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Separation of Selenium Species in Japanese Littleneck Clam 'Asari' (*Ruditapes philippinarum*) and *In Vitro* Assessment of Their Bioavailability

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Elemental selenium, an essential element for humans and animals is totally obtained from foods and used for the synthesis of selenoproteins, such as glutathione peroxidases (GPx). Fish and shellfish are selenium-rich foodstuffs, and are the major dietary source of selenium for the Japanese population. However, the chemical structure and bioavailability of selenium species from seafood materials have hardly been elucidated. The Japanese littleneck clam (Asari) is the most popular consumed seawater bivalve in Japan. In this study, the selenium species in Asari were separated and assessed as a nutritional selenium source using cultured cells. The selenium content in the lyophilized Asari edible meat was 4.34±0.49 μg/g. The selenium extraction rate into water was 17−24% (1.07±0.14 μg/g-dry Asari). Based on the results that selenium in the Asari water-extract was mostly retained on both cationic Q Sepharose and anionic SP Sepharose columns, the selenium species in the Asari water-extract appeared to possess an amphoteric character. Selenium in the Asari water-extract mostly passed through a membrane with the molecular mass cutoff of 5000. After lyophilization, the resulting filtrate was subjected to *in vitro* assessment of the selenium bioavailability. HepG2 and HeLa cells were cultured in a basal medium containing the filtrate. The selenium content and GPx activity of the HepG2 cells increased with the increasing selenium content in the medium and incubation time, which suggested that the selenium species in the Asari water-extract were utilized for the synthesis of the GPxs. Overall, these results demonstrated that Asari contains nutritionally effective selenium species.

Key words selenium, clam, bioavailability, HeLa cell, HepG2 cell

INTRODUCTION

Selenium plays critical roles in various physiological processes in the human body.[1)] It had been only recognized as a toxic element since discovered by Berzelius until its requirement for animals was reported by Schwarz and Foltz.[2)] The occurrence of Keshan disease and Kashin-Beck disease (an endemic cardiomyopathy and osteoarthropathy) in China are invariably associated with a selenium deficiency.[3,4)] In areas that the diseases occurred, the selenium concentrations in the soil (0.1−0.2 mg/kg) were half of the values in normal areas or less.[5)] Since selenium is not uniformly distributed in solid earth, the selenium concentrations in the soil of certain areas in Europe and Oceania are also relatively low, which causes the decreases in the blood selenium concentration of the local residents.[6)]

Currently, selenium is known to be involved in various physiological processes in organisms, particularly as a critical factor of antioxidative systems.[1,7,8)] Many studies have also demonstrated the relationships between the selenium status in the human body and various diseases such as cancer, cardiac disease, infectious disease and neurodegenerative disease.[9–11)] The physiological functions of selenium in organisms are thought to be exhibited by selenoproteins in which selenium is incorporated as selenocysteine (SeCys).[12)] SeCys is referred to as the 21st proteinogenic amino acid that contains the selenium atom in place of the sulfur atom in cysteine. SeCys in selenoproteins is coded by UGA, which usually acts as a stop codon and requires specific translational mechanisms composed of the SeCys insertion sequence (SECIS) in the 3' untranslated region of mRNA, selenocysteine specific tRNA and several proteins. Recently, Ingold *et al*. reported that selenium-dependent glutathione peroxidase (GPx) 4 is a vital enzyme for cells to protect from ferroptosis, which is a nonapoptotic cell death dependent on cytosolic iron.[13)]

Selenium needed for human health is only obtained from foods. The bioavailability of selenium is dependent on the chemical form of selenium. For example, inorganic selenious acid (SA) is highly bioavailable and utilized for the treatment and prevention of selenium deficiency, although its level to cause acute toxicity is generally much lower than those of organic selenium species.[14)] Selenomethionine (SeMet) is one of the major organic selenium species. Although the acute toxicity of SeMet is lower than that of SA, a large scale clinical trial revealed that the long-term daily intake of SeMet (400 μgSe/day) significantly increased the risk of type 2 diabetic mellitus.[15)] Therefore, it is important to assess the toxicity and efficiency of selenium species to be consumed.

