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Simultaneous tracing of multiple precursors each labeled with a different homo-elemental isotope by speciation analysis: Distribution and metabolism of four parenteral selenium sources*

Kazuo T. Suzuki‡, Chiaki Doi, and Noriyuki Suzuki

Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan

Abstract: The availability, distribution, and metabolism of four typical selenium sources [inorganic selenite and selenate, and organic selenomethionine (SeMet) and methylselenocysteine (MeSeCys)] were compared by administering them simultaneously through a parenteral route. The four selenium sources were each labeled with a different enriched selenium isotope (^{82}Se, ^{78}Se, ^{77}Se, and ^{76}Se, respectively), and administered intravenously at the dose of 25 μg Se/kg body weight each to rats that had been depleted of natural abundance selenium with a single isotope, ^{80}Se, by feeding ^{80}Se-selenite in drinking water and a selenium-deficient diet. At 1 h post-injection, the amounts of the four tracers recovered from major organs and blood comprised around 70, 55, and 50 % of the doses for selenite, MeSeCys and SeMet, and selenate, respectively, being most abundant in the liver. The intact precursors, except for selenite, were recovered from all organs. ^{77}Se and ^{76}Se of SeMet and MeSeCys origin, respectively, were much more efficiently recovered from the pancreas than selenite and selenate, in forms mostly bound to proteins together with intact forms, suggesting that SeMet and MeSeCys are preferentially distributed directly to the pancreas. The incorporations of selenium into selenoprotein P (Sel P) and selenosugars were most efficient from selenite and less efficient from SeMet, suggesting that selenite was most efficiently utilized for the syntheses of selenoproteins and selenosugars. Although selenate was partly excreted into the urine in its intact form, it was retained longer in the plasma in its intact form than the other selenium sources. The advantage of simultaneous administration of multiple precursors each labeled with a different enriched isotope to depleted hosts followed by simultaneous tracing of the labeled isotopes over the conventional method with a single tracer is emphasized together with cautions that may occur with the new multiple tracer method.

Keywords: metabolomics; metallomics; methylselenocysteine; parenteral nutrition; selenate; selenite; selenium; selenomethionine; selenosugar; speciation; stable isotopes; trimethylselenonium.

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‡Corresponding author: E-mail: ktsuzuki@p.chiba-u.ac.jp

INTRODUCTION

Inorganic selenium (selenite and selenate) and organic selenoamino acids [selenomethionine (SeMet) and Se-methylselenocysteine (MeSeCys)] are readily available and chemically stable nutritional and supplemental selenium sources [1–3]. At the same time, these typical selenocompounds can be used as selenium sources for parenteral alimentation [4,5]. Therefore, the relative availability, distribution, metabolism, and excretion of these selenocompounds administered orally and parenterally into the body have to be assessed, and their characteristics have to be determined for their safe application.

We developed a new tracer method that can be applied effectively to compare the availability and metabolism of multiple selenium sources simultaneously using multiple enriched stable isotopes as tracers [6,7]. Namely, multiple selenium sources are each labeled with a different enriched stable isotope, and then the cocktail of the labeled precursors (selenium sources) is administered to host animals that have been depleted of natural abundance selenium with single enriched selenium. This new tracer method makes it possible to trace and compare multiple precursors simultaneously using exactly identical host animals under the same sampling and tracer detection conditions. With this new tracer method, metabolic differences between multiple inorganic and organic selenium sources were compared simultaneously, revealing precise characteristics of different selenium sources under the same experimental conditions within a single experiment [8–10]. However, so far this tracer method has only been applied for oral treatment to compare the differences in the availability and distribution among organs, and the excretion metabolites of nutritional sources of selenium, other administration routes not having been examined yet.

Parenteral nutrition is an alternative route for the supply of nutritional sources for patients having restrictions as to oral administration [11], and this administration route may differ from oral administration in availability, distribution, metabolism, and excretion. In addition, different selenium sources have to be compared to recommend a better selenium source for parenteral use. In the present study, we administered four selenium sources each labeled with a different labeled selenium isotope simultaneously through a parenteral (intravenous) route for the first time; inorganic (selenite and selenate) and organic selenium sources (MeSeCys and SeMet). Host rats that had been depleted of natural abundance selenium with a ^{80}Se-enriched isotope by feeding ^{80}Se-selenite were administered the same doses of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite simultaneously, and the body fluids (blood and urine) and organs/tissues (liver, kidneys, lungs, heart, brain, spleen, pancreas, and muscles) were obtained at various times after the administration, and the concentrations and chemical species of each labeled isotope were determined simultaneously by inductively coupled argon plasma mass spectrometry (ICP-MS) and high-performance liquid chromatography (HPLC)-ICP-MS, respectively [12,13].

In our previous experiments involving this new tracer method, we observed that the pancreas preferentially took up SeMet and also MeSeCys compared with selenite, selenate, and methylseleninic and methylselenonic acids through the oral route, and the labeled selenium was bound to proteins in the pancreas [8–10]. In the present study, we also intended to determine whether selenium of both SeMet and MeSeCys origin could be taken up by the pancreas and bound to proteins on intravenous administration. In particular, we have been interested in detecting a response at an early time after administration. This is because it is not clear whether pancreatic proteins labeled with selenium are gene products (synthesized de novo through selenide) or enzyme products (SeMet and MeSeCys residues or their metabolites bound to specific proteins).

One of the inorganic selenium forms, selenite, has been proposed to be taken up selectively and rapidly by red blood cells (RBCs) through the band 3 protein on the RBC membrane, reduced to selenide in the RBCs, and then excreted into the bloodstream with the assistance (presence) of albumin [14–18]. The selenide bound to albumin is considered to be transferred to and taken up by the liver, where selenide is most efficiently incorporated into selenoprotein P (Sel P). Even if selenite is administered directly into the bloodstream as in the case of intravenous injection, selenite is not excreted in

its intact form into the urine. Contrary to selenite, selenate is partly excreted directly into the urine in its intact form when it is administered into the bloodstream [19]. Selenate administered orally to rats is also partly excreted into the urine in its intact form. Nevertheless, oral selenate is incorporated into Sel P with comparable or better efficiency than other selenium sources [9]. Thus, direct comparison of the availability, distribution, metabolism, and excretion of four typical selenochemicals (selenium sources) is required to select a better selenium source for parenteral nutrition.

Furthermore, although the mechanism underlying the selective accumulation and excretion of SeMet in pancreas is not known, it was suggested to be applicable as a clinical test for pancreas [20,21]. We have been interested in revealing why and how selenium of SeMet and MeSeCys origin is preferentially taken up by the pancreas [22–25]. In the present study, we intended to determine whether selenium in both selenoamino acids is taken up by the pancreas preferentially compared to inorganic sources, and also to compare the availability of the two selenoamino acids in the pancreas.

