotide sequences.

rgpA of HG66 is absent in *rgpB* (Fig. 2A). The close similarity between *rgpA* and *rgpB* suggests their evolutionary relationship (*31*). It is thought that they have been generated *via* duplication of an ancestral *rgp* gene, insertion of the hemagglutinin domain region into one of the two copies, or deletion of the region from one copy, and homologous recombination between the proteinase domain regions of the two *rgp* genes. Subsequently, *rgpB* homologs (*rgp-2* and *prtRII*) have been cloned from different strains (*32*, *16*).

The function of the amino-terminal propeptide remains to be clarified, but it is plausible that the propeptide is required for stabilization of the gene product during transport. The carboxy-terminal adhesin domains, most parts of which are absent in the *rgpB* gene product, consist of four domains (HGP44, HGP15, HGP17, and HGP27) (*24*). The domain protein HGP15 was found to be a hemoglobin receptor (HbR) responsible for hemoglobin adsorption and heme accumulation (*33*). The other domain proteins, HGP44, HGP17, and HGP27, were demonstrated to be involved in hemagglutination of *P. gingivalis* (*15*). These adhesin proteins are associated with the catalytic domain protein in a non-covalent form even after proteolytic processing, making protein-complex forms with high molecular mass. The multiple isoforms of Rgp include (i) RgpA and RgpB, soluble, monomeric forms consisting of the catalytic proteinase domains of *rgpA* and *rgpB*; (ii) c-RgpA, soluble, hetero-dimeric or multimeric complex forms comprising the catalytic proteinase domain in non-covalent association with adhesin(s) of *rgpA*; and (iii) m-Rgp, membrane-associated forms of these monomers and multimers. It has recently been reported that RgpA and RgpB, especially m-Rgp, are modified by carbohydrates and that the extent and composition of the sugars differ in these isoforms (*34*). The sugar moieties may affect the conformation of the proteinase itself and/or the interaction of the proteinase with the cell-surface molecule(s), which may change localization of the enzyme, *i.e.*, on the cell surface or secreted.

Five genes products that possess lysine-specific proteinase activity have been deposited in the databases: *kgp* from 381 (*35*) and HG66 (*36*), *prtP* from W12 (*37*) and W83 (*38*), and *prtK* from W50 (*39*). Comparison of these genes revealed that they are almost identical, with only minor alterations. Their names have recently been unified to *kgp* (*28*). The basic structure of *kgp* is similar to that of *rgpA*, consisting of four domains: the signal sequence, the amino-terminal propeptide, the catalytic proteinase domain, and the carboxy-terminal adhesin domain (Fig. 2A). Comparison of the *kgp* genes from different strains showed high homology in the propeptide and catalytic proteinase domains, but several variations in the carboxy-terminal adhesin domains. The *kgp* (*prtP*) gene product of W12, porphypain, was suggested to have dual specificity for arginine and lysine (*40*), whereas other *kgp* gene products revealed only a lysine-specific proteinase activity. However, a *kgp* (*prtP*)-null mutant of W12 showed no lysine-specific proteinase activity but the same level of arginine-specific proteinase activity as the wild-type parent (*41*), which was consistent with the properties of a *kgp*-null mutant of ATCC 33277 (*23*). Furthermore, the heterogeneous expression of *kgp* (*prtP*) in *Bacteroides* species has demonstrated that the recombinant Kgp (PrtP) has not arginine-specific but lysine-specific proteolytic activity (*41*). These results indicate that the single *kgp* gene is responsible for the lysine-specific proteinase activity. The carboxy-terminal domain region of *kgp* is highly similar to that of *rgpA*, although their amino-terminal prodomains and catalytic proteinase domains are widely diverse (*35*). The *rgpA* and *kgp* genes can be divided into six DNA regions on the basis of the similarity of the nucleotide sequences (Fig. 2B). The P region, which includes the prepropeptide and most of the proteinase domain, shows little similarity (only 32% identity) between *rgpA* and *kgp*. The following CP region, which corresponds to the carboxy-terminal region of the proteinase domain, shows close similarity between them (94% identity). The A2 and A4 regions, covering most parts of the carboxy-terminal adhesin domain, also show close similarity (98 and 96% identities, respectively). Accordingly, the Rgp and Kgp proteinases from the *rgpA* and *kgp* genes are quite different,

but the hemagglutinins (HGP44, HGP17, and HGP27) and the hemoglobin receptor protein (HGP15) can be produced from both genes. Interestingly, the A3 region of *rgpA* is almost identical (99%) to the A1 region of *kgp*. These close similarities in the carboxy-terminal domain regions of *rgpA* and *kgp* suggest the occurrence of recombinational rearrangements such as transposition or gene conversion in the area. Several nucleotide repeats occur in this region, and these may have facilitated the rearrangements. Kgp can thus make a protein complex with the carboxy-terminal adhesin(s) as Rgp.

