ANALYTICAL SCIENCES SEPTEMBER 2008, VOL. 24

Distribution and Dynamic Pathway of Selenium Species in Selenium-deficient Mice Injected with ^{82}Se-enriched Selenite

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In order to elucidate Se metabolism in a living body, ^{82}Se-enriched selenite was injected intravenously into mice fed Se-adequate and -deficient diets. We studied the time-dependent changes in the distribution of the labeled Se in organs, red blood cells, and plasma. The total Se was determined by flow-injection ICPMS, and Se speciation analysis was conducted by micro-affinity chromatography coupled with low-flow ICPMS. Total Se in almost all organs, including liver, showed the maximum at 1 h after injection. From speciation analysis, exogenous ^{82}Se as Se-containing proteins other than selenoprotein P (Sel-P) (selenium containing albumin (SeAlb) and extra cellular glutathione peroxidase (eGPx)), peaked at 1 h and quickly decreased from 1 to 6 h after injection, whereas that as Sel-P, peaked at 6 h, and gradually decreased from 6 to 72 h after injection. We found that there were two pathways for the transfer of Se in mice; one was as SeAlb until 1 h after injection, and the other was as Sel-P from 6 to 72 h after injection.

(Received June 30, 2008; Accepted July 28, 2008; Published September 10, 2008)

Introduction

Selenium (Se) is an essential element for humans and other animals with a narrow adequate range between deficient and excessive doses, and has functions in antioxidant effect,[1] alleviating the toxicity of heavy metals,[2] and sperm production.[3] Se deficiency induces arterial sclerosis,[4] cardiac myopathy,[5] and infectious disease.[6] On the other hand, Se is known to be a highly toxic element, and Se excess leads to hair and nail loss as well as brittleness, gastrointestinal problem, and nervous system abnormalities.[7] Therefore, the recommended daily intake of Se for human health is strictly defined.[8]

The metabolic pathway of Se, such as absorption, metabolism, and excretion, has been elucidated by studies of Se compounds related to human Se metabolism.[9,10] Many tracer experiments with a stable Se isotope were performed to elucidate the metabolic pathway of Se.[11-13] Suzuki *et al.* revealed a metabolic pathway of selenite in the blood stream by Se speciation studies.[11,12] Selenite taken up by red blood cells (RBCs) is reduced to selenide, and transferred to the liver in a form bound to albumin (Alb). Selenide is taken up by the liver, and is utilized for the synthesis of selenoproteins, such as selenoprotein P (Sel-P) and cellular glutathione peroxidase (cGPx). However, the metabolic fates of Sel-P and Se as Sel-P are not known. In addition, changes in a metabolic pathway of Se, such as absorption, metabolism, and excretion, under various Se nutritional statuses have not been investigated.

There are three major Se-containing proteins in human plasma: Sel-P, extra cellular GPx (eGPx), and selenium containing albumin (SeAlb).[14,15] Sel-P is the most common Se-containing protein in human plasma, and its concentration level is a good biochemical marker for Se status.[16,17] A recent supplementation study found that Sel-P in plasma is a more accurate marker for Se saturation levels than the previously used eGPx.[18] Animal experiments using Sel-P-knockout mice revealed that Sel-P plays a pivotal role in delivering hepatic Se to target tissues, such as brain and testis.[19,20] In order to investigate the transport of Se to tissues as Sel-P, it is important to measure the Se concentration in various organs.

Chromatographic separation coupled with ICPMS has been used for Se speciation analysis.[21] Sel-P speciation is studied by means of size-exclusion[11-13] and affinity chromatography.[22-24] One difficulty with conventional HPLC-ICPMS systems is that they require relatively large (sub-ml) sample volumes. In our previous work, it was possible to determine Sel-P in sub-µl samples of human plasma by coupling micro-affinity chromatography to ICPMS using a total consumption nebulizer.[25]

To investigate the time-dependent metabolism of Se, which we called a "dynamic" pathway, ^{82}Se-enriched selenite was injected intravenously into mice, and endogenous and exogenous ^{82}Se in blood and twelve different organs were traced during whole the metabolic pathway, from 1 to 72 h after injection.

