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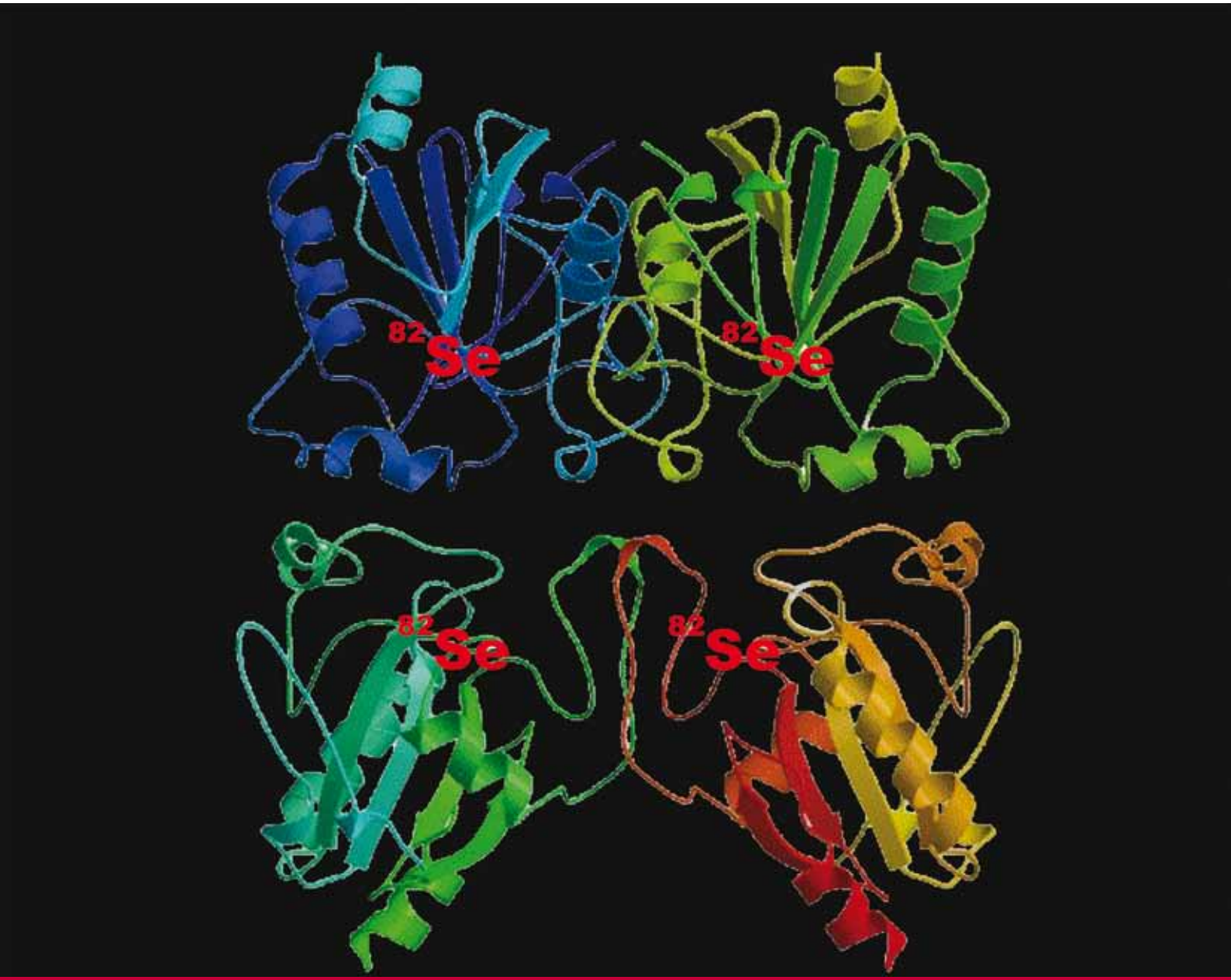

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Dynamic pathways of selenium metabolism and excretion in mice under different selenium nutritional statuses†

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The selenoprotein, cellular glutathione peroxidase (cGPx), has an important role in protecting organisms from oxidative damage through reducing levels of harmful peroxides. The liver and kidney in particular, have important roles in selenium (Se) metabolism and Se is excreted predominantly in urine and feces. In order to characterize the dynamics of these pathways we have measured the time-dependent changes in the quantities of hepatic, renal, urinary, and fecal Se species in mice fed Se-adequate and Se-deficient diets after injection of ^{82}Se-enriched selenite. Exogenous ^{82}Se was transformed to cGPx in both the liver and kidney within 1 h after injection and the synthesis of cGPx decreased 1 to 6 h and continued at a constant level from 6 to 72 h after injection. The total amount of Se associated with cGPx in mice fed Se-deficient diets was found to be less than in mice fed Se-adequate diets. This finding indicated that cGPx synthesis was suppressed under Se-deficient conditions and did not recover with selenite injection. Excess Se was associated with selenosugar in liver and transported to the kidney within 1 h after injection, and then excreted in urine and feces within 6 h after injection. Any excess amount of Se was excreted mainly as a selenosugar in urine.

