VIP Mass Spectrometry

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

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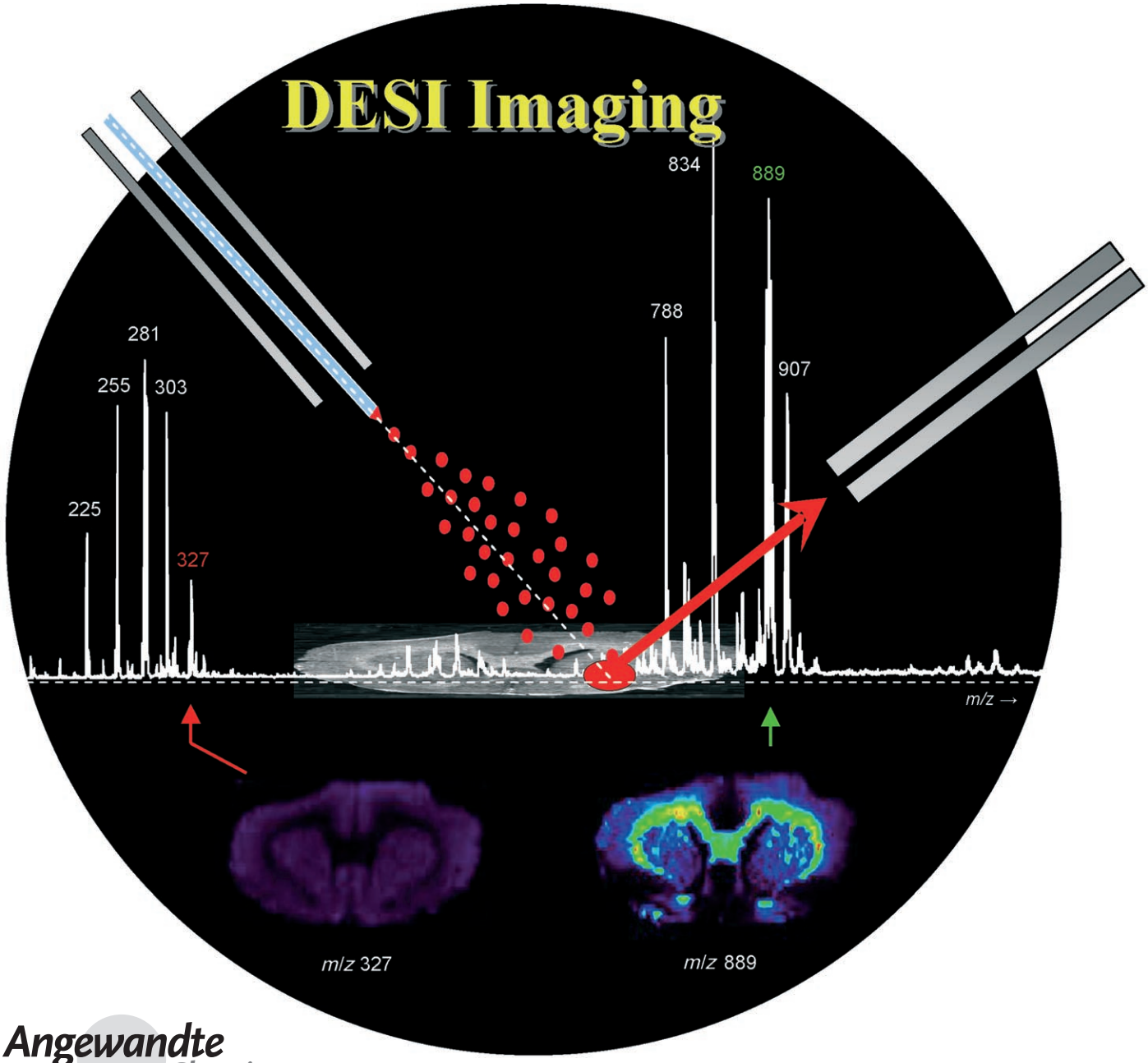

