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Biological tissue imaging with a hybrid cluster SIMS quadrupole time-of-flight mass spectrometer

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ABSTRACT

A 20 keV C_{60}^+ ion source was mounted onto a commercial MALDI/electrospray orthogonal ToF mass spectrometer. Cross-sectional mouse brain and lung slices between 5 and 10 μm prepared by cryostat sectioning were successfully imaged using a DC C_{60}^+ primary ion beam at a spot size of 100 μm. Analysis was performed at room temperature following vacuum drying. An abundance of ions were mapped in all samples, many whose identity can only be found using the MS/MS functionality. We have successfully identified and imaged localizations of diacylglycerol (DAG) ions – 1-palmitoyl-2-oleoyl-glycerol (m/z^+ 577.5) and 1,2-dioleoyl-glycerol (m/z^+ 603.5) – in lung tissue. The mouse brain slice revealed strong, distinct localizations of many ions revealing the potential for this technique for biological imaging. Ions throughout the mass range of m/z^+ 50–800 were collected in sufficient quantities to permit unambiguous chemical mapping. Mass resolutions of 12,000 or greater were routinely obtained allowing for more accurate ion mapping than typically seen with ToF-SIMS image analysis.

1. Introduction

Molecular imaging of biological samples with SIMS is particularly challenging due to the intrinsic complexity of the mass spectra associated with biomaterials and to the fact that many key components are present in low concentrations. The emergence of cluster ion sources has certainly allowed the quality of the spectra and the sensitivity limits to be greatly improved [1], but detailed interpretation of spectra as well as low secondary ion flux remains problematic. Here we investigate the utility of a novel imaging SIMS instrument employing a cluster ion source on a hybrid quadrupole orthogonal time-of-flight analyzer. This combination enables the cluster ion beam to operate in a DC mode, taking advantage of the cluster ion sources ability impart less surface damage to the sample when compared to liquid metal ion sources [1]. This can increase the incident ion fluence by four orders of magnitude over traditional ToF experiments resulting in significantly higher secondary ion counts provided the sample thickness allows it. In addition, this instrument provides tandem mass spectrometry capability for identification of unknown mass peaks. In the current state, this instrument has a secondary ion transmission efficiency approaching that of a high performance ToF-SIMS instrument, yet allows mass resolution greater than 12,000 with a few ppm mass accuracy. The high mass resolution is valuable in separating meaningful peaks from background ions as we have demonstrated previously with polydimethylsiloxane (PDMS) contaminant at m/z^+ 147.065 and an important cholesterol fragment at m/z^+ 147.117 [2]. We illustrate the capabilities of this approach by imaging a variety of mass ions associated with lung and rat brain slices over several mm^2 fields of view.

2. Experimental

2.1. Instrumentation

The front end of a QSTAR® XL system, a hybrid LC/MS/MS instrument originally designed for MALDI and electrospray ionization mass spectrometry (Applied Biosystems/MDS Sciex) was modified to fit a 20 keV C_{60}^+ source by Ionoptika Ltd. Details of the QSTAR® XL system and the 20 keV C_{60}^+ source can be found elsewhere [2–4, this issue]. Briefly, the QSTAR® XL system is a tandem quadrupole orthogonal time-of-flight mass spectrometer.

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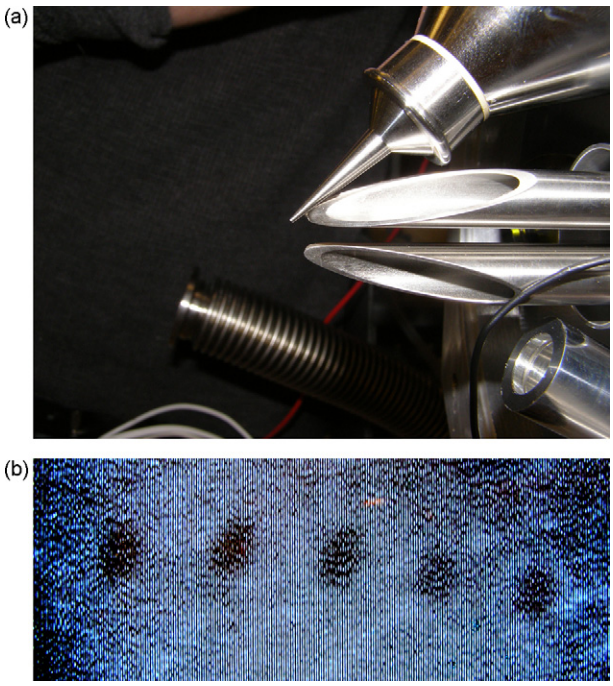

