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Review

Vibrio cholerae hemagglutinin(HA)/protease: An extracellular metalloprotease with multiple pathogenic activities

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ABSTRACT

Vibrio cholerae of serogroup O1 and O139, the etiological agent of the diarrheal disease cholera, expresses the extracellular Zn-dependent metalloprotease hemagglutinin (HA)/protease also reported as vibriolysin. This enzyme is also produced by non-O1/O139 (non-cholera) strains that cause mild, sporadic illness (i.e. gastroenteritis, wound or ear infections). Orthologs of HA/protease are present in other members of the *Vibrionaceae* family pathogenic to humans and fish. HA/protease belongs to the M4 neutral peptidase family and displays significant amino acid sequence homology to *Pseudomonas aeruginosa* elastase (LasB) and *Bacillus thermoproteolyticus* thermolysin. It exhibits a broad range of potentially pathogenic activities in cell culture and animal models. These activities range from the covalent modification of other toxins, the degradation of the protective mucus barrier and disruption of intestinal tight junctions. Here we review (i) the structure and regulation of HA/protease expression, (ii) its interaction with other toxins and the intestinal mucosa and (iii) discuss the possible role(s) of HA/protease in the pathogenesis of cholera.

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1. Introduction

Cholera is an acute, water-borne diarrheal disease caused by the facultative, Gram-negative bacterium *Vibrio cholerae* of serogroup O1 of the classical and El Tor biotype and serogroup O139, which originated from the El Tor biotype and exhibits a distinct lipopolysaccharide (Albert, 1994). The O1 *V. cholerae* serogroup contains a common A antigen and can be subdivided in Ogawa and Inaba serotypes on the basis of serotype-specific antigens B and C, respectively (Kaper et al., 1995). Mankind has experienced seven recorded cholera pandemics. The seventh and current pandemic is characterized by the predominance of O1 strains of the El Tor biotype with sporadic emergence of serogroup O139. Approximately 5 million cases of cholera and 130,000 deaths occur annually (http://www.cdc.gov/cholera/general). Endemic cholera continues to be a major public health problem in vast regions of South Asia and Africa. Introduction of virulent *V. cholerae* O1 in non-endemic areas with low sanitation can result in rapidly spreading outbreaks as occurred in 2010 in Haiti (Ali et al., 2011). The typical symptoms of this illness include a profuse rice-watery diarrhea and vomiting. If untreated, this condition can lead to severe dehydration, electrolyte imbalance, and death.

V. cholerae O1 and O139 strains expresses two major virulence factors: (i) cholera toxin (CT) and (ii) the toxin co-regulated pilus (TCP). The TCP is a type IV pilus that mediates adherence and microcolony formation and is required for intestinal colonization in neonate mice and humans (Herrington et al., 1988; Tacket et al., 1998; Thelin and Taylor, 1996). CT is an ADP-ribosyltransferase responsible for the profuse rice-watery diarrhea typical of this disease (Kaper et al., 1995; Finkelstein and Dorner, 1985; Finkelstein and LoSpalluto, 1972). It is composed of one A subunit, which catalyzes NAD-dependent ADP-ribosylation of host adenylate cyclase and five B subunits that carry the ganglioside GM_1 receptor binding site (Griffiths et al., 1986). CT is well recognized as the major secretogenic factor causing the clinical symptoms of cholera. However, *V. cholerae* produces additional toxic factors, such the zonula occludens toxin (Zot) (Fasano et al., 1991, 1995), the accessory cholera enterotoxin (Ace) (Trucksis et al., 1993), the repeat toxin (RTX) (Lin et al., 1999), hemolysin (HlyA) (Nagamune et al., 1995) and the metalloprotease hemagglutinin (HA)/protease (Hase and Finkelstein, 1991). The contributions of these secondary factors to the pathogenesis of cholera has been difficult to dissect due to strain diversity and the lack of a single animal model fully mimicking the disease as it occurs in humans. It is likely, however, that expression of the above auxiliary toxins could modulate the course of an infection, which is known to vary in clinical symptoms from asymptomatic or mild to severe and life-threatening.