MATERIALS AND METHODS

Chemicals

Metallic forms of ^{76}Se (99.8 % enriched), ^{77}Se (99.9), ^{78}Se (99.9), ^{80}Se (99.5), and ^{82}Se (98.9) were purchased from Isoflex USA, San Francisco, USA. Other organic and inorganic chemicals of the highest grade were purchased from Wako Pure Chemicals, Tokyo, Japan, and used without further purification.

Preparation of ^{76}Se-methylselenocysteine (MeSeCys), ^{77}Se-selenomethionine (SeMet), ^{78}Se-selenate, and ^{80}Se- and ^{82}Se-selenite

^{76}Se-MeSeCys was prepared by reacting metallic ^{76}Se-selenium powder with CH_3Li, followed by reacting the resulting $CH_3{}^{76}SeLi$ with 3-chloro-L-alanine hydrochloride, as described elsewhere [10]. ^{77}Se-SeMet was prepared by reacting $CH_3{}^{77}SeLi$ with α-amino-γ-bromobutyric acid hydrobromide, which was prepared from (*S*)-(–)-α-amino-γ-butyrolactone hydrochloride, as described elsewhere [7,26]. ^{80}Se- and ^{82}Se-selenite were prepared by dissolving the metallic forms (^{80}Se and ^{82}Se, respectively) in concentrated nitric acid, followed by adjustment to neutral pH with 0.1 M NaOH [15]. ^{78}Se-selenate was prepared by dissolving the metallic form (^{78}Se) in concentrated nitric acid in the presence of hydrogen peroxide, followed by adjustment to neutral pH with 0.1 M NaOH [15].

Experimental animals and animal experiments

All animal experiments were carried out according to the "Principles of Laboratory Animal Care" (NIH version, revised in 1996) and the Guidelines of the Animal Investigation Committee, Graduate School of Pharmaceutical Sciences, Chiba University, Japan.

Pregnant Wistar rats (gestation day 2), 12 weeks of age, were purchased from Clea Japan Inc., Tokyo, Japan, and maintained individually in plastic cages at 22 ± 2 °C, with a light/dark cycle of 12/12 h, and fed a selenium-deficient diet (Oriental Yeast Co., Ltd.; Se <0.02 μg/g diet), and drinking water comprising ^{80}Se-selenite at a concentration of 1.0 μg Se/mL in distilled water ad libitum until weaning. The weanling rats were also fed the same selenium-deficient diet and drinking water containing ^{80}Se-selenite until 16 weeks of age, and the rats were fed the selenium-deficient diet and selenium free-distilled water for 2 weeks.

^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite were dissolved in saline at 25.0 μg Se/mL each. The mixed solution, 25.0 μg Se/kg body weight for each labeled selenium source, was administered intravenously to rats that had been depleted of endogenous natural abundance selenium with ^{80}Se. Three rats/group were sacrificed at 1/6, 1/2, 1, 6, and 24 h after the administration. Three rats not

administered the mixed solution were used as controls (time point 0). Blood (heparinized for plasma and non-heparinized for serum) was obtained under light ether anesthesia, the heparinized and the clotted blood being centrifuged at 1600 *g* for 10 min to obtain plasma and serum, respectively. Three additional rats were housed individually in metabolic cages to collect urine at three time points, 3 (0–3 h), 6 (3–6 h), and 24 h (6–24 h), after the injection. Organs/tissues were excised after whole body perfusion, and portions of them were ashed with concentrated HNO_3/H_2O_2 (v/v = 1) and then subjected to quantification of selenium by ICP-MS. Portions of livers, kidneys, and pancreases were homogenized with a Polytron homogenizer (Kinematica, Switzerland) in 4 volumes of a 50 mM Tris–HCl buffer solution, pH 7.4, under an atmosphere of nitrogen, and then centrifuged at 105 000 *g* for 60 min to obtain supernatant fractions. Equal volumes of the supernatants from three rats in each group were combined for HPLC-ICP-MS analysis.

ICP-MS with an octopole reaction system

An Agilent 7500cs ICP-MS (Yokogawa Analytical Systems, Hachioji, Japan) equipped with an octopole reaction system (ORS) was used with a D_2 gas flow of 2.0 mL/min [27]. The ICP-MS was operated independently or coupled to an HPLC system as a detector.

HPLC

The HPLC system consisted of an on-line degasser, an HPLC pump (PU713; GL Science Co., Ltd., Tokyo), a Rheodyne six-port injector with 20- and 200-μL sample loops, and a column. Gel filtration columns, Shodex Asahipak GS520 7G (7.5 i.d. × 500 mm, with a guard column, 7.5 i.d. × 75 mm, Showa Denko, Tokyo) for the separation of serum, and liver, kidney, and pancreas supernatants, and GS320 HQ (7.5 i.d. × 300 mm, with a guard column, 7.5 i.d. × 75 mm, Showa Denko) for the separation of urine, were used. The former was injected with 200 μL aliquots of serum and supernatants, and then eluted with 50 mM Tris–HCl, pH 7.4, at a flow rate of 1.0 mL/min, whereas the latter was injected with a 20 μL aliquot of urine and eluted with 50 mM ammonium acetate, pH 6.8, at a flow rate of 0.6 mL/min. The eluate was introduced directly into the Babington nebulizer of the ICP-MS.

RESULTS

Figure 1 shows the changes in the recovery of the four labeled selenium isotopes summed up for major organs and blood without that in the urine at each time point after simultaneous administration of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite. The recoveries of the labeled selenium isotopes originating from the selenoamino acids, ^{76}Se-MeSeCys (A) and ^{77}Se-SeMet (B), were similar to each other, being maximal at 1 h after the injection. On the other hand, although those of the inorganic ones, ^{78}Se-selenate (C) and ^{82}Se-selenite (D), showed similar patterns but with different recovery rates at each time point, that of selenate being lower than that of selenite. The four panels suggest that a selenium isotope of selenite origin can be most efficiently recovered from major organs/body fluids, and one of selenate origin is less efficiently recovered. The lower recovery for selenate was expected from the direct excretion into the urine (19), while the higher recovery of selenite at an early time after intravenous injection was expected from the efficient uptake of selenite into RBCs [15,16].