Experimental

Chemicals

The ^{82}Se-enriched selenite solution used for the tracer experiments was prepared by dissolving ^{82}Se-enriched Se metal (97.19% ^{82}Se-enriched, Oak Ridge National Laboratory, Oak Ridge, TN, USA) in nitric acid (electronic laboratory grade, Kanto Chemical Co., Inc., Tokyo, Japan) and then neutralizing

with 1 mol l^{-1} NaOH.[26]

Samples were digested with nitric acid (70%) and hydrogen peroxide (30%) (both were of electronic laboratory grade, Kanto Chemical Co., Inc., Japan). The digested samples were diluted with ultrapure water (>18.4 MΩ cm^{-1}) (Japan Millipore K.K., Tokyo, Japan). Standard solutions for calibration were prepared by diluting a standard solution of Se (1000 μg ml^{-1}; AAS grade, Kanto Chemical Co., Inc., Japan) with 0.1 mol l^{-1} nitric acid. Indium (1000 μg ml^{-1}; AAS grade, Kanto Chemical Co., Inc., Japan) was used as an internal standard. KCl and KBr (both are of suprapur, Merck Ltd., Darmstadt, Germany) were used to correct for spectral interferences. Oxalic acid (special reagent grade, Kanto Chemical Co., Inc., Japan) was used to correct for signal enhancement of Se due to carbon (C).

To validate the analytical procedures, we analyzed biological certified reference materials of NIST bovine liver CRM 1577a (National Institute of Standards and Technology, Gaithersburg, MD, USA), Seronorm® trace elements human serum 203105 and seronorm® trace elements human urine 201205 (Sero As, Bllingstad, Norway).

Buffer solutions for Se speciation were prepared by dissolving tris(hydroxymethyl)aminomethane (ultra grade, Sigma-Aldrich Japan K.K., Tokyo, Japan) in acetic acid (99.8%) and ammonia (29%) (both are of are special reagent grade, Kanto Chemical Co., Inc., Japan).

Animal experiments

Thirty male mice (Crlj: CD1(ICR)), 4 weeks old, were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan), and had received a Se-adequate control diet (0.45 μg Se g^{-1}; 2.25 μg Se day^{-1}) for 1 week before the beginning a mouse experiment. One group of mice was fed a Se-adequate control diet (0.45 μg Se g^{-1}; 2.25 μg Se day^{-1}); the other group of mice was fed a Se deficient diet (Se: <0.05 μg g^{-1}; <0.25 μg Se day^{-1}) for the following 4 weeks. These diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

After the 4-week feeding period, 0.2 ml of enriched solution (containing $Na_2{}^{82}SeO_3$ as 11.25 μg Se ml^{-1}) were injected intravenously into mice. Blood and twelve different organs (liver, kidneys, spleen, pancreas, heart, lungs, brain, thymus, submandibular glands, testes, epididymides, and seminal vesicles) were collected and weighted at the beginning of the mouse experiment (0), 1, 6, 24, or 72 h after injection. These organs are illustrated in Fig. 1. Three samples of each group were collected. Liver perfusion was carried out with 5% glucose (special reagent grade, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Blood samples were heparinized and centrifuged at 1500*g* for 10 min at 4°C, and separated into plasma and RBCs. RBCs were washed twice with 5% glucose. The hematocrit values were obtained from a small portion of a heparinized sample, which were used to calculate the volumes of both the plasma and RBCs.

Determination of total Se concentration in the mouse plasma, RBCs, and organs

To determine the total Se, an aliquot of plasma, RBCs, organs (about 0.1 g) were digested using a microwave oven (MLS-1200 MEGA; rotor, MDR-300/s; Milestone General, Tokyo, Japan) after the addition of 400 μl HNO_3 and 200 μl H_2O_2. After samples were completely digested, indium was added to a final concentration of 50 ng ml^{-1} for use as an internal standard, and the weight was adjusted to 1.5 g (a dilution factor of 15) with ultra pure water.

Se and other elements (Mn, Cu, Zn, As) were determined with an ICPMS instrument (HP4500, Agilent Technologies Japan Ltd., Tokyo, Japan). In this case of determining total Se, a conventional nebulizer (AR35-1-FC1, Glass Expansion, West Melbourne, VIC, Australia) was used.

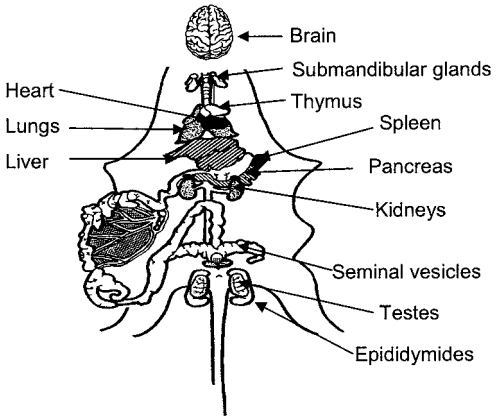

Fig. 1 Twelve mouse organs analyzed in this study.

Since sample volumes were limited in this study, Se concentration in 100-μl samples was measured by flow injection ICPMS (FI-ICPMS). Carbon (C) concentration in the digested samples was determined by ICPAES (SPS5100, SII Nano Technology Inc., Tokyo, Japan) to correct for the enhancement effect due to co-existing organic carbon.

