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MAPPING STEROLS IN A MARINE FLATWORM-ALGAE-SYSTEM USING MALDI-2 TIMS MS-IMAGING

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Introduction

Waminoa sp. acoel flatworms hosting *Symbiodiniaceae* and the related *Amphidinium* dinoflagellate algae are an interesting model system for symbiosis in the marine environment. While the host provides a microhabitat and safety, the algae power the system by photosynthesis. In other related symbioses such as in corals, a "currency" in symbiosis is known to be the transfer of sterols between guest and host, including cholesterol and numerous phytosterols. These compounds are produced by the symbiotic dinoflagellates, but their transfer to and fate within the sterol-auxotrophic *Waminoa* worm host as well as their role in its metabolism are unknown.

For the analysis of sterols, mass spectrometry (MS) is one of the most widely used techniques and more recently, MALDI-MS has been employed. However, poor ion yields complicate or even hinder the analysis of neutral sterols like cholesterol. Thus, to improve the ion yields for sterols in a MALDI-MS imaging experiment, laser post-ionization (MALDI-2) is employed.

In addition to the low sensitivity, another challenge lies in the annotation of the different known species due to the common occurrence of isomeric ion species in the natural sterol diversity. A powerful extension of MS imaging is the on-line combination with trapped ion mobility spectrometry (TIMS), because it introduces an additional layer of separation in analyzing highly complex samples. With the ability of TIMS, ions can be separated based on their collisional cross section prior to MS analysis.

Methods

Symbiotic systems of *Waminoa* sp. flatworms hosting intracellular symbiotic *Amphidinium* and *Symbiodiniaceae* dinoflagellates were cultured in artificial seawater aquaria. They were embedded in a mixture of 5% 2-hydroxyethylcellulose / 10% gelatin prior to cryosectioning to 14 µm thickness. DHAP matrix was applied by sublimation.

MALDI-2-MS images were acquired on a timsTOF fleX that has been modified to allow for MALDI-2 at 266 nm and 1 kHz repetition rate (10 µs delay) at 5 µm pixel size. For TIMS imaging $1/K_0$ values were measured between 0.6 and 1.6 with a ramp time of 659 ms. Data analysis was performed using SCiLS Lab (2020b) and TDF viewer software.

Results

Characteristics of Sterol Signals Detected with MALDI-2 TIMS MSI

In positive ion mode and using MALDI-2, sterols are predominantly detected as protonated species with the loss of water ($[sterol\text{-}H_2O+H]^+$). Sterol signals are expected in the *m/z*-region between about *m/z* 250-500. MALDI-2-MSI spectra generated directly from tissue in this *m/z*-region are generally highly complex due to matrix-, tissue- and fragment-derived signals. Therefore, TIMS is included as an additional orthogonal separation technique to aid with tentative assignment of molecular IDs. Using TIMS, these sterol ion species produce characteristic peculiar feature shape in the mobility dimension. Compared to phospholipid or matrix-derived ions, peak shapes are considerably broadened for all tested sterols. This characteristic feature shape appears exclusive to sterols as demonstrated in Fig. 1.

