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Bacterial serine protease HtrA as a promising new target for antimicrobial therapy?

Silja Wessler[1*], Gisbert Schneider[2] and Steffen Backert[3]

Abstract

Recent studies have demonstrated that the bacterial chaperone and serine protease high temperature requirement A (HtrA) is closely associated with the establishment and progression of several infectious diseases. HtrA activity enhances bacterial survival under stress conditions, but also has direct effects on functions of the cell adhesion protein E-cadherin and extracellular matrix proteins, including fibronectin and proteoglycans. Although HtrA cannot be considered as a pathogenic factor per se, it exhibits favorable characteristics making HtrA a potentially attractive drug target to combat various bacterial infections.

Background

HtrA proteins and their orthologues represent an important class of heat-shock-induced serine proteases and chaperones protecting protein structures. They are expressed in both prokaryotic and eukaryotic species, including plants and humans [1–3]. Whereas HtrA orthologues commonly display proteolytic activities against multiple target proteins, their structural architecture and physiological functions are rather miscellaneous and differ between species. In many bacteria, HtrA proteases are composed of an N-terminal signal peptide, followed by a trypsin-like serine protease domain and one or two C-terminal PDZ (postsynaptic density protein [PSD95], *Drosophila* disc large tumor suppressor [Dlg1], and zonula occludens-1 protein [ZO-1]) modules which permit intermolecular protein-protein interactions [4, 5] (Fig. 1). In Gram-negative bacteria, HtrA proteases are generally transported into the periplasm, where they form proteolytic active multimers with known functions in protein quality control. The best characterized HtrA proteins are the *Escherichia coli* DegP, DegQ, and DegS orthologues [6, 7]. All these different HtrAs display a high degree of sequence identity in their protease domain, but exhibit numerous specific features and activities [6]. DegP and DegQ harbor two PDZ domains, while DegS often contains a transmembrane domain and only one PDZ domain [1, 8] (Fig. 1). DegP is well characterized as a protease with ATP-independent chaperone functions. Its active oligomers assemble upon target binding and hydrolyze unfolded or misfolded proteins into small peptides [9, 10]. DegS represents a regulatory protease which cleaves the anti-sigma factor RseA, while the physiological functions of DegQ are not fully understood [11]. Inactivation of the *htr*A gene by mutation causes an increased sensitivity to stress, e.g., elevated temperature, of all bacteria investigated to date [12–18].

(Patho)-physiological function of bacterial HtrA

Until recently, it has been commonly accepted that HtrA family members of bacteria are strictly acting inside the periplasm. However, we have recently unraveled a hitherto unknown function of HtrA during bacterial infection. *Campylobacter jejuni* and its close relative *Helicobacter pylori* actively secrete HtrA proteins in the extracellular environment, where they target host cell factors [19–21]. HtrA was also identified in outer membrane vesicles released by *C. jejuni*, *H. pylori*, *Vibrio cholera*, *Chlamydia muridarum* or *Borrelia burgdorferi* [22–26]. Infection experiments with polarized cell monolayers in vitro suggested that *H. pylori* and *C. jejuni* HtrA can disrupt the epithelial barrier by opening cell-to-cell junctions. This remarkable effect is achieved by cleaving-off the extracellular domain of the surface adhesion protein and

* Correspondence: silja.wessler@sbg.ac.at
[1]Department of Molecular Biology, Division of Microbiology, Paris-Lodron University of Salzburg, Billroth Str. 11, A-5020 Salzburg, Austria
Full list of author information is available at the end of the article

