Review

REGULATION OF BACTERIAL PROTEASE ACTIVITY [#]

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Abstract: Proteases, also referred to as peptidases, are the enzymes that catalyse the hydrolysis of peptide bonds in polipeptides. A variety of biological functions and processes depend on their activity. Regardless of the organism's complexity, peptidases are essential at every stage of life of every individual cell, since all protein molecules produced must be proteolytically processed and eventually recycled. Protease inhibitors play a crucial role in the required strict and multilevel control of the activity of proteases involved in processes conditioning both the physiological and pathophysiological functioning of an organism, as well as in host-pathogen interactions. This review describes the regulation of activity of bacterial proteases produced by dangerous human pathogens, focusing on the *Staphylococcus* genus.

Key words: Protease, Protease inhibitor, Zymogen, Operon, *Staphylococcus*

Abbreviations used: α2M – alpha2-macroglobulin; aa – amino acid; *agr* – accesory gene regulator; AprA – alkaline protease from *Pseudomonas aeruginosa*; *aur* – aureolysin gene; Clp – bacterial proteolytic system analogous to eukaryotic proteasome; ClpP – proteolytic core of the Clp; DegP – conserved heat shock protein; LasB – elastase from *P. aeruginosa*; RBS – ribosome binding site; *sar* – staphylococcal accessory regulator; *scp* – staphylococcal cysteine protease operon; SpeB – streptopain, cysteine protease form *Streptococcus pyogenes*; Spi – specific inhibitor of SpeB; *spl* – serine protease-like operon; *ssp* – staphylococcal serine protease operon; VVP – *Vibro vulnificus* protease

INTRODUCTION

Proteases catalyse the cleavage of peptide bonds in proteins and peptides. Depending on the reaction they catalyse, proteases are divided into endopeptidases (also named proteinases), which preferentially hydrolyse peptide bonds in the inner regions of peptide chains, and exopeptidases, whose activity is directed at the amino- or carboxyl-termini of proteins. Based on their catalytic mechanism, proteases are divided into seven groups: aspartic acid proteases, cysteine proteases, glutamic acid proteases, metalloproteases, serine proteases, threonine proteases, and the seventh group, which constitutes a number of peptidases that cannot yet be assigned to any particular catalytic type. Among prokaryotic organisms, proteases of 6 of the catalytic types (except glutamic acid proteases) are common (for review see: [1]). In terms of enzyme specificity, the cleavage of peptide bonds by proteases may be specific, e.g. limited proteolysis, which depends on the amino acid sequence surrounding the hydrolysed site, or unspecific, which results in complete degradation of proteins to oligo-peptides and/or amino acids.
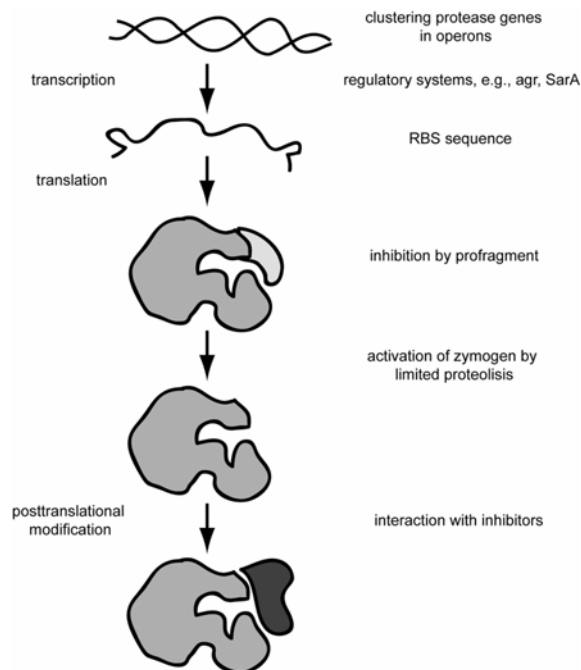

Fig. 1. The levels of regulation of protease expression and activity.