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Selenoamino acids and their derivatives were detected in selenium-enriched plant foods such as selenized yeast, mushroom and wheat.[16–18] SeCys is predicted to be a major chemical form in meat and fish. It still remains uncertain that the chemical form of the selenium species in selenium-enriched foods are the same as those in the non-enriched natural foods. In contrast to plant food, there are a few reports about the selenium species in fish and shellfish, because of the difficulty of generating selenium-enriched species. Additionally, several papers have described that seafood contains several uncharacterized selenium species.[19,20] Selenoneine (2-selenyl-*Nα*,*Nα*,*Nα*-trimethyl-L-histidine) was recently reported to be in the blood of the blue fin tuna and the liver of sea turtles.[21,22] This compound is thought to be a major selenium species in fish and shellfish.

Fish and shellfish are reported as the major selenium sources in the dietary habits of the Japanese.[23] The authors previously reported that selenium in a processed Japanese anchovy, Niboshi, effectively restored the hepatic selenium content and the GPx activity in dietary selenium-deficient mice.[24] This study addressed the selenium species in the Japanese littleneck clam, Asari (*Ruditapes philippinarum* Adams & Reeve, Veneridae) that is one of the most popular clams found in the seashore of Japan. The selenium species in Asari edible meat were characterized by several analytical techniques and their bioavailability was assessed using cultured cells.

MATERIALS AND METHODS

Preparation of Asari Samples Fresh Asari were purchased at local grocery stores in Nagasaki and used immediately for the sample preparation. After soaking in a 3% NaCl aqueous solution for 3 h, the shells were removed from the edible meat of the fresh Asari and washed with water (Fig. S1A, B). The clam meats were lyophilized by a VD-800F freeze dryer (Taitec Corp., Saitama, Japan), and ground into a powder by a food processor (Tescom Co., Ltd., Tokyo, Japan).

Preparation of Asari Extracts About 2 g of the lyophilized Asari meat was boiled in 20 mL of water for 10 min followed by centrifugation at 7500 rpm (5100×*g*), 20 °C for 30 min. The Asari water-extract was prepared by filtration of the obtained supernatant using a disk filter with the membrane pore size of 0.45 μm, and the addition of water to make a final volume of 20 mL (Fig. S1C).

The lyophilized Asari samples were also extracted with several organic solvents. About 2 g of the sample was immersed in the organic solvents, such as acetonitrile, chloroform, ethanol, ethyl acetate and hexane, at 25 °C for 5 h. After the filtration using paper, the solvent used for extraction was added to make a final volume of 20 mL.

The concentrations of the selected substances in the Asari extracts were calculated by the following equation: =[(amount of substances in the extract used for analysis)/(volume of the extract used for analysis)]･[(total volume of the extract prepared)/(weight of the Asari used for extraction)] (ng or μg/g-Asari). A Milli-Q Biocel system (Millipore Corp., Billerica, MA, U.S.A.) was utilized to generate the water (>18 MΩ·cm) throughout this study.

Determination of Selenium Content Samples were digested with the 5:1 mixture by volume of nitric and perchloric acids before the determination of selenium with 2,3-diaminonaphthalene (DAN, Tokyo Chemical Ind., Tokyo, Japan).[25] The fluorescent intensity of the piaselenol (the excitation wavelength: 375 nm, the emission wavelength: 520 nm) generated by the reaction of DAN with the digested sample in a 0.1 M HCl solution was measured by a FP-6600 spectrofluorometer (JASCO, Tokyo, Japan). Selenium (IV) dioxide in 0.1 M nitric acid (1000 ppm, Kanto Chemical Co., Inc., Tokyo, Japan) was used as the selenium standard solution for the preparation of the calibration curve.

Determination of Protein Concentrations The protein concentration in a specimen was determined by Lowry's method after the appropriate dilution with water.[26] A five-fold volume of a 10:1:1 mixture of 2% Na_2CO_3-0.1 M NaOH, 1% $CuSO_4 \cdot 5H_2O$ and 2% sodium (+)-tartrate dihydrate was mixed with the diluted sample and allowed to react for 10 min. A half volume sample of Folin-Ciocalteu's reagent solution diluted 2-fold with water was added and allowed to react for another 30 min. A V-660 UV-Visible spectrophotometer (JASCO, Tokyo, Japan) was used to monitor the absorbance at 650 nm and a calibration curve was made with bovine serum albumin as the reference (working concentration range: 0.01−0.5 mg/mL).

Ultrafiltration An Amicon Ultra-4 (Merck Millipore, Darmstadt, Germany) and Amicon Stirred Cell (EMD Millipore Corporation, Billerica, M.A. U.S.A.) were used for ultrafiltration of the Asari water-extract after the appropriate dilution with water. The regenerated cellulose membranes with a molecular mass cutoff (MMCO) of 5, 10, 30 and 100 kDa were used. The sample solutions were centrifuged in Amicon Ultra-4 at 7500 rpm (5100×*g*) and 20 °C. The selenium and protein contents in the obtained filtrate were determined by the DAN and Lowry's methods, respectively.

