International Journal of Mass Spectrometry 348 (2013) 15–22

Contents lists available at SciVerse ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Direct imaging of plant metabolites in leaves and petals by desorption electrospray ionization mass spectrometry

Bin Li, Steen Honoré Hansen, Christian Janfelt*

Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 16 April 2013
Accepted 16 April 2013
Available online 24 April 2013

Keywords:
Desorption electrospray ionization
Mass spectrometry imaging
Hypericum perforatum
Very long chain fatty acids
Plant metabolites

ABSTRACT

Two different approaches to direct imaging of plant material with desorption electrospray ionization (DESI) mass spectrometry are presented and demonstrated on leaves and petals of *Hypericum perforatum*. The direct imaging approaches are in contrast to previous DESI imaging studies where indirect analysis via imprints were used in order to overcome the morphological barrier presented by the layer of cuticular waxes covering the surface of a leaf or a petal. In order to enable direct imaging of such plant materials, a new ternary solvent system is introduced, providing a higher and more stable signal from soft plant materials than the binary solvent systems typically used in DESI. With this ternary solvent system, it was possible to image a number of very long chain fatty acids (VLCFAs), a significant class of metabolites located in the cuticle layer in leaves and petals, as well as other plant metabolites. In the case of the petals of *H. perforatum*, all common metabolites could be imaged directly using the ternary solvent, whereas in the case of leaves from the same plant, only some of the metabolites were accessible, even with the ternary solvent system. For these samples, the leaves could be imaged with direct DESI after chloroform had been used to remove most of the cuticle, thus exposing lower layers in the leaf structure. A number of considerations regarding selection of samples and instrumental parameters that must be made in direct DESI imaging of plant materials are discussed.

1. Introduction

In recent years, the new mass spectrometry imaging (MSI) techniques have presented a significant potential within plant science, allowing us to explore secondary metabolites distribution in plant tissues and even in cells [1–3]. Mass spectrometry imaging studies can provide us with new knowledge concerning the physiological role and fate of metabolites during plant growth and development, as well as in defense reactions as a response to environmental challenges. In the group of MSI techniques, desorption electrospray ionization (DESI) imaging [4,5] is special compared to e.g. matrix assisted laser desorption ionization (MALDI) imaging [6] and secondary ion mass spectrometry (SIMS) imaging [7,8] in its simplicity and the fact that a DESI imaging source for a relatively low cost can be coupled to most of electrospray mass spectrometers installed in laboratories worldwide. While DESI imaging in general offers a quite modest spatial resolution, typically about 100 μm and upwards, DESI has the advantage that it requires little or no sample preparation and no application of matrix.

DESI imaging has become an attractive tool for visualizing spatial location of various chemical components, e.g. lipids [9–11], metabolites and drug substrates in animal and human tissues [12,13]. From the aspect of plant materials, direct non-imaging DESI analysis secondary metabolites has been published [14–16], whereas direct DESI imaging has only been presented on seaweed [17] and stripped epidermis of barley leaves [18]. This is mainly due to the layer of cuticular waxes, which covers the leaves and petals of plants to protect them from drying out, but also makes the analytes in the plant tissue less accessible for example, DESI imaging; the problem is thus not present in the analysis of marine plants such as seaweed, which need no such protection layer. In an imaging experiment the surface is scanned at a constant speed, and a spot on the sample is interrogated for less than a second, typically less time than it takes the spray to penetrate the cuticle. In order to produce a good image, the signal stability must be very high, resulting in very few bad spectra as they cause disturbing pixels in the image. One method that overcomes this cuticular barrier related to soft plant tissues is the indirect DESI imaging method, which was introduced a few years ago. With this method, the plant material is imprinted onto a porous Teflon surface from which the analytes of interest are easily imaged [19].

The cuticle of plants predominantly consists of significant secondary metabolites, for example very long chain fatty acids (VLCFAs) with aliphatic tails longer than 22 carbons. VLCFAs, which are synthesized in the epidermal cells, are vital precursors of

* Corresponding author. Tel.: +45 35 33 65 57; fax: +45 35 33 60 30.
E-mail address: christian.janfelt@sund.ku.dk (C. Janfelt).

1387-3806/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijms.2013.04.018

aldehydes, primary and secondary alcohols, alkanes, ketones and esters [20]. Interest in VLCFAs has increased greatly since their specific biofunction in the human brain is closely related to the disorder of peroxisomal metabolism [21]. In addition, the plant cuticle also includes triterpenoids and minor secondary metabolites, such as sterols and flavonoids [22].

In this paper we explore and develop the methods for the direct imaging of soft plant tissues by the optimizing and modifying the DESI spray solution. It is the first time a ternary spray solution with the specific combination, chloroform–acetonitrile–water, was used to capture in situ ion images of VLCFAs and other secondary metabolites from the surface of *Hypericum perforatum* leaves and petals. Various DESI spray solutions have been presented over the years, e.g. non-aqueous spray solutions [23,24], surfactant spray solutions [25], reactive additive spray solutions [26] and dilution protein spray solution [27], all developed in order to broaden the detection range for different types of chemical compounds and improve the detection limits. Amongst them the non-aqueous spray solutions were evaluated and shown to offer better selectivity and intensity for the hydrophobic compounds due to the solubility effect when used in DESI [23]. Yet, DESI is normally used for desorbing polar compounds from tissues, and no method for the detection and imaging of very non-polar metabolites coated on the surface of plant tissues has been reported so far. With a starting point in the non-aqueous spray solutions we have developed a ternary spray solution that increases the sensitivity towards this class of compounds (VLCFAs) by 4–10 times and also enables imaging of other metabolites. Regardless of the spray solvent, DESI is an energetically very soft ionization/desorption technique less capable of penetrating the wax layer, compared to e.g. the laser ablation electrospray ionization (LAESI) [28] technique. Thus, in order to image the metabolites located under the wax layer directly, we treated the leaf with chloroform for some seconds, an approach that has previously been successfully used in GALDI (graphite assisted laser desorption ionization) imaging of *Arabidopsis* leaves [29]. Furthermore, non-imaging DESI analysis of *Cercidiphyllum japonicum* leaves has been performed after treatment with chloroform and dichloromethane [30]. After similar treatment with chloroform, we were able to perform direct DESI imaging of all the well-known metabolites in the different compartments of the *H. perforatum* leaves.

