Enhanced Coverage of Insect Neuropeptides in Tissue Sections by an Optimized Mass Spectrometry Imaging Protocol

In this application note, we describe the sample preparation and imaging analysis of insect neuropeptides using the rapifleX MALDI-TOF.

This method allows for increased neuropeptide coverage content and high spatial resolution localization, as demonstrated using the retrocerebral complex of the American cockroach, *Periplaneta americana*.

Introduction

Neuropeptides are signaling molecules produced by neurosecretory cells of the central nervous system and regulate several physiological functions in multicellular organisms. As neuropeptides control most aspects of insect physiology (e.g. feeding, reproduction), they are targets for novel insecticide development. By understanding neuropeptide processing,

Keywords: neuropeptides, MALDI Imaging, rapifleX, MALDI-TOF, SCiLS

Authors: Lapo Ragionieri[1], Sander Liessem[1], Alice Ly[2], Reinhard Predel[1];
[1] Cologne Biocenter, Institute for Zoology, University of Cologne, Cologne, Germany; [2] Bruker Daltonik GmbH, Bremen, Germany

distribution and their alterations in response to insecticides, we can improve prediction of insect behavior and develop substances which are detrimental to pests while harmless to beneficial species. The retrocerebral complex (RCC) is a major insect neuroendocrine organ and consists of different sections: a glandular part of the *corpora cardiaca*, producing and releasing adipokinetic hormones (AKHs), a neurohemal part of the *corpora cardiaca* which mostly stores and releases neuropeptides produced in the brain and gnathal ganglion, and the endocrine *corpora allata* which synthesize and release juvenile hormones. All of these tissues are crossed by axons projecting from neurosecretory cells of the CNS and thus it is possible to detect several neuropeptides [1]. The study of insect neuropeptide interactions is typically carried out using a combination of mass spectrometry and immunohisto-chemistry (IHC). While mass spectrometry is potentially able to identify all peptide products of a single gene as well as from multiple genes, the spatial