Ion-Exchange Chromatography Q Sepharose and SP Sepharose [particle size: 45–165 μm in wet state, capacity: 0.18−0.25 meq/mL, Sigma Co., St. Louis, MO, U.S.A.] were used for the ion-exchange chromatography. The Q Sepharose [functional group: $-[OCH_2CH(OH)CH_2]N^+(CH_3)_3 \cdot X^-$] was packed into a glass column and conditioned with 0.1 M NaOH to make the OH form (X=OH) followed by thoroughly washing with water until the pH of the elute became neutral. The SP Sepharose [functional group: $-(CH_2)_3SO_3^- \cdot Y^+$] packed column was also prepared by a similar method using 0.1 M HCl. A PUMP 560 and a prep･UV254 monitor (Yamazen, Kyoto, Japan) were used for the chromatographic separations.

Cell Culture The HeLa cells and HepG2 cells were purchased from the JCRB Cell Bank (Tokyo, Japan) and maintained in a 10-cm culture dish (Nippon Genetics Co., Ltd., Tokyo, Japan) with 10% fetal bovine serum (FBS)-containing Dulbecco's modified Eagle medium (DMEM) under humidified 5% CO_2-95% air. The cells were subcultured at 50−80% confluence. Phosphate-buffered saline (PBS, calcium and magnesium free) was used for the cell rinsing throughout this study.

Assessment of Selenium Absorption Behavior by HeLa Cells and HepG2 Cells The Asari water-extract was partially purified by ultracentrifugation at 60000 rpm (330105×*g*), 4 ºC for 1 h, then by ultrafiltration with the membrane MMCO 5 kDa. The lyophilized filtrate was dissolved in 5% FBS-containing DMEM to make a selenium concentration of 0.05−0.25 μM followed by filtration using a sterilized disk filter with the pore size of 0.20 μm. Selenious acid (SA, H_2SeO_3) and seleno-L-methionine (SeMet) were dissolved in PBS, then added to the 5% FBS-containing DMEM to make the selenium concentrations of 0.05−0.25 μM. For the assessment of

the selenium bioavailability, cells were seeded at 5×10^5 cells/dish and cultured in 10% FBS-containing DMEM. After 24 h, the medium was changed to the selenium species-supplemented 5% FBS-containing DMEM and subsequently incubated for the indicated times.

Determination of Cellular GPx Activity After incubation with the selenium species for the indicated time, the cells were washed with PBS. The mixed solution of 0.25% trypsin and 0.02% EDTA in PBS was utilized for cell detachment, and the detached cells were collected by centrifugation at 1000×*g*. The cells were washed three times by gentle pipetting in PBS. For the osmotic cell lysis, 150 µL of water (>18 MΩ·cm) was added to the cell suspensions, and then sonicated in a bath-type sonicator for 5 min and vortexed for another 5 min. After the centrifugation at 2000×*g* for 10 min, the obtained supernatant was used for the determination of the GPx activity and protein concentration.

The GPx activity was determined according to a previously reported method.[27] The cytosolic fraction was combined with the NADPH solution (final concentration 0.2 mM), reduced glutathione (GSH) solution (1 mM) and glutathione reductase solution (1 unit/mL) in a 66 mM phosphate buffer (pH 7.4). The reaction was initiated by the addition of a hydrogen peroxide solution (0.25 mM) containing sodium azide (5 mM). The absorbance at 340 nm due to the NADPH was monitored every 1 min for 3 min just after mixing by inversion. The GPx activity was calculated using equation (1) as µmoles NADPH oxidized per minute, where ΔA_{SAM} is the decrease in the absorbance at 340 nm of the sample solutions for 1 min between 15 and 195 s after the addition of the substrates, ΔA_{BLK} is the decrease in absorbance at 340 nm per minute of the solutions using water instead of the sample solutions, 20.6 is the dilution factor, ε_{mM} is the extinction coefficient for the 1 mM NADPH solution [6.22/(mM·cm)], and c is the protein content (µg/mL).[28]

$$\text{GPx activity}=(\Delta A_{SAM}-\Delta A_{BLK})\times 20.6/\varepsilon_{mM}/c \quad \cdots\cdots (1)$$

