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Mass spectrometric characterization of the crustacean neuropeptidome

*Qing Yu, Chuanzi OuYang, Zhidan Liang, Lingjun Li**

School of Pharmacy and Department of Chemistry, University of Wisconsin – Madison, Madison, WI, USA

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ABSTRACT

Neuropeptides (NPs) are the largest class of signaling molecules used by nervous systems. Despite their functional importance, numerous challenges exist to characterize the full complement of NPs – the neuropeptidome. In this review, we discuss recent advances in MS-based techniques for NP identification and quantitation, as well as sample preparation strategies for various applications in several crustacean model organisms. By surveying published examples of crustacean neuropeptidomic analyses, we highlight challenges and progress in this dynamic field, and summarize the current state of knowledge about crustacean NPs and MS-based methodologies for NP analysis.

1. Introduction

Animal behaviors are precisely controlled by the nervous system which employs a myriad of signaling molecules to mediate this control process. One of the most diverse signaling molecules are neuropeptides (NPs), which are synthesized as larger precursor proteins, and undergo extensive processing and modification to become bioactive NPs to participate in cell–cell signaling. Since they are directly involved in modulating many physiological processes, such as feeding, pain sensing and reproduction [1,2], there is growing interest in studying their structures, functions and distributions. Because of the relatively simple nervous system and accessible electrophysiology at the single-cell and neural circuit level, decapod crustacean has long been used as an attractive model preparation for neuromodulation and NP research [3,4], as evidenced by the large number of NPs discovered since 2000 (Table 1).

Traditional studies on NPs often use biochemical techniques that can be imprecise and cumbersome. Mass spectrometry (MS) has evolved as a powerful tool to characterize NPs, due to its capability to precisely determine the identity and primary structure of a NP as well as its ability to quantify these signaling molecules in a complex mixture. A new concept, peptidomics, was introduced in 2001, largely due to the rapid growth of MS-based large-scale peptide and protein analysis [5–7]. By exploring the utility of MS based methods, various NP families with multiple isoforms that differ from each other by a single amino acid can be distinguished in contrast to antibody-based immunochemical techniques. The combined use of MS and MS/MS scans enable the discovery of novel members of established NP families and the identification of new peptide families, significantly expanding the neuropeptidome in a model organism [3,8]. With their structural information available, the next step for NP study is to determine its function during various physiological processes.

* *Corresponding author at*: School of Pharmacy and Department of Chemistry, University of Wisconsin – Madison, 777 Highland Avenue, Madison, Wisconsin 53705-2222, USA. Tel.: +1 608 265 8491; fax: +1 608 262 5345.
E-mail address: lli@pharmacy.wisc.edu (L. Li).

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Table 1 – Crustacean neuropeptidomic studies by mass spectrometric techniques.

Species	Tissues studied	MS instrumentation	References
Ghost crab *Ocypode ceratophthalma*	Eyestalk ganglia (including sinus glands), brain, pericardial organ	MALDI-TOF/TOF ESI-Q-TOF	[32]
Jonah crab *Cancer borealis*	Hemolymph, stomatogastric nervous system, pericardial organ, thoracic ganglia	MALDI-TOF/TOF ESI-Q-TOF MALDI–FTMS	[39,45,91,104,115,187]
American lobster *Homarus americanus*	Brain, pericardial organ, stomatogastric ganglion	MALDI–FTMS ESI-Q-TOF MALDI TOF/TOF MALDI LTQ Orbitrap	[36,37,40,89,135,188]
Blue crab *Callinectes sapidus*	Pericardial organ, stomatogastric nervous system, sinus gland	MALDI–FTICR ESI-Q-TOF MALDI TOF/TOF	[42,46]
Water flea *Daphnia pulex*	Brain-optic ganglia, brain	MALDI TOF/TOF	[189]
White shrimp *Litopenaeus vannamei*	Sinus gland, brain	MALDI-FTMS ESI-Q-TOF	[44]
European green crab *Carcinus maenas*	Brain, thoracic ganglia, sinus gland, pericardial organ	MALDI-FTMS ESI-Q-TOF	[116]
Spinycheek crayfish *Orconectes limosus*	Sinus gland	ESI-Q-TOF	[38]
Red swamp crayfish *Procambarus clarkii*	Brains, ventral nerve cord	ESI-Q-TOF MALDI TOF/TOF	[135,190]
Giant tiger prawn *Penaeus monodon*	Eyestalk, brain, thoracic ganglia	ESI-Q-TOF	[191–194]
Common yabby *Cherax destructor*	Stomatogastric nervous system	MALDI TOF/TOF	[195]
Red rock crab *Cancer productus*	Sinus gland, pericardial organ	MALDI-FTMS ESI-Q-TOF	[196]

The advancement of MS-based tools and tandem MS capability coupled with isotope labeling strategies and label-free approaches have accelerated several large-scale comparative neuropeptidomic analyses, enabling a global view of coordinated changes of NPs related to a physiological process.

2. Sample preparation

2.1. *Overview of sampling strategies*

Because of the biochemical complexity, high-salt contents, and endogenous proteases, sample preparation is often critical to produce the desired outcome of a peptidomic experiment. Depending on the goals and requirements of specific experiments, various sample preparation methods have been developed. As outlined in Fig. 1, samples can be prepared by homogenization and extraction of tissues, analyzed by direct tissue experiments or collected by microdialysis techniques followed by liquid chromatography (LC) coupled to MS or MS/MS analysis.

2.2. *Sample preparation for direct tissue analysis*

For tissue-based study, it is crucial to avoid delocalization and degradation of analytes. Proper tissue harvest, stabilization and preservation are all important aspects in the sample preparation process. After animal sacrifice, snap-freezing dissected tissue in powdered dry ice, liquid nitrogen or liquid nitrogen-chilled isopentane would quickly preserve the structural integrity. Storage at −80 °C is required to minimize the degradation until use [9]. Longer freezing method could be achieved by loosely wrapping the tissue in aluminum foil and gently placing it into liquid nitrogen, ice-cold ethanol or isopropanol bath for 30–60 s [10].

Tissue preservation is required to prevent post mortem proteome degradation and slow down sample aging before actual MS experiments. Common methods include focusing microwave irradiation [11] and thermal denaturation by Stabilizor T1, which extensively denature active proteolytic enzymes [12,13]. Other popular method such as formaldehyde-fixed paraffin-embedding (FFPE) is not very suitable with MS imaging because of the cross-linking it caused between peptides and proteins [14]. A modified approach of formalin-fixed paraffin embedding used ethanol rather than formalin to fix the tissue. MS images generated in this way were significantly improved [15].

One significant advantage of direct tissue analysis over liquid phase LC–MS method is the ability to preserve the structural integrity and morphology of the tissue. In order to get smooth thin tissue sections around 10–20 μm in cryostat, tissues are usually embedded in supporting media such as optimal cutting temperature (OCT) compound, Tissue-tek and carboxymethylcellulose (CMC) [10]. However, these polymer-based materials which contain polyethylene glycol (PEG), polyvinyl alcohol (PVA), or both, can cause strong background signals which mask the detection of peptides and proteins of interest. In order to eliminate the interference from