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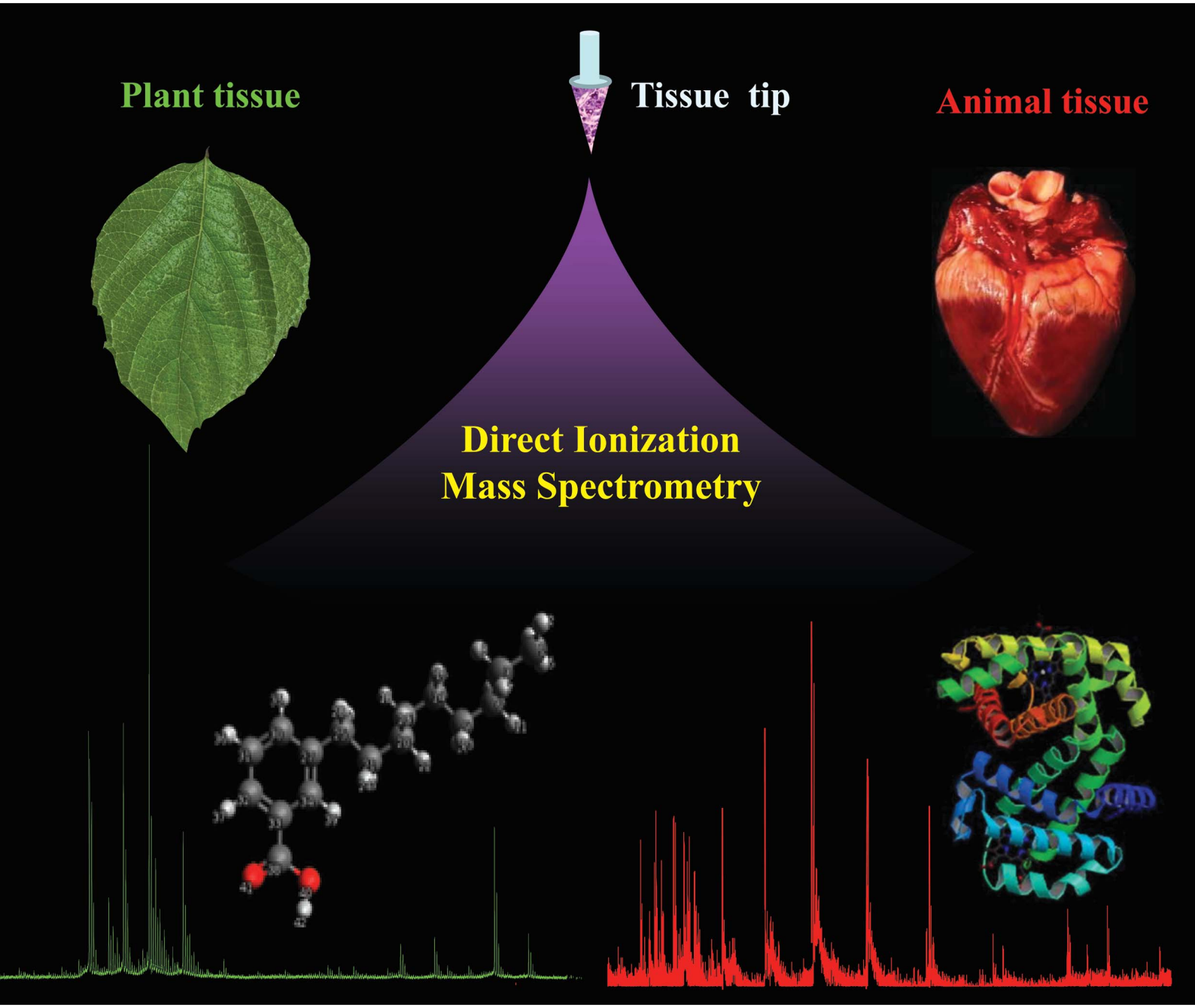

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COMMUNICATION

Direct ionization of biological tissue for mass spectrometric analysis†

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Analysis of biological tissue at a molecular level is of great importance in biological, medical and clinical studies. In this manuscript, we report that both plant and animal tissues can be directly ionized and analyzed by mass spectrometry under ambient conditions. By adding some solvents and applying a high voltage, spray ionization can be induced at the tip of biological tissue and a mass spectrum can be observed. Various plant and animal tissues have been tested and compounds such as lipids, alkaloids, glucosides, lignans, pharmaceuticals and proteins could be detected in the spectra. This new technique provides a simple and rapid method for tissue analysis and allows observation of compounds that cannot be detected by other ionization techniques.