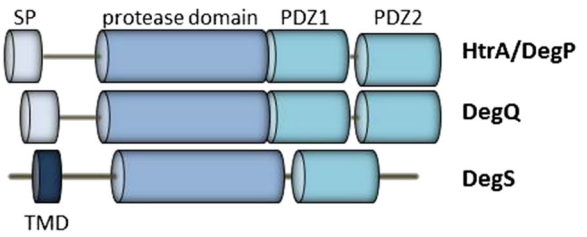

Fig. 1 Domain structure of HtrA proteins in Gram-negative bacteria. Monomeric DegP and DegQ proteins harbor an N-terminal signal peptide (SP), an ATP-independent trypsin-like protease domain followed by two PDZ domains. Many DegS proteins are composed of a transmembrane domain (TMD), protease domain and one PDZ domain

tumor suppressor E-cadherin, and probably other junctional proteins by HtrA, followed by paracellular bacterial transmigration [20, 21]. The deletion of the *htrA* gene in *C. jejuni* led to a defect in E-cadherin shedding and causes impaired transmigration of the bacteria across monolayers of polarized epithelial cells in vitro [19, 21].

In particular, E-cadherin showed to be an important factor for establishing and maintaining epithelial integrity in the host. E-cadherin is a single transmembrane protein, which consists of an intracellular domain (IC), a transmembrane domain (TD), and five extracellular domains (EC) [27]. EC domains establish homophilic interactions in *cis* and *trans* that require calcium binding to the linker region between the EC domains. We have recently identified the cleavage sites of *H. pylori* HtrA in E-cadherin. Mass-spectrometry-based proteomics and Edman degradation revealed three signature motifs containing the [VITA]-[VITA]-x-x-D-[DN] sequence pattern as preferentially cleaved by HtrA [28]. The results of our studies also suggest that the presence of calcium ions blocks HtrA-mediated cleavage by interfering with the accessibility of calcium-binding regions between the individual EC domains harboring the HtrA cleavage sites [29]. Investigating *C. jejuni* Δ*htrA* deletion mutants in in vivo studies, it was demonstrated that HtrA plays a crucial role during infection by triggering host cell apoptosis and immunopathology in mice [30, 31]. Similarly, HtrA is critical for the virulence of many other pathogens including *Brucella abortus* [32], *Yersinia enterocolitica* [33], *Salmonella enterica* [34], *Legionella pneumophila* [13], *Shigella flexneri* [35], *Klebsiella pneumoniae* [14], *Listeria monocytogenes* [36], *Burkholderia cenocepacia* [17], *Chlamydia trachomatis* [37], *Borrelia burgdorferi* [23], *Mycobacterium tuberculosis* [38] and *Haemophilus parasuis* [39]. In contrast, the deletion of the *htrA* gene in *H. pylori* has not yet been reported, and the generation of Δ*htrA* knockout mutants was found to be lethal [40, 41]. Given the fact that *H. pylori htrA* is an essential bifunctional gene with crucial intracellular and extracellular functions, it may be justified to consider HtrA as a new target for future anti-bacterial therapy.

Why is HtrA inhibition a step forward in the fight against pathogens?

With the exception of *Mycoplasma genitalium* and *Methanococcus janaschii*, it seems that all bacterial pathogens and commensals in the microbiota express HtrA proteins; a fact that evades the classical and precise definition of virulence or pathogenic factors [42]. Consequently, this observation leads to the question if such a factor might also serve as a potent macromolecular drug target? In fact, targeting HtrA offers some potential advantages:

(i.) it is secreted into the extracellular micro-milieu or presented on the bacterial cell surface and therefore accessible to drug compounds [43, 44],
(ii.) it has a defined enzymatic active site and substrate recognition [19, 20, 45, 46],
(iii.) it cleaves E-cadherin, proteoglycans and fibronectin as host factors with important functions for bacterial pathogenesis [19–21, 47], and
(iv.) it is an essential enzyme in *H. pylori* physiology [40, 41].

These characteristics make HtrA a potentially attractive candidate for novel therapeutic approaches to treat bacterial pathogenesis.