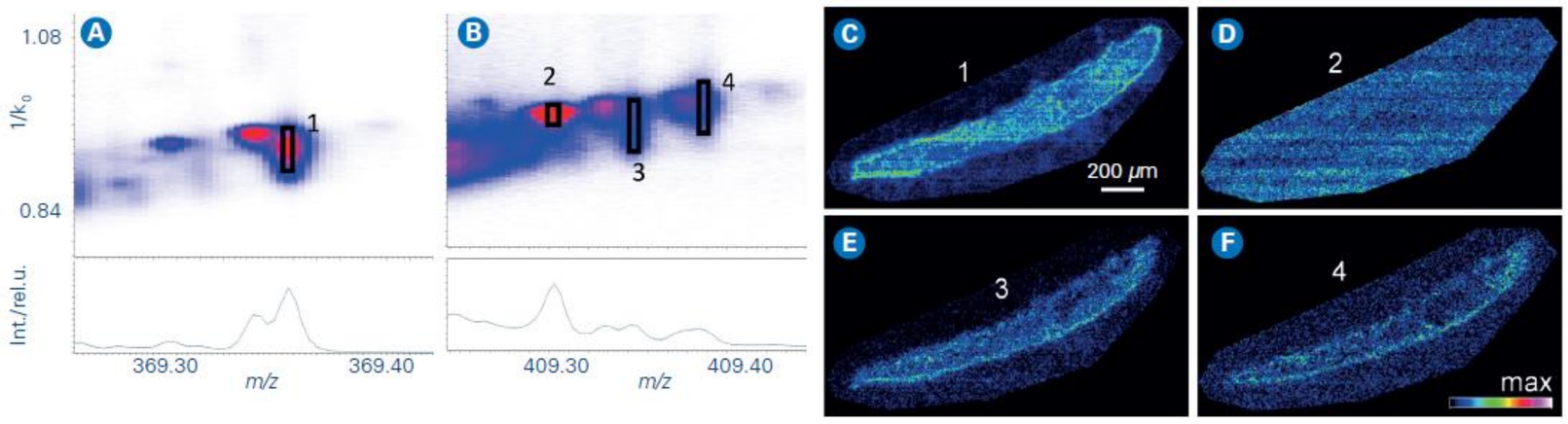

Fig. 1. TIMS heatmaps of cholesterol (A, 1), sterol $C_{29}H_{46}O_2$ (B, 3) and gorgosterol (B, 4). Also shown is an exemplary feature assigned to matrix-derived chemical noise (B, 2). C-F show the respective ion images. Other than sterols, matrix- and non-sterol derived endogenous signals showed compact features.

MS imaging of Sterol Species Detected from the Symbiotic System

Typical dimensions of *Waminoa* flatworms are 4 mm length, 2 mm width, and 200 µm thickness. Imaging at 5 µm resolution allows for identifying the algae and their direct surroundings as the dinoflagellate cells have diameters of approx. 9-13 µm. Apart from the variety of sterols, numerous signals are identified in the MSI outlining the morphology of the flatworm.

Fig. 2 d-f shows the distribution of cholesterol ($C_{27}H_{46}O$), stigmasterol ($C_{29}H_{48}O$), and saringosterol ($C_{29}H_{48}O_2$) in three sections of three individual host flatworms recorded using MALDI-2-MSI. The approximate positions of the sections and a schematic of the dinoflagellate symbionts within the animal are shown in Fig. 2A. While cholesterol is distributed almost homogenously throughout the tissue, the cross-section through the center of one of the animals (top) reveals a higher concentration of stigmasterol within an area corresponding to the syncytial gut, possibly containing gonads. Saringosterol is similarly distributed to cholesterol in the center of the animal but shows depleted signal intensity in the outer parts.

Interestingly, three groups of spatial features, putatively assigned to the single-cellular algae by their shape and size, can clearly be discerned based on signals correlating with the dinoflagellates (Fig. 2C, insets). The most intense of these signals can be assigned to the photosynthetic molecule chlorophyll *a*, which appears to undergo in-source fragmentation.

