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Speciation and metabolism of selenium injected with ^{82}Se-enriched selenite and selenate in rats

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Abstract

Selenate and selenite injected intravenously into rats were speciated by the HPLC–ICP MS method with use of an enriched stable isotope as the tracer. In dose–relation experiments, ^{82}Se-enriched selenate or selenite was injected intravenously into male Wistar rats of 8 weeks of age (three rats/group) at single doses of 10, 25, 50, 100 and 200 μg/kg body weight for the selenate group, and 2, 5, 10, 25 and 50 μg/kg body weight for the selenite group. The animals were sacrificed 1 or 24 h later, and the concentrations and distributions of ^{82}Se in the liver, kidneys, serum, and urine remaining in the bladder or 24-h urine were determined. In time-course experiments, ^{82}Se-enriched selenate and selenite were injected at doses of 50 and 10 μg/kg body weight, respectively, and the animals were sacrificed 5, 15, 30, 60 and 180 min later. It was suggested that selenate is directly taken up by the liver with an efficiency of approximately 1/2 compared with selenite, the latter being taken up by the liver after being metabolized to selenide in red blood cells. Although selenate and selenite were metabolized differently in the bloodstream, and also a part of only selenate was excreted directly into the urine, the ^{82}Se taken up by the liver was shown to be metabolized in a manner indistinguishable between selenate and selenite. ^{82}Se of selenite origin but not of selenate origin was suggested to undergo redox reaction in the bloodstream. These results suggest that although parenteral selenate is utilized less efficiently by the body, it is utilized in the liver in a similar manner to selenite much more safely.

Keywords: Speciation; Selenium; Selenite; Selenate

1. Introduction

Selenium (Se) is an essential micronutrient, and both inorganic and organic forms of Se can be utilized as the nutritional source [1]. Selenite and selenate are the inorganic forms, and both are used as an additive in the treatment of patients with a nutritional Se deficiency [2,3], and as a total parenteral nutrition source [4].

Our recent studies revealed that selenite and selenate are metabolized differently in the bloodstream; selenite is rapidly and selectively taken up by red blood cells (RBCs) [5,6], reduced in the RBCs, and then transported into the plasma in the form of selenide, where Se is bound selectively to albumin [5,7,8], and transferred to and taken up by the liver [9,10]. On the other hand, selenate is taken up directly by the liver without being processed in the bloodstream [6].

The present study was carried out to reveal the difference in the metabolism of selenite and selenate

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with use of an enriched stable isotope. The goal of the present study is to find a way of supplementing Se efficiently and safely in parenteral nutrition based on the mechanism underlying the metabolic pathway by the speciation method.

Dose and time-dependent changes in the recovery and distribution of exogenous Se in serum and urine, and in supernatants of the liver and kidneys were determined by mass spectrometry (MS) with ionization with inductively coupled argon plasma (ICP), and by HPLC with in-line detection of Se by ICP MS (HPLC–ICP MS method) [11–13], respectively. A stable chemical isotope, ^{82}Se, was used to trace Se of selenite and selenate origin, and the exogenous Se in the liver, kidneys, serum and urine was speciated simultaneously with endogenous Se [7,10].

2. Materials and methods

2.1. Chemicals

^{82}Se-enriched selenate and selenite were prepared by oxidation of ^{82}Se-enriched metallic Se (97.02% enriched; Oak Ridge National Laboratory, Oak Ridge, TN, USA). Oxidation of the metal was achieved by dissolving it in concentrated metal-free nitric acid for selenite [7], and in concentrated metal-free nitric acid containing hydrogen peroxide (30% H_2O_2–conc. HNO_3, 4:1 v/v) for selenate, and subsequent neutralization with 1 *M* NaOH. The ^{82}Se-enriched oxidation products were identified as selenite and selenate by mixing with their naturally occurring authentic samples on a GS 520 (7.6×500 mm, with a guard column, 7.6×50 mm; Showa Denko, Tokyo) column by the HPLC–ICP MS method.