Introduction

Selenium (Se) is an essential element for humans and other animals and plays important roles in (i) producing an antioxidant effect,[1] (ii) alleviating the toxicity of heavy metals[2] and (iii) in sperm production.[3] It is already known that the adequate concentration range between deficient and excessive doses of Se is very narrow. The recommended daily intake of Se for human health is strictly defined[4] and it is important to elucidate Se metabolism. Se deficiency induces arterial sclerosis,[5] cardiac myopathy[6] and exacerbates infectious disease.[7] The data of epidemiological study suggested that dietary Se deficiency is associated with an increased risk of cancer.[8,9] Therefore, it is important to investigate the changes in a metabolic pathway of Se, such as absorption, metabolism and excretion, under Se deficient statuses.

To characterize the Se metabolism, tracer experiments with the stable ^{82}Se isotope and Se speciation analysis using high performance liquid chromatography (HPLC) coupled with inductively-coupled plasma mass spectrometry (ICPMS) were performed.[10–14] Suzuki *et al.* have previously used Se speciation studies to identify a metabolic pathway for selenite in the blood stream.[10,12,13] Circulating selenite was found to be taken up by red blood cells (RBC) and reduced to selenide, which circulates in the blood stream bound to albumin (Alb). Selenide is taken up by the liver, where it is utilized for the synthesis of selenoproteins including, selenoprotein P (Sel-P) and cellular glutathione peroxidase (cGPx). Alternatively, the Se can be methylated and excreted. The regulation of Se metabolic pathways under various Se nutritional statuses has not been previously investigated. However, most of their works focused on the time dependent change of Se metabolism and the data were not quantitative.

In our previous study, the distribution of ^{82}Se in plasma as Se-containing proteins other than Sel-P, including Se-bound Alb (SeAlb) and extra cellular glutathione peroxidase (eGPx), peaked at 1 h and quickly decreased from 1 to 6 h after ^{82}Se injection.[15] By comparison, ^{82}Se in the form of Sel-P peaked at 6 h and gradually decreased from 6 to 72 h after injection.[15] From these data, we propose that there are two pathways for the transport of Se in mice. One Se transport pathway utilizes SeAlb in the short term up to 1 h after injection and the other utilizes Sel-P from 6 to 72 h after injection. About a 1.5-times increase of exogenous ^{82}Se associated with Sel-P was observed in mice fed deficient diets compared with mice fed adequate diets. Suzuki's group also administered Se to rats under Se deficient conditions. Their experiments were aimed at the exchange rates of exogenous Se after oral administration of ^{82}Se-enriched selenite[16] and the transformation of selenosugar after intravenous injection of ^{76}Se-enriched selenosugar.[17] But Se usage to selenium-containing protein under Se deficient conditions is as yet unclear.

In this study, we focused on exogenous ^{82}Se distribution to the liver and kidney as well as ^{82}Se excretion in urine and feces

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for mice fed either Se-adequate or Se-deficient diets. To investigate the time-dependent or "dynamic" pathway of Se metabolism, we injected ^{82}Se-enriched selenite intravenously into mice, and the chemical species associated with endogenous Se and exogenous ^{82}Se in the liver, kidney, urine, and feces were quantified from 1 to 72 h after injection.

Experimental

Chemicals

The ^{82}Se-enriched selenite solution used as a tracer 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), which was then neutralized with 1 mol L^{-1} NaOH.

Animal experiments

Animal experiments were performed under the ethical approval from Animal Experiment Ethical Committee, Juntendo University School of Medicine (approval number is 210068). 30 male 4 week old mice (Crlj: CD1(ICR)), were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan), and received a maintenance Se-adequate diets (0.45 μg Se g^{-1}; 2.25 μg Se day^{-1}) for 1 week before beginning the dietary modification experiment. One group of mice continued to be fed Se-adequate diets (Se-adequate mice); the other group was then fed Se-deficient diets (Se: <0.05 μg Se g^{-1}; <0.25 μg Se day^{-1}) for the following 4 weeks (Se-deficient mice). The mouse diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). After the 4-week feeding period, 0.2 mL of an enriched ^{82}Se solution (containing 11.25 μg ^{82}Se mL^{-1} as $Na_2{}^{82}SeO_3$) were injected intravenously into mice. The mice continued to receive the Se-adequate and Se-deficient diets respectively for 72 h after injection.

After euthanized with diethyl ether, three mice of each group were dissected just before the injection (0 h), 1, 6, 24, and 72 h after injection, and liver and kidney were collected. The livers were perfused with 5% glucose (special reagent grade, Wako Pure Chemical Industries, Ltd., Osaka, Japan) prior to further analysis. The urine and feces samples were separately collected using metabolic cages (Type; MC, Sugiyama-Gen Iriki Co., Ltd, Japan).

