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SHORT COMMUNICATION

Application of MALDI-MSI for detection of antimicrobial peptides in tissues of the marine invertebrate *Arenicola marina*

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Abstract

MALDI imaging mass-spectrometry (MALDI-MSI) is a highly informative approach combining morphology with molecular data. It is widely applied in neuroscience, plant science, cancer-biology, biomedicine, including clinical, and preclinical studies, but not for investigation of endogenous peptides/proteins or metabolites in marine invertebrates. We examined the informativeness of MALDI-MSI for analysis of distribution of antimicrobial peptides (arenicins) in the polychete *Arenicola marina* and concluded that it can be successfully used as a primary rough express screening method.

Key Words: MALDI-MSI; antimicrobial peptides; polychete immunity; *Arenicola marina*

Introduction

Antimicrobial peptides (AMPs) are key players in innate immunity of diverse organisms, contributing to both effector and regulatory functions. They are among immune effectors most intensively studied during last 30 years (Harder and Schröder, 2016). AMPs are relatively small (not exceeding 100 amino acids) usually cationic polypeptidic molecules with a prominent inhibitory potential against various microbial pathogens. Although AMPs were identified in a wide range of organisms including plants, vertebrate and invertebrate animals, protists and prokaryotes (Boman, 2003; Reddy *et al.*, 2004; Yount *et al.*, 2006; Otero-González *et al.*, 2010; Pasupuleti *et al.*, 2012; Harder and Schröder, 2016) different taxa are still very unequally studied in terms of AMPs diversity and functioning, and immunology in general. The wide distribution of AMPs makes them an attractive object for comparative immunology and description of AMPs functioning in less studied taxa (among which many marine invertebrates, *e.g.*, polychetes) is in high demand.

Being very important in respect of both practical and theoretical points of view, AMPs attract attention in different aspects: structure, structure-functional interrelation, mechanism of action, spatiotemporal pattern of expression and mechanism of its regulation. In this respect, the widening of methodological background for AMPs investigation in different taxa is an urgent task.

MALDI imaging mass-spectrometry (MALDI-MSI) is a comparatively young and highly informative approach superposing morphological and molecular data, which was called "molecular histology" (Stoeckli *et al.*, 2001; Walch *et al.*, 2008). It allows characterization of spatial distribution of a wide spectrum of components (such as proteins, peptides, lipids, glycanes, hormones, secondary metabolites) *in situ* in a crude tissue material label free, without laborious procedures of processing histochemical slides, obtaining antibody and staining. The pioneering applications of MALDI-MSI for analysis of specific peptides in whole cells were performed on neuronal tissue of snails (Jimenez *et al.*, 1994; Dreisewerd *et al.*, 1997). Nearly in that period, the effectiveness of MALDI-MSI usage in fresh tissue sections was proved (Caprioli *et al.*, 1997). Since then MALDI-MSI became a routine method in neuroscience, plant science, cancer-biology, biomedicine, including clinical and preclinical studies (*e.g.*, Baluff *et al.*, 2011; Salzet *et al.*, 2012). Recent advances include *in situ* trypsinization for protein profiling, even in formalin-fixed tissues (De Sio *et al.*, 2015), the use of specialized matrices for small molecules (Shanta *et al.*, 2012) and *in situ* derivation for direct detection of some neuromediators (Shariatgorji *et al.*, 2015). However, the applications of MALDI-MSI for investigation of endogenous peptides/proteins or metabolites in invertebrates are still not so numerous (*e.g.,* Esquenazi *et al.*, 2008; Bruand *et*

