doi:10.1016/j.jmb.2006.03.028 J. Mol. Biol. (2006) 359, 322–330

JMB

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Solution Structure of Monomeric BsaL, the Type III Secretion Needle Protein of *Burkholderia pseudomallei*

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Many Gram-negative bacteria that are important human pathogens possess type III secretion systems as part of their required virulence factor repertoire. During the establishment of infection, these pathogens coordinately assemble greater than 20 different proteins into a macromolecular structure that spans the bacterial inner and outer membranes and, in many respects, resembles and functions like a syringe. This type III secretion apparatus (TTSA) is used to inject proteins into a host cell's membrane and cytoplasm to subvert normal cellular processes. The external portion of the TTSA is a needle that is composed of a single type of protein that is polymerized in a helical fashion to form an elongated tube with a central channel of 2–3 nm in diameter. TTSA needle proteins from a variety of bacterial pathogens share sequence conservation; however, no atomic structure for any TTSA needle protein is yet available. Here, we report the structure of a TTSA needle protein called BsaL from *Burkholderia pseudomallei* determined by nuclear magnetic resonance (NMR) spectroscopy. The central part of the protein assumes a helix-turn-helix core domain with two well-defined α-helices that are joined by an ordered, four-residue linker. This forms a two-helix bundle that is stabilized by interhelix hydrophobic contacts. Residues that flank this presumably exposed core region are not completely disordered, but adopt a partial helical conformation. The atomic structure of BsaL and its sequence homology with other TTSA needle proteins suggest potentially unique structural dynamics that could be linked with a universal mechanism for control of type III secretion in diverse Gram-negative bacterial pathogens.

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Keywords: type III secretion; BsaL; needle protein; NMR; pathogenesis

Introduction

Many Gram-negative bacterial pathogens of plants and animals, including a number of potential bioterrorism agents, use type III secretion systems (TTSSs) as key virulence factors for the subversion of target eukaryotic cell functions.[1] Examples of these pathogens are *Shigella flexneri* (dysentery), *Salmonella* spp. (gastroenteritis and typhoid fever), *Burkholderia pseudomallei* (mellioidosis), and *Yersinia* spp. (plague and gastroenteritis). *Shigella* and *Salmonella* are major causes of diarrheal illness in the developing world and industrialized nations, but each has also been implicated in intentional exposure and infection within the United States.[2,3] *Burkholderia* and *Yersinia* are considered even more serious bioterrorism threats and are included as CDC and USDA select agents. All of these organisms use TTSSs as essential components of their virulence arsenals and a better understanding of all aspects of type III secretion would be useful for improving both public health and national security.

B. pseudomallei is the causative agent of mellioidosis, an illness that can manifest itself in humans as an acute, sub-acute or chronic infection.[4] Infection by this saprophytic bacterium is endemic to southeastern Asia and northern Australia, and has the potential for global spread.[4] Acute mellioidosis is a potentially fatal septicemic infection,

Abbreviations used: HMQC, heteronuclear multiple quantum coherence; HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; TTSA, type III secretion apparatus; TTSS, type III secretion system.

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while the sub-acute form of the disease can lead to the formation of systemic abscesses in multiple organ systems.[5] Asymptomatic infections can progress to clinical mellioidosis as is often observed in endemic regions.[6] Acute and sub-acute mellioidosis can also give rise to *B. pseudomallei* latency and the potential for delayed, and often fatal, relapse[5] despite appropriate antibiotic therapy during the initial infection.[7] *B. pseudomallei* and its close relative *Burkholderia mallei* (causative agent of glanders) are considered important bioterrorism agents due to their ready world-wide availability and their ability to be transmitted by aerosol.[8]

B. pseudomallei possesses at least one genetic region that encodes a TTSS resembling that of the plant pathogens *Ralstonia solanacearum* and *Xanthomonas* spp.[8] A separate TTSS (the *bsa* locus) encoded within a recently identified *B. pseudomallei* pathogenicity island[8] is similar to those encoded by the *Shigella* virulence plasmid (the Mxi/Spa TTSS) and *Salmonella* pathogenicity island SPI-1 (Inv/Spa TTSS).[8] These genetic elements encode proteins such as invasins (Inv), membrane excretion of invasins (Mxi), and surface presentation of antigens (Spa), required by *Shigella* and *Salmonella* to become virulent. The importance of the Bsa TTSS for *B. pseudomallei* virulence and pathogenesis is not yet entirely clear; however, it has been predicted that cellular invasion and survival in macrophages is an important aspect of *B. pseudomallei* pathogenesis.[9] Furthermore, Stevens *et al*. showed that the Bsa TTSS is critical for *B. pseudomallei*'s ability to invade and replicate in J774.2 murine macrophages and for vacuolar escape following host cell invasion.[8]