Sample preparation

A portion of liver and kidney was homogenized with a glass-teflon homogenizer (Model HOM, As One Co., Osaka, Japan) in 2 volumes of 50 mmol L^{-1} Tris-HNO_3 buffer solution (pH 7.4) while being chilled in an ice bath. The homogenate was then ultracentrifuged at 105 000 × g for 60 min at 4 °C and the cytosol fraction was recovered.

An aliquot of 1 mL of ultrapure water was added to 0.10 g of mouse feces and the sample was homogenized with the same glass-teflon homogenizer and then processed to sonication while being chilled in an ice bath. The homogenate was then centrifuged at 1500 × g for 30 min at room temperature and the supernatant was recovered. An aliquot of 1 mL of ultrapure water was added to the pellet, which was extracted again by the same method. This procedure was repeated three times and 3 mL of supernatant was collected. The collected extract was lyophilized and diluted to 0.4 g with ultrapure water.

All samples were filtrated through a nitrocellulose membrane filter with a pore diameter of 0.45 μm (Nihon Millipore K. K., Tokyo, Japan) prior to the Se speciation analysis by HPLC-ICPMS.

Determination of total Se concentration in the mouse organs, body fluids, and feces

To improve exogenous ^{82}Se recovery, we determined the total Se in urine, feces and muscle in addition to 12 organs, plasma and red blood cells analyzed in previous studies.[15] Following the same analytical procedures described in the previous paper,[15] about 0.1 g of urine, feces and muscle of mice 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 with ultra pure water. Se was determined after correcting for spectral interferences and signal enhancement by carbon, and conducting internal standardization as described in the previous paper.[15] Total muscle weight was calculated by multiplying 0.4 to mouse body weight.

Se speciation analysis using HPLC-ICPMS

An HPLC system was used for sample analysis and consisted of a degasser (DG-158053, Jasco Co., Tokyo, Japan), an HPLC pump (PU-1580i, Jasco Co., Tokyo, Japan) and a sample injector (Model 9725i, Rheodyne Inc., CA, USA). An ICPMS (HP 4500, Agilent Technologies, Tokyo, Japan) was used for the measurement of ^{77}Se and ^{82}Se. A conical concentric nebulizer (AR35-1-FC1, Glass Expansion, West Melbourne, VIC, Australia) was also used.

A size exclusion column (Asahipak GS-520 7G, 7.5 mm internal diameter (i.d.) × 500 mm long, Showa Denko K.K., Tokyo, Japan) with a guard column (Asahipak GS-2G 7B, 7.5 mm i.d. × 50 mm long, Showa Denko K.K., Tokyo, Japan) was used for the separation of hepatic and renal cytosol. A 100 μL aliquot of hepatic and renal cytosol was injected onto this column and 50 mmol L^{-1} Tris-HNO_3 buffer solution (pH 7.4) at a flow rate of 1.0 mL min^{-1} was used for elution.

A size exclusion column (Asahipak GS 320, 7.5 mm i.d. × 300 mm long, Showa Denko K.K., Tokyo, Japan) with a guard column (Asahipak GS-2G 7B, 7.5 mm i.d. × 50 mm long, Showa Denko K.K., Tokyo, Japan) was used for the Se speciation studies of urine and fecal extracts. A 20 μL aliquot of samples was injected onto this column and 50 mmol L^{-1} Tris-HNO_3 buffer solution (pH 7.4) at a flow rate of 0.6 mL min^{-1} was used for elution.

The eluates from the size exclusion column were introduced directly to an ICPMS. The concentrations of Se compounds were determined by one point standard calibration method using analytical standards of cGPx (Sigma-Aldrich, Saint Quentin Fallavier, France), selenite (Nacalai Tesque Inc.,

Kyoto, Japan), 1β-methylseleno-*N*-acetyl-D-galactosamine (selenosugar) (kindly provided by Prof. Kazuo T. Suzuki and Prof. Yasumitsu Ogra) and trimethyl selenonium ion ($TMSe^+$) (Tri Chemical Lab., Yamanashi, Japan) after signals were integrated by ICP-MS ChemStation (B.03.05, Agilent Technologies Inc., Tokyo, Japan).

The concentrations of endogenous and exogenous Se were calculated from m/z counts of ^{77}Se and ^{82}Se as described in a previous study.[10] The relationship 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:

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

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

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

$$\text{Total Se} = \text{Exogenous } ^{82}\text{Se} \times 1.03 + \text{Endogenous } ^{82}\text{Se} \times 11.5$$

To clarify the difference between endogenous and exogenous Se in one figure, endogenous and exogenous ^{82}Se was used in this paper.

