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Researchers publish new studies and findings in the area of anthrax

New findings from the United States describe advances in anthrax.

Study 1: Recent research from the United States published in the *Journal of Molecular Biology* documented the acid-induced unfolding of the amino-terminal domains of anthrax toxin.

"The two enzymatic components of anthrax toxin, lethal factor (LF) and edema factor (EF), are transported to the cytosol of mammalian cells by the third component, protective antigen (PA). A heptameric form of PA binds LF and/or EF and, under the acidic conditions encountered in endosomes, generates a membrane-spanning pore that is thought to serve as a passageway for these enzymes to enter the cytosol. The pore contains a 14-stranded transmembrane beta-barrel that is too narrow to accommodate a fully folded protein, necessitating that LF and EF unfold, at least partly, in order to pass.

"Here," wrote B.A. Krantz and colleagues, Harvard University, "we describe the pH-dependence of the unfolding of LFN and EFN, the 30 kDa N-terminal PA-binding domains, and minimal translocatable units, of LF and EF."

"Equilibrium chemical denaturation studies using fluorescence and circular dichroism spectroscopy show that each protein unfolds via a four-state mechanism: N \leftrightarrow I \leftrightarrow J \leftrightarrow U. The acid-induced N \leftrightarrow I transition occurs within the pH range of the endosome (pH 5-6)," the authors reported.

"The I state predominates at lower pH values, and the J and U states are populated significantly only in the presence of denaturant. The I state is compact and has characteristics of a molten globule, as shown by its retention of significant secondary structure and its ability to bind an apolar fluorophore.

"The N \rightarrow I transition leads to an overall 60% increase in buried surface area exposure. The j state is expanded significantly and has diminished secondary structure content," they explained.

"We analyze the different protonation states of LFN and EFN in terms of a linked equilibrium proton binding model and discuss the implications of our findings for the mechanism of acidic pH-induced translocation of anthrax toxin.

"Finally," Krantz and coinvestigators concluded, "analysis of the structure of the transmembrane beta-barrel of PA

shows that it can accommodate alpha-helix, and we suggest that the steric constraints and composition of the lumen may promote alpha-helix formation."

Krantz and colleagues published their study in the *Journal of Molecular Biology* (Acid-induced unfolding of the amino-terminal domains of the lethal and edema factors of anthrax toxin. *J Mol Biol*, 2004;344(3):739-756).

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Study 2: Researchers have conducted a Western blot analysis of the exotoxin components from *Bacillus anthracis* separated by isoelectric focusing gel electrophoresis.

"The components of the *Bacillus anthracis* exotoxins, protective antigen (PA), lethal factor (LF), and edema factor (EF), from 24 isolates were separated by isoelectric focusing gel electrophoresis and detected by Western blot with monoclonal antibodies. Only two isoforms each were observed for PA and EF. Four isoforms were identified for LF," scientists in the United States report.

"The biological activities of both lethal toxin and edema toxin were measured by using in vitro cell-based assays. This study provides another method of characterizing various isolates of *B. anthracis* by determining the isoelectric points of the exotoxin components and

may be useful in the development of protective vaccines against *B. anthracis* infection," wrote Stephen F. Little at the United States Army Medical Research Institute of Infectious Diseases.

Little published his study in *Biochemical and Biophysical Research Communications* (Western blot analysis of the exotoxin components from *Bacillus anthracis* separated by isoelectric focusing gel electrophoresis. *Biochem Biophys Res Commun*, 2004;317(1):294-300).

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Study 3: Anthrax lethal factor-cleavage products of mitogen-activated protein kinase (MAPK) kinases exhibit reduced binding to their cognate MAPKs.

According to published research from the United States, "Anthrax lethal toxin is the major cause of death in systemic anthrax. Lethal toxin consists of two proteins: protective antigen and LF (lethal factor). Protective antigen binds to a cell-surface receptor and transports LF into the cytosol.

"LF is a metalloprotease that targets MKKs [MAPK (mitogen-activated protein kinase) kinases]/MEKs [MAPK/ERK (extracellular-signal-regulated kinase) kinases], cleaving them to remove a small N-terminal stretch but leaving the bulk of the

protein, including the protein kinase domain, intact," stated A. Jane Bardwell and colleagues at the University of California-Irvine. "LF-mediated cleavage of MEK1 and MKK6 has been shown to inhibit signaling through their cognate MAPK pathways. However, the precise mechanism by which this proteolytic cleavage inhibits signal transmission has been unclear.

"Here we show that the C-terminal LF-cleavage products of MEK1, MEK2, MKK3, MKK4, MKK6, and MKK7 are impaired in their ability to bind to their MAPK substrates, suggesting a common mechanism for the LF-induced inhibition of signaling," concluded Bardwell and her collaborators.

Bardwell and her coauthors published their findings in the *Biochemical Journal* (Anthrax lethal factor-cleavage products of MAPK (Mitogen-activated protein kinase) kinases exhibit reduced binding to their cognate MAPKs. *Biochem J*, 2004;378(Part 2):569-577).

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The information in this article comes under the major subject areas of Anthrax, Biowarfare, Bacteriology, and Proteomics.

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