The current model of HtrA function in bacterial pathogenesis is based on the hypothesis that HtrA-mediated E-cadherin cleavage represents a central step in bacterial pathogenesis prior to and/or after the interference of virulence factors (e.g., effector proteins, cytotoxins, adhesins) with the integrity of the polarized epithelium [48, 49]. These complex pathogen-host interactions require sophisticated and coordinated mechanisms to provide access to laterally expressed E-cadherin and subsequently to basolaterally presented host cell receptors or circulating cells of the immune system in deeper regions of the tissues. In principle, the opening of tight junctions has been shown to be HtrA-independent in *H. pylori* [20] and *C. jejuni* [21], indicating that additional bacterial factors are involved in the disruption of the epithelial polarity. In *H. pylori* infections, soluble factors such as vacuolating cytotoxin A (VacA), cytotoxin-associated gene A (CagA) and urease were previously described to open up tight junctions [50–52], underlining that the interplay of various pathogenic factors and HtrA is responsible for disrupting the lateral junctions between epithelial cells. The mechanism by which *C. jejuni* opens tight junctions is yet unknown. For both pathogens, an HtrA-mediated transmigration process was observed [20, 21, 28], enabling bacterial contact with

basolaterally expressed receptors, such as α5β1 integrins or fibronectin [53, 54], but also allowing the bacteria to directly interact with cells of the immune system. It is currently being investigated whether *C. jejuni* prefers the transcellular migration or paracellular route, or whether this pathogen combines two pathways to overcome the epithelial barrier [48]. However, HtrA-mediated E-cadherin cleavage in concert with activated host proteases has been shown to promote pathogenesis in vitro for *H. pylori* [20, 55, 56] and in *C. jejuni* animal models [30, 31], which has been summarized in several review articles [49, 57]. Beta1-integrins and fibronectin have already been identified as important binding partners for a number of additional pathogens including *Yersinia pseudotuberculosis, Staphylococcus aureus*, *Klebsiella pneumoniae*, and others [58], indicating the importance of opening intercellular adhesion complexes. The observation that additional gastrointestinal pathogens (*Shigella flexneri*, enteropathogenic *Escherichia coli* [EPEC], *Yersinia enterocolitica*, *Salmonella enterica* sub. Enterica) utilize the HtrA homologs DegP and DegQ for E-cadherin cleavage during infection of cultured epithelial cells and in vitro underlines a function of HtrA proteins as "virulence- or pathogenicity-promoting" factors [19, 59]. Based on this hypothesis, it is enticing to surmise that pharmacological inhibitors blocking extracellular HtrA activity could stop bacterial transmigration and tissue invasion in vivo, while leaving the microbiota unaffected. Consequently, selective pharmacological inhibition of HtrA might facilitate antibiotic treatment by preventing bacterial access to deeper regions of gastrointestinal tissues. Possibly, bacterial HtrAs could also target additional substrates. For *Chlamydia trachomatis*, it was demonstrated that HtrA is secreted into the chlamydia-containing vesicles and into the host cytoplasm. Although substrates for HtrA were not identified, inhibition of HtrA efficiently affected the bacterial life cycle and survival [60, 61]. With the availability of high-resolution structural models of the various HtrAs from relevant pathogens, structure-based inhibitor design should become feasible (Fig. 2).

In contrast to other investigated bacterial species, *H. pylori* HtrA synthesis appears to be crucially important for bacterial physiology and survival since any intervention via mutagenesis or deletion of the *htrA* gene in the genome of *H. pylori* has not been successful up to date [20, 40, 41]. Correspondingly, a naturally occurring *htrA*-negative *H. pylori* isolate was not found in a comprehensive screening of more than 990 samples [41]. These observations point to the question whether pharmacological inhibition of HtrA could tackle *H. pylori* physiology specifically? *Helicobacter* HtrA inhibitor (HHI) was the first described small molecule compound inhibiting *H. pylori* HtrA [20], which blocked HtrA-mediated E-cadherin cleavage and subsequent bacterial transmigration across a polarized epithelial monolayer. However, HHI did not affect the bacterial survival [20] and it is unknown, whether HHI is actually taken up by the bacteria. A first step in the direction of a future targeted *H. pylori* therapy has recently been made by demonstrating that compound 1 drastically affected *H. pylori* survival and/or growth [41, 62]. The data obtained suggest that compound 1 penetrates the bacterial cell wall to block periplasmic HtrA activity and subsequently *H. pylori* survival. Further research will be necessary to identify and optimize small molecule HtrA inhibitors as anti-*H. pylori* pharmacological lead compounds.