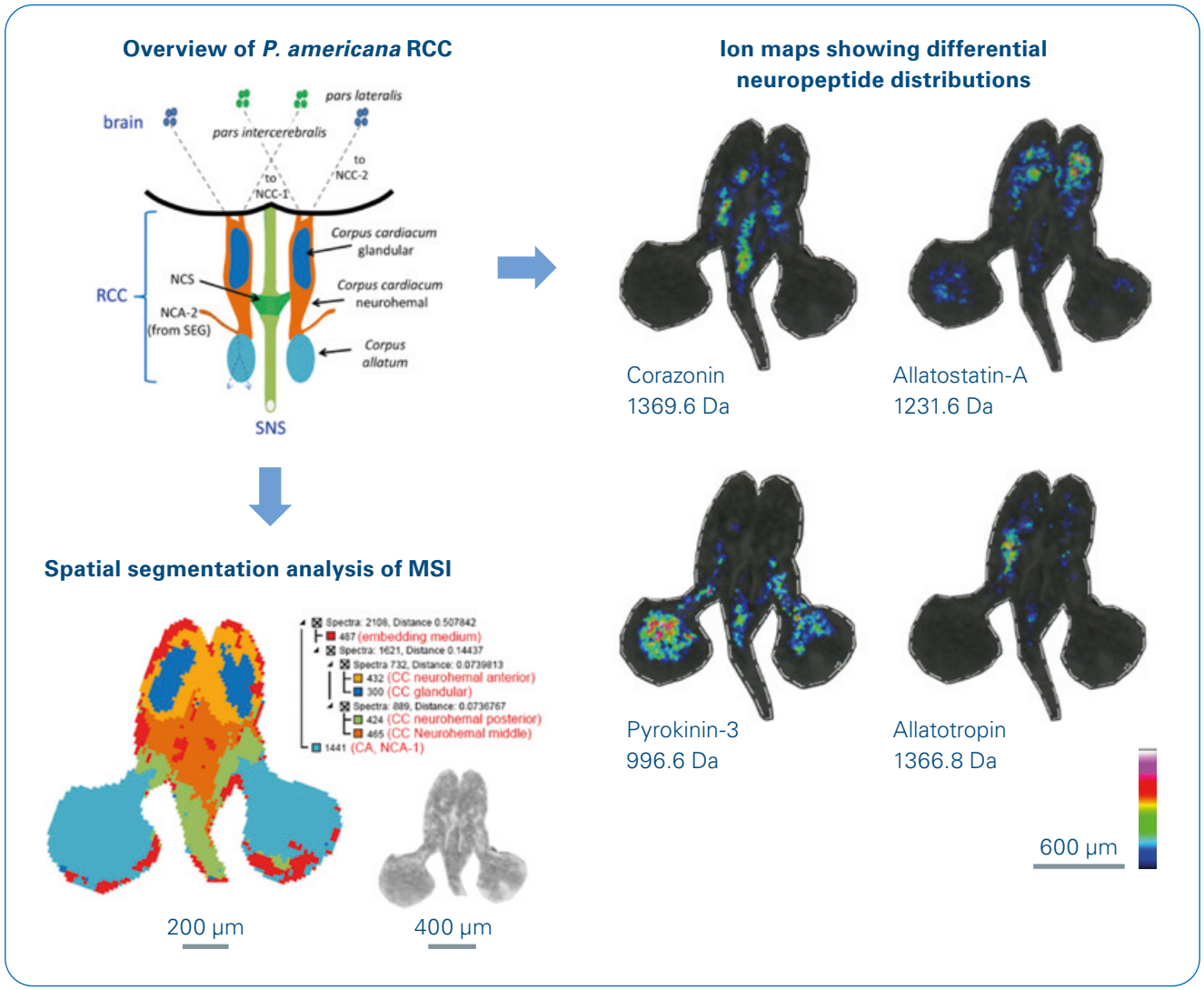

Figure 1: Neuropeptide distributions in the P. americana *RCC. Top Left: Schematic of the RCC and junctions with brain and stomatogastric nervous system (SNS). Neurosecretory cells in the* pars intercerebralis *and* pars lateralis *of the brain are indicated by green and blue circles, respectively. Dotted lines represent the respective pathways leading to the* nervi corporis cardiaci. *NCC,* nervus corporis cardiaci*; NCA,* nervus corporis allati*; NCS,* nervus cardiostomatogastricus*; SEG, subesophageal ganglion. Bottom Left: Spatial segmentation analysis of MSI data from a single RCC section divides the RCC into distinct regions corresponding to the* corpora allata *(CA),* nervi corporis allati-1 *(NCA-1), the glandular* corpora cardiaca *(CC) and neurohemal CC. The neurohemal CC is further subdivided into three subcompartments. Right: MALDI-MSI ion maps showing the distribution of four different neuropeptides within the RCC.*

