Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.2, No.3, 95-99, 2015

Original Article

Metabolism of trimethylselenonium ion in selenium accumulator, *Allium sativum*

Yasumitsu Ogra[1], Yurie Ogihara[2] and Yasumi Anan[2]

[1]Department of Toxicology and Environmental Health, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-41 Inohana, Chuo, Chiba 260-8675, Japan
[2]Laboratory of Chemical Toxicology and Environmental Health, Showa Pharmaceutical University, 3-3165 Higashi-Tamagawagakuen, Machida, Tokyo 194-8543, Japan

(Received June 3, 2015; Accepted June 4, 2015)

ABSTRACT — To understand selenium (Se) circulation in the biosphere, the metabolism of organic Se, in particular, Se metabolites, in animals and plants should be elucidated. In this study, garlic*, Allium sativum,* a well-known Se accumulator with high Se metabolic ability, was hydroponically cultivated and then exposed to trimethylselenonium ion (TMSe), a urinary metabolite. Thereafter, the Se concentration in several parts of garlic, such as roots, bulbs, and leaves, was determined. To reveal the metabolic pathway of TMSe, the Se species in *A. sativum* were investigated by speciation using HPLC hyphenated with an inductively coupled plasma mass spectrometer (LC-ICP-MS). Se was mostly accumulated in the roots. TMSe was detected in the extract of each plant part. However, the amount of Se incorporated from the medium was not completely recovered in the garlic, suggesting that a part of TMSe was metabolized into volatile Se. Consequently, we conclude that the majority of TMSe incorporated into garlic is accumulated as is, the rest is partially desmethylated to form a volatile Se compound, such as a dimethylated Se compound.

Key words: Selenium, Garlic, Trimethylselenonium, ICP-MS, Speciation

INTRODUCTION

Selenium (Se) belongs to group 16 in the periodic table, which includes oxygen, sulfur, and tellurium, and Se, sulfur, and tellurium are called chalcogens. Se possesses unique physicochemical properties and is widely used in several industries, such as glass production and electrowinning. From the viewpoint of biology, Se has ambivalent characteristics: It is an essential element in animals, but can be highly toxic when the amount ingested exceeds the nutritional level. As an essential element in animals, Se is required as the active center of selenoproteins that function as an antioxidant and participate in thyroid hormone production, DNA synthesis, and fertilization (Suzuki and Ogra, 2002; Whanger, 2002; Bock *et al.*, 2007; Burk and Hill, 2005). Se is mostly excreted into urine. The first urinary Se metabolite to be identified was trimethylselenonium ion (TMSe) (Byard, 1969). TMSe is simply the methylated compound of Se. Basically, TMSe appears in urine when Se exceeding the nutritional level is ingested (Byard, 1969; Kraus *et al.*, 1985; Francesconi and Pannier, 2004; Janghorbani *et al.*, 1999). On the other hand, *Se*-methylseleno-*N*-acetyl-galactosamine (selenosugar, MeSeGalNAc) was the major metabolite identified in the urine of animals that ingested Se within the nutritional level to the low-toxicity level (Kobayashi *et al.*, 2002). Hence, TMSe and selenosugar are released by animals to the environment. In contrast, Se is not an essential element in plants and exists as a "bystander" mineral. However, its beneficial effects on plant growth have been reported (Freeman *et al.*, 2007; Djanaguiraman *et al.*, 2005). Some plants, such as garlic (*Allium sativum*) and Indian mustard (*Brassica juncea*), are known as Se accumulators because of their large Se metabolic capacity and high Se metabolic ability (Montes-Bayón *et al.*, 2002). It has been shown that these plants can metabolize inorganic Se, such as selenite and selenate, into a specific selenoamino acid. However, considering Se circulation in the biosphere, an investigation of the metabolism of animal Se metabolites in plants seems to be important. In our previous work, we found that Indian mustard metabolized MeSeGalNAc but not TMSe (Ogra *et al.*, 2013).

