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Mass spectrometry strategies for venom mapping and peptide sequencing from crude venoms: Case applications with single arthropod specimen

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Abstract

Due to their complexity and diversity, animal venoms represent an extensive source of bioactive compounds such as peptides and proteins. Conventional approaches for their characterization often require large quantities of biological material. Current mass spectrometry (MS) techniques now give access to a wealth of information in a short working time frame with minute amounts of sample. Such MS approaches may now be used for the discovery of novel compounds, and once their structure has been determined they may be synthesized and tested for functional activity. Molecular mass fingerprints of venoms allow the rapid identification of known toxins as well as preliminary structural characterization of new compounds. De novo peptide sequencing by tandem mass spectrometry (MS/MS) offers rapid access to partial or total primary peptide structures. This article, written as a tutorial, also contains new material: molecular mass fingerprint analysis of *Orthochirus innesi* scorpion venom, and identification of components from bumblebee *Bombus lapidarius* venom, both collected from one single specimen. The structure of the three major peptides detected in the *Bombus* venom was fully characterized in one working day by de novo sequencing using an electrospray ionization hybrid quadrupole time-of-flight instrument (ESI-QqTOF) and a matrix-assisted laser desorption ionization time-of-flight instrument (MALDI-LIFT-TOF-TOF). After presenting the MS-based sequence elucidation, perspectives in using MS and MS/MS techniques in toxinology are discussed.

Keywords: Mass spectrometry; MALDI-LIFT-TOF-TOF; Nano-ESI-MS/MS; MS/MS de novo sequencing; Venom mapping; *Orthochirus* venom; *Bombus* venom; Toxins; Alpha-helical amphipathic peptide; Mass fingerprint; Bioactive peptide; Peptidomics

1. Introduction

Venoms of animal origin represent a rich source of bioactive compounds yet most of the components remain to be discovered and investigated. Venom complexity in terms of peptides and proteins content together with the number of venomous species indicate that only a small proportion (less than 1%) of the bioactive molecules has been identified and characterized to date, and little is known at the genomic level of venomous organisms (Ménez et al., 2006). Several venom components have already been successfully used for biomedical research and for the development of new research tools, diagnostic reagents and therapeutic drugs (Lewis and Garcia, 2003). Regularly, additional biomolecules originating from venomous animals appear very promising in terms of medical application. These discoveries mainly rely on a conventional approach using biological activity to follow the purification and structural characterization of the bioactive molecule. Nevertheless, this strategy requires large quantities of product for biological activity screening and is thus restrained to a

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few venomous species. Moreover, obtaining large quantities of venom may require venom gland dissection and extraction, and is thus a destructive method with negative impact on animal biodiversity, in particular, since some promising biological models can be rare specimens. It may also imply keeping live animals and collect venom for weeks or months, to obtain sufficient quantities of venom. In order to overcome such problems in the discovery of novel bioactive molecules from venoms, alternative strategies were developed. We and others have chosen to turn the discovery process from being a biologically driven screening approach into a structurally driven strategy, with the objective to gain structural information and better exploit the potential of biocomputing. Getting information from complex matrices such as venoms has been made possible in the last decade through the use of soft ionization MS. This analytical tool has been extensively used over the past 20 years, mainly to assist structural investigations and to control purity during sample fractionation and purification. MS has evolved and become a reference analytical tool for the study of proteins. One consequence was the emergence of proteomic sciences in the field of toxinology (Fox et al., 2002; Stöcklin and Favreau, 2002).

The use of different types of mass spectrometers gives access to a wealth of information going from simple molecular masses of intact components to primary sequences of peptides. For example, on-line liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS) or matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis of crude venoms give a molecular mass fingerprint of the venom components. In many cases, this has allowed identification of species, which has become a useful chemotaxonomic tool, also for biodistribution and ecological studies (Escoubas et al., 1997; Stöcklin et al., 2000). Further structural characterization, such as primary structure elucidation, requires the use of specific tandem MS equipment. Usually fitted with ESI or MALDI sources, these instruments include various combinations of mass analyzers such as ion traps, triple quadrupole, hybrid quadrupole time-of-flight (QqTOF), hybrid quadrupole/linear ion trap (Q-TRAP), Fourier transform-ion cyclotron resonance (FT-ICR) or time-of-flight/time-of-flight (TOF–TOF) instruments for the fragmentation of the peptide of interest. As an example, ESI-QqTOF mass spectrometers provide high signal resolution and mass accuracy for sequence characterization and is well adapted for peptide characterization. With the advantages of a convenient sample preparation and high throughput ability, MALDI-LIFT-TOF-TOF offers a rapid and more sensitive detection method. After a brief overview of the 'structure to function' strategy and its implications, we will emphasize the usefulness of MALDI-TOF, ESI-QqTOF and MALDI-LIFT-TOF-TOF analyses: model examples will be presented using two arthropod venoms from *Orthochirus innesi* (order: scorpions, family: Buthidae) and from *Bombus lapidarius* (order: Hymenoptera, family: Apidae).

2. From structure to function

The search for novel bioactive molecules aiming at drug discovery is commonly driven by the identification of a particular target through a biological screening assay. Identification of a target further leads to the purification and characterization of the active component. However, this approach is often time consuming and only appropriate when large quantities of starting material are available to perform biological screening during the entire process of the purification of the compound of interest. In addition, the validation of a biomolecule as a possible lead compound often requires finding variants in order to match its active site and to map essential residues for activity or residue-driven selectivity between different targets. This can be achieved via synthetic approaches through the preparation of synthetic libraries of venom components and analogues thereof. In view of the recent drastic reduction of peptide production costs, such an approach now becomes possible, even though it requires time and a rational design by bioinformatic tools. However, as a complementary approach, we and others tend to believe that nature, aided by evolution, may often be just the most efficient source of new drug candidates, in particular if we consider that the genome does not waste energy in producing molecules that do not interact with biological systems (Harvey, 1999). Finding novel natural isoforms or variants from a known type of venom peptide is thus particularly well adapted to the approach considered here. Venom molecular mass mappings allow a rapid and tentative identification of known groups of toxins based on the molecular mass and thus gives a useful survey of what can be expected from a given venom in terms of variant complexity and novelty.

This strategy has already been successfully applied to the discovery of novel venom components. Sarafotoxins are potent vasoconstrictors that are partly responsible for the venom toxicity by causing cardiac dysfunctions. The peptides of this group are 21-residues long and contain two internal disulfide bridges. Being similar to the endothelins that are naturally occurring in mammalian vascular systems, the sarafotoxins have thus contributed to the study of the different endothelin receptors in mammals (Ducancel, 2005). In order to find novel natural variants of sarafotoxins, the venom from the snake *Atractaspis microlepidota microlepidota* was investigated by on-line LC-ESI-MS (Hayashi et al., 2004). The mass pattern corresponding to the known sarafotoxins at 2400–2600 Da was shifted to a new set of masses at 2800–3000 Da corresponding to potentially related peptides. Analysis by tandem mass spectrometry led to the discovery of a new set of sarafotoxins with the three additional residues Asp-Glu-Pro residues at the C-terminal end. Recently, additional