Phytochemistry 139 (2017) 72–80

Contents lists available at ScienceDirect

Phytochemistry

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

Visualizing spatial distribution of small molecules in the rhubarb stalk (*Rheum rhabarbarum*) by surface-transfer mass spectrometry imaging

Joanna Nizioł*, Justyna Sekuła, Tomasz Ruman

Rzeszów University of Technology, Faculty of Chemistry, 6 Powstańców Warszawy Ave., 35-959, Rzeszów, Poland

ARTICLE INFO

Article history:
Received 15 October 2016
Received in revised form
7 April 2017
Accepted 11 April 2017
Available online 17 April 2017

Keywords:
Rheum rhabarbarum
Polygonaceae
Rhubarb
Mass spectrometry imaging
Low molecular weight compounds
Gold nanoparticles

ABSTRACT

Laser desorption/ionization mass spectrometry imaging (LDI-MSI) with gold nanoparticle-enhanced target (AuNPET) was used for visualization of small molecules in the rhubarb stalk (*Rheum rhabarbarum* L.). Analysis was focused on spatial distribution of biologically active compounds which are found in rhubarb species. Detected compounds belong to a very wide range of chemical compound classes such as anthraquinone derivatives and their glucosides, stilbenes, anthocyanins, flavonoids, polyphenols, organic acids, chromenes, chromanones, chromone glycosides and vitamins. The analysis of the spatial distribution of these compounds in rhubarb stalk with the nanoparticle-rich surface of AuNPET target plate has been made without additional matrix and with minimal sample preparation steps.

1. Introduction

Rhubarb (*Rheum rhabarbarum* L.) is the common name for approximately 50 various kinds of *Rheum* species of plants in the family *Polygonaceae*, some of which have been domesticated as medicinal rhubarb (e.g., *Rheum officinale* B. and *Rheum palmatum* L.) and others are considered as vegetable rhubarb [*Rheum rhabarbarum* (syn. *undulatum*) L.]. Furthermore, some of them (e.g. *Rheum rhaponticum* L.) are used both as food and as raw material for medicinal purposes (Chin and Youngken, 1947). Rhubarb (*R. rhabarbarum*) is one of the oldest and most well-known traditional Chinese herbal medicine and has been widely used for more than thousands of years most frequently in China for the treatment of a variety of diseases including gastro-intestinal hemorrhage, constipation, inflammation, ulcers and jaundice (Xiao et al., 1984; Duke, 2002; Wu et al., 1995). The root and the dried rhizome are still being used as ingredients of some drugs.

Recently, many research groups focused on the pharmaceutical applications of rhubarb which include not only purgation, analgesic, antibacterial, antitumor and antispasmodic effects, but its ingredients may be of use in case of renal disorders (Tang and Eisenbrand, 1992; Huang, 1993).

Till now, a large number of compounds have been isolated from *Rheum* species. The major biologically active compounds in rhubarb are anthraquinone derivatives including emodin (1,3,8-trihydroxy-6-methylanthraquinone), aloe-emodin (1,8-dihydroxy-3-hydroxymethylanthraquinone), physcion (1,8-drihydroxy-3-methyl-6-methoxyanthraquinone), chrysophanol (1,8-dihydroxy-3-methylanthraquinone), rhein (1,8-dihydroxy-3-carboxyanthraquinone), danthron (1,8-dihydroxy-9,10-anthraquinone) and their glucosides (Cai et al., 2004; Huang et al., 2007). What is interesting, biologically active compounds found in this plant belong into different classes of chemical compounds such as dianthrones, stilbenes, anthocyanins, flavonoids, polyphenols, organic acids, chromenes, chromone glycosides and vitamins classes (Agarwal et al., 2001).