Fig. 1. (a) Nose cone modification to the C_{60}^+ ion source to reduce the distance travelled by the primary ions through high N_2 pressure. The sample is situated approximately 4 mm in front of the nose cone when the MALDI attachment is in place. (b) Optical image of sputter craters formed by C_{60}^+ at, from left to right, 5×10^{-5}, 1×10^{-3}, 3×10^{-3}, 5×10^{-3}, and 8×10^{-3} Torr. Craters are approximately 100 μm in diameter.

In this instrument, N_2 gas is used for collisional cooling and collisional focusing. The pressure of N_2, on the order of 5 mTorr near the sample and collisional focusing quadrupole region (Q_0), is critical for efficient operation [5,6]. In this configuration, the ion source operates efficiently with an extraction potential of only a few volts. The instrument is capable of MS/MS experiments as well as ion trapping for increased sensitivity.

Imaging is accomplished through movement of the sample stage up to 50 mm in either direction either in a stepped motion down to 10 μm or in a continuous motion. During stepped stage motion, the ion source beam is pulsed off with each stage movement. Data analysis is handled by Analyst® QS 2.0 software and sample stage motion is controlled by oMALDI™ Server 5.0 software, both by Applied Biosystems/MDS Sciex. Further image processing was completed with BIOMAP version 3.7.5.4 software.

The C_{60}^+ ion source for these experiments was operated with 10–15 pA current on the sample in DC mode, however the current can be reduced to static levels if sample damage or depletion is a concern. In order to minimize C_{60}^+ collisions with N_2, the beam region of the source was fitted with a sleeve that facilitated differential pumping to below 1×10^{-6} Torr. Estimations based on the diameter of C_{60}^+, as well as work done by others shows that C_{60}^+ remains largely intact at this pressure [7]. In addition, an extended nose cone with a 100-μm final aperture was fabricated to reduce the distance between the ion source and the sample to approximately 4 mm and to aid in maintaining relatively low pressures in the beam region. This configuration allows a maximum of several hundred pA of primary beam current to be delivered to the target with a spot size that is adjustable from 30 to 200 μm. While this ion source was designed to be capable of a 3-μm beam size, the larger beam size described in this prototype instrument is due primarily to the change in geometry between the sample and the final focusing lens brought about by the addition of the narrow nose cone. It is expected that this number can be significantly reduced by alteration of the final focusing lens as well as adjustment of the working distance.

