Phospholipids

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Mass Spectrometric Profiling of Intact Biological Tissue by Using Desorption Electrospray Ionization**

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Imaging mass spectrometry has emerged as a powerful technique based on time-of-flight secondary-ion mass spectrometry (TOF-SIMS)[1] and matrix-assisted laser desorption/ionization (MALDI).[2,3] However, until now, the mass-spectrometric analysis of biological tissues has been limited to techniques that require sample preparation after which the sample is confined to the high-vacuum region of the instrument, thus making this approach inaccessible to further physical or chemical manipulations. Herein, direct chemical profiling of biological tissues is achieved under ambient conditions at atmospheric pressure by desorption electrospray ionization mass spectrometry (DESI-MS),[4] which is a new and versatile method for the creation of ions from surfaces outside of the mass spectrometer. The profiling of lipids in biological tissues by using DESI-MS provides highly sensitive and chemically specific information almost instantaneously. This capability is illustrated by examples in which phospholipid profiles of mouse pancreas, rat brain, and metastatic human-liver adenocarcinoma tissues are obtained without any sample pretreatment.

Biological membranes serve as crucial mediators in cellular differentiation and proliferation; the former being important in tumor formation, the latter in tumor progression. Phospholipids play important roles in maintaining the structural integrity of the cell by forming of the characteristic bilayer of the membrane and by serving as key mediators in important cellular events, for example, cell signaling and protein sorting on so-called lipid rafts. As such, the chemical and physical properties of the cell membrane are determined by the phospholipid composition. Alterations in the phospholipid composition of tissues have been reported for certain diseases, including cancer and Alzheimer's disease.[5] Importantly, the abundance of certain phospholipids and their enzymatic by-products has been associated with malignant transformations in some tissues.[6] These findings emphasize the importance of the determination of the composition of phospholipids in biological tissues, information which may serve as prognostic variables in diseased and nondiseased tissues.

Mass spectrometry has been used extensively to investigate lipids (e.g., phospholipids),[7] although not under ambient conditions. MALDI-TOF has been used for the analysis of lipids in lens tissue[8] and has emerged as a particularly powerful technique for profiling the spatial distributions of peptides and proteins in biological tissues.[3,9] An alternative molecular imaging method, TOF-SIMS, has been applied to the investigation of the phosphocholine distribution in *Tetrahymena* mating junction by monitoring the phosphocholine head group at m/z 184.[10] In another report, TOF-SIMS imaging of lipids in mouse-brain sections was performed and various sulfatides, as well as phosphatidylinositol, phosphatidylcholine, and cholesterol, were identified and their spatial distributions determined.[11]

DESI belongs to the family of spray ionization methods, such as electrospray ionization (ESI),[12] as well as to the family of desorption ionization methods.[13] DESI allows direct and rapid analysis of surfaces without sample preparation or the need to introduce the sample into a vacuum system.[4] The methodology has been applied to the analysis of trace levels of many classes of compounds, including peptides, proteins, nucleotides, nitroaromatic compounds, and others.[14] Semiquantitative results have been obtained from

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the crude profiling of plant tissue for alkaloid content,[15] whereas a computer-controlled moving stage has been used for the direct analysis of dyes and other compounds on chromatography plates.[16] In another example, DESI was combined with an ion-mobility time-of-flight mass spectrometer to probe the conformations of proteins desorbed from an insulating surface.[17]