Mutagenesis of *rgp* and/or *kgp* genes

In vitro studies with Rgp and Kgp revealed these enzymes to be potent virulence factors of *P. gingivalis*, which can cause destruction of host tissue components and impairment of host defense mechanisms. However, to what extent they contribute to the virulence of *P. gingivalis* and whether they have physiological significance remained to be answered. To gain some insights into these questions, the *rgp* and *kgp* mutants of *P. gingivalis* were constructed by gene inactivation with the suicide plasmid systems and characterized (*23*, *30*, *42*) (Table I).

Although the arginine-specific proteolytic activity was decreased in the *rgpA* and *rgpB* single mutants, significant activity was still found in both culture supernatants and cell extracts of these mutants (*30*). The *rgpA rgpB* double mutant showed a complete loss of the arginine-specific activity in both fractions, demonstrating that the arginine-specific proteinase activity of *P. gingivalis* was derived from these two genes. The proteolytic activity of the culture supernatant of the *rgpA rgpB* double mutant toward protein substrates, casein and hemoglobin, was only 5–10% of the activity of the wild-type parent, indicating that Rgp is the major extracellular proteinase of *P. gingivalis*. The chemiluminescence (CL) response of polymorphonuclear leukocytes, which is closely related to the bactericidal activity of the cells, was drastically inhibited by the culture supernatant of the wild-type strain. But the culture supernatant of the *rgpA rgpB* double mutant hardly inhibited the response (Table I). The insertional disruption of the *kgp* gene on the chromosome of *P. gingivalis* ATCC 33277 completely eliminated the lysine-specific proteinase activity from the bacterium (*23*), indicating that the *kgp* gene is responsible for all of this activity in the bacterium. The *kgp* mutant retained the strong inhibitory activity toward the CL response of polymorphonuclear leukocytes (Table I), but it showed a marked decrease in degradation of fibrinogen.

The culture supernatant and cell extract of the *rgpA rgpB kgp* triple mutant contained neither Rgp nor Kgp activity (*42*). The culture supernatant also had no proteolytic activity toward bovine serum albumin or gelatin derived from human type I collagen (Table I). Importantly, the triple mutant could not grow in a defined medium which contained bovine serum albumin as a sole carbon/energy source, whereas the wild-type strain, *rgpA*, *rgpB*, *rgpA rgpB* double, and *kgp* mutants grew well in the medium. These results indicate that the extracellular proteolytic activities of *P. gingivalis*, which are essential for its growth, are attributable to Rgp and Kgp, and that the collagenolytic activity of the organism is derived from these proteinases. These findings show that Rgp and Kgp not only contribute significantly to the virulence of the bacterium but also have important physiological functions for its viability and growth.

Physiological roles of Rgp and Kgp in *P. gingivalis*

Protoheme is an absolute requirement for growth of *P. gingivalis* (*43*, *44*), and it is probably derived from erythrocytes in periodontal pockets. Therefore, it is important for the organism to agglutinate and lyse erythrocytes in order to survive *in vivo*. The hemagglutinating activity of the *rgpA rgpB* double mutant was markedly decreased (*30*) (Table I), indicating hemagglutination activity is closely associated with the *rgp* genes. The hemagglutinating activity of the *kgp* mutant was slightly decreased (*23*). As shown in Fig. 2A, the *rgpA* and *kgp* genes encode hemagglutinins and the hemoglobin receptor protein in their carboxy-terminal regions. A part of the carboxy-terminal DNA region is also present in *hagA* and *tla*, which encode the surface hemagglutinin A and a TonB-linked adhesion protein involved in capture or utilization of heme, respectively; however, expression of *tla* may be very little according to Northern analyses (*45*, *46*). The *rgpA kgp hagA* triple mutant exhibited neither hemagglutinating activity nor immunoreaction with a monoclonal antibody that reacts with the hemagglutinins. The *rgpA rgpB kgp* triple mutant exhibited no hemagglutinating activity but produced proteins with high molecular masses that immunoreacted with the antibody (*42*). Taken together, these findings indicate that the hemagglutinating activity of *P. gingivalis* probably depends on the hemagglutinins encoded by *rgpA*, *kgp*, and *hagA*, and Rgp and Kgp proteinases are indispensable for generation of the hemagglutinins by proteolytic processing.

Rgp and Kgp have recently been shown to function as processing enzymes for various cell-surface proteins of *P. gingivalis* (*47*). The cleavage sites of several bacterial proteins processed by Rgp and Kgp are summarized in Fig. 3. *P. gingivalis* is known to have a number of curly fimbriae on the cell surface, and these are thought to be important for the initial interaction between the bacterium and host cells and for the its colonization on gingival tissues (*48*, *49*).

