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# "GINGIPAINS"- A Pathogenic Virulent Factor For Periodontal Diseases

KEYWORDS: Porpyromonas gingivalis, Proteolytic enzymes, Periodontitis, Gingipains

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**ABSTRACT** *Porphyromonas gingivalis has been considered as a major etiological agent in the pathogenesis of chronic periodontitis. A number of potential virulence factors associated with this disease may contribute to its initiation. Proteolytic enzymes produced by P.gingivalis being of particular interest because of their production in large quantities. These enzymes participate in the degradation of periodontal tissue either directly or indirectly, as inducers and activators of host proteinases.*

## INTRODUCTION

Porphyromonas gingivalis is a Gram negative, asacchorolytic anaerobe. It is a frequent isolate from the dentogingival crevice of individuals with periodontal disease. It is generally believed to play a major role in the pathogenesis of chronic periodontitis [1].

The association of Porphyromonas gingivalis with periodontitis is based on microbiological and serological studies in humans and non-humans primates [2].

Porphyromonas gingivalis produces several virulence factors like outer membrane vesicles, adhesins, lipopolysaccharides (LPS), hemolysins and proteinases that enable this organism to cause disease [3,4]. Among them, cysteine proteases referred as Gingipains have received considerable attention [2].Cysteine proteses are thiol proteinases and comprise the group of endopeptidases whose members rely for their catalytic activity on the presence of thiol group of a cysteine residue in the enzyme molecule. Cysteine proteinases have a strong ability to degrade a broad range of host proteins [5].

## GINGIPAINS

The gingipains constitute a group of cysteine endopeptidases that are responsible for at least 85% of the general proteolytic activity and 100% of the so-called "trypsin-like activity" produced by Porphyromonas gingivalis [2].

They are expressed on the outer membrane of Porphyromonas gingivalis and may be released with vesicles or as soluble proteins. Historically these proteinases were poorly classified and denoted by variety of generic names like trypsin-like proteinases, arginine specific protease, lysine specific protease etc. But now Arg- gingipain and Lys-gingipain are the 2 main endopeptidases produced by Porpyhromonas gingivalis and are both extracellular and cell-bound [6].

## STRUCTURE OF GINGIPAINS

Arg-gingipain **(Rgp)** is encoded by two genes namely, **rgpA and rgpB ,** while Lys-gingipain **(Kgp)** is encoded by one gene kgp [7]. RgpA and Kgp are made of a catalytic domain and a hemagglutinin/adhesion domain while RgpB does not possess the latter [8]. Because of their large activity spectrum, RgpA, RgpB, and Kgp are thought to play key roles in the pathogenesis of Periodontitis [9].

## GINGIPAINS R STRUCTURE

RgpA gene comprises 3 domain regions: The amino-terminal propeptide, the catalytic proteinase domain and the carboxy terminal adhesion domain. The adhesion domain consists of four COOH-terminal subdomains i.e. HA1, HA2, HA3 and HA4. These initial protein products undergo posttranslational processing that involves cleavage of the protein and addition of the carbohydrate group to generate multiple isoforms. The simplest form, denoted $RgpA_{9cat)}$, corresponds to the catalytic domain alone and is generated by either aberrant proteolytic processing of the initial polyprotein or by an interrupted transcription process [5]. In addition, a catalytic domain heavily modified with lipopolysaccharide can be found in the cell envelope fraction and is referred to as mt-$RgpA_{(cat)}$ (for membrane type) [10]. In contrast, rgpB is missing in almost the entire section encoding the hemagglutinin/ adhesion domains except for a small carboxy-terminal segment, which is a single chain enzyme containing only a catalytic domain. Apart from this difference, the translated polypeptides of the rgpA and rgpB share 72%, 93% and 40% identity within the profragments, the catalytic domains and the C-terminal extensions, respectively. The rgpB gene is expressed simply as a precursor that requires posttranslational modification by proteolytic cleavage of the profragment. In addition, attachment of lipopolysaccharide to this form of the proteinase apparently leads to the generation of a membrane associated form of the enzyme reffered to as mt-RgpB [10].

## GINGIPAINS K STRUCTURE

The basic structure of kgp is similar to that of rgpA, consisting of 3 domains: the amino-terminal peptide, the catalytic proteinases domain and the carboxy-terminal adhesion domain[2]. The adhesion domain region of kgp is highly similar to that of rgpA although their prodomains and catalytic domains are widely diverse.

