INTACT PROTEIN ANALYSIS OF SNAKE VENOMS WITH CZE-MS

Gayatri Vishwakarma[1], Melinda Andrási[1], Ruben Szabó[1], Péter Hajdú[2], Vladimir Petrilla[3,4] Monika Petrillová[5], Jaroslav Legath[6,7], Attila Gáspár[1]

[1]Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem ter 1., Debrecen 4032, Hungary
[2]Department of Dental Biochemistry and Department of Biophysics and Cell Biology, University of Debrecen, Egyetem tér 1., Debrecen 4032, Hungary
[3]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy, Košice, Slovakia
[4]Zoological Department, Zoological Garden Košice, Košice-Kavečany, Slovakia
[5]Department of General Competencies, University of Veterinary Medicine and Pharmacy, Košice, Slovakia
[6]Department of Pharmacology and Toxicology, University of Veterinary Medicine and Pharmacy, Košice, Slovakia
[7]Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland.
e-mail: gayatri.vishwakarma@science.unideb.hu

Abstract
In this research work we demonstrated the potential analytical performance of capillary zone electrophoresis coupled with mass spectrometry (CZE-MS) for the intact protein analysis of similar venom samples. Using 1 M formic acid (pH=1.9) as BGE, minimal adsorption and narrow peaks shapes - thus good separation efficiencies - were obtained for the protein components of the venom samples.

Introduction
Venoms consist of several biologically active components, primarily peptides and proteins. these toxin components have the potential to cause lethal effects [1,2]. Top-down mass spectrometric technique when combined with capillary zone electrophoresis, becomes highly effective in studying the structural characteristics of intact proteins. This methodology can be utilized for the analysis of complex protein molecules such as snake venom [3].

Experimental
Analyses were conducted using a 7100 model CE instrument (Agilent) with UV and MS (maXis II UHR ESI-QTOF MS instrument, Bruker) detection. Fused silica capillaries of 85 cm x 50 μm I.D. and 370 μm O.D. was used. UV detection was carried out by on-capillary photometric measurement (detection wavelength: 200 nm). Background electrolytes were 1 M formic acid (pH=1.8), sheath liquid: 0.1% formic acid in 1:1 isopropyl alcohol.

Results and discussion
The precision of migration times and peak areas were 1.9-2.8 RSD% and 0.8-7.2 RSD%, respectively and the theoretical plate numbers were 32000-238000 for peaks having signal-to-noise ratio (S/N) larger than 50. More than 250 different toxin components (7-10 kDa) were detected in the venoms obtained from snakes of 9 different subspecies (belonging either to Naja or Dendroaspis species). The protein contents of the venoms of the same subspecies collected from different geographical regions are similar and differ only in a few (less than 10%)

components. However, the venom samples collected from different organisms (within the same species) exhibit very different protein patterns.

Conclusion

Our study utilizes fused silica capillaries for their simplicity and cost-effectiveness, employing background electrolytes with extremely low pH conditions to separate and characterize various components in snake venom samples. Notably, our findings revealed discrete protein patterns among venoms from different subspecies, highlighting the unique fingerprinting potential of venom in various snake populations.

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