Statistical Analysis Data were presented as the mean and standard deviation (S.D.) (n=2−4). Statistical analyses were performed using PRISM 4 (GraphPad Software, Inc., La Jolla, CA, USA). The multiple mean values were compared by a one-way ANOVA with a Tukey post-hoc test. Comparisons were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Selenium in Asari and its Extract The selenium concentration of the lyophilized Asari meat (an edible part) was 4.34±0.48 µgSe/g which was higher than that of Niboshi, processed Japanese anchovy (1.21±0.03 µgSe/g), Shijimi clam (3.51±0.12 µgSe/g) or other foodstuffs.[29–31] The determination of selenium in lyophilized Asari meat indicated the dry-mass dependent increase of the total selenium amount in the Asari meat (Fig. S2). The bioavailability of selenium in seafood has been thought to be lower than those in terrestrial plant or animal foods because selenium can interact with several heavy metals such as mercury. The formation of the insoluble complexes with heavy metals reduces the toxicity of these metals for organisms, although the bioavailability of selenium will also be reduced.[32] The total mercury content in the lyophilized Asari meat was determined by the gold amalgam method. Asari meat contained 0.06 µg-mercury/g, thus the molar ratio of mercury to selenium (Hg/Se) was 0.02. Such a biologically unavailable selenium complex with mercury was thought to be negligible in Asari. The distribution of selenium in the Asari meat was further evaluated at the level of organs. Selenium in the Asari meat was mostly detected in the internal organs such as the mid-gut gland and stomach, heart and intestine (Table 1). This suggested that the selenium in Asari comes from its food such as plankton and suspended organic substances and/or their metabolites. The selenium distribution characteristics, such that the entrails contained a higher amount of selenium than the flesh, is similar to those in other seafoods and livestock.[14,33] The internal organs of large fish are usually thrown away, although these parts are rich in selenium. On the other hand, small fish and whole clams are edible without removing their internal organs. Small seafood used in our dietary habit is thought to be an effective source of selenium.

The optimal conditions for effectively extracting selenium from the lyophilized Asari meat were determined. To find the suitable extraction solvents, the lyophilized Asari meat was immersed in several kinds of solvents including water. The selenium in Asari meat was poorly extractable by the organic solvents, such as ethanol, hexane and chloroform (Table 2). We further optimized the extraction conditions into water with respect to the extraction time and temperature (Fig. 1). The amount of selenium extracted from the Asari meat into water increased with the increases in time and temperature. The Asari water-extract used for the following experiments was prepared by the 10-min boiling of the lyophilized Asari meat at 100 °C. The selenium content in the water-extract was 0.66±0.05 µg/g-dry mass (indicated as the amount of selenium extracted from the lyophilized Asari meat used for extraction) and this corresponded to approximately 23% of the selenium in the lyophilized Asari meat.

Separation of Selenium Species in the Asari Water-Extract The water-extract of Asari was separated by ultra-

Table 1. Selenium Concentrations in Organ/Tissue of Lyophilized Asari

Organ/Tissue	Selenium Concentration (µg/g)	Selenium Distribution (%)
Adductor Muscle	0.798 ± 0.070	3.72
Leg	0.847 ± 0.077	4.05
Mantle	1.037 ± 0.108	8.45
Heart	4.622 ± 1.145	10.20
Intestine	3.308 ± 0.540	14.45
Mid-Gut Gland and Stomach	5.436 ± 2.143	9.62
Gill and Siphon	2.704 ± 0.572	28.47
Others	0.931 ± 0.240	18.04
Total	1.964 ± 0.162	97.00

Values are mean ± S.D. (n = 3)

Table 2. Selenium Extraction Rates into Various Solvents

Solvent	Selenium concentration (µg/g-dry Asari)	Selenium extracted (%)
Hexane	0.006	0.23 ± 0.08
Chloroform	0.023	0.68 ± 0.23
Ethyl acetate	0.007	0.31 ± 0.14
Acetonitrile	0.006	0.21 ± 0.06
Ethanol	0.026	1.21 ± 0.62
Water	0.660	21.10

Values are mean ± S.D. (n = 2−6)

