THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Gingipains: The Virulent Invaders

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Abstract:
Although nearly 400 different bacterial taxa have been identified in dental plaque samples, only a few species have been implicated as periodontal pathogens. This group of bacteria includes Porphyromonas gingivalis, Treponema denticola, Bacteroides forsythus and Actinobacillus actinomycetemcomitans. All of these organisms, with the exception of A. actinomycetemcomitans, are known to produce significant levels of proteolytic activity which, by virtue oftheir ability to cleave the trypsin-specific substratebenzoyl-L-arginine-p-nitroanilide or benzoyl-L-arginine- p-napthyl amide, have been collectively referredto as trypsin-like proteinases. Among several proteinases produced by P. gingivalis, the cysteine proteinases, referred to as gingipains, are clearly in the spotlight. Gingipains seem to be key players in subvertinghost defense systems with, significantly, the complementand neutrophils being the main target. Gingipains are potent virulence factors of P.gingivalis and are likely to be associated with development of periodontitis, therefore gingipain inhibition by vaccination and gingipain-specific inhibitors is a useful therapy for adult periodontitis caused by P.gingivalis infection.

Keywords: *trypsin-like proteinases, heamagglutination, proteolysis,kallikrein-kinin pathway*

1. Introduction

Periodontal diseases represent infections that are associated with inflammation of the gingiva, destruction of periodontal tissue, pocket formation and alveolar bone resorption. If untreated, they eventually lead to exfoliation of teeth. Severe periodontitis may predispose to more serious systemic conditions such as cardiovascular diseases and the delivery of preterm infants. The severity of periodontitis correlates with the presence of specific bacteria that trigger inflammatory host responses which, together with the bacterial virulence factors, cause the majority of tissue destruction.[1]

Porphyromonas gingivalis, a Gram-negative, anaerobic, black-pigmented bacterium, has been implicated as the major aetiological agent in the initiation and progression of adult periodontitis (Holt et al., 1988).It possesses virulence factors such as proteinases, fimbriae, lectin-type adhesins and haemagglutinating factors, which enable it to colonize periodontal pockets (Cutler et al., 1995; Lamont and Jenkinson, 1998)[2].

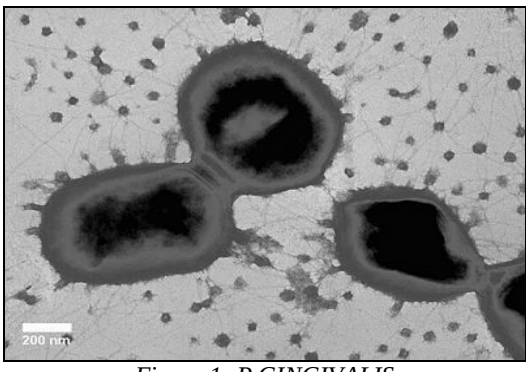

Figure 1: P.GINGIVALIS

It is generally accepted that the proteolytic enzymes of this organism play a central role in the pathogenesis of periodontitis. Although several different types of proteinases are expressed by P.gingivalis, cysteine proteinases with Arg– and Lys– specificity,

referred to as gingipains R and K, respectively, are responsible for at least 85% of the overall proteolytic activity and are recognized as the major virulence factors of this periodontal pathogen[3] (Potempa and Travis, 1996; Kuramitsu, 1998).
PROTEINASES : One of the potentially significant virulence characteristics of *P.gingivalis* is the large no. of hydrolytic, lipolytic and proteolytic enzymes.These enzymes are exposed at the surface of the bacterium where they are able to come in contact with host cells and tissues.Proteolytic enzymes that break peptide bonds.
The classifications of proteinases are relied upon their catalytic functions.Two types – Endopeptidases, Exopeptidases.
Endopeptidases - Enzymes acting on central peptide linkage to split protein into small polypeptides.and Exopeptidases - Small polypeptidase or oligopeptidases are broken into their constituent amino acids by action of Exopeptidases.
2 types of Exopeptidases – Amino peptidases – act on amino terminal residues
Carboxy peptidases – act on carboxyl terminal residues.[4]

2. Gingipains
Endopeptidases produced by P.Gingivalis.Responsible for 85% of the general proteolytic activity and 100% of so called 'Trypsin like activity' produced by P.Gingivalis.Other names – Trypsin like protease, arginine Specific protease, Lysine specific Protease, Protease I, Protease II, Gingipain, Porphypain, Arginipain.
Gingipains are products of 3 genes encoding Cysteine proteases and referred to as Gingipain R and Gingipain K according to the hydrolysis of Arg-Xaa or Lys- Xaa respectively
The gene encoding gingipain R with hemagglutinin/adhesion domains should be referred to as *rgpA.*
The gene that encodes gingipain R without this carboxy-terminal domain should be referred to as *rgpB.*
The name *kgp* was suggested as a reference to the gene encoding gingipain K[5]