Mass spectrometry (MS) is an increasingly indispensable tool in the biological sciences. Innovations in methods of creating, analyzing (mass), dissociating, reacting, and collecting ions are allowing the identification of increasingly complex biomolecules and molecular complexes, and some of these innovations hold the promise of implementation in a clinical setting. We report on one such new development, imaging of biological tissue sections at atmospheric pressure without prior chemical treatment of the tissue. The experiment uses a new mass spectrometry technique, desorption electrospray ionization (DESI),[1,2] introduced as one of a family of methods that have extended the applications of mass spectrometry to ordinary samples in the ambient environment. The DESI methodology is demonstrated here to allow mapping of the distribution of specific lipids directly from thin histological sections of rat brain with spatial resolution of less than 500 μm and with unit-mass resolution.

DESI is a novel ionization technique that permits the analysis of surfaces without the addition of a matrix or other treatment while the sample is held under ambient conditions.[1] It is a desorption ionization (DI) method in which the surface being analyzed is impacted by charged droplets of solvent generated from an electrospray. With this method, high-throughput surface analysis is achieved at atmospheric pressure. An analogous method uses charged droplets of glycerol and ammonium acetate, although the sample is maintained under vacuum.[3]

The potential of DESI for molecular imaging was recognized early.[4] Direct analysis of histological tissue sections was demonstrated recently,[5] where profiling of liver adenocarcinoma tissue sections revealed differences in the phospholipid profiles along a scan line extending from the tumor to nontumor regions. The presence of specific sphingomyelins marked the tumor margin, and this has implications for the underlying biochemistry of sphingomyelin metabolism in adenocarcinomas.

The present study reports the first 2D molecular images of biological samples using ambient mass spectrometry. Elemental or molecular images of samples, including biological tissue samples examined under vacuum, have been reported using DI techniques, specifically secondary-ion mass spectrometry (SIMS) and matrix-assisted laser desorption ionization (MALDI). The earliest experiments[6] used finely focused primary-ion beams as probes, and these were rastered across a surface to record images showing elemental or small-molecule distributions. At the end of 1970s, SIMS studies in this lab successfully mapped cocaine and cinnamoylcocaine in whole coca plant tissues[7] and muscarine in fungal tissues.[8] Recently, imaging mass spectrometry has emerged as a powerful technique in the biological sciences and is based on MALDI[9,10] and SIMS.[11] SIMS has the advantage of very high (micron scale) spatial resolution, while MALDI is applicable to larger molecules and is faster but gives lower resolution and requires the addition of a matrix to the sample. MALDI imaging of histological sections of biological tissues has been demonstrated previously, especially in the case of peptides and proteins.[12] The method, as developed by Caprioli et al.,[9,13] permits the analysis of peptides and proteins directly from tissue sections after matrix deposition and introduction of the sample into the vacuum system. Thus the quality of data obtained in these studies depends on the choice of the matrix compound, the matrix solvent, and the homogeneity of the matrix coating. There would be advantages to methods that require no sample treatment prior to sample analysis to allow a true in situ measurement; DESI-MS provides this capability. DESI is also complementary to MALDI imaging in that lipids are more readily ionized by DESI while peptides and proteins are more readily examined by MALDI (although in neither case do these represent intrinsic restrictions in the methodology).

The spot size in the DESI-MS imaging experiment depends on the capillary diameter and the tip-to-surface distance, among other parameters.[14] However, the achievable spatial resolution appears to be significantly poorer than the impacting spot diameter; in some experiments a spatial resolution of 250 μm is achieved at a signal/noise ratio (S/N) of 10:1 (see Supporting Information). In the DESI imaging experiment the sample (placed onto a target such as a microscope slide, or it is analyzed in situ) is moved under the fixed spray nozzle to study an area of interest. This process generates a mass spectrum for each exposed area or pixel on the surface. Ion images of the sampled area showing the spatial (2D) distribution of the intensity of a selected ion (or set of ions) are created from the data.