In recent years, many techniques have been reported for the separation and determination of active compounds in rhubarb. However, the analysis of the spatial distribution of molecules of interests in rhubarb tissue has not yet been done. The visualization of distribution of biomolecules in organs and organelles of biological samples has until recently mainly been done with conventional techniques, such as fluorescence microscopy with proper labelling or staining techniques. There is also a need to increase the knowledge regarding analysis of the patterns of temporal-spatial distribution of specific molecules in the plant metabolome that are induced by environmental stresses. To achieve mentioned aims,

* Corresponding author.
E-mail address: jniziol@prz.edu.pl (J. Nizioł).

http://dx.doi.org/10.1016/j.phytochem.2017.04.006
0031-9422/

laser desorption/ionization mass spectrometry imaging (LDI-MSI) methods are currently the best choice.

LDI-MSI is a two-dimensional mass spectrometry based molecular imaging technique which allows visualization of the spatial distribution of many molecules in tissues sections without extraction, purification, separation, or labelling. Over the preceding decade, LDI-MSI has been widely adopted in several scientific fields including the medicinal, pharmaceutical, and botanical research communities (Svatoš, 2010). Laser-based mass spectrometry imaging is a powerful analytical tool that allows for the analysis of hundreds to thousands of compounds in a single experiment. MSI produces important information concerning primary metabolism, natural products, plant defense, plant response to abiotic and biotic stress.

In the recent years, several MS techniques have been reported for imaging of a wide range of materials. Among MSI techniques, matrix assisted laser-desorption ionization (MALDI) (Norris and Caprioli, 2013), secondary-ion mass spectrometry (SIMS) (Belu et al., 2003), and desorption electrospray ionization (DESI) (Heyman and Dubery, 2016; Thunig et al., 2011) are most commonly used. Spatial analysis of biological tissues by MSI is commonly performed using thin tissue sections performed in a cryostat with a microtome. The sections are then moved in two dimensions while the mass spectra are recorded (Norris and Caprioli, 2013). Alternatively, newer approaches applying variations of blotting or imprint techniques where the chemicals from biological samples are initially transferred to another surfaces were shown. This relatively new approach has been successfully applied in several MSI techniques including MALDI (Tucker et al., 2011), SIMS (Sjövall et al., 2003), DESI (Watrous et al., 2010) and nano-assisted laser desorption-ionization (Vidová et al., 2010).

LDI-MSI is expected to open a new frontier in plant science. In particular, to our best knowledge, the spatial distribution of metabolites in rhubarb stalk have never been examined previously. Herein, we present examples of imaging of biological tissue represented by cross-sections of rhubarb stalk, which was imprinted onto gold nanoparticle enhanced target (AuNPET) and followed by LDI-MSI analysis (Sekuła et al., 2015a, 2015b).

2. Results and discussion

Gold nanoparticle-enhanced target (AuNPET) was used previously for LDI-MS analysis of low molecular weight (LMW) compounds in biological objects (Sekuła et al., 2015a). AuNPET target plate was shown in our recent work to be a promising alternative to traditional MALDI targets (Sekuła et al., 2015b).

The list of compounds shown in Table 1 was created based on both MS imaging but also literature data. All of presented compounds were previously found in rhubarb tissue, usually in relatively large quantities. The identity of majority of compounds was confirmed with LIFT® MS/MS experiments (Supplementary Information). Figures presented within this work contain ion images generated for all compounds/ions listed in Table 1. Additional information regarding MS data is shown in Supplementary Information. LDI-MSI experiment data was performed by measuring series of high-resolution MS spectra with 40 × 40 μm lateral resolution of part of rhubarb stalk imprint of *ca.* 5 × 3 mm size made on AuNPET target plate. This methodology was previously shown to give excellent results (Nizioł et al., 2016).

