INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Development Of An Lc-ms/ms-based Integrated Analysis Pipeline For The Detection Of Trace Protein And Small Molecule Toxins In Complex Matrices

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A key feature of biological threat deterrence and response is the ability to perform rapid molecular-scale detection and characterization of trace hazardous agents in complex matrices. As a complement to DNA sequencing, proteomic and metabolomic profiling can provide information for characterization, response and attribution, particularly for hazards such as toxins that can lack genetic material for sequencing. Our objective is to develop a fully integrated analysis pipeline that is capable of detecting and characterizing toxins of multiple classes from a trace and complex sample. In addition to detecting and identifying known toxins in a threat environment, the purpose of this method is to characterize unknown toxins in order to identify potential novel toxin threats that may not currently exist in a database. Taking the protein sequence(s) obtained from our analysis method as input, we are using computational approaches such as modeling of protein folding and substrate binding to predict whether unknown proteins exhibit characteristics of a toxin. These computational methods, which are described in a separate abstract, are also under development as part of this program.

To date, we have developed and optimized methods to characterize the mass spectrometric signatures of a broad array of trace protein toxins including ricin, abrin, staphylococcus enterotoxin (SEB), botulinum neurotoxin (BoNT), and multiple conotoxins using a proteomics sample preparation procedure followed by peptide sequencing using liquid chromatography – tandem mass spectrometry (LC-MS/MS). To ensure our procedure is amenable to nucleic acids, we successfully conducted DNA and RNA analysis of a known sample using our developed extraction conditions. Figures of merit for the protein analysis method including limits of detection (LOD) of the method and percent recovery from complex matrices such as soil, food, and protein shake have been established. Comprehensive method LODs (not instrument LODs) have been measured as low as 2.8 µg/mL for ricin, SEB, and BoNT, 2.5 µg/mL for Ω-Conotoxin MVIIC, and 5 µg/mL for µ-Conotoxin PIIIA. This is an improvement over previously reported method LODs for ricin at 21.3 µg/mL1. Percent recoveries of ricin reach 58% from a protein shake (200 ng ricin spiked into 100 µL protein shake) and 31.1% from soil (2 µg ricin spiked onto 100 mg soil, with 200 ng ricin sampled for analysis). This leads to a slight worsening of the LODs when in the presence of a complex matrix: ricin LODs are 4.9 µg/mL in a protein shake sample, and 9.2 µg/mL in a soil sample. This sensitive and precise method for detecting and characterizing known and novel trace proteins in complex matrices has relevance across operational conditions. In addition, we are currently developing methods for characterizing small molecule toxins and active pharmaceutical ingredients (API) that will improve the DoD’s ability to characterize and respond to biological threats.

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