Herein, DESI-MS is used to investigate the lipid distributions in mouse-pancreas, rat-brain, and metastatic human-liver adenocarcinoma tissue; additional data is presented for whole tissue analysis. These experiments represent the first application of DESI to biological-tissue imaging. The secondary-ion signals in DESI-MS are affected by solvent composition, that is, polarity, pH value, salt concentration, applied voltage, backing pressure in the source, and surface chemistry, as well as other factors.[4,14] These parameters can be adjusted to enhance signals from other compound classes. Direct investigation (without tissue preparation except for microtoming) of biological tissues by using DESI-MS showed very intense peaks because of the phospholipids present. Strong ion signals from phosphatidylcholine (PC) species are consistent with the fact that these species constitute the majority of biological membranes (Figure 1 a). The mass-to-charge (m/z) range 700–900 in Figure 1 a shows the phospholipids detected in untreated mouse-pancreas tissue. The phospholipids appear as potassium adducts as a result of endogenous potassium in the tissue (Figure 1 b). Chemical assignments were confirmed by tandem mass spectrometry (Figure 1 a, inset). Similar studies on rat-brain sections were performed and gave results typified by those shown in Figure 2. As expected,[11] the rat-brain sections produced phospholipid profiles composed mainly of saturated fatty acids, specifically palimitate and stearate.

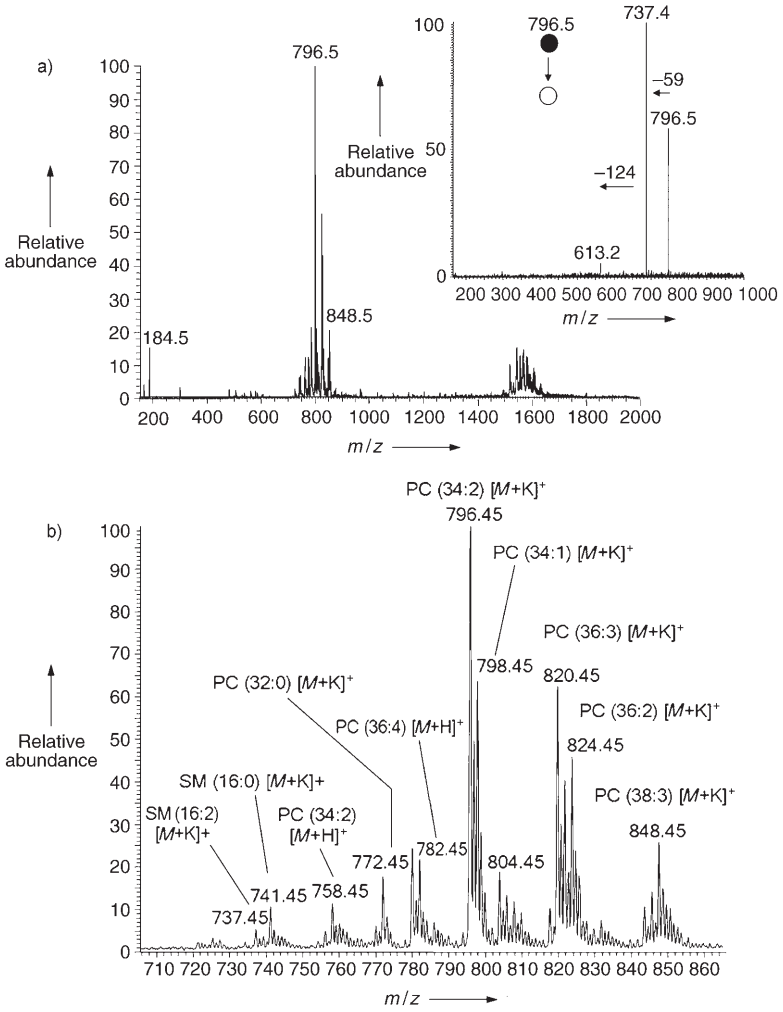

Figure 1. a) Positive-ion DESI mass spectrum recorded on mouse-pancreas tissue with methanol/water (1:1) with 1% acetic acid as the spray solvent at a flow rate of 3 μL min^{-1} and a nebulizing N_2 back pressure of 7 bar. The ions at m/z 184 represent the phosphatidylcholine (PC) head group, thus indicating the presence of free phosphocholine. The ions present at m/z 1550–1650 are phospholipid dimers. The inset shows the MS/MS spectrum of the base peak, and the characteristic neutral losses that were observed make its identification as PC (34:2; $[M+K]^+$) unequivocal. b) An expanded view between m/z 700 and 900 which shows individual phospholipid species and their potassium adducts. The ratio of number of carbon atoms/number of double bonds is given in brackets.