Nitric acid and hydrogen peroxide for metal analysis were purchased from Wako Pure Chemicals (Osaka, Japan). Tris[hydroxymethyl]aminomethane (Trizma® Base) was purchased from Sigma (St. Louis, MO, USA). Both Tris–HNO_3 and Tris–HCl buffers were prepared by mixing Trizma Base and nitric acid, and Trizma Base and hydrochloric acid, respectively, at 25°C. Other chemicals, such as glucose, were of analytical grade. The standard Se solution for ICP–atomic emission spectrophotometry (1,000 μg/ml; Kanto Chemicals, Tokyo) was used after appropriate dilution with 0.1 *M* nitric acid.

2.2. Animals

Male rats of the Wistar strain were purchased from Clea Japan, Tokyo, at 7 weeks of age, fed a standard diet (CE-2, Clea Japan) and tap water ad libitum for 1 week, and then divided into six groups (three rats/group) for each experiment. The animals were maintained in an animal facility at a room temperature of 22±2°C with a 12-h light–dark cycle. The animals were sacrificed by exsanguination under ether anesthesia. Serum was obtained after clotting by centrifugation at 1600 *g* for 15 min at 4°C.

2.3. Determination of Se concentrations

Livers and kidneys were homogenized in 4 vols of 50 m*M* Tris–HCl buffer, pH 7.4, at 25°C, containing 0.25 *M* glucose, with a Polytron homogenizer (Kinematica, Lutzern, Switzerland) under an atmosphere of nitrogen with ice-water cooling. The homogenates were centrifuged at 105 000 *g* for 1 h at 4°C to obtain the supernatant.

2.4. Analytical procedures

A 0.5-ml portion of each homogenate and supernatant of liver and kidneys, and 0.2 ml of serum, and 0.1 ml of urine were wet-digested with 0.6 ml of nitric acid and 0.2 ml of hydrogen peroxide. The concentrations of Se were determined by ICP MS (HP4500; Yokogawa Analytical Systems, Musashino, Japan) at m/z=77 and 82 by the standard addition method. The quality control was carried out using a reference sample (NIES human serum, NIES, Tsukuba, Japan; Although the certified value is not given, the acceptable value for Se is given to be 0.14 μg/ml).

2.5. HPLC–ICP MS

A 0.1-ml aliquot of a supernatant of an organ, serum or urine was applied to a size-exclusion HPLC column (GS 520 or GS 320 column, 7.6×500 mm, with a guard column, 7.6×50 mm; Showa Denko), and then the column was eluted with 50 m*M* Tris–

HCl or Tris–HNO_3 buffer, pH 7.4, at the flow-rate of 1.0 ml/min on an HPLC (PU 610; GL Sciences Co., Tokyo). The latter elution conditions were used for the samples in Fig. 4b, and the chromatograms were compared with those obtained with the former elution conditions. The eluent was monitored at 280 nm with an ultraviolet (UV) detector, and then introduced directly into the nebulizer of the ICP MS to detect Se (HPLC–ICP MS method). The endogenous Se in the control profile was detected at $m/z=77$. The exogenous (labeled) ^{82}Se was also detected at $m/z=82$ and expressed after subtracting the ^{82}Se of endogenous origin.

3. Results

3.1. Dose-related changes in the recovery and distributions of ^{82}Se 1 h after the injection

Our preliminary experiments suggested that the recovery of exogenous ^{82}Se in selenoprotein P (Sel P) in serum for selenate is approximately the same as that for selenite when a 4- or 5-fold dose of selenate relative to that of selenite was injected intravenously. Based on this result, three independent experiments were carried out to reveal the dose-related changes in the recoveries and distributions of ^{82}Se of selenate and selenite origin.

In the dose–recovery experiments, ^{82}Se-enriched selenate and selenite were singly injected intravenously into male Wistar rats of 8 weeks of age at doses of 10, 25, 50, 100 and 200 μg Se/kg body weight, and 2, 5, 10, 25 and 50 μg Se/kg body weight, respectively, and the animals were sacrificed 1 h later by exsanguination (Figs. 1 and 2). The liver and kidneys were dissected out, and the urine remaining in the bladder was collected.

The concentrations of exogenous ^{82}Se in the serum increased with the dose (Fig. 1a). The concentrations of exogenous ^{82}Se in the liver (Fig. 1b) and kidneys (data not shown) increased likewise with the dose. When the amounts of exogenous ^{82}Se present in these organs and serum were expressed as the recovery relative to the dose, the recovery stayed almost constant for all doses (between 25–37%). Although the urine remaining in the bladder was collected in some rats, quantitation was not possible.