By cleaving peptide bonds, proteases may change the biological properties of polypeptide chains. Specific proteolysis often results in protein and peptide activation or inactivation, and unspecific proteolysis in their degradation, and

thus these enzymes are potentially dangerous for cells, and may alter their environment. Therefore, organisms have developed a wide range of mechanisms to control protease activity. This regulation may take place at any level of gene expression, from the clustering of protease genes in operons, through their modulation at the transcription and translation levels, to post-translational modifications and interactions with protease inhibitors and other proteins (see Fig. 1).

The genetic organization of bacterial protease genes
The extracellular proteases produced by various pathogens are often accessory proteins, not fundamental for cell growth and division, but considered virulence factors. These proteins are not produced constitutively, but regulated in response to various environmental and cellular stimuli. The genetic determinants for these virulence factors are frequently associated with mobile genetic elements such as plasmids, pathogenecity islands and integrated phages. Clustering of protease genes in larger transcriptional units (operons) allows their coordinated expression, which in turn may imply cooperation of the produced proteins.
A good example of the diverse organization of genes is the genes coding for epidermolytic toxins from *Staphylococcus aureus*, the most virulent and important pathogen of the staphylococci. The genes for these proteins are spread all over the genome of the bacterium. The *eta* gene, encoding epidermolytic toxin A, is located in a prophage genome integrated in the *S. aureus* chromosome [2], while the *etb* gene, coding for epidermolytic toxin B, is located on a plasmid [3]. It was also shown by Yamaguchi *et al.* that the epidermolytic toxin D gene (*etd*) is placed tandemly in a DNA block together with the ADP-ribosyltransferase gene (*edin-B*), forming a pathogenicity island on the chromosome [2].
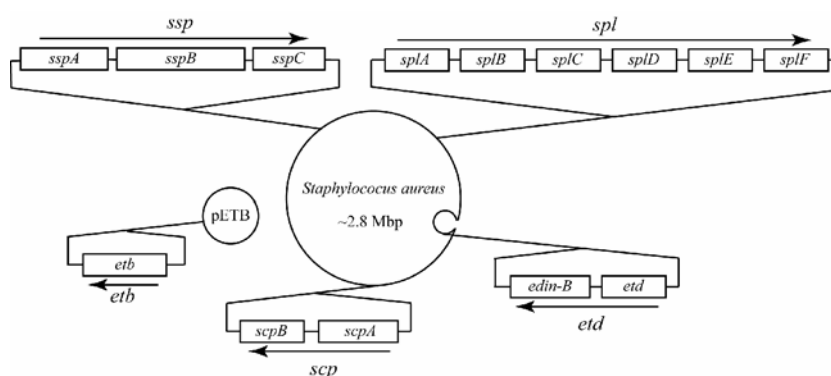

Fig. 2. The schematic genetic organization of *Staphylococcus aureus* proteases genes. *Ssp* (staphylococcal serine protease), *spl* (serine-like protease), *etd* (epidermolytic toxin D), and *scp* (staphylococcal cysteine protease) operons are spread all over the bacterial chromosome. The *etb* (epidermolytic toxin B) gene is located on the plasmid pETB.

Genes of other extracellular staphylococcal proteases are clustered in operons (see Fig. 2). In *S. aureus*, there is a tricistronic operon of staphylococcal serine protease (*ssp*) consisting of a gene (*sspA*) encoding glutamyl endopeptidase, also referred to as V8 protease, a gene (*sspB*) coding for cysteine protease, staphopain B, and a gene (*sspC*) for staphostatin B, a specific endogenous intracellular inhibitor of the latter protease [4-6]. The staphylococcal cysteine protease (*scp*) operon comprises a staphopain A gene (*scpA*) and a gene (*scpB*) coding for staphostatin A, an inhibitor of the protease [6-8]. The pathogenic strain *S. aureus* CH-91 produces a third cysteine protease with a high sequence homology to staphopain A [9, 10]. It was recently shown that a gene coding for the protease is clustered in a bicistronic operon together with a gene encoding a specific inhibitor of the protease [11]. A similar organization of the staphopain and staphostatin genes was also found in the genome of *S. epidermidis* [12], and in partially sequenced genomes of *S. carnosus* [13] and *S. warneri* [11]. In the case of the latter, genes coding for staphopain and staphostatin are preceded by a gene encoding an orthologue of the *S. aureus* serine protease. Moreover, in the majority of *S. aureus* strains, a unique operon (*spl*) coding for six serine protease-like proteins was identified. The encoded Spl proteins share 44 to 95% amino acid sequence identity with each other and 33 to 36% sequence identity with V8 protease. The *spl* operon is transcribed on a 5.5-kb transcript, but several non-random degradation products of this transcript were also identified [14]. Transcriptional coupling of protease and inhibitor genes is not limited to the *Staphylococcus* genus. In *Pseudomonas aeruginosa*, a major cause of nosocomial (hospital acquired) infections, the *aprA* gene, coding for an alkaline protease, is located in an operon together with *aprI*, encoding a specific inhibitor of this protease, and genes with products involved in the secretion of the protease [15]. Recently, a novel protease-inhibitor pair from *Streptococcus pyogenes*, an important human pathogen, was discovered [16]. However, contrary to staphostatins sharing a lipocalin-like fold [17], the inhibitor (Spi) encoded downstream of the streptopain (SpeB) gene possesses 70% sequence similarity to the SpeB propeptide [16].