## FUNCTIONS OF GINGIPAINS

**Housekeeping or Physiological functions of gingipains**

Gingipains are the major processing enzymes for various cell surface proteins in Porpyhromonas gingivalis. In addition to participating in the maturation of the hemoglobin-binding receptor protein domain and hemagglutining activity from the hagA gene product, they process the immunogenic 75-kDa cell surface protein, profimbrillin & are probably responsible for their own processing [11]. Gingipains may also carry out the processing of the Tpr proteinase and periodontain, two other cysteine proteinases produced by Porpyromonas gingivalis [12].

Protoheme, which is a main requirement for the growth of Porphyromonas gingivalis, is probably derived from erythrocytes in periodontal pockets. It has been recently established that a 15 kDa part of the hemagglutinin/adhesion domain of HRgpA and Kgp, referred to as HA2, is involved in hemoglobin binding and may directly participate in the acquisition of heme from erythrocytes [13]. In addition to these functions, the hemagglutinin/adhesion domain of the gingipains har-

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bors strong epitopes, for the B cells of host immune systems [14].

**Involvement of gingipains in fimbria-mediated adhesion activity of P.gingivalis**

Fimbriae, the cell surface appendages of most gram-negative bacteria, often play an important function in microbial pathogenicity. In P.gingivalis, fimbriae consisting of a 43-kDa subunit protein (fimbrillin) are believed to act as key factors facilitating the initial interaction between this organism and host cells, especially during epithelial cell invasion. In this regard, the involvement of gingipains R in profimbrillin processing, which is a prerquisite for fimbriae assembly, is pivotal for the expression of virulence by P.gingivalis [9].

**Direct effects of gingipains on the integrity of the connective tissue**

The list of host proteins degraded by gingipains in vitro include extracellular matrix components such as laminin, fibronectin, and collagen type III, IV, and V. Although degradation of these proteins in infected periodontal sites may lead to damage of basement membranes, extracellular matrix and host cells it would be rather insufficient to directly cause the major connective tissue destruction associated with progression of periodontitis [15].

Despite some reports, it is clear that gingipains are unable to cleave native type 1 collagen, a major constitiuent of collagen fibers that accounts for approximately 60% of the gingival connective tissue volume. This effect, however, can be achieved indirectly by stimulation of the release of matrix degrading proteinases [MMPs] by host cells resident in connective tissue [16].

**Pathological functions of gingipains**

**Dysregulation of the coagulation cascade**

Activation of coagulation cascade in the host response to bacterial invasion is a recognized pathophysiological reaction that plays an important function in both the confinement of infection and enhancement of phagocytosis. In particular, thrombin, the key enzyme of the clotting cascade exerts a multitude of diverse biological activities that include the stimulation of prostaglandin and platelet activating factor synthesis, as well as the production of interleukin-1 by endothelial cells and macrophages in response to lipopolysaccharide. Prostaglandins and interleukin-1 are considered predominant factors in the tissue destruction process of periodontal disease and, therefore, uncontrolled generation of thrombin by gingipains may significantly increase their contribution to this pathological process. In this regard, it is remarkable that gingipains R are potent modulators of the tightly regulated coagulation cascade through their uncontrolled activation of factor X, prothrombin, and protein C resulting in increased vascular permeability and leukocyte chemotaxis.

**Activation of the kallikrein/kinin pathway**

Bradykinin, a potent mediator for inflammation is responsible for pain and local extravasation leading to edema at local inflammatory sites and enhances the development of hypotension and shock at systemic level.

This potent mediator is released from high-molecular-weight kininogen by plasma kallikrein which, in turn, is generated from prekallikrein by activated Hageman factor (activated factor XII, XIIa). Despite the tight regulation of this pathway, bradykinin generation by pathogenic proteinases is a universal event occurring during most bacterial infections and can greatly enhance pathogen dissemination from the local site of infection into the systemic circulation [13].