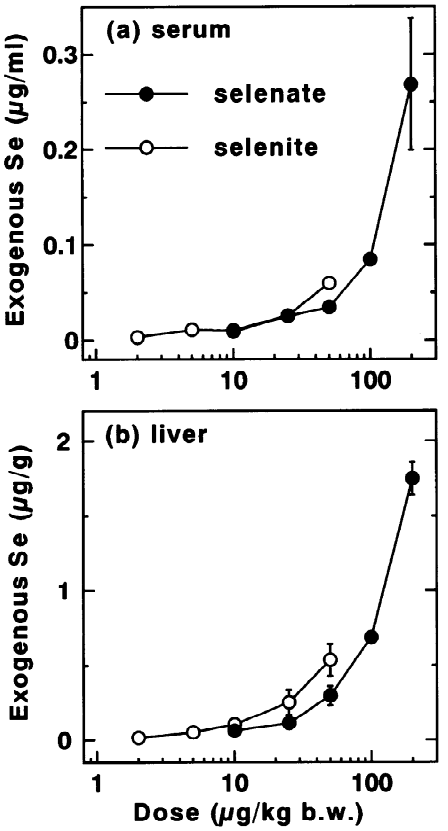

Fig. 1. Dose-related changes in the concentrations of ^{82}Se in the serum and liver 1 h after a single intravenous injection of ^{82}Se-labeled selenate or selenite into rats. Male Wistar rats of 8 weeks of age (three rats/group) were injected with different doses of ^{82}Se-labeled selenate (10, 25, 50, 100 and 200 μg/kg body weight) (closed circles) or ^{82}Se-labeled selenite (2, 5, 10, 25 and 50 μg/kg body weight) (open circles), sacrificed 1 h after the injection by exsanguination, and then their livers were dissected out. Serum was obtained after clotting by centrifugation. The concentrations of Se in the serum and liver were determined by ICP MS.

The distributions of exogenous ^{82}Se in serum and liver supernatant were determined on a gel filtration column by the HPLC–ICP MS method (Fig. 2). The distribution of endogenous Se was determined for non-treated (control) rats, as shown at the top of each panel (Fig. 2a–d). Extracellular glutathione peroxidase (eGPx, 9.6 min) and Sel P (12.0 min) were the major endogenous Se-containing constituents in the serum, as observed previously (Fig. 2a and c) [6,7,10]. Exogenous ^{82}Se peaks were detected at retention times of 12.0 and 15.0 min, which corre-