Transcriptional and translational control of protease expression
Genes in operons are usually co-transcribed. However, the transcription process itself is dependent on a set of factors within the cell and/or its environment that regulate the initiation and intensity of the process. In *S. aureus*, the expression of extracellular proteases is controled at the transcription level by two main global regulatory systems: *agr* (accessory gene regulator) [18] and *sar* (staphylococcal accessory regulator) [19]. The *agr* locus is composed of two divergent transcriptional units, an operon *agrBDCA* under the control of the P2 promoter, and the P3 operon, which specifies a 0.5 kb transcript, RNA III. This last is the actual effector of the *agr* response, and, incidentally, encodes the agr-regulated peptide delta-hemolysin [20]. The action of the *agr* system is manifested at the end of the exponential phase of bacterial growth as a result of the accumulation

of an autoinducer peptide, encoded in *agrD*. As a consequence, surface-protein gene expression is repressed, and genes encoding secretory proteins, including proteases, are subsequently activated [21]. Gambello *et al.* described a similar two-component autoinducing system in *P. aeroginosa*, where both the 27-kDa LasR protein and pseudomonal autoinducer N-(3-oxododecanoyl)-L-homoserine lactone are necessary for efficient transcription of the *aprA* gene [22]. The LasR and the autoinducer form a complex, which subsequently binds to the DNA structure motif ("lux box"), and stimulates transcription of the protease gene among others [23].

In *S. aureus*, the *sar* locus codes for three copies of a transcription factor, SarA [24]. The factor may operate either indirectly, acting on promoters of the *agr* system [25], or directly regulate the transcription process of particular genes interacting with their promoters [26]. It was shown that SarA strongly inhibits the transcription of genes encoding extracellular proteases [8, 27]. Apart from the mentioned systems, other factors are also engaged in the regulation of protease gene transcription. The regulator Rot, which is claimed to be a SarA homologue, negatively controls the transcription of *ssp* and *spl* operons [28, 29]. Moreover, it was demonstrated that *ssp*, *aur* and *scp* transcription is repressed by σ^B, an alternative subunit of RNA polymerase [8, 30]. Hence, through the co-operation of the systems, the activity of different groups of genes, the products of which are indispensable in subsequent phases of bacterial growth and infection, is coordinated. Induction of extracellular protease expression in the beginning of the postexponential phase of growth *in vitro* is suspected to reflect the situation *in vivo*, in which the enzymes degrade host tissues supplying nutrients to the bacteria. Moreover, the proteases support the spread of the infection and dissemination of the bacteria into deeper tissue through the shedding of cell surface adhesion molecules and inactivation of the components of the host immune system [8].

Clustering genes in operons allows for coordinated transcription. However, regulation of the expression intensity of the particular components of the operon is provided at the translation level, as a sequence disparity of the ribosome binding site (RBS). Such a situation is observed in the case of staphylococcal *ssp* and *scp* operons, where the RBS following a start codon for the staphostatin coding sequence diverges significantly from the TGGAGG(A/T) motif characteristic for RBS in gram-positive bacteria, including *S. aureus*. As a result, staphostatins, although indispensable for the bacteria, are produced in amounts at least a thousand times lower than staphopains, encoded in the same operon [6, 31].