Introduction

Analysis of biological tissue at a molecular level is an important task in biological, medical and clinical studies.[1–3] Understanding molecular compositions of tissue allows us to monitor growth, development and variation of biological individuals, discover markers for disease diagnosis and gain insight into the mechanism of diseases. Conventional approaches for tissue analysis typically involve homogenization, extraction and analysis of extracts, and are usually time-consuming and labor intensive.

Mass spectrometry (MS) is a rapid and sensitive tool for qualitative and quantitative analyses of various samples. The ambient ionization techniques introduced in recent years have greatly facilitated sample preparation for MS analysis.[4–7] Direct analysis of tissues by MS has been achieved mainly with techniques including secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI) and matrix-assisted laser desorption/ionization (MALDI).[8,9] These techniques employ high energy ions,[10] charged microdroplets[11] and laser (with the assistance of a matrix)[12] respectively to desorb and ionize analytes on a tissue surface, and can be used for tissue imaging. Among these three techniques, DESI imaging can be performed at atmospheric pressure.[13] Other techniques for ambient imaging include electrospray-assisted laser desorption/ionization (ELDI)[14] and probe electrospray ionization (PESI).[15] In ELDI, analytes on a tissue surface are desorbed by laser then postionized by an ESI fume;[14] while in PESI, a tissue surface is probed by a solid needle, and the trace biological fluid adhering to the needle is then analyzed in a way similar to ESI.[15] Recently, paper spray was also used for tissue analysis.[16] In this method, a tissue sample is loaded on a paper. When solvent is added and a high voltage is applied to the paper, spray ionization occurs at the paper tip, and compounds such as hormones, lipids and therapeutic drugs could be detected from animal tissue.

We recently reported the electrospray ionization using wooden tips.[17] Upon applying a high voltage, sample solution adhering to a wooden tip (toothpick) can be sprayed out to generate a characteristic mass spectrum. Wood is a plant material in nature. The successful utilization of wooden tips for ionization led us to further investigate direct ionization (DI) of plant tissue and other similar materials. In this study, we report that both plant and animal tissues can be directly ionized and analyzed by mass spectrometry.

Experimental methods

Materials

Herbal medicines, including *Coptis chinensis* Franch, *Schisandra sphenanthera*, *Schisandra chinensis*, and crude and processed *Polygonum multiflorum*, were purchased from pharmacy stores in Hong Kong. Spinach leaves and animal tissues used in this study were purchased from supermarkets in Hong Kong. Methanol, acetone and formic acid were purchased from Fisher Scientific (New Hampshire, U.S.), α-cyano-4-hydroxycinnamic acid (CHCA) from Fluka, losartan from Gracure Pharmaceuticals Ltd. (New Delhi, India), and filter paper from Macherey Nagel (Düren, Germany). Extraction of spinach leaf and herbal medicines was performed by vortexing 10 mg of the homogenized sample and 500 μl of methanol–water (1/1, v/v) for 1 min, and the supernatants were used for analysis.

Setup for direct ionization analysis of tissue samples

The experimental setup for DI analysis of tissue samples is shown in Fig. 1. A tissue sample was held typically with a clip connected to the

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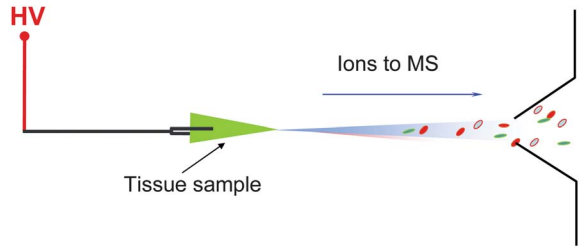

Fig. 1 Experimental setup for DI analysis of biological tissue by MS.

high voltage supply of a mass spectrometer. The tissue sample had been cut to produce a sharp end, which was then placed pointing to the MS inlet (see Fig. S1 in the ESI† for photos of the experimental setup for analysis of tissue samples). The shape and size of the analyzed tissue sample and the holding and connecting device are variable, as long as the tissue sample can be steadily held with a sharp end pointing to the MS inlet and is effectively connected to the high voltage. After adding some solvents (usually 2 μL, may skip this for very wet samples) and applying a high voltage, a plume of spray was induced at the sharp end of the analyzed tissue, and a mass spectrum could be observed. The high voltage applied was typically 3 kV, under which desirable mass spectra could be generally obtained. Further increasing the high voltage was found to increase the tendency of undesirable electric discharge.

