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Molecular imaging of alkaloids in khat (*Catha edulis*) leaves with MeV-SIMS

Boštjan Jenčič [a,*], Luka Jeromel [a], Nina Ogrinc Potočnik [a,b], Katarina Vogel-Mikuš [a,c], Primož Vavpetič [a], Zdravko Rupnik [a], Klemen Bučar [a], Matjaž Vencelj [a], Mitja Kelemen [a], Jiro Matsuo [d], Masakazu Kusakari [d], Zdravko Siketić [e], Muhammad A. Al-Jalali [f], Abdallah Shaltout [f,g], Primož Pelicon [a]

[a] *Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*
[b] *M4I Institute, Maastricht University, 6229 ER Maastricht, The Netherlands*
[c] *University of Ljubljana, Biotechnical Faculty, Dept. of Biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia*
[d] *Quantum Science and Engineering Center, Kyoto University, Uji, Kyoto, Japan*
[e] *Ruđer Bošković Institute, P.O. Box 180, 10000 Zagreb, Croatia*
[f] *Physics Department, Faculty of Science, Taif University, 21974 Taif, P.O. Box 888, Saudi Arabia*
[g] *Spectroscopy Department, Physics Division, National Research Center, El Behooth Str., 12622 Dokki, Cairo, Egypt*

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ABSTRACT

Imaging Mass Spectroscopy (IMS) is a unique research tool providing localization and identification of a wide range of biomolecules as essential data to understand biochemical processes in living organisms. Secondary Ion Mass Spectrometry with high-energy heavy ions (MeV-SIMS) is emerging as a promising IMS technique for chemical imaging of biological tissue.

We measured the molecular mass spatial distributions in leaves of khat (*Catha edulis*). Khat is a natural drug plant, native to eastern Africa and the Arabian Peninsula. In these countries, fresh leaves are being chewed by significant part of population. It was reported that 80% of the adult population in Yemen chew the khat leaves. The main stimulating effects of khat are induced by a monoamine alkaloid called cathinone. During leaf ageing, cathinone is further metabolised to cathine and norephedrine. Earlier studies identified the alkaloids in khat, however little is known on their spatial distribution, reflecting the biosynthesis and accumulation in the tissue.

Chemical mapping of alkaloids on cross-sections of khat leaves by MeV-SIMS was done at JSI by a pulsed 5.8 MeV $^{35}Cl^{6+}$ beam, focused to a diameter of 15 μm, using a linear time-of-flight (TOF) spectrometer with a mass resolution of 500. In addition, measurements of MeV-SIMS mass spectra were performed at Kyoto University by a continuous broad beam of 6 MeV $^{63}Cu^{4+}$ ions at an orthogonal TOF spectrometer with a high mass resolution of 11,000. Sections of leaves were analysed and mass spectra obtained at both MeV-SIMS setups were compared. Tissue-level distributions of detected alkaloids are presented and discussed.

1. Introduction

Molecular imaging plays an important role in modern biomedical research, providing both identification and lateral distribution of molecules in living organisms. Among the techniques of Imaging Mass Spectroscopy (IMS), the Secondary Ion Mass Spectrometry [1,2] with high-energy heavy ions (MeV-SIMS) [3] is emerging as a novel tool for molecular imaging at intact, chemically non-processed sections of biological materials. In contrast with the conventional Secondary Ion Mass Spectroscopy (SIMS) method, which uses ions with energies ranging between 0.5 and 30 keV, it provides high yields of non-fragmented molecular ions due to specific desorption process during the collision of swift ion with insulating material, governed by electron stopping process [4,5].

There are also several other molecular imaging techniques, such as MAALDI (Matrix-assisted laser desorption/ionization) and DESI (Desorption electrospray ionisation), which have their own advantages over conventional SIMS method. However, the application of matrix for MAALDI method results in diffusion of analyst and the method is therefore not as suitable for imaging of organic tissues as SIMS. DESI can be more widely used for larger scale samples,

* Corresponding author.
E-mail address: bostjan.jencic@ijs.si (B. Jenčič).

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due to much poorer imaging capabilities (sub micron resolution with SIMS and 200–500 μm resolution with DESI) [6].

MeV-SIMS imaging method has been developed at Jožef Stefan Institute (JSI) nuclear microprobe during the last five years as a complementary molecular imaging method [7,8] to the elemental imaging executing extensively for biomedical community by micro-PIXE [9–13].

The 2 MV tandetron accelerator at JSI is providing ^{35}Cl ions with charge states of 5+, 6+ or 7+, with the resulting energies between 5 MeV and 12 MeV. The charge state and the corresponding energies of chlorine ions are selected for MeV-SIMS in compromise between the demand for magnetic rigidity low enough to enable focusing with Oxford Microbeam triplet lens, yet high enough electronic stopping to enable optimal MeV-SIMS conditions characterized by soft sputtering process and high yields of non- fragmented molecular ions. Standard mode of operation provides a pulsed primary ion beam, which bombards the sample residing in vacuum of about 5×10^{-7} mbar. The pulsing is achieved by parallel plate deflector, with one of the plates constantly biased at 900 V, while the other is switching from 0 to 900 V for short periods, resulting in effective pulse duration of appox. 30 ns. Frequency of pulses is 10 kHz. The start of the voltage pulse also serves as the reference for measuring the time-of-flight of the sputtered molecular ions. Their velocity depends on the applied bias on the sample holder, which is usually set to 3 kV. The TOF telescope operates reliably with the bias values between 300 V and 5 kV in a positive extraction mode, as well as with biases of −2 kV to −5 kV in a negative extraction mode. The MCP detector at the end of 1 m long drift tube provides the stop signal for time-of-flight measurement. The Einzel lens, installed at the beginning of the drift tube, focuses the secondary ions to the MCP detector and enhances the secondary ion yield by a factor of 10 in comparison with the operation without bias.