Post-translational modifications

Many organisms, including bacteria, commonly utilize post-translational modifications to modulate the activity of the expressed proteins. Of the enzymes engaged in these processes, proteases play a significant role. For example, methionyl aminopeptidases remove a formylated methionine residue from newly

synthesized polypeptide chains (for review see: [32]), while signal peptidases cleave off signal sequences during protein translocation through the cytoplasmic membrane (for review see: [33]). In turn, proteases themselves are produced as inactive precursors, also referred to as zymogens. Subsequently, their activity is liberated by the post-translational removal of profragments of various lengths and locations in the precursor [34].

Several functions have been demonstrated for the propeptides during the processing of the protease precursors. The pro-sequence may be required for proper folding or to maintain the protease in an inactive form until its release from the cell, and for temporary docking of the molecule into the membrane. In some cases, propeptides have been shown to play multiple roles. The precursor for subtilisin, a serine endopeptidase produced by a variety of a *Bacillus* species, is a good example of a broad-function proenzyme. It is synthesized as a preproenzyme consisting of a typical signal peptide of 29 residues followed by a propeptide of 77 residues preceding the 275 residue segment of mature subtilisin [35]. First, the subtilisin propeptide restricts the proteolytic activity of the proenzyme. Second, it promotes the correct folding of the mature protease processes. Finally, it temporarily anchors the protein in the plasma membrane [36].

Major staphylococcal extracellular proteases are produced as zymogens, followed by a signal sequence. The aureolysin precursor consists of 509 amino acids, of which (aa), the first 27 residues comprise a signal peptide removed during the secretion of the protein. Extracellularly, the 181 aa profragment is proteolytically detached, which results in the formation of a mature enzyme, constituting 301 aa [37]. The protease responsible for the removal of the profragment remains unknown. It was suggested that, similarly to other metalloproteases from the thermolysin family, the profragment is removed autocatalytically [38]. Recent studies, however, refute such a mechanism [8]. V8 protease zymogen consists of a 29 aa signal peptide, a 39 aa profragment and an around 280 aa part comprising the enzymatically active part. The heterogeneity of the mature form arises from the variable number of three amino acid repeats (Pro-Asn/Asp-Asn) in the C-terminal part of the protein, which is a result of *sspA* gene polymorphism [39, 40]. Additional variation arises from autodigestion at the glutamic acid residue in the C-terminal part of the molecule [41]. The zymogen undergoes proteolytic activation *via* cleavage by aureolysin [42]; however, an alternative activation mechanism was also suggested, since in culture fluids of mutants deficient in metalloprotease, the mature form of V8 protease was also observed [8, 43]. Staphopain B (SspB) is expressed as a preproenzyme containing a 36 aa signal sequence. After extracellular release, V8 protease, encoded in the same operon, cleaves a 183 aa profragment which results in the formation of a mature, fully active protease [5]. However, contrary to the commonly accepted rule that profragments inhibit the proteolytic activity of zymogens, a slight enzymatic activity of prostaphopain B against a synthetic low molecular weight substrate was observed. Moreover, the lack of degradation

of the profragment by SspA and SspB suggest that the propeptide does not function as an inhibitor of that enzyme [5]. However, studies performed by Filipek *et al.* based on the structural and biochemical analysis of prostaphopain B contradict the above hypothesis [44]. It is of note that in the latter studies, the used zymogen differed slightly in the amino acid sequence of the profragment, which among other things, meant the affinity of the propeptide for the mature form of the protease was twice as high. However, both authors agree that activation of the zymogen is not autocatalytic, and that it goes through sequential events of limited proteolysis, where aureolysin activates V8 protease, which in turn releases an active staphopain B from its zymogen. The detailed mechanism of staphopain A post-translational activation remains unknown. One may suspect that the 25 aa signal peptide is removed during protein translocation through the cytoplasmic membrane. Recent studies showed that neither metalloprotease nor V8 protease and staphopain B are engaged in excising the 189 aa profragment [8]. Similarly, in the case of staphopain A2, a homologue of staphopain A from the *S. aureus* CH-91 strain, the activation mechanism is obscure. Analysis of the encoding gene suggests that the protease is produced as a 399 aa preproenzyme. From the culture fluids, however, a 174 aa mature protease was isolated [9, 10]. The unusual method of post-translational activation was observed in a group of Spl proteases. They do not posses a typical profragment, only a 36 aa signal peptide [14]. This part of the molecule, however, is responsible for inhibiting the protease activity. The proteases are maintained latent until the signal peptide is removed by a signal peptidase. It was shown that additional, artificial amino acids at the N-terminal of the molecule are responsible for the lack of enzymatic activity of recombinant forms of the protease. Therefore, here the signal peptide fulfils a dual function, not only leading the protease through the cytoplasmic membrane but also preventing its proteolytic activity inside the cell [45].

