CHAPTER 2

THE LYSINE-SPECIFIC GINGIPAIN OF *PORPHYROMONAS GINGIVALIS*

Importance to Pathogenicity and Potential Strategies for Inhibition

Tang Yongqing,[1] Jan Potempa,[2] Robert N. Pike*,[1]
and Lakshmi C. Wijeyewickrema[3]

[1]Department of Biochemistry and Molecular Biology and CRC for Oral Health Sciences, Monash University, Clayton, Victoria, Australia; [2]Oral Health and Systemic Disease Research Facility, University of Louisville School of Dentistry, Louisville, Kentucky, USA; [3]Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
**Corresponding Author: Robert N. Pike— Email: rob.pike@monash.edu*

Abstract: Periodontitis is a disease affecting the supporting structures of the teeth. The most severe forms of the disease result in tooth loss and have recently been strongly associated with systemic diseases, including cardiovascular and lung diseases and cancer. The disease is caused by biofilms of predominantly anaerobic bacteria. A major pathogen associated with severe, adult forms of the disease is *Porphyromonas gingivalis*. This organism produces potent cysteine proteases known as gingipains, which have specificity for cleavage after arginine or lysine residues. The lysine-specific gingipain, Kgp, appears to be the major virulence factor of this organism and here we describe its structure and function. We also discuss the inhibitors of the enzyme produced to date and the potential pathways to newer versions of such molecules that will be required to combat periodontitis.

INTRODUCTION: PERIODONTAL DISEASE—SIGNIFICANCE AND AETIOLOGY

Periodontal disease is a complex disorder involving Gram-negative anaerobic bacteria interacting with host cells with the combined effect leading to the destruction of the supporting structures of teeth. The supporting structures include the gingiva

Cysteine Proteases of Pathogenic Organisms, edited by Mark W. Robinson and John P. Dalton.

(gums), the periodontal ligament between the cementum and the junctional epithelium and the alveolar bone. Periodontal disease affects approximately 30% of the adult population and is amongst the most prevalent oral diseases contributing to tooth loss in adults.[1,2] Furthermore, epidemiological evidence from the past few decades has pointed to a correlation between periodontal disease and several systemic disorders, including circulatory diseases, respiratory infections, premature delivery and low birth weight of infants, neuronal damage, renal malfunction, certain cancers and autoimmune diseases.[3-10] Although the mechanisms underlying this correlation may vary among systemic conditions, insult by periodontopathic bacteria and their toxic products and/or the hyper-inflammatory effects of the host immune response are likely to be involved.[11]

Periodontal disease is an inflammatory disorder instigated and mediated by multiple bacterial species, which usually change dynamically during different states of disease and differ among individuals.[12] The disease starts in the marginal gingiva as a reversible inflammatory state, called gingivitis, resulting from the excessive accumulation of bacterial dental plaque reaching deep into the gingival sulcus.[13-15] This generates a local environment prone to colonization by and the proliferation of anaerobic periodontopathogenic bacteria. As the infection progresses, some of the harmful bacterial species induce damage to the sub-gingival tissues such as the periodontal ligament and the junctional epithelium, leading to loss of attachment and the formation of periodontal pockets. These pathogenic changes are accompanied by massive destruction of the soft tissues and permanent alveolar bone resorption.[14,16] This more advanced stage is defined as periodontitis,[15] which eventually leads to tooth loss due to the lack of periodontium support.[13,17] Periodontal disease is a multifactorial disease, in which the onset and outcome are affected by a variety of risk factors involving the host, bacteria and environment.[14] Of the 300~400 bacterial species residing in the periodontal environment, only 10~20 may be periodontopathic,[12] including, but not limited to the cluster of 'red complex' bacteria comprising *Tannarella forsythia*, *Treponema denticola* and *Porphyromonas gingivalis,* often found in large numbers in the biofilms of adult periodontitis patients.[18,19]

Porphyromonas gingivalis

Among the putative periodontopathic bacteria, *P. gingivalis* is believed to be one of the major pathogens involved in the progression of periodontal disease, based on the observation that increased *P. gingivalis* levels were associated with an increased severity of periodontal disease.[20] The results of experimental oral infection of primates with *P. gingivalis* strongly support this hypothesis, by showing that the sub-gingival inoculation of the bacterium into *P. gingivalis*-free monkeys resulted in the development of periodontitis. Significantly, in this model, the levels of the bacterium in the sub-gingival area closely correlated with the aggravation of periodontitis, marked by an exacerbated loss of alveolar bone.[21]

P. gingivalis is a nonmotile, Gram-negative obligatorily anaerobic coccobacillus, typically 0.5~3.5 μm in diameter[22] that is a late colonizer of the dental bacterial plaque. It utilizes bacteria already resident in the oral cavity for initial settlement.[23] The bacterium has a hemolytic nature and exhibits a characteristic black pigment that contains the metabolic derivatives of heme, extracted from hemoglobin or other heme-containing proteins, on the cell surface and in the centre of aged colonies grown on blood agar.[24,25] Metabolically, *P. gingivalis* is an asaccharolytic species which generates energy and obtains carbon by fermentation of peptides and amino acids.[24,26] Different *P. gingivalis*

strains can be categorised according to their pathogenicity, which is evaluated by the severity of the inflammatory responses in mice following subcutaneous injection of bacteria.[27] Virulent strains such as W50 can cause severe spreading abscesses and induce fatal sepsis in murine models after 2~4 days, while less-virulent strains such as W186 may only result in localized pustules.[27]

P. gingivalis exerts its pathogenic effects via the production of a variety of virulence factors, including the capsule, outer membrane vesicles and abundant adhesive structures, such as fimbriae, hemagglutinin/adhesins and lipopolysaccharide (LPS).[24,28-32] More importantly, the virulence factors of *P. gingivalis* also comprise a group of proteolytic endopeptidases, of which cysteine proteases with trypsin-like activity are closely associated with tissue damage and host immunity disruption in periodontitis.[23,33-35] The enzymes, referred to as gingipains,[36] are responsible for the vast majority of the extracellular proteolytic (85%) and trypsin-like amidolytic activities (99%) of *P. gingivalis* and hence are believed to be critical for the virulence of this pathogen.[37] Indeed, a few spontaneous mutants of the virulent *P. gingivalis* strain W50 with lowered trypsin-like amidolytic activities have been found to be avirulent in murine models.[38,39]

GINGIPAINS

Members of gingipains (family C25) strictly cleave peptide bonds with either Arg-Xaa or Lys-Xaa at the cleavage site and the responsible enzymes are referred to as Arg-gingipain and Lys-gingipain, respectively.[37,40,41] Arg-gingipains include two members, Arg-gingipain A (RgpA) and Arg-gingipain B (RgpB), encoded by two closely related genes, *rgpA* and *rgpB*, respectively. Lys-gingipain (Kgp) is encoded by a single gene, *kgp*.[42,43] A number of studies have shown that *P. gingivalis* mutants with a deficiency of one or more of the gingipain genes exhibited lower pathogenic potential in mice than wild type bacteria, hence confirming that the gingipains are the main contributors to the virulence of *P. gingivalis*.[44-47] Furthermore, O'Brien-Simpson et al[47] compared the virulence of *P. gingivalis* isogenic mutants defective in the individual gingipain gene in a murine lesion model, to estimate the extent of contribution of each of the three gingipain proteases to the pathogenic potential of the bacterium. They reported that Kgp contributed more to pathogenicity than the two Arg-gingipains and thus this gingipain can be considered to be the major virulence factor of *P. gingivalis*.[47] This finding has been further confirmed by Pathirana et al who conducted a similar experiment with a recently developed murine periodontitis model and found that Kgp contributed more to pathogenicity than RgpB and much more than RgpA. Therefore, Kgp represents one of the major determinants of the virulence of *P. gingivalis*.[48]