Results and discussion

Recovery of exogenous ^{82}Se

We determined Se in 13 different tissues (liver, kidneys, spleen, pancreas, heart, lungs, brain, thymus, submandibular glands, testes, epididymides, seminal vesicles and muscle), 3 kinds of body fluid (plasma, red blood cells and urine) and feces in present and previous studies.[15] With addition of ^{82}Se amounts in muscle, ^{82}Se recoveries were improved from 71 to 77% for Se-adequate mice and from 79 to 86% for Se-deficient mice within 24 h after injection. In this study, we could explain 70 and 62% of the injected ^{82}Se by analysis of liver, kidney, urine and feces of Se-adequate and Se-deficient mice, respectively.

Identification of Se species using HPLC-ICPMS

Two major Se peaks were observed in the hepatic cytosol of mice, at 1 h after ^{82}Se injection, at retention times of 780 s and 1180 s (Fig. S1a, ESI†). The first Se peak at 780 s was the predominant peak at 24 h after injection (Fig. S1b, ESI†) and comparison with standard Se-containing molecules identified it as cGPx (Fig. S1c, ESI†). The second peak at 1180 s, was similarly identified as selenosugar (Fig. S1d, ESI†). The profile of Se-containing molecules identified in renal cytosol was similar to that of liver (data not shown). In addition to the retention time matching, we confirmed each peak by standard addition (Fig. 1). From these results, we integrated signals from 590 to 990 s for cGPx and from 1080 to 1280 s for selenosugar, respectively.

Three peaks at 960 s, 1160 s and 1350 s were observed in the size exclusion chromatogram of mouse urine collected at 0 to 24 h after injection (Fig. S2a, ESI†). These peaks were identified as selenite, selenosugar and $TMSe^+$, respectively, by comparison with the retention times of standards (Fig. S2b, S2c and S2d, ESI†). A similar profile of Se-containing molecules was identified in fecal extracts (data not shown). In addition to the retention time matching, we confirmed each peak by standard addition (Fig. 2). From these results, we integrated signals from 870 to 1110 s for selenite, from 1110 to 1290 s for selenosugar, and from 1290 to 1430 s for $TMSe^+$, respectively.

Two major Se-containing molecules *in vivo* were cGPx and selenosugar. cGPx is one of the major antioxidant proteins in mammals. It is a tetrameric glycoprotein with four identical subunits, each of which contains one selenocysteine residue and has a molecular mass of 22 to 23 kDa.[18,19] An activated Se metabolite, such as glutathione selenopersulfide (GS-SeH), is transferred to the sugar moiety (1β-*N*-acetyl-D-galactosamine) and then it is methylated to form 1β-methylseleno-*N*-acetyl-D-galactosamine (selenosugar).[13,20]

The distribution of Se in cGPx and in selenosugar, as well as the Se amounts in whole tissues, and the extraction efficiency of Se in the cytosol fraction of liver and kidney are summarized in Table 1. Se extraction efficiencies in the hepatic and renal cytosol of Se-deficient mice were lower than those of Se-adequate mice. It is known that cGPx is localized to the cytosol, whereas Sel-P associates with the plasma membrane.[21,22] When Se species in the liver and kidney were extracted, cGPx in the cytosol should be easily extracted, whereas Sel-P in plasma membrane may not be extracted into the cytosol. As a result, the amount of Sel-P in the extract may be under-representative of the total amount of Sel-P. Under conditions of Se-deficiency, cGPx mRNA expression is inhibited, whereas the inhibition of Sel-P mRNA expression is more moderate.[23–25] Therefore, in Se deficiency, the available Se is preferentially associated with Sel-P, which plays an important role in delivering hepatic Se to other tissues.[26] This hypothesis agrees well with the experimental results, which showed a 1.5-fold increase in exogenous ^{82}Se associated with Sel-P in Se-deficient mice than in Se-adequate mice.[15] Therefore, the extracted Se in the cytosol fraction was lower in the case of Se-deficient mice.

Dynamic pathways of Se metabolism in mouse liver and kidney

The amounts of endogenous and exogenous ^{82}Se associated with cGPx and selenosugar in the hepatic cytosols were assayed for mice fed Se-adequate diets (Fig. 3a and b) and Se-deficient diets (Fig. 3c and d). Exogenous ^{82}Se was associated with cGPx and selenosugar in liver extracts of mice within 1 h after ^{82}Se injection and remained associated with cGPx in the liver from 6 to 72 h after injection for both Se nutritional statuses. In the mice fed the Se-adequate diets, the amount of exogenous ^{82}Se associated with cGPx peaked at 1 h after injection and rapidly decreased from 1 to 6 h after injection. In mice fed the Se-deficient diets, the amount of exogenous ^{82}Se associated with cGPx peaked at 1 h and slightly decreased from 1 to 6 h after injection. Under the Se