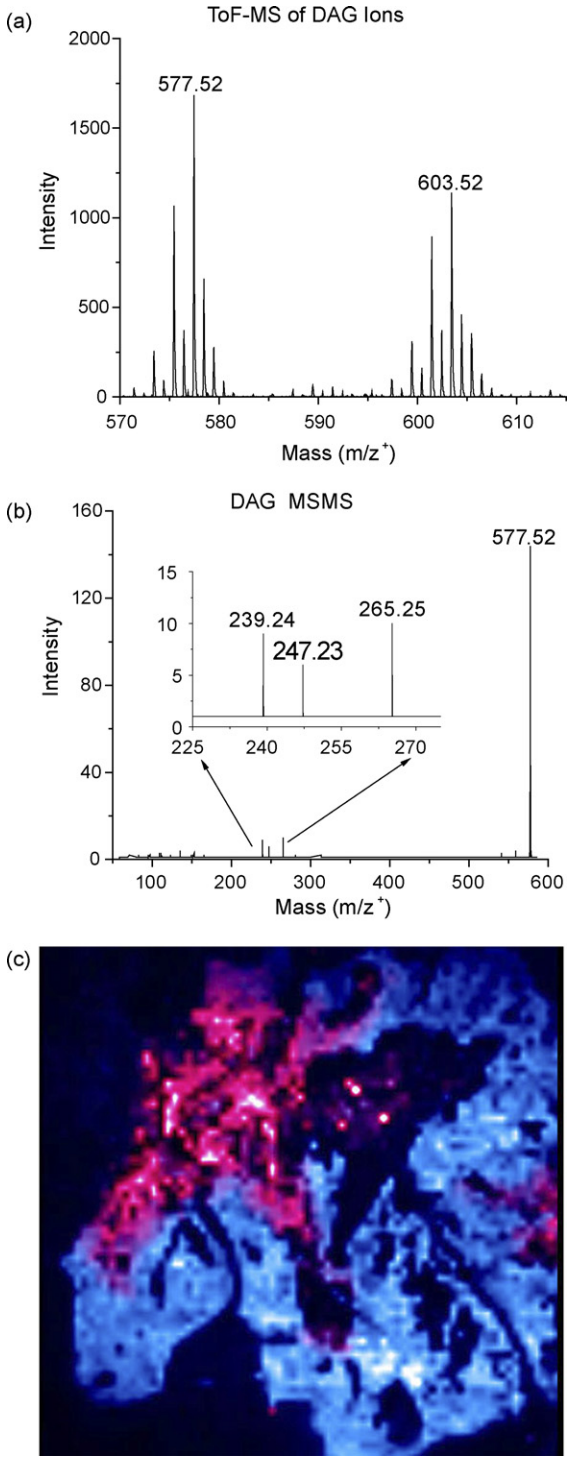

Fig. 2. (a) ToF-MS spectrum showing 1-palmitoyl-2-oleoyl-glyceride (m/z^+ 577.5) and 1,2-dioleoyl-glyceride (m/z^+ 603.5) in the mouse lung. (b) The tandem MS spectrum of molecular ion 577.5 with 30 V collision energy produced three major fragments m/z^+ 239.2, m/z^+ 265.2 and m/z^+ 247.2. (c) Selected ion image of lung tissue showing the 1-palmitoyl-2-oleoyl-glycerol ion (m/z^+ 577.5) and phosphocholine head group (m/z^+ 184) are represented in pink and blue, respectively. The dark areas are substrate. (For interpretation of the references to color in the text and figure caption, the reader is referred to the web version of the article.)

2.2. Materials and sample preparation

The partial sagittal mouse brain sample was stored at 190 K and sectioned in ice with a cryostat at 250 K to a thickness of 10 μm onto a glass cover slip. It was then dried in a dessicator for 24 h and remained at room temperature for several days during shipping. Without further preparation, it was placed on a MALDI sample plate for analysis.

For the mouse lung study, a mouse lung was embedded in optimal cutting temperature (OCT) compound, kept at 200 K until cryostat slicing, and sectioned at 250 K onto conductive indium tin oxide (ITO) slides. The samples were kept at 250 K until washing with 0.9% saline solution plus Girards T derivitizing agent in an attempt to enhance cholesterol signal in the sample [8]. They were then dried under vacuum for 2 h. All samples were then shipped in dry ice, and stored for several weeks at 273 K until analysis.

The thin film used for the spot size vs. pressure investigation was prepared by drop drying several drops of a 10-mg/ml solution of progesterone in acetone directly onto a MALDI sample plate and allowing it to dry to a hazy film. Progesterone was used because it dried in a fairly uniform opaque film that allowed for visualization of crater formation.

3. Results and discussion

3.1. C_{60}^+/N_2 interaction

Deleterious effects of high N_2 pressure on the C_{60}^+ primary ion beam were a significant concern with this instrument design. In efforts to understand and characterize the effectiveness of the modifications to the primary ion source in regard to beam size, five holes were sputtered in a thin film of progesterone at pressures ranging from $\sim 5 \times 10^{-5}$ to 8×10^{-3} Torr. It should be noted that the lowest pressure is an estimate. The nose cone modification as well as an optical image of the sputter craters formed with C_{60}^+ at varying pressures is shown in Fig. 1. There was no noticeable increase in beam size due to N_2 pressure. These results were verified with AFM analysis (data not shown).