The pulsed-beam operating mode of MeV SIMS enables chemical mapping of biological samples at moderate lateral resolution of 5 μm × 5 μm for $^{35}Cl^{5+}$ primary ions and only to 15 μm × 15 μm for more energetic $^{35}Cl^{7+}$ ions due to lower beam flux and, correspondingly, widely opened slit system at the microprobe. The mass resolution is limited mainly by the duration of the pulse, and the typical values are approximately m/dm = 500 for molecules with m/z between 100 u and 500 u. The upper mass limit of non-fragmented detected molecule is not determined yet. Heaviest identified non-fragmented molecular ion is currently angiotensin II (human) with a mass of 1046 u.

2. Khat imaging

Catha edulis Forsk., with a common name of khat, is a flowering plant native in the east Africa and the Arabian Peninsula. In both areas, khat chewing has a history as a social custom [14,15], dating back thousands of years, especially in Djibouti, Kenya, Ethiopia, Somalia and Yemen, where khat is not considered a drug of abuse and is therefore legal.

The main stimulant in khat is a monoamine alkaloid cathinone (Fig. 1), which causes mild-to-moderate dependence (less than tobacco or alcohol) [16,17]. Cathinone (m/z = 149.2), however, is a very unstable molecule and quickly beaks down producing cathine and norepinephrine (m/z for both 151.2). In dried khat leaves, cathinone decomposes within 48 h, leaving behind the milder chemical, cathine [18–20].

The khat leaves tissue was prepared for chemical imaging by MeV-SIMS at Biotechnical faculty of University of Ljubljana. Small pieces of fresh leaf tissue were embedded into Tissue-Tek (Leica), shock frozen in propane cooled with liquid nitrogen [21], and cut into 20 μm thick slices, freeze-dried and deposited on blank silicon wafer.

The spectra show presence of typical molecules for organic samples (Fig. 2). As usually found in positive MeV-SIMS spectra of plant tissue, we observed abundance of Na^+ (m/z = 23) and K^+ (m/z = 39) ions, which can be used in imaging mode to recognize the tissue morphology, as well as to distinguish it from supporting media, such as Tissue-Tek and other sample preparation substances.

Significant differences were observed between the spectra, obtained at JSI with focused (7 × 7 μm) $^{35}Cl^{5+}$ beam at 5 MeV with mass resolution m/dm = 500, and the spectra from Kyoto, where broad continuous 6 MeV $^{63}Cu^{4+}$ beam was applied over khat leaf tissue with much better mass resolution than the one of JSI (around m/dm = 11,000). The Kyoto measurements were also performed under different pressure conditions. The sample was residing in a very rough vacuum of about 20 mbar. The pressure is then decreasing along the ToF tunnel, reaching 10^{-7} mbar in the orthogonal ToF analyser. Therefore, the differences between the two spectra might be partially attributed to different vacuum conditions.

However, the different intensity of several peaks, such as m/z = 28–34 and m/z = 90 might be mainly attributed to simultaneous coverage of the leaf tissue and its surrounding media composed of Tissue-Tek. The spectra obtained at JSI feature higher yield of sample related molecules, such as Na, K and others. Set of peaks at m/z = 147, m/z = 222 and m/z = 282 is a result of well-known and undesired effect of PDMS contamination of our samples [22]. In order to get rid of these contaminating peaks, we recently switched to the use of PDMS-free sample containers.

The detailed look into spectra (Fig. 3) shows, that although cathinone is expected to decompose within 48 h after drying leaves of Khat, its peak is still significant, much higher than the one of cathine. The sample protocol included shock freezing and freeze-drying which apparently enabled good preservation of molecular structure when compared to air drying the plant shoots undergo quickly after collection from the plant and selling at the market. The spectra from Kyoto show the presence of peak at m/z = 148, while the Cathine peak is insignificant. The JSI results also show the dominating peak of protonated cathinone molecule at m/z = 150, while they also hint the presence of molecules with mass 152, which corresponds to the protonated cathine molecule.

The difference between Kyoto and JSI spectra might be also explained by broad Kyoto beam, which irradiated more back-

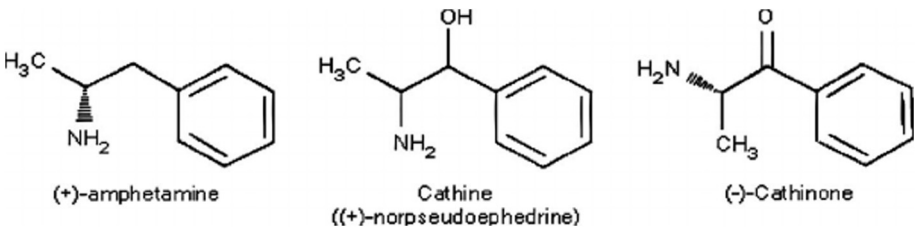

Fig. 1. Chemical structures of basic molecule, amphetamine (m = 135.2 u) and its derivatives, Cathine (m = 151 u) and Cathinone (m = 149.2 u) [19].

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