One of the most important classes of chemical compounds found in rhubarb species are anthraquinones and their glycosides due to their medical applications. They are phenolic-type compounds naturally occurring in all species of rhubarb (Tang and Eisenbrand, 1992; He and Luo, 1980). Compounds of this class were believed to be the active laxative agents used in Chinese traditional medicine. Purgative effect of rhubarb has been attributed to the significant amount of anthraquinone derivatives and their glycosides (Qu et al., 2008; Yang et al., 2011). The most important pharmaceutically relevant anthraquinone derivatives in rhubarb are emodin and aloe-emodin. These compounds have been determined to have a variety of additional therapeutic effects such as anti-inflammatory (Ghosh et al., 2010; Choi et al., 2013), anti-diabetic (Wang et al., 2006; Zhao et al., 2009) and anti-tumor properties (Srinivas et al., 2007; Tabolacci et al., 2010; Wei et al., 2011).

Our LDI-MSI studies on the constituents of rhubarb stalk have revealed the presence of a variety of anthraquinone derivatives i.e. emodin, aloe-emodin, chrysophanol, chrysophanic acid, 2-(hydroxymethyl)anthraquinone and citreorosein (Table 1). All of mentioned compounds have previously been isolated from different species of rhubarb (Oshio, 1978; Tutin and Clewer, 1908; Chopra et al., 1978).

Mass spectrometry imaging results of the rhubarb stalk imprint suggest, that the ions of *m/z* 271.0601 (Fig. 1D) corresponds to $[M+H]^+$ form of ion of emodin and isomeric aloe-emodin. As noticeable on the ion image, these ions are located almost exclusively in ground tissue near epidermal cells. Moreover, high abundance of ions was observed from vascular bundle region of the plant situated near the epidermis.

Image of ion distribution shown in Fig. 1E presents the lateral distribution of ion of *m/z* 255.0652 assigned to proton adduct of chrysophanol. In Chinese traditional medicine, the antraquinones have been used as a laxative, but pharmacological studies have credited antraquinones with hemostatic and bactericidal properties (Lu et al., 2010). Another ion image concerns the distributions of the potassium adduct (Fig. 1F) of 2-(hydroxymethyl)anthraquinone at *m/z* 277.0262 with highest abundance near epidermis cells. 2-(Hydroxymethyl)anthraquinone is one of the most important biologically active constituents found in rhubarb species, with anti-inflammatory activity (Dzoyem et al., 2016) and strong activity against *Helicobacter pylori* (Park et al., 2006).

In contrast to above-discussed ion localization, the ions at *m/z* 287.0550 (Fig. 1G) appears to be exclusively localized within the skin of the rhubarb stalk section. This has been tentatively identified as the citreorosein, which was found to have oestrogenic and tyrosinase-inhibitory activities (Lu et al., 2012).

Anthraquinone glucosides were found almost in all species of rhubarb (Okabe et al., 1973). Spatial distribution of sodium adducts of emodin and aloe-emodin glucoside (*m/z* 455.0949, Fig. 1H), presents the highest abundance near the edge of the stalk. Similarly, ions assigned to protonated adducts of sennoside E and F (*m/z* 847.2080, Fig. 1I) were found almost exclusively near the epidermis. Mentioned glycosides of emodin and aloe-emodin were previously described as the important constituents of rhubarb (Wagner et al., 1963). The presence of isomeric sennosides E and F was also reported in rhubarb species (Oshio et al., 1972). Anthraglycosides, similarly to antraquinones, show purgative activity but it was concluded that anthraquinones are less active than their glycosides (Oshio et al., 1974).

Rhubarb species produce a variety of stilbene derivatives. The most important stilbenes analyzed in this work include important anti-oxidant resveratrol, *trans*-stilbene, rhaponticin and piceatannol 4'-galloylglucoside. The presence of these compounds has been reported in various rhubarb species (Kashiwada et al., 1984; Matsuda et al., 2001). It can be judged from Fig. 1I that the highest concentration of resveratrol in form of sodium adduct at *m/z* 251.0679 is in ground tissue near epidermal cells.

Resveratrol is a naturally occurring phytoalexin produced by some of higher plants. It was found to show cancer chemopreventive activity (Jang et al., 1997). Therapeutic potential of this