Correspondence: Yasumitsu Ogra (E-mail: ogra@chiba-u.jp)

In this study, garlic, *A. sativum,* which is expected to have higher Se metabolic ability than Indian mustard, was cultivated in TMSe-containing medium and examined for its ability to accumulate Se. Then, Se species found in the plant and the cultivation medium were speciated by LC-ICP-MS. Finally, we evaluated whether or not garlic can metabolize TMSe, and discussed the significance of TMSe in environmental toxicology.

MATERIALS AND METHODS

Chemicals

Ammonium acetate, hydrogen peroxide, sodium hydroxide, nitric acid, and standard solutions of Se and tellurium (Te) (each 1,000 μg/mL) were purchased from Wako (Osaka, Japan). Trimethylselenonium iodide (TMSe) was obtained from Tri Chemical Laboratories, Inc. (Yamanashi, Japan). Otsuka salt mixture-A was purchased from Otsuka AgriTechno Co., Ltd. (Tokyo, Japan). All reagents were of the highest or analytical grade. Deionized water (18.3 MΩ·cm) was used throughout.

Plant growth and sample preparation

Garlic bulbs were purchased from Sawada Farm, Inc. (Aomori, Japan). The bulbs were germinated and hydroponically grown in a solution of Otsuka salt mixture-A in a growth chamber (LH-55-RDS; Nihonikakikai, Osaka, Japan) with a photoperiod of 14 hr light (8000 lux)/10 hr dark at 25.0°C for two weeks. The cultivation medium was sterilized prior to use. The plants were exposed to a 250 mL portion of a solution of Otsuka salt mixture-A containing TMSe at the concentration of 30 μM for 7 days. The cultivation medium was collected daily to check the Se concentration. After the exposure, the plants were immediately harvested, gently washed with fresh medium, and then divided into roots, bulbs, and leaves. Those parts were lyophilized and milled to obtain the homogenized powder. A 100 mg portion of each of the lyophilized powder of garlic leaves, bulbs, and roots was mixed with a 1.0 mL aliquot of deionized water, and the mixture was incubated for 1 hr at room temperature. Water extracts were obtained by centrifugation of the mixture at 105,000 x *g* for 60 min and filtration through a 0.45 μm membrane filter.

Determination and speciation analysis of Se in extracts of garlic leaves and roots by LC-ICP-MS

A 100 mg portion of each the lyophilized powder of garlic leaves, bulbs and roots was digested in a microwave oven with nitric acid in a Teflon PTFE tube. The digest was diluted with deionized water and Te standard solution was added as the internal standard (10 ng Te/mL at the final concentration). The concentration of Se in each part of garlic plant was measured with an ICP-MS at *m/z* 82 and 128 for Se and Te, respectively (Agilent 7500ce, Agilent Technologies, Hachioji, Japan).

A 20 μL aliquot of the water extract and the hydroponic medium was applied to an HPLC coupled with an ICP-MS (LC-ICP-MS) to analyze the distribution of the metalloids. The HPLC system (Prominence, Shimadzu, Kyoto, Japan) consisted of an on-line degasser, an HPLC pump, a Rheodyne six-port injector, and a multi-mode size exclusion column (Shodex Asahipak GS-320HQ, 7.5 i.d. x 300 mm with a guard column; Showa Denko, Tokyo). The column (GS-320HQ) was eluted with 50 mM ammonium acetate, pH 6.5, at the flow rate of 0.6 mL/min. Then, the eluate was introduced into the nebulizer of the ICP-MS to detect Se at *m/z* 82. To evaluate the recovery of Se from the column, the eluate was collected and Se concentration in the eluate was determined.