Although the precise reason for the low recovery (not reaching 100 % of the dose) at an early time after the administration is not known, it may be due to uneven distributions of the selenium sources within big organs/tissues such as muscle (50 % of the weight being assumed), and thus the concentrations do not represent the whole organs/tissues. In fact, although the concentration of each labeled selenium isotope in muscle was low, the whole content in each tissue (μg/whole tissue) was high but with large deviation. It is also not known why the labeled selenium was not detectable at a comparable level as to the dose at an early time after injection, or why the labeled selenium that disappeared from the

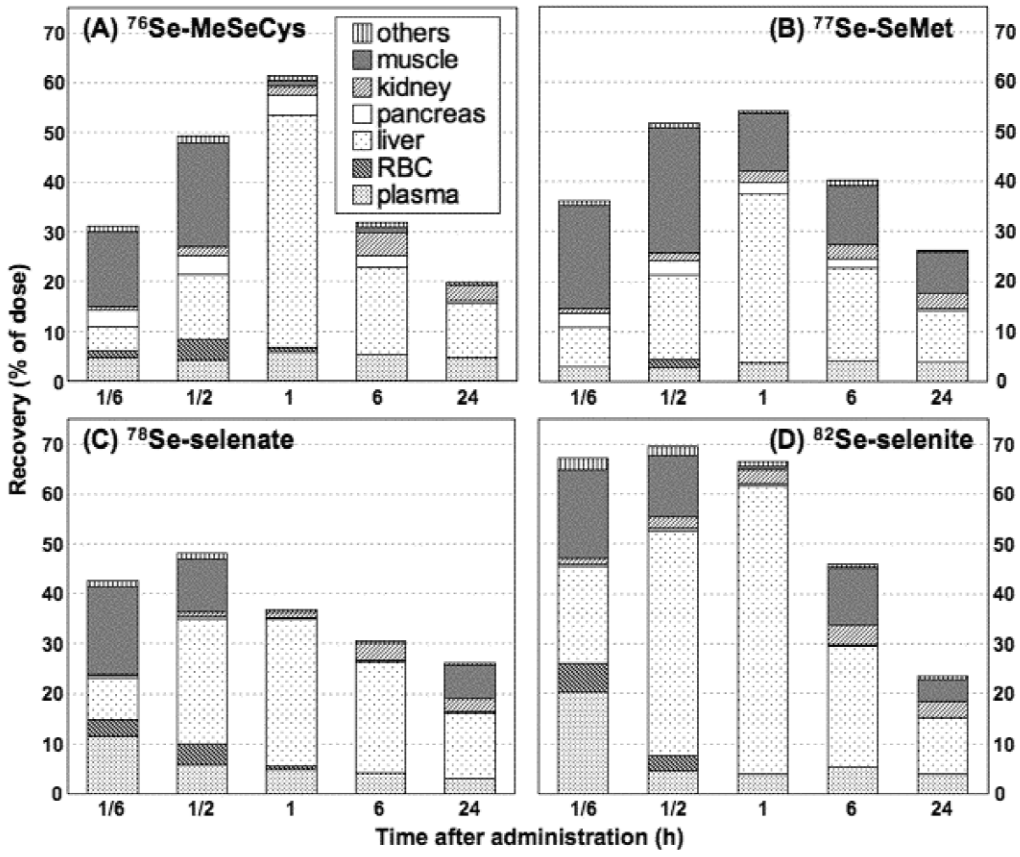

Fig. 1 Time-related changes in the recovery of four labeled selenium isotopes in blood and organs/tissues. ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite were administered simultaneously to rats that had been depleted of natural abundance selenium with ^{80}Se-enriched selenium at the dose of 25 μg Se/kg body weight each. The rats (n = 3) were exsanguinated 1/6, 1/2, 1, 6, and 24 h later, and their hearts, brains, lungs, spleens, kidneys, pancreases, and livers, and blood (RBCs, plasma, and serum) were obtained. The mean concentrations of each labeled selenium isotope in organ and body fluid samples were summed up at each time point.

bloodstream was redistributed to the major organ liver with time, the maximum distribution being reached at .5–1 h after the injection for all four selenium sources. Thus, it is not known where the labeled selenium sources are distributed immediately after the injection.

Figure 2 shows the changes in the distributions of the four labeled selenium isotopes in the serum with time after the simultaneous injection of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite. The ^{80}Se and ^{74}Se profiles represent the distributions of the main endogenous and residual selenium isotopes, respectively, after the depletion. The ^{80}Se profiles show the distinct peaks corresponding to extracellular glutathione peroxidase (eGPx) at the retention time of 11.2 min and Sel P at 13.5 min. On the other hand, these two peaks were not detectable in the ^{74}Se profile because of the depletion of natural abundance selenium. However, a distinct and constant peak was detected throughout the observation time at 20.6 min in the ^{74}Se profile which was assigned as contaminating ^{74}Ge from the plasticware used in the experiment, as also supported by the corresponding ^{76}Ge peak according to the natural abundance ratio of germanium [9].

In the ^{82}Se profiles in Fig. 2, the eGPx and Sel P peaks detected were much bigger compared with the natural abundance ratio, as typically represented in the control panel, where the ^{82}Se peaks of eGPx and Sel P should be smaller than the ^{78}Se peaks, reflecting the natural abundance ratio (23.77 % for