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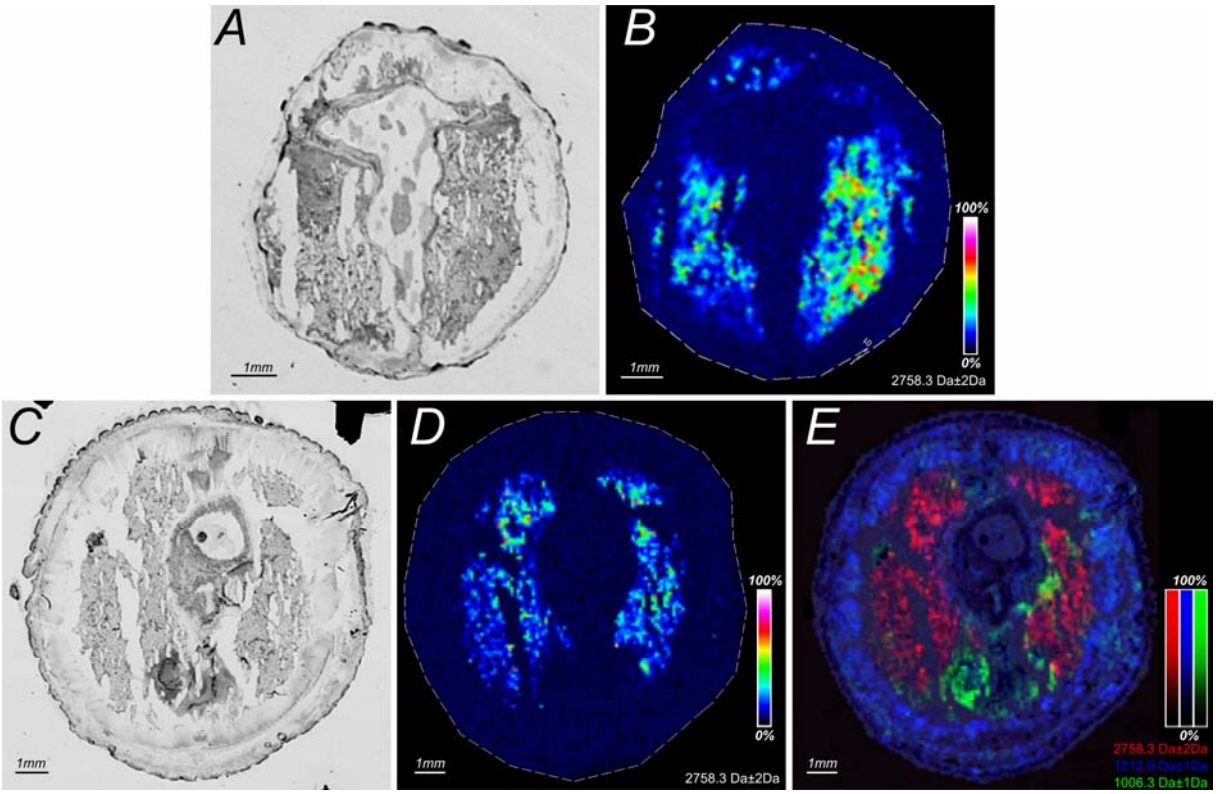

Fig. 1 MALDI-MSI visualization of arenicins (2,758.3 Da ± 2Da) in anterior (A, B) and main body (C, D, E) parts. A, C - densitometry imaged slices; B, D - arenicin-positive MS-signal in anterior or main body parts, respectively; E - representation of principal body compartments by filtered MS-signals: celomic fluid (arenicins 2,758.3 Da ± 2Da, red channel), epithelium of body wall (m/z 1,212.9 ± 1, blue channel) and celothelium (1,006.3 ± 1, green channel).

al., 2011); in respect to AMPs of invertebrates they are unique (Kuhn-Nentwig *et al.*, 2014).

This motivated us to examine the applicability and informative value of MALDI-MSI for characterization of distribution of AMPs (arenicins) in the marine invertebrate *Arenicola marina*.

Material and Methods

Animals

Adult lugworms (approx. 15 cm length) *Arenicola marina* were collected from wild populations in the intertidal zone in the vicinity of the White Sea Marine biological station of Saint Petersburg University (Russia). Animals were maintained in permanently aerating static tanks with marine water for 5 - 7 days.

Tissue preparation

For preparation of cryo-slides worms were frozen in liquid nitrogen after being anesthetized in 5 % $MgCl_2$ in sterile marine water. Immediately after freezing, cross-sections (15 µm thick) of anterior (first chaetigerous segments) and middle body (gill-carrying segments) parts were prepared with Cryomicrotome (Leica CM 3050S) at -18 °C. Sections were thaw mounted onto indium tin oxide coated glass slides and drayed under vacuum in Labconco SpeedVac for 30 min. Fiducial points for microscopic and MALDI-MSI co-registration were drawn with Edding 750 white marker and preparations were subsequently imaged with Bio-Rad GS-800 calibrated densitometer at 600 dpi. For calibration purposes, Bruker Peptide Calibration Standard II was deposited near feducual points. Sections were matrix-coated in automated matrix sprayer Bruker ImagePrep (program version 2.0.1) using build-in “HCCA_nsh04” protocol with matrix solution containing 7 g/l a-Cyano-4-hydroxy-cinnamic acid (CAS 28166-41-8), 50 % v/v acetonitrile and 0.2 % v/v trifluoroacetic acid.

