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The Research of Selenium Speciation in Se-enriched Kelp by HPLC-ICP-MS

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The speciation of selenium in selenium-enriched kelp (*Laminaria japonica*) was studied by using chromatographic separation coupled with mass spectrometry detection (HPLC-ICP-MS). The kelp was cultivated hydroponically for 60 hours in seawater containing selenite (200 mg/dm^3 Na_2SeO_3) and N and P (150 mg/ dm^3 $NaNO_3$ + 25 mg/ dm^3 NaH_2PO_4) nutrient substance. Biotransformation of selenium in the kelp was studied by measuring the concentration of selenium in biological samples. The Selenium-enriched kelp samples were divided into upper, middle and lower parts, and the Selenium values in different parts (dry weight) were 2.26mg/g, 2.29mg/g and 1.89mg/g respectively. The different parts were extracted with water and methanol (90:10), then shook automatically for 24h, selenium speciation was carried out by high performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The result was that different organic Se was transformed from inorganic Se in different parts of kelp samples. The species of Se in upper, middle and lower parts in kelp samples were found as three, five and six respectively. Some organic Se was unknown because lack of standards, and further study was needed to identify their structure by high performance liquid chromatography coupled with electro-spray ionization time-of-flight mass spectrometry (HPLC-ESI-TOF-MS).

Key words: HPLC-ICP-MS; kelp; Selenium, Speciation.

Selenium (Se) is a key trace element required in small amounts in humans and animals for the function of a number of selenium-dependent enzymes, such as glutathione peroxidase (GPX)(B Hymer, C.,2000) and thioredoxin reductase(Block, E.2001), however, this element can also be toxic in larger doses, Bianchini, F., 2001. Both the beneficial and the toxic effects of selenium are based on concentration ingested and its chemical forms (Bird, S. M.,1997). The toxicity of inorganic Se is higher than that of organic Se.

Kelps are kinds of large economic algae, and they have been a part of the human diet for thousands of years. They also have been used frequently in Chinese medicine. Kelps also have been proven to be good sources of many mineral elements, such as I, Ca, P, and Zn. This would suggest that algae possess an effective mechanism that enables them to take up some trace elements from ocean more readily. The different Se transformation in kelps will be studied by HPLC-ICP-MS. The Se-enriched kelp samples were divided into upper, middle and lower parts in order to study transformation from inorganic selenium into organic selenium of different parts.

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EXPERIMENTAL

Instrumentation

An Agilent Technologies series 1100 instrument equipped with a manual sampler was used for chromatographic separation of Se. The chromatographic column was C_8 Alltima (150×4.6 mm, 5¼m particle). An Agilent 7500a inductively coupled plasma mass spectrometer (ICP-MS) connected to a Babington nebulizer and quarts double-pass spray chamber was used for selenium-specific detection in effluent after chromatographic separation. The column effluent was introduced on-line to ICP-MS. The ICP-MS and HPLC conditions are given in Table 1 and Table 2. Speed wave MW-3$^+$microwave digestion system (Berghof, Germany) was employed to digest the kelp samples.

Table 1. ICP-MS conditions

Parameter	Value
RF power (W)	1350
Sampling depth (mm)	6.5
Nebuliser gas flow(L/ min)	1.1
Make-up gas flow(L/ min)	0
Chamber temperature	2°C
Cones	Ni

Table 2. HPLC conditions

Parameter	Value
Column	Alltima C_8 (250mm×4.6mm,5µm)
Mobile phase	CH_3OH:H_2O: CH_3COOH =10:90:0.1(v/v/v)
Volume injected	100µL
Flow rate	0.7 mL/min

Reagents

Analytical reagent grade chemicals and HPLC-grade methanol (Merck, Germany) were used. Seleno-DL-methionine, Se-methylseleno-L-cysteine and sodium selenite were purchased from Aldrich (USA), and the stock solutions containing 1 mg ml^{-1} selenium compound were prepared in double deionized water and stored frozen. Working solutions were prepared daily by appropriate dilution. For the determination of total selenium, the working solutions were prepared daily by appropriate diluting the standard solution of Agilent multi-element standard Partÿ5183-4688. Nitric acid 65% (Suprapure) was from Merck (Germany), deionized water from Milli-Q system (Millipore, USA, 18 MX).

