Sinapine Detection in Radish Taproot Using Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry

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Ⓢ *Supporting Information*

ABSTRACT: Plant research and natural product detection are of sustainable interests. Benefited by direct detection with no sample preparation, sinapine, a bioactive chemical usually found in various seeds of *Brassica* plants, has been unambiguously detected in radish taproot (*Raphanus sativus*) tissue using a liquid-assisted surface desorption atmospheric pressure chemical ionization mass spectrometry (DAPCI-MS). A methanol aqueous solution (1:1) was nebulized by a nitrogen sheath gas toward the corona discharge, resulting in charged ambient small droplets, which affected the radish tissue for desorption/ionization of analytes on the tissue surface. Thus, sinapine was directly detected and identified by tandem DAPCI-MS experiments without sample pretreatment. The typical relative standard deviation (RSD) of this method for sinapine detection was 5−8% for six measurements (S/N = 3). The dynamic response range was $10^{-12}-10^{-7}$ g/cm^2 for sinapine on the radish skin surface. The discovery of sinapine in radish taproot was validated by using HPLC-UV methods. The data demonstrated that DAPCI assisted by solvent enhanced the overall efficiency of the desorption/ionization process, enabling sensitive detection of bioactive compounds in plant tissue.

KEYWORDS: DAPCI, sinapine, radish taproot, plant tissue, tandem mass spectrometry, LTQ-MS

INTRODUCTION

By creating ions of analytes without laborious matrix cleanup, ambient mass spectrometry[1−5] enables characterization of complex samples at the molecular level with high sensitivity, high specificity, and high throughput and has been increasingly used for trace analysis of various raw materials. For example, trace analytes involved in drug discovery,[6−8] organic chemistry,[9,10] material science,[11] biology,[12,13] environmental science,[14] etc., can be rapidly detected/imaged using ambient mass spectrometry. Another remarkable merit of ambient mass spectrometry is that the raw sample is accessed at atmospheric pressure during mass spectrometric analysis. This facilitates many applications in which samples are not conveniently compatible with the vacuum environment. Similar to conventional mass spectrometry, ambient mass spectrometric measurement provides abundant chemical information, which cannot be fully paralleled by other analytical methods. These features render ambient mass spectrometry a very attractive tool for the investigation of extremely complicated biological systems such as living objects.[15−18] Efforts have been made for In Vivo breath examination,[19,20] skin analysis,[18,21,22] and microorganism[18,23] differentiation using ambient mass spectrometry.

Plants dominate a large part of the biosphere and play essential roles in many aspects of human society. In-depth knowledge of plants provided by advanced analytical techniques is highly desirable for better usages of plant resources, but this has far

yet to be achieved.[24−27] For example, even the flavor of fruits such as grapes and strawberries, considered by people as daily foods, present many chemical mysteries, which challenge analytical science for better understanding.[24,28] As part of the drug discovery strategy, natural plants are of increasing importance to serve as natural resources of pharmaceutical products.[29] Definitely, information on living plants obtained with molecular scale resolution can promote the understanding of the chemical mechanism of plant life and return enhanced benefits to human society. Surface desorption atmospheric pressure chemical ionization−mass spectrometry (DAPCI-MS) has been demonstrated with satisfactory sensitivity and high throughput for the detection of various compounds in complex matrices.[12,21,30−33] Samples originated from natural plants (e.g., tea products[33]) and animal tissues (e.g., sea cucumber products,[30] chicken egg[12]) have been successfully characterized by using DAPCI-MS, showing the potential utilization of DAPCI-MS for fast analysis of natural products.