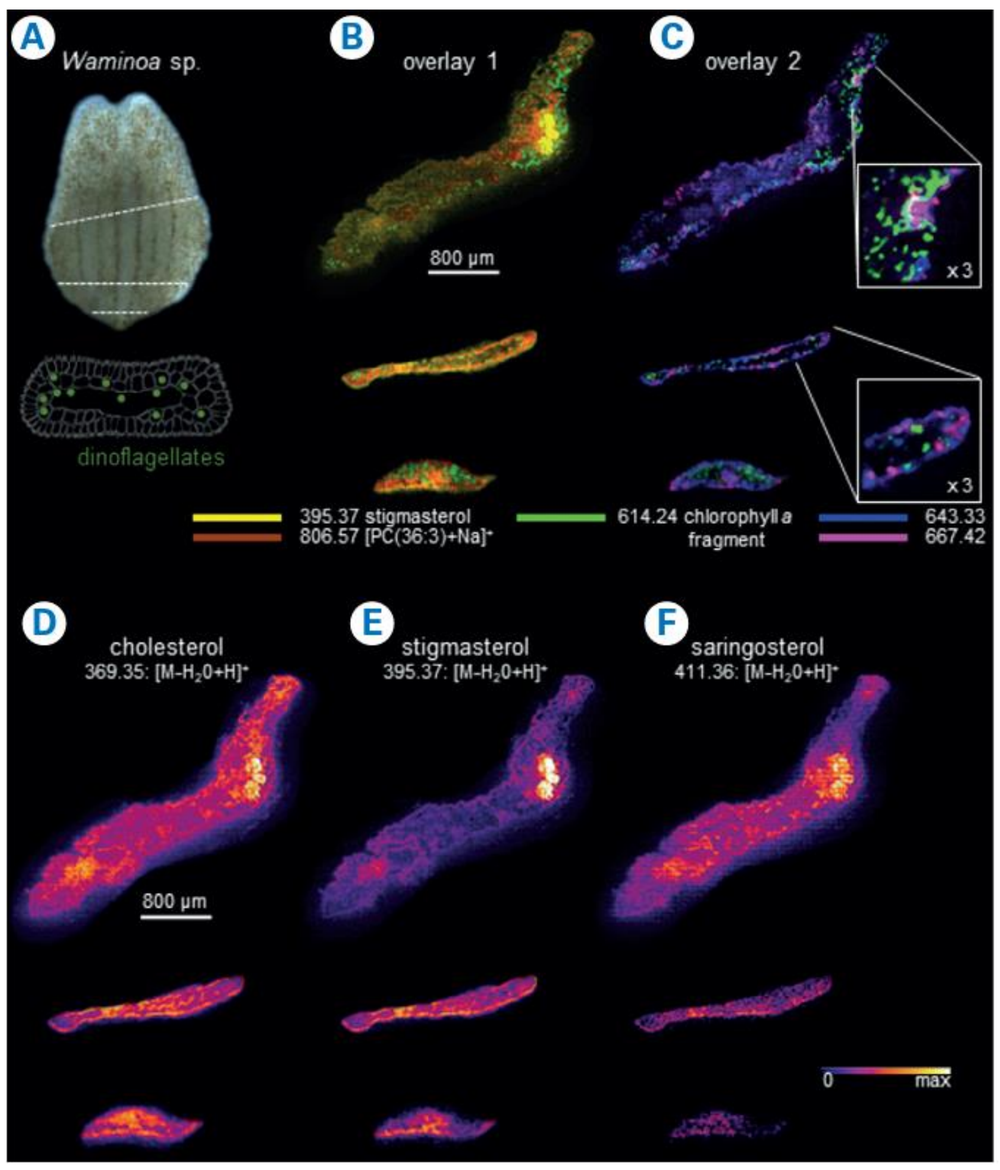

Fig. 2. MALDI-2 MSI of three *Waminoa* flatworms. (A) *Waminoa* flatworm with approx. positions of the sections, and schematic of a cross-section with symbiotic dinoflagellates. (B) Overlay of stigmasterol (yellow) with PC(36:3) (brown), and a chlorophyll *a* fragment (green), representative of the two algae species. (C) Overlay of the chlorophyll *a* fragment and two unknown species. MALDI-2 MS images of cholesterol (D), stigmasterol (E), and saringosterol (F).

This work demonstrated that the distribution of symbiont-produced sterols in Waminoa flatworms can be visualized at high spatial resolution. In addition, the transfer of sterols between the host and symbionts can be easily seen.

Conclusion

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