THE BIOLOGICAL FUNCTIONS OF KGP

Kgp participates in many *P. gingivalis*-mediated pathogenic processes by binding various targets and importantly, cleaving multiple proteins (Fig. 1).[49] Kgp can bind red blood cells and many heme-containing proteins, including hemoglobin and acts as a major hemolytic enzyme to produce and store iron/heme, which is a vital growth factor essential for the survival and function of *P. gingivalis*.[50-52] The importance of Kgp in providing iron/heme for *P. gingivalis* has been demonstrated by the observation that

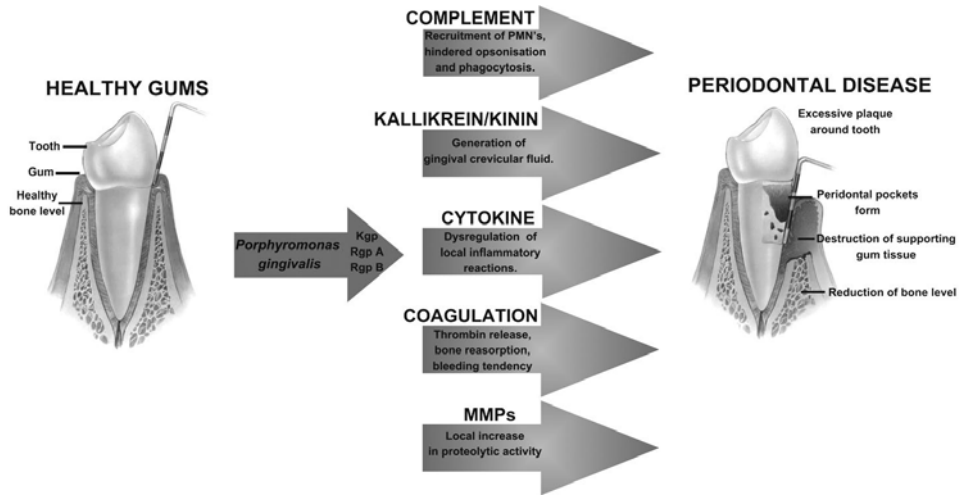

Figure 1. Diagrammatic overview of the postulated contributions of the gingipain enzymes from *Porphyromonas gingivalis* to the progression of periodontitis. A healthy tooth and surrounding oral tissue, is converted to one exhibiting the symptoms of periodontitis as shown by the summarised clinical indicators on the right of the diagram. Sites of periodontitis show destruction of supporting tissue, lowered levels of the alveolar bone socket and enhanced depth of probing (visualized by the metal probe on the right). The gingipains of *P. gingivalis* are postulated to contribute to the progression of the disease by affecting the indicated systems to dysregulate normal responses to the bacterium and thus enhance the clinical indications and contribute to disease.

Kgp-null *P. gingivalis* strains lacked the black pigment characteristic for this species and were less virulent.[46,53] In concert with the two Rgp members, Kgp also directly binds and cleaves fibrinogen and indirectly breaks down collagens via the activation of the matrix metalloproteinase system, resulting in an increased bleeding tendency and tissue damage at the infected periodontal site.[54-57] Whilst the proteolytic activity of Kgp is important for generating peptides/amino acids as the energy and carbon source suitable for the asaccharolytic nature of *P. gingivalis*, it is also utilized by *P. gingivalis* to manipulate and evade the host immune response via complex mechanisms,[58-62] mainly involving the degradation of surface molecules of immune cells [63-66] and interference with the cytokine system of the host.[61,67] In addition, Kgp can also bind host epithelial cells and other bacteria, providing a biological basis for the attachment and colonization of *P. gingivalis*.[49,68] Cumulatively, Kgp is critical for the survival and pathogenicity of *P. gingivalis* and hence is a promising target for inhibitors to control periodontal disease.

THE STRUCTURAL CHEMISTRY OF KGP

The precursor of Kgp is a polyprotein consisting of domain components evolutionarily related to those of RgpA and RgpB[17,37,49,69] (Table 1). The RgpB polyprotein does not have HA domains[70] and is 72%, 99%, 52% and 51% identical in the sequences of the pro-domain, catalytic sub-domain, IgSF sub-domain and C-terminal domain, respectively, to those of the RgpA polyprotein.[71] However, Kgp_{cat} only shares 27% sequence identity to $RgpA_{cat}$ and RgpB.[72] The C-terminal HA domains of Kgp polyprotein are highly similar

Table 1. The domain structure of RgpA-, RgpB- and Kgp-polyproteins

Polyprotein	Domain Structure (from N- to C-terminus)	Descriptions	References
RgpA (1706 residues)	Signal peptide	23 residues	17, 37, 49, 69
	Pro-domain	204 residues	
	Arg-specific catalytic subdomain	Together forming a 45 kDa Arg-specific protease domain ($RgpA_{cat}$)	
	IgSF* subdomain		
	$RgpA_{A1}$	RgpA hemagglutinin/adhesin (HA) domain 1, formerly rHGP-44*	
	$RgpA_{A2}$	RgpA HA domain 2, formerly rHGP-15	
	$RgpA_{A3}$	RgpA HA domain 3, formerly rHGP-17	
	$RgpA_{A4}$	RgpA HA domain 4, formerly rHGP-27	
	C-terminal domain	70 residues	
RgpB (736 residues)	Signal peptide	24 residues	49
	Pro-domain	205 residues	
	Arg-specific catalytic subdomain	Together forming a 45 kDa Arg-specific protease domain ($RgpB_{cat}$)	
	IgSF subdomain		
	C-terminal domain	71 residues	
Kgp (1723 or1732 residues)	Signal peptide	19 residues	37, 40, 49, 76-78
	Pro-domain	209 residues	
	Lys-specific catalytic subdomain	Together forming a 48 kDa Lys-specific protease domain (Kgp_{cat})	
	IgSF subdomain		
	$Kgp_{A1, 2, 3...}$(see Table 2)	Kgp HA domains, formerly kHGP*	
	C-terminal domain	70 residues	

* IgSF = immunoglobin superfamily; rHGP = Arg-specific high molecular weight gingipain; kHGP = Lys-specific high molecular weight gingipain. The HA domains of RgpA and Kgp were formerly named by their molecular weights.

in sequence to those of RgpA polyprotein, in particular Kgp_{A2}, which, apart from two residues, is identical to $RgpA_{A2}$.[72,73] Several repetitive Adhesin Binding Motifs (ABMs) were identified in the HA domains of Kgp and RgpA. These ABMs connect the HA domains in terms of their sequence similarity and link the HA domains to other proteins,[74] for example, the ABM2 (SYTYTVYRDGTKIKEGLTATTFEEDGVAA) is responsible for the interaction between Kgp and extracellular matrix proteins,[49,75] while ABM3 (VTLKWDAPNGTPNPNPNPNPNPGTTTLSESF) is critical for attachment of Kgp to hemoglobin and red blood cells.[49,75] These HA domains of the Kgp polyprotein vary