Determination of Sel-P and other proteins in mouse plasma

Prior to Se speciation analysis, the plasma was filtered through a 0.45-μm nitrocellulose membrane filter (Nihon Millipore K. K., Tokyo, Japan). We used a capillary liquid chromatographic system consisting of a degasser (DG661, GL Sciences Inc., Tokyo, Japan), a capillary pump (MP710i, GL Sciences Inc., Japan), and a sample injector (sample volume: 0.5 μl; Model C4-1344, Valco Instruments Co., Houston, TX, USA). In order to operate at a low flow rate (6 μl min^{-1}), the micro affinity column, which packed by heparin, was scaled down to 0.5 mm i.d. × 35 mm long (AFpak AHR-894, Showa Denko K.K., Tokyo, Japan). In the case of speciation analysis of Se, a total consumption nebulizer (CEI-100 CETAC, Omaha, NE, USA) was used. Fused-silica tubes (50 μm i.d., 375 μm o.d.; GL Sciences Inc., Tokyo, Japan) were used for all connections.

Sel-P in mouse plasma was determined using micro-affinity chromatography coupled with low-flow ICPMS, which was developed in our previous work.[25] This system enabled speciation analysis of Sel-P in sub-μl plasma samples. It has been reported that only Sel-P among all of the Se-containing proteins in plasma is selectively retained on heparin.[15,22] Histidine residues, which are a constituent of the heparin-binding site of selenoprotein P, are important in the binding.[27] To elute the absorbed Sel-P, the mobile phase was switched from equilibration buffer (0.02 M Tris-HNO_3; pH 7.4) to elution buffer (0.02 M Tris-HNO_3 + 1.4 M ammonium acetate; pH 7.4) by a step gradient at 180 s. The chromatographic peaks were integrated, and calibrated relative to the signal of a standard selenite solution (100 ng ml^{-1}) obtained by FI-ICPMS.

Results and Discussion

Validation of analytical procedures

To validate the analytical results, we analyzed a bovine liver

Table 1 Determination of selenium in certified reference materials of bovine liver (NIST CRM 1577a), human serum (STE 203105), and human urine (STE 201205)

	Mass (*m/z*)	Bovine liver (NIST CRM 1577a)/ ng g^{-1}	Human Serum (STE 203105)/ ng ml^{-1}	Human Urine (STE 201205)/ ng ml^{-1}
Measured value	77	1690 ± 70	259 ± 28	84.3 ± 3.3
	82	1730 ± 50	175 ± 3	68.0 ± 4.1
Correction for spectral interferences	77	1690 ± 70	195 ± 10	64.0 ± 2.5
	82	1680 ± 50	172 ± 2	59.6 ± 5.0
Correction for signal enhancement	77	979 ± 42	158 ± 8	60.9 ± 2.4
due to organic carbon	82	1010 ± 30	142 ± 2	56.9 ± 4.8
(Carbon in digested samples)		(4230)[a]	(1350)[a]	(253)[a]
Internal-standard correction	77	985 ± 66	150 ± 7	59.3 ± 2.8
	82	1020 ± 50	134 ± 1	55.5 ± 4.9
Certified		1100 ± 100	136 ± 9	58.6 ± 3.1

a. Carbon concentration (μg ml^{-1}) in digested samples was measured after dilution of 40, 15, 15 times for bovine liver, human serum and human urine, respectively.

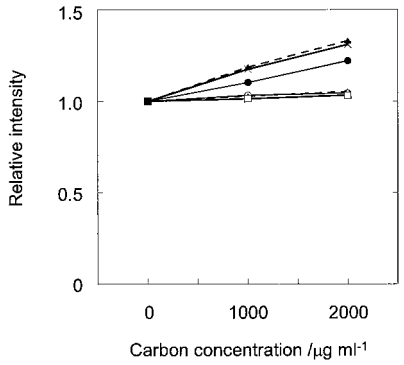

Fig. 2 Effect of the C concentration on the relative intensity of: ○, ^{55}Mn; △, ^{65}Cu; □, ^{66}Zn; ●, ^{75}As; ▲, ^{77}Se; ×, ^{82}Se.

(NIST CRM 1577a), human serum (STE 203105) and human urine (STE 201205). Biological samples include large amounts of inorganic salt, organic compounds, such as protein and amino acid, which induce spectral interferences due to the formation of polyatomic ions, non-spectral interferences due to matrix. In this study, the spectral interferences of ^{40}Ar^{37}Cl and ^{81}Br^{1}H on ^{77}Se and ^{82}Se, respectively, and non-spectral interferences of C derived from organic compounds were corrected for, and finally internal standardization was adapted.