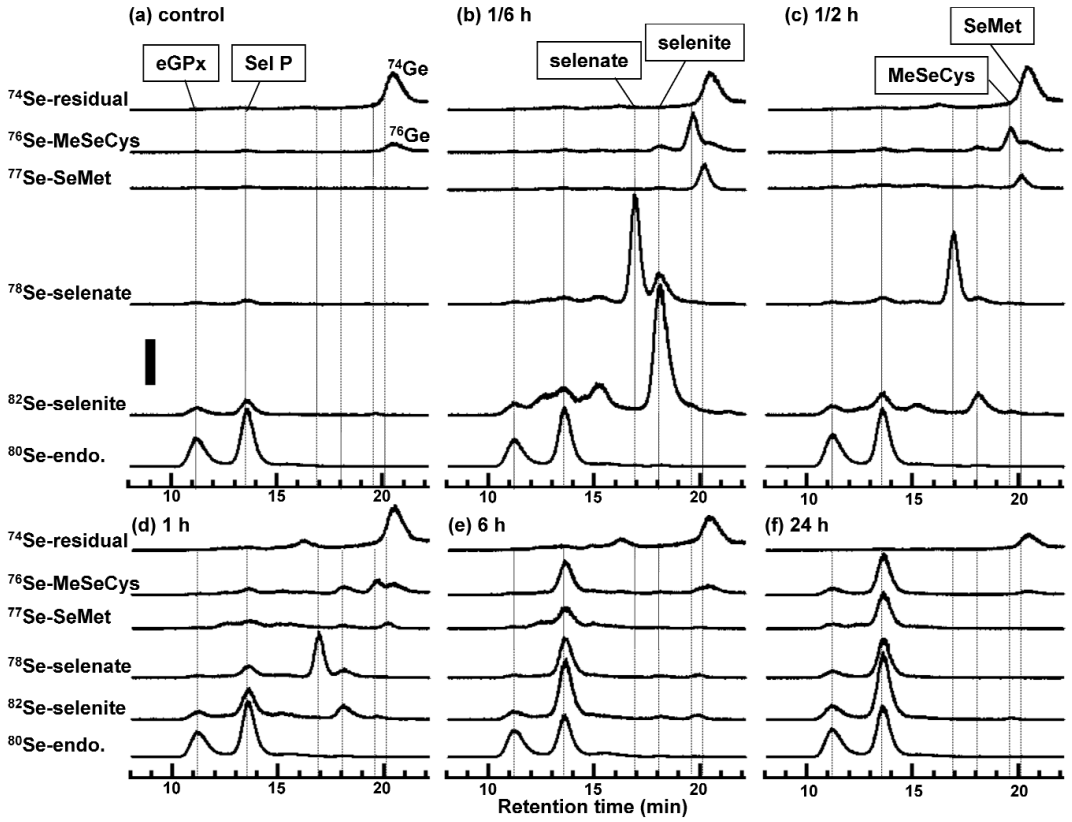

Fig. 2 Time-related changes in the distributions of four labeled selenium isotopes in serum. The serum obtained from blood of rats at five time points after intravenous administration of the four selenium sources each labeled with a different enriched stable isotope was subjected to HPLC-ICP-MS analysis on an Asahipak GS520 7G gel filtration column. The vertical bar shows the detection level of each labeled selenium isotope (20 ng/mL) except for ^{80}Se (120 ng/mL). The ^{74}Se-profile shows the distribution of ^{74}Se selenium remaining after the present depletion conditions, while the ^{80}Se-profile shows the distribution of the endogenous selenium.

^{78}Se, 8.73 % for ^{82}Se). This increased size of the two peaks is mainly due to the $^{80}SeD^+$ peak in addition to the residual ^{82}Se peak with the use of deuterium collision gas [27]. Thus, when major endogenous ^{80}Se peaks such as those of eGPx and Sel P in serum are present, they are reflected in the corresponding ^{82}Se peaks due to the occurrence of $^{80}SeD^+$ peaks in the D_2 collision mode.

A distinct ^{76}Se peak of ^{76}Se-MeSeCys origin at 19.6 min was detected at 10 min after the injection and decreased with time, which was assigned as intact MeSeCys, suggesting a rapid disappearance from the plasma (Fig. 2). The Sel P peak at 13.5 min started to increase slowly after 30 min, and appeared more distinct after 6 h, while the eGPx peak at 11.2 min increased much more slowly and was marginally detected after 24 h, reflecting the rapid synthesis of Sel P [9,10].

Similar to MeSeCys, a distinct but smaller ^{77}Se peak of ^{77}Se-SeMet origin was detected at 20.1 min at 1 h after the injection, which then decreased with time (Fig. 2). Although small peaks such as those at 12.7 and 15.3 min were detected in the ^{82}Se profiles, they could not be assigned in the present study.

In the ^{78}Se profiles in Fig. 2, a distinct selenate peak was detected at 16.9 min at 1 h after the injection, which decreased with time like the intact selenate peak together with the reduced selenite peak at 18.1 min. The Sel P peak at 13.5 min increased with time after 30 min.

The labeled selenium recovered in the urine amounted up to 12–13 % of the doses for all labeled isotopes by 24 h except for SeMet, the amount of which recovered amounted to only 5–6 % of the dose (data not shown). Figure 3 demonstrates the distributions of the four labeled selenium isotopes in the urine. In the control profile, no selenium isotope peaks were detected, indicating that the rats were not fed sufficient selenium after the depletion. In the first 3-h urine (collected 0–3 h after the injection), a distinct selenate peak at 15.1 min was detected in the ^{78}Se profile. A small but distinct selenosugar peak was detected at 19.8 min in the four labeled isotope profiles and also in the endogenous ^{80}Se profile; in decreasing order of the intensity, ^{82}Se > ^{76}Se = ^{78}Se > ^{77}Se. These results suggest that selenate but not selenite was excreted in its intact form into the urine shortly after the injection, and SeMet and MeSeCys were not excreted in their intact forms into the urine. The major excretion metabolite was selenosugars for all four selenium sources, and trimethylselenonium (TMSe) was detected in the MeSeCys profile [9,10].

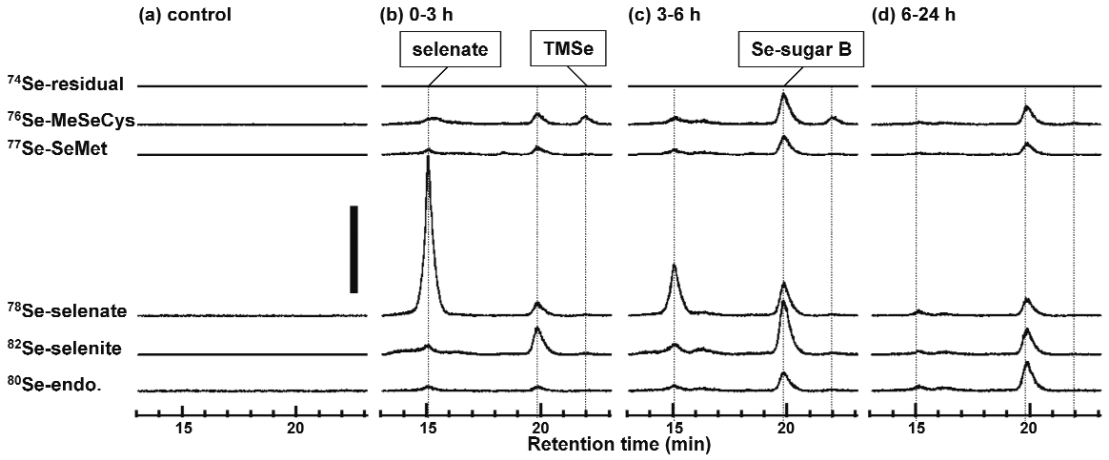

Fig. 3 Time-related changes in the distributions of four labeled selenium isotopes in urine. The urine collected for 3 (0–3 and 3–6 h) and 18 h (6–24 h) from the same three rats was combined in each time point group, and subjected to HPLC-ICP-MS analysis on an Asahipak GS320 HQ gel filtration column. The vertical bar shows the detection level of each selenium isotope (500 ng/mL).