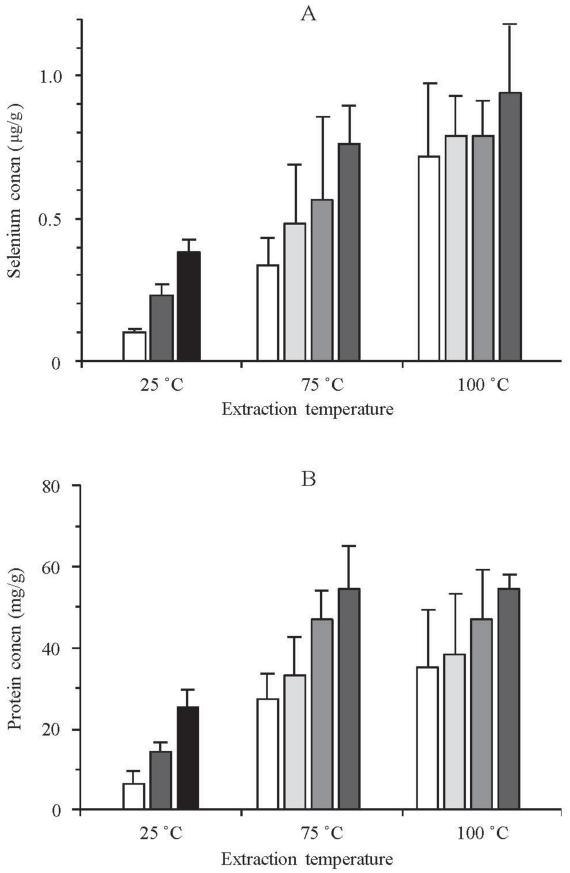

Fig. 1. Selenium (A) and Protein (B) Concentrations of Asari Water-Extracts at Elevated Temperature and Extraction Time

Extraction time (min): □, 10; ▒, 30; ▒, 60; ■, 90; ■, 300. Values are mean ± S.D. (n = 3)

filtration to estimate the molecular mass of the selenium species in the extract. The membranes with MMCO 5 or 30 kDa were utilized. The diluted extract was filtered by centrifugation at 7500 rpm, 20 ºC for 30 min. The selenium and protein concentrations in the filtrate were determined and proved that more than 90% of the selenium in the Asari water-extract passed through the membrane with an MMCO 5 kDa (Table 3). This indicated that the molecular mass of the major selenium species in the Asari water-extract was less than 5000. Since the molecular mass of known selenoproteins was higher than 9 kDa,[12] the selenium species in the Asari water-extract was postulated to be not an intact selenoprotein but fragments of the selenoproteins and/or small molecular-mass selenium species such as selenoamino acids.

The Asari water-extract was separated by ion-exchange chromatography to characterize the ionic property of the selenium species in the extract. A 20-fold concentrated Asari water-extract was applied to the anion-exchange Q Sepharose column in the OH form [-$(OCH_2CH(OH)CH_2)N^+(CH_3)_3$·$OH^-$] and the column was washed out of the unbound species with water. Besides anionic compounds, amphoteric compounds, such as amino acids and/or proteins, can be retained on the column because of the dissociation of the acidic groups in the amphoteric compounds in a high pH environment. Under these separation conditions, most of the components in the Asari water-extract were retained, then eluted with 0.1 M HCl (Fig. 2A). The selenium species were mostly detected in the fractions eluted during the retention times of 60−75 min that

Table 3. Selenium and Protein Filtration Rate of Asari Water-Extract

MMCO (kDa)	Selenium (%)	Protein (%)
5	95.7 ± 17.6	20.4 ± 4.3
30	96.7 ± 13.6	51.6 ± 8.1

Values are mean ± S.D. (n = 3)

Table 4. Selenium and Protein Contents in Fractions Separated by Q Sepharose and SP Sepharose Ion-Exchange Chromatography

Q Sepharose	Selenium eluted (%)	Protein eluted (%)
Eluted with water (Cationic/Nonionic Fraction)	5.34 ± 3.83	1.99 ± 0.65
Eluted with 0.1 M HCl (Anionic/Amphoteric Fraction)	60.44 ± 1.56	72.62 ± 0.69
SP Sepharose		
Eluted with water (Anionic/Nonionic Fraction)	14.18 ± 2.04	11.69 ± 1.99
Eluted with 0.1 M NaOH (Cationic/Amphoteric Fraction)	73.15 ± 3.31	89.19 ± 5.40