Mass spectrometry

Mass spectra were acquired on a QToF II mass spectrometer (Waters, Milford, MA) using positive ion mode unless specified elsewhere. DESI experiments were performed using a home-made DESI ion source with spray solvent methanol–water–formic acid (1/1/0.1%) at a flow rate of 5 μL min^{-1}. Paper spray experiments were performed in a way similar to the literature[16,18] and with methanol–water–formic acid (1/1/0.1%) as the solvent. Capillary voltages were typically set at 3.0 kV for direction ionization, 3.5 kV for DESI, 3.5 kV for paper spray, and 1.5 kV for nano-ESI. These voltages were chosen after optimization of each technique. LC-MS experiments were performed using the CapLC liquid chromatography (Waters, Milford, MA) coupled to the Q-ToF II mass spectrometer. Other settings were similar to those for normal ESI analysis.[17]

MALDI spectra were obtained using a MALDI Micro-MX time-of-flight mass spectrometer equipped with a 337 nm UV laser source (Waters, Milford, MA). For MALDI analysis of spinach leaf, a small piece of spinach leaf was attached to a target plate with double-faced adhesive tape, and a matrix solution of CHCA was loaded on the leaf. The plate was introduced into the mass spectrometer for MALDI analysis after the matrix solution became dry. Other experimental settings were similar to those for normal MALDI analysis.[19]

Results and discussion

The DI mass spectrum obtained from a fresh spinach leaf with methanol–water (1/1) as the added solvent is shown in Fig. 2a. The spectrum was dominated with peaks that were identified as monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), the two most abundant membrane glycerolipids in higher plant tissue,[20] based on their masses and MS/MS mass spectra (Fig. S2 in the ESI†). These glycerolipids, however, could not be detected by direct analysis of the spinach leaf (or the scratched spinach leaf) using DESI, MALDI, paper spray (Fig. 2b–d) or desorption atmospheric pressure chemical ionization (DAPCI).[21] DESI[9] and DAPCI[21–23] are usually used to detect analytes on surfaces and DAPCI[21–23] is more suitable for analysis of relatively volatile compounds. The endogenous glycerolipids may have strong affinity inside the spinach leaf and thus could not be desorbed and ionized by these two techniques. DAPCI has been reported to detect some pigments from the spinach leaf. The undetectability of the glycerolipids by MALDI and paper spray was probably due to the signal suppression. Analysis of the spinach leaf extract using MALDI, paper spray (data not shown) or nano-electrospray ionization (nano-ESI) (Fig. 2e) was also not able to detect the glycerolipids. These results reveal that DI analysis of tissue is more straightforward and allows detection of some compounds that cannot be detected by other techniques of direct analysis. MGDG and DGDG play important roles in plant photosynthesis.[24] Our results indicated that DI could be a simple and rapid method for detection and monitoring of these glycerolipids in plant growth and development.

Other chemical constituents, mainly including plant pigments, commonly detected for spinach using LC-MS[25–28] were not observed with DI. The spinach extract, obtained using extraction solvents of methanol–H_2O (1/1), the same solvent system used in DI, was analyzed by LC-MS for a comparison in this study. In addition to those glycerolipids observed with DI, more glycerolipids and a range of plant pigments, such as phenophytin a and pyropheophorbide a, were observed by LC-MS (Fig. S3 in ESI†), indicating that LC-MS analysis gave more complete information about the composition of spinach leaf. The reason why glycerolipids were predominately detected in the DI spectrum could be that in response to cutting, the glycerolipids significantly accumulated at the wounded region of the leaf,[29] where the ionization occurred. Further investigation is required for more detailed understanding of the DI mechanism.

Coptis chinensis Franch is a herbal medicine with biological effects such as detoxification and prevention of sepsis and its complications.[30,31] A piece of dried *Coptis chinensis* Franch root was analyzed by the DI method. The spectrum obtained (Fig. 3a) was very similar to that obtained by analysis of *Coptis chinensis* Franch tissue using MALDI,[32] DESI and paper spray, respectively (Fig. 3b and c), and was in good agreement with the results obtained by analysis of the *Coptis chinensis* Franch extract using LC-MS[33] and nano-ESI (Fig. 3d) respectively. The predominant peaks at *m/z* 320, 336, and 352 correspond to alkaloids coptisine, berberine/epiberberine, and palmatine, respectively,[32,33] which can be easily ionized.[24] Our results suggested that for dried tissue samples enriched with easily ionized species, their DI spectra might be similar to those obtained by other methods. The signal intensity of the major peaks obtained by DI was comparable to those obtained by paper spray and nano-ESI, but higher than that obtained by DESI (Fig. 3).

