NANOSCALE CHARACTERIZATION OF SPIDER VENOM PEPTIDES BY HIGH RESOLUTION LC-MS/MS ANALYSIS.

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Animal venoms are natural libraries of biologically active peptides that encompass a wide variety of structures and pharmacological activities and represent an enormous resource of novel molecules to be used as therapeutics and drug models. However, the obstacle of sample size is daunting as many venomous species are of a size which is too small for classical bioassay-guided fractionation and biochemical characterization. High resolution mass spectrometry can be used as an alternative technology for peptide sequence determination and sequence tag generation to permit the use of cDNA libraries. Venom profiling can be used for species identification and to indicate the presence of potentially unknown toxins. We demonstrate that *de novo* sequencing at the nanoscale level is applicable to venomics research.

Crude venoms from an Australian funnel-web spider (*Hadronyche infensa*: Orchid beach) (Hexathelidae, Hi:OB) and a small jumping spider (Salticidae, Sp004, body size 4 mm) were first analyzed by high resolution nano-LC-MS/MS for intact mass determination. Analysis of reduced and alkylated venoms permitted the determination of disulfide bridge number and offered improved fragmentation in a systematic MS/MS analysis of fractions. Full scan spectra were deconvoluted to obtain the exact masses of native and alkylated venom peptides. Detected peptides were selected for Collision Induced Dissociation (CID), Higher energy Collisional Dissociation (HCD) and Electron Transfer Dissociation (ETD) fragmentation. *De novo* sequencing was performed using MS/MS information and sequence tags were used to search for homologies in specific venom peptide and nucleotide databases.

Venom peptide masses ranged from 1000 to 15000 Da with 75% of the peptides in the 4000 to 8000 Da range. 479 peptide masses were detected in the Hi:OB venom and 377 peptides in the small jumping spider venom sample. In the Hi:OB sample, numerous toxins could be identified based on the match of exact mass with masses of known toxins. The exact mass of alkylated peptides was correlated to the mass of crude

peptides to determine the number of cysteines and further validate peptide identifications.

A combination of CID, HCD and ETD was used to further characterize unknown venom peptides. HCD spectra were of particular use since they contain immonium ions that are useful for *de novo* sequencing. ETD was expected to be very efficient for the fragmentation of large peptides and small proteins. For this study, it allowed the generation of numerous sequence tags for unknown peptides, that could be matched to peptide and nucleotide sequences from venoms. Sequence homologies were also obtained for the jumping spider sample demonstrating the efficiency of the technique for the characterization of long peptides from very small amounts of biological material, and the feasibility of "nano-venomics".

Spider venomics will be further developed to allow deep mining of the enormous biological resource represented by small venomous animals. Massive peptide libraries based on venoms will be of major importance in the discovery of the drugs of the future.