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Speciation of cationic selenium compounds in *Brassica juncea* leaves by strong cation-exchange chromatography with inductively coupled plasma mass spectrometry

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Abstract

Strong cation-exchange chromatography (SCX-HPLC) was used in conjunction with inductively coupled plasma mass spectrometry (ICP-MS) to investigate cationic selenium species present in leaf extract of wild-type *Brassica juncea* supplemented with selenite. Total amount of Se accumulated by the leaves was found to be 352 $\mu g\,g^{-1}$. Cation-exchange solid-phase extraction (SCX-SPE) was used to pre-concentrate the cationic species present in the leaf extract. Methylselenomethionine (MeSeMet) and dimethylselenoniumproprionate (DMSeP) were synthesized and characterized by electrospray quadrupole time-of-flight MS (ESI-QTOF-MS). Laboratory synthesized and commercially available standards were used in chromatographic studies to identify the Se species in the leaf extract through retention time comparisons and standard addition method. Major cationic selenium species identified in the present study were MeSeMet and methylselenocysteine (MeSeCys) while selenomethionine (SeMet) was found in minor quantities.

Keywords: Se speciation; *Brassica juncea*; Strong cation-exchange chromatography-ICP MS; Cation-exchange solid-phase extraction

1. Introduction

Selenium is a naturally occurring element widely distributed in almost all natural forms on Earth [1]. Soil Se (usually as selenate) results from parent marine materials containing high amounts of Se [1]. Other sources of Se in the environment are due to oil refining [2] and mining [3] in the form of selenite (SeO_3^{2-}) and fossil fuel combustion in the form of elemental Se and selenium dioxide (SeO_2) [4]. Se levels above 2 ppm are found in seleniferous soils of western USA, Ireland, Australia, Israel and other countries [5]. Concern over Se as a contaminant in water and soils has increased in the past few years [6] requiring remedial actions at some point.

Phytoremediation is suggested as a low cost and environmentally friendly technology to clean up metal contaminated soil and water, using plants [7]. Plants that are suitable for Se phytoremediation must grow rapidly, tolerate salinity and other toxic conditions, produce high biomass with substantial uptake of metal for accumulation and volatilization of relatively large amounts of Se and ultimately produce forage for Se-deficient livestock [1]. *Brassica juncea* is one plant that has been identified for such characteristics and has been extensively studied for its phytoremediation [8] implications with fewer studies at the molecular level chemistry.

The amount of Se transported and/or localized in the plants depends on the chemical form of Se that the plant is exposed to [9]. In previous studies, when *Brassica* plants were treated with Se(VI), it was observed that the plant predominantly accumulated Se(VI). However, *Brassica* plants grown in a Se(IV) enriched medium, metabolized inorganic selenium to organic forms [10]. It would be of interest to know the nature of Se species accumulated in *Brassica* leaves as they are included in human diet [11] and also as fodder [7]. There is a very narrow range between Se as a nutrient and toxicant for human and animal species [12]. It is, therefore, important to know the total amount of Se accumulated and nature of various Se species

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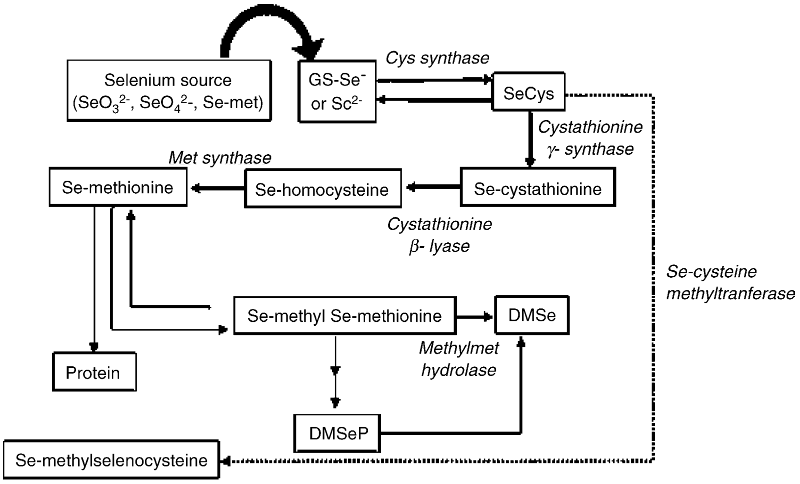

Fig. 1. Schematic diagram of possible Se cycle in plants [13]; the dotted line suggests an alternative pathway.

present in different parts of the plant that are used as Se supplement.