Table 2. The HA domains of Kgp-polyproteins from different *P. gingivalis* strains

P. gingivalis Strain	HA Domain Structure	Former Name	References
381	Kgp_{A1}	kHGP-39	78
	Kgp_{A2}	kHGP-15	
	Kgp_{A3}	kHGP-44	
W50	Kgp_{A1}	kHGP-39	37
	Kgp_{A2}	kHGP-15	
	Kgp_{A3}	Collectively kHGP-44	
	Kgp_{A4}		
	Kgp_{A5}		
HG66 and ATCC33277	Kgp_{A1}	Collectively kHGP-44	40
	Kgp_{A2}		
	Kgp_{A3}	kHGP-15	
	Kgp_{A4}	kHGP-17	
	Kgp_{A5}	kHGP-27	
W12 and W83	Kgp_{A1}	kHGP-44	76,77
	Kgp_{A2}	kHGP-15	
	Kgp_{A3}	Collectively kHGP-44	
	Kgp_{A4}		

in number and order among *P. gingivalis* strains. Four Kgp variants have been identified to date[37,40,76-78] (Table 2). It is interesting to note that recently Li et al[79] have postulated a different set of protein boundaries for the HA domains of Kgp and RgpA and proceeded to successfully express and purify a so-called K2 domain which is largely equivalent to the domain termed Kgp_{A2} or kHGP-15 previously (Table 2). Bioinformatic analysis suggests that the domains belong to the *Cleaved_adhesin* domain family, with affiliation to the galactose-binding domain-like superfamily. They also solved the structure of this protein (Fig. 2) and showed that it was a β-strand rich structure in a "jelly-roll" fold, with numerous loops connecting the β-strands, resembling carbohydrate binding domains and so-called MAM domains found in receptor-type tyrosine-protein phosphatases and ephrin type A/B receptors. They demonstrated that the domain was able to weakly bind to galactose containing carbohydrates and that it could act as a hemolysin in its uncleaved form. This study most likely will constitute a breakthrough in our understanding of the structure and function of the HA domains of the gingipains and further work in this regard will be of great interest.

Similar to other gingipains, the mature Kgp exists extracellularly in multiple isoforms, including monomeric soluble forms, membrane-associated forms of monomers and RgpA-Kgp complexes.[71,73,80-87] The processes of secretion and maturation of Kgp are complex and not fully understood. Generally glycosylation at the C-terminal domain is believed to provide the anchor for the membrane-associated Kgp isoforms,[81,88] and the maturation of Kgp requires N- and C-terminal modifications in the intra-domains of

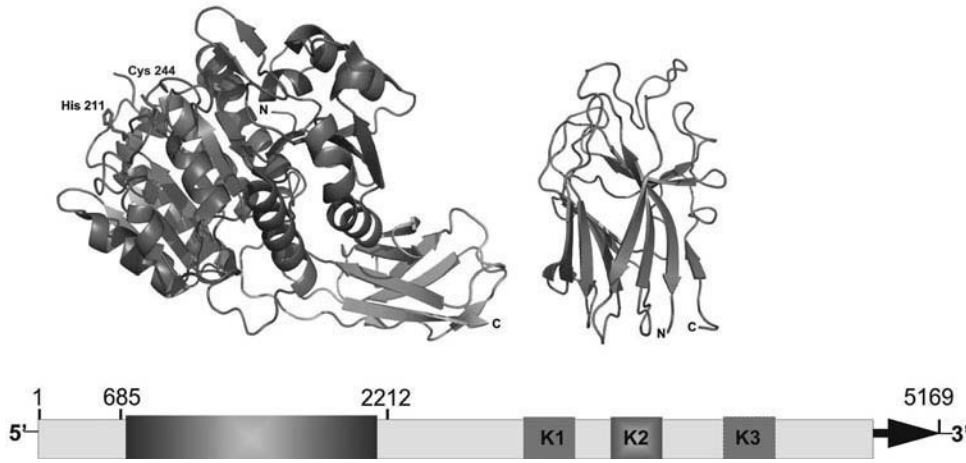

Figure 2. The domain structure of Kgp and known three-dimensional structures for the domains. The gene sequence for Kgp is shown as a grey bar overlaid with the translated domain structure as recently elucidated by Li et al.[79] The structure of the catalytic region of Kgp (blue bar) is postulated to be similar to that solved for RgpB,[92] the three-dimensional structure of which is shown as a ribbon diagram. The catalytic residues, Cys_{244} and His_{211}, are shown in pink ball and stick to indicate the position of the active site. The recently determined structure of the K2 haemagglutinin/adhesin domain of Kgp is shown as a ribbon diagram in light green.

Kgp polyprotein. Interestingly, these intra-domain modifications were found to require the activities of both Rgp and Kgp.[71] Furthermore, the Kgp polyprotein in an Rgp-null *P. gingivalis* mutant was found in the fully mature form,[89] and the introduction of RgpB into a *P. gingivalis* mutant lacking all three gingipain members was found to result in mature RgpB.[49] These phenomena of auto-processing of the gingipains may explain the initial source of active gingipains, which participate in the proteolytic modification of subsequently produced Kgp polyprotein.

The crystal structure of Kgp is yet to be determined, but despite the fact that the identity between the catalytic domains of Kgp and RgpA/RgpB is only around 27%, there is a strong likelihood that Kgp_{cat} will have a similar conformation to RgpB, for which a crystal structure is available.[90,91] Topologically, the crystal structure of RgpB displays an N-terminal domain and a C-terminal domain (Fig. 2).[92] The N-terminal domain is comprised of A- and B-sub-domains, each of which has a characteristic α/β motif that is made up of a central β-sheet sandwiched by α-helices. This α/β-sandwich structure can also be seen in caspase-1 and -3.[92] The B-sub-domain encompasses the catalytic residues of the Cys-His dyad and hence the N-terminal domain represents the catalytic domain. The C-terminal IgSF domain is composed entirely of β-sheets, which is a topological homologue to an IgG domain (Fig. 2).[92]

As mentioned, Kgp cleaves Lys-Xaa bonds and RgpB cleaves Arg-Xaa bonds exclusively, meaning a strict preference for Lys and Arg as the P1-residue (note that the nomenclature for proteases[93] indicates that cleavage occurs in substrates between the P1 and P1′ residues, with substrate residues N-terminal to P1 labelled P2, P3 etc and those C-terminal to P1′ labelled P2′, P3′ etc; subsites binding the substrate residues in the enzymes are correspondingly labelled, eg. the S1 subsite binds the P1 residue etc.) for Kgp and RgpB,