RESULTS AND DISCUSSION

Se was detected in garlic roots, bulbs, and leaves 7 days after the exposure (Fig. 1). This indicates that TMSe incorporated into garlic is retained in large amounts in the roots and minimally distributed to other parts of the plant. The TMSe standard was eluted at the retention time

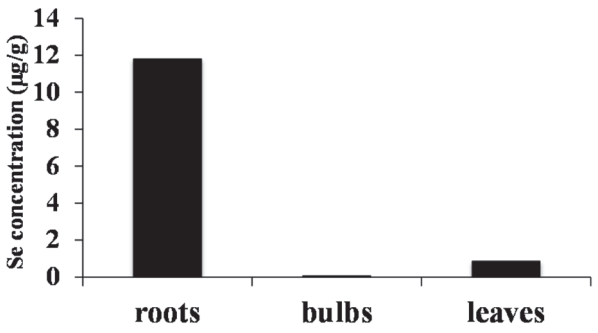

Fig. 1. Concentrations of Se in roots, bulbs, and leaves of *A. sativum* exposed to TMSe. *A. sativum* was hydroponically cultivated and then exposed to TMSe at the concentration of 30 μM for 7 days. The roots, bulbs, and leaves were lyophilized and milled to obtain the homogenized powder. A 100 mg portion of the lyophilized powder was digested in a microwave oven with nitric acid in a Teflon PTFE tube. The digest was diluted with deionized water, and Te standard solution was added as the internal standard (10 ng Te/mL at the final concentration). The concentration of Se in each part of garlic plant was measured with an ICP-MS at *m/z* 82 and 128 for Se and Te, respectively.