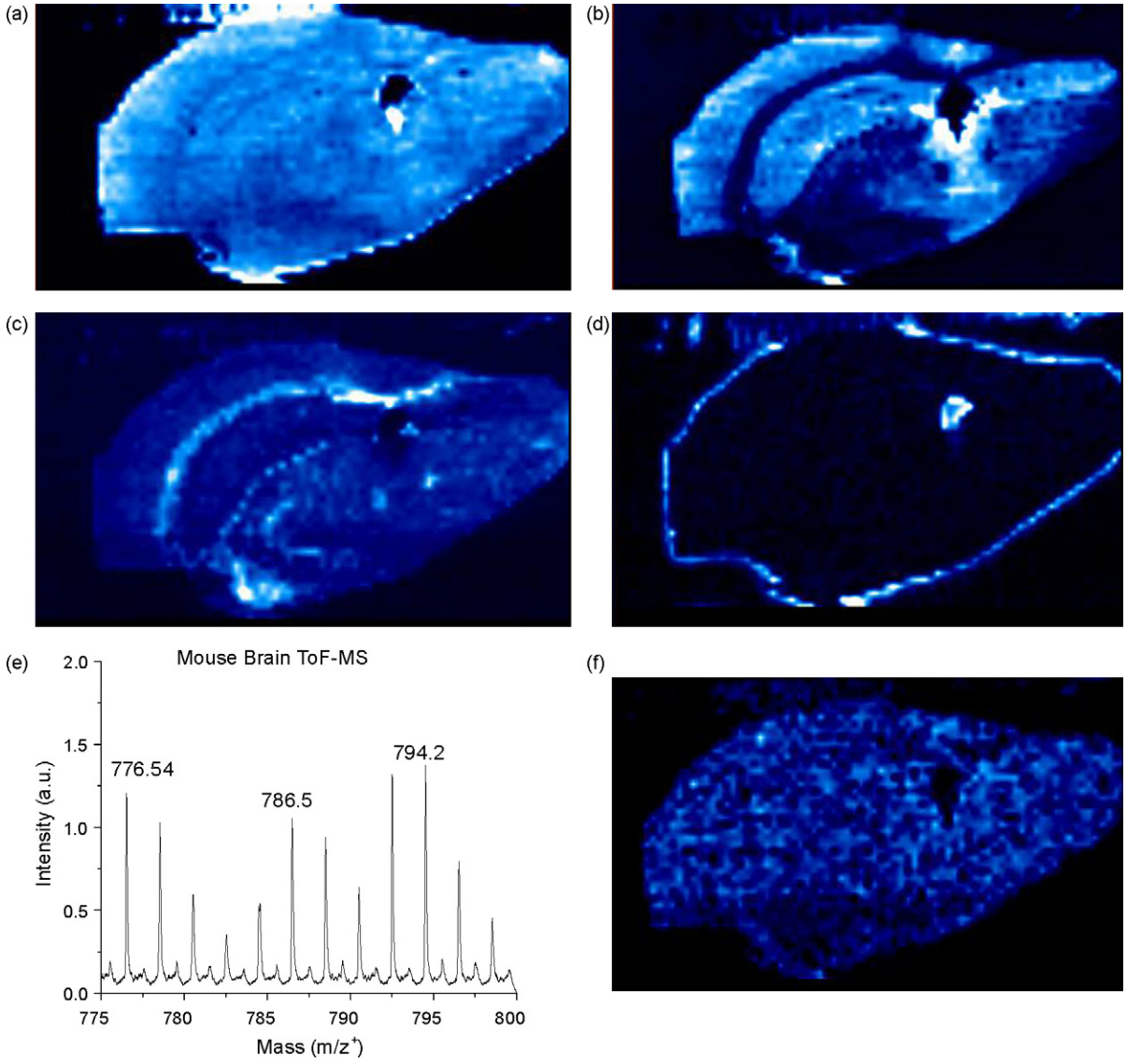

Fig. 3. Selected ion images and high mass spectrum of a partial sagittal mouse brain cross-section. (a) m/z^+ 184 distributed uniformly over the tissue. (b) m/z^+ 234.83 localized primarily in the hippocampus, cortex, and striatum. (c) m/z^+ 178.08 localized in the corpus callosum. (d) m/z^+ 439.31 localized to the periphery of the slice. (e) High mass spectrum showing high signal to noise ratio as well as abundant counts. (f) Selected ion image of m/z^+ 794.52 (shown in (e)).

