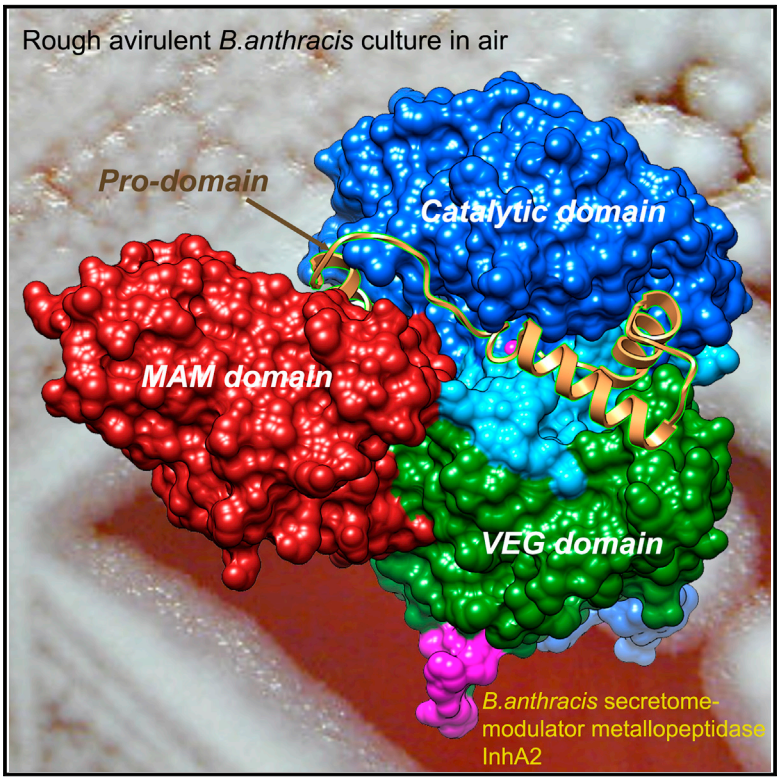
Article

Structure

Structural Basis for Latency and Function of Immune Inhibitor A Metallopeptidase, a Modulator of the *Bacillus anthracis* Secretome

Graphical Abstract



Highlights

- First structure of a *Bacillus anthracis* secretome-modulator InhA metallopeptidase
- Latency exerted via a unique N-terminal pro-domain blocking the active-site cleft
- Multi-domain protein with a VEG domain and a MAM domain found only in eukaryotes
- MAM domain grafted into the VEG domain is involved in folding and stability E-TOC

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In Brief

Immune inhibitor A(InhA)-type metallopeptidases (MPs) are secreted by *Bacillus cereus* group bacteria. *Bacillus anthracis* has *Ba*InhA1 and *Ba*InhA2, which degrade host tissue proteins. *Ba*InhA2 is a ~750-residue four-domain structure featuring a pro-peptide, a catalytic domain, a domain reminiscent of viral envelope glycoproteins, and a MAM domain, which is required for proper protein expression. Latency is exerted by the N-terminal segment of the pro-peptide, which binds the catalytic zinc.

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Structural Basis for Latency and Function of Immune Inhibitor A Metallopeptidase, a Modulator of the *Bacillus anthracis* Secretome

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SUMMARY

Immune inhibitor A(InhA)-type metallopeptidases are potential virulence factors secreted by members of the *Bacillus cereus* group. Two paralogs from anthrax-causing *Bacillus anthracis* (*Ba*InhA1 and *Ba*InhA2) were shown to degrade host tissue proteins with broad substrate specificity. Analysis of their activation mechanism and the crystal structure of a zymogenic *Ba*InhA2 variant revealed a ~750-residue four-domain structure featuring a pro-peptide, a catalytic domain, a domain reminiscent of viral envelope glycoproteins, and a MAM domain grafted into the latter. This domain, previously found only in eukaryotes, is required for proper protein expression in *B. anthracis* and evinces certain flexibility. Latency is uniquely modulated by the N-terminal segment of the pro-peptide, which binds the catalytic zinc through its α-amino group and occupies the primed side of the active-site cleft. The present results further our understanding of the modus operandi of an anthrax secretome regulator.

INTRODUCTION

Bacillus anthracis is a facultative anaerobic Gram-positive spore-forming bacterium that belongs to the *Bacillus cereus* group (BCG) of Bacillales (Rasko et al., 2005). In 1876, Robert Koch identified it as the etiologic agent of anthrax (Koch, 1876), an ancient disease believed to have originated in Egypt and Mesopotamia around 1250 BC, which might have been among the ten plagues of Egypt (Sternbach, 2003). Anthrax is an acute, rapidly progressing infectious disease that affects humans and other animals (Baillie and Read, 2001), and infection occurs through the spores, which produce three clinical pictures depending on the entry pathway: cutaneous, inhalational, or gastrointestinal anthrax. The former leads to skin ulcers, which are generally easy to treat. In contrast, inhalational and gastrointestinal anthrax are invasive and systemic, and often fatal. Accordingly, novel therapeutic approaches targeting *B. anthracis* are of importance and currently under investigation (Artenstein and Opal, 2012).

In addition to the major virulence factors of *B. anthracis* identified, namely the plasmid-encoded anthrax toxin and the poly-D-glutamic acid capsule, other proteins contribute to virulence and disease, and may provide targets for therapy against anthrax (Artenstein and Opal, 2012). Among candidate virulence factors are immune inhibitor A (InhA) peptidases, which belong to the thuringilysin family within the metzincin clan of metallopeptidases (MPs) (Cerdà-Costa and Gomis-Rüth, 2014). They have also been ascribed to family M6 in the MEROPS proteolytic enzyme database (http://merops.sanger.ac.uk). Metzincins are characterized by a globular catalytic domain (CD) spanning ~130–270 residues, which consists of a structurally conserved N-terminal upper subdomain (NTS) and a lower, structurally more disparate C-terminal subdomain (CTS). The two subdomains are separated by the active-site cleft. NTSs span a (mostly) five-stranded β sheet, a backing helix, and an active-site helix, which contains an extended metal-binding motif, **H***E*XX**H**XX(G,N)XX(**H,D**). This motif encompasses three metal-binding residues (in bold) and the catalytic general base/acid glutamate (in italics) required for reaction. Some metzincins also include an additional adamalysin helix, first described in adamalysins/ADAMs (Cerdà-Costa and Gomis-Rüth, 2014). Downstream CTSs mainly share a conserved loop with a central methionine, the Met-turn, which provides a hydrophobic basis for the metal-binding site (Tallant et al., 2010), and a C-terminal helix. Among the 12 metzincin families structurally characterized are the toxilysins (Cerdà-Costa and Gomis-Rüth, 2014; Gomis-Rüth, 2013; Ng et al., 2013), which are part of the bacterial AB_5 toxins (Beddoe et al., 2010). Like the protective antigen and lethal factor tandem in *B. anthracis* anthrax toxin, AB_5 toxins from *Escherichia coli* (EcxAB) and *Citrobacter freundii* (CfxAB) consist of a subunit for host-cell invasion (pentameric subunit B) and an active MP that subverts intracellular functions (subunit A) (Beddoe et al., 2010).

The thuringilysins are one of the oldest known MP families, as the founding member, InhA1 from *Bacillus thuringiensis*, was identified in the mid-1970s as an immune inhibitor that interferes