Both *Schisandra sphenanthera* and *Schisandra chinensis* are *Fructus Schisandrae*. *S. chinensis* is mainly distributed in northern China, while *S. sphenanthera* is mainly distributed in southern China.[31] The dried ripe fruits of these two plants have long been used as herbal medicines, but their quality is different due to their differences in contents of lignans.[34] Differences in lignan constituents, *e.g.*, Schisandrin and Schisandrol B, between *Schisandra sphenanthera* and *Schisandra chinensis* could be easily observed by DI analysis of the dried fruits of these plants (Fig. 4). The spectra obtained were very similar to those

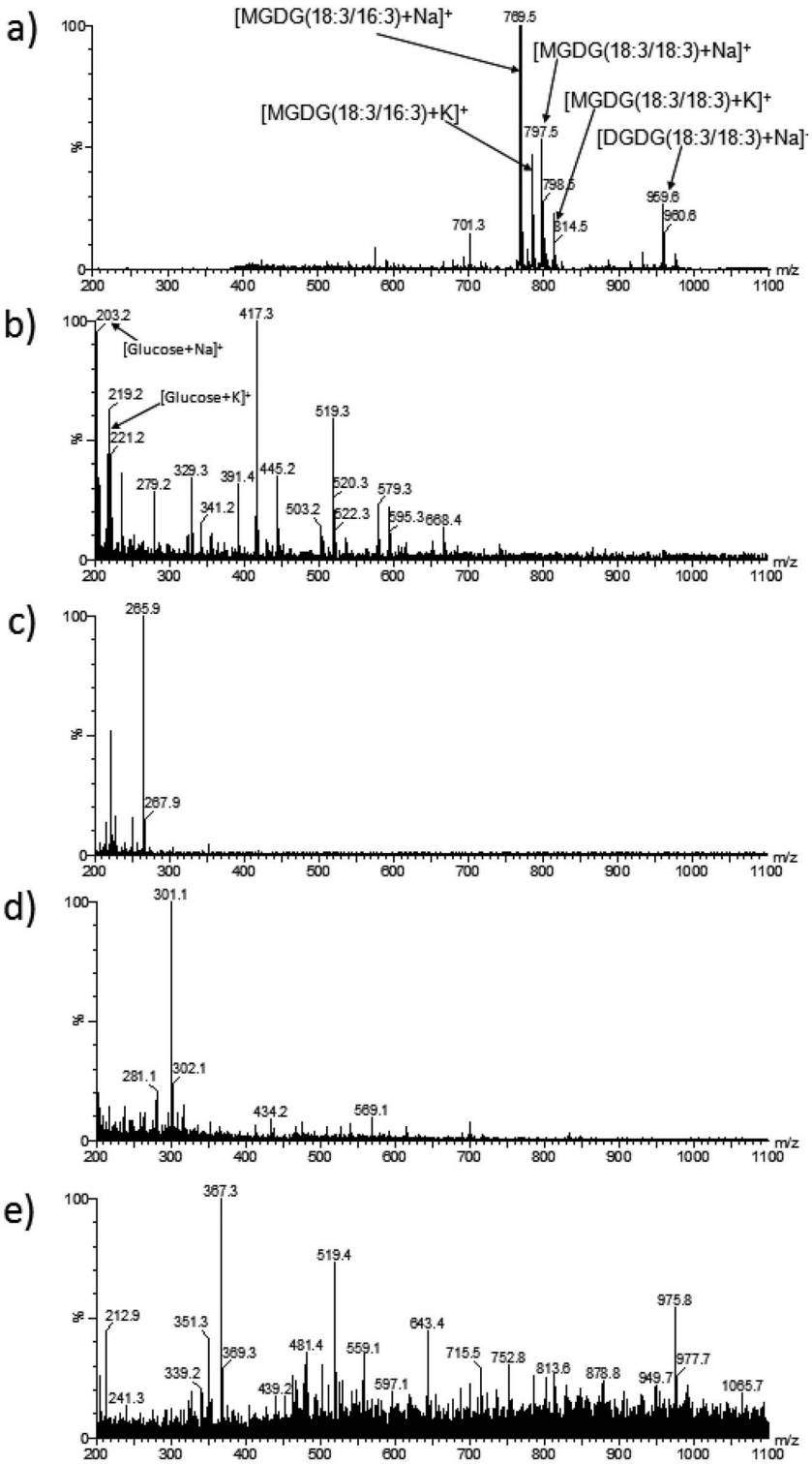

Fig. 2 Mass spectra of the fresh spinach leaf obtained by (a) DI, (b) DESI, (c) MALDI, (d) paper spray, and (e) nano-ESI (analysis of extract).