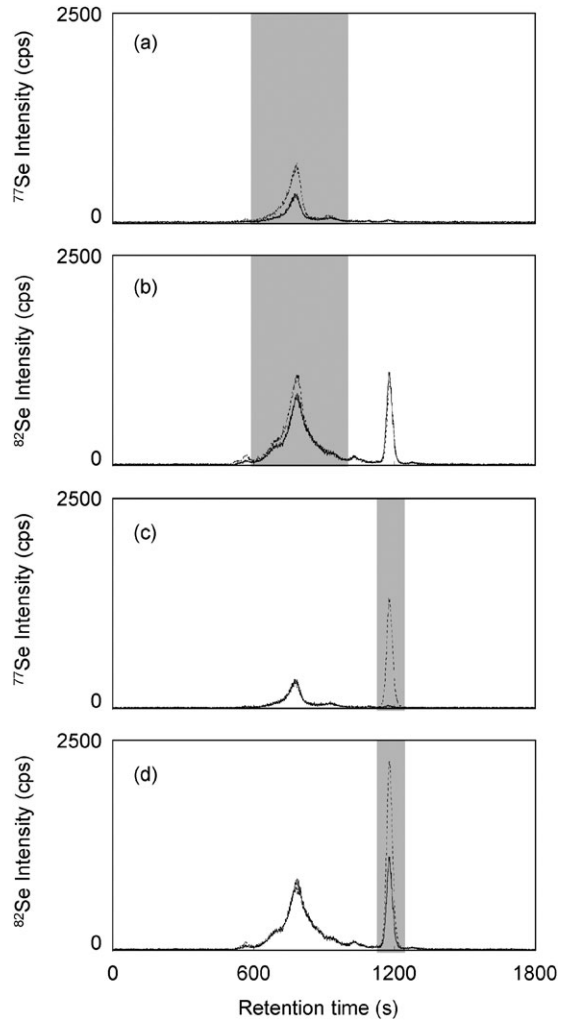

Fig. 1 Size exclusion chromatograms of hepatic cytosol for mice fed Se-adequate diets at 1 h after injection before/after standard addition of cGPx (a, ^{77}Se; b, ^{82}Se) and selenosugar (c, ^{77}Se; d, ^{82}Se). Equal volume of 230 ng Se ml^{-1} cGPx or 500 ng Se ml^{-1} selenosugar was added to hepatic cytosol. The solid and dotted lines indicate chromatograms before and after standard addition, respectively.

deficient status, the amount of exogenous ^{82}Se associated with cGPx was lower than that in mice fed Se-adequate diets at 1 h after injection because of inhibition of cGPx mRNA expression as described before. The amount of exogenous ^{82}Se associated with cGPx remained at a constant level from 6 to 72 h after injection for both nutritional statuses. These data indicated that exogenous ^{82}Se transport to the liver continued for 72 h after injection.

The amounts of endogenous and exogenous ^{82}Se associated with cGPx and selenosugar in the renal cytosols were assayed for mice fed Se-adequate diets (Fig. 4a and b) and Se-deficient diets (Fig. 4c and d). In the kidney, the time-dependent changes in ^{82}Se species were the same as those observed for the liver. The amount of exogenous ^{82}Se associated with cGPx peaked at 1 h after injection, decreased from 1 to 6 h and remained at a constant level from 6 to 72 h after injection for both Se nutritional statuses. It was also shown that decrease of the exogenous ^{82}Se amount associated with cGPx