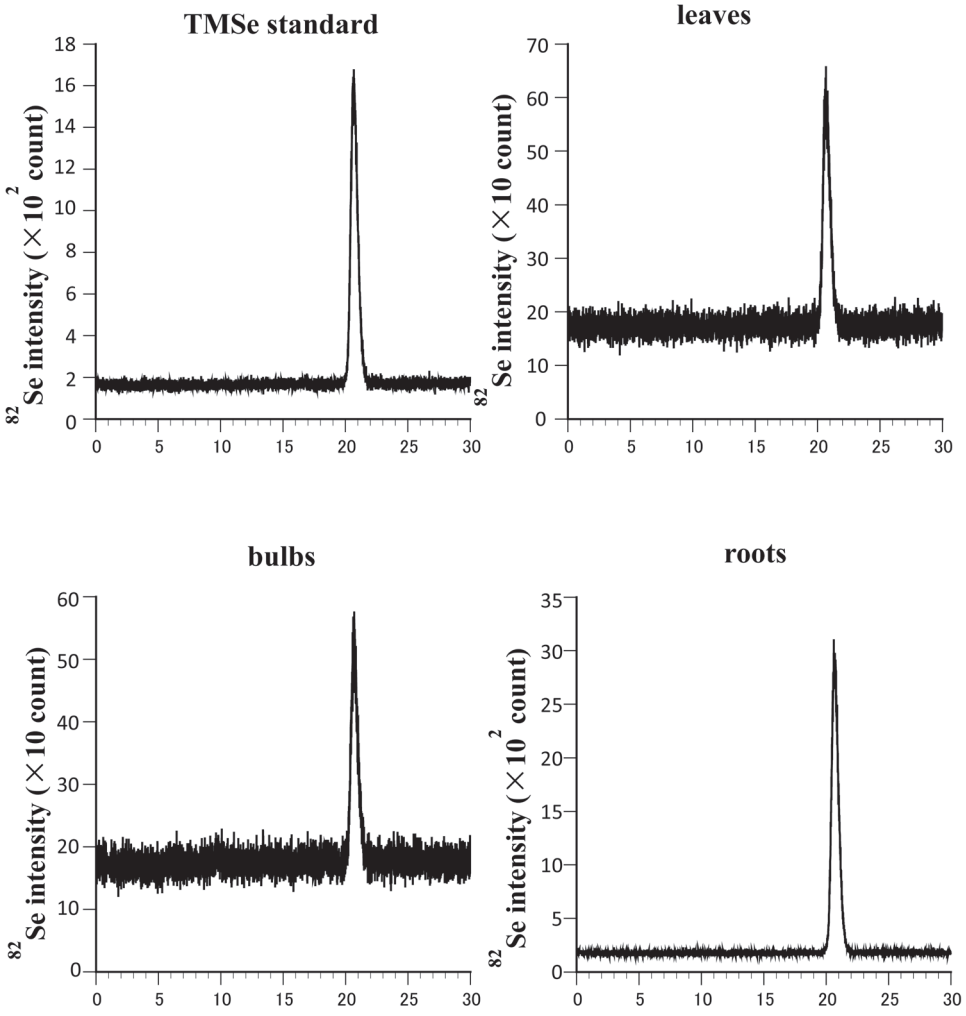

Fig. 2. Elution profiles of Se in water extracts of roots, bulbs, and leaves of *A. sativum* exposed to TMSe. *A. sativum* was hydroponically cultivated and then exposed to TMSe at the concentration of 30 μM for 7 days. A 20 μL aliquot of TMSe standard solution (1.0 μg Se/mL) and the water extract were applied to the HPLC equipped with a multi-mode size exclusion column, GS-320HQ. The column was eluted with 50 mM ammonium acetate, pH 6.5, at the flow rate of 0.6 mL/min. The eluate was directly introduced into the nebulizer of the ICP-MS to detect Se at *m/z* 82.

of 20.6 min from the column (Fig. 2a). Only one Se peak was detected in the extracts prepared from the three garlic parts, and the retention time of Se in the extracts matched that of TMSe standard (Figs. 2b-2d). These results suggest that TMSe is not metabolized into any other Se species and is accumulated in the garlic plant as is. TMSe was not also metabolized into any Se species in *Brassica rapa* var. *peruviridis*, another Se accumulator (Ogra *et al.*, 2013). Although garlic is expected to be one of the plants that have the highest metabolic ability for chalcogens, such as sulfur, Se and tellurium, TMSe is not metabolized in garlic (Ogra *et al.*, 2010; Anan *et al.*, 2013). However, Se concentration in the cultivation medium was decreased in a time-dependent fashion (Fig. 3). No other Se species except TMSe were detected in the cultivation medium (Fig. 4). Although TMSe was actually accumulated in the garlic plant, the amount of Se recovered from the garlic was 54.2% of the amount of Se that was decreased in the medium. TMSe did not spontaneously change into volatile forms in the absence of garlic (data not shown). We speculated the reason for the discrepancy of Se mass balance, as follows. A part of TMSe incorporated in the garlic plant would be partially desmethylated and transformed into a dimethylated Se compound, *i.e.*,

dimethylselenide. As dimethylselenide is a volatile compound, it cannot be eluted from the HPLC column under the conditions used in this study, and this results in the low column recovery. This explains why Se concentration in the cultivation medium is decreased. It was reported that biogenic Se metabolites, such as selenoamino acids and selenosugars other than TMSe, were metabolized into a major botanical Se metabolite, *Se* methylselenocysteine (MeSeCys) (Ogra *et al.*, 2013). Other biogenic Se metabolites commonly have a monomethylated selenyl group in their molecules; hence, this indicates that trimethylated Se cannot be incorporated into the metabolic pathway of Se in plants. If TMSe were converted into monomethylated Se compounds, such as monomethylselenol and dimethyldiselenide, MeSeCys would be detected in the garlic plant exposed to TMSe. Consequently, we conclude that the majority of TMSe incorporated into the garlic plant is accumulated as is, the rest is partially desmethylated to form a volatile dimethylated Se compound. The dimethylated Se compound should be directly detected in our future work by means of appropriate techniques to detect volatile Se compounds, such GC-ICP-MS.

