Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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69451 Weinheim, Germany

Tissue Imaging at Atmospheric Pressure using Desorption Electrospray Ionization (DESI) Mass Spectrometry

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[**]This work was supported by Prosolia, Inc., Indianapolis, IN, and the Office of Naval Research and performed in the Bindley Biosciences Center. The authors gratefully thank Candice Kissinger, James Woods, and Peter Kissinger of Bionalytical Systems Inc., West Lafayette, IN for advice and for providing rat brain specimens for these studies. The authors also thank Shannon Cornett of Vanderbilt University for information on data conversion. In addition, we thank Dominik Lenz at Purdue University Bindley Bioscience Center for providing optical images of rat brain sections.

keywords: lipids; molecular imaging; microscopy; tandem mass spectrometry; phospholipids;

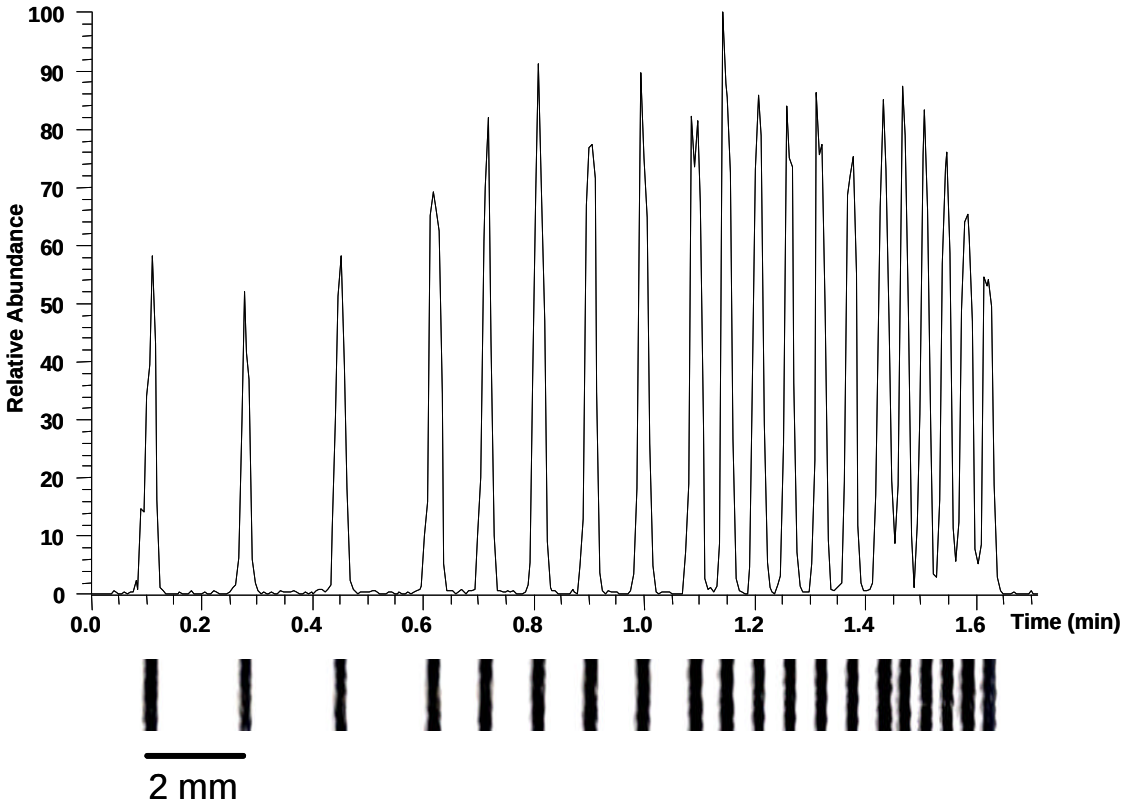
Lateral Spatial Resolution

The lateral spatial resolution available was tested by printing parallel lines onto photographic paper using black ink (Figure S1). The lines were spaced in successively smaller increments from 2 mm to 450 μm, center to center. DESI was used to scan across the surface at a velocity of 200 μm/s while monitoring the ion current from m/z 372.4 (± 0.8) in the single ion monitoring mode. The result of scanning over the pattern is shown in the figure. In this favorable case the achievable lateral spatial resolution was 250 μm, representing the blank spaces between the lines in the last set of lines (450 μm, center to center), while achieving a signal to noise ratio (S/N) of 10:1.

Lateral spatial resolution data achieved with biological samples is not as good as this; in this data recorded here it is estimated as better than 500 μm based on the ability to resolve

certain sub-structures in the brain (i.e. lateral ventricles). The ion images are presented as interpolated plots of pixels in the image as opposed to plotting the data as individual pixels. The interpolated plot allows a better visual comparison of the data with the optical image.

Figure S1



Experimental

Mass Spectrometry

DESI MS spectra were acquired using a Thermo Finnigan LTQ linear ion trap mass spectrometer equipped with a custom-built, automated DESI ion source. The DESI ion source used in these studies consists of an inner capillary (fused silica, 50µm i.d., 150 µm o.d.) (Polymicro Technologies, AZ) for delivering the spray solvent and an outer capillary (250µm i.d., 350µm o.d.) for delivering the nebulizing gas. The surface moving stage includes two motorized (x, y)

translational stages (Princeton Research Instruments, Inc., Princeton, NJ) capable of 3 in. x 2 in. travel and a 3 in. x 3 in. polycarbonate block capable of securing a 3 in. x 1 in. microscope glass slide. The DESI ion source is mounted onto an x, y, z manual translational moving stage coupled with a 360 degree rotational stage in order to adjust the incident impact angle. A 0.5 mm i.d. stainless steel capillary was utilized for ion collection and transport into the LTQ mass spectrometer. The stainless steel capillary extended over the surface allowing the surface stage to be moved in two-dimensions under the ion source. Mass spectra were acquired in the profile mode and the AGC (automatic gain control) feature was turned off. The MS injection time was set at 250 ms and two consecutive scans were summed for each pixel in the image. See Table S1 for the complete list experimental parameters.