MALDI-pictures obtaining and processing.

Spectra were obtained on MALDI-TOF Bruker Ultraflextreme mass-spectrometer with Bruker FlexControl v. 3.3 and FlexImaging v. 3.0 software. Laser intensity was set to 60 %, laser spot size was set to “small”, raster size 100 µm. Actual laser spot size measured by matrix ablation was around 10 µm. Spectra were collected in reflected mode with positive polarity, mass window 700 - 3,500 Da, pulsed ion extraction delay 80 ns, matrix ions with masses less than 700 Da were deflected. The preparation was scanned in bidirectional mode with 1000 shoots per pixel and random walk pattern inside pixel. MALDI-MSI and optical images were aligned in FlexImaging by previously marked fiducial points. Method was calibrated on spots previously

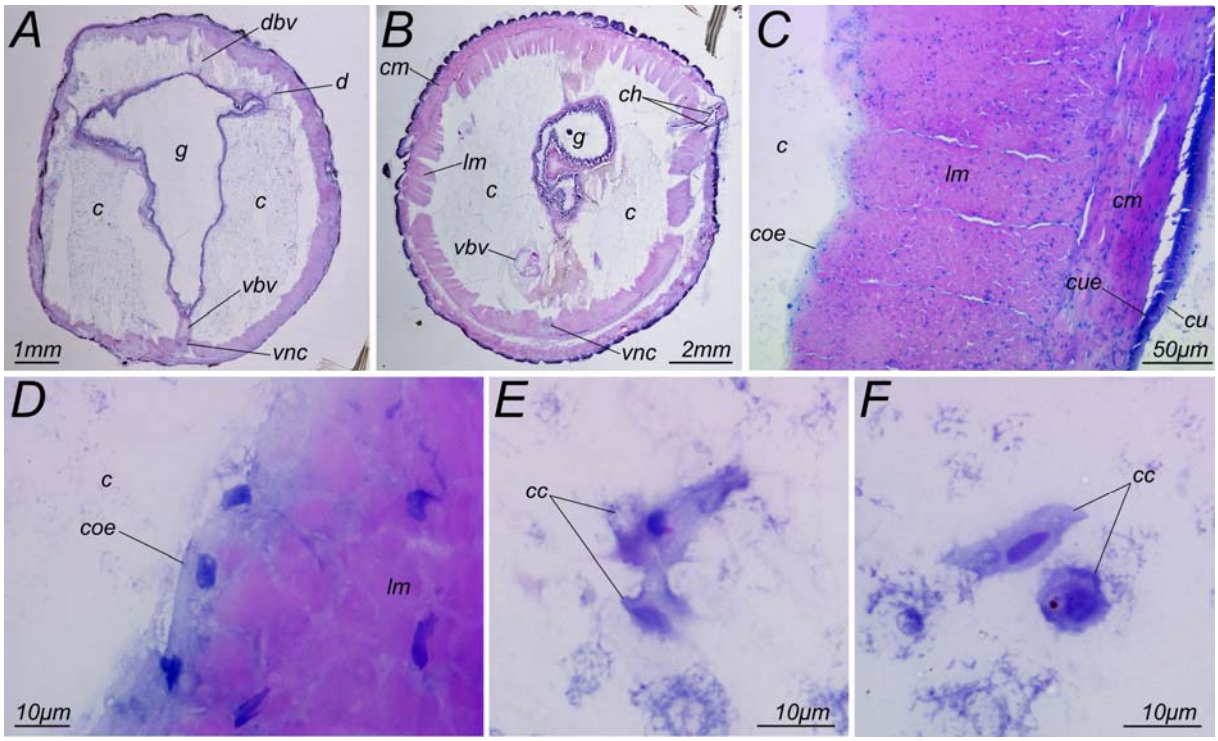

Fig. 2 Hematoxylin-eosin staining after MALDI-imaging. A, B - cross-sections through anterior (A) and middle (B) parts of the body; C - fragment of the body wall; D - longitudinal body musculature and celothelium; E, F - celomocytes. Abbreviations: c - celomic cavity, cc - celomocytes, ch - chaetae, cm - circular body musculature, coe - celomic epithelium, cu - cuticle, cue - cuticular epithelium, d - dissepiment, dbv - dorsal blood vessel, g - gut, lm - longitudinal body musculature, vbv - ventral blood vessel, vnc - ventral nerve cord.

deposited with Bruker Peptide Calibration Standard II. Spectra were preprocessed by baseline subtraction using TopHat algorithm, no smoothing was done, spectra were normalized by total ion count (TIC), peak-picking was done with SNAP algorithm.