S. pyogenes expresses an extracellular cysteine protease (SpeB) with a well-documented role in pathogenesis (for review see: [46]; Tab. 1). The enzyme is produced as a zymogen where the prosegment inserts into the protease active site cleft and keeps the proenzyme inactive [47]. The activation occurs under reducing conditions by auto-proteolytic removal of the propeptide, resulting in the release of the 28-kDa mature form of the protease [48]. In several cases of metalloproteases from the thermolysin family, extracellular autoprocessing to the mature form of enzyme was also suggested. For instance, the 33-kDa elastase (LasB) from *P. aeruginosa* is initially synthesized as a 54-kDa preproenzyme with a typical 3-kDa N-terminal signal peptide, which is cleaved during its passage through the inner membrane. Then, in the periplasma, the 18-kDa N-terminal propeptide is cut off by an autocatalytic mechanism and the mature elastase is released. The N-terminal propeptide forms a complex with mature elastase and protects the cell against intracellular proteolysis. Outside of the bacteria cell, the propeptide dissociates from the complex and is finally digested [49]. *Vibro vulnificus*, an emerging pathogen of humans which can

invade the bloodstream either through a wound or from the gastrointestinal tract, causing a severe and life-threatening illness called primary septicemia, produces a precursor of metalloprotease (VVP) that includes not only a signal peptide and an N-terminal propeptide, but also an additional C-terminal peptide. It was shown that the C-terminal propeptide is autocatalytically removed upon incubation at 37ºC. The additional C-terminal sequence is necessary for efficient attachment of the metalloprotease to protein substrates and the erythrocyte membrane [50].

Tab. 1. Extracellular bacterial proteases and their roles in pathogenesis.

Protease	Catalytic class	Organism	Postulated role in pathogenesis	References
Aureolysin	metal	*S. aureus*	Modulation of immunogenic reactions by affecting the stimulation of lymphocytes and inhibiting immunoglobulin production; deregulation of host proteolytic activity through inactivation of plasma serpins	[51-53]
Epidermolytic toxins	serine	*S. aureus*	Cleavage of desmoglein 1; causative agents of staphylococcal scalded skin syndrome	[54]
Glutamylendo-peptidase (V8 protease)	serine	*S. aureus*	Interference with the host defense mechanisms through inactivation of plasma serpins and degradation of immunoglobins	[55] and ref. within
Staphopains A and B	cysteine	*S. aureus*	Tissue invasion and destruction associated with staphylococcal ulceration; sepsis development through liberation of kinins from kininogen	[56, 57]
Staphopain A2	cysteine	*S. aureus* CH-91	Agent of atopic dermatitis in chickens	[9]
SplA - SplF	serine	*S. aureus*	Unknown, but a high titer of anti-Spl antibodies correlates with edocarditis	[58]
Elastase (LasB)	metal	*P. aeruginosa*	Laminin degradation leading to pseudomonal keratitis	[59] and ref. within
Alkaline protease (AprA)	metal	*P. aeruginosa*	Laminin degradation leading to pseudomonal keratitis	[59] and ref. within
Streptopain (SpeB)	cysteine	*S. pyogenes*	Host immune system and matrix protein degradation, liberation of interleukin-1 and kinins from their precursors leading to inflammation and severe streptococcal infections	[46] and ref. within, [66]
VVP	metal	*V. vulnificus*	Vascular permeability enhancement; hemorrhagic tissue damage; causative factor for edematous skin lesions	[38] and ref within
56-kDa protease	metal	*S. marcescens*	Degradation of defense-oriented humoral proteins and tissue constituents, leading to serratial pneumonia and keratitis	[59] and ref. within