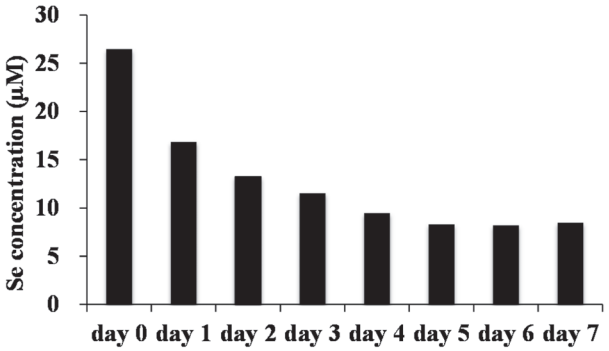

Fig. 3. Changes in the concentration of Se in the hydroponic medium. A 1.0 mL portion of the medium containing TMSe was collected daily during the exposure period. Te was used as the internal standard. The concentration of Se in the medium was measured with an ICP-MS at *m/z* 82 and 128 for Se and Te, respectively.

In contrast to plants, it was reported that TMSe was utilized in animals, although the utilization was less efficient than those of other Se compounds, such as inorganic Se salts and monomethylated and dimethylated Se compounds (Vadhanavikit *et al.*, 1993; Suzuki *et al.*, 2005). Because animals require Se as an essential element, animals can assimilate all biogenic Se compounds, including TMSe. Indeed, no biogenic Se compounds that cannot be utilized for the biosynthesis of selenoproteins are known. TMSe is excreted into urine when animals ingest Se beyond the nutritional requirement (Suzuki *et al.*, 1995). In other words, TMSe is produced when excess amounts of Se are ingested. TMSe is less efficiently metabolized than other Se metabolites in animals and plants, indicating that excess amounts of Se are excluded and preserved in the form of TMSe in the biosphere.

ACKNOWLEDGMENTS

This study was supported by JSPS KAKENHI Grant Numbers 25870739, 26293030, and 15K14991, and the

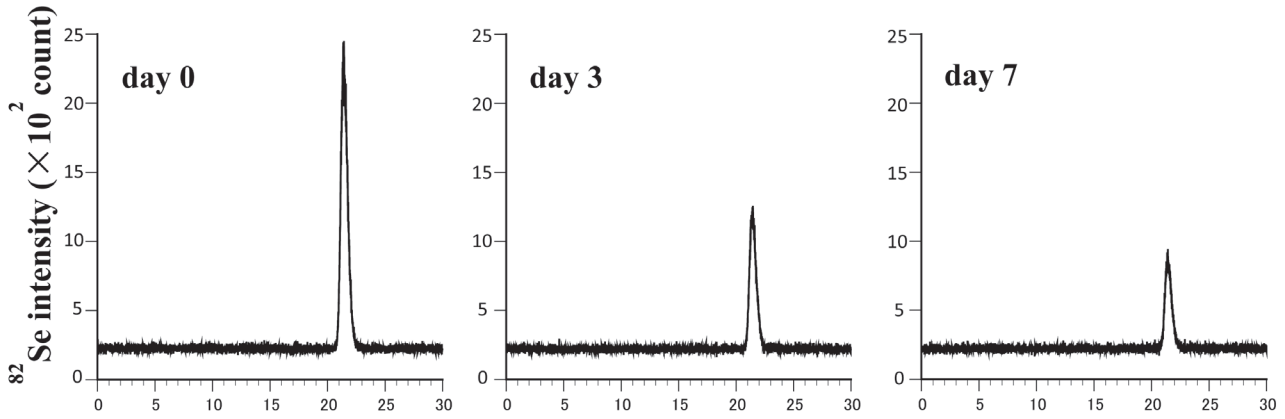

Fig. 4. Elution profiles of Se in the hydroponic medium. A 1.0 mL portion of the medium containing TMSe was collected daily during the exposure period. A 20 μL aliquot of the hydroponic medium collected on days 0, 3, and 7 was applied to the HPLC equipped with a multi-mode size exclusion column, GS-320HQ. The column was eluted with 50 mM ammonium acetate, pH 6.5, at the flow rate of 0.6 mL/min. The eluate was directly introduced into the nebulizer of the ICP-MS to detect Se at *m/z* 82.

MEXT-Supported Program for the Strategic Research Foundation at Private Universities (2013-2017).