The pathway of Se biotransformation in plants is believed to be along the same lines as that of sulfur [9]. When plants are supplied with Se, most of the Se absorbed in the root is directly transported to the shoots and is converted to organic Se forms by enzymes present in chloroplast. Conversion of Se to selenocysteine (SeCys) is the first step in formation of organoselenium compounds. SeCys is quickly converted to a number of Se compounds such as the Se analog of glutathione, selenomethionine (SeMet), Se-cystathionine, Se-homocysteine to name a few (Fig. 1, shows a schematic diagram of possible Se cycle in plants [13] where the dotted line suggests an alternate pathway).

Although the entire biochemical pathway for volatilization of Se is not fully understood, it is believed that selenium species undergo transformation to form volatile dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) [13]. One intermediate believed to be present in halophytes for the formation of the volatile Se species, DMSeP, is believed to act as an osmoprotectant [14]. A previous report [15] on possible presence of DMSeP in *B. juncea* leaves treated with Se(VI) was intriguing. It would be interesting to extend this finding to *B. juncea* plants supplemented with Se(IV).

The aim of the current study was identification and characterization of cationic selenium species in *Brassica* leaves when plants are grown hydroponically in a Se(IV) enriched medium. Various studies on speciation of Se in natural products and plants deal with liquid chromatography in conjunction with ICP-MS for sensitive and selective Se monitoring [13]. Previous studies relating separation of Se standards were performed using cation-exchange chromatography [16]. A good number of these standards are the species involved in Se metabolic pathway, hence cation-exchange chromatography was the preferred method for analysis of *B. juncea* leaf extract in the present study. The leaf extract was subjected to pre-concentration by SCX-SPE and later analyzed for cationic selenium species by SCX-HPLC coupled to ICP-MS. Since dimethylselenonium propionate and methylselenomethionine (MeSeMet) are two immediate precursors of volatile dimethylselenide, it would be worth investigating their possible presence in the leaves of *B. juncea*. These standards were synthesized in the laboratory and their molecular identity was confirmed by ESI-QTOF-MS. The two selenium compounds synthesized and other commercially available standards were then used for assignment of peaks observed in SCX-HPLC-ICP-MS chromatogram of leaf extract.

2. Experimental

2.1. Instrumentation

Chromatographic separations were carried out using an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, an autosampler, a vacuum degasser system, a thermostated column compartment and a diode array detector. The ICP-MS detector was an Agilent 7500ce (Agilent Technologies, Tokyo, Japan). The ICP-MS detector was equipped with an octopole reaction cell that can be operated with or without the reaction gas. A conventional Meinhard nebulizer, a Peltier-cooled spray chamber (2 °C) and a shielded torch constitute the sample introduction system under standard plasma conditions. The instrumental operating conditions are summarized in Table 1.

The chromatographic column (Phenosphere SCX, 125 mm × 4.0 mm I.D. with 5 μm particle size) used was strong cation-exchange column (Phenomenex, Torrance, CA, USA). The chromatographic runs were conducted using step elution program of pyridinium formate buffer, as outlined in Table 1.

ESI-MS used in the present study was a quadrupole time-of-flight (QTOF) system from Micromass (Platform, Micromass, Manchester, UK). The instrument was operated in positive ion mode. The applied voltage to the capillary was 30 V with N_2 as nebulizing gas. Operating parameters for ESI-MS are delineated in Table 1.

2.2. Chemicals and reagents

All commercial chemicals were of analytical grade and were used without further purification. All solutions were