Figure 4 shows the time-related changes in the distributions of the four labeled selenium isotopes in the liver supernatants. In the control profile, a broad but distinct peak was detected at 13.5 min for the endogenous ^{80}Se together with ^{78}Se and ^{82}Se peaks, the ^{82}Se peak appearing bigger than the ^{78}Se peak, reflecting the $^{80}SeD^+$ peak detected in the a D_2 collision mode in the presence of a big ^{80}Se peak [9]. The big peak at 13.5 min was tentatively assigned as cellular GPx (cGPx) [8–10]. A selenosugar A peak (17.7 min) followed by a selenosugar B peak (19.8 min) was detected for all four labeled isotopes, the former peak being eluted overlapping with glutathione-conjugated selenide [8], and the latter peak being eluted closely with SeMet (eluted slightly faster than selenosugar B) and MeSeCys (eluted slightly slower than selenosugar B), which was confirmed on a reversed phase column, as shown in Fig. 7. The complex peaks appearing between cGPx and selenosugar A were not examined further in the present study. The distribution profiles of the four labeled isotopes in Fig. 4 suggest that most of the ^{77}Se peaks of SeMet origin were smaller than those of the other three isotope peaks, reflecting the lower incorporation of selenium of SeMet origin into selenoenzymes and selenosugars than that of selenium of other source origins in the liver. TMSe was not detected even in the profile of ^{76}Se of MeSeCys origin in the liver (data not shown).

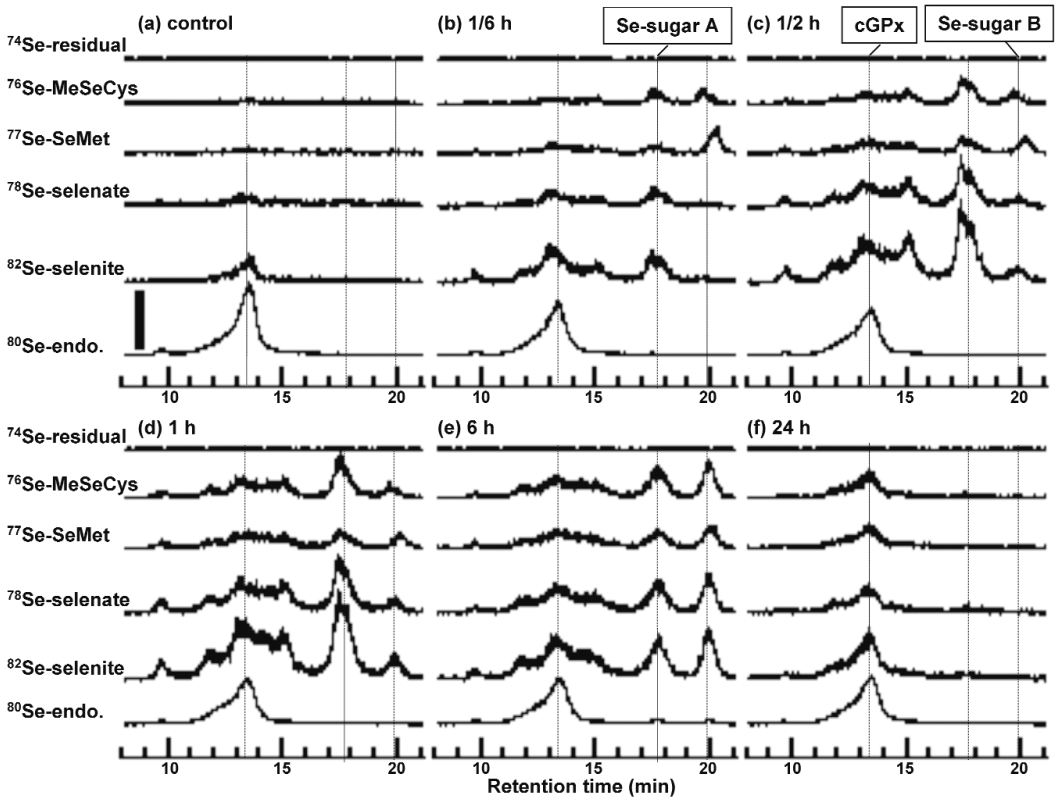

Fig. 4 Time-related changes in the distributions of four labeled selenium isotopes in liver supernatants. Livers were obtained from three rats sacrificed at five time points after the administration of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite, as described in the legend to Fig. 1, and then 20 % supernatants (w/v) were subjected to HPLC-ICP-MS analysis on an Asahipak GS520 7G gel filtration column. The vertical bar shows the detection level of each selenium isotope (10 ng/mL) except for endogenous ^{80}Se (100 ng/mL).

Figure 5 demonstrates the time-related changes in the distributions of the four labeled selenium isotopes in the kidney supernatants. A similar relation to that in the liver supernatants was observed as to the cGPx peaks in the ^{82}Se and ^{78}Se profiles in the controls (Fig. 5a) reflecting $^{80}SeD^{+}$. Differently from in the liver supernatants in Fig. 4, although the selenosugar B peak (MeSeCys/selenosugar B/SeMet peaks) was distinct, the selenosugar A one (selenosugar A/GSH-conjugated selenide) was not evident. The selenosugar B peak was confirmed on a reversed-phase column, and the ^{76}Se and ^{77}Se peaks were mostly of MeSeCys and SeMet origin, respectively, indicating that selenoamino acids were more efficiently taken up by the kidneys than by the liver. TMSe was detected at 21.8 min only in the profile of ^{76}Se of MeSeCys origin. Selenate seems to be incorporated less efficiently into seleno-enzymes and selenosugars because of direct excretion into the urine in its intact form.