Histology and light microscopy

After MALDI imaging slides were washed in ethanol to remove the matrix, stained with Ehrlich's hematoxylin-eosin following standard protocol and mounted in DPX mounting medium. The slides were examined with Leica DM2500 light microscope equipped by Leica DFC495 camera.

Results and Discussion

Arenicins are short (21 residue peptides, 2.8 kDa) peptides with strong antimicrobial activity, originally isolated from motile phagocytic cells of celomic fluid - celomocytes (Ovchinnikova *et al.*, 2004). The spatiotemporal pattern of arenicins expression in lugworm body was later thoroughly characterized with immunohistochemistry and semi-quantitative RT-PCR. Although the expression of arenicins takes place in different compartments, the strongest signal was detected in cells of celomic fluid (Maltseva *et al.*, 2014). This allows to compare present results with earlier work to determine the applicability and scope of the approach chosen here.

MALDI-MSI is used in either linear or reflected mode, the later with higher resolution. Arenicins are short enough to be analyzed in reflected mode of TOF/MS with resolution up to 20,000. Identification of arenicins was done by previously known by LC-ESI-qTOF mass of 2,758.3 Da with mass window ± 2Da (Figs 1B, D, E - red channel). For better structure representation automatic mass list filtering was done revealing two m/z of 1,212.9 ±1 and 1,006.3 ±1 colocalized with epithelium of body wall and celothelium respectively (Fig.1E - blue and green channels).

Correspondence of MS-signals to particular tissues/cells was established on the basis of histological staining of MALDI-scanned slides (Fig. 2). The staining indicates acceptable preservation of all compartments from which reliable signals were detected - epithelium and musculature of the body wall (2C), celothelium (2D) and its derivates, gut (2A, B), celomocytes (2E, F).

Figure 1 shows that arenicin-positive signal in both anterior (1a, b) and main body (1C, D, E) parts was detected in celomic fluid, where strong arenicins expression was described. No specific signal was obtained from other body compartments, where arenicins were reported to be expressed (epithelium of the body wall and gut, extravasal

tissue, cuticle, ventral nerve cord (Maltseva *et al.*, 2014).

The result obtained with MALDI-MSI only partially reproduced the previously reported data. There could be several possible not mutually exclusive explanations for this.

(1) Differential abundance of arenicins in different tissues. Previous results demonstrated that the arenicins are more abundant in celomocytes than in other cells types (Maltseva *et al.*, 2014). The strength of the signal from body cavity was not maximal, but moderate (which could be the negative consequence of salt presence in tissues of marine animals). This allows to suspect that in the other compartments the abundance of arenicins was below the limit of detection. Similarly, only AMPs ctenidins highly abundant within granules of spider hemocytes were detectable by MALDI-MSI, but not defensins, stored in small amounts (Kuhn-Nentwig *et al.*, 2014).

(2) Differential accessibility of arenicins in different tissues. Celomocytes of polychetes are rather fragile cells. Thus, they are easily destroyed during contact with exogenous material as was reported, *e.g.,* for celomocytes of *Nereis diversicolour* during the process of implant encapsulation (Porchet-Hennere *et al.*, 1987). This is also the case for *A. marina* celomocytes (our own observation). So, possibly arenicins are more easily extracted into the matrix from celomocytes than from other tissues - this makes them detectable in these cells. This explanation does not exclude the first one.

Conclusion

The first application of MALDI-MSI for study AMPs in marine invertebrates was performed and reported here. The obtained data additionally confirm that celomocytes are the major compartment of arenicins expression. Although the present results only partially reproduced the output of alternative methods, the main compartment of arenicins expression was correctly detected. Consequently, MALDI-MSI can be successfully used as primary rough express screening approach for characterization of distribution of AMPs in tissues of marine invertebrates.

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References

Balluff B, Schöne C, Höfler H, Walch A. MALDI imaging mass spectrometry for direct tissue analysis: technological advancements and recent applications. Histochem. Cell Biol. 136: 227-244, 2011.

Boman HG. Antibacterial peptides: basic facts and emerging concepts. J. Internal. Med. 254: 197-215, 2003.

Bruand J, Sistla S, Mériaux C, Dorrestein PC, Gaasterland T, Ghassemian M, *et al*. Automated querying and identification of novel peptides using MALDI mass spectrometric imaging. J. Proteome Res. 10: 1915-1928, 2011.