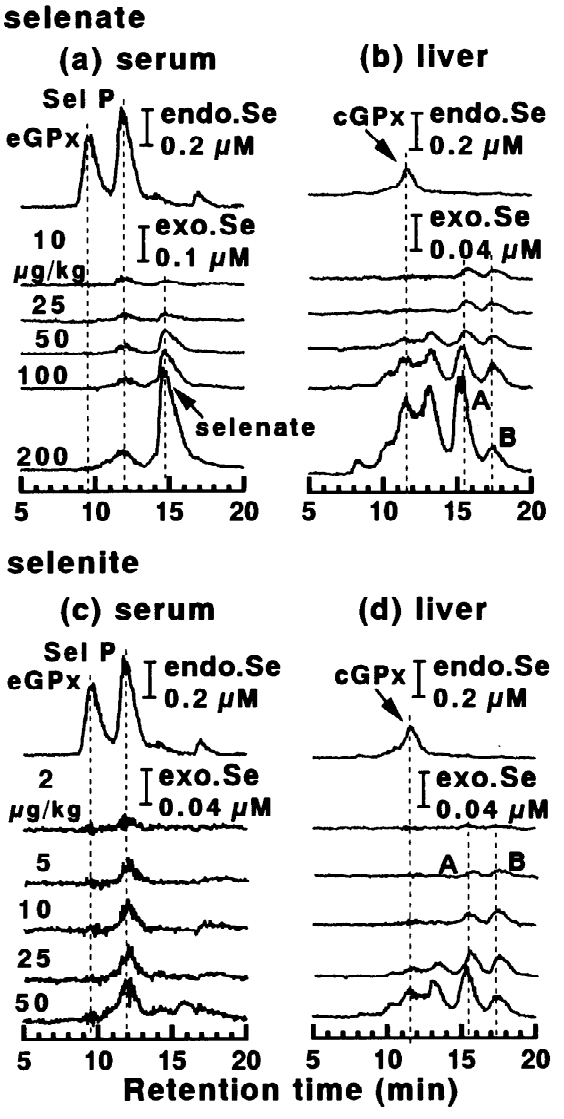

Fig. 2. Dose-related changes in the distribution of ^{82}Se in the serum and liver supernatant 1 h after a single intravenous injection of ^{82}Se-labeled selenate or selenite into rats. The livers were homogenized in 4 vols of the extraction buffer and the homogenates were ultracentrifuged to obtain supernatants. The serum and liver supernatant were subjected to the HPLC–ICP MS analysis on a gel filtration HPLC GS 520 column. The vertical bars indicate the detection levels.

spond to those of Sel P and selenate, respectively (Fig. 2a and c).

The major endogenous Se in the liver supernatant was cellular GPx (cGPx), as shown on the top profile in of Fig. 2b. The exogenous ^{82}Se peaks at 15.6 and 17.3 min increased in intensity with the dose, and have been named tentatively peaks A and B, respectively, the former (peak A) being transformed in vitro to peak B, the major urinary methylated product (peak B) [6,10]. The exogenous ^{82}Se peaks at 10.6, 11.6 and 13.2 min also increased with the dose, although these materials have not been characterized yet.

The distributions of endogenous and exogenous ^{82}Se in the kidney supernatant are similar to those in the liver supernatant, although the intensity of the exogenous ^{82}Se peak for the kidney supernatant was much lower than that for the liver supernatant (data not shown).

In the dose–recovery experiment, ^{82}Se-enriched selenite was injected in the same manner as selenate at doses of 2, 5, 10, 25 and 50 μg Se/kg body weight. The concentrations of exogenous ^{82}Se in the serum (Fig. 1a), the liver (Fig. 1b), and kidneys (data not shown) were increased with the dose at 1 h after the injection. The recovery of exogenous ^{82}Se in these organs and serum relative to the dose stayed almost constant (between 47–51%).

The distributions of exogenous tracer ^{82}Se were determined, as shown in Fig. 2. The lower concentration of labeled ^{82}Se in the serum in the selenite group than in the selenate group was attributed to the absence of the injected form of Se. The labeled eGPx and Sel P peaks in Fig. 2c are comparable with those in Fig. 2a except for the absence of the selenate peak (Fig. 2c). The distributions of exogenous ^{82}Se in the liver (Fig. 2d) and kidney (data not shown) supernatants were also similar to those in the selenate group in Fig. 2b. The distribution of exogenous ^{82}Se in urine was also similar to that in the selenate group, when the major selenate peak was neglected and peaks are expressed on the same scale (data not shown).

3.2. Dose-related changes in the recovery and distribution of ^{82}Se 24 h after the injection

In the dose–recovery experiments mentioned above, quantitative data for urinary excretion could not be obtained owing to the short observation period. Therefore, an experiment to obtain the urin-

ary data was carried out. The 24-h urine was collected after the injection of different doses of selenate into rats, and the corresponding data for serum, liver and kidneys were obtained, as shown in Fig. 3.

The exogenous ^{82}Se present in serum, liver, and kidneys increased with the dose, but were less than the corresponding levels at 1 h after the injection, as shown in Fig. 1, the extent of the decrease with time being most significant in the liver. As a result, the recovery of exogenous ^{82}Se in blood, liver and kidneys decreased with time. Instead, the recovery in urine seems to increase as if the ^{82}Se present in the liver at 1 h were excreted into the urine. The overall recovery was calculated to be between 39 and 50%, as shown in Fig. 3.