previously obtained by analysis of methanol extracts of two herbs using ESI-MS.[35] These lignan constituents, however, were not observed in a recent DAPCI-MS study, in which only volatile compounds, *e.g.*, terpenoids, on the herb surface could be detected.[36]

The rhizome of *Polygonum multiflorum* is another common herbal medicine. The crude rhizome of *P. multiflorum* is toxic and needs to be processed before it can be used as a medicine. The processing involves hydrolysis of toxic glucoside compounds in the crude rhizome of *P. multiflorum* into nontoxic deglycosylated compounds.[37,38] DI mass spectra of crude and processed rhizomes of *P. multiflorum* are shown in Fig. 5. Abundant peaks of 2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside were observed in the spectrum

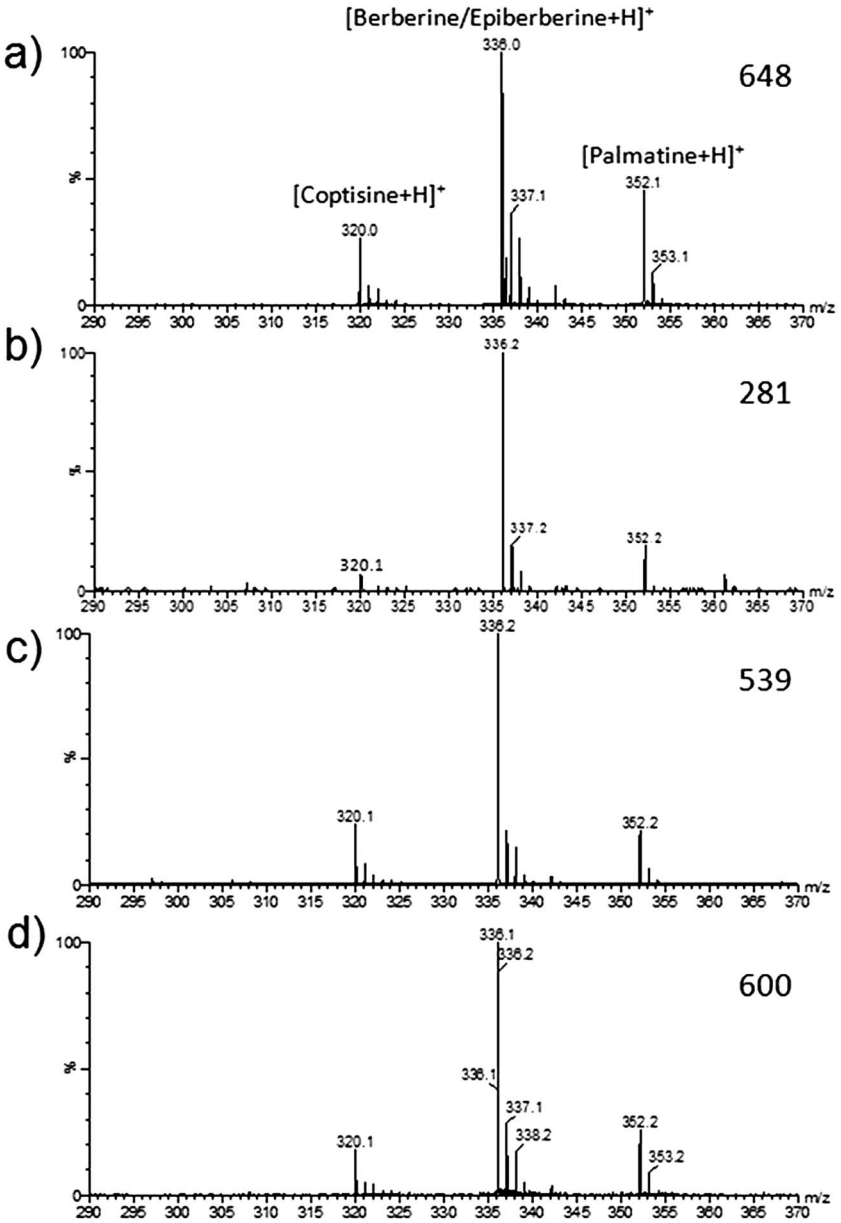

Fig. 3 Mass spectra of *Coptis chinensis* Franch obtained by (a) DI, (b) DESI, (c) paper spray, and (d) nano-ESI (analysis of extract). Intensity of each spectrum is labelled on the upper right corner.