Plant growth and samples

The experiment was conducted on kelp (*Laminaria japonica*). The kelp was prepared for the experiments in order to stimulate the growth of plants. The kelp was grown hydroponically in containers of 50L full of seawater at room temperature. N and P (150 mg/ dm^3 $NaNO_3$ + 25 mg/ dm^3 NaH_2PO_4) were added as nutrient substance and selenite (200 mg/dm^3 Na_2SeO_3) was added as measured substance. The kelp was cultivated about 60h and rinsed with tap water and distilled water, then were divided into upper, middle and lower parts. The different parts were frozen and dried, then all parts of kelp were homogenized.

Total selenium determination

For the determinations of total selenium with ICP-MS, the microwave digestion was employed. Approximately 0.2 g of dried kelp (upper, middle and lower parts) was digested by using 1 ml H_2O_2 (30%) and 5 ml HNO_3 (65%) . The end-solutions were diluted with double distilled water up to 25 ml. Then the standard solution and reagent blanks were digested just like this. The total selenium concentration was directly determined by ICP-MS.

Extraction procedures

About 0.2 g of dried and powdered kelp was weighted precisely in the plastic tubes, then the extraction was carried out with 3 ml of water: methanol (90:10). The samples were shook mechanically for 24h and then centrifuged (20 min, 8000 rpm). The Supernatants were filtered by 0.45µm membrane and analyzed by HPLC-ICP-MS.

RESULTS AND DISCUSSION

Total selenium determination

The selenium in different parts of kelp was tested by measuring the total selenium content. The results are listed in Table 3. All concentration values presented in this paper are calculated for dry weight of the sample. The total selenium concentration was determined by ICP-MS. The detector of Agilent 7500a ICP-MS was quadrupole detectorÿSo the major disruptions in the course of analysis were isobars interference, oxide ion

interference, double charged ions interference and polyatomic ions interference from aqueous solution and carrier gas. There are several major Se isotopes, and their qualities were 77, 78, 80 and 82 respectively. ^{77} Se, ^{78} Se and ^{80} Se were interfered by $^{40}Ar^{37}Cl^{+}$, $^{39}Ar^{39}Ar^{+}$and $^{40}Ar^{40}Ar^{+}$, and therefore ^{82}Se isotope was chosen to measure. From Table 3, it was showed that Se could be accumulated in upper, middle and lower parts of kelp. There was no obvious difference in Se concentration in every part of kelp.

Table 3. The result of Selenium in kelps

Sample	Upper(mg/g)	Middle(mg/g)	Lower (mg/g)	No enriched (mg/g)
^{82}Se	2.26	2.29	1.89	0.07

Chromatographic speciation studies

Retention time of Se speciations

Single Se speciation standard was measured by HPLC-ICP-MS and retention times of three Se speciations were obtained. The retention time of sodium selenite, Seleno-DL-methionine and Se-methylseleno-L-cysteine were respectively 254, 314 and 384s. The HPLC-ICP-MS chromatographic spectrum of three mixed Se standards in ultra pure water was shown in Figure 1.Three Se standards were separated absolutely.

Se speciation in different parts of kelp

The HPLC-ICP-MS chromatographic spectrums of common kelp and three parts of Se-enriched kelp were shown as from Figure 2 to figure 5. There was only one kind of selenium compound

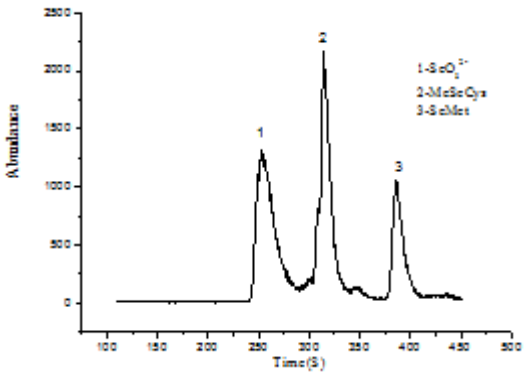

Fig. 1. The HPLC-ICP-MS chromatographic spectrummixed Se standards in ultra pure water