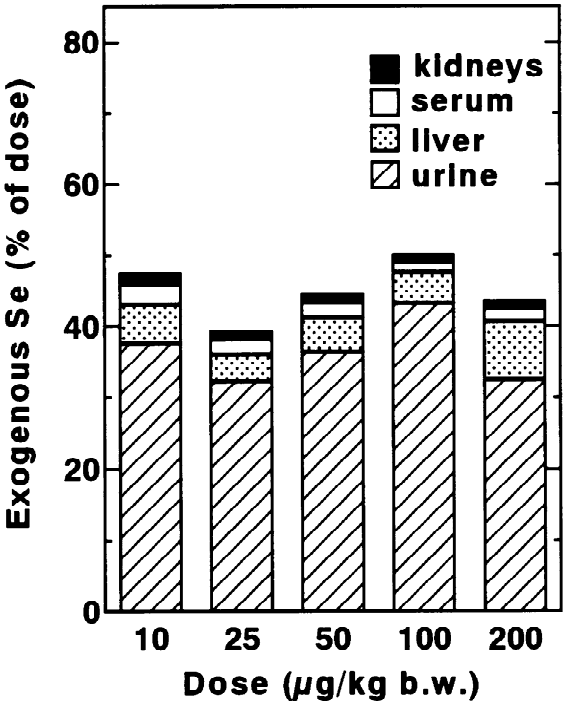

Fig. 3. Dose-related changes in the recovery of ^{82}Se in the serum, liver, kidneys, and 24-h urine after a single intravenous injection of ^{82}Se-labeled selenate into rats. Male Wistar rats of 8 weeks of age (three rats/group) were injected with different doses of ^{82}Se-labeled selenate (10, 25, 50, 100 and 200 μg/kg body weight), and 24-h urine was collected individually with metabolic cages. The animals were sacrificed 24 h after the injection by exsanguination, and then their livers and kidneys were dissected out. Serum was obtained after clotting by centrifugation. The concentrations of Se in the serum, liver, kidneys and urine were determined by ICP MS.

The exogenous ^{82}Se was detected as distinct peaks of eGPx and Sel P for serum (Fig. 4a), and of cGPx for the liver (Fig. 4b) and kidney supernatants (data not shown), the intensities increasing with the dose. The intensity of the selenate peak relative to the methylated metabolite B peak became comparable with all doses for urine (the relative peak intensities being 17.1, 17.8, 19.5, 19.7 and 11.7% for the doses of 10, 25, 50, 100, 200 μg/kg body weight, respectively) (Fig. 4c), suggesting that the ratio between the uptake by the liver and the filtration by the glomeruli seems to be constant on any dose of selenate.

3.3. Time-related changes in the recovery and distributions of ^{82}Se

The time-courses of the recovery of the exogenous ^{82}Se of selenate origin were determined for the dose of 50 μg Se/kg body weight, as shown in Fig. 5. The concentration in the serum decreased with time (Fig. 5a), while that in the liver increased at the beginning and peaked at 1 h (Fig. 5b), and that in the kidneys started to increase after 1 h (data not shown),

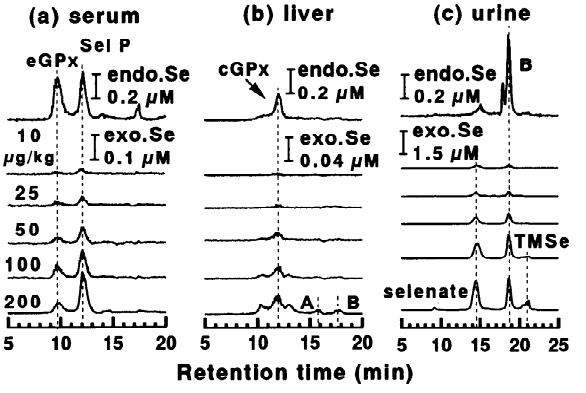

Fig. 4. Dose-related changes in the distribution of ^{82}Se in the serum, liver supernatants and urine 24 h after a single intravenous injection of ^{82}Se-labeled selenate into rats. The livers were homogenized in 4 vols of the extraction buffer and the homogenates were ultracentrifuged to obtain supernatant fractions. The serum, liver supernatants and urine were subjected to HPLC–ICP MS analysis on a gel filtration HPLC column (GS 520 column for serum and liver supernatants, and GS 320 column for urine). The vertical bars indicate the detection levels.