Sinapine (*O*-sinapoylcholine), a bioactive antioxidant[34,35] found in canola and rapeseed, is a crucial component, with important medicinal value, of many dietary and medicinal plants. Sinapine is sensitive to heat and oxygen, and decays quickly when
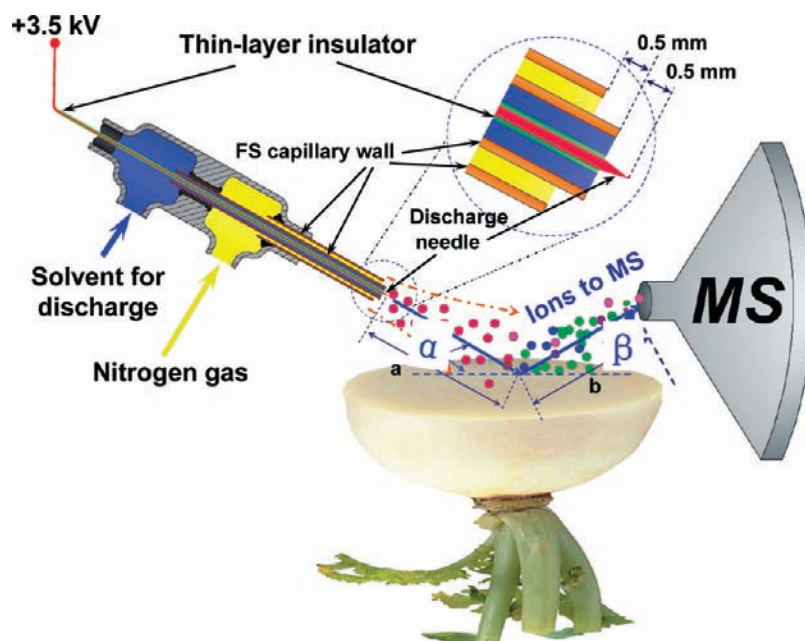
Figure 1. Schematic diagram of the solvent-assisted DAPCI source for detection of sinapine in radish taproot tissues.

exposed to air. Usually, sinapine has to be extracted from the plant seeds before it can be detected by means of chromatography,[36−38] mass spectrometry,[36,39] electrochemical analysis,[40] and optical spectroscopy.[41−43] According to our knowledge, sinapine is merely found in seeds such as seeds of Brassicaseae plants. However, as demonstrated here by the experimental data, sinapine can be positively detected and identified from the taproot of radish (*Raphanus sativus*) plant by using DAPCI-MS without sample pretreatments such as grinding, extraction, and separation. Because radish taproot has much wider availability than its seeds, these findings significantly increase the resource of sinapine. The experimental results also suggest that DAPCI-MS can be a useful tool for the sensitive detection of bioactive components in living biological tissues and might open possibilities for In Vivo plant metabolomics studies and plant physiological applications.

■ EXPERIMENTAL PROCEDURES

Instrumental Setup. A modified DAPCI source built in our laboratory was interfaced to a commercial linear ion trap mass spectrometer (LTQ-XL, Finnigan, San Jose, CA) and operated in the positive ion detection mode for direct analysis of radish tissue. Unlike previously reported DAPCI experiments,[12,21,30−33,44] a methanol/water solution (1:1, v/v), infused through a fused silicon capillary (i.d. = 0.25 mm, o.d. = 0.40 mm) at a rate of 7 μL/min, was nebulized by using a sheath gas flow to generate fine droplets, which were then efficiently charged by the corona discharge. The charged droplets impacted the sample surface, with momentum/charge transfer and extraction of analytes on the surface, and then the droplets scattered back to the air and produced ions of analytes at ambient conditions. The discharge electrode (i.d. = 0.15 mm, length = 10 cm) with a sharp tip (i.d. = ca. 0.015 mm) was isolated from the solution using a thin layer (ca. 0.02 mm) insulator (Figure 1), so that the high voltage (4 kV) could not be grounded through the methanol/water mixture. The discharge needle was exposed to air for 0.5 mm without coverage by the insulator. The nitrogen sheath gas (1.8 MPa, measured at the outlet of the nitrogen tank) was guided through a Teflon tube (i.d. = 0.5 mm, o.d. = 0.76 mm), which connected

to the DAPCI source assembly by using a 3-way T connector. The sheath gas capillary, the solvent capillary, and the discharge tip were carefully arranged to make an interval gap of 0.5 mm, as shown in the inset of Figure 1. The modified DAPCI source was coupled to an LTQ mass spectrometer, allowing a 2 mm distance (a) between the discharge tip and the plant tissue surface and 15 mm (b) from the instrument inlet. The freshly cut radish slices were directly supplied for the DAPCI source, where the sample spot was carefully centered under the tip of the DAPCI discharge needle. The angle (α) formed between the discharge needle and the sample surface was 45°, and the angle (β) formed by the ion entrance capillary with respect to the sample holder was 15−30°. The primary ions impacted the radish tissue surface for desorption/ionization, so that the sinapine ions created at the ambient pressure were then introduced through the ion guide system into the LTQ mass analyzer for mass analysis. The temperature of the heated capillary of the LTQ instrument was maintained at 275 °C. No further optimization of the LTQ instrument was performed. All of the full-scan mass spectra were collected with an average time of 1 min and background subtracted. For tandem mass spectrometry experiments, the parent ions of interest were selected with a mass-to-charge window width of 1.4 units. The collision-induced dissociation (CID) experiments were performed with 10−35 units of collision energy (CE) and 30 ms duration. The mass spectrum recorded from a clean, dry radish skin surface was used as the blank spectrum for background subtraction. For reference experiments, authentic sinapine (50 ng) was deposited on the intact radish skin surface and then detected after drying in the air.