TABLE I. **Summary of characteristic properties of Rgp-, Kgp-, and Rgp Kgp-deficient mutants.**

	Growth in defined medium	Color of colony on blood agar plate	Degradation of collagen	Degradation of immunoglobulins	Inhibition of CL response[a]	Hemagglutinating activity[b]
Wild type	+	black	++	++	100%	1/32
Rgp-deficient mutant	+	black	+	+	30	1/4
Kgp-deficient mutant	+	cream	+	+	80	1/16
Rgp Kgp-deficient mutant	No	white	No	No	0.0	No

[a]Inhibition of chemiluminescence (CL) response is represented as a percentage of the inhibitory activity of the wild-type strain against the CL response of guinea pig polymorphonuclear leukocytes. [b]*P. gingivalis* cells were suspended in PBS at an optical density at 540 nm of 0.4, and a series of 2-fold dilutions was tested for the activity to agglutinate sheep erythrocyte suspension. Hemagglutinating activity is represented as the highest dilution exhibiting full agglutination.

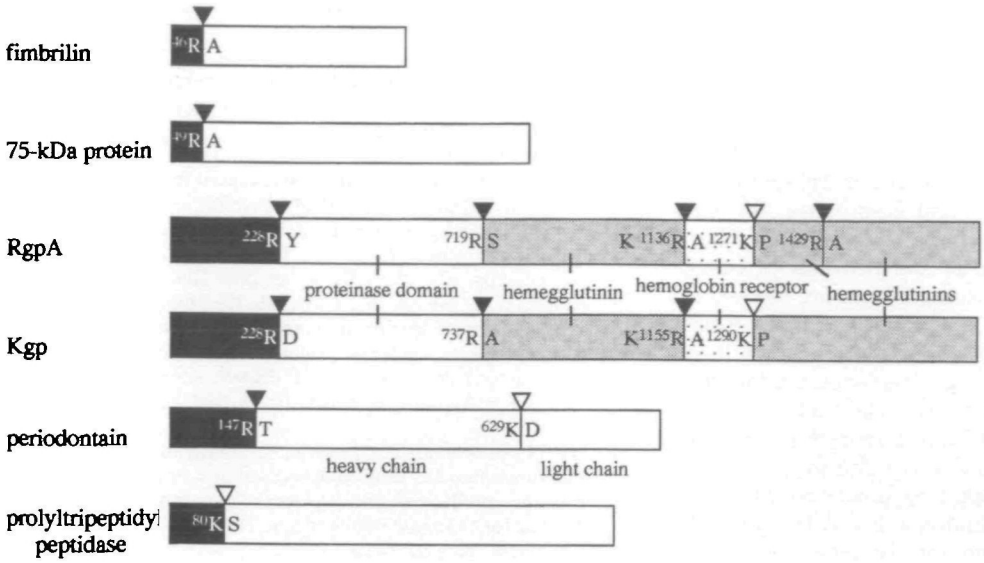

Fig. 3. **Processing of *P. gingivalis* bacterial proteins by Rgp and Kgp.** The processing sites were determined by analysis of the amino-terminal amino acid sequences in the wild-type parent and Rgp- and Kgp-deficient mutants of *P. gingivalis*. The closed arrowheads indicate the cleavage sites by Rgp, and the open arrowheads indicate the cleavage sites by Kgp.

Electron microscopic study has revealed that the wild-type parent and *kgp* mutant have fimbriae on the cell surfaces, whereas the *rgpA rgpB* double mutant, exhibited little fimbriation (*50*). Fimbrilin, a 43-kDa major component of fimbriae (*51*), appeared not to be processed at the amino-terminal region in the *rgpA rgpB* double mutant, as revealed by immunoblot analysis and amino-terminal amino acid sequencing. The precursor form of fimbrilin expressed in *Escherichia coli* was converted to the mature fimbrilin by incubation with Rgp *in vitro*. Since the maturation of prefimbrilin requires the specific cleavage between the Arg^{48}-Ala^{47} bond (Fig. 3), Rgp is likely to be responsible for the cleavage. Analysis of binding of bacterial cells to anti-fimbrilin antibody by surface plasmon resonance revealed that the *rgpA rgpB* double and *kgp* mutants had the same ability to bind the antibody as the wild-type strain, but the *rgpA rgpB kgp* triple mutant had no binding ability. Fimbrial formation may depend on Kgp to a lesser extent than Rgp. Similarly, the 75-kDa cell surface protein (*52*), which is thought to be a component of minor fimbriae of *P. gingivalis* (*53*), did not mature in the *rgpA rgpB* double mutant. Taken together, these results indicate that maturation of both subunits of the two types of fimbriae is regulated by proteolytic processing of Rgp and Kgp.