of the crude medicinal herb. These peaks almost totally disappeared in the spectrum for the processed rhizome of *P. multiflorum*, and the corresponding deglycosylated products could be observed. These results demonstrated that DI is a simple, rapid and effective method for detection of major constituents of various plant tissues and for monitoring changes of these constituents, and can be used for differentiation of plants, *e.g.*, herbal medicines, from different sources and different processing methods.

Some other common plant tissues were also investigated. As shown in the preliminary results depicted in Fig. S4†, various chemical constituents, such as sugars and amino acids, could be detected in different plant materials. Further refinement of the method will be performed to detect a broader range of chemicals in plant materials of different texture and morphology.

DI analysis of animal tissue was also tested in this study. Animal tissue is usually much softer than plant tissue and was held by a stainless steel needle for DI analysis in this study (see Fig. S1b†). Fig. 6a is the spectrum obtained for porcine heart using methanol–water (1/1) as the added solvent. Similar to the results obtained by DESI[11] or paper spray[16] for animal tissue analysis, phospholipids such as phosphatidylcholine (PC) were predominantly observed in this spectrum, and in the spectra of other animal tissues such as porcine liver, porcine kidney, porcine spleen, porcine medulla, porcine lung, bovine muscle, fish gill and fish heart (Fig. S5†). Lipids are important compounds for energy storage and construction of cell membranes, and are potential biomarkers for some diseases.[39,40] These results demonstrated that the DI method could be used for rapid detection of lipids from various animal tissues.

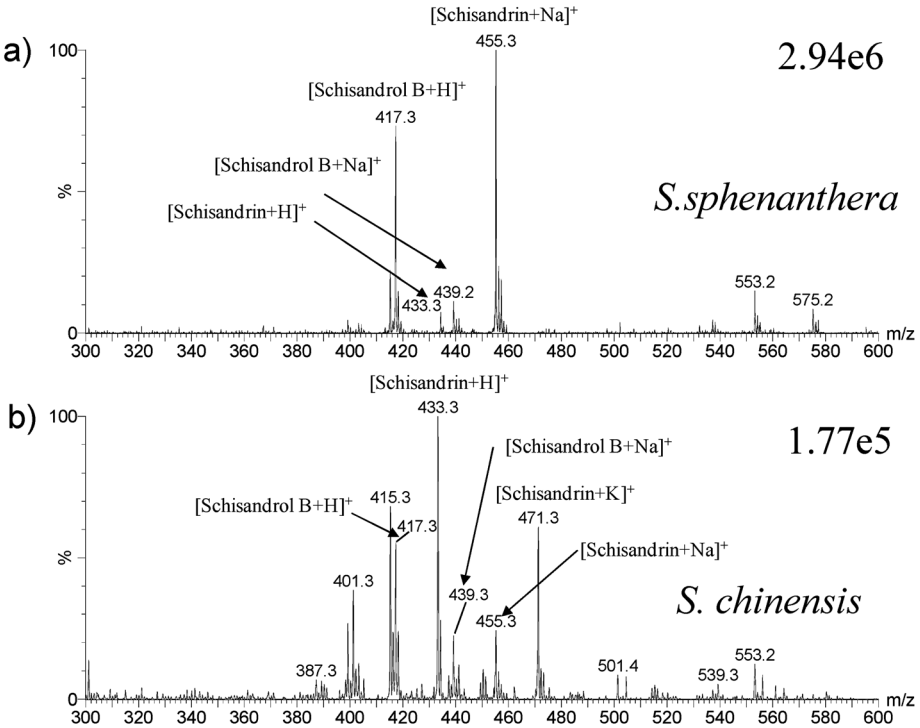

Fig. 4 DI mass spectra of *S. sphenanthera* and *S. chinensis* fruits, acquired on a triple-quadrupole mass spectrometer (Quattro Ultima, Waters, Milford, MA), with methanol–water (1/1) as the added solvent. Intensity of each spectrum is labeled on the upper right corner.