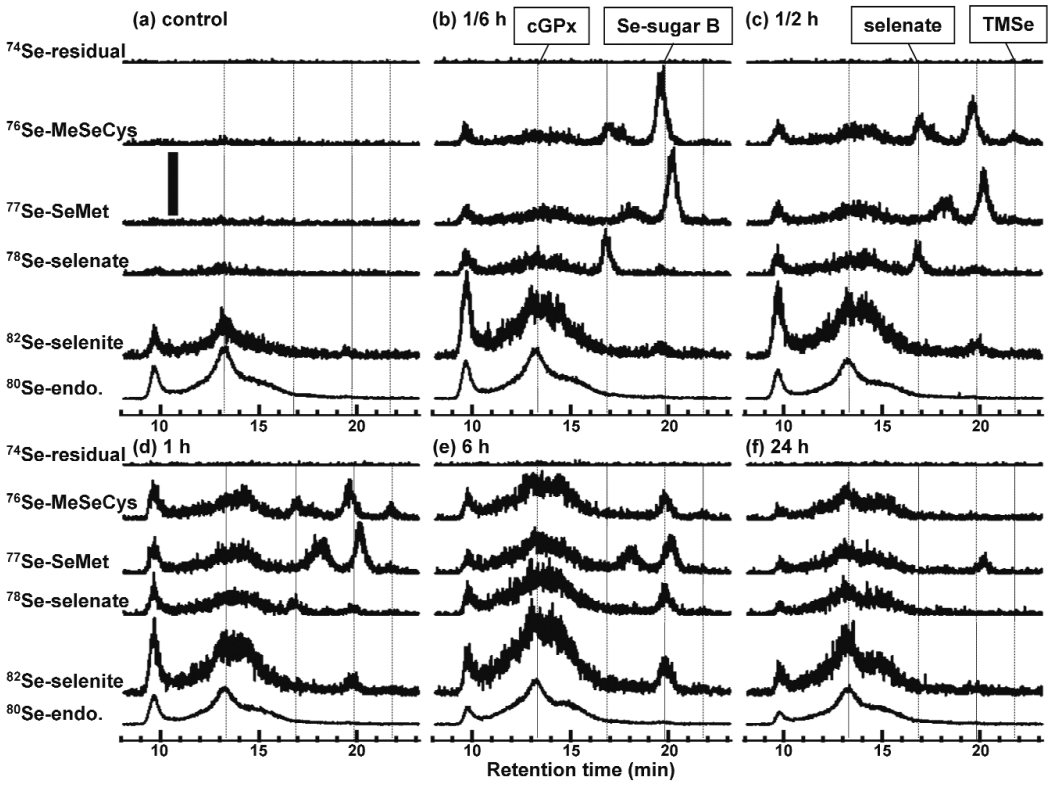

Fig. 5 Time-related changes in the distributions of four labeled selenium isotopes in kidney supernatants. Kidneys were obtained from three rats sacrificed at five time points after the administration of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite, as described in the legend to Fig. 1, and then 20 % supernatants (w/v) were subjected to HPLC-ICP-MS analysis on an Asahipak GS520 7G gel filtration column. The vertical bar shows the detection level of each selenium isotope (2 ng/mL) except for endogenous ^{80}Se (38 ng/mL).

Figure 6 shows the time-related changes in the distributions of the four labeled selenium isotopes in the pancreas supernatants. MeSeCys and SeMet were taken up efficiently by the pancreas in their intact forms. However, selenosugar A was not detected and selenosugar B was only marginally detected in the pancreas supernatants. Instead, differently from in the liver and kidneys, ^{76}Se of MeSeCys and ^{77}Se of SeMet origin were taken up much more efficiently than selenite and selenate, and they were sequestered by proteins (14.0 min) that were eluted at a slightly slower retention time than that of pre-existing proteins (corresponding to cGPx at 13.5 min) immediately after the injection (by 10 min). The incorporation of the two selenoamino acids into proteins was much more rapid than that into Sel P in the liver, kidneys, and pancreas in Figs. 4–6, respectively. It is not known yet whether ^{76}Se of MeSeCys and ^{77}Se of SeMet were incorporated into the new selenium-binding proteins in their intact forms (MeSeCys and SeMet residues) or through selenide (as a gene product). However, it was revealed that MeSeCys and SeMet origin were efficiently taken up by the pancreas and incorporated into the new selenium-binding proteins irrespective of routes of administration (per oral or parenteral). On the other hand, inorganic selenium was not or much less efficiently taken up by the pancreas than by the liver and kidneys. The results as to the uptake and distribution of selenium sources in the pancreas suggest that selenium sources, especially selenoamino acids, and also inorganic selenium are handled differently from in other organs/tissues.

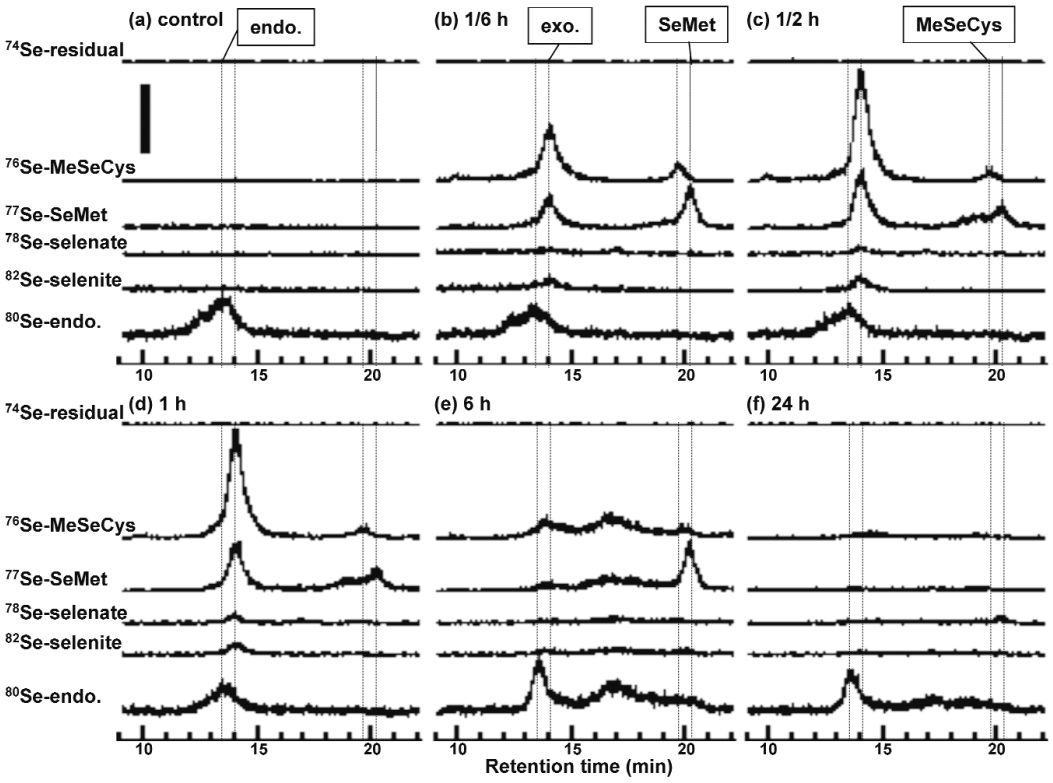

Fig. 6 Time-related changes in the distributions of four labeled selenium isotopes in pancreas supernatants. Pancreases were obtained from three rats sacrificed at five time points after the administration of ^{76}Se-MeSeCys, ^{77}Se-SeMet, ^{78}Se-selenate, and ^{82}Se-selenite, as described in the legend to Fig. 1, and then 20 % supernatants (w/v) were subjected to HPLC-ICP-MS analysis on an Asahipak GS520 7G gel filtration column. The vertical bar shows the detection level of each selenium isotope (5 ng/mL).