The negative-ion DESI mass spectrum from a rat brain section is shown in Figure 1 a and represents the average mass spectrum recorded from the tissue section. In the low mass/charge region ($m/z < 400$), ions corresponding to deprotonated free fatty acids palmitate (m/z 255), oleate (m/z 281), stearate (m/z 283), arachidonate (m/z 303), and docosohexaenoate (DHA; m/z 327) are present, along with other ions. In the high m/z region ($m/z > 600$), ions corresponding to the deprotonated forms of various lipids are present. The lipid species detected directly from the brain tissue in the m/z range 600–900 consist mainly of sulfatides, phosphatidylserines, and phosphatidylinositols. The less abundant species detected in the m/z range 700–800 are identified as phosphatidylethanolamines. The inset of Figure 1 a displays an MS/MS product-ion mass spectrum of the peak at m/z 885.6 which

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

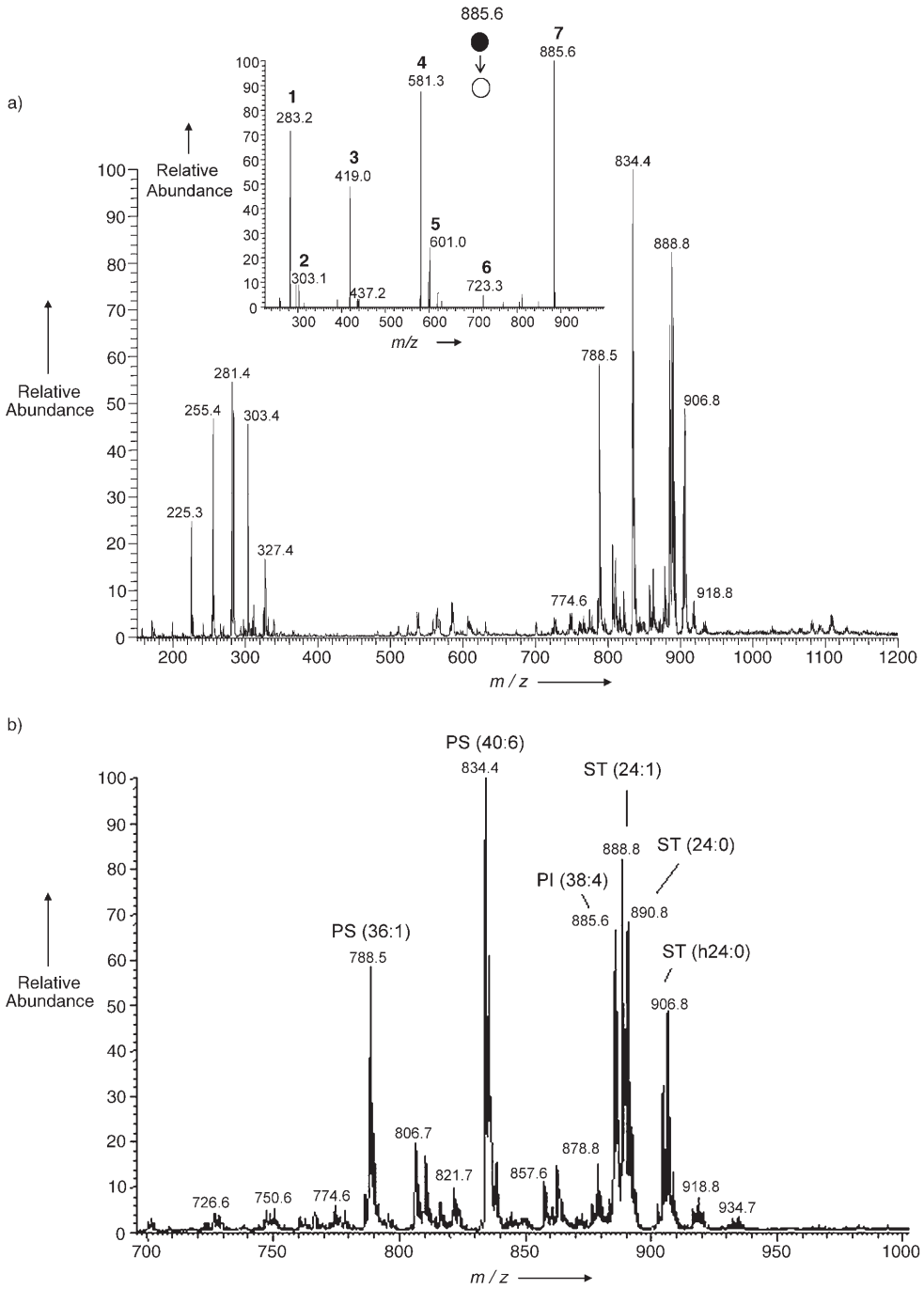

Figure 1. Negative-ion DESI mass spectrum recorded on a 4-μm thick coronal rat brain section examined using methanol/water (1:1 v/v) as the spray solvent. a) Full mass spectrum. The inset shows the MS/MS investigation of phosphatidylinositol (38:4). 1) $[C_{17}H_{36}COO]^-$; 2) $[C_{19}H_{32}COO]^-$; 3) $[M-H-(C_{17}H_{36}CH{=}C{=}O)-\text{Inositol}]^-$; 4) $[M-H-(C_{19}H_{32}COOH)]^-$; 5) $[M-H-(C_{17}H_{36}COOH)]^-$; 6) $[M-H-\text{Inositol}-H_2O]^-$; 7) $[M-H]^-$. b) Expanded view of the mass range from *m/z* 700–1000 including the molecular ion, $[M-H]^-$, signals from several specific phosphatidylserines (PSs), sulfatides (STs), and phosphatidylinositols (PIs).

shows fragment ions that identify it as phosphatidylinositol (38:4). Figure 1b shows an expanded view of the DESI mass spectrum in the *m/z* range 700–1000. Confirmation of the lipid assignments was achieved by tandem mass spectrometry (MS/MS), spectral comparisons with authentic lipid standards, and comparison to existing ESI mass spectra.[15] As the fragmentations of many different lipid species have been studied extensively,[15] lipids detected directly from the tissue surface could be identified through the MS/MS data. (A summary of the peak assignments and relative abundances for negative ions detected from the rat brain is provided in the Supporting Information.)

