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RESEARCH PAPER

Direct Molecular Analysis of Garlic Using Internal Extractive Electrospray Ionization Mass Spectrometry

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Abstract: The internal extractive electrospray ionization mass spectrometry (iEESI-MS) has been applied to direct molecular analysis of garlic tissues. An extraction solvent (e.g., methanol) under a high voltage (+4.5 kV) was injected into the fused silicon capillary whose far end penetrated into the bulk tissue sample. The chemicals in the bulk tissue were selectively extracted by the working solvent and carried along the electric field toward the apex of the sample, to generate electrospray in front of an ion trap mass spectrometer. Without time-consuming sample pretreatment, active garlic substances such as organosulfur compounds (e.g., alliin, allicin), amino acids (e.g., arginine) and saccharides (glucose, polysaccharides) were successfully detected and directly identified via collision-induced dissociation (CID) in positive ion detection mode. Mass spectral fingerprints of different kinds of garlic cloves (24 samples), as well as various post-treated ones (36 samples) in the range of *m*/*z* 50–2000 Da, were classified via principal component analysis (PCA). The experimental results indicated that iEESI-MS allowed direct identification of chemical components in garlic tissue and rapid recognition of metabolic changes in the garlic tissue subjected to various external stimuli, with the analytical advantages such as simplicity, rapidity (less than 2 min per sample), good specificity, and minimal disturbance to the bioactivity of analytes.

Key Words: Internal extractive electrospray ionization; Garlic; Direct analysis; Mass spectrometry

1 Introduction

Recently, ambient mass spectrometric methods have become important for the analysis of complex raw samples, which commonly pose analytical challenges in the scientific community such as life science, chemistry, material science and forensic analysis[1,2]. Thanks to the remarkable merits of ambient ionization techniques, chemical substances spanning a wide concentration range can be directly analyzed with no or minimal sample pretreatment[3]. In the past decade, more than 50 ambient ionization techniques have been invented for practical applications[3]. Techniques such as desorption electrospray ionization (DESI)[4], low temperature plasma probe (LTP)[5], direct analysis in real time (DART)[6], microwave plasma torch (MPT)[7], air flow-assisted ionization (AFAI)[8], surface desorption atmospheric pressure chemical ionization (SDAPCI)[9] have been applied to surface analysis, later extended to imaging studies. For liquid or gaseous substances, paper spray (PS)[10] and extractive electrospray ionization (EESI)[11] are available. Owing to laser desorption, analytes located tens of micrometres underneath the sample surface were available for MS interrogation using techniques such as ambient surface-assisted laser desorption/ionization (ambient SALDI)[12] and laser ablation electrospray ionization (LAESI)[13]. Recently emerging leaf spray ionization[14] made plant tissue analysis even easier. However, while analyzing biological samples, degradation and inactivation of bio-active chemicals may occur due to the destructive sampling of intact sample bulk, possibly affecting the measurement accuracy. Therefore, a simple and effective method is urgently needed to

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meet the requirements for the rapid analysis of substances inside intact solid samples.

Garlic bulb, a spicy, common vegetable in daily life, contains rich bioactive constituents such as organic sulfides, amino acids and polysaccharides. Most analytical method have limited capabilities for the rapid analysis of multi-component garlic tissue with high molecular specificity[15,16]. In present study, garlic bulb was chosen as a representative sample. Chemical constituents such as active organosulfur compounds (e.g., alliin, allicin), amino acids (e.g., arginine), and sugars (glucose, polysaccharides) were *in situ* identified by internal extractive electrospray ionization mass spectrometry (iEESI-MS)[17,18] without sample pretreatment. By analyzing chemical fingerprints of garlic bulb with principal component analysis (PCA), an MS strategy for the rapid characterization of garlic tissue components at the molecular level was established.

2 Experimental

2.1 Instruments and reagents

MS experiments were carried out using an LTQ-XL MS instrument equipped with Xcalibur data processing system (Thermo Scientific, CA). A homemade iEESI apparatus was coupled in the front of MS inlet. The LTQ-XL MS instrument was set at positive ionization detection mode with a mass range of 50–2000 Da. The ion introduction capillary temperature was 150 °C. In the collision-induced dissociation (CID) experiments, the precursor ions of interest were isolated with a window width of 1.5 Da and the collision energy was set at 10%–30% in an arbitrary unit. Other parameters were set as default values and no further optimization was performed.

