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Selenium speciation in dill (*Anethum graveolens* L.) by ion pairing reversed phase and cation exchange HPLC with ICP-MS detection

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Abstract

In the present work, speciation of selenium in dill (*Anethum graveolens* L.), supplemented with sodium selenite during its growth, was performed using ion pairing reversed phase and cation exchange chromatography. Heptafluorobutyric acid (HFBA) was used as the ion-pairing agent in reversed phase chromatography. In cation exchange chromatography, two different gradient programs were employed for the identification of selenospecies using pyridinium formate as the mobile phase. Low molecular weight selenocompounds were extracted from root, stem and dill leaf with 0.1 M HCl. Enzymatic digestion was used for the extraction of selenospecies related to high molecular weight compounds. The chromatograms obtained from different parts of the plant revealed major differences in the type of selenospecies as well as their concentrations. The major selenospecies found in different parts of the plant is *Se*-methyl-selenocysteine (MeSeCys). Another major Se species identified is *Se*-methyl-selenomethionine (MeSeMet), which has the highest relative concentration in the root indicating possible Se volatilization from that part of the plant. Selenomethionine (SeMet) is present in minor quantities in all parts of the plant.

Keywords: Dill (*Anethum graveolens*); IP-RP-HPLC; SCX-HPLC; ICP-MS

1. Introduction

Selenium is an essential trace element for humans and animals. Essentiality of Se was established in the observation of some deficiency symptoms, which were reduced after Se supplementation [1]. Although the metabolism and requirements of Se in the organisms are not yet completely understood, it is known to have both structural and enzymatic roles [2]. At least, 15 selenoproteins and/or selenoenzymes have been identified in biological systems [2,3]. There is strong evidence that Se or some of its compounds are effective against different types of cancer [4]. Clark et al. showed that dietary supplementation of 200 μg Se day^{-1} is capable of decreasing the overall cancer morbidity and mortality by nearly 50% with certain tumors [5].

High doses of Se have been known to cause major health problems in livestock and humans for more than a century [1]. Therefore, Se contaminated soil, which is the primary source for Se to enter food chain, must be treated. Phytoremediation has been suggested as a low cost, environmentally friendly and efficient cleanup procedure of Se laden/polluted natural bodies [6]. Plants, especially Se accumulators, are very effective in removing Se from soil and water and metabolize it to volatile, relatively non-toxic selenium forms, such as dimethylselenide (DMSe) [7]. Zayed and Terry [8] observed that volatilization of Se occurs primarily in the roots.

Coupling of inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC) is an excellent technique for the speciation of Se and has been used successfully for a number of different sample types [9,10]. However, molecular information about the compounds is not provided by ICP-MS. Identification of species is achieved by retention time matching with available standards utilized in a standard addition mode.

Green leafy vegetables are good source of minerals as well as vitamins. Dill (*Anethum graveolens* L.) is a green leafy vegetable that belongs to the carrot family and has an attractive flavor. It has been used as a basic component in canning, soups and sauces

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and also flavoring salads and seafood [11,12]. Recently, it has been reported that it is a potential source of antioxidant and also has anti-microbial properties [13]. Due to various above-stated applications of dill, it would be interesting to investigate the selenium species in this plant. The purpose of this study is to investigate selenium species in different parts of dill (*A. graveolens* L.) that is grown in a controlled environment and supplemented with Se in the form of sodium selenite. The current study involves separation of low molecular weight selenium species using ion pairing reversed phase chromatography and cation exchange chromatography. Although there are a few studies on the determination of total Se in dill [14,15] to the best of our knowledge, Se speciation in this plant has not yet been reported.

2. Experimental

2.1. Instrumentation

Chromatographic separation was achieved using an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary HPLC pump, a vacuum degasser, an autosampler and a multi wavelength UV detector. The system was coupled to an Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan). The ICP-MS was equipped with the Agilent octapole reaction system and H_2 was used as reaction gas. The outlet of UV detector was connected to sample inlet of the ICP-MS nebulizer using a 0.25 mm i.d. polyether ether ketone (PEEK) tubing of 30 cm in length.