The spatial images of specific lipids (distributions of signal intensity) in the rat brain tissue are shown in Figure 2. For comparison, an optical image is provided in Figure 2a and major structural features are indicated. The negative ions detected from the tissue were deprotonated molecules of the form $[M-H]^-$. The ion images of the phospholipids phosphatidylinositol (PI), phosphatidylserine (PS), and sulfatide (ST) corresponding to peaks at *m/z* 885.6 (PI), *m/z* 834.4 and 788.5 (PS), and *m/z* 888.8 and 906.8 (ST), respectively, represent specific lipids at or near the surface of the tissue. The image of the ion at *m/z* 885.6, identified as PI 38:4, shows a relatively homogeneous distribution across the entire brain tissue section, except in the lateral ventricles where no signal is observed (Figure 2b). The ventricles in the rat brain are clearly resolved in the corresponding ion images, indicating that a lateral spatial resolution of less than 500 μm is achieved. The ion image of the peak at *m/z* 834.4, identified as PS 40:6 (Figure 2c), shows a homogeneous distribution in areas of the brain corresponding to gray matter (i.e. cerebral cortex). Its signal is less intense in the areas corresponding to white matter (e.g. corpus callosum). In contrast, the ion image in Figure 2f, corresponding to the peak at *m/z* 788.4 (PS 36:1), shows complementary localization with the ion image of PS 40:6. Distinctly different spatial intensity distributions are observed for the sulfatide peaks at *m/z* 888.8 and 906.8 (Figure 2d,e) than seen for the PI 38:4 ion image in Figure 2b. The sulfatide distributions show complementary localization with PS 40:6, and their intensity distributions resemble the distinctive corpus callosum and striatum anatomic structures in the brain section. In addition, the sulfatide intensity distributions (Figure 2d,e) are also localized in an anatomic substructure of the brain representing the anterior commissure.