The spectral interferences of ^{40}Ar^{37}Cl and ^{81}Br^{1}H on ^{77}Se and ^{82}Se, respectively, were corrected for mathematically by interference correction factors (IFs). Solutions of Cl and Br were prepared with concentration matching to their levels in bovine liver (Cl 50 μg ml^{-1}, Br 0.05 μg ml^{-1}), human serum (Cl 250 μg ml^{-1}, Br 0.05 μg ml^{-1}), and human urine (Cl 250 μg ml^{-1}, Br 0.05 μg ml^{-1}) after 15 times dilution. The solutions were measured daily for calibrating IFs as described elsewhere.[28] In the case of bovine liver, correction equations are expressed as follows:

$$^{77}Se_{\text{corrected for interferences}} = {}^{77}Se_{\text{measured}} - \text{IFs } (0.00013) \times {}^{37}Cl \quad (1)$$

$$^{82}Se_{\text{corrected for interferences}} = {}^{82}Se_{\text{measured}} - \text{IFs } (0.0056) \times {}^{81}Br \quad (2)$$

Standard solutions of 20 ng ml^{-1} Se, 200 ng ml^{-1} Mn, Cu, Zn, As in 0, 1000, 2000 μg ml^{-1} C were used for the matrix effect on the analyte signal. The ratios of the Se signal enhancement were calculated in each level of the C concentration, are plotted in Fig. 2. Since the Se intensity ratio proportionally increased along with an increase in the C concentration, it was decided that the Se signal enhancement needed to be corrected. In order to determine the signal enhancement correction factors (SFs), the C in digested samples was determined by using FI-ICPAES. The Se signal enhancement was corrected for mathematically by SFs obtained from the C concentration in each digested sample. In the case of bovine liver, the correction equations are expressed as follows:

$$^{77}Se_{\text{corrected for signal enhancement}} = {}^{77}Se_{\text{corrected for interferences}}/\text{SFs } (1.72) \quad (3)$$

$$^{82}Se_{\text{corrected for signal enhancement}} = {}^{82}Se_{\text{corrected for interferences}}/\text{SFs } (1.66) \quad (4)$$

Finally, the analytical value was determined using an internal-standard method. The results of a CRM analysis are summarized in Table 1; the concentrations of ^{77}Se and ^{82}Se agreed well with the certified values within the analytical error. These results confirmed that the ICPMS method used was suitable for the determination of Se in biological samples.

Calculation of endogenous and exogenous Se

In mice, Se isotopes (74, 76, 77, 78, 80, and 82) exist in their natural abundances (Fig. 3a). After the intravenous injection of ^{82}Se-enriched selenite, exogenous and endogenous Se were distributed throughout the mouse body (Fig. 3b). The concentrations of endogenous and exogenous Se were calculated from *m/z* counts of 77 and 82.[11] To clarify the representations of endogenous and exogenous Se in the figures, endogenous and exogenous ^{82}Se are used in this paper. The relationships between the concentration of exogenous Se and exogenous ^{82}Se, and between those of endogenous Se and endogenous ^{82}Se, respectively, are expressed as follows:

$$\text{Exogenous } {}^{82}Se = \text{Exogenous Se} \times {}^{82}\text{Se isotopic abundance } (0.9719) \quad (5)$$

$$\text{Endogenous } {}^{82}Se = \text{Endogenous Se} \times {}^{82}\text{Se isotopic abundance } (0.0873) \quad (6)$$

Therefore, exogenous Se is almost the same as exogenous ^{82}Se (multiplied by 1.03); whereas endogenous Se can be calculated as multiplying 11.5 by the endogenous ^{82}Se.

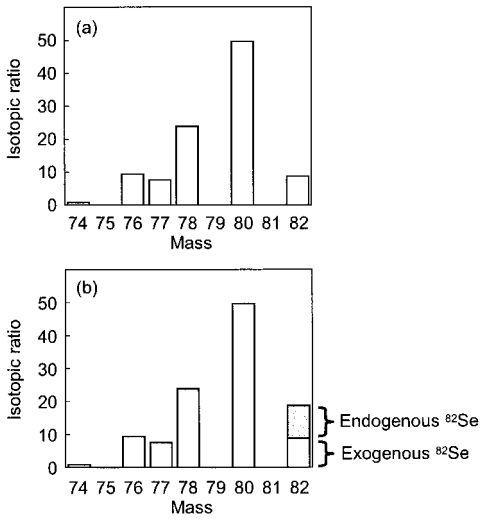

Fig. 3 Se isotope patterns (m/z = 74, 76, 77, 78, 80, and 82): (a) before and (b) after ^{82}Se-enriched selenite was added.