Gingipains R, which is a potent vascular permeability enhancement factor, induces this activity through plasma prekallikrein activation and subsequent bradykinin release. In contrast, gingipain K, by itself, was not able to induce vascular permeability enhancement in human plasma; however, working synergistically with gingipains R, the pair efficiently released bradykinin directly from high-molecular-weight kininogen, thus mimicking the action of kallikrein. In summary,both gingipains are important vascular permeability enhancement factors and significantly contribute to gingival fluid production at periodontitis sites infected with Porphyromonas gingivalis [15]. In addition, bradykinin also participates in the process of alveolar bone loss through activation of prostaglandin synthesis in human periodontal-ligament cells and osteoblasts.

**Evasion of Host Defense system**

Gingipains play a pivotal role in the evasion of host defense systems. They degrade CD14 and LBP (major receptors for bacterial LPS) on human monocytes and human gingival fibroblasts, leading to LPS hypo responsiveness [17]. This explains the defective elimination of Porphyromonas gingivalis from the host, consequently the chronic inflammation at periodontitis sites.

Park et al (2005) demonstrated that Rpg enhances the expression of IL-6 and human b-defensins (hBDs) from oral epithelial cells by activating the G-protein-coupled protease-activated receptors-1 and -2 (PAR-1 and PAR-2) [18].

Tada et al (2003) showed that gingipains reduced the expression of ICAM-1 from human oral epithelial cells, and degraded these molecules on the cell membranes and consequently disrupted the PMN–oral epithelial cell interaction.

**Dysregulation of complement pathway**

The complement system consists of about 20 glycoproteins that circulate in the extracellular fluid compartment. If stimulated, they interact in a precise sequence of reactions that result in the production of biologically active cleavage fragments that promote phagocyte accumulation, opsonization and phagocytosis as well as direct cell damage. Pathogenic proteinases degrades the complement factors accumulated on bacterial surface and preclude the formation of membrane attack complex.

Porphyromonas gingivalis efficiently employs this tactic and numerous studies have indicated that attenuation of the complement-dependent bactericidal activity is due to degradation of C3, C4, C5 & factor B [2,5].

Wingrove et al (1992) showed in vitro that C3 is highly susceptible to degradation by RgpA, which converted this complement factor in a stepwise manner first to C3a-like and C3b- like fragments, followed by further extensive degradation of the C3a-like portion of the molecule.

**Desensitization of Neutrophils**

Porphyromonas gingivalis is well equipped to passively resist phagocytosis, but its ability to render neutrophils locally dysfunctional has, potentially, a much greater impact on disease development because it would also protect other subgingival microorganisms. In this regard, proteinases seem to be potent offensive weapons, as indicated by an ever-growing number of recent reports.

-gingipains R cleaves the proteinases-activated-receptor-2 (PAR-2), a G-protein-coupled receptor present on the surface of neutrophils, leading to their activation as indicated by an increase in the intercellular calcium concentration.

-Gingipain K and a non-tryptic serine proteinases associated with vesicles were found to contribute significantly to evasion of neutrophils phagocytosis through cleavage of the C5a receptor

**GINGIPAINS AS TARGETS FOR PERIODONTITIS THERAPY**

The potential contribution of gingipains to the pathophysiology of periodontitis suggests availability of the enzymes as targets for the therapy of periodontal disease. It can be

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achieved by two ways

**Vaccination using gingipains:**
Genco et al (1998) showed that immunization of mice with a peptide derived from the amino terminal sequence of the catalytic domain of gingipains R resulted in protection from P.gingivalis invasion, subsequent cachexia and death [19].

Gibson et al (2001) showed that immunization with RgpA stimulates the production of hemagglutinin domain-specific antibodies, which contribute to the prevention of P.gingivalis mediated oral bone loss [20].

**Specific inhibitors for gingipains**
Ciancio et al (1994) observed improved clinical parameters with tetracycline and its analogues. However, this treatment did not affect the P.gingivalis load at the periodontal sites[21].

Matsuhita et al (2003) showed that DX-9065a, a proteinases inhibitor primarily specific for activated coagulation factor X, selectively reduced P.gingivalis growth.

Although the trial for gingipain inhibitors has just started, this approach may contribute to the therapy of periodontal disease.

**SUMMARY**
Rgp and Kgp have been found to be the major proteinases of Porphyromonas gingivalis, which have various properties closely related to the virulence. The activities of Rgp and Kgp not only lead to the destruction of periodontal tissue but also disruption of host defense mechanisms. Thus, both 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.

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