HPLC Analysis. Briefly, the HPLC measurements were carried out using a HPLC LC-20A instrument (Shimadzu, Japan) with a VP-ODS Shimadzu column and UV detector by following the procedure modified from the literature.[38] For sample preparation, an amount of 500 g of freshly harvested radish taproot was ground thoroughly in a mortar until a paste was formed. The slurry was then subjected to centrifugation (6000 rpm) for 10 min. To separate most of the sugar/proteins, pure ethanol was added into the resulting liquid (1:5, v/v). This mixture was stored at −5 °C for 2 h before it was centrifuged (10000 rpm) for 10 min, and the resulting supernatant was ready for HPLC analysis.

The KH$_2$PO$_4$ aqueous solution (0.08 mol/mL) and pure acetonitrile mixture (15:85, v/v) was used as the mobile phase. The flow rate of the mobile phase was set at 1.0 mL/min. Before the experiments were run,
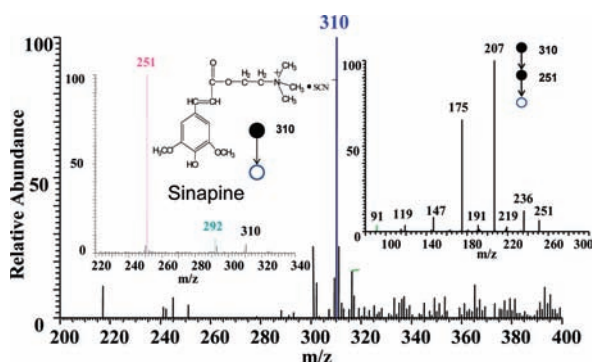
Figure 2. DAPCI-MS spectra of authentic sinapine deposited on the skin surface of radish taproot. (Insets) MS/MS and MS/MS/MS spectra of sinapine signals.

the mobile phases were first filtered through qualitative filter paper (Baxter, McGaw Park, IL) and then degassed in an ultrasonic bath. For the reference experiment, the authentic sinapine compound was directly dissolved in the mobile phase solution ($10\,\mu$g/mL) and then delivered to HPLC analysis to record the retention time of sinapine at the experimental conditions. The column temperature was set at 25 °C. The detection wavelength was selected at 318 nm.

Materials and Reagents. Sinapine, with thiocyanate counterion (purity > 99%), was purchased from EPL Bio Analytical Services (Niantic, IL) and used immediately when it was diluted to low concentration levels. Other chemicals such as methanol (HPLC grade), acetonitrile (HPLC grade), and potassium dihydrogen phosphate (Analytical Reagent grade) were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without pretreatment.

Taproots of radish plants (*R. sativus* L.) were randomly selected from those ripe autumn radish plants (100 days for cultivation, full length of 25−28 cm, individual weight of 0.7−1.0 kg) in the local cultivation area (Fuzhou, China). All of the radish taproots were properly cleaned by normal water washing process immediately after they were harvested. The radish taproot samples were kept in normal storage conditions (25 °C, air exposure, 45−60% RH) for 0−7 days. No pretreatment except for tissue slicing was performed on the radish taproot before sinapine detection by DAPCI-MS.