2.1. Gingipain - R Structure
The translated product of *rgpA* consists of a profragmentwith a signal sequence, a catalytic domain, and a hemagglutinin /adhesion domain

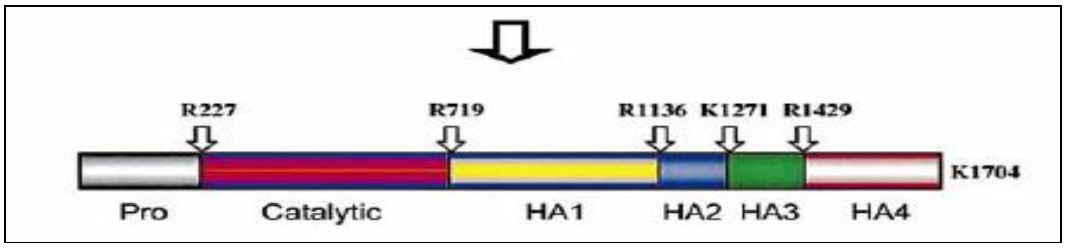

Figure 2

3 different molecular forms of the enzyme
RgpA(cat) is a form of the catalytic domain alone and is made by either aberrant proteolytic processing of the initial protein or by an interrupted transcription process
A membrane associated form in which the catalytic domain is modified with lipopolysaccharides is mt-RgpA(cat)
HRgpA is the non-covalent but very stable complex of the catalytic domain and a hemagglutinin/adhesin domain(s)
RgpB is a product of *rgpB* that is missing almost the entire section encoding the hemagglutinin/ adhesin domains except for a small carboxy -terminal segment, which is a single chain enzyme containing only a catalytic domain .[6]

2.2. Gingipain K Structure
kgp gene encodes a polyprotein consisting of a typical leader sequence, a profragment, a catalytic domain, and hemagglutinin/adhesin domains

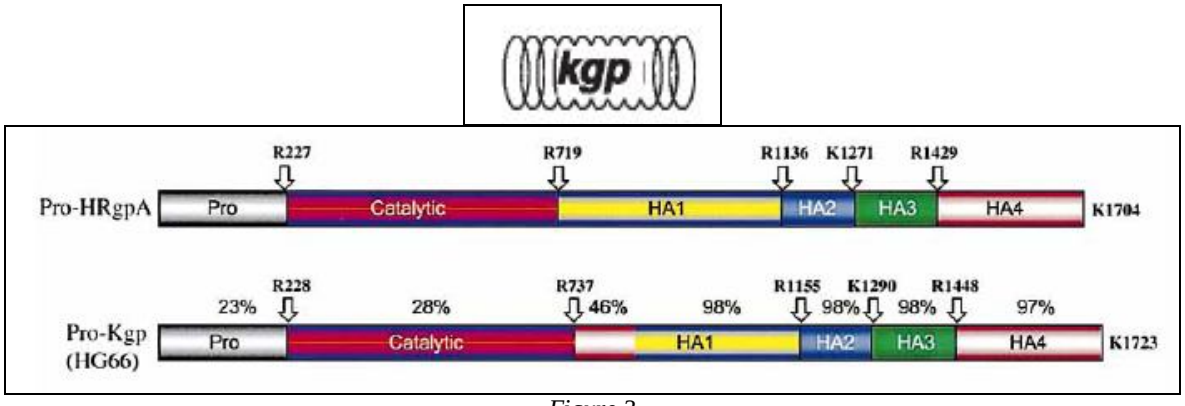

Figure 3

2.2.1. Functions of P.Gingivalis Proteinases

Adherence to host cells, Adherence to other bacteria in the host environment to supporting in vivo growth of P.gingivalis as well as to inhibiting selected host defense mechanisms. They might also be involved in direct tissue destruction. Gingipains are shown to be potent vascular permeability up regulators, being capable of inducing vascular permeability in human plasma and cleaving bradykinin directly from the high – molecular weight kininogen.[7]

This process results in increased gingival vascular permeability and increased gingival fluid flow.

Since the gingipains are chemotactic for polymorphonuclear leukocytes, there is an increased concentration of these host cells at sites of potential tissue and bone destruction.