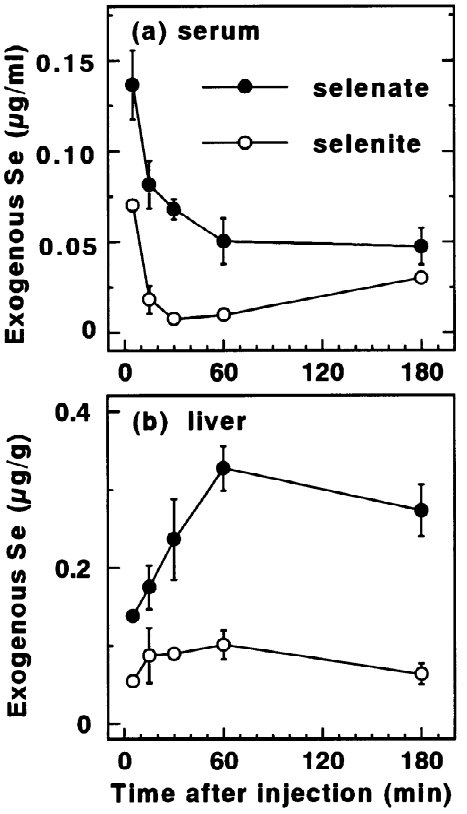

Fig. 5. Time-related changes in the concentration of ^{82}Se in the serum and liver after a single intravenous injection of ^{82}Se-labeled selenate or selenite into rats. Male Wistar rats of 8 weeks of age (three rats/group) were injected with ^{82}Se-labeled selenate at the dose of 50 μg/kg body weight (closed circles) or ^{82}Se-labeled selenite at the dose of 10 μg/kg body weight (open circles) and were sacrificed 5, 15, 30, 60 and 180 min after the injection. The concentrations of Se in the serum and liver were determined by ICP MS.

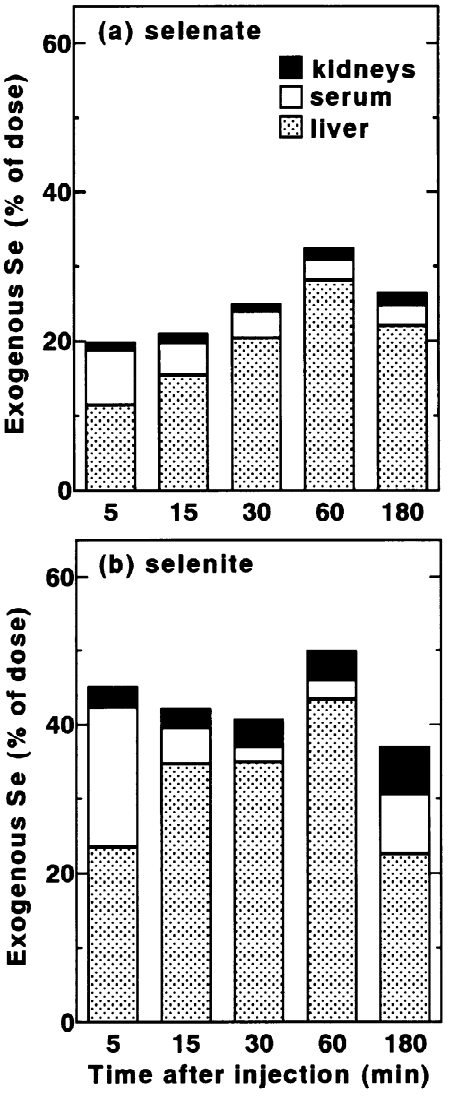

Fig. 6. Time-related changes in the recovery of ^{82}Se in the serum, liver and kidneys after a single intravenous injection of ^{82}Se-labeled selenate or selenite into rats. Male Wistar rats of 8 weeks of age (three rats/group) were injected with ^{82}Se-labeled selenate and selenite at the dose of 50 and 10 μg/kg body weight, respectively, and were sacrificed 5, 15, 30, 60 and 180 min after the injection. The concentrations of Se the serum, liver and kidneys were determined by ICP MS.

suggesting that selenate is not taken up directly in its form. The recovery of ^{82}Se in these organs and serum was 32% at the highest point at 1 h after the injection (Fig. 6a).

The concentration of ^{82}Se in the serum had decreased rapidly by 15 min, and then started to increase 30 min after the injection of ^{82}Se-selenite at the dose of 10 μg Se/kg body weight (Fig. 5a). The concentration of ^{82}Se in the liver stayed constant for 15–60 min (Fig. 5b), while that in the kidneys increased gradually with time (data not shown). The recovery of ^{82}Se in the serum, liver and kidneys was higher in the selenite group than in the selenate group, as shown in Fig. 6.