■ RESULTS AND DISCUSSION

Optimization of DAPCI Conditions for Sinapine Detection. Trace amounts of authentic sinapine were deposited on a clean, dry radish taproot surface, which was subjected to DAPCI-MS analysis. Under the positive ion detection mode, sinapine was detected as cations (m/z 310, the positive counterions of the sinapine molecule) among other signals of low abundance (Figure 2). Upon CID, the precursor ion (m/z 310) generated ionic species of m/z 292 and 251 (the left inset of Figure 2), by the loss of water and $N(CH_3)_3$, respectively. The abundant peak at m/z 251 shows that the loss of $N(CH_3)_3$ is much more favored than the loss of water under the experimental conditions. The product ions of m/z 251 produced major fragments of m/z 236, 219, 207, and 191, probably by the loss of CH_3, CH_3OH, CO_2, and CH_3COOH, respectively (right inset of Figure 2); the ionic fragment of m/z 207 further fragmented into ions of m/z 175, 147, 119, and 91, by the loss of CH_3OH, CH_2CH_2, CH_2CH_2, and CO, successively. The characteristic fragmentation pattern provides the fundamental chemistry evidence for specific detection of sinapine in plant tissue.

The DAPCI conditions were chosen by optimizing the signal intensity of sinapine (m/z 251) in the MS/MS experiments. As shown in Figure 3a, the signal intensity increased along the discharge voltage and reached its maximal level at 4 kV; the signal level decreased significantly when the discharge voltage was >4.5 kV. Figure 3b shows the signal responses to the angles (α) formed between the discharge tip and the sample surface. The maximal signal intensity was obtained at α = 40−45°. For the highest signal intensity, a methanol/water mixture (1:1, v/v) was selected as the spray solvent (Figure 3c), probably because this solvent improved the desorption/ionization efficiency. Given the nebulizer gas pressure of 1.8 MPa, the flow rate of the spray solvent was optimized to be 7 μL/min (Figure 3d), because a stable spray for better ionization efficiency was produced under these conditions. The distance between the discharge tip and the ion entrance was selected as 1.5 cm, as shown in Figure 3e. The temperature of the heated capillary was optimized to be 275 °C, probably because this temperature improved the desolvation process and prevented thermal decomposition of the sinapine ions (Figure 3f). The default values for other working parameters of the LTQ-XL instrument were directly used as recommend by the manufacturer. The following measurements were carried out under the optimized conditions.

DAPCI Mass Spectrum of Radish Taproot. Under the optimized conditions, sinapine was detected as cations of m/z 310 in the DAPCI-MS spectrum recorded from a freshly cut radish taproot tissue (Figure 4a). In comparison with Figure 2a, many more peaks were detected in the range of 50−1000 Da (data partly shown in Figure 4a) of the DAPCI-MS spectrum. Most likely, the signals detected corresponded to small molecules such as metabolites on the radish tissue surface, showing that DAPCI-MS can be useful for plant metabolite profiling without sample pretreatment. The signal detected at m/z 310 generated major fragments of m/z 293, 292, and 251 in the MS/MS experiments (insets of Figure 4a). Clearly, the fragments of m/z 293, 292, etc., were irrelevant to sinapine, indicating that more compounds than sinapine were detected at m/z 310 in the full scan mass spectrum. Thus, the product ions of m/z 251 were isolated for MS^3 experiments, which generated the identical fragmentation patterns observed using the authentic sinapine compounds (insets of Figure 4a). Therefore, the fragment of m/z 251 derived from the precursor ions (m/z 310) detected from the radish taproot tissue was of 100% purity of sinapine and thereby confirmed the successful detection of natural sinapine from the radish taproot tissue.