In vitro p.gingivalis is capable of degrading complement C3, and interfering with the accumulation of these neutrophils in the confines of the developing periodontal pocket.

2.3. Functions of Gingipains

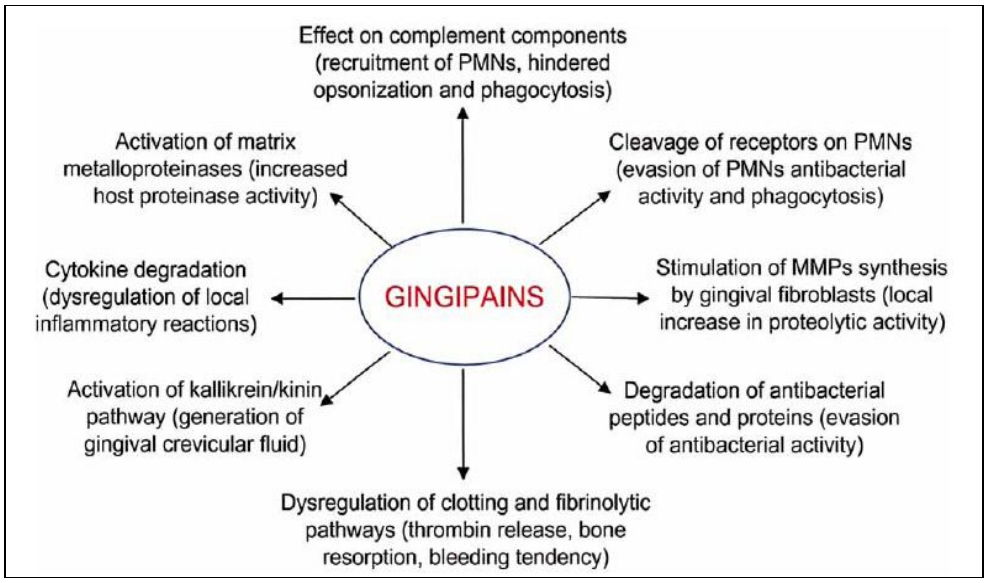

Figure 4

2.2.2. Adherence to Host Cells

Gingipains function as Bacterial Hemagglutinins, adhesins and hemoglobin-binding proteins.

HRgpA and Kgp assist bacterial cells with an hemagglutinin/adhesion ability that is a prerequisite for colonization of host tissues (Okuda & Takazoe,1974) .*P. gingivalis* could bind specifically and with high affinity to components of the connective tissue, including fibronectin and fibrinogen, and then degrade these proteins.*P. gingivalis* whole cells or purified HRgpA generates a major IgG response targeted to epitopes within the hemagglutinin/adhesion domain of gingipains, and only a very weak response against the catalytic domain.Part of the hemagglutinin/adhesion domain HA2 may directly participate in the acquisition of heme from erythrocytes.[8]

2.4. Housekeeping Functions of Gingipains

Major processing enzymes for various cell surface proteins.Process both the immunogenic 75-kDa cell surface protein, profimbrilin and pro-Kgp, and are probably responsible for their own processing. Processing of the Tpr proteinase and periodontain, two other cysteine proteinases produced by P. gingivalis.Fimbriae, the cell surface appendages of most gram-negative bacteria, often play an important function in microbial pathogenicity .Gingipain R enhanced the binding of fimbriae to cultured fibroblasts or extracellular matrix proteins immobilized on a microtiter plate (Kontani M 1996).Kgp and HRgpA, themselves, have a strong affinity for extracellular matrix proteins and that the latter enzyme is much more active in the degradation of laminin and fibronectin.[9]

2.5. Effects of Gingipains on Connective Tissue

Host proteins degraded by gingipains in vitro include extracellular matrix components such as laminin, fibronectin, and collagen type III, IV, and V

Insufficient to directly cause any damage to Connective tissue for progression of periodontitis.

Cleavage of Collagen type I of, however, can be achieved indirectly by stimulation of the release of matrix-degrading proteinases by host cells resident in connective tissue (MMP 1,3,9)

2.6. Dysregulation of Complement Pathway

Attenuation of the complement-dependent bactericidal activity is due to degradation of C3, C4, C5 and factor B

Certain proteinase mimic the activity of complement factor D, thus enabling the assembly of a fluid-phase C3 convertase whose activity interferes with the significant accumulation of C3 on the bacterial cell surface- bacteriocidal activity is reduced.