respectively and a broad spectrum of residues at the P1′ position for both gingipains.[37,93] The structural basis for this substrate specificity of Kgp can be inferred based on that of RgpB from its crystal structure. On the surface of the B-sub-domain of RgpB, adjacent to the catalytic dyad (His_{211} and Cys_{244}), a deep S1 pocket is formed by the peptide segments: Tyr_{283}~Met_{288}, Thr_{209}~His_{211} and Val_{242}~Cys_{244}. Of these, Met_{288} and Val_{242} were hypothesized to be important in determining the P1-Arg specificity of RgpB by participating in lining the side and bottom of the S1 pocket, respectively.[92] This S1 pocket is not only an optimal conformational fit for the side chain of the P1-Arg, but also contains a negatively-charged Asp_{163} at its bottom, contributing an electrostatic stabilisation for the bound P1-Arg.[92] Compared to this, in the modelled S1-pocket of Kgp, Asp_{288} and Glu_{287} form a small negatively-charged patch,[40] which may be important for binding the positively-charged Lys; Phe_{242} in Kgp is a bigger residue than Val_{242} in RgpB and thus may contribute to altering the shape of the S1 pocket to exclusively accommodate Lys residues. On the other hand, outside the S1 pocket of RgpB, around the catalytic dyad, there is a relatively flat and open surface, which can accommodate different types of amino acids.[92] Based on the topological and functional similarities and molecular modelling experiments, a similar flat and open area is also expected to exist outside the S1-pocket of Kgp, around its catalytic dyad (His_{217} and Cys_{249}).[40] Therefore, for all members of the gingipain family, in contrast to the high specificity for the P1-residue of the substrate, less specificity is predicted for the residues closely flanking the P1-residue, including the P3-, P2-, P1′- and P2′-residues.[92]

THE CATALYTIC MECHANISM OF KGP

A detailed understanding of the mechanism by which Kgp interacts and cleaves its substrates is critical for the development of Kgp inhibitors. Since members of the gingipain family (C25) share the characteristic catalytic dyad motif, His-Gly-X-Ala-Cys, with all other familial members of the CD clan of cysteine proteases, including family 14 (caspases), family 11 (bacterial clostripains), family 13 (plant and animal legumains) and family 50 (separase), the principal catalytic mechanism is therefore likely to be shared by all members of cysteine proteases in the CD clan, including Kgp.[49,94] This mechanism, in general, is similar to that exerted by papain-like (Clan CA) cysteine proteases.

In this catalytic mechanism (Fig. 3), under activated conditions, the negatively-charged Sγ atom of the catalytic Cys first attacks the carbonyl carbon of the scissile bond of the substrate, forming an enzyme-substrate complex.[95,96] The resulting proximity of the substrate and the catalytic dyad leads to a nucleophilic interaction between the N atom of the scissile bond and the Nδ atom of the catalytic His. Consequently, the scissile bond breaks, releasing the prime side of the substrate in the form of an amide.[97] The remaining acyl-enzyme intermediate is the subject of nucleophillic attack by a water molecule, which attacks the carbonyl carbon from the previous scissile bond, resulting in the release of the remaining N-terminus of the substrate and the functional protease molecule.[95,96]

Based on the catalytic mechanism and the P1 specificity of Kgp, it is possible to attenuate the proteolytic activity of Kgp via the inhibition of the catalytic Sγ atom, by introducing a peptidyl inhibitor containing a P1-Lys and close to it, one or more functional groups such as an aldehyde group, which can tightly bind to the Sγ atom.[98] In this scheme, the interaction of the peptide chain with the catalytic site of Kgp, including the binding of P1-Lys to the S1-pocket, brings the functional group into close proximity to the catalytic Sγ atom. Consequently, a covalent Sγ-functional group bond is formed, interrupting the

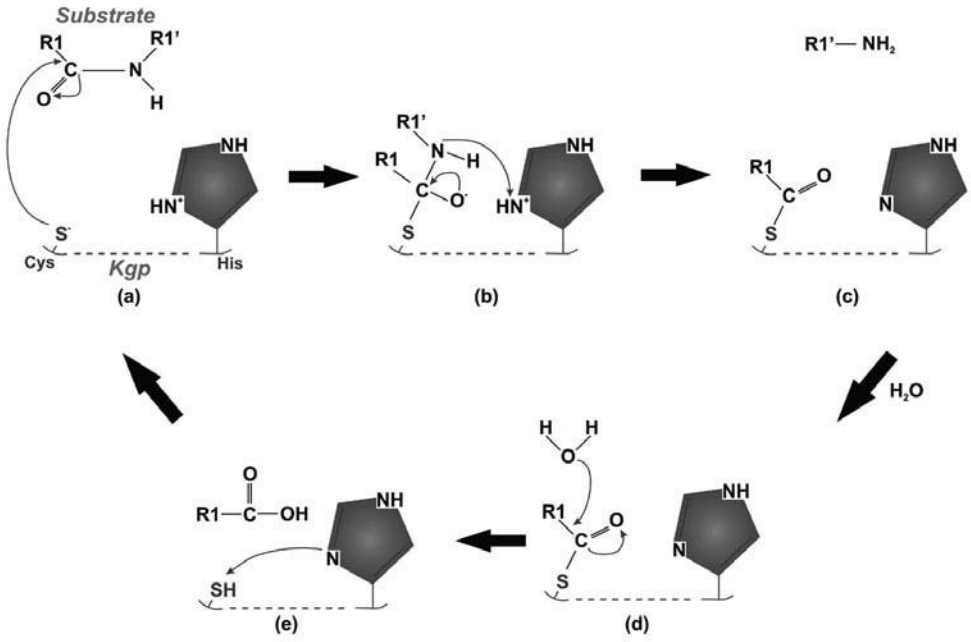

Figure 3. The catalytic mechanism of cysteine proteases. The catalytic dyad is illustrated in blue. The red arrows indicate the movement of protons. A) The catalytic Sγ atom attacks the carbonyl carbon of the scissile bond of a substrate, forming (B) an enzyme-substrate complex. In this complex, the N atom of the scissile bond attacks the Nδ atom of the catalytic His residue, resulting in the breakage of the scissile bond and the release of the prime side of the substrate in the form of an amine. C) An acyl-enzyme intermediate is formed. D) A water molecule attacks the acyl-enzyme complex, leading to (E) the release of the N-terminus of the substrate in the form of a carboxylic acid and a functional enzyme molecule. Under activated conditions, the catalytic dyad of the cysteine protease switches into the active or charged form (A).

catalytic function of Sγ.[97,98] Furthermore, it can be postulated that the efficacy of a peptidyl Kgp inhibitor would be greatly dependent on the effectiveness of the interaction between the peptide chain and the Kgp catalytic site. This interaction is not only affected by the binding of the P1-Lys to the S1 pocket, but also by the interaction amongst the residues flanking P1-Lys, including the P3-, P2-, P1′- and P2′-residues, with their corresponding sub-sites on Kgp. Supporting this hypothesis, Abe et al found that there was a decrease in cleavage of Lys-Xaa bonds by Kgp when the P2-residue is an Arg or Lys, although the amino acid specificity for P2-residue is theoretically broad.[99] Moreover, in another study, Abe et al found that Kgp was able to cleave the peptidyl substrate Z-His-Glu-Lys-MCA nearly 20-fold more effectively than Boc-Val-Leu-Lys-MCA, indicating that there may be preferences for the P3- and/or P2-position of substrates cleaved by Kgp.[100] Thus, it is possible that peptides of certain amino acid sequence may be more preferred by Kgp for interaction and cleavage. These peptides therefore would constitute optimal scaffolds for developing effective Kgp inhibitors. However, the overall specificity of Kgp at the S3, S2, S1′ and S2′ sub-sites, where the P3-, P2-, P1′- and P2′-residues of the substrates or inhibitors bind, has never been fully profiled.