The distribution of exogenous ^{82}Se of selenate origin in the serum (Fig. 7a) changed in a similar

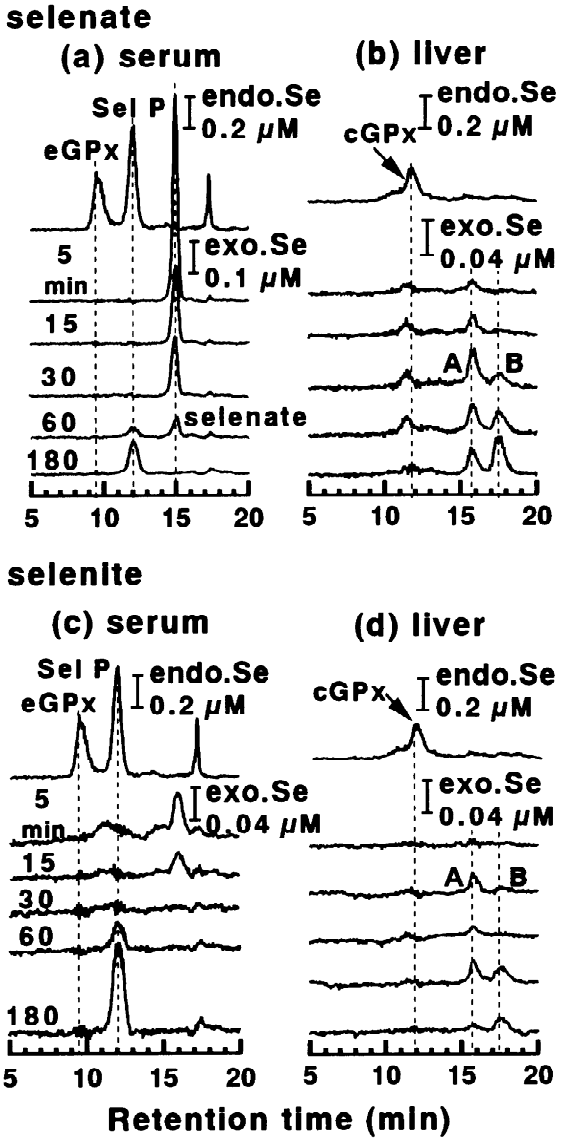

Fig. 7. Time-related changes in the distribution of ^{82}Se in the serum and liver supernatant after a single intravenous injection of ^{82}Se-labeled selenate or selenite into rats. The serum and liver supernatants were subjected to HPLC–ICP MS analysis on a gel filtration HPLC GS 520 column. The vertical bars indicate the detection levels.

pattern to that already reported for a high dose of non-labeled selenate [6]; the selenate peak decreased and the Sel P peak increased with time. For the liver supernatant, peak A started to increase immediately after the injection and peaked 30 min after the injection, while peak B continued increasing during the observation period (Fig. 7b). For the kidney supernatant, a small selenate peak was detected at the beginning and peak B was detected later than 3 h after the injection (data not shown). The selenate peak was detected throughout, this peak decreasing and peak B increasing with time in the urine (data not shown).

^{82}Se of selenite origin is known to be taken up rapidly by RBCs [5,6], and reduced to selenide [14], and then the selenide is transported into the plasma [15] where it is bound selectively to albumin [5,7,8,16] and transferred to the liver within 10–30 min [7,9]. However, in the present profiles, the ^{82}Se was detected at the selenite peak of 15.6 min in the serum at 5 and 15 min after the injection (Fig. 7c). A separate experiment suggested that this difference in the distribution is due to the oxidation of the ^{82}Se bound to albumin during the storage of the serum samples (data not shown). The unstable form of Se bound to albumin in the plasma was observed only shortly after the injection of selenite and an artifact peak of Se was produced even when the plasma/serum sample was stored frozen (unpublished observation). Taking these observations into consideration, although ^{82}Se in the serum was detected as selenite at 5 and 15 min after the injection, this selenite was concluded to be produced during storage through oxidation of the Se bound to albumin, and the distribution of ^{82}Se changed in a similar way as already reported elsewhere (Fig. 7c) [7]. The distributions in the liver (Fig. 7d) and kidney (data not shown) supernatants were similar to those in Fig. 7b, suggesting that although Se is taken up in the different forms, i.e. intact selenate and selenide in the case of selenite, the Se is transformed to the common intermediates in the liver.