Similar measurement was also done using other techniques, including the gasless DAPCI source[12,21,30−33,44] and open-air DESI source.[1,8,45−47] For authentic sinapine compound (≥ 50 ng) deposited on the root skin surface, these techniques generated comparable mass spectra, where sinapine was detected at m/z 310 and could be identified by MS^2 and MS^3 experiments. For sinapine naturally originating from the radish taproot tissue, the gasless DAPCI source as well as the open-air DESI source was not sensitive enough to generate trustable signals at m/z 251 in the MS^2 experiments, and thus no sinapine could be identified by matching the characteristic fragmentation pattern obtained in the MS^3 experiments using the authentic sinapine compound. For the modified DAPCI source, no signal at all was recorded once the sheath gas was off, probably because the liquid solution hanging on the tip of the discharge needle prevented the primary ion production. Therefore, the solvent nebulized by the sheath gas was required for direct detection of sinapine in the radish
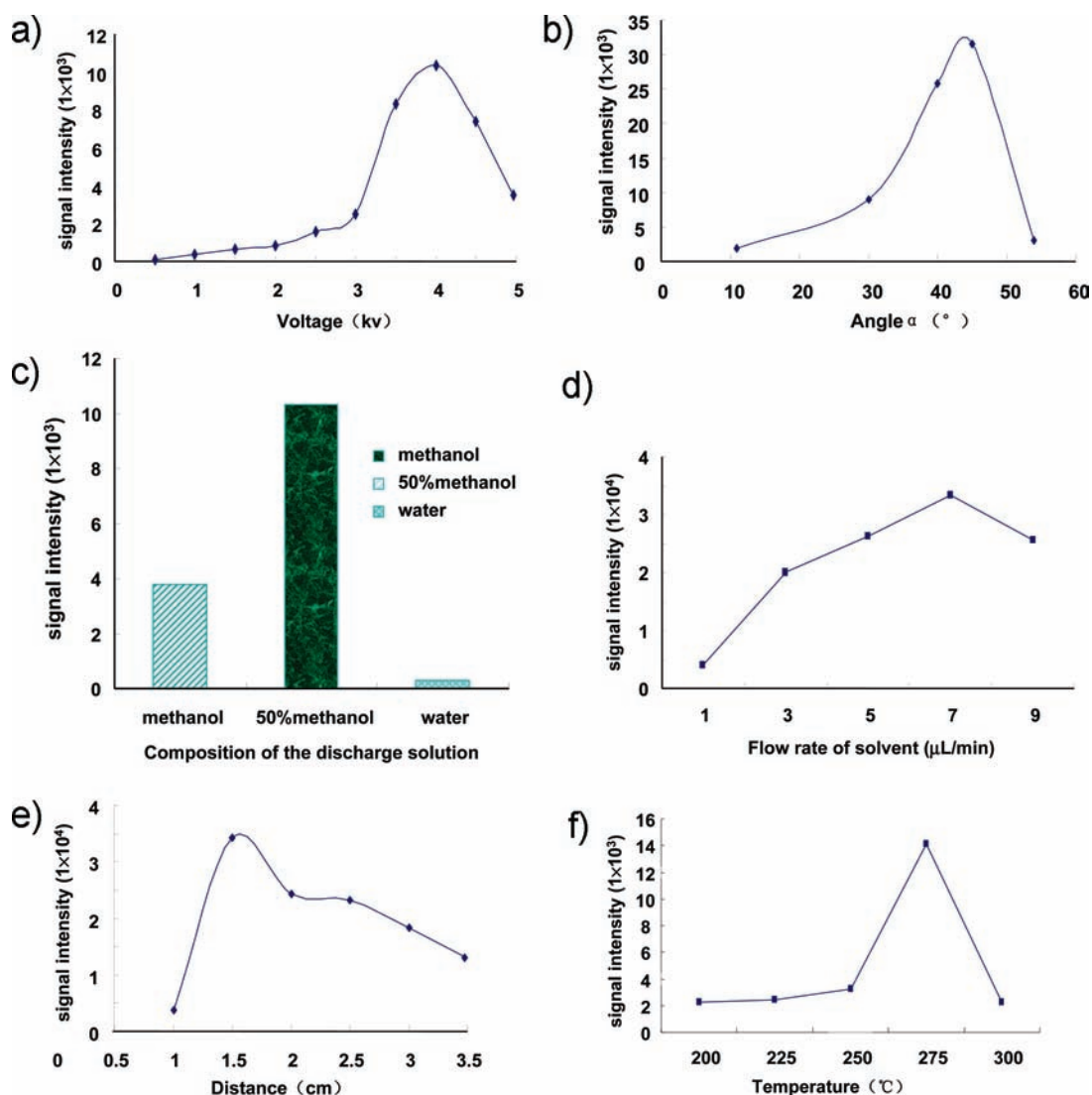
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Figure 3. Optimization of DAPCI source conditions for sinapine detection in the MS/MS experiments: (a) effect of the discharge voltage on the signal intensity of the peak at m/z 251; (b) effect of the angles (α) formed between the discharge tip and the sample surface on the signal intensity of the peak at m/z 251; (c) effect of the solvent composition on the signal intensity of the peak at m/z 251; (d) effect of infusion rate on the signal intensity of the peak at m/z 251; (e) effect of the distance between the discharge tip and the ion entrance on the signal intensity of the peak at m/z 251; (f) effect of the temperature of the heated capillary on the signal intensity of the peak at m/z 251. Each point designates an average of six measurements.

tissue. This was possibly attributed to the physiological state of sinapine in the plant tissue, in which the sinapine might strongly bind to the plant cells.[48] A systematic study addressing the mechanistic difference between the solvent-assisted DAPCI and the gasless DAPCI is under way.