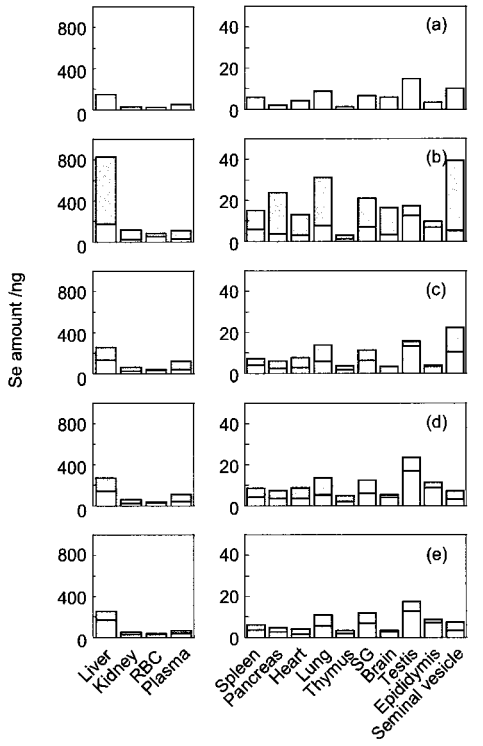

Fig. 4 Distribution of endogenous and exogenous ^{82}Se in mice fed the control diet: (a) 0, (b) 1, (c) 6, (d) 24, and (e) 72 h after injection. □, Endogenous ^{82}Se; ■, exogenous ^{82}Se.

Distribution of endogenous and exogenous ^{82}Se in mouse organs, RBCs, and plasma under different Se nutritional statuses

The concentrations of C in digested organs, RBCs, and plasma were measured by FI-ICPAES, and the results were 1200 – 6900 μg ml^{-1}, 3600 μg ml^{-1}, 1400 μg ml^{-1}, respectively. SFs were calculated from both data shown in Fig. 2, and the concentration of C in digested samples. Spectral interferences and the signal enhancement due to organic carbon were corrected for as mentioned in the previous section. After performing internal standardization, endogenous and exogenous ^{82}Se were

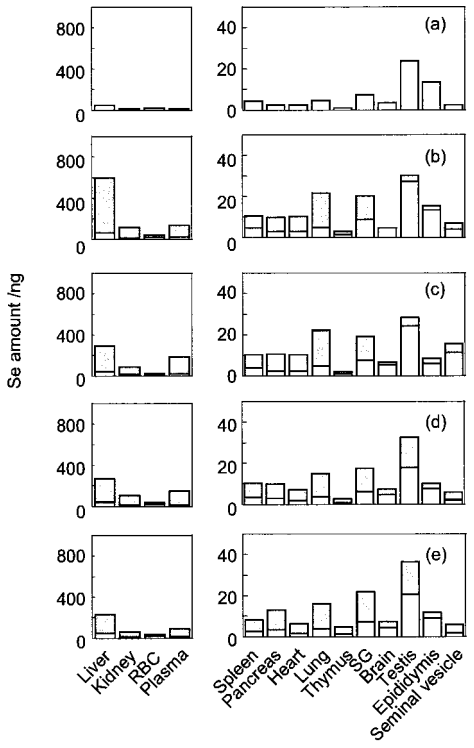

Fig. 5 Distribution of endogenous and exogenous ^{82}Se in mice fed the deficient diet: (a) 0, (b) 1, (c) 6, (d) 24, and (e) 72 h after injection. □, Endogenous ^{82}Se; ■, exogenous ^{82}Se.

determined. The distribution of endogenous and exogenous ^{82}Se in mice at different intervals is shown for mice fed Se-adequate and -deficient diet in Figs. 4 and 5, respectively.

At the beginning of tracer experiment (Figs. 4a and 5a), endogenous ^{82}Se in the liver of mice fed the Se-deficient diet was 3 times less than those of mice fed the control diet. The lack of Se in the liver resulted in a lowering of endogenous ^{82}Se in the kidneys, RBCs, and plasma of mice fed the deficient diet. Considering that Se absorbed in the liver is utilized for the synthesis of Sel-P and cGPx,[9] the decreased hepatic Se concentration in mice fed the deficient diet likely reflects the insufficient supply of Se, and is consistent with the decreased levels of Se circulating in the mouse body. However, this reduction of endogenous ^{82}Se in the testis and epididymis was relatively small compared with that of other organs. Since Se plays an important role in sperm production,[3] we assume that some metabolic pathways work to maintain the Se concentration. As shown in Fig. 4, exogenous ^{82}Se was mainly distributed in the liver, kidney, RBCs, and plasma during the experiment period. The exogenous ^{82}Se dramatically increased in liver during the first hour following injection. The injected Se was absorbed by the liver more efficiently than by other organs, and was supposed to be utilized for the synthesis of selenoproteins, or to be excreted from the mouse body. On the other hand, the distribution of exogenous ^{82}Se was observed in almost all other organs at 1 h after injection. It is likely that there is another pathway whereby the injected ^{82}Se-enriched selenite is transferred to other organs without passing through the liver. Exogenous ^{82}Se absorbed in the organs of mice fed the control diet steadily decreased from 1 to 72 h after injection, and the relative Se distribution recovered to that observed at the beginning of the experiment. The excess Se was excreted from the mouse body because sufficient Se was present in the mice