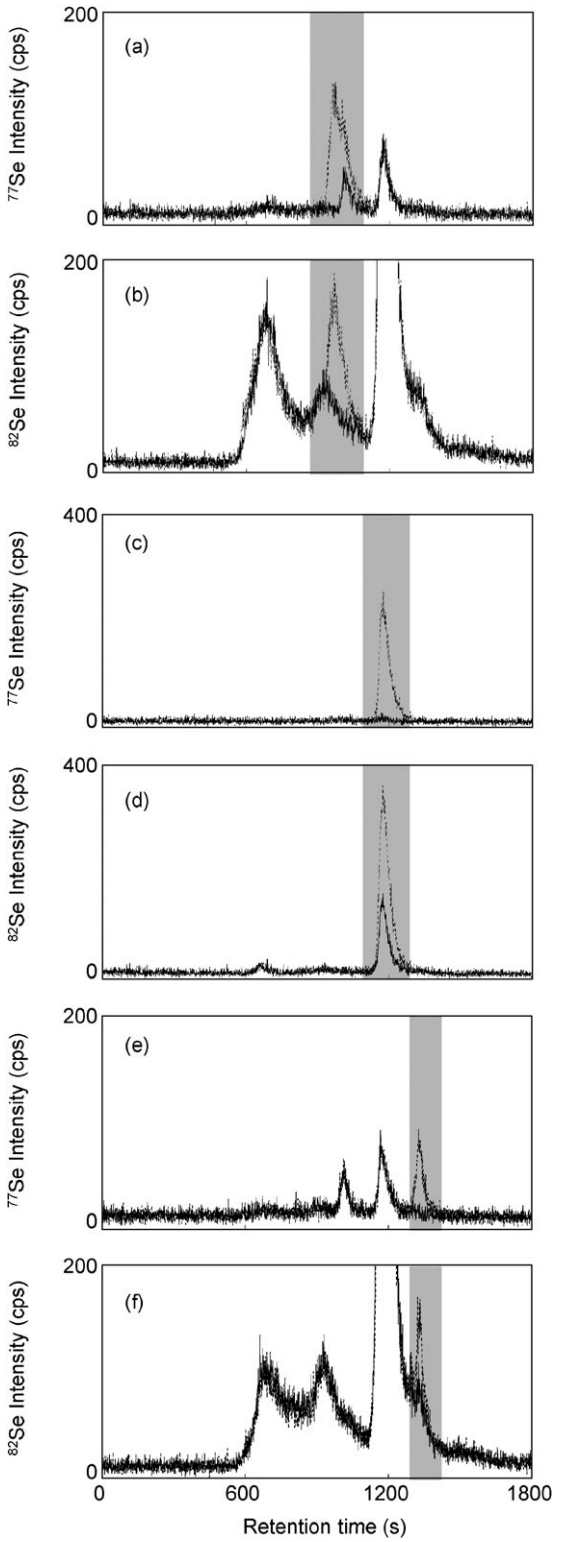

Fig. 2 Size exclusion chromatograms of urine for mice fed Se-adequate diets at 0–24 h after injection before/after standard addition of selenite (a, ^{77}Se; b, ^{82}Se), selenosugar (c, ^{77}Se; d, ^{82}Se) and $TMSe^+$ (e, ^{77}Se; f, ^{82}Se). Equal volumes of 420 ng Se ml^{-1} selenite or 2.5 ng Se ml^{-1} $TMSe^+$ was added to 2 times diluted urine and that of 1900 ng Se ml^{-1} selenosugar was added to 20 times diluted urine. The solid and dotted lines indicate chromatograms before and after standard addition, respectively.

Table 1 Total Se amount associated with cGPx and selenosugar, total Se amount in whole tissue, and extraction efficiency in the liver and kidney for mice fed Se-adequate and -deficient diets

Liver

	Adequate				Deficient				Deficient/Adequate
Time after injection/h	cGPx/ng	Selenosugar/ng	Total Se in whole tissue[a]/ng	Extraction efficiency[b] (%)	cGPx/ng	Selenosugar/ng	Total Se in whole tissue[a]/ng	Extraction efficiency[b] (%)	Decrease ratio of cGPx (%)
0	951	0	1720	55	178	0	560	32	19
1	1310	252	2690	58	383	152	1250	43	29
6	1300	2	1620	80	280	0	750	37	22
24	930	1	1760	53	241	0	690	35	26
72	1160	2	2020	57	224	0	722	31	19
Average				61				36	23

Kidney

	Adequate				Deficient				Deficient/Adequate
Time after injection/h	cGPx/ng	Selenosugar/ng	Total Se in whole tissue[a]/ng	Extraction efficiency[b] (%)	cGPx/ng	Selenosugar/ng	Total Se in whole tissue[a]/ng	Extraction efficiency[b] (%)	Decrease ratio of cGPx (%)
0	134	0	369	36	30	0	182	16	22
1	155	34	395	48	46	18	273	24	30
6	141	2	302	47	39	1	226	17	28
24	144	0	303	48	37	0	261	14	26
72	130	0	366	36	35	0	184	19	27
Average				43				18	26

[a] Total Se amount in the whole tissue were in previous study.[13] [b] Extraction efficiency was calculated as follow: $\frac{\text{cGPx+Selenosugar}}{\text{Total Se in whole tissue}} \times 100$.

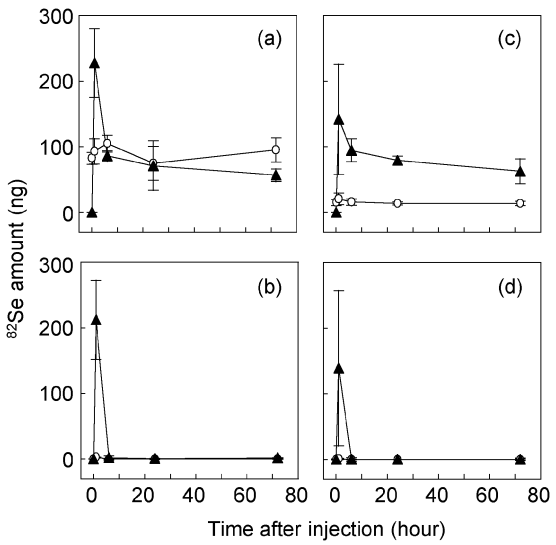

Fig. 3 Time dependent changes of ^{82}Se species in mouse hepatic cytosol: (a) cGPx and (b) selenosugar in mice fed the Se-adequate diets, (c) cGPx and (d) selenosugar in mice fed the Se-deficient diets. Error bars indicate standard deviation. ○, endogenous ^{82}Se; ▲, exogenous ^{82}Se.