The TTSA of Gram-negative bacterial pathogens is a macromolecular protein assembly that allows physical contact between the pathogen and its target cell.[1] The TTSA functions somewhat like a syringe, wherein bacterial proteins are delivered directly into the host cell membrane to form a translocon pore through which other proteins are then translocated into the host cytoplasm.[10] A major component of the TTSA is an external needle that extends from a basal structure and forms a conduit for translocating proteins into the target cell. For *B. pseudomallei*, the TTSA needle is presumably formed by the assembly of multiple copies of a single protein, BsaL, which is a homologue of MxiH in *Shigella*, PrgI in *Salmonella*, YscF in *Yersinia*, and other putative type III needle proteins (see Figure 1).

The *Shigella* MxiH needle, for which more extensive data are available, consists of a tube-like structure with a 70 Å outer and a 25 Å inner diameter channel that extends 500 Å from the base of the TTSA.[11] A similar structure exists at the surface of *Salmonella*,[12] *Yersinia*[13] and *Pseudomonas aeruginosa*.[14] In *Shigella*, the needle monomer (MxiH) is arranged in a helical pattern with ~24 Å pitch and 5.6 units per turn.[15] Recent mutational analysis has implicated MxiH in controlling the induction of type III secretion in *Shigella*[16] with related findings reported for YscF of *Yersinia pestis*.[17] These reports implicate the TTSA needle and any associated extracellular proteins in sensing host cell contact and the transmission of type III secretion signals.

Sequence alignment of the needle proteins from various bacterial species shows higher sequence conservation in the middle part of the proteins compared to their amino-terminal regions (Figure 1). Secondary structure predictions indicate a predominantly α-helical secondary content with some possible β-strand structure.[16,18] Circular dichroism (CD) spectroscopy confirms the α-helical content of the *Shigella* needle protein MxiH[18] as well as PrgI[18] and BsaL (R. Kenjale *et al.*, unpublished results). The three-dimensional atomic structure of any needle protein, however, is currently unknown. Because of the potential for needle monomer self-association[19] or aggregation (R. Kenjale *et al.*, unpublished results), handling recombinant needle proteins *in vitro* must be considered in such studies. Previous studies have shown that short C-terminal deletions render at least some TTSA needle proteins unable to polymerize on the bacterial surface.[16] Such deletions yielded soluble and monomeric forms of MxiH[18] and PrgI,[18] as well as BsaL (R. Kenjale *et al.*, unpublished results). These needle protein monomers retain all of their native secondary structure and tend to display readily reversible thermal unfolding[18] (R. Kenjale *et al.*, unpublished results).

Here, we report the high-resolution solution structure of the needle protein of *B. pseudomallei*,

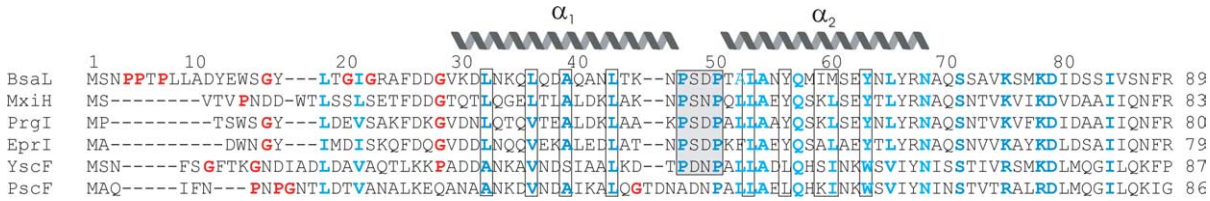

Figure 1. The TTSS needle proteins of different bacteria show sequence conservation, especially within the core structure where helix α_1 and α_2 of BsaL are located. The conserved P-(S/D)-(D/N)-P motif that forms the inter-helical turn in BsaL is boxed. The residues at the interface of the BsaL helix α_1 and α_2, which are involved in stabilizing the structure of the core domain, are also conserved among bacterial species (also boxed). Conserved and identical residues are in blue, and the glycine and proline residues preceding helix α_1, are in red. Sequences are from Swiss-Prot, and the bacterial species and its associated needle protein are: *B. pseudomallei* (BsaL), *S. flexneri* (MxiH), *S. typhimurium* (PrgI), *E. coli O157:H7* (EprI), *Y. pestis* (YscF), and *P. aeruginosa* (PscF).