PREVIOUSLY DEVELOPED KGP INHIBITORS

A few Kgp inhibitors from natural sources have been tested (Table 3)[101-105], however, they only showed limited inhibitory activity. Furthermore, a number of enzyme-substrate-based Kgp inhibitors have been developed (Table 4)[41,106-109]. For example, the ketopeptide inhibitor KYT-36 (carbobenzoxy-Glu(NHN(CH_3)Ph)-Lys-CO-NHCH$_2$Ph) was designed based on the sequence of histatin 5, a natural gingipain inhibitor in human saliva.[108] It displayed modest inhibitory effects in keeping with the weak inhibition of Kgp by histatin 5. In stark contrast, an acyloxymethyl ketone inhibitor (carbobenzoxy-Phe-Lys-CH_2OCO-2,4,6-Me_3Ph) and an aza-peptide Michael acceptor inhibitor (PhCH$_2$CH$_2$CO-Leu-ALys-CH=CHCOOEt) for Kgp have shown much higher inhibitory potency for Kgp, with k_{ass} values (the second-order rate constant of an inhibitory reaction, which indicates how fast an effective inhibition occurs) in the range of 10^6 $M^{-1}s^{-1}$.[41] As mentioned, since the amino acid specificity of Kgp at the positions closely flanking the S1-pocket has not been fully profiled, there may be some optimal

Table 3. Kgp inhibitors from natural sources

Inhibitor	Source	Inhibition or Association Rate Constants***	Reference
Histatin 5	Human saliva	$IC_{50} = 1.4 \times 10^{-5}$ M	101
CrmA (Asp>Lys)	Cowpox virus	$k_{ass} = 2.1 \times 10^5$ $M^{-1}s^{-1}$	102
p35	Baculovirus	$Ki = 2 \times 10^{-10}$ M	102
Cranberry nondialysable fraction	Cranberry	not determined	103
Cranberry polyphenol fraction	Cranberry	not determined	104
Pancreatic trypsin inhibitor	Pancreas	$k_{ass} = 2.0 \times 10^4$ M^{-1}	105

***Inhibition or association rate constants:
IC_{50}, the half maximal inhibitory concentration, is the amount of inhibitor taken to effectively inhibit half of the initial amount of enzyme. IC_{50} indicates the functional strength of an inhibitor against an enzyme, not the affinity of binding between them.
k_{ass}, the second-order rate constant of an inhibitory reaction, indicates how fast an effective inhibition occurs.
Ki is the dissociation constant of an inhibitory reaction. It indicates the affinity of the binding between inhibitor and enzyme.

Table 4. Synthetic Kgp inhibitors

Inhibitor	Chemical Property	Inhibition or Association Rate Constants	Reference
Cbz-Phe-Lys-CH_2O-CO-2,4,6-Me_3-Ph	Acyloxymethyl ketone	$k_{ass} = 4.2 \times 10^6$ $M^{-1}s^{-1}$	41
Chlorhexidine	Chlohexidine	$Ki = 1.65 \times 10^{-4}$ M	106
A71561	Amide	$Ki = 9 \times 10^{-10}$ M	107
KYT-36	Ketopeptide	$Ki = 1.3 \times 10^{-10}$ M	108
aza-peptide Michael acceptor (with Lys)	aza-peptide Michael acceptor	$k_{ass} = 3.3 \times 10^6$ $M^{-1}s^{-1}$	109
Rational-designed inhibitors	Chloromethyl ketone	$k_{ass} = 10^6 \sim 10^7$ $M^{-1}s^{-1}$	110

enzyme-substrate-based Kgp inhibitors, which achieve a higher range of k_{ass} values, yet to be developed. In agreement with this hypothesis, some small dipeptide chloromethyl ketone Kgp inhibitors, recently developed by Białas et al by structure-based rational design, for the first time were found to exhibit rapid interaction with Kgp, with k_{ass} values reaching 10^7 $M^{-1}s^{-1}$.[110] Importantly, Białas et al also highlighted the critical role of the P2-residue, which acts as a hydrophobic interactant in the Kgp inhibitors.[110] This finding reflected the necessity of determining the full specificity of the active site of Kgp to allow the development of optimal Kgp inhibitors.

CONCLUSION

It is evident that the lysine-specific gingipain, Kgp, is a major virulence factor of the anaerobe, *P. gingivalis*, which in turn is a major pathogen of adult periodontitis. The development of inhibitors of this enzyme will therefore be of critical importance to combat this disease. Understanding of the catalytic mechanism, transition state intermediates and the full specificity of the enzyme appear to be prerequisites for the development of further selective, potent inhibitors of Kgp.

REFERENCES

1. Brown LJ, John BA, Wall TP. The economics of periodontal diseases. Periodontol 2000. 2002; 29:223-234.
2. Spencer AJ, Wright FAC, Brown DF et al. A socio-dental study of adult periodontal health: Melbourne 1985. Melbourne: The University of Melbourne, 1988.
3. Shultis WA, Weil EJ, Looker HC et al. Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes. Diabetes Care 2007; 30:306-311.
4. DeStefano F, Anda RF, Kahn HS et al. Dental disease and risk of coronary heart disease and mortality. Br Med J 1993; 306:688-691.
5. Jeffcoat MK, Hauth JC, Geurs NC et al. Periodontal disease and preterm birth: result of a pilot intervention study. J Periodontol 2003; 74:1214-1218.
6. Scannapieco FA. Role of oral bacteria in respiratory infection. J Periodontol 1999; 70:793-802.
7. Shapira L, Ayalon S, Brenner T. Effects of Porphyromonas gingivalis on the central nervous system: Activation of glial cells and exacerbation of experimental autoimmune encephalomyelitis. J Periodontol 2002; 73:511-516.
8. Teng YT, Taylor GW, Scannapieco F et al. Periodontal health and systemic disorders. J Can Dent Assoc 2002; 68:188-192.
9. Mercado FB, Marshall RI, Bartold PM. Inter-relationship between rheumatoid arthritis and periodontal disease. A review. J Clin Periodontol 2003; 30:761-772.
10. Meyer MS, Joshipura K, Giovannucci E et al. A review of the relationship between tooth loss, periodontal disease and cancer. Cancer Cause Control 2008; 19:895-907.
11. Seymour GJ, Ford PJ, Cullinan MP et al. Relationship between periodontal infections and systemic disease. Clin Microbiol Infect 2007; 13:3-10.
12. Socransky S, Haffajee A. Evidence of bacterial aetiology: a historical perspective. Periodontol 2000 1994; 5:7-25.
13. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 2004; 34:9-12.
14. Kinane DF. Causation and pathogenesis of periodontal diseases. Periodontol 2000 2001; 25:8-20.
15. Armitage GC. Classifying periodontal diseases—a long-standing dilemma. Periodontol 2000 2002; 30:9-23.
16. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994; 5:78-111.
17. Fitzpatrick RE, Wijeyewickrema LC, Pike RN. The gingipains: scissors and glue of the periodontal pathogen, Porphyrmonas gingivalis. Future Microbiol 2009; 4:471-487.
18. Kasuga Y, Ishihara K, Okuda K. Significance of detection of Porphyromonas gingivalis, Bacteroides forsythus and Treponema denticola in periodontal pockets. Bull Tokyo Dent Coll 2000; 41:109-117.