Alltima C8 column (Alltech Associates, Deerfield, IL, USA) (250 mm × 4.6 mm i.d. with 5 μm particle size) and a Phenomenex (Torrance, CA, USA) silica-based strong cation exchange column (Phenosphere SCX, 150 mm × 3.2 mm i.d. with 5 μm particle size) were used for separations.

Total digestions of samples were performed using a closed-vessel CEM discover–explorer microwave digestion system (CEM Corporation, Matthews, NC, USA). Flow injection analysis was employed for the determination of total Se and HPLC pump was used for pumping the carrier solution. A rheodyne injector with a 50 μL sample loop was used for sample injection. A Sorvall centrifuge (Kenro Laboratory Products, Newtown, CT, USA) was used for the centrifugation of plant extracts.

2.2. Reagents

All reagents used throughout the experiments were of analytical grade. Deionized water (18 MΩ cm) was obtained from Barnstead deionization system (Sybron, Barnstead, Boston, MA, USA). The extractions were carried out using 0.1 M HCl prepared from 36% (w/w) HCl (Fischer Scientific, Fair Lawn, NJ, USA) by diluting with deionized water. Pyridinium formate buffer was prepared by dissolving required amount of pyridine (Fischer Scientific, Fair Lawn, NJ, USA) in water and adjusting pH of the resultant solution to 3.0 by addition of formic acid (J.T. Baker, Phillipsburg, NJ, USA). Methanol (Tedia Company, Fairfield, OH, USA) was added to this solution to give a final methanol concentration of 3% (v/v). This was used as mobile phase in cation exchange chromatography. In reversed phase experiments, 0.15% (v/v) heptafluorobutyric acid (HFBA) (Sigma, St. Louis, MO, USA) containing 5% (v/v) methanol was used as mobile phase (pH 2.1).

Standard stock solutions were prepared from sodium selenite (ICN Biomedicals Inc., OH), sodium selenate (Aldrich, Milwaukee, MO), MeSeCys and SeMet (Sigma, St. Louis, MO) by dissolving in water and storing at −21 °C. Working solutions were prepared from stock solutions by serial dilution with water to obtain a final concentration of 100 ppb. Since MeSeMet and trimethylselenonium iodide ($TMeSe^+I^-$) are not commercially available, they were synthesized in the laboratory according to the procedure described by Wrobel et al. [16].

2.3. Sample pretreatment

Dill samples, grown in pots, were purchased from a local market. They were kept untreated for the first 2 days and fed with DI water only. From third day onwards, the plants were supplemented with 100 mL of 10 mg L^{-1} selenite solution over a period of 2 weeks. A control sample was also grown under the same conditions as Se treated plants, but watered with DI water only. After harvesting, the plants were washed several times with deionized water to remove the soil and other material from the surface and then separated into root, stem and leaf. They were ground manually under liquid N_2 using mortar and pestle and then stored at −21 °C until extraction.

2.4. Total digestion

For determining total Se concentration, the samples were treated using suprapure HNO_3 (Pharmaco, Hartford, CT, USA) and microwave digested. A 0.050 g of different plant compartments (root, stem and leaf) was weighed and placed in a glass digestion vessel with teflon lined caps supplied from CEM. After addition of 1.0 mL of concentrated HNO_3 to remove initially formed gaseous products, the vials were kept uncapped for 0.5 h, capped, and placed in the digestion system. The digestion program involved three steps. In the first step, the temperature was increased to 80 °C in 10 min, which was increased to 90 °C in 10 min in the second step. Finally in the third step, the temperature was maintained at 90 °C for 30 min. During all these steps, a pressure below 250 psi was maintained. The clear digests were diluted with deionized water for the ICP-MS measurements.

2.5. Extraction of Se species

During extraction, about 0.250 g of each of different plant parts were placed into glass vials and 3.0 mL of 0.1 M HCl was added. In the enzymatic extraction, 0.125 g of root was combined with 1.5 mL of deionized water and 0.020 g of Protease XIV. The mixtures of both acid and enzymatic hydrolysis were stirred for 24 h at room temperature. During extraction, the vials were closed to prevent contamination and/or evaporation of extraction solution. The solutions were then centrifuged at 5000 rpm and the supernatant solution was transferred to 1.5 mL eppendorf tubes and centrifuged at 12,000 rpm for 20 min. The centrifugation step was repeated until no observable particulate material