Extracellular metalloproteases are widely distributed among bacteria and pathogenic vibrios (reviewed in (Hase and Finkelstein, 1993; Shinoda and Miyoshi, 2011; Miyoshi, 2013)). In this article we concisely summarize our knowledge of the structure, regulation and pathogenic activities of HA/protease, a Zn-dependent metalloprotease with mucinase activity (Hase and Finkelstein, 1991; Booth et al., 1983; Finkelstein et al., 1983). We show that HA/protease exhibits a broad range of potentially pathogenic activities in cell culture and animal models. These activities include the covalent modification of other toxins, the degradation of the protective mucus barrier and disruption of intestinal tight junctions. A critical assessment of published *in vitro* and *in vivo* studies suggests that HA/protease can enhance the pathogenesis of cholera by (i) increasing the activity of other toxic factors (ii) providing access of vibrios or their toxic factors to the microvilli underlying the protective mucus barrier and (iii) facilitating the dissemination of infecting vibrios along the gastrointestinal tract.

2. Structure and substrate specificity of HA/protease

An extracellular protein with hemagglutinating and proteolytic activities was initially purified from *V. cholerae* strain CA401 and denoted cholera lectin (Finkelstein and Hanne, 1982). The protease was subsequently demonstrated to be a metalloprotease (Booth et al., 1983) acting on several physiologically relevant substrates such as fibronectin and mucin and was also shown to cleave lactoferrin and nick the A subunit of the *Escherichia coli* heat labile toxin (LT) (Finkelstein et al., 1983). Cloning and sequencing of the *hapA* gene encoding HA/protease showed that the protein is highly homologous to *Pseudomonas aeruginosa* elastase (LasB) (Hase and Finkelstein, 1990, 1991), a metalloprotease known to degrade components of the extracellular matrix during acute and chronic *P. aeruginosa* infection, breach epithelial cell tight junctions and cleave pulmonary surfactants (Kuang et al., 2011). HA/protease also shows significant amino acid sequence homology to *Vibrio vulnificus* elastase (VvpE), which contributes to local tissue damage during vibriosis caused by this human pathogen (Jones and Oliver, 2009). The domain structure of HA/protease is shown in Fig. 1. The amino acid sequence of HA/protease begins with a signal peptide followed by a propeptide. The propeptide includes a fungalysin/thermolysin propeptide (FTP) domain and a PepSY domain. These domains are suggested to have chaperone and/or a protease inhibitor functions that prevent the activation of the enzyme prior to its secretion into the extracellular milieu. N-terminal sequencing of HA/protease indicated that the propeptide is cleaved to generate a mature protease starting at A196. The amino acid sequence of the mature protein places HA/protease within the M4 thermolysin family of Zn-dependent secreted eubacterial endopeptidases containing a HEXXH motif, which has been shown in crystallographic studies to form part of the metal-binding site. Finally, the amino acid sequence of HA/protease ends with a prepeptidase C-terminal domain. This domain is found at the C-terminus of secreted bacterial peptidases and is commonly not present in the active enzyme. The difference between the predicted molecular weight of the mature HA/protease (47 kDa) and the actual molecular weight of purified HA/protease (32 kDa) suggests that the prepeptidase C-terminal domain is indeed removed during HA/protease maturation (Hase and Finkelstein, 1991). The crystal structure of HA/protease has been deduced by homology modeling with *P. aeruginosa* LasB (Lutfullah et al., 2008).

3. Regulation of HA/protease expression

3.1. Transcription regulation

In V. cholerae, the transcription of hapA is activated under culture conditions that integrate nutrient limitation, entry into stationary phase and high cell population density (Fig. 2). Transcription of hapA requires the quorum sensing regulator HapR (Jobling and Holmes, 1997; Silva and Benitez, 2004) and the RNA polymerase alternative sigma factor RpoS (σ^S) (Silva and Benitez, 2004; Yildiz and Schoolnik, 1998). The cAMP receptor protein (CRP) acts upstream of HapR and RpoS to integrate the nutrient limitation and cell density stimuli (Silva and Benitez, 2004; Benitez et al., 2001; Liang et al., 2007). The regulatory circuit responsible for HA/protease expression is shown in Fig. 3. Briefly, conditions of nutrient limitation result in elevation of the intracelllular cAMP pool and activation of RpoS and CRP (Silva and Benitez, 2004). Activation of CRP furtther enhances the transcription of rpoS (Silva and Benitez, 2004). In parallel, CRP activates HapR expression to integrate nutritional and population cell density signals (Benitez et al., 2001; Liang et al., 2007, 2008). HapR activates the transcription of hapA directly and indirectly by increasing the