H. perforatum was chosen as the imaging subject due to its specific structure containing translucent glands and dark or red nodules on the leaves and petals. In a number of studies, various traditional separation methods have been used to analyze the secondary metabolites in *H. perforatum* [31–33]. Recently, matrix-free UV-laser desorption/ionization (LDI) MSI [34] as well as indirect DESI MSI [19,35] were applied for the location of secondary metabolites in *H. perforatum* leaves and/or petals. Both techniques offer obvious advantages in studies where the metabolites are unknown. This is however the first time that VLCFAs are detected and directly imaged on the surface of *H. perforatum* leaf and petal with DESI MS.

2. Experimental

2.1. Chemicals

LC–MS grade acetonitrile was purchased from VWR International (Herlev, Denmark), chloroform was purchased from Merck (Germany) and water was prepared with a Millipore Direct-Q3 UV system (Billerica, MA, USA). All standard compounds were purchased from Sigma–Aldrich (Copenhagen, Denmark). A 10 μg/mL melissic acid (C30:0) stock solution was prepared in pure chloroform and stored at 4 °C.

2.2. Plant material and preparation

Plants of *H. perforatum* were collected during the period July–September 2012 in Denmark. The dorsal side of leaves and the upper side of petals were attached onto glass slides with double-sided tape and subjected to direct DESI imaging analysis. In the experiments where the wax layer was removed, the blade tip (ca.1/2 of the leaf) of a fresh *H. perforatum* leaf was totally dipped into the chloroform for ca. 30 s and dried in air for immediate subsequent imaging.

2.3. DESI-MSI instrumentation and analysis parameters

The imaging was carried out on a Thermo Fisher Scientific LTQ XL Linear Ion Trap Mass Spectrometer (San Jose, CA, USA) equipped with a custom-built DESI ion source described in detail elsewhere [19]. The mass spectra were collected and converted to imzML files [36] using an imzML converter (www.maldi-msi.org), and images were created with Data Cube Explorer (AMOLF, Amsterdam, Netherland). The optimized MS instrumental parameters in the negative-ion mode used were as follows: 10 bar nebulizer gas (N_2) pressure, 400 °C capillary temperature, −5 kV spray voltage, −30 V capillary voltage and −100 V tube lens voltage. The ion injection time was 100 ms (automatic gain control was disabled), and 4 microscans were averaged for each pixel in the images. Imaging experiments were performed by continuously scanning the plant tissue surface in the *x*-direction at surface velocity of 168 μm/s for the petal, 253 μm/s for the non-treated leaf and 202 μm/s for the half treated leaf in full scan mode. Correspondingly, each step in the *y*-direction was between 100 and 150 μm, depending on which pixel size was desired in the images. The DESI geometry parameters were optimized, resulting in a tip-to-surface distance of 1.5–2 mm, a tip-to-inlet distance of 4–5 mm, an incident angle of 50°–60° and a collection angle of 10°. A 50 μm ID-150 μm OD fused silica capillary (SGE, Austin, TX, USA) was used for the solvent, surrounded by a 250 μm ID-350 μm OD fused silica capillary (SGE) carrying the nebulizer gas in the sprayer assembly. Optical images of the sections were obtained by use of a digital camera. The identification of all the compounds mentioned in the article is based on MS/MS fragmentation and comparison with standard compounds presenting the same fragment ions or fragmentation data reported in the literature.

A new ternary spray solution was developed not only to penetrate the cuticle for analysis of compounds below the cuticle, but also analysis of the cuticle itself. For this purpose we initially tested the non-aqueous spray solutions which had previously been introduced for analysis of non-polar compounds. Although the non-aqueous $CHCl_3$–ACN spray solution could be used to provide spectra of the surface metabolites, the resulting signal intensities were too low to be used for DESI imaging. Thus, in order to improve ionization efficacy, spray stability, as well as extraction amount of hydrophilic compounds, which might co-localize with the waxes or be locating next to the wax layer, we added a small amount of water (200 μL–10 mL $CHCl_3$–ACN (1:1) close to what can be dissolved in the chloroform/acetonitrile mixture), resulting in a ternary spray solution composed of $CHCl_3$–ACN–H_2O (1:1:0.04). This ternary spray solution maintains the good properties of the non-aqueous spray solutions, and improve the ion generation, probably due to the water acting as proton acceptor in the negative-ion mode. It is likely that the addition of an acid or base to the non-aqueous spray solutions would have the similar effect in the positive and negative ion mode, respectively.

In this work, the initial DESI parameters were tested and optimized with standard solutions deposited onto glass slides, resulting in a nebulizer gas pressure of 8 bar, 300 °C capillary temperature and 3 μL/min solvent flow rate. When these parameters were used