Selenium and protein contents in Asari water-extract applied to the columns were defined as 100%. Values are mean ± S.D. (n = 3)

corresponded to 60.4% of the total selenium applied to the column (Table 4). These results suggested that the selenium species in the Asari water-extract possess an anionic and/or amphoteric character. Subsequently, a cation-exchange SP Sepharose column in the H form [-$(CH_2)_3SO_3^-$·H^+] was utilized for the separation of the Asari water-extract. Under this separation condition, besides cationic compounds, amphoteric compounds can be retained on the column because of the dissociation of basic groups in the amphoteric compounds in a low pH environment. As shown in Fig. 2B, a considerable amount of substances was eluted without retention by the flushing of water and the washout was suggested to contain anionic species. The amount of selenium in these fractions was 14.2% of the total selenium applied to the column. The retained substances were eluted with 0.1 M NaOH and cationic and/or amphoteric species were involved in the eluted fraction. The amount of selenium eluted during the retention times of 95–115 min was 73.2% of the total selenium applied to the column (Table 4). Elution profiles of the proteinous substances were similar to those of the selenium species in both the Q and SP Sepharose columns. Based on the results of the ion-exchange chromatographic separation, most of the selenium species in the Asari water-extract were proposed to be amphoteric and a small amount of anionic species was also involved. These selenium species might be selenoamino acids and/or their derivatives or selenium containing proteins.

We reported that Shijimi (*Corbicula japonica*), a typical clam eaten in Japan, contains selenium species with the ^{80}Se ion peak at *m/z* 534 that was separated by the ion-pair extraction.[30] This selenium species was found in the visceral sites including the digestive tract and the mid-gut gland by imaging mass spectrometry. The characteristics of the selenium species in the Asari water-extract were very similar to those of the Shijimi. As the ion-pair extraction of selenium species in the Asari water-extract by organic solvents was unsuccessful, we couldn't obtain any selenium isotopic pattern detected by mass spectrometry. The ionic selenium species in the Asari water-extract seem to possess different characteristics from those in the Shijimi water-extract.

***In Vitro* Assessment of Bioavailability of Selenium Species in the Asari Water-Extract** Human cervical carci-

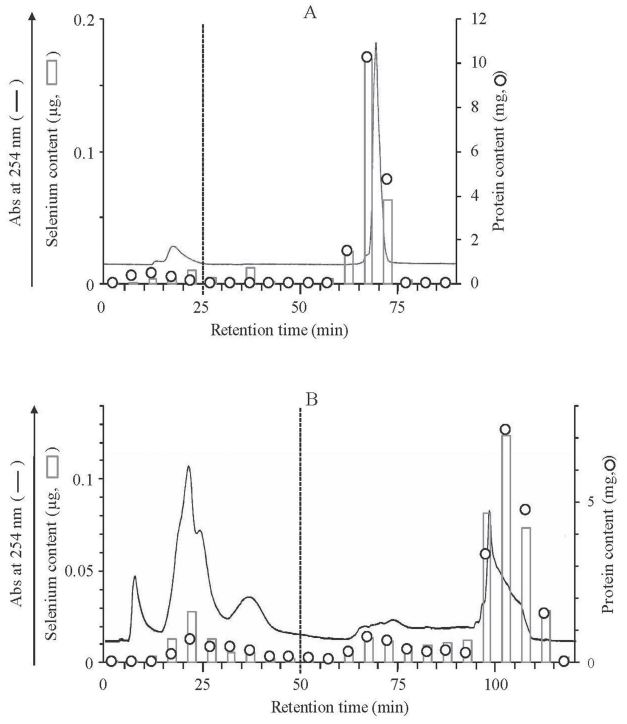

Fig. 2. Ion-Exchange Chromatographic Separation of the Asari Water-Extract on Q-Sepharose (A) and SP-Sepharose (B) Columns

Column dimension: 1.1 i.d. × 30 cm, Mobile phase: (A) 0−25 min, water; 25−100 min, 0.1 M HCl, (B) 0−50 min, water; 50−120 min, 0.1 M NaOH, Flow rate: 2.0 mL/min, Fraction volume: 10 mL, Injection sample volume: 1.0 mL.