19. Holt SC, Ebersole JL. Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. Periodontol 2000 2005; 38:72-122.
20. White D, Mayrand D. Association of oral bacteroides with gingivitis and adult periodontitis. J Periodontal Res 1981; 16:259-265.
21. Holt SC, Ebersole J, Felton J et al. Implantation of Bacteroides gingivalis in nonhuman primates initiates progression of periodontitis. Science 1988; 239:55-57.
22. Gibson FC, Genco CA. The genus Porphyromonas. In: The Prokaryote. New York: Springer, 2006:428-454.
23. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. Microbiol Mol Biol Rev 1998; 62:1244-1263.
24. Mayrand D, Holt SC. Biology of asaccharolytic black-pigmented Bacteroides species. Microbiol Rev 1988; 52:134-152.
25. Smalley JW, Silver J, Marsh PJ et al. The periodontopathogen Porphyromonas gingivalis binds iron protoporphyrin IX in the m-oxo dimeric form: an oxidative buffer and possible pathogenic mechanism. Biochem J 1998; 331:681-685.
26. Shah HN, Williams RAD. Utilization of glucose and amino acids by Bacteroides intermedius and Bacteroides gingivalis. Curr Microbiol 1987; 15:241-246.
27. van Steenbergen TJM, Kastelein P, Touw JJA et al. Virulence of black-pigmented Bacteroides strains from periodontal pockets and other sites in experimentally induced skin lesions in mice. J Periodont Res 1982; 17:41-49.
28. Amano A, Shizukuishi S, Horie H et al. Binding of Porphyromonas gingivalis fimbriae to proline-rich glycoproteins in parotid saliva via a domain shared by major salivary components. Infect Immun 1998; 66:2072-2077.
29. Holt SC, Kesavaku L, Walker S et al. Virulence factors of Porphyromonas gingivalis. Periodontol 2000 1999; 20:168-238.
30. Lamont RJ, Yilmaz O. In or out: the invasiveness of oral bacteria. Periodontol 2000 2002; 30:61-69.
31. Lamont RJ, Bevan CA, Gil S et al. Involvement of Porphyromonas gingivalis fimbriae in adherence to Streptococcus gordonii. Oral Microbiol Immunol 1993; 8:272-276.
32. Isogai H, Isogai E, Yoshimura F et al. Specific inhibition of adherence of an oral strain of Bacteroides gingivalis 381 to epithelial cells by monoclonal antibodies against the bacterial fimbriae. Arch Oral Biol 1988; 33:479-485.
33. Birkedal-Hansen H, Taylor RE, Zambon JJ et al. Characterization of collagenolytic activity from strains of Bacteroides gingivalis. J Periodont Res 1988; 23:258-264.
34. Bleeg HS, Polenik P. Sodium dodecyl sulfate potentiates collagen degradation by proteases from Porphyromonas gingivalis. FEMS Microbiol Ecol 1991; 85:125-132.
35. Kesavalu L, Holt SC, Ebersole JL. Trypsin-like protease activity of Porphyromonas gingivalis as a potential virulence factor in the murine lesion model. Microb Pathog 1996; 20:1-10.
36. Chen Z, Potempa J, Polanowski A et al. Purification and characterization of a 50-kDa cysteine proteinase (gingipain) from Porphyromonas gingivalis. J Biol Chem 1992; 267:18896-18901.
37. Pike R, McGraw W, Potempa J et al. Lysine- and arginine-specific proteinases from Porphyromonas gingivalis. Isolation, characterization and evidence for the existence of complexes with hemagglutinins. J Biol Chem 1994; 269:406-411.
38. Kesavalu L, Holt SC, Ebersole JL. Porphyromonas gingivalis virulence in a murine lesion model: effects of immune alterations. Microb Pathog 1997; 23:317-326.
39. McKee AS, McDermid AS, Wait R et al. Isolation of colonial variants of Bacteroides gingivalis W50 with reduced virulence. J Med Microbiol 1988; 27:59-64.
40. Pavloff N, Pemberton PA, Potempa J et al. Molecular cloning and characterization of Porphyromonas gingivalis lysine-specific gingipain. J Biol Chem 1997; 272:1595-1600.
41. Potempa J, Pike R, Travis J. Titration and mapping of the active site of cysteine proteinases from Porphyromonas gingivalis (gingipain) using peptidyl chloromethanes. J Biol Chem 1997; 378:223-230.
42. Curtis MA, Aduse-Opoku J, Rangarajan M. Cysteine Proteases of Porphyromonas Gingivalis. Crit Rev Oral Biol M 2001; 12:192-216.
43. Curtis MA, Kuramitsu HK, Lantz M et al. Molecular genetics and nomenclature of proteases of Porphyromonas gingivalis. J Periodontal Res 1999; 34:464-472.
44. Tokuda M, Duncan M, Cho MI et al. Role of Porphyromonas gingivalis protease activity in colonization of oral surfaces. Infect Immun 1996; 64:4067-4073.
45. Tokuda M, Karunakaran T, Duncan M et al. Role of Arg-gingipain A in virulence of Porphyromonas gingivalis. Infect Immun 1998; 66:1159-1166.
46. Shi Y, Ratnayake DB, Okamoto K et al. Genetic analyses of proteolysis, hemoglobin binding and hemagglutination of Porphyromonas gingivalis: construction of mutants with a combination of rgpA, rgpB, kgp and hagA. J Biol Chem 1999; 274:17955-17960.