Interactions with inhibitors

Proteolytic activation of zymogens is an irreversible process. This creates a need for additional mechanisms regulating post-translationally activated proteases. Modulation by specific inhibitors is the most common of these. However, in bacteria, endogenous proteins inhibiting the proteases are relatively rare. Higher organisms have developed various inhibitors of proteolytic enzymes, not only to control self-produced proteases. Protease inhibitors are also an important element of the host anti-pathogen defence system. Mammal defence protease inhibitors belong to two classes: the active-site inhibitors, represented by a superfamilies of serpins and cystatins; and the α2-macroglobulins. The members of the former group inactivate enzymes by binding to the active site, the latter act as molecular traps for the proteases. However, some bacteria have learned to utilize the inhibitors to regulate the activity of their extracellular proteases for their own purposes.

Research on the mechanism of staphopain B maturation revealed the presence of a 109-amino acid inhibitor of the enzyme, encoded in one operon together with the mentioned protease [5]. Further papers report that the staphopain A operon also contains a similar inhibitor. The mentioned inhibitors were named staphostatins because of their extraordinary specificity to staphopains, and not to other papain superfamily members [6]. *In vitro*, staphostatins form tight noncovalent equimolar complexes with the target proteases, totally abolishing their peptidase activity. It was shown that a properly formed active site of the enzyme is not necessary for the binding since the proteolytically inactive mutants of staphopains still bind the inhibitor [6, 11]. Structural studies performed both with x-ray crystallography and NMR methods revealed that staphostatins are β-barrels formed by a three-stranded mixed β-sheet and a five-stranded antiparallel β-sheet. Such a fold is closely related to lipocalins [7, 17, 60]. Further analysis shows that a loop composed of 5 amino acid residues (from 97 to 101), but particularly a 98-glycine residue adopting an unusual conformation which prevents G98-T99 peptide bond cleavage, is responsible for staphostatin inhibitory activity. This observation was confirmed by the construction of the G98 mutants. Since other amino acids cannot, for steric reasons, adopt the conformation taken by glycine, the molecules lost their inhibitory functions and were efficiently cleaved downstream of the mutated residue [61, 62]. It is worth noticing that staphostatins are localized intracellulary, whereas staphopains are secreted outside the cell. Therefore, the role of the former is in protection against autoproteolysis caused by misdirected staphopains which, instead of being secreted, remain inside the cell [6]. This suggestion was supported by both indirect and direct studies. The former evidence comes from the studies of the expression of staphopain A in *Escherichia coli*. The enzyme is toxic and may be produced only while coexpressed with its inhibitor staphostatin A [63]. Direct evidence was also presented by the construction of the staphostatin B knockout strain, which exhibits a wide range of striking pleiotropic alterations in its phenotype,

including a defect in growth [31]. It should also be noted that functional staphostatins were found in *S. epidermidis*, *S. warneri* and the pathogenic strain *S. aureus* CH-91 [11, 12]. This type of protection against secreted proteases prematurely activated in the cytoplasm is not limited to the *Staphylococcus* genus: recently, an intracellular inhibitor of cysteine protease from *Streptococcus pyogenes* was identified [16]. The inhibitor (Spi), unlike the staphostatins, is strikingly homologous in sequence to the SpeB propeptide, with 37% residue identity and 70% sequence similarity. Thus, although Spi was also proposed to protect cytoplasmic proteins from the activity of SpeB, the inhibitor is likely to act in a manner similar to SpeB propeptide [16]. Although a lot is known about staphostatins and Spi inhibition of their target proteases, it remains unknown whether the process *in vivo* is reversible, or whether the prematurely activated proteases trapped in the complex with the inhibitors undergo degradation.