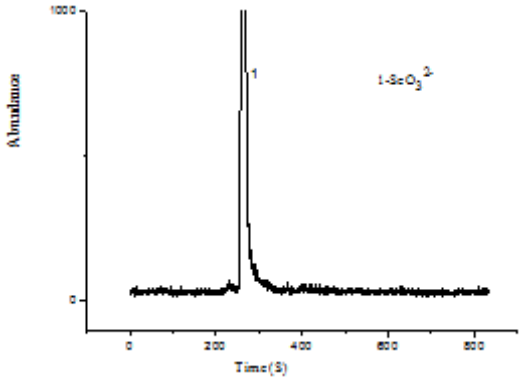

Fig. 2. The HPLC-ICP-MS chromatographic spectrums of Se in common kelp

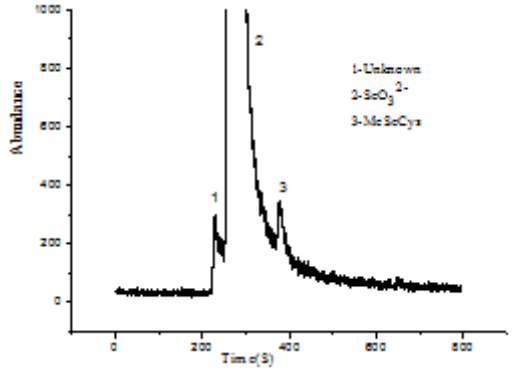

Fig. 3. The HPLC-ICP-MS chromatographic spectrums of Se in upper parts of Se-enriched kelp

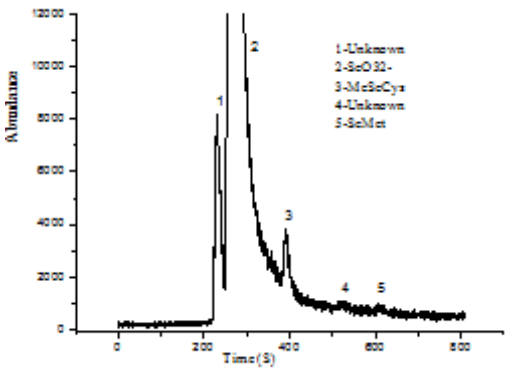

Fig. 4. The HPLC-ICP-MS chromatographic spectrumsin middle parts of Se-enriched kelp

in common kelp, which was SeO_3^{2-} concluded by its retention time. The species of Se in upper, middle and lower parts in kelp samples respectively were 3, 5 and 6. There was a kind of organic selenium Se-methylseleno-L-cysteine in upper parts of Se-enriched kelp observed from Figure 3. There were two kinds of organic selenium Se-methylseleno-L-cysteine and Seleno-DL-methionine in the middle and lower parts of Se-enriched kelp observed From Figure 4 and 5. The kelp samples can effectively transform inorganic selenium into organic selenium through metabolism. Different organic Se was transformed from inorganic Se in different parts of kelp samples. Some organic Se was unknown because of the lack of standards, and further study was need to identify their structure by HPLC-ESI-TOF.

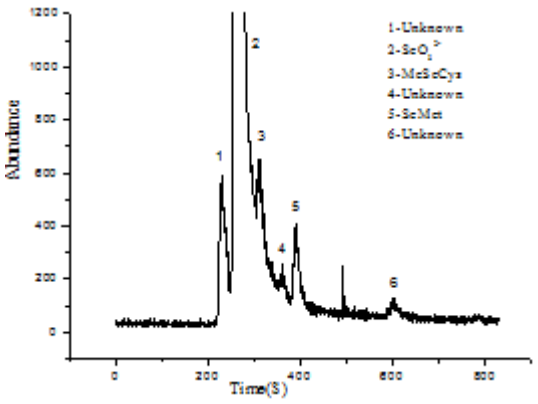

Fig. 5. The HPLC-ICP-MS chromatographic spectrums of Se in lower parts of Se-enriched kelp

CONCLUSIONS

Different kinds of organic Se were transformed from inorganic Se in different parts of kelp samples. The species of Se in the upper, middle and lower parts of kelp samples were three, five and six respectively. Some organic Se was unknown because lack of standards, and further study were need to identify their structure by high performance liquid chromatography coupled with electro-spray ionization time-of-flight mass spectrometry (HPLC-ESI-TOF-MS).

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