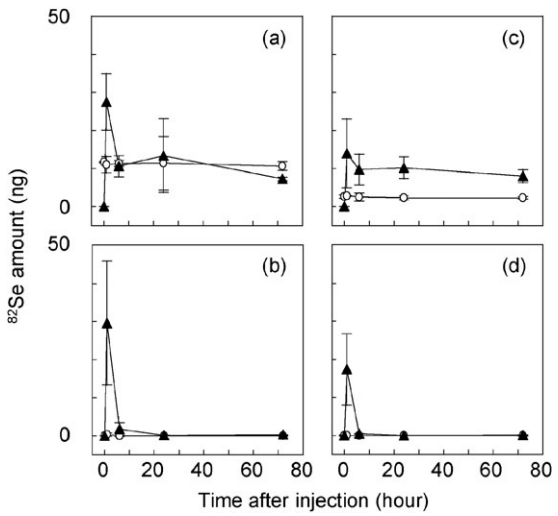

Fig. 4 Time dependent changes of ^{82}Se species in mouse renal cytosol: (a) cGPx and (b) selenosugar in mice fed the Se-adequate diets, (c) cGPx and (d) selenosugar in mice fed the Se-deficient diets. Error bars indicate standard deviation. ○, endogenous ^{82}Se; ▲, exogenous ^{82}Se.

in Se-deficient mice at 1 h after injection. These data indicate that exogenous ^{82}Se was also transported to the kidney during the period from 6 to 72 h after injection for both Se nutritional statuses in a similar fashion to its transport to the liver.

We identified that exogenous ^{82}Se was transported to both liver and kidney up to 72 h after injection. In our previous study, exogenous ^{82}Se was transported bound predominantly to SeAlb in plasma from 1 to 6 h after injection and to Sel-P from 6 to 72 h after injection.[15] The amount of exogenous ^{82}Se associated with Sel-P in the plasma of mice fed Se-deficient diets was 1.5 times higher than that of mice fed Se-adequate diets. The total amount of Se associated with cGPx in liver for the Se-deficient mice was 23% of that for the Se-adequate dietary mice and in kidney it was 26% of that for the Se-adequate dietary mice (Table 1).

It has been observed that Sel-P mRNA expression is elevated during the statuses of Se deficiency[27] and others have reported that Se deficiency induces down-regulation of cGPx mRNA expression and decreased cGPx activity.[23–25,28] Moreover, the recovery of cGPx activity in Se-deficient cells by dietary supplementation with Na_2SeO_3 was only partial in comparison with the Se-adequate cells.[29] In the present study, the total amount of Se associated with cGPx in Se-deficient

mice decreased to 23 and 26% of that in Se-adequate mice for liver and kidney, respectively. Thus, we propose that cGPx mRNA expression is decreased in Se-deficient mice and not recovered by selenite injection.

The amount of exogenous ^{82}Se associated with selenosugar in the hepatic and renal cytosols rapidly increased within 1 h after injection and rapidly decreased within 6 h after injection. It has been reported that selenosugar is produced less efficiently in the kidneys than in the liver.[11] Therefore, rapid production of selenosugar occurs in the liver within 1 h, which is then transported to the kidney and excreted within 6 h after injection. We found greater amounts of selenosugar in the liver and kidney of Se-adequate mice than in the Se-deficient mice. These data are consistent with a greater amount of selenosugar synthesized in liver, which is then transported to kidney for excretion, in the Se-adequate mice than in the Se-deficient mice.

Se excretion to urine and feces

The time dependent changes of endogenous and exogenous ^{82}Se in urine as three species were shown for mice fed Se-adequate diets (Fig. 5a, b and c) and Se-deficient diets (Fig. 5d, e and f). Most of the exogenous ^{82}Se was excreted as selenosugar within 24 h after injection regardless of the Se nutritional statuses and a small amount of exogenous ^{82}Se was excreted as selenite and $TMSe^+$. The amount of exogenous ^{82}Se excreted as selenosugar in the Se-adequate mice was greater than that in the Se-deficient mice.