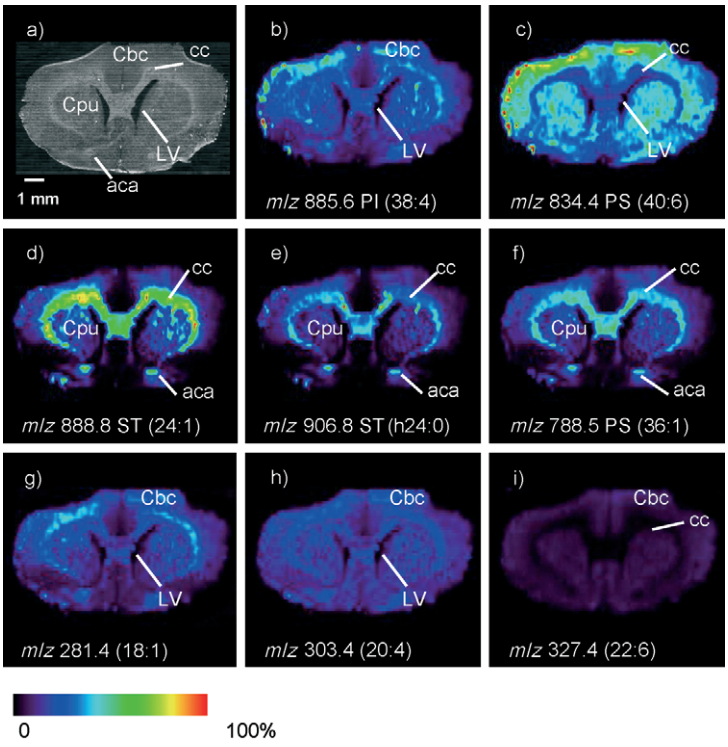

Figure 2. Selected molecular ion $[M-H]^-$ images of specific lipids from analysis of a 13×10 mm^2 area of rat brain tissue section. a) Optical image of the coronal section of the rat brain prior to analysis. cc = corpus callosum; CPu = striatum; Cbc = cerebral cortex; LV = lateral ventricle; aca = anterior part of anterior commissure. b–i) Ion images of PI (38:4; b), PS (40:6; c), ST (24:1; d), ST (h24:1; e), PS (36:1; f), oleate (18:1; g), arachidonate (20:4; h), and docosahexaenoate (22:6; i).

Homogeneous spatial intensity distributions across the entire tissue section are observed for the deprotonated ions of oleic acid (18:1; Figure 2g) and arachidonic acid (20:4; Figure 2h). However, the intensity of the ion at *m/z* 327 corresponding to DHA (22:6), an *n*−3 polyunsaturated fatty acid, is lower in the region of the brain corresponding to the corpus callosum.

These results are in qualitative agreement with the reported literature on lipid composition and distribution in brain tissue, suggesting that the spatial signal intensity distributions reflect relative concentration differences of the lipids in the brain tissue.[16] Specifically, sulfatides are known to be enriched in white matter but also present in neurons and astrocytes, and represent approximately 5% of the total lipids in adult brain myelin.[17] The selected ion images for the long-chain fatty-acid-containing sulfatides reflect these concentration differences in the brain and directly correlate with anatomic features corresponding to the corpus callosum and the striatum.

The results presented here represent the first application of DESI-MS to two-dimensional molecular-ion imaging of biological tissues. The capability of DESI-MS in providing spatial information about specific molecules on the tissue surface was demonstrated with a spatial resolution of less than 500 μm and with unit-mass resolution. The resulting ion images reflect the relative concentrations of the species detected from the tissue surface. The spatial resolution in these initial experiments is not as good as in MALDI and significantly poorer than in SIMS imaging, however, there are offsetting advantages in terms of ease of implementation and ambient analysis. Note also that the unit-mass resolved imaging data is complemented by tandem mass spectrometry information, which is used to confirm the identities of individual compounds or even to scan the tissue for groups of compounds that have a functional group of interest. These capabilities open the way to studies of drug distributions in tissue, diagnostic applications, biochemical investigations of anatomic substructures in tissue, and eventually to in vivo mass spectrometry.

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