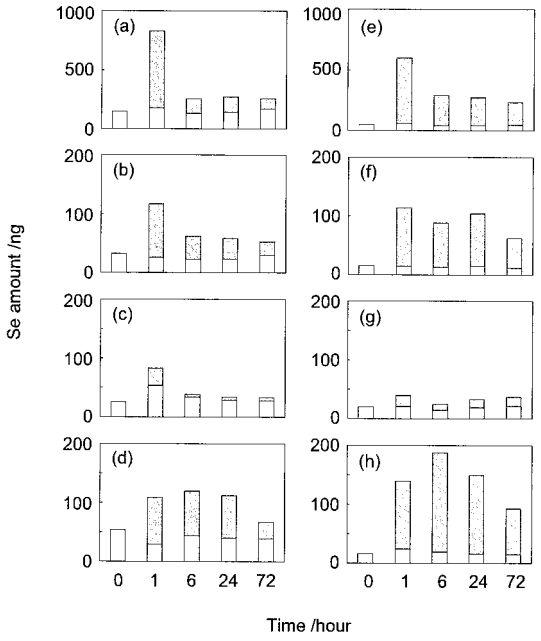

Fig. 6 Time-dependent changes of endogenous and exogenous ^{82}Se in (a) liver, (b) kidneys, (c) RBCs, and (d) plasma of mice fed the control diet, (e) liver, (f) kidney, (g) RBCs, and (h) plasma of mice fed the deficient diet. □, Endogenous ^{82}Se; ■, exogenous ^{82}Se.

fed the control diet.

As shown in Fig. 5, at 1 h after injection, the distribution of exogenous ^{82}Se in the liver of mice fed the deficient diet was observed to be at the same level as that of mice fed the control diet. Almost of the injected ^{82}Se-enriched selenite was initially absorbed in the liver regardless of the Se nutritional status. At 6 h after injection, the decrease of exogenous ^{82}Se in mice fed the deficient diet was less than that in mice fed the control diet, and the amount of exogenous ^{82}Se in organs remained higher than that in mice fed the control diet. We assume that the excretion of Se was suppressed, and the Se level was maintained in each organ.

The variation of endogenous and exogenous ^{82}Se in liver, kidney, RBCs, and plasma, was plotted against time for Se-adequate and -deficient statuses (Fig. 6). From 1 to 6 h after injection, exogenous ^{82}Se in the livers and kidneys of mice fed the control diet dramatically decreased (Figs. 6a and 6b), whereas that in mice fed the deficient diet remained constant (Figs. 6e and 6f). The absorption of exogenous ^{82}Se into the blood stream of mice fed the deficient diet was higher than that in mice fed the control diet. In particular, at 6 h after injection, the amount of Se was 2.5 times as much as that in mice fed the control diet (Figs. 6d and 6h). We assume that the amount of Se in the plasma increased in the deficient mice so that Se could be supplied to other organs.

Investigation of dynamic pathways of Se species in mouse plasma

To elucidate the dynamic pathways of Se species in plasma, we analyzed Se speciation by means of micro-affinity chromatography coupled with low-flow ICPMS. As shown in Fig. 7, two Se peaks were observed in the plasma of mice fed the deficient diet. The first peak, which was not absorbed by heparin, corresponds to Se-containing proteins other than Sel-P (SeAlb and eGPx), and the second peak, which was eluted by switching from equilibration buffer to elution buffer,

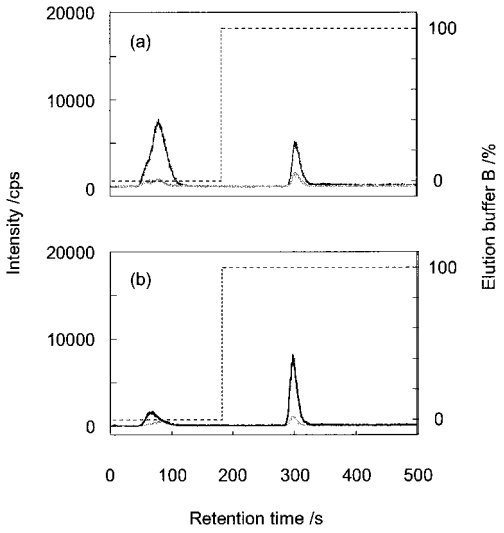

Fig. 7 Affinity chromatogram of plasma for (a) mice fed the control diet and (b) mice fed the deficient diet at 24 h after injection. The black and grey lines indicate ^{82}Se and ^{77}Se, respectively. The broken line indicates the mixing ratios of the elution buffer.