47. O'Brien-Simpson NM, Paolini RA, Hoffmann B et al. Role of RgpA, RgpB and Kgp proteinases in virulence of Porphyromonas gingivalis W50 in a murine lesion model. Infect Immun 2001; 69:7527-7534.
48. Pathirana RD, O'Brien-Simpson NM, Brammar GC et al. Kgp and RgpB, but not RgpA, are important for Porphyromonas gingivalis virulence in the murine periodontitis model. Infect Immun 2007; 75:1436-1442.
49. O'Brien-Simpson NM, Veith PD, Dashper SG et al. Porphyromonas gingivalis gingipains: The molecular teeth of a microbial vampire. Curr Protein Pept Sci 2003; 4:409-426.
50. Barua PK, Dyer DW, Neiders ME. Effect of iron limitation on Bacteroides gingivalis. Oral MicroImmunol 1990; 5:263-268.
51. Shizukuishi S, Tazaki K, Inoshita E et al. Effect of concentration of compounds containing iron on the growth of Porphyromonas gingivalis. FEMS Microbiol Lett 1995; 131:313-317.
52. Lewis JP, Dawson JA, Hannis JC et al. Hemoglobinase activity of the lysine gingipain protease (Kgp) of Porphyromonas gingivalis. J Bacteriol 1999; 181:4905-4913.
53. Okamoto K, Nakayama K, Kadowaki T et al. Involvement of a lysine-specific cysteine proteinase in hemoglobin adsorption and heme accumulation by Porphyromonas gingivalis. J Biol Chem 1998; 273:21225-21231.
54. Pike RN, Potempa J, McGraw W et al. Characterization of the binding activities of proteinase-adhesin complexes from Porphyromonas gingivalis. J Bacteriol 1996; 178:2876-2882.
55. Ryan ME, Golub LM. Modulation of matrix metalloproteinase activities in periodontitis as a treatment strategy. Periodontol 2000 2000; 24:226-238.
56. Decarlo Jr AA, Windsor LJ, Bodden MK et al. Activation and novel processing of matrix metalloproteinases by a thiol-protease from the oral anaerobe Porphyromonas gingivalis. J Dent Res 1997; 76:1260-1270.
57. Imamura T, Potempa J, Pike RN et al. Effect of free and vesicle-bound cysteine proteinases of Porphyromonas gingivalis on plasma clot formation: implications for bleeding tendency at periodontitis sites. Infect Immun 1995; 63:4877-4882.
58. Fletcher J, Reddi K, Poole S et al. Interactions between periodontopathogenic bacteria and cytokines. J Periodont Res 1997; 32:200-205.
59. Yun PLW, DeCarlo AA, Hunter N. Modulation of major histocompatibility complex protein expression by human gamma interferon mediated by cysteine proteinase-adhesin polyproteins of Porphyromonas gingivalis. Infect Immun 1999; 67:2986-2995.
60. Oleksy A, Banbula A, Bugno M et al. Proteolysis of interleukin-6 receptor (IL-6R) by Porphyromonas gingivalis cysteine proteinases (gingipains) inhibits interleukin-6-mediated cell activation. Microb Pathog 2002; 32:173-181.
61. Yun PLW, Decarlo AA, Collyer C et al. Hydrolysis of interleukin-12 by Porphyromonas gingivalis major cysteine proteinases may affect local gamma interferon accumulation and the Th1 or Th2 T-cell phenotype in periodontitis. Infect Immun 2001; 69:5650-5660.
62. Liu RK, Cao CF, Meng HX et al. Polymorphonuclear neutrophils and their mediators in gingival tissues from generalized aggressive periodontitis. J Periodontol 2001; 72:1545-1553.
63. Sugawara S, Nemoto E, Tada H et al. Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from Porphyromonas gingivalis leading to lipopolysaccharide hyporesponsiveness. J Immunol 2000; 165:411-418.
64. Kitamura Y, Yoneda M, Imamura T et al. Gingipains in the culture supernatant of Porphyromonas gingivalis cleave CD4 and CD8 on human T-cells. J Periodont Res 2002; 37:464-468.
65. Kitamura Y, Hirofuji T, Yoneda M et al. Cleavage of surface proteins on human T-cells by the culture supernatant of Porphyromonas gingivalis 381. Dent J 2000; 36:109-112.
66. Fishburn CS, Slaney JM, Carman RJ et al. Degradation of plasma proteins by the trypsin-like enzyme of Porphyromonas gingivalis and inhibition of protease activity by a serine protease inhibitor of human plasma. Oral Microbiol Immunol 1991; 6:209-215.
67. Uehara A, Imamura T, Potempa J et al. Gingipains from Porphyromonas gingivalis synergistically induce the production of proinflammatory cytokines through protease-activated receptors with Toll-like receptor and NOD1/2 ligands in human monocytic cells. Cell Microbiol 2008; 10:1181-1189.
68. Huang GTJ, Kim D, Lee JKH et al. Interleukin-8 and intercellular adhesion molecule 1 regulation in oral epithelial cells by selected periodontal bacteria: multiple effects of Porphyromonas gingivalis via antagonistic mechanisms. Infect Immun 2001; 69:1364-1372.
69. Pavloff N, Potempa J, Pike RN et al. Molecular cloning and structural characterization of the arg-gingipain proteinase of Porphyromonas gingivalis. J Biol Chem 1995; 270:1007-1010.
70. Mikolajczyk-Pawlinska J, Kordula T, Pavloff N et al. Genetic variation of Porphyromonas gingivalis genes encoding gingipains, cysteine proteinases with arginine or lysine specificity. Biol Chem 1998; 379:205-211.
71. Potempa J, Sroka A, Imamura T et al. Gingipains, the major cysteine proteinases and virulence factors of Porphyromonas gingivalis: Structure, function and assembly of multidomain protein complexes. Curr Protein Pept Sci 2003; 6:443-450.
72. Slakeski N, Cleal SM, Bhogal PS et al. Characterization of a Porphyromonas gingivalis gene prtK that encodes a lysine-specific thiol proteinase and multiple adhesins. Oral Microbiol Immunol 1999; 14:92-97.

73. Slakeski N, Bhogal PS, O'Brien-Simpson NM et al. Characterization of a second cell-associated Arg-specific cysteine proteinase of Porphyromonas gingivalis and identification of an adhesin binding motif involved in association of the prtR and prtK proteinases and adhesins into large complexes. Microbiol 1998; 144:1583-1592.
74. O'Brien-Simpson NM, Paolini RA, Reynolds EC. RgpA-Kgp peptide-based immunogens provide protection against Porphyromonas gingivalis challenge in a murine lesion model. Infect Immun 2000; 68:4055-4063.
75. O'Brien-Simpson NM, Pathirana RD, Paolini RA et al. An immune response directed to proteinase and adhesin functional epitopes protects against Porphyromonas gingivalis-induced periodontal bone loss. J Immunol 2005; 175:3980-3989.
76. Barkocy-Gallagher GA, Han N, Patti JM et al. Analysis of the prtP gene encoding porphypain, a cysteine proteinase of Porphyromonas gingivalis. J Bacteriol 1996; 178:2734-2741.
77. Lewis JP, Macrina FL. IS195, an insertion sequence-like element associated with protease genes in Porphyromonas gingivalis. Infect Immun 1998; 66:3035-3042.
78. Okamoto K, Kadowaki T, Nakayama K et al. Cloning and sequencing of the gene encoding a novel lysine-specific cysteine proteinase (lys-gingipain) in Porphyromonas gingivalis: Structural relationship with the arginine-specific cysteine proteinase (arg-gingipain). J Biochem 1996; 120:398-406.
79. Li N, Yun P, Nadkarni MA et al. Structure determination and analysis of a haemolytic adhesin domain from Porphyromonas gingivalis. Mol Microbiol 2010; 76:861-873.
80. Bhogal PS, Slakeski N, Reynolds E. Characterization of a cell-associated, protein complex of Porphyromonas gingivalis W50 composed of Arg- and Lys-specific cysteine proteinases and adhesins. Microbiol 1997; 143:2485-2495.
81. Curtis MA, Thickett A, Slaney JM et al. Variable carbohydrate modifications to the catalytic chains of the RgpA and RgpB proteases of Porphyromonas gingivalis W50. Infect Immun 1999; 67:3816-3823.
82. Rangarajan M, Smith SJM, U S et al. Biochemical characterization of the arginine-specific proteases of Porphyromonas gingivalis W50 suggests a common precursor. Biochem J 1997; 323:701-709.
83. Seers CA, Slakeski N, Veith PD et al. The RgpB C-terminal domain has a role in attachment of RgpB to the outer membrane and belongs to a novel C-terminal-domain family found in Porphyromonas gingivalis. J Bacteriol 2006; 188:6376-6386.
84. Pathirana RD, O'Brien-Simpson NM, Veith PD et al. Characterization of proteinase-adhesin complexes of Porphyromonas gingivalis. Microbiol 2006; 152:2381-2394.
85. Fujimura S, Nakamura T. Isolation and characterization of a protease from Bacteroides gingivalis. Infect Immun 1987; 55:716-720.
86. Bedi GS. Comparative study of four proteases from spent culture media of Porphyromonas gingivalis (FAY-19M-1). Prep Biochem 1995; 25:133-154.
87. Takii R, Kadowaki T, Baba A et al. A functional virulence complex composed of gingipains, adhesins and lipopolysaccharide shows high affinity to host cells and matrix proteins and escapes recognition by host immune systems. Infect Immun 2005; 73:883-893.
88. Shoji M, Ratnayake DB, Shi Y et al. Construction and characterization of a nonpigmented mutant of Porphyromonas gingivalis: cell surface polysaccharide as an anchorage for gingipains. Microbiol 2002; 148:1183-1191.
89. Aduse-Opoku J, Davies NN, Gallagher A et al. Generation of Lys-gingipain protease activity in Porphyromonas gingivalis W50 is independent of Arg-gingipain protease activities. Microbiol 2000; 146:1933-1940.
90. Potempa J, Banbula A, Travis J. Role of bacterial proteinases in matrix destruction and modulation of host responses. Periodontol 2000 2000; 24:153-192.
91. Okamoto K, Misumi Y, Kadowaki T et al. Structural characterization of argingipain, a novel arginine-specific cysteine proteinase as a major periodontal pathogenic factor from Porphyromonas gingivalis. Arch Biochem Biophys 1995; 316:917-925.
92. Eichinger A, Beisel HG, Jacob U et al. Crystal structure of gingipain R: an Arg-specific bacterial cysteine proteinase with a caspase-like fold. EMBO J 1999; 18:5453-5462.
93. Schechter I, Berger A. On the size of the active site in proteases. I. Papain. Biochem Biophys Res Commun 1967; 27:157-162.
94. Chen JM, Rawlings ND, Stevens RA et al. Identification of the active site of legumain links it to caspases, clostripain and gingipains in a new clan of cysteine endopeptidases. FEBS Lett 1998; 441:361-365.
95. Howard AE, Kollman PA. OH- versus SH- nucleophilic attack on amides: dramatically different gas-phase and solvation energetics. J Am Chem Soc 1988; 110:7195-7200.
96. Arad D, Langridge R, Kollman PA. A simulation of the sulfur attack in the catalytic pathway of papain using molecular mechanics and semiempirical quantum mechanics. J Am Chem Soc 1988; 112:491-502.
97. Shi Y. Caspase activation: revisiting the induced proximity model. Cell 2004; 117:855-858.
98. Albeck A, Kliper S. Mechanism of cysteine protease inactivation by peptidyl epoxides. Biochem J 1997; 322:879-884.