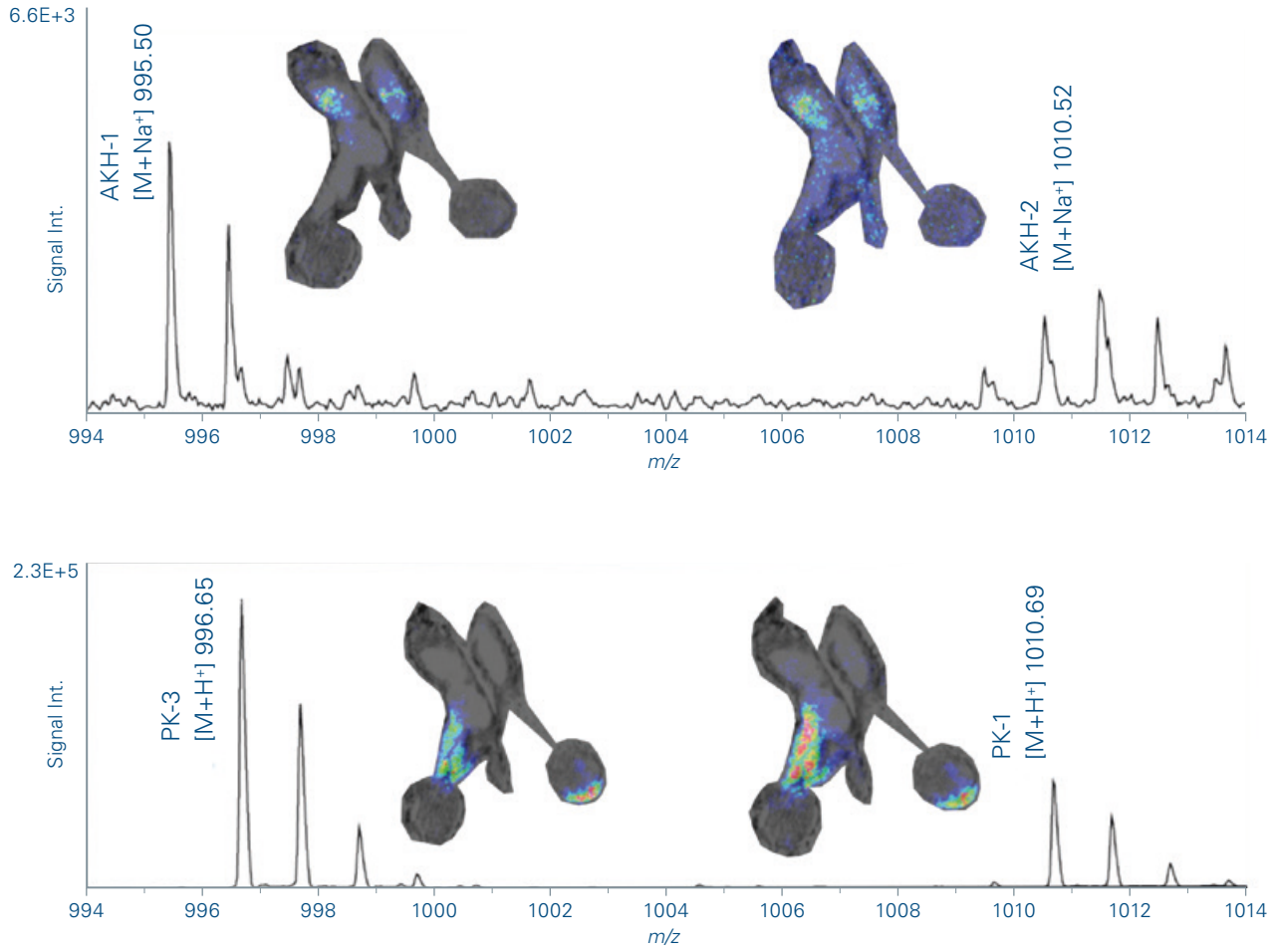

Figure 2: Discrimination between mass-similar neuropeptides Adipokinetic hormones (AKHs) and pyrokinins (PKs). Top: Ion maps of AKH-1 (995.50) and AKH-2 (1010.53) with single spectra from region prominent in AKHs. Bottom: Ion maps of PK-3 (996.73) and PK-1 (1010.67) with single spectra from region prominent in PKs. AKHs accumulate in the glandular CC while PKs are distributed in the neurohemal CC. Despite the similarity in masses, it is possible to discriminate between ion signals with a difference of only 0.2 Da.

localization of neuropeptides in the CNS is usually studied by means of IHC, however, this technique is unable to discriminate between many different neuropeptide precursor products.

In comparison, MALDI-MSI allows for the simultaneous visualization of the distribution of numerous molecules in a single tissue section. We developed an optimized protocol to study the spatial distribution of neuropeptides in the RCC of the American cockroach, *Periplaneta Americana* (*P. americana)* [2]. Using this protocol, it is possible to obtain a near-complete coverage of insect neuropeptides from a single tissue section; thus allowing us to reconstruct the compartmentalization of the RCC, and to detect differential neuropeptide processing. This protocol allows not only investigation of neuropeptide distributions but also analysis of differences in the neuropeptidome, e.g. in response to insecticide exposure or environmental stress in general.

Methods

We used the American cockroach *P. americana*, a model organism for the study of insect neuropeptides. Retrocerebral complexes were extracted and embedded in 120 mg/mL gelatin/deionized water. Tissue samples were frozen at -50°C and sectioned with a thickness between 14 and 20 µm on a cryomicrotome, and thaw mounted on ITO slides. Tissue sections were washed at room temperature in 70% (v/v) ethanol/water and 100% ethanol for 20 s each with an interval of five seconds drying time. Slides were then dried under vacuum, then coated with 5 mg/mL CHCA in 50% acetonitrile/water with 2% TFA using a SunCollect Dispenser System (SunChrom, Friedrichsdorf, Germany). MS data was acquired on a rapifleX MALDI-TOF mass spectrometer in positive reflector mode over a mass range m/z 600-3200. Pixel size was set to 15 µm and laser repetition rate was set to 5 kHz. Data were analyzed using flexImaging 5.0, flexAnalysis 4.0 and SCiLS Lab 2018a. All neuropeptide identifications were confirmed via published lists [3].