Localization and detection of therapeutic drugs and their metabolites in animal tissue are important in pharmacokinetic studies.[16] DI was attempted for such an analytical purpose in this study. One ng of losartan, a drug for treatment of high blood pressure,[41] was spiked onto 5 mg of porcine kidney. After solvent vaporization, the tissue sample was analyzed by the DI approach with addition of 1 μL of

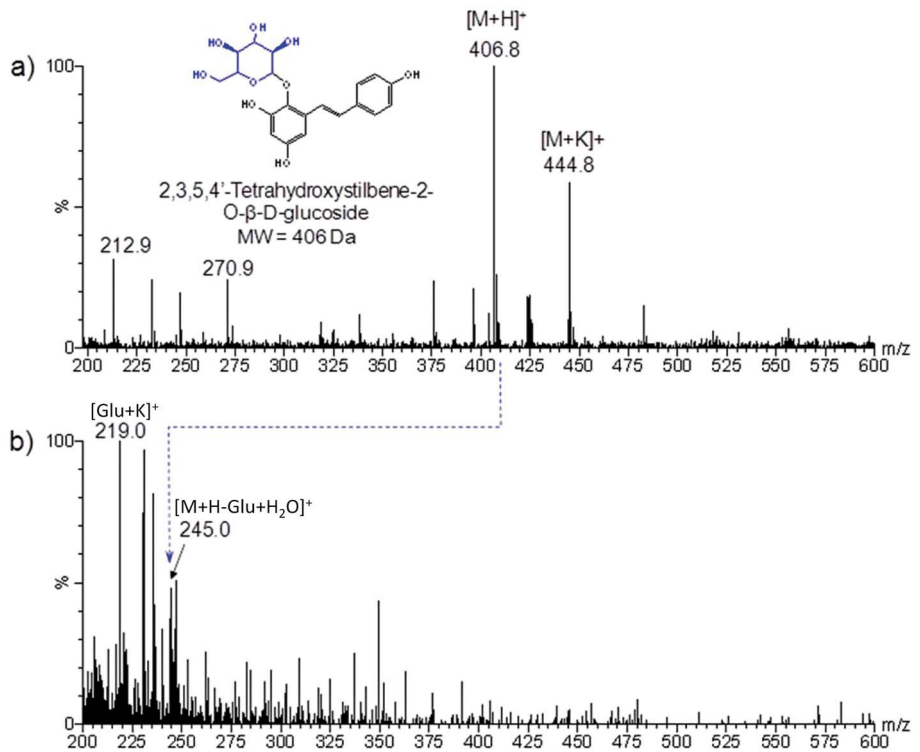

Fig. 5 DI mass spectra of (a) crude and (b) processed rhizomes of *P. multiflorum*, obtained with methanol–water (1/1) as the added solvent.

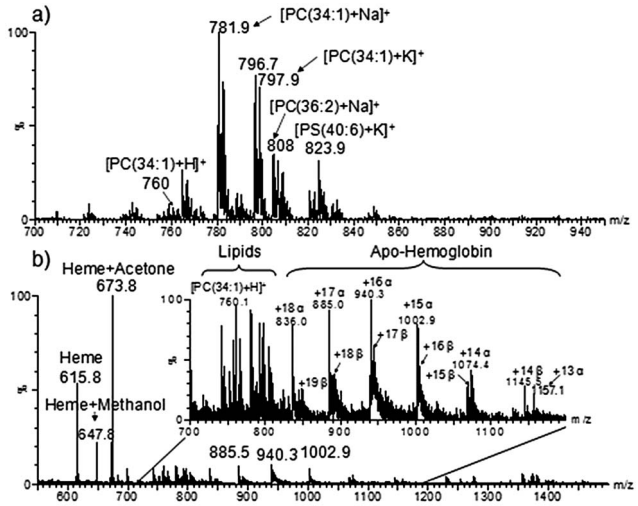

Fig. 6 DI mass spectra of porcine heart. The two spectra were acquired with (a) methanol–water (1/1) and (b) methanol–acetone (1/1) containing 0.1% formic acid as the added solvents respectively.