The fused silicon capillary (inner diameter 0.25 mm, outer diameter 0.30 mm) was purchased from Agilent (USA). HPLC grade methanol and acetic acid were from ROE Scientific® Inc. Newark, USA. The double-distilled water was used throughout the experiment. Two kinds of garlic bulbs (common garlic bulb and single-clove garlic bulb) were purchased from a local supermarket.

2.2 Experimental method

The conceptual illustration of iEESI-MS was shown in Fig.1. A fused silicon capillary was inserted into the bulk sample in parallel with the sample surface, allowing a gap of ca. 2 mm between the capillary orifice and the edge of sample apex. The apex of the tissue was intentionally pointed toward the ion entrance of the mass spectrometer, keeping a distance of ca. 5 mm in between. An extraction solvent (e.g., methanol) biased with high voltage (+4.5 kV) was injected into the fused silicon capillary at a flow rate of 2 μL min^{-1}. The chemicals in the bulk tissue were selectively extracted by the working solvent

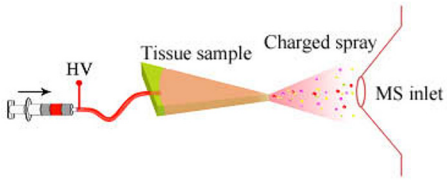

Fig.1 Schematic diagram of the internal extractive electrospray ionization process

and carried along the electric field toward the apex of tissue sample, where an electro-spraying was induced. The charged micro-scale droplets were subsequently desolvated, producing gaseous ions ready for MS analysis.

Garlic cloves with similar size were chosen, and triangular-shaped tissues were sampled from the similar part of each garlic clove, in order to assure the repeatability. In the case of characterization of different kinds of garlic, two sets of garlic cloves (12 common garlic cloves and 12 single-clove garlic cloves) were analyzed in triplicate. In addition, three sets of garlic cloves (untreated garlic cloves, frozen garlic cloves and greened garlic cloves) were measured with 12 garlic cloves in each set, respectively. The frozen garlic cloves were stored in the refrigerator (–18 °C) for 6 h, and the greened ones were soaked in 10% acetic acid aqueous solution for 6 days. Fresh ones were taken as the control group. All of the three groups of garlic cloves were used directly without further treatments, and each garlic clove was analyzed once by iEESI-MS. All the mass spectral fingerprint data were further exported to Excel software for principal component analysis (PCA) performed using the Matlab (version 7.8.0, Mathworks, Inc., Natick, MA, USA).

3 Results and discussion

3.1 Analysis of different kinds of garlic cloves

Two sets of garlic cloves (common garlic and single-clove garlic) were subjected to iEESI-MS analysis and the chemical fingerprints were shown in Fig.2. Due to the inherent nature of iEESI extraction, rich inorganic ions in garlic tissue (e.g., Na^+, K^+) contributed in the ionization reaction, $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$ and $[M + NH_4]^+$. The precursor ions of interest were isolated in the following collision-induced dissociation (CID) experiments, and characteristic fragment ions were obtained for the identification purpose. Based on characteristic fragments and the results of relevant literatures[15,19], active organosulfur compounds in the garlic tissue were identified. Peaks such as *m/z* 178 $[alliin + H]^+$, *m/z* 216 $[alliin + K]^+$, *m/z* 163 $[allicin + H]^+$, *m/z* 180 $[allicin + NH_4]^+$, *m/z* 185 $[allicin + Na]^+$, and *m/z* 201 $[allicin + K]^+$ were assigned to ionization products of alliin (M_w 177) and allicin (M_w 162), respectively (Fig.2a). Moreover, MS/MS spectra of *m/z* 201 $[allicin + K]^+$ and *m/z* 216 $[alliin + K]^+$ were exhibited in Fig.3a and Fig.3b,