Caprioli RM, Farmer TB, Gile J. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. Analyt. Chem. 69: 4751-4760, 1997.

De Sio G, Smith AJ, Galli M, Garancini M, Chinello C, Bono F, *et al*. MALDI-Mass Spectrometry Imaging method applicable to different formalin-fixed paraffin-embedded human tissues. Mol. Biosyst. 11: 1507-14, 2015.

Dreisewerd K, Kingston R, Geraerts WP, Li KW. Direct mass spectrometric peptide profiling and sequencing of nervous tissues to identify peptides involved in male copulatory behavior in *Lymnaea stagnalis*. Int. J. Mass Spectrom. Ion Processes 169: 291-299, 1997.

Esquenazi E, Coates C, Simmons L, Gonzalez D, Gerwick WH, Dorrestein PC. Visualizing the spatial distribution of secondary metabolites produced by marine cyanobacteria and sponges via MALDI-TOF imaging. Mol. BioSyst. 4: 562-570, 2008.

Harder J, Schröder JM (eds) Antimicrobial peptides: role in human health and disease, Springer International Publishing, Switzerland, 2016.

Jimenez CR, Van Veelen PA, Li KW, Wildering WC, Geraerts WPM, Tjaden UR, *et al.* Neuropeptide expression and processing as revealed by direct matrix-assisted laser desorption ionization mass spectrometry of single neurons. J. Neurochem. 62: 404-407, 1994.

Kuhn-Nentwig L, Kopp LS, Nentwig W, Haenni B, Streitberger K, Schürch S, *et al.* Functional differentiation of spider hemocytes by light and transmission electron microscopy, and MALDI-MS-imaging. Dev. Comp. Immunol. 43: 59-67, 2014.

Maltseva AL, Kotenko ON, Kokryakov VN, Starunov VV, Krasnodembskaya AD. Expression pattern of arenicins - the antimicrobial peptides of polychaete *Arenicola marina*. Front. Physiol. 5: 497-503, 2014.

Otero-González AJ, Magalhães BS, Garcia-Villarino M, López-Abarrategui C, Sousa DA, Dias SC, *et al*. Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. FASEB J. 24: 1320-1334, 2010.

Ovchinnikova TV, Aleshina GM, Balandin SV, Krasnosdembskaya AD, Markelov ML, Frolova EI, *et al*. Purification and primary structure of two isoforms of arenicin, a novel antimicrobial peptide from marine polychaeta *Arenicola marina*. FEBS Lett. 577: 209-214, 2004.

Pasupuleti M, Schmidtchen A, Malmsten M. Antimicrobial peptides: key components of the innate immune system. Crit. Rev. Biotechnol. 32: 143-171, 2012.

Porchet-Henneré E, M'Berri M, Dhainaut A, Porchet M. Ultrastructural study of the encapsulation response of the polychaete annelid *Nereis diversicolor*. Cell Tissue Res. 248: 463-471, 1987.

Reddy KVR, Yedery RD, Aranha C. Antimicrobial peptides: premises and promises. Int. J. Antimicrobial Agents 24: 536-547, 2004.

Salzet M, Mériaux C, Franck J, Wistorski M, Fournier I. MALDI imaging technology application in neurosciences: from history to perspectives. In: Karamanos Y. (ed.), Expression profiling in neuroscience, neuromethods, Springer Science, pp 181-223, 2012.

Shanta SR, Kim TY, Hong JH, Lee JH, Shin CY, Kim KH, *et al*. A new combination MALDI matrix for small molecule analysis: application to imaging mass spectrometry for drugs and metabolites. Analyst 137: 5757-5762, 2012.

Shariatgorji M, Nilsson A, Källback P, Karlsson O, Zhang X, Svenningsson P, *et al.* Pyrylium salts as reactive matrices for MALDI-MS imaging of biologically active primary amines. J. Am. Soc. Mass Spectrom. 26: 934-939, 2015.

Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM. Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. Nat. Med. 7: 493-496, 2001.

Walch A, Rauser S, Deininger SO, Höfler H. MALDI imaging mass spectrometry for direct tissue analysis: a new frontier for molecular histology. Histochem. Cell Biol. 130: 421-434, 2008.

Yount NY, Bayer AS, Xiong YQ, Yeaman MR. Advances in antimicrobial peptide immunobiology. Pept. Sci. 84: 435-458, 2006.