Kgp activity of the *rgpA rgpB* double mutant was lower than that of the wild-type parent (*30*), and Kgp purified from the mutant had three additional amino acids at the amino-terminus (*47*). These results suggest that Rgp also acts as a processing enzyme of the Kgp precursor protein.

When *P. gingivalis* is grown on blood agar, it accumulates heme on the cell surface, resulting in black pigmentation of the colonies, one of the characteristic features of the bacterium. Interestingly, the *kgp* mutant did not form black-pigmented colonies (*23*). Western blot analysis showed that expression of the hemoglobin receptor protein (HGP15) was greatly retarded in the *kgp* mutant. Since HGP15 protein is intragenically encoded in the *rgpA*, *kgp*, and *hagA* genes, generation of HGP15 domain protein from their initial translation products may require Kgp activity. As a matter of fact, HGP15 has a lysine residue at the carboxy terminus of the protein, suggesting the potential role of Kgp as an

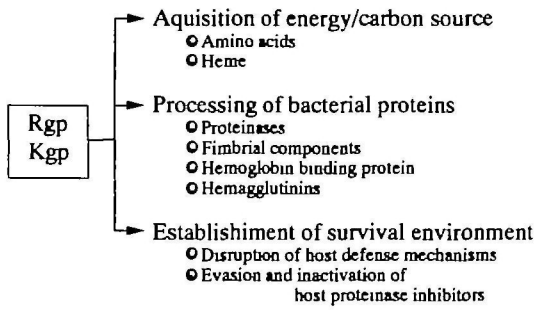

Fig. 4. **Physiological functions of Rgp and Kgp in *P. gingivalis*.**

enzyme processing HGP15 at the carboxy terminus. As the amino terminus of HGP15 from the wild type started with Ala^{1137}, a peptide bond between the Arg^{1136} and Ala^{1137} bond has to be cleaved. On the other hand, the amino-terminus of HGP15 from the *rgpA rgpB* double mutant started with the Arg^{1136} residue, indicating the involvement of Rgp in cleavage of the peptide bond. These results also indicate that Kgp may be involved in the amino-terminal cleavage of HGP15 in the absence of Rgp activity, since a Lys residue is located just before Arg^{1136}. Therefore, Kgp seems to play important roles in hemoglobin binding and heme accumulation of *P. gingivalis*. Periodontain, an α1-proteinase inhibitor–degrading cysteine proteinase of *P. gingivalis*, is thought to mature by processing at the Arg^{147}-Thr^{148} bond in its amino-terminal region and at the Lys^{629}-Asp^{630} bond in its carboxy-terminal region, resulting in production of the heavy and light chains (*54*). Cleavage specificity of these two peptide bonds suggests involvement of Rgp and Kgp in maturation of these proteinase. The prolyl tripeptidyl peptidase of *P. gingivalis* requires cleavage at the Lys^{80}-Ser^{81} bond for its maturation, suggesting involvement of Kgp (*55*). Figure 4 summarizes the physiological functions of Rgp and Kgp in the organism.

Conclusions

P. gingivalis has been implicated as one of the major

causative agents of periodontal diseases including chronic adult periodontitis, refractory periodontitis, generalized juvenile periodontitis, and periodontal abscess formation. However, factors and mechanisms of virulence of the bacterium remained unestablished. In the last decade, Rgp and Kgp have been found to be the major proteinases of *P. gingivalis*, which have various properties closely related to the virulence. The activities of Rgp and Kgp cause not only destruction of periodontal tissue but also disruption of host defense mechanisms. Amino acids and peptides produced by proteolytic degradation of host proteins are necessary for the bacterium to survive *in vivo*. In addition to the role as producers of energy/carbon sources, Rgp and Kgp have several important functions as processing enzymes for various proteins of the bacterium. The bacterial proteins which are subjected to processing by Rgp and/or Kgp are also important for the bacterium to survive and grow in periodontal regions, and some of them may have harmful activities to host cells. From these findings, it can be said that Rgp and Kgp are key determinants in the growth and virulence of *P. gingivalis*. Therefore, it is likely that virulence of *P. gingivalis* can be attenuated by inactivation of Rgp and Kgp with proteinase inhibitors or antibodies specific to Rgp and Kgp. In addition, Rgp and Kgp may be useful as a vaccine against periodontal diseases. In view of the specificity of the proteolytic action of Rgp and Kgp and the uniqueness of these proteinases in the cysteine proteinase family, inhibitors of Rgp and Kgp have the potential to prevent or cure periodontal diseases. Construction of synthetic inhibitors with high specificity towards Rgp and Kgp is now in progress at our laboratory.

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