Quantification of Sinapine in Radish Taproot. A calibration curve for the quantification of sinapine in radish root tissue was made by using the most abundant characteristic fragment (m/z 251) obtained in the MS/MS experiments. The linear dynamic response range was about 5 orders of magnitude in the logarithmic scales (Figure 4b), showing the corresponding equation of $y = 0.1438x$ ($-\log$ C, g/cm^2) $+ 2.3959$ ($R^2 = 0.989$). This calibration curve shows the sinapine in the radish tissue can be quantitatively detected without sample pretreatment. Each data point designates the averaged value of six measurements. Typical relative standard deviation (RSD) was about 4−8% for each data point. Table S1 of the Supporting Information shows the repeatable measurements for sinapine at different concentration

levels. For sinapine on the surface of radish taproot tissue, the limit of detection (LOD) was found to be 1.3×10^{-13} g/cm^2 (S/N = 3, n = 10) without matrix cleanup.

Method Validation by HPLC Measurement. According to our knowledge, sinapine has been found in seeds of Brassicaceae plants. The detection of sinapine by DAPCI-MS/MS was featured by the high sensitivity and high speed, showing potential interest for fast screening of sinapine in plant tissues. To validate the discovery of sinapine in radish taperoot, the extracts of radish taproot were analyzed by using high-performance liquid chromatography (HPLC) methods. Figure 5a shows the chromatogram of a standard sinapine methanol solution (10 μg/mL, 10 μL). The signal of sinapine appeared at the retention time of 7.082 min. Under the same conditions, a small peak was detected at the same retention time once a freshly prepared radish juice sample was analyzed by HPLC-UV (Figure 5b). These data show that the detection of sinapine by DAPCI-MS was correctly validated by using conventional HPLC
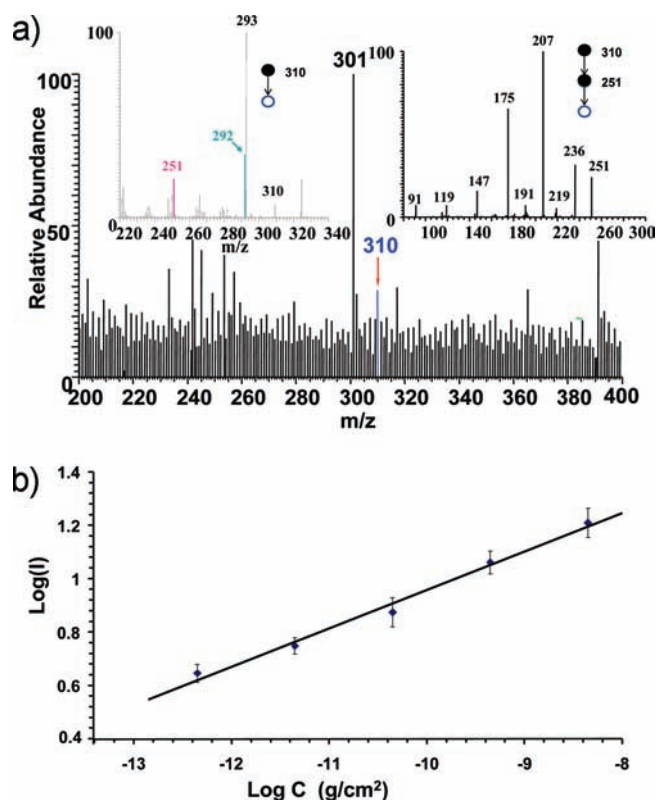
Figure 4. Detection of sinapine from radish taproot tissue: (a) DAPCI-MS spectrum of radish taproot tissue (insets show MS^2 and MS^3 spectra, respectively); (b) signal responses of sinapine detected from the skin surface of radish taproot.

Figure 5. Detection of sinapine using HPLC method: (a) chromatogram of a standard sinapine methanol solution (10 μg/mL, 10 μL); (b) chromatogram of the supernatant prepared from radish taproot samples; (c) degradation curve of sinapine in the supernatant at 4 °C.

method, with sample pretreatment steps, such as separation and preconcentration.