3.2. Mouse lung imaging

Tissue imaging is challenging since ion intensity is observed at virtually every mass. Lung tissue is of interest since the surface chemistry can be modified by exposure to exogenous agents such as ozone. A preliminary example of lipid diversity in this tissue is illustrated by the information shown in Fig. 2. The SIMS analysis of mouse lung tissue reveals diacylglyceride (DAG) ions, 1-palmitoyl-2-oleoyl-glyceride (m/z^+ 577.52) and 1,2-dioleoyl-glyceride (m/z^+ 603.52), the spectra of which can be seen in Fig. 2a. The identification of the DAG ions was confirmed via MS/MS structure analysis and was consistent with previous studies [9]. Tandem MS analysis of the 577.5 peak produced three major fragments; the palmitate chain (m/z^+ 239.24), the oleate chain (m/z^+ 265.25) and the dehydrated oleate chain (m/z^+ 247.23) (Fig. 2b). Both DAG ions revealed the strongest intensity in the same area of the lung. The localization of m/z^+ 577.52 can be seen in pink in Fig. 2c with the phosphocholine head group ion m/z^+ 184.07 in blue. Due to the discontinuous nature of the lung cross-section, much of the underlying substrate can be seen. The image was taken with 100 μm step size, image dimensions of 7.8 mm × 7.4 mm, and C_{60}^+ primary ion dose of 2.8×10^{12} ions/cm^2.

Currently, the origin of these DAG ions is unclear. It is possible they are native to the tissue, or they could have been formed by the fragmentation of lipids due to primary ion collisions. Work done previously in this lab on lipid thin films shows a significantly higher lipid parent to daughter ion ratio than seen in the current analysis, indicating the high concentrations of m/z^+ 577.52 and m/z^+ 603.52 in these samples is not from collisional fragmentation of lipids. Future work in this area will include precursor ion analysis [10] of the lung tissue to determine the parent molecule, if any, as well as further evaluation of neat thin films.

3.3. Mouse brain imaging

The chemical and structural heterogeneity found in rodent brains analyzed previously with SIMS [11] makes these samples an obvious choice for initial investigation of cluster SIMS imaging of biological samples with a DC primary ion beam. As noted earlier, this is an incomplete sagittal cross-section, however it is sufficient to show the imaging characteristics of this prototype SIMS/MALDI instrument. Beam size as well as step size is 100 μm, C_{60}^+ current is 14 pA, and accumulation time per pixel is 3 s resulting in a dose of 3×10^{12} ions/cm^2. Total image size is 4.4 × 9.1 mm. Image acquisition time is approximately 6 h. Shown in Fig. 3 are selected ion images of a sagittal mouse brain slice. As commonly found with SIMS analysis of tissue slices, phosphocholine head group ion, m/z^+ 184.07 shown in Fig. 3a was one of the most intense ions collected, and it was fairly uniformly distributed across the sample. More interestingly, images generated by mapping masses m/z^+ 234.83, 178.08, and 439.31 (Fig. 3b–d, respectively) among others show distinct localizations.

While the identity of most of these masses is currently unknown, the images revealed by mapping them suggest they are biologically relevant and are worthy of further investigation. We are currently pursuing the identification of these masses through further ToF-MS experiments as well as MS/MS analysis. A direct benefit of high primary ion doses can be seen in Fig. 3e. Even masses up to m/z^+ 800 have relatively high signal to noise ratios and intensities sufficient for mapping as shown in Fig. 3f, m/z^+ 794.52. As an illustration of the advantage of using a DC primary ion beam, an image of this sample acquired with the same primary ion dose using typical pulsed beam instrumental parameters would have taken 2.2 years.

4. Conclusions

Despite the great progress in the field of SIMS analysis of biological samples brought about in large part by cluster ion sources, acquiring sufficient ion intensities for unambiguous chemical imaging remains one of the most significant challenges in this field. An orthogonal ToF instrument fitted with a cluster ion source allows for a significantly higher secondary ion collection rate due to its ability to operate with a DC primary ion beam. In addition, high mass resolution, MS/MS analysis, and ion trapping capabilities provided by orthogonal ToF tandem quadrupole systems like the QSTAR® XL system provide powerful tools for interpreting complicated organic data sets. Preliminary results obtained from this prototype instrument are encouraging, and improvements, primarily in regards to imaging capabilities, will be a focus in future works. Approaches to reduce beam size as well as a concurrent increase in rastering and data collection efficiency are being explored. Advanced analysis methods such as looped experiments and multiple daughter ion precursor scans, capabilities that are native to the QSTAR® XL system, will play a larger role in sample analysis with this instrument.

Acknowledgments

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