Figure 7 shows the time-related changes in the distributions of the four labeled selenium isotopes in the soluble fractions of heat-denatured liver (Fig. 7a), kidneys (7b), and pancreas (7c) supernatants on a reversed phase column. These profiles confirm that ^{76}Se of MeSeCys and ^{77}Se of SeMet origin are present in the liver, kidneys, and pancreas in response to these selenium sources with different patterns depending on the organs. Namely, both selenoamino acids taken up by the liver decreased with time, and instead selenosugar B increased, SeMet being taken up more than MeSeCys, but selenosugar B being produced more from SeMet (Fig. 7a). Although both selenoamino acids also decreased with time in the kidneys, selenosugar B was detected less than in the liver (Fig. 7b). In the pancreas, SeMet was taken up more than MeSeCys, and little selenosugar B was produced from any selenium sources. Thus, it was confirmed that both selenoamino acids were distributed to all organs/tissues in their intact forms. It was also confirmed that selenosugar B was detected less in the kidneys and pancreas than in the liver, suggesting that the selenosugar is produced more in the liver.

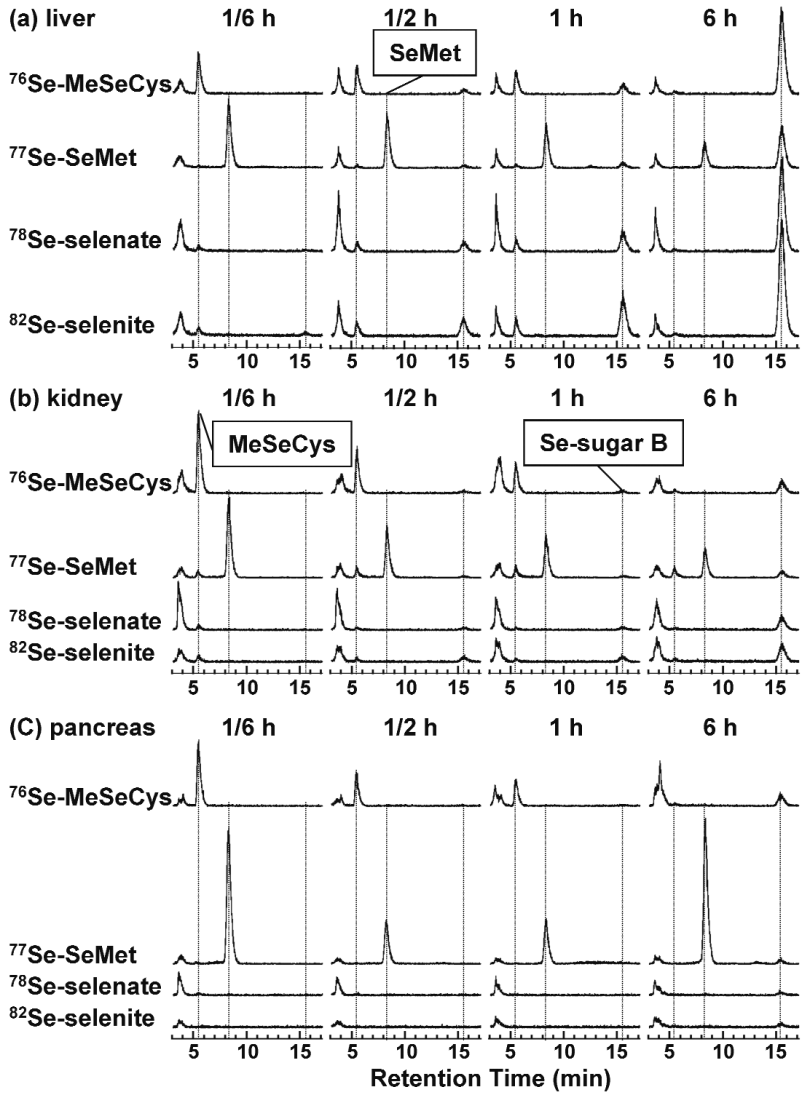

Fig. 7 Distributions of selenosugars, SeMet, and MeSeCys in liver, kidney, and pancreas supernatants on a reversed-phase Inertsil ODS-3 column. The liver, kidney, and pancreas supernatants at time points 3, 6, and 9 h in Figs. 4–6 were heat-treated at 97 °C for 3 min to remove heat-denatured proteins, and then the heat-stable soluble fractions were subjected to HPLC-ICP-MS analysis on an Inertsil ODS-3 column (4.6 × 250 mm; GL Sciences Inc., Tokyo) with elution with 1 % methanol (injection volume, 200 μL; flow rate, 0.8 mL/min; column oven, 40 °C).

DISCUSSION

The availability, distribution, and metabolism of four typical selenium sources (selenite, selenate, MeSeCys, and SeMet) were compared by administering them each labeled with a different enriched selenium isotope simultaneously into the same animals that had been depleted of natural abundance selenium with a single isotope (^{80}Se), followed by determination of their concentrations by ICP-MS and speciation analysis by HPLC-ICP-MS. Thus, the four selenium sources were compared in a single experiment using the same animals, and the same sample preparation and analytical conditions.

The dose applied in the present experiment was 25 μg Se/kg body weight for each selenium source, which seems to be high as a dose for a parenteral route, i.e., as a single intravenous injection. The rats used as the present hosts were depleted of endogenous natural abundance selenium with a single enriched selenium isotope, and four precursors each labeled with a different enriched stable isotope were injected simultaneously. As a result, the four labeled selenium sources could be detected approximately 10-fold more sensitively than on detection with the natural abundance forms, and could be detected as absolute rather than relative labels. However, the detection sensitivity for selenium may not be sufficient for a speciation study. Therefore, a relatively high dose of each precursor was applied at the same dose for each as in the case of oral administration [8–10]. As TMSe was not detected in the urine except in the distribution profile of selenium of MeSeCys origin, this dose seems to be within the capacity to regulate selenium through normal handling, i.e., without excretion of TMSe due to the excess dose [28,29].

As the present animals were depleted of natural abundance selenium with a single enriched isotope, the four selenium isotopes used as tracers for the four selenium sources were each utilized as an absolute label rather than a relative label. The present method can be explained as follows; simultaneous administration of multiple precursors (selenium sources) each labeled with a different enriched stable isotope into animals that had been depleted of endogenous natural abundance selenium with a single enriched stable isotope, followed by tracing of the labeled multiple isotopes simultaneously with ICP-MS and HPLC-ICP-MS. This new tracer method makes it possible to compare the availability, distribution, and metabolism of multiple precursors (selenium sources) simultaneously in a single experiment using the same hosts, and the same sample preparation and detection conditions. Furthermore, in a conventional tracer experiment involving an enriched stable isotope, the enriched isotope used as a tracer is a relative label and cannot be discriminated from the corresponding endogenous isotope. Thus, the present method provides highly reliable data in a self-verifiable manner. This method can be applied widely to elements consisting of multiple stable isotopes, and the replacement of endogenous natural abundance isotopes makes it possible to use isotopes other than those used for replacement as absolute labels without considering the corresponding endogenous isotopes.