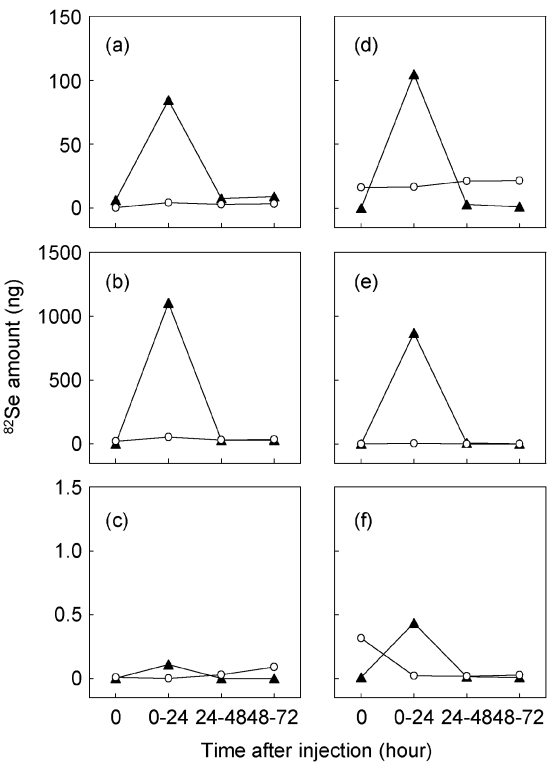

Fig. 5 Time dependent changes of ^{82}Se species in urine: (a) selenite, (b) selenosugar and (c) $TMSe^+$ in mice fed the Se-adequate diets, and (d) selenite, (e) selenosugar and (f) $TMSe^+$ in mice fed the Se-deficient diets. ○, endogenous ^{82}Se; ▲, exogenous ^{82}Se.

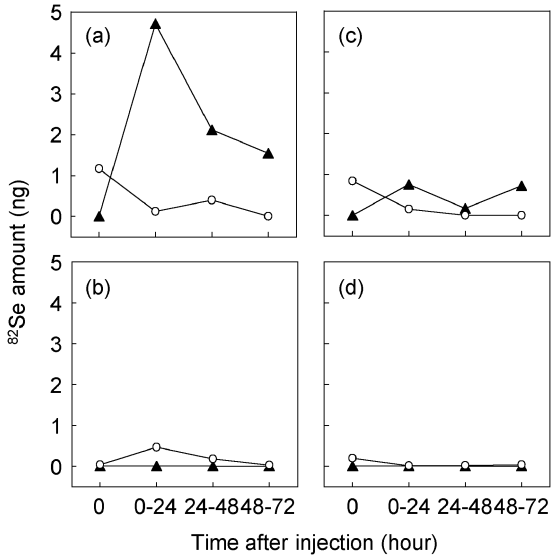

Fig. 6 Time dependent changes of ^{82}Se species in feces extract: (a) selenosugar and (b) $TMSe^+$ in mice fed the Se-adequate diets, (c) selenosugar and (d) $TMSe^+$ in mice fed the Se-deficient diets. ○, endogenous ^{82}Se; ▲, exogenous ^{82}Se.

The time-dependent changes in the excretion of endogenous and exogenous ^{82}Se species in feces were observed for mice fed either Se-adequate diets (Fig. 6a and b) or Se-deficient diets (Fig. 6c and d). Exogenous ^{82}Se was excreted into the feces predominantly as selenosugar and most exogenous ^{82}Se was excreted within 24 h after injection regardless of Se nutritional statuses. Exogenous ^{82}Se in the form of selenite was detected in urine but not in feces, possibly as a result of the excretion of a small fraction of the selenite into urine before reduction by RBC. Regardless of the Se nutritional statuses of the mice, the amount of exogenous ^{82}Se excreted into feces was less than that excreted into urine. The total excretion of exogenous ^{82}Se into urine was greater for Se-adequate mice than for the Se-deficient mice. From these data, it appears that excessive amounts of exogenous ^{82}Se were excreted into both urine and feces more effectively in Se-adequate than in Se-deficient mice.

The amount of exogenous ^{82}Se in the kidney in the form of selenosugar increased within 1 h after injection and rapidly decreased within 6 h after injection in both nutritional statuses (Fig. 4). Therefore, mice excreted most of the exogenous Se into urine in the form of selenosugar within 6 h regardless of their Se nutritional statuses, but the amount of Se excretion was dependent on their Se nutritional statuses.

Conclusions

In this study, 77 and 86% of injected ^{82}Se were recovered for Se-adequate and Se-deficient mice, respectively, 24 h after injection. And 70 and 62% of injected ^{82}Se could be explained for Se-adequate and Se-deficient mice, respectively, by analysis of liver, kidney, urine and feces.

Extraction efficiencies of total Se to hepatic and renal cytosols of Se-deficient mice were decreased to 23 and 26% of those of Se-adequate mice, respectively, due to the suppression of cGPx synthesis.

The amount of exogenous ^{82}Se associated with cGPx in liver and kidney peaked at 1 h after injection, decreased from 1 to 6 h, and remained at a constant level from 6 to 72 h after injection. This indicates that exogenous ^{82}Se transport to the liver and kidney continued for 72 h. The amounts of total Se associated with cGPx were suppressed under Se-deficient conditions and not recovered to the level of Se-adequet conditions by selenite injection.

Regardless of the Se nutritional statuses of the mice, excess amounts of exogenous ^{82}Se were excreted mainly into urine as selenosugar within 6 h after injection. Excess Se was transformed to selenosugar in the liver and transported to the kidney within 1 h after injection. It was then excreted in urine and feces within 6 h after injection. The amounts of Se excretion remained dependent on Se nutritional statuses of the mouse.

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