It was also found that sinapine degraded quickly in the prepared extracts even cooled at 4 °C, probably because it reacted with oxygen in the air. Figure 5c shows the degradation curve against storage time at 4 °C, showing a first-order degradation equation, which was fitted as ln $C = -0.02t$ (min) $+ 11.98$, $R^2 = 0.977$, where C is the instant sinapine concentration (μg/mL) at time t (min) in the radish extract. According to this equation, no sinapine was detectable from the extract after storage for about 600 min. Our experiments demonstrated, however, no signal was detected at the retention time of 7.082 from the radish extract sample stored for 80 min at 4 °C, probably because the sinapine concentration was lower than the limit of detection of the HPLC method. Note that the degradation constant varied under the storage conditions; for example, sinapine was not detected by HPLC from the extract stored at room temperature (25 °C) for 15 min. For most HPLC procedures, the sample pretreatment steps occur at room temperature and take >20 min. This could be why no sinapine was previously detected from radish taproot using HPLC methods.

Distribution of Sinapine in Radish Taproot. Taking advantage of DAPCI for direct analysis of plant tissue without sample pretreatment, the distribution of sinapine in radish taproot was investigated using DAPCI-MS/MS. Figure 6a shows the picture of a radish taproot on which four individual locations were measured using DAPCI-MS/MS. Figure 6b shows the sinapine concentrations measured at four sample spots (i.e., S1−S4) located at one-fourth, half, and three-fourths of the full length
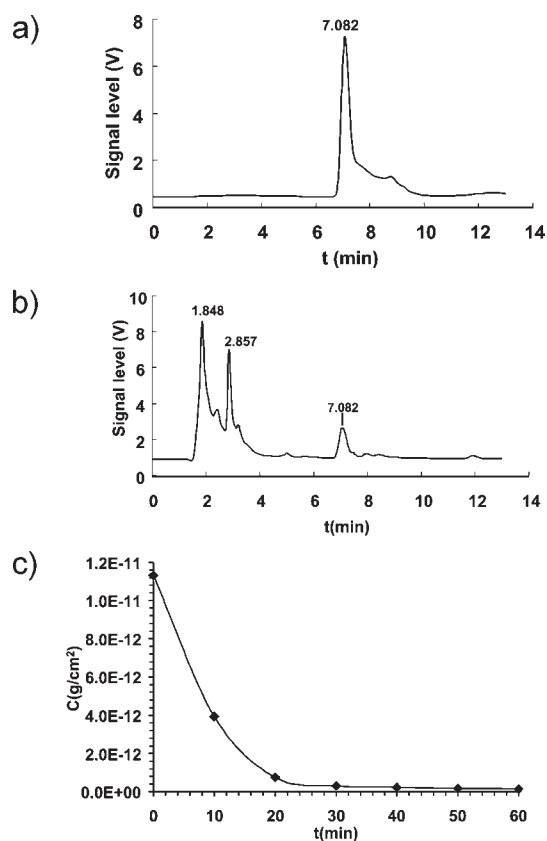
of radish taproot. On the basis of preliminary data (Figure 6b), sinapine was distributed unevenly in the taproot tissue, but most data points were located within a relatively narrow distribution range from 1.5 to 2.2 pg/cm^2. For the concentration of sinapine found at the places (S1 series) along the axis of the radish taproot, the concentration of sinapine found at one-fourth of the length was generally higher than those found at half and three-fourths of the length, probably because the biosynthesized sinapine was somehow accumulated along the axis of the taproot. For the other data series, no clear trend was found for sinapine distribution on the basis of the samples tested. However, the mean value of the sinapine concentration was altered within the range of 1.60−1.80 pg/cm^2 for all of the samples, showing not much dependence on the length of taproot.

Measurement of Sinapine in Radish Taproot. After harvest, radish is usually transported for a long distance to serve final consumers at different locations. It is thus desirable to probe the degradation kinetics of sinapine under normal storage conditions. As for the first trial, freshly harvested radish taproots were kept intact at room temperature (25 °C, air exposure, 45−60% RH) for 0−7 days. It was found that the sinapine concentrations maintained almost the same levels at different measuring spots for the first 2 days. However, the sinapine concentration levels continuously decreased after 48 h of storage (Figure 7). The half-life time of sinapine decay was found to be 2 days after storage. The sinapine degraded slowly to about 25% of the original levels within 4 days and then maintained almost the same levels for the rest of the time tested. Note that for each concentration level at

2152

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Figure 6. Distribution of sinapine in radish taproot samples: (a) ripened radish plant and radish taproot on which four spots on three sliced sections were measured using DAPCI-MS/MS; (b) distribution of sinapine in radish taproot samples.