99. Abe N, Kadowaki T, Okamoto K et al. Biochemical and functional properties of Lysine-specific cysteine proteinase (Lys-gingipain) as a virulence factor of Porphyromonas gingivalis in periodontal disease. J Biochem 1998; 123:305-312.
100. Abe N, Baba A, Kadowaki T et al. Design and synthesis of sensitive fluorogenic substrates specific for Lys-gingipain. J Biochem 2000; 128:877-881.
101. Gusman H, Travis J, Helmerhorst EJ et al. Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease. Infect Immun 2001; 69:1402-1408.
102. Snipas SJ, Stennicke HR, Riedl S et al. Inhibition of distant caspase homologues by natural caspase inhibitors. J Biochem 2001; 357:575-580.
103. Bodet C, Piché M, Chandad F et al. Inhibition of periodontopathogen-derived proteolytic enzymes by a high-molecular-weight fraction isolated from cranberry. J Antimicrob Chemother 2006; 57:685-690.
104. Yamanaka A, Kouchi T, Kasai K et al. Inhibitory effect of cranberry polyphenol on biofilm formation and cysteine proteases of Porphyromonas gingivalis. J Periodont Res 2007; 42:589-592.
105. Bania J, Kubiak A, Wojtachnio K et al. Pancreatic secretory trypsin inhibitor acts as an effective inhibitor of cysteine proteinases gingipains from Porphyromonas gingivalis. J Periodont Res 2008; 43:232-236.
106. Cronan CA, Potempa J, Travis J et al. Inhibition of Porphyromonas gingivalis proteinases (gingipains) by chlorhexidine: synergistic effect of Zn(II). Oral Microbiol Immunol 2006; 21:212-217.
107. Curtis MA, Aduse-Opoku J, Rangarajan M et al. Attenuation of the virulence of Porphyromonas gingivalis by using a specific synthetic Kgp protease inhibitor. Infect Immun 2002; 70:6968-6975.
108. Kadowaki T, Baba A, Abe N et al. Suppression of pathogenicity of Porphyromonas gingivalis by newly developed gingipain inhibitors. Mol Pharmacol 2004; 66:1599-1606.
109. Ekici OD, Götz MG, James KE et al. Aza-peptide Michael acceptors: a new class of inhibitors specific for caspases and other clan CD cysteine proteases. J Med Chem 2004; 47:1889-1892.
110. Białas A, Grembecka J, Krowarsch D et al. Exploring the Sn binding pockets in gingipains by newly developed inhibitors: structure-based design, chemistry and activity. J Med Chem 2006; 49:1744-1753.
111. Ally, N. The specificity of proteinase-adhesins from Porphyromonas gingivalis. PhD thesis. Melbourne: Monash University, 2003.
112. Thomas DA, Francis P, Smith C et al. A broad-spectrum fluorescence-based peptide library for the rapid identification of protease substrates. Proteomics 2006; 6:2112-2120.
113. Birkett AJ, Soler DF, Wolz RL et al. Determination of enzyme specificity in a complex mixture of peptide substrates by N-terminal sequence analysis. Anal Biochem 1991; 196:137-143.
114. Petithory JR, Masiarz FR, Kirsch JF et al. A rapid method for determination of endoproteinase substrate specificity: Specificity of the 3C proteinase from hepatitis A virus. Proc Natl Acad Sci USA 1991; 88:11510-11514.
115. Turk BE, Cantley LC. Using peptide libraries to identify optimal cleavage motifs for proteolytic enzymes. Methods 2004; 32:398-405.
116. Meldal M, Svendsen I, Breddam K et al. Portion-mixing peptide libraries of quenched fluorogenic substrates for complete subsite mapping of endoprotease specificity. P Natl Acad Sci USA 1994; 91:3314-3318.
117. Leon S, Quarrell R, Lowe G. Evaluation of resins for on-bead screening: a study of papain and chymotrypsin specificity using PEGA-bound combinatorial peptide libraries. Bioorg Med Chem Lett 1998; 8:2997-3002.
118. Harris JL, Backes BJ, Leonetti F et al. Rapid and general profiling of protease specificity by using combinatorial fluorogenic substrate libraries. P Natl Acad Sci USA 2000; 97:7754-7759.
119. Matthews DJ, Well JA. Substrate phage: selection of protease substrates by monovalent phage display. Science 1993; 260:1113-1117.
120. Deng SJ, Bickett DM, Mitchell JL et al. Substrate specificity of human collagenase 3 assessed using a phage-displayed peptide library. J Biol Chem 2000; 275:31422-31427.
121. Le Bonniec BF, Myles T, Johnson T et al. Characterization of the P2′ and P3′ specificities of thrombin using fluoresence-quenched substrates and mapping of the subsites by mutagenesis. Biochem 1996; 35:7114-7122.
122. Ally N, Whisstick JC, Sieprawska-Lupa M et al. Characterization of the specificity of Arginine-specific gingipain from Porphyromonas gingivalis reveals active site differences between different forms of the enzymes. Biochem 2003; 42:11693-11700.