Because considerable information on the disease state of a tissue can be obtained by determination of the distribution of marker compounds, such as phospholipids, investigations of biological tissue utilizing DESI-MS were carried out on metastatic human-liver adenocarcinoma tissue. Lipid distributions in cancerous tissues are known to vary in composition from noncancerous tissue.[18] It has been shown in some cases that elevated levels of phosphatidylcholine species are linked to malignant transformations in cancer cells.[6] In addition, cell-signaling molecules, such as ceramides, derivatives of the sphingomyelin species, are involved in cell-signaling cascades that are directly related to tumor survival. In some cases, extracellular vesicles rich in sphingomyelin have been reported to induce angiogenesis in tumor-proliferating cells.[19] These prior findings emphasize the importance of the determination of the composition of phospholipids in biological tissues and indicate that such information may serve as a prognostic variable in diseased and nondiseased tissues.

Herein, spot sizes are less than 1 mm and the tissue is untreated and examined in the ambient environment. Approximately 50 scans (25 s) were taken on average per spot to obtain high-quality data; however, chemically specific information can be obtained in less than 5 s per spot, including the use of MS/MS.[15] Figure 3 shows a comparison of DESI mass spectra collected from two different regions of metastatic human-liver adenocarcinoma tissue. In the nontumor regions, the lipids are predominantly in the form of palmitic acid containing phospholipids (Figure 3 a). In the transition region from nontumor tissue to cancerous tissue, the lipid distribution is composed of mostly unsaturated fatty acids containing phospholipids (Figure 3 b), whereas the cancerous portions of the tissue showed elevated levels of sphingomyelin (Figure 3 c), which may be associated with a dysfunctional ceramide-mediated apoptosis pathway.[20] In the case of healthy cells, sphingomyelinase can be activated, which results in lower sphingomyelin concentrations.

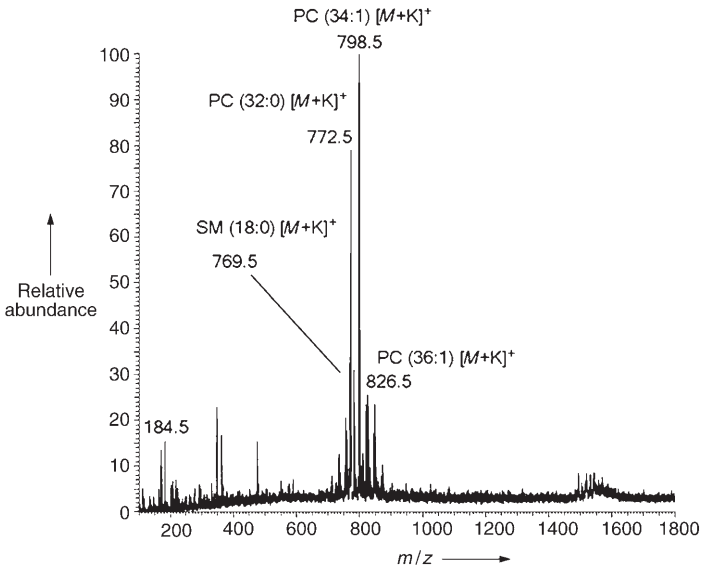

Figure 2. Positive-ion DESI mass spectrum recorded on rat-brain tissue with methanol/water (1:1) with 1% acetic acid as the spray solvent at a flow rate of 3 $\mu L\,min^{-1}$ and a nebulizing N_2 gas back pressure of 7 bar. The base peak is identified as the potassium form of phosphatidylcholine (34:1). The ratio of number of carbon atoms/number of double bonds is given in brackets

Intact nonsectioned tissues can also be examined by DESI. Results from the analysis of adipose tissue in situ surrounding a chicken heart are presented in Figure 4. The mass spectrum indicates the presence of free fatty acids on the tissue surface, whereas other spectra collected from a freshly cut surface of chicken heart feature small peptide components, as well as constituents of hemoglobin (heme and Hb (α_a, α_d, and β chains)).