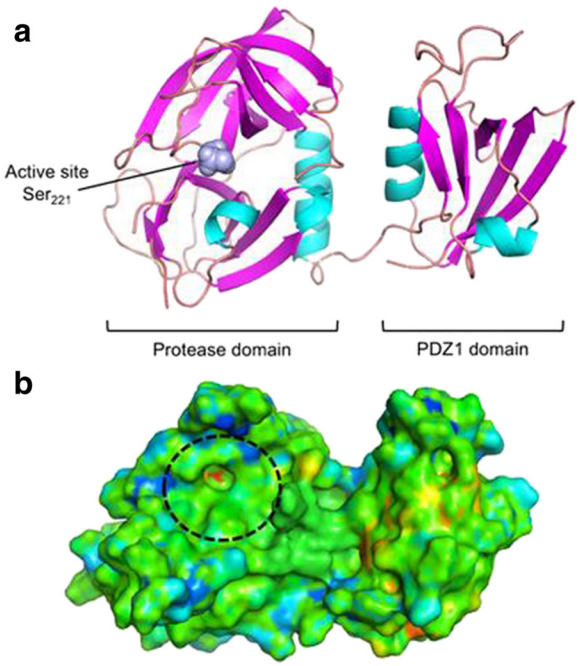

Fig. 2 Structural model of the *H.pylori* HtrA monomer. The model is based on a preliminary X-ray crystal structure of the *apo*-enzyme containing one of the two PDZ domains [63]. The cartoon structure (**a**) shows the protease domain with the catalytic residue Ser_{221} highlighted. The interface between the protease domain and PDZ1 is mediated by helix-helix interactions. The surface representation (**b**) has the same orientation as in (**a**). Temperature coloring is according to the computed "ligandability" [45, 64]. A potential ligand interaction "hot spot" is predicted inside the active site (approximated by the *dashed circle*). This model and related computational analyses support the design of *H. pylori* HtrA inhibitors. The graphics were prepared with MacPyMol (v1.7, Schrödinger LLC, New York, NY, USA)

Conclusions

New strategies are urgently needed to combat bacterial infections. At the first glance, targeting a widespread bacterial enzyme does not appear to be straightforward. However, considering the HtrA-mediated host cell factor processing as a central step in the pathogenesis of many different infectious bacteria opens up a new perspective.

Inhibiting extracellular HtrA by compounds that do not penetrate the bacterial membrane will likely not affect the colonization and survival of commensals; thus solely interference of pathogens with their individual virulence/pathogenic factors with the epithelium will be limited. Potent HtrA inhibitors penetrating the periplasm of *H. pylori* might pave the way towards a targeted anti-*H. pylori* treatment owed to the fact that *H. pylori* physiology essentially requires functional HtrA activity. While many of the current antibiotics affect all bacteria independently of assets and drawbacks for the colonized host, pathogen-selective HtrA inhibitors might present a drug discovery opportunity.

Abbreviations
DegP/Q: Periplasmic serine endoproteases; HtrA: High temperature requirement A; PDZ: Postsynaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (ZO-1).

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Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions
Wrote the paper: SW, GS, SB. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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Author details
[1]Department of Molecular Biology, Division of Microbiology, Paris-Lodron University of Salzburg, Billroth Str. 11, A-5020 Salzburg, Austria. [2]Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), Vladimir-Prelog-Weg 4, CH-8093 Zürich, Switzerland. [3]Division of Microbiology, University of Erlangen-Nuremberg, Staudtstr. 5, D-91058 Erlangen, Germany.

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