Figure 7. Sinapine degradation curve obtained by using DAPCI-MS/MS measurements. Note that the samples were stored under normal conditions for 0−7 days before measurement.

three locations (i.e., one-fourth, half, and three-fourths of the length) was averaged from 240 data points, which were obtained from 4 sample spots (i.e., S1−S4) × 6 measurements for each spot × 10 individual radish taproots. The degradation process was also fitted into a first-order linear equation, $\ln C = -0.5831t$ (day) + 26.67, $R^2 = 0.946$. In comparison with the degradation process where the sinapine was extracted and exposed to air, the

degradation of sinapine was much slower when the intact radish taproot was kept for storage at normal conditions. This indicates that, to gain the maximal benefit of the sinapine, radish taproot is desired to be consumed within 2 days after its harvest or to be kept intact for storage of >2 days.

Besides acting as an active antioxidant,[36,43,49,50] sinapine also plays important roles in many biological processes. For example, once the phenylpropanoid pathway is specifically blocked, the sinapine content of the mutants can be significantly altered.[51] Here we show the preliminary results that sinapine in radish taproots responds to UV light exposure, resulting in decreased sinapine contents in the radish taproots irradiated by UV light (253.7 nm, 18 W) for 30 min. It is already known that many plants react with UV light exposure and activate a self-protection mechanism to avoid the damage.[52−55] This process involves many biological chemical reactions, resulting in changes in many aspects such as metabolites.[56] For example, the DAPCI-MS spectral fingerprints recorded from a radish taproot before and

after UV light exposure are shown in Figure S1 of the Supporting Information, panels a and b, respectively. Clearly, the mass spectral pattern was changed, providing evidence at the molecular level to show the plant response to UV irradiation. For sinapine, the levels were significantly decreased after the UV irradiation; only 43−80% of the sinapine remained in the root tissue after the UV light exposure (Figure S2 of the Supporting Information), depending on how much of the dose given. These findings indicate that sinapine might undergo photolysis to protect the plant tissue from damage caused by high-energy irradiation. By measurement of sinapine in plants, it may be possible to monitor sinapine biosynthesis pathways, which opens possibilities to modulate a physiological process of plants and helps to develop new methods probing biological activities such as seed germination[48,57] for better understanding.

Analysis Speed. Traditionally, multistep sample pretreatment is required prior to actual plant sample analysis using classic HPLC methods or conventional mass spectrometry-based techniques. Particularly, for trace detection of natural products, sample pretreatment including sample cutting, tissue crushing, extraction, separation, and preconcentration is usually required using many techniques. The multistep sample pretreatment is generally laborious and time-consuming and may introduce chemical interference from the environment, especially for some bioactive compounds, which degrade quickly when they are exposed to ambient air. Thus, sensitive analysis is highly desirable for these samples. As demonstrated in this study, natural products such as sinapine in radish taproots were directly detected by using DAPCI-MS with minimal sample pretreatment. A single sample analysis was completed in about 30 s using MS[3]. Within such a short time period, bioactive compounds such as sinapine could not decay significantly, so that it was detected using DAPCI-MS/MS experiments.

Products originating from plants are increasingly used nowadays. For better utilization of plant resources, analytical techniques probing the fundamental chemical mechanism of plant life mysteries are thus of paramount importance. Taking advantage of DAPCI for direct detection with no sample preparation, sinapine, a bioactive chemical found in seeds of *Brassica* plants, has been unambiguously detected in radish taproot (*R. sativus*) tissue at several pg/mm^2 levels. Because radish taproot is of wide availability, the discovery of sinapine in it increases the resources of sinapine and promotes better usage. Furthermore, the current study demonstrates that DAPCI-MS can be a promising tool for the sensitive detection of bioactive compounds in plant tissue. This may inspire advanced applications of ambient ionization techniques in plant science research.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Table S1, repeatable measurements for sinapine at different concentration levels; Figure S1, DAPCI mass spectral fingerprints responded to UV light exposure; Figure S2, sinapine levels found before and after UV light irradiation for 30 min. This information is available free of charge via the Internet at http://pubs.acs.org.

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2156

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