These results serve as the basis for in vivo/in situ applications to tissue under ambient conditions, a topic with implications for basic biochemistry, as well as pathology, food safety, and real-time diagnosis during surgery. In summary, DESI allows: 1) the direct and rapid analysis of intact biological tissues, including whole organs; 2) sensitive and specific molecular detection; 3) adequate secondary-ion yields for chemical confirmation by tandem mass spectrometry; 4) samples to be used that do not need to be confined under high vacuum or subjected to any other preparation procedure; 5) chemical imaging with spot sizes of less than 1 mm.

Experimental Section

All experiments were performed on a Thermo Finnigan LTQ mass spectrometer (San Jose, CA) equipped with a desorption electrospray ion source, (prototype Omni Spray Ion Source, Prosolia Inc., Indianapolis, IN) which is described elsewhere.[4,14] After dissection, the mouse-pancreas, rat-brain, and human-liver tissues were frozen in liquid nitrogen and stored at −80 °C. Prior to analysis, the tissue sections were prepared by cryosectioning with a Leica CM3050 S (Leica Microsystems, Inc., Bannockburn, IL) to a thickness of 16 μm, thaw mounted directly onto a microscope glass slide, and stored under vacuum until analyzed. No further treatment of the tissue samples was necessary. DESI-MS analysis of the tissues was carried out by directing the

