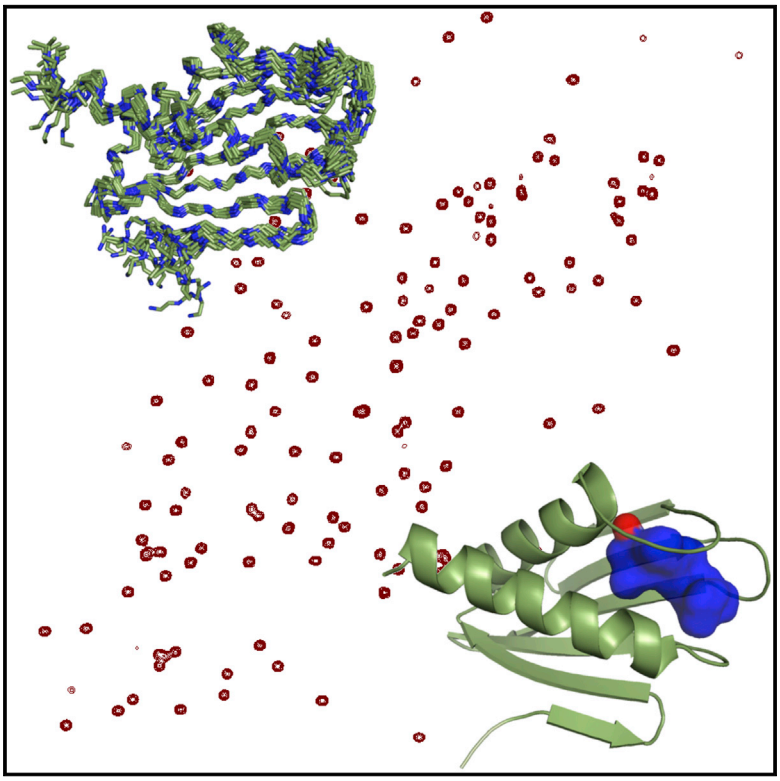
Short Article

Structure

NMR Structure of *Francisella tularensis* Virulence Determinant Reveals Structural Homology to Bet v1 Allergen Proteins

Graphical Abstract



Authors

James Zook, Gina Mo, ...,
Wade D. Van Horn, Petra Fromme

Correspondence

petra.fromme@asu.edu (P.F.),
wade.van.horn@asu.edu (W.D.V.H.)

In Brief

Flpp3, a virulence determinant for the pathogen *F. tularensis*, is a possible target for vaccine and drug development. Zook et al. report the structure of Flpp3, revealing for the first time the internal binding cavity. Flpp3 is structurally homologous to the Bet v1 family of proteins, responsible for many allergic reactions.

Highlights

- Solution NMR structure of novel *F. tularensis* virulence determinant, Flpp3
- Flpp3 displays internal binding cavity for possible drug design
- Electrostatically polarized surface provides insight on membrane association
- Initial NMR relaxation studies suggest slow timescale protein dynamics

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NMR Structure of *Francisella tularensis* Virulence Determinant Reveals Structural Homology to Bet v1 Allergen Proteins

James Zook,[1,2] Gina Mo,[1] Nicholas J. Sisco,[1,4,5] Felicia M. Craciunescu,[2,3] Debra T. Hansen,[1,2,3] Bobby Baravati,[1,2] Brian R. Cherry,[4] Kathryn Sykes,[2,3] Rebekka Wachter,[1,2] Wade D. Van Horn,[1,4,5,*] and Petra Fromme[1,2,*]
[1]Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA
[2]Center for Membrane Proteins in Infectious Diseases, Arizona State University, Tempe, AZ 85287, USA
[3]Center for Innovations in Medicine, Biodesign Institute, Arizona State University, Tempe, AZ 85281, USA
[4]Magnetic Resonance Research Center, Arizona State University, Tempe, AZ 85287, USA
[5]The Virginia G. Piper Center for Personalized Diagnostics, Arizona State University, Tempe, AZ 85281, USA
*Correspondence: petra.fromme@asu.edu (P.F.), wade.van.horn@asu.edu (W.D.V.H.)
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SUMMARY