The interaction of proteases with protease inhibitors does not always result in the inactivation of the former. Many peptidases, especially those produced by pathogenic microorganisms, are able to escape the inhibition, and moreover, efficiently degrade potential inhibitors. For example, staphylococcal extracellular proteases are able to cleave human blood serpins and kininogens, releasing kinins from the latter [52, 53, 57]. Streptococcal protease SpeB liberates bradykinin from human and mouse kininogens, instead of being inhibited. It is believed that it helps streptococci survive in sepsis and streptococcal toxic shock syndrome [64]. The only inhibitors capable of inhibiting proteases secreted by pathogens are α2-macroglobulins, which operate by the unique process of molecular entrapment. In this process, the protease, regardless its catalytic mechanism, is enclosed in the molecular cage of the α2-macroglobulin (α2M) polypeptide chain [65]. Protease entrapment disables the enzyme's ability to hydrolyse macromolecular substrates too large to fit into the α2M cage, but leaves intact the ability of the entrapped enzyme to hydrolyse low molecular mass substrates small enough to enter the α2M cage and interact with the active site of the protease [66]. Protease entrapment is initiated by the proteolytic cleavage of a peptide bond in a specialized bait region of the α2M polypeptide, which triggers the molecular compaction that entraps the protease molecule [67]. Trapping of the protease by α2M results in complex structural changes on the surface of the inhibitor where the specific receptor recognition site is exposed. The protein complex is then delivered to a receptor-mediated endolytic system and degraded in secondary lysosomes. Receptors for α2M have also been found on fibroblasts and macrophages. Nevertheless, extracellular protease (56 kDa) from *Serratia marcescens*, a pathogen associated with urinary and respiratory tract infections, makes use of a semistable complex with α2M, which is internalised *via* receptors on fibroblasts, where the gradually regenerated active protease causes damage to the cells. Such a mechanism may also contribute to the cytotoxicity of *Pseudomonas* [68]. The unique properties of the inhibitor were probably utilized by *S. pyogenes* to regulate the activity of

its extracellular protease SpeB, and in turn its defence against antimicrobal peptides. Most strains of the bacteria express GRAB, an α2-macroglobulin-binding cell wall-attached protein. SpeB is captured by the bound inhibitor and retained at the bacterial surface, where it efficiently degrades the antibacterial peptide LL-37 attacking the bacterial cell membrane [69].

Other factors controlling the activity of proteases
Under stress conditions, the stability of proteins is frequently upset. Misfolded or unfolded proteins appear. Therefore, among the factors involved in controlling the activity of proteases, a very important function is fulfilled by environmental factors like pH, ionic strength, temperature, co-factors, housekeeping proteins and small molecular elements.
Cysteine proteases utilize a catalytic cysteine residue in the form of a thiolate as a nucleophile in proteolytic reactions; therefore, at least *in vitro*, they require reducing agents for their activity [70]. Co-factors are also indispensable for the activity of metalloproteases. Zinc is required for aureolysin catalytic activity, but it can be substituted with cobalt, producing a protease that is more active than the native enzyme [42]. However, the structural stability of the protease is maintained by calcium ions. Conformational changes in the tertiary structure of the Ca^{2+}-depleted metalloprotease have been confirmed [71]. This not only leads to the enzyme's inactivation, but also makes it susceptible to degradation by other proteases [72].
The bacterial proteolytic system Clp has analogous structure and function to the 26S subunit of the eukaryotic proteasome. The Clp complex consists of a proteolytic core (ClpP) flanked by Clp ATPases (ClpA or ClpX) (for review see: [73]). When ClpP is not associated with Clp ATPase, it is able to degrade only small peptides. The whole complex is responsible for specific proteolysis of protein substrates, where subunits with ATPase activity play an important role in the unfolding and transport of proteins to the proteolytic chamber of ClpP [74]. Recent reports have confirmed the importance of Clps in thermal, osmotic and pH tolerance, as well as in the virulence of pathogenic bacteria, including *S. aureus* [75-77]. Furthermore, it was shown that acyldepsipeptides isolated from the culture medium of *Streptomyces hawaiiensis* cause non-specific activation of ClpP, which leads to uncontrolled degradation of bacterial proteins. Moreover, synthetic derivatives of acyldepsipeptides were successfully used as antibiotics against dangerous human pathogens, especially methicillin-resistant strains of *S. aureus* [78].
The widely conserved heat shock protein DegP is a part of a large family of ATP-independent serine proteases, the members of which are found in most organisms. They are typically localized in extracytoplasmic compartments such as the periplasma of Gram-negative bacteria. The DegP of *E. coli* has both chaperone and protease activities, which are switched in a temperature-dependent manner [79]. The protease activity is most apparent at higher

temperatures. In the chaperone conformation, the protease domain of DegP exists in an inactive form [80].

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