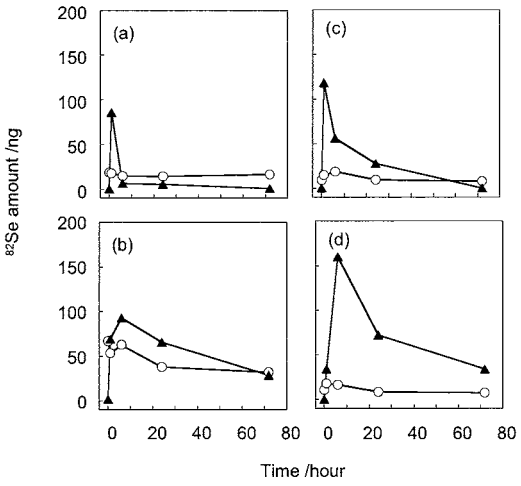

Fig. 8 Time variation of Se species in mouse plasma: (a) Se-containing proteins (SeAlb and eGPx), (b) Sel-P in mice fed the control diet; (c) Se-containing proteins (SeAlb and eGPx), and (d) Sel-P in mice fed the deficient diet. ○, Endogenous ^{82}Se; ▲, exogenous ^{82}Se.

corresponds to Sel-P. At 1 h after injection (Fig. 7a), exogenous ^{82}Se was mainly distributed to Se-containing proteins other than Sel-P. However at 6 h after injection (Fig. 7b), exogenous ^{82}Se was distributed to Sel-P.

As shown in Figs. 8a and 8b, in plasma of mice fed the control diet, exogenous ^{82}Se of Se-containing proteins other than Sel-P peaked at 1 h after injection, and quickly decreased from 1 to 6 h after injection, whereas exogenous ^{82}Se of Sel-P peaked at 6 h after injection and gradually decreased from 6 to 72 h after injection. Suzuki and Itoh reported that ^{82}Se enriched selenite was mainly combined with Alb at 10, 30, and 60 min after injection.[11] Thus, we suspected that Se-containing proteins other than Sel-P observed at 1 h after injection consisted mainly of SeAlb. These results suggest that almost all of the ^{82}Se-enriched selenite was transformed into SeAlb within 1 h, and

was absorbed by the liver, where Sel-P in mouse plasma was synthesized within 6 h. There are two pathways whereby Se was transferred to organs; one was as SeAlb until 1 h after injection, and the other was as Sel-P from 6 to 72 h.

As shown in Figs. 8c and 8d, in mice fed the deficient diet, the increase of exogenous ^{82}Se as Se-containing proteins other than Sel-P was almost comparable to that in mice fed the control diet, although about a 1.5-times increase of exogenous ^{82}Se as Sel-P was observed compared with mice fed the control diet. It was reported that Sel-P mRNA in Se deficiency remained higher relative to the control.[29] Their finding agreed with our results that Sel-P was produced more efficiently in Se deficiency than the control. As shown in Fig. 5, the experimental results that exogenous ^{82}Se in organs remained higher than that in mice fed the control diet could be explained by the continuous supply of Se as a chemical form of Sel-P in plasma from 6 to 72 h after injection. It is suspected that Sel-P plays an important role in Se transport to organs.

Conclusions

Most ^{82}Se-enriched selenite was transferred to the liver within 1 h after intravenous injection, and was utilized for the synthesis of selenoproteins, regardless of the Se nutrition status. We suspect that ^{82}Se-enriched selenite was transformed into SeAlb within 1 h, and was mainly absorbed by the liver, where Sel-P was produced within 6 h. Sel-P gradually decreased from 6 to 72 h as Se was transported to other organs. These results suggest that there are two pathways for Se metabolism in mice. One is a pathway for Se transfer in the chemical form SeAlb until 1 h after injection; and the other is a pathway for Se transfer in the chemical form Sel-P, from 6 to 72 h. Because the exogenous ^{82}Se in the plasma of mice fed the deficient diet increased in the chemical form Sel-P to 1.5 times high as much as that of mice fed the control diet, it appears that Sel-P played an important role in the transport of Se to Se-deficient organs.

Acknowledgements

This research was conducted under a research project entitled "Development of a highly sensitive Eco/Bio-ICPMS system for speciation analysis" at the Institute of Science and Engineering, Chuo University. We are grateful to Kazunori Iwata (Showa Denko K.K.) for providing an affinity column.