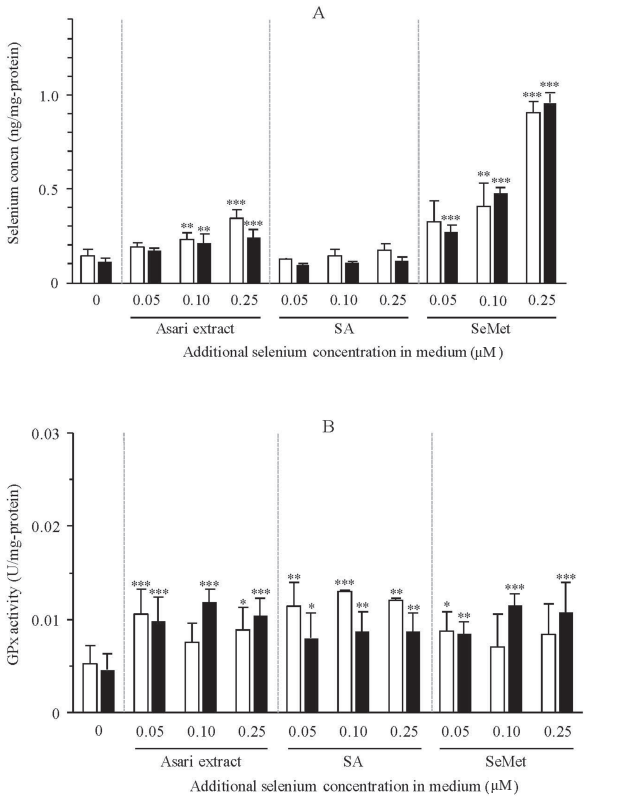

Fig. 3. Selenium Content (A) and GPx Activity (B) of HeLa Cells after Incubation in Asari Water-Extracts-Supplemented Culture Medium for 48 or 72 h

Selenium concentrations added to the basal 5% FBS-containing DMEM, 0.05, 0.1 and 0.25 μM. Incubation time: ▭,48 h; ▬, 72 h. Values are mean ± S.D. (n=2−12). *, **, ***, Significantly different from supplement-free control groups with $P < 0.05$, 0.01, 0.001.

noma (HeLa) cells and hepatoma (HepG2) cells were maintained in 10% FBS-containing DMEM (selenium concentration: ≈0.02 μM) before exposure to the Asari water-extract. For the assessment of the bioavailability of selenium in the Asari water-extract, the concentration of FBS in the incubation media was lowered to 5%. No significant changes in the selenium content and GPx activity due to the reduction of the FBS concentration were observed for both the HeLa and HepG2 cells (Fig. S3). The HeLa and HepG2 cells were incubated in the Asari water-extract, SA or SeMet-supplemented 5% FBS-containing DMEM for several days, then their selenium contents and GPx activities were determined (Figs. 3 and 4). These cells incubated in the supplement-free 5% FBS-containing DMEM were used as the control. When the HeLa and HepG2 cells were cultured in the medium containing 0.5 μM selenium from the Asari water-extract, deformation and detachment of the cells from the culture dish were, in part, observed. Such morphological changes were presumably caused by the high concentrations of inorganic salts and amino acids resulting from the concentrated Asari water-extract. Thus, the maximum selenium concentration supplemented to the culture media was 0.25 μM. Similar changes in the cells were hardly observed in the SA and SeMet-supplemented media up to 0.5 μM.

As is shown in Fig. 3A, the selenium contents in the HeLa cells incubated with the increasing SA concentration in the culture medium were almost the same as that with the supplement-free medium. On the other hand, the selenium contents increased with the increasing selenium concentration from the Asari water-extract. A similar trend was observed when incubated with SeMet. A higher amount of selenium from the SeMet-supplemented medium could be retained in the HeLa cells compared to those from the Asari water-extract and SA-supplemented media. As shown in Fig. 3B, the GPx activity of the HeLa cells incubated with the Asari water-extract became approximately twice as high as that of the control without additional selenium. In the HeLa cells, the GPx activity did not necessarily seem to correspond to the selenium contents, selenium species and incubation time. Although the selenium content in the HeLa cells with 0.25 μM SeMet was over 4-fold higher than that with 0.25 μM SA, there was no significant difference in the GPx activity between the two cells. The selenium content in the HeLa cells with the Asari water-extract was lower than that with SeMet, but higher than that with SA. These results demonstrated that selenium from the Asari-extract was taken up into the HeLa cells and utilized for the biosynthesis of GPx, although the expression of GPx appeared to be almost saturated with 0.05 μM selenium in the culture medium.