4. Discussion

The present study precisely revealed the dose- and time-related changes in the recoveries and distributions of exogenous Se injected intravenously into rats in the form of ^{82}Se-enriched selenate and selenite. Especially, the HPLC–ICP MS method was effectively applied to characterize the difference in the

metabolic pathway between the two nutritional Se species that can be used in the supplementation of this essential element. The enriched ^{82}Se aided the speciation of metabolites as the tracer and enhanced the mass spectrometric detection, which made it easier to determine intermediates/metabolites in the metabolic pathway. The present results help not only determining the clinical/nutritional application of selenate/selenite but also revealing the metabolic regulation of Se by the body as discussed below.

Although selenate and selenite are metabolized quite differently in the bloodstream, once they are taken up by the liver, they seem to be metabolized mostly in the same manner with similar efficiency. Se of selenate and selenite origin was utilized in the liver in an indistinguishable manner for the synthesis of selenoproteins and also for the excretion of methylated metabolites, the former being detected as the incorporation of the labeled ^{82}Se into eGPx and Sel P in the plasma, and into cGPx in the liver and kidneys, while the latter being detected as the incorporation into the peak B compound. However, it was found that there are several distinct differences in the metabolism between selenate and selenite:

(1) Selenate is transferred directly to and taken up by the liver, while selenite is metabolized (reduced) in the bloodstream to selenide and then transferred to the liver in a form bound to albumin [6,10].

(2) The reduced form of Se bound to albumin is susceptible to oxidation, yielding selenite in vitro and probably in vivo. In the latter case, selenite seems to circulate between the plasma and RBCs, continuously producing an active reducing agent ($H_2Se \rightarrow SeO_3^{2-}$, i.e. $Se^{2-} \rightarrow Se^{4+}$), which seems to produce reactive oxygen species [17–21]. This may explain the more toxic action of selenite than that of selenate, especially in the bloodstream.

(3) Although selenate seems to be transferred directly to and taken up in the intact form by the liver, while selenite is taken up in the reduced form, both Se compounds seem to be utilized with similar efficiency for either utilization in the form of selenoproteins or excretion in the form of methylated metabolites in the liver. Although selenite is reduced to selenide by thiol groups such as glutathione (GSH), selenate is not reduced to selenite by GSH. Therefore, the questions as to when, where and how selenate is reduced to selenite remained unanswered.

(4) Selenate is either taken up by the liver or filtered out by the glomeruli. The former selenate is utilized for the synthesis of selenoproteins or excreted after being methylated. On the other hand, the latter selenate can be excreted directly into the urine in the form of selenate. The ratio of the uptake by the liver and the filtration by the kidneys was shown to be almost constant for different doses. The mechanism remains unknown, too.

(5) Selenite is taken up selectively and rapidly by RBCs, reduced to selenide and then transferred in a form bound to albumin, and then is taken up selectively by the liver. As a result, selenite injected intravenously into rats is not filtered out by the glomeruli and not excreted directly into the urine in the form of selenite.

The more efficient utilization of selenite than selenate is explained by the points 4 and 5, i.e. efficient uptake of Se of selenite origin by the liver. Although the overall recovery of exogenous ^{82}Se was not sufficient in any experiment in the present study, it was suggested that selenite is taken up by the liver two times more efficiently than selenate. This observation indicates that selenite and selenate should be carefully used in total parenteral nutrition, especially from the viewpoint of dosage.

It has been reported that selenite is more toxic than selenate in the presence of sulfhydryl compounds [20,21]. From the metabolic viewpoint, Se of both selenate and selenite origin is metabolized in the same manner after being taken up by the liver. However, their metabolism in the bloodstream is quite different, and only selenide can easily be oxidized back to selenite. This facile oxidation by recycling back to RBCs followed by reduction to selenide may explain the toxic action of selenite. Further experiments are in progress to explain the toxic action of selenite through the production of reactive oxygen species.

Selenate is utilized less efficiency, approximately 1/4 compared with selenite in the whole body, in parenteral administration. Selenite but not selenate may produce reactive oxygen species in the bloodstream. Taking these observations into consideration, it is concluded that although selenite is more efficiently utilized by the body, selenate can be used more safely in total parenteral nutrition. It should be noted that selenate and selenite are not the same as to metabolism and utilization by the body.

Acknowledgements

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