Image Acquisition

Imaging experiments were performed by continuously scanning the surface (area 13.2 mm x 10.2 mm) at a surface velocity of 200 µm/s while acquiring mass spectra every 0.67s. Each step in the direction parallel to the inlet of the MS was 300µm resulting in the collection of 3366 mass spectra in an array of 99 by 34 data points; thus, each pixel in the image is 300µm x 134µm. Negative ion detection was used and methanol:water (1:1 v/v) was used as the spray solvent.

Data Analysis

BioMap (freeware, www.msi.maldi.org) was used to generate the spatial intensity distributions of selected ions based on the mass spectrometry data. BioMap is an image analysis software platform that can be used with a variety of imaging tools. An import filter for data conversion of Xcalibur™ raw data files acquired on the Thermo Finnigan LTQ into ANALYZE™ format was written in-house. The mass spectrometry ion images are presented as interpolated plots of the image data.

Tissue Preparation

Male Sprague-Dalley rats (Harlan Industries, IN) were euthanized using pentabarbitol (390mg/kg) and were decapitated upon cessation of respiration. The rat brains were removed from the skull and immediately frozen at -80°C. All procedures herein abided by the Care and Use of Laboratory Animals (NIH). Frozen rat brain was cut into thin, 4µm sections, at Bregma from ~ 1.0 to 2.0 using a Shandon Cryostat (Thermo Electron, San Jose CA) at -19°C. The digital photograph in Figure 2a represents a Bregma of ca. 1.5. Serial sections were made and directly thaw mounted onto a general microscope glass slide. After sectioning, the tissue sections were stored at -80C until DESI analysis. Prior to analysis the sections were dried in a vacuum dessicator for at least 2 hours.

Table S1 Experimental Parameters

Parameter	Value
Spray voltage	5000 V
Solution flow rate	1.25 µL/min
Spray solvent	Methanol/Water (1:1 v/v) [a] Methanol (100%) [b]
N_2 sheath gas pressure	7 bar
Incident angle (a)	54°
Tip-to-surface (d_1)	2 mm
Tip-to-inlet (d_2)	4 mm
MS acquisition time/pixel	670 ms
Vertical step size	300 µm [c]
Image size	13.2 mm x 10.2 mm [c]
Surface velocity	200 µm/s

[a] DESI spray solvent used to record molecular ion image of rat brain tissue.
[b] DESI spray solvent used to record the ion chromatogram resulting from scanning the surface of the photographic paper.
[c] Details specific to the tissue imaging experiment.

Extension to Non-Lipid Components

Our studies have so far focused on lipids. This is the result of the spray conditions selected and the fact that we have chosen to examine negatively-charged not positively-charged ions. In other unpublished work peptides and proteins have been detected from the tissue surface of rat brain. It remains to be seen how well compound classes other than lipids can be seen and whether or not there is significant ionization suppression under the conditions of these experiments.

Supporting Information

Table S2

Lipid Molecular Species Detected from Rat Brain Tissue in Negative Ionization Mode

Mass-to-charge ratio (m/z)	Molecular species[a]	Percent (%) Relative intensity in corpus callosum[c]	Percent (%) Relative intensity in cerebral cortex[c]
225.5	Myristoleic acid (14:1)	7.2	49.3
255.4	Palmitic acid (16:0)	13.6	53.1
281.4	Oleic acid (18:1)	25	43.1
283.4	Stearic acid (18:0)	19	53.1
303.4	Arachidonic acid (20:4)	24.1	39.8
327.4	Docosahexaenoic acid (22:6)	3.1	20.9
700.6	plasmenyl-PE (34:1)	3.2	<2
726.6	plasmenyl-PE (36:2)	5.1	<2
750.6	plasmenyl-PE (38:4)	3.4	<2
768.3	PE (38:3)[b]	<2	<2
774.6	plasmenyl-PE (40:6)	2.8	7.1
788.5	PS (36:1)	79.7	21.2
794.4	PE (40:4)	2	2.8
806.8	ST (h18:0)	31	6.4
810.5	PS (38:4)	23.8	8.8
821.7	LPA (40:6)[b]	8.5	3.2
834.4	PS (40:6)	38	100
857.6	PI (36:4)	6.3	10.6
862.8	ST (22:0)	20.7	4.1
878.8	ST (h22:0)	22.5	6
885.6	PI (38:4)	40.4	51.6
888.8	ST (24:1)	100	17.6
890.8	ST (24:0)	83.2	15.9
904.8	ST (h24:1)	45	8.9
906.8	ST (h24:0)	62.1	15.5
916.7	ST (25:0)	6.5	<2
918.8	ST (h25:1 or 26:0)[b]	9.5	<2
920.8	ST (h25:0)	5	<2
932.8	ST (h26:1)	3.5	<2
934.7	ST (h26:0)	4	<2

[a] (X:Y) represents the different number of carbon atoms and the different number of double bonds in the fatty acid chains. The notation (hX:Y) denotes a hydroxylated sulfatide species; ST sulfatide; PI phosphatidylinositol; PE phosphatidylethanolamine; PS phosphatidylserine; LPA lysophosphatidic acid

[b] Tentative assignment

[c] Percent relative intensity based on the centroid m/z value and relative to the base peak in each column