Subsequently, the HepG2 cells were incubated with the Asari water-extract under the same conditions used for the HeLa cells (Fig. 4). The selenium contents in the HepG2 cells significantly increased with the increasing concentration of selenium from the Asari water-extract. Increases in the selenium content were also observed for the HepG2 cells with SA and SeMet. No significant differences in the selenium content due to the incubation time were observed. The GPx activities of the HepG2 cells were quite dependent on the supplied

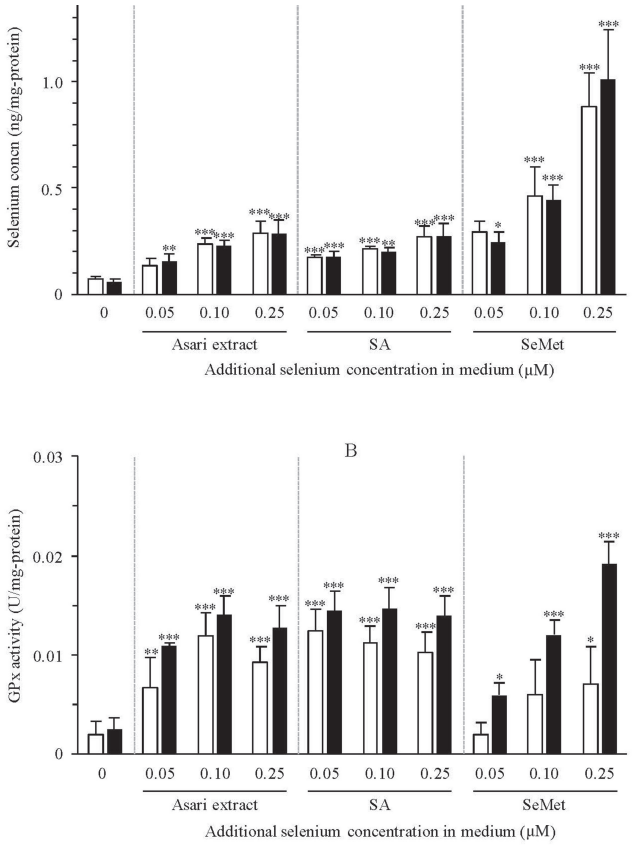

Fig. 4. Selenium Content (A) and GPx Activity (B) of HepG2 Cells after Incubation in Asari Water-Extracts-Supplemented Culture Medium for 48 or 72 h

Selenium concentrations added to the basal 5% FBS-containing DMEM, 0.05, 0.1 and 0.25 μM. Incubation time: ▭, 48 h; ▬, 72 h. Values are mean ± S.D. (n=2−12). *, **, ***, Significantly different from supplement-free control groups with $P < 0.05$, 0.01, 0.001.

selenium species. SA was the most effective to raise the GPx activity at 0.05 μM selenium in the medium. The GPx activity of the HepG2 cells increased with an increase in the selenium concentration from the Asari water-extract. In addition, the GPx activity of the HepG2 cells after incubation for 72 h was higher than those at 48 h. These trends were remarkable in the HepG2 cells with SeMet, and the highest GPx activity was observed for 0.25 μM SeMet at 72 h. The GPx activity of the HepG2 cells with the Asari water-extract was almost comparable to that with SA. These results demonstrated that the bioavailability of selenium from the Asari water-extract was comparable to that from SA in the HepG2 cells. Because the amount of inorganic selenium with oxidation state IV in the Asari water-extract was less than 6% (data not shown), some organic selenium species in the Asari water-extract could possibly contribute to the rise of the GPx activity in HepG2 cells.

The bioavailability of selenium species in the Asari water-extract was compared to those of the SA and SeMet. When the HeLa cells were incubated with an increasing SA concentration, the GPx activity increased, but the selenium content was not significantly changed. The absorbed SA and/or its metabolites might be effluxed from the cells and poorly retained in the HeLa cells probably due to their toxicity. On the contrary, a larger amount of selenium from SeMet was taken up into both the HeLa and HepG2 cells than those from the Asari water-extract and SA, although the SeMet supplement did not effectively improve the GPx activity at its low concentration. SeMet is known to be non-specifically incorporated into proteins in place of methionine but cannot be effectively used for the biosynthesis of selenoproteins.[33]

CONCLUSION

The selenium content in the lyophilized Asari meat was approximately 4.3 μg/g and 23% of that able to be extracted into water under certain conditions. Most of the selenium species in the Asari water-extract was found to be organic, hydrophilic and amphoteric with a molecular mass less than 5000. The selenium contents in the HeLa and HepG2 cells increased when these cells were cultured in the culture media supplemented with selenium from the Asari water-extract. The GPx activities of the HeLa and HepG2 cells incubated with the Asari water-extract were comparable to those with SA. The selenium species from the Asari are proposed to be utilized for the biosynthesis of selenoproteins in these cells as well as SA. Overall, the selenium species from the Asari were concluded to be a nutritionally effective selenium source.

Supplementary Materials The online version of this article contains supplementary materials.

Conflict of interest The authors declare no conflict of interest.

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