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Anan, Y., Yoshida, M., Hasegawa, S., Katai, R., Tokumoto, M., Ouerdane, L., Lobinski, R. and Ogra, Y. (2013): Speciation and identification of tellurium-containing metabolites in garlic, Allium sativum. Metallomics: integrated biometal science, **5**, 1215-1224.

Bock, A., Flohe, L. and Kohrle, J. (2007): Selenoproteins - biochemistry and clinical relevance. Biol. Chem., **388**, 985-986.

Burk, R.F. and Hill, K.E. (2005): Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. Annu. Rev. Nutr., **25**, 215-235.

Byard, J.L. (1969): Trimethyl selenide. A urinary metabolite of selenite. Arch. Biochem. Biophys., **130**, 556-560.

Djanaguiraman, M., Durga Devi, D., Shanker, A.K., Annie Sheeba, J. and Bangarusamy, U. (2005): Selenium – an antioxidative protectant in soybean during senescence. Plant Soil, **272**, 77-86.

Francesconi, K.A. and Pannier, F. (2004): Selenium metabolites in urine: a critical overview of past work and current status. Clin. Chem., **50**, 2240-2253.

Freeman, J.L., Lindblom, S.D., Quinn, C.F., Fakra, S., Marcus, M.A. and Pilon-Smits, E.A. (2007): Selenium accumulation protects plants from herbivory by Orthoptera via toxicity and deterrence. New Phytol., **175**, 490-500.

Janghorbani, M., Xia, Y., Ha, P., Whanger, P.D., Butler, J.A., Olesik, J.W. and Daniels, L. (1999): Quantitative significance of measuring trimethylselenonium in urine for assessing chronically high intakes of selenium in human subjects. Br. J. Nutr., **82**, 291-297.

Kobayashi, Y., Ogra, Y., Ishiwata, K., Takayama, H., Aimi, N. and Suzuki, K.T. (2002): Selenosugars are key and urinary metabolites for selenium excretion within the required to low-toxic range. Proc. Natl. Acad. Sci. USA, **99**, 15932-15936.

Kraus, R.J., Foster, S.J. and Ganther, H.E. (1985): Analysis of trimethylselenonium ion in urine by high-performance liquid chromatography. Anal. Biochem., **147**, 432-436.

Montes-Bayón, M., Grant, T.D., Meija, J. and Caruso, J.A. (2002): Selenium in plants by mass spectrometric techniques: developments in bio-analytical methods. J. Anal. At. Spectrom., **17**, 1015-1023.

Ogra, Y., Katayama, A., Ogihara, Y., Yawata, A. and Anan, Y. (2013): Analysis of animal and plant selenometabolites in roots of a selenium accumulator, Brassica rapa var. peruviridis, by speciation. Metallomics: integrated biometal science, **5**, 429-436.

Ogra, Y., Okubo, E. and Takahira, M. (2010): Distinct uptake of tellurate from selenate in a selenium accumulator, Indian mustard (*Brassica juncea*). Metallomics: integrated biometal science, **2**, 328-333.

Suzuki, K. T., Itoh, M. and Ohmichi, M. (1995): Selenium distribution and metabolic profile in relation to nutritional selenium status in rats. Toxicology, **103**, 157-165.

Suzuki, K.T., Kurasaki, K., Okazaki, N. and Ogra, Y. (2005): Selenosugar and trimethylselenonium among urinary Se metabolites: dose- and age-related changes. Toxicol. Appl. Pharmacol., **206**, 1-8.

Suzuki, K.T. and Ogra, Y. (2002): Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. Food Addit. Contam., **19**, 974-983.

Vadhanavikit, S., Ip, C. and Ganther, H.E. (1993): Metabolites of sodium selenite and methylated selenium compounds administered at cancer chemoprevention levels in the rat. Xenobiotica, **23**, 731-745.

Whanger, P.D. (2002): Selenocompounds in plants and animals and their biological significance. J. Am. Coll. Nutr., **21**, 223-232.