References

1. H. Tapiero, D. M. Townsend, and K. D. Tew, *Biomed. Pharmacother.*, **2003**, *57*, 134.
2. C. Chen, H. Yu, J. Zhao, B. Li, L. Qu, S. Liu, P. Zhang, and Z. Chai, *Environ. Health Perspect.*, **2006**, *114*, 297.
3. K. Renko, M. Werner, I. Renner-Müller, T. G. Cooper, C. H. Yeung, B. Hollenbach, M. Scharpf, J. Köhrle, L. Schomburg, and U. Schweizer, *Biochem. J.*, **2008**, *409*, 741.
4. G. A. Agbor, J. A. Vinson, S. Patel, K. Patel, J. Scarpati, D. Shiner, F. Wardrop, and T. A. Tompkins, *J. Agric. Food Chem.*, **2007**, *55*, 8731.
5. B. Dalir-Naghadeh and S. A. Rezaei, *Am. J. Vet. Res.*, **2008**, *69*, 659.
6. E. S. Wintergerst, S. Maggini, and D. H. Hornig, *Ann. Nutr. Metab.*, **2007**, *51*, 301.
7. S. B. Goldhaber, *Regul. Toxicol. Pharm.*, **2003**, *38*, 232.
8. World Health Organization and Food and Agriculture Organization of the United Nations, "*Vitamin and mineral requirements in human nutrition*", 2nd ed., **2004**, WHO, Geneva, 206.
9. K. T. Suzuki, *J. Health Sci.*, **2005**, *51*, 107.
10. B. Gammelgaard, C. Gabel-Jensen, S. Stürup, and H. R. Hanse, *Anal. Bioanal. Chem.*, **2008**, *390*, 1691.
11. K. T. Suzuki and M. Itoh, *J. Chromatogr., B*, **1997**, *692*, 15.
12. K. T. Suzuki, K. Ishiwata, and Y. Ogra, *Analyst*, **1999**, *124*, 1749.
13. K. T. Suzuki, C. Doi, and N. Suzuki, *Toxicol. Appl. Pharmacol.*, **2006**, *217*, 185.
14. H. Koyama, K. Omura, K. Ejima, and Y. Kasanuma, C. Watanabe, and H. Satoh, *Anal. Biochem.*, **1999**, *267*, 84.
15. I. Harrison, D. Littlejohn, and G. S. Fell, *Analyst*, **1996**, *121*, 189.
16. M. Persson-Moschos, W. Huang, T. S. Srikumar, B. Aakesson, and S. Lindeberg, *Analyst*, **1995**, *120*, 833.
17. J. Neve, *Nutr. Rev.*, **2000**, *58*, 363.
18. Y. Xia, K. E. Hill, D. W. Byrn, J. Xu, and R. F. Burk, *Am. J. Clin. Nutr.*, **2005**, *81*, 829.
19. L. Schomburg, U. Schweizer, B. Holtmann, L. Flohé, M. Sedtner, and J. Köhrle, *Biochem. J.*, **2003**, 370, 397.
20. K. E. Hill, J. Zhou, W. J. McMahan, A. K. Motley, J. F. Atkins, R. F. Gesteland, and R. F. Burk, *J. Biol. Chem.*, **2003**, *278*, 13640.
21. C. B'Hymer and J. A. Caruso, *J. Chromatogr., A*, **2006**, *1114*, 1.
22. L. H. Reyes, J. M. Marchante-Gayon, J. I. Garcia Alonso, and A. Sanz-Medel, *J. Anal. At. Spectrom.*, **2003**, *18*, 1210.
23. P. Jitaru, M. Prete, G. Cozzi, C. Turetta, W. Cairns, R. Seraglia, P. Traldi, P. Cescon, and C. Barbante, *J. Anal. At. Spectrom.*, **2008**, *23*, 402.
24. P. Jitaru, G. Cozzi, A. Gambaro, P. Cescon, and C. Barbante, *Anal. Bioanal. Chem.*, **2008**, *391*, 661.
25. K. Shigeta, K. Sato, and N. Furuta, *J. Anal. At. Spectrom.*, **2007**, *22*, 911.
26. K. T. Suzuki, S. Yoneda, M. Itoh, and M. Ohmichi, *J. Chromatogr., B: Biomed. Appl.*, **1995**, *670*, 63.
27. R. J. Hondal, S. Ma, R. M. Caprioli, K. E. Hill, and R. F. Burk, *J. Biol. Chem.*, **2001**, *276*, 15823.
28. P. Jitaru, K. Tirez, and N. D. Brucker, *At. Spectrosc.*, **2003**, *24*, 1.
29. K. E. Hill, P. R. Lyons, and R. F. Burk, *Biochem. Biophys. Res. Commun.*, **1992**, *185*, 260.