Tularemia is a potentially fatal bacterial infection caused by *Francisella tularensis*, and is endemic to North America and many parts of northern Europe and Asia. The outer membrane lipoprotein, Flpp3, has been identified as a virulence determinant as well as a potential subunit template for vaccine development. Here we present the first structure for the soluble domain of Flpp3 from the highly infectious Type A SCHU S4 strain, derived through high-resolution solution nuclear magnetic resonance (NMR) spectroscopy; the first structure of a lipoprotein from the genus *Francisella*. The Flpp3 structure demonstrates a globular protein with an electrostatically polarized surface containing an internal cavity—a putative binding site based on the structurally homologous Bet v1 protein family of allergens. NMR-based relaxation studies suggest loop regions that potentially modulate access to the internal cavity. The Flpp3 structure may add to the understanding of *F. tularensis* virulence and contribute to the development of effective vaccines.

INTRODUCTION

Francisella tularensis is the causative agent of the severely debilitating disease tularemia. It is an aerobic, Gram-negative coccobacillus, and a potentially fatal bacterial pathogen that is highly infectious and easily aerosolized. Consequently, the bacterium is sufficiently dangerous to be classified as a class A bioterrorism agent. The type A subspecies, exemplified by the SCHU S4 strain, is an especially potent infectious pathogen given that fewer than ten cells can cause fatality in humans (Dennis et al., 2001). *F. tularensis* is endemic in North America and in many parts of Europe and Asia, but cases of tularemia are relatively low and outbreaks generally only occur in regions with poor sanitation and inaccessibility to modern health care. One of the largest outbreaks in recent history was in postwar Kosovo between October 1999 and May 2000, with 327 confirmed cases (Reintjes et al., 2002).

Development of a vaccine is desirable, since treatment of *F. tularensis* is intensive (Parra et al., 2010) and may be unavailable in undeveloped areas where outbreaks tend to occur. At present only live attenuated vaccines based on the type B live vaccine strain (LVS) are available for *F. tularensis* immunization. The limitations of the LVS vaccine preclude its use in the United States, due to cases of human infection as a result of the improper administration of the vaccine and insufficient acquired immunity. The lack of acquired immunity from the current LVS vaccines has been reported to leave 20%–30% of the population susceptible to infection (Isherwood et al., 2005; McCrumb, 1961; Saslaw et al., 1961).

The protein derived from *F. tularensis* LVS open reading frame (ORF) FTL_0645 was identified to cause an immunogenic response in mice through the toll-like receptor 2 pathway, and therefore is likely a target for a subunit vaccine (Parra et al., 2010). The amino acid sequences of the proteins FTL_0645 from LVS and FTT1416c from SCHU S4 are identical. FTL_0645/FTT1416c is a lipoprotein localized to the outer membrane. It possesses a signal sequence and a lipobox consensus sequence (Ile-Ser-Gly-Cys), and has been named Flpp3 (*Francisella* lipoprotein 3) (Parra et al., 2010). Flpp3 has also been identified as a virulence determinant via mutagenesis studies: a significant decrease in *F. tularensis* virulence via respiratory infection was observed upon insertion-mutation of the ORF encoding Flpp3 (FTT1416c) (Su et al., 2007). Structural information may also shed light on the precise function of Flpp3, which is currently unknown.

No atomic resolution structures yet exist for any lipoprotein from the genus *Francisella*. In this study, we obtained the structure of the entire soluble domain of Flpp3 (Flpp3sol), comprising residues D26 to the C-terminal residue T137. Structural determination of the soluble domain independently from the transmembrane region can provide the first important structural and potentially functional information on this membrane protein. Furthermore, still under discussion is the question whether the mature protein is anchored to the membrane by a single transmembrane domain or by a lipid. It has been proposed that the