Results

Using this protocol it is possible to detect more than 100 peptides including around 60 putative bioactive neuropeptides from 15 precursor genes in a single tissue section; thus obtaining a near-complete coverage of insect neuropeptides by MSI. The results were also corroborated with extract analyses of neuropeptides from the RCC [2].

The distributions of different neuropeptide gene products within the RCC are shown in Figure 1. The obtained ion maps differed dramatically between different neuropeptides. Corazonin and allatostatin-A peptides are both expressed in neurosecretory cells of the *pars lateralis* of *P. americana* brain with projections via *nervus corporis cardiac* (NCC)-2 into the RCC and the stomatogastric nervous system (SNS). Corazonin was detected in the neurohemal part of the *corpora cardiaca* and in the *nervus cardiostomatogastricus*, while allatostatin-A signals were present in the *corpora cardiaca* (CC) and along the *corpora allata* (CA). In contrast, pyrokinins, which are mostly expressed from neurosecretory cells of the gnathal ganglion, is detected in the CA and in the SNS [2]. In addition, we also observed in the CC the distribution of neuropeptides that were not previously described with either immunohistochemistry or mass spectrometry in the RCC of *P. americana*, such as Allatotropin. Across different preparations we detected ion signals in the SNS and in the CC, but rarely in the CA. Notwithstanding, it is unknown how allatotropin enters the RCC, based on MSI analyses, it is possible that allatotropin reaches the *corpora cardiaca* via the NCC-1.

Bioinformatic analysis of the acquired spectra allowed us to reconstruct the main compartments within the RCC, the spatial-segmentation analysis of a single RCC section (Figure 1). This analysis uses MSI experiments without any *a priori* knowledge and clearly discriminates between different regions within the *P. americana* RCC, corresponding to CA and adjoining *nervi corporis allati-1*, the glandular CC, the neurohemal CC which is further differentiated in three sub-compartments, and the SNS. Moreover, the dendrogram, based on more than 2000 spectra, showed that the posterior and middle portions of the CC are more closely related to each other than to the neuroglandular area.

This method could be used to simultaneously identify mature products of multiple copies (paracopies) precursor proteins, such as allatostatin-A and pyrokinins – a clear advantage compared to IHC which is not able to discriminate between paracopies of precursor proteins. Using our approach, it is possible to discriminate between ion signals differing for only 0.2 Da, such as pyrokinin-1 and mass of sodium-adduct ion of Adipokinetic hormone-2 (Figure 2). As can be observed in the ion maps of a single RCC section, it is sufficient to obtain two completely different ion maps which perfectly match with the expected distribution of this neuropeptides in the RCC despite this small mass difference.

Conclusion

Using the optimized protocol it was possible:

- To obtain a comprehensive neuropeptidome of the RCC of *P. americana* with high reproducibility, signal quality, and spatial resolution.
- To describe distinct accumulation of different neuropeptides in the RCC of *P. americana.*
- To separate the distribution of neuropeptides with similar molecular masses.
- To reconstruct the compartmentalization of the RCC of *P. americana* using a combination of mass spectrometry and bioinformatic analyses.

Based on our results, MSI can be incorporated into neuroscience-related topics such as complex changes in the insect neuropeptidome, including several model organisms, that might be associated with development or adaptations induced by environmental stress (e.g., xenobiotics).

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com/rapiflex

References

[1] Gundel M, Penzlin H (1980). Cell Tissue Res., **208**, 283–297

[2] Ly A, Ragionieri L, et. al. (2019). Anal Chem., **91**, 1980-1988. doi: 10.1021/acs.analchem.8b04304.

[3] Neupert S, Fusca D, et al. (2012). J Comp Neurol., **520**, 694-716. doi: 10.1002/cne.22745.

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com