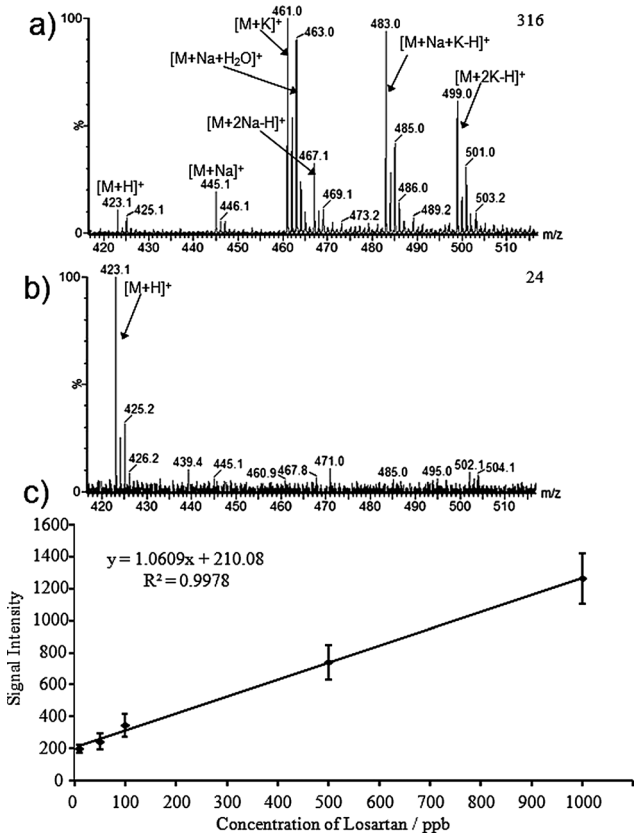

Fig. 7 Mass spectra of porcine kidney after spiking with losartan obtained by (a) DI and (b) LC/MS. Intensity of each spectrum is labeled on the upper right corner. (c) Linear relationship between signal intensity of the losartan ion and concentration of the spiked losartan solution was observed when 2 μL of losartan solution was spiked onto 5 mg of porcine heart tissue. Signal intensity of the losartan ion was measured using selected reaction monitoring (*m/z* 423 > *m/z* 207) on the triple-quadrupole mass spectrometer.

methanol. Protonated molecules and salt adducts of losartan were clearly observed in the spectrum (Fig. 7a) and further confirmed by the MS/MS study (data not shown). When a similar size of tissue sample spiked with the same amount of losartan was extracted with a minimal volume of methanol solvent, *i.e.*, 20 μL, and the extract was analyzed by LC-ESI-MS, only protonated molecules of the target compound were observed and the ion intensity obtained was significantly lower than that in DI (Fig. 7b). These data suggested that DI analysis was more straightforward and could offer higher sensitivity than LC-MS. As shown in Fig. 7c, the signal intensity of losartan was found to have a linear relationship with the spiked amount of the pharmaceutical compound over two orders of magnitude of sample concentrations. The precision of quantitative data achieved was 30%, comparable to quantitative analysis using DESI in some applications.[42] Although further investigation is still needed for the real application and improvement of reproducibility and linear dynamic range of the method, these preliminary results indicated that the DI method has potential application in qualitative and quantitative analyses of therapeutic drugs in animal tissue.

Detection of proteins directly from animal tissue by MS is of significant interest due to the important roles of proteins in biological processes. However, such detection can only be achieved by very few mass spectrometric techniques[4] such as MALDI[12] and ELDI-MS.[14] In this study, by using methanol–acetone (1/1) containing 0.1% formic acid as the added solvent, protein signals were successfully observed in the DI mass spectrum of fresh porcine heart. As shown in Fig. 6b, α and β subunits of hemoglobin were observed, along with abundant peaks of heme at the low mass region. The more hydrophobic solvents favor the observation of proteins, indicating that DI analysis is a solvent-dependent extraction process. The detection of proteins from animal tissue suggested that the DI technique could be a potential tool for diagnosis of diseases such as hemoglobinopathy.[43]

Conclusions

In summary, we have demonstrated that both plant and animal tissues can be directly ionized and characteristic mass spectra can be generated under ambient conditions. The experimental setup of this new technique is very simple and analysis of one tissue sample can be completed within one minute. Various plant and animal tissues have been tested and compounds such as lipids, alkaloids, glucosides, lignans, pharmaceuticals and proteins were observed in the spectra. DI analysis can be considered as a complementary tool to tissue imaging. In DI analysis, spray ionization directly occurs on the tissue sample. The analysis is straightforward and allows us to observe some compounds that cannot be detected by other direct MS techniques. Although further investigation about the detailed mechanism of this new technique is still required, our preliminary results indicate that the directly applied high voltage, the added solvents, the textile structure of tissue, and the properties and distribution of analytes inside tissue are important factors for the spectra observed. Further applications of this new technique are being studied.

Notes

The study in this paper was originally reported in an academic meeting in May 2011.[44] During the submission of this paper, we noticed two latest publications reporting direct mass spectrometric analysis of plant tissue[45] and animal tissue.[46]

Acknowledgements

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