Degradation and/or fluid-phase consumption of this factor may significantly affect P. gingivalis survival in periodontal pockets. C3 activation is a central event. C3-derived opsonins are crucial in facilitating the recognition and engulfment of bacterium by neutrophils. Depletion of C3 would hinder the generation of C5-convertase. Hamper phagocyte accumulation. Assembly of the membrane attack complex, which is directly responsible for the killing of gram-negative bacteria. C3, with C5 , is a key factor in the activation of the complement pathway, and it is also a target for P. gingivalis proteinases.C5 proteolysis is producing an initial internal cleavage of the α-chain to generate 30- and 86-kDa fragments. Further degradation released C5a fragment which activated neutrophils.Despite dysregulation of the complement pathway neutrophil movement would still remain intact responsible for the massive accumulation of neutrophils in inflamed periodontal tissue.[10]
P. gingivalis in this system -Resistant to phagocytosis, Asaccharolytic organism it requires peptides as a source of carbon and energy, Neutrophils may provide the proteolytic power required for the degradation of the constituents of both connective tissue and plasma exudate, in this manner nursing bacteria and, at the same time, causing serious damage to the periodontal tissues

2.7. Activation of the Kallikrein/Kinin Pathway
Ability of *P. gingivalis* proteinases to activate the kallikrein/kinin pathway was first described by Hinode et al(1992)
Gingipains R are very potent vascular permeability enhancement factors, inducing this activity through plasma prekallikrein activation and subsequent bradykinin release
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.[11]

2.8. Gingipain Action on Bradykinin

2.8.1. Dysregulation of the Cytokine Networking Systems
Apart from the induction of pro- and anti-inflammatory mediators by cell membrane components, periodontopathogenic bacteria have developed various mechanisms that can alter cytokine activities and, their proteinases must play an important role in the dysregulation of the cytokine network at the site of inflammation.
IL – 8 and ICAM 1 are the two key molecules that help in the recruitment of neutrophils to the site of infection.P.gingivalis possesses the novel ability to downregulation of IL – 8 protein and ICAM – 1.The regulation of IL – 8 at mRNA levels suggests that P.gingivalis is able to modulate the intercellular signaling pathways of the host epithelial cells, which govern gene regulation. Activation and deactivation of IL-8 depends on Gingipain concentration.[12]
More neutrophils – more tissue destruction – more proteins released – more nutrition for bacteria

2.9. Gingipains in Protection against P.Gingivalis Infection
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 achieved in two ways:
Specific inhibitors for gingipains.
Vaccination using gingipains.
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 periodontitis sites.
Matsushita et al (2003). found that DX-9065 a, a proteinase inhibitor primarily specific for activated coagulation factor X, selectively reduced *P. gingivalis* growth
Vaccination therapy using Gingipains:
Genco et al (1998) showed that immunization of mice with a peptide derived from the amino – terminal sequence of catalytic domain of gingipains R resulted in protection from P.gingivalis invasion.
This indicates that antibodies directed to the amino terminal region of the catalytic domain of protective immune respose against P.gingivalis
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.[13]
Recently accumulated large bodies of evidence have strongly implicated proteolytic enzymes released by subgingival plaque bacteria in the pathogenicity of periodontal disease. With regard to proteolytic power, however, the contribution from different microbial species considered as periodontal pathogens is not equal. Two of these bacteria, *P. gingivalis* and *T. denticola*, have developed an elaborate proteolytic systems composed of several surface-located or secreted enzymes, which apparently serve a role to provide bacteria with nutrients in the form of small peptides and amino acids. Of these two species, proteinases of *P. gingivalis* are the most intensively studied, and during the last decade an impressive array of information has been accumulated with respect to the biochemical characterization of purified proteinases and structure of the genes encoding them, the regulation of expression and the effects of these enzymes on host systems.[14] In addition, studies on proteinase-deficient isogenic mutants has shedlight on both their housekeeping functions and potential role(s) in the pathogenicity of periodontitis. Among several proteinases produced by *P. gingivalis*, the cysteine proteinases, referred to as gingipains, are clearly in the spotlight. They are the subject of several recent reviews and generally considered as the major virulence factors of this periodontal pathogen Gingipains seem to be key players in subverting host defense systems with, significantly, the complement and neutrophils being the main target. In addition, through uncontrolled activation of kallikrein/ kinin pathway and coagulation cascade they contribute to local

generation of bradykinin and thrombin, two synergistically working pro-inflammatory reagents with a strongly, although indirectly, stimulatory effect on bone resorption.[15] Furthermore, the ability to interact with the cytokine networking systems has the potential to dysregulate the local inflammatory reaction. Finally, gingipains have a strong effect on mechanisms controlling host matrix.

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