Simultaneous detection of multiple homo-elemental stable isotopes can be verified by avoiding various interfering ions on MS detection. Selenium consists of six stable isotopes, as shown in Table 1. ^{74}Se is the lowest ratio isotope and the best isotope as a tracer because of the lowest interference from the corresponding endogenous natural abundance isotope, which may make it possible to use the stable isotope as an absolute label rather than a relative one. Thus, the lowest natural abundance isotope was the best isotope for the tracer experiment. However, the best isotopes cannot be used sometimes due to the interfering ions and/or other reasons. In the case of selenium, the best isotope is ^{74}Se, and the second best ones are ^{76}Se, ^{77}Se, and ^{82}Se from the viewpoint of the natural abundance ratio. If the endogenous natural abundance ratio is lowered to a negligible level, the corresponding enriched stable isotope can be used as an absolute label, which also raises the detection sensitivity. The endogenous natural abundance ratio could be decreased by replacing the endogenous natural abundance selenium with a single stable isotope, i.e., stable isotopes other than that used for replacement can be used as an absolute label. For example, if endogenous natural abundance selenium in the host animals is replaced with the highest natural abundance isotope ^{80}Se or the second highest natural abundance one ^{78}Se, the other five isotopes can be used as absolute labels.

Table 1 Potential interfering species in the determination of Se by ICP-MS.

Selenium species	Abundance (%)	Interfering species	
^{74}Se	0.89	$^{74}Ge^+$, $^{38}Ar^{36}Ar^+$	$^{152}Gd^{2+}$
^{76}Se	9.37	$^{76}Ge^+$, $^{40}Ar^{36}Ar^+$, $^{75}AsH^+$	$^{152}Gd^{2+}$
^{77}Se	7.63	$^{40}Ar^{37}Cl^+$, $^{76}SeH^+$, $^{75}AsD^+$	$^{154}Gd^{2+}$, $^{155}Gd^{2+}$
^{78}Se	23.77	$^{78}Kr^+$, $^{40}Ar^{38}Ar^+$, $^{77}SeH^+$, $^{76}SeD^+$	$^{155}Gd^{2+}$, $^{156}Gd^{2+}$, $^{157}Gd^{2+}$
^{80}Se	49.61	$^{80}Kr^+$, $^{40}Ar^{40}Ar^+$, $^{79}BrH^+$	$^{160}Gd^{2+}$
^{82}Se	8.73	$^{82}Kr^+$, $^{40}Ar^{42}Ca^+$, $^{81}BrH^+$, $^{80}SeD^+$	

However, to detect multiple isotopes simultaneously, it is recommended to avoid interfering ions. There are many interfering ions, as itemized in Table 1, in the detection of multiple selenium isotopes [30–33]. In relation to the detection of six selenium isotopes, the most significant interference is due to ions from the plasma source argon, $^{40}Ar^{36}Ar^+$ for *m*/*z* 76, $^{40}Ar^{38}Ar^+$ for *m*/*z* 78 and $^{40}Ar^{40}Ar^+$ for *m*/*z* 80, which can be avoided or reduced in the H_2 or D_2 collision mode. However, new interfering ions arise on these collisions, as shown in Table 1. In the present case, we used the D_2 collision mode rather than the H_2 collision mode because of the less noise occurring on D_2 collision [27]. However, another interfering ion, $^{80}SeD^+$ for the detection of ^{82}Se was revealed, which inhibits the precise detection of ^{82}Se, as observed in Figs. 3 and 4, and also pointed out in Table 1. Other possible interfering ions are also listed in Table 1. Based on our results, we recommend the following criteria; deplete natural abundance selenium with ^{78}Se, and use precursors labeled with ^{76}Se, ^{77}Se, and ^{82}Se, and then trace the labeled isotopes including the endogenous ^{78}Se in the D_2 collision mode. This combination of selenium isotopes seems to minimize the interference due to interfering ions listed in Table 1.

Although ^{77}Se of SeMet origin was distributed at comparable levels in organs (Fig. 1) compared with ^{76}Se of MeSeCys origin, it was incorporated less efficiently into seleno-enzymes, most typically into Sel P (Fig. 2), and also into selenosugars. Furthermore, intact SeMet was detected less than MeSeCys. These observations suggest that SeMet is present in organs/tissues in forms other than seleno-enzymes, selenosugars, and the intact form, i.e., probably in the form of SeMet residues in general proteins [SeMet residues incorporated into general proteins without being discriminated from methionine (Met)] [34]. Although this form of SeMet may not be recognized as a selenochemical, it can be recognized as one when it is transformed into free SeMet or further intermediates in the trans-selenation pathway during the turnover of general proteins. Therefore, the lower distribution of selenium of SeMet origin in organs/tissues/body fluids may not suggest that the availability/distribution of SeMet is lower than selenium of other origins. Instead, it may suggest that SeMet is a safe storage form for selenium in the body, and excess free SeMet is buffered by transformation of it into SeMet residues in general proteins [35]. Similar apparent low incorporation of selenium of SeMet origin has been observed with oral administration, too [10].

Although hexavalent selenate and methylselenonic acid have been shown to be partly excreted into the urine in their intact forms even on oral ingestion [9], the present intravenous injection caused more rapid and significant excretion of selenate into the urine. Thus, hexavalent selenium sources are not efficiently absorbed through a parenteral route compared with the per oral route.

Inorganic selenite and selenate, and organic methylseleninic and methylselenonic acids were not or only slightly distributed to or taken up little by the pancreas [9]. Contrary to these selenochemicals, selenoamino acids SeMet and MeSeCys are known to be taken up and be present in forms bound to proteins [9,10]. However, it is not known why and how SeMet and MeSeCys are selectively taken up by the pancreas and are present in forms bound or incorporated into proteins. The present study involving an injection of SeMet and MeSeCys was the first such one, and it was shown that both SeMet and MeSeCys are taken up by the pancreas directly in their intact forms, the latter selenoamino acid more efficiently. Thus, although SeMet was more abundantly detected in its intact form than MeSeCys, selenium of MeSeCys origin was more efficiently incorporated into proteins than SeMet. However, it is not

known how these selenoamino acids are incorporated into proteins. The present experiment revealed that both SeMet and MeSeCys are delivered to the pancreas, and then transformed into pancreas proteins rather than these selenoamino acids are transformed into proteins and then transferred to the pancreas.

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