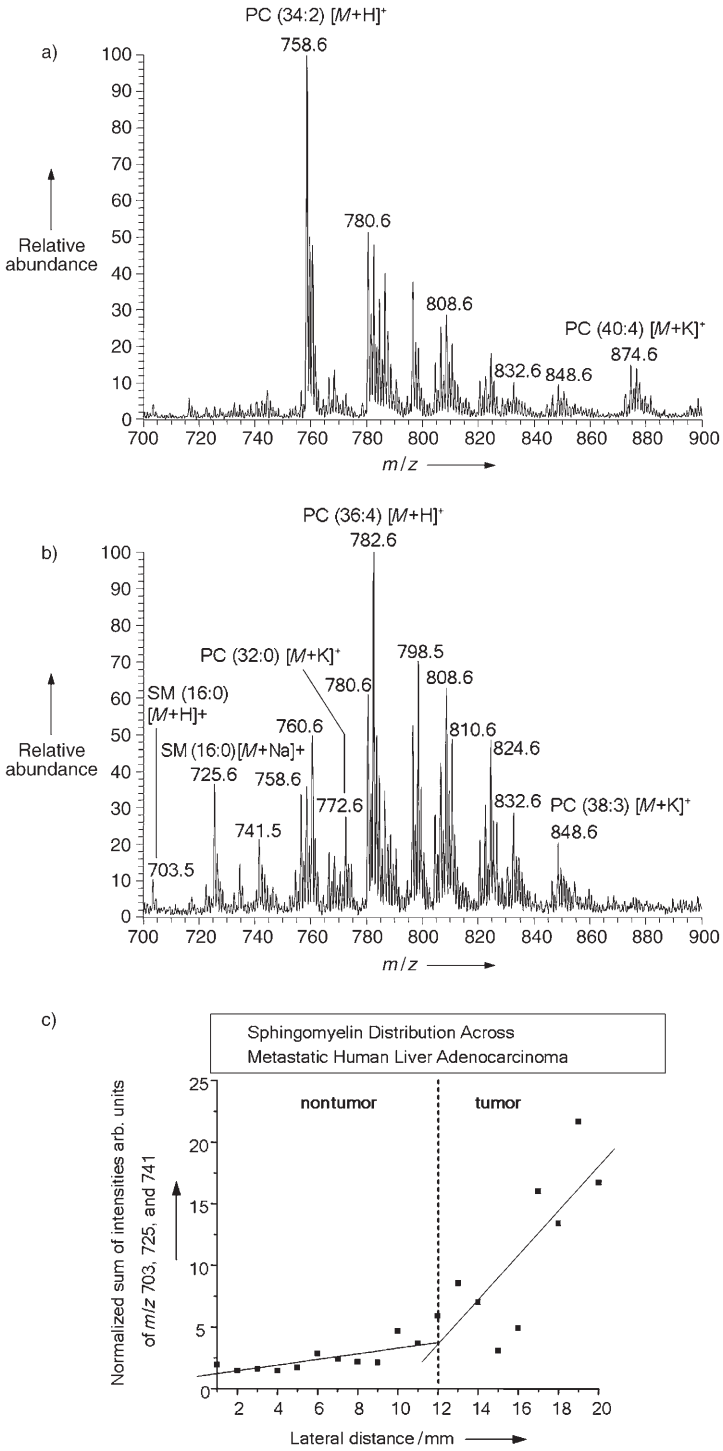

Figure 3. Positive-ion DESI mass spectra recorded on metastatic human-liver adenocarcinoma tissue. The experiment was conducted with methanol/water (1:1, v/v) with 0.1% NH_4OH added. Line scans were performed by moving the sample stage in 1 mm increments across the tissue at 50 scans $step^{-1}$ (25 s $step^{-1}$). a) DESI mass spectrum representative of the nontumor region of the tissue. b) DESI mass spectrum representative of the cancerous region of the tissue. Enhanced signals from sphingomyelin species at *m/z* 703, 725, and 741 are detected in this region of the tissue. c) Ion-intensity distribution of the sum of the *m/z* values 703, 725, and 741 which correspond to the protonated, sodium, and potassium forms of sphingomyelin (16:0), respectively, versus position in the tissue. The intensities were normalized to the ion intensity at *m/z* 184, which corresponds to phosphocholine. The ratio of number of carbon atoms/number of double bonds is given in parentheses.

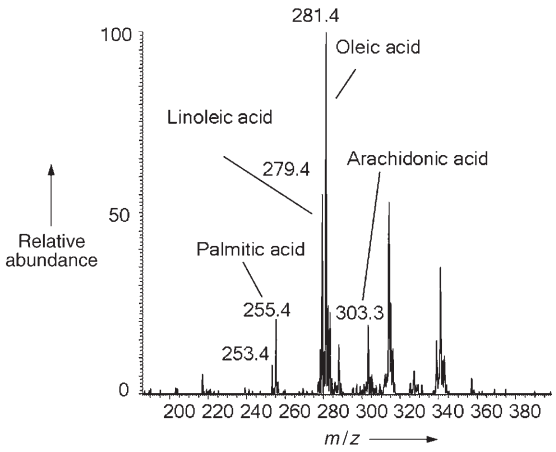

Figure 4. A negative-ion DESI mass spectrum recorded on adipose tissue that surrounds gallus heart with methanol/water (1:1) with 1% acetic acid as the spray solvent at a flow rate of 3 μL min^{-1} and a nebulizing N_2 gas back pressure of 7 bar.

electrospray (5 kV), composed of either methanol/water/acetic acid (50:49:1 v/v) or methanol/water/ammonium hydroxide (50:49.9:0.1 v/v), at an incident angle of approximately 50° toward the tissue. The volumetric flow rate and the back pressure in the source were 3 μL min^{-1} and 7 bar, respectively. Ions generated from the surface were collected using a standard heated capillary interface with an orifice diameter of 500 μm at approximately 10°.

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