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Binding of selenium (administered as selenite) to albumin after efflux from red blood cells

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Abstract

The role of albumin in the metabolism of inorganic selenium (Se) was studied in vivo and in vitro using a HPLC–ICP-MS method. Although Se injected in the form of selenite binds selectively to albumin after being reduced to selenide and then being effluxed into the plasma, Se was shown to be metabolized normally in the absence of albumin. The reduced form of Se, selenide, bound selectively to albumin but only to a percentage of it. The thiol group and the intermolecular disulfide group at the 34th cysteinyl residue of albumin were not responsible for the selective binding of Se to albumin. Selenide was suggested to be bound to a disulfide not a thiol group, i.e., to one of the 17 disulfide bonds in a conformationally different isoform of albumin.

Keywords: Selenium; Albumin; Selenite; Selenide

1. Introduction

Both inorganic and organic selenium (Se) can be utilized as a nutrient by the body [1]. Selenite (SeO_3^{2-}) is the commonest chemical form of inorganic Se, while selenocysteine (SeCys) and selenomethionine (SeMet) are the most typical organic forms. Animals are able to synthesize SeCys de novo from inorganic Se and also from SeMet. However, they cannot synthesize SeMet, and SeMet taken up by the body is incorporated either into general proteins without being distinguished from methionine or into selenoproteins after being metabolized to the key intermediate, selenide, in the liver [2]. In fact, concentrations of Se increased in various organs of rats that were fed a diet containing SeMet [3].

Selenite is utilized for the synthesis of selenoproteins only after being metabolized to selenide in the bloodstream and then transferred to the liver. Selenite injected intravenously (i.v.) into rats was shown to be taken up selectively by red blood cells (RBCs) within 1 min through the anion-exchange channel (band 3 protein) [4], and then reduced by glutathione (GSH) in the RBCs [5]. The reduced form of Se was proposed to be effluxed from RBCs into the plasma [6], and then bound selectively to albumin [7,8]. However, the mechanisms underlying the efflux and binding of Se to albumin are not known yet.

Although oxidized forms of Se including selenite can be reduced in the liver in the presence of abundant GSH [5] and then utilized for the synthesis of Se-containing proteins (selenoproteins) [9], sele-

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nite taken up as a nutrient and transferred to the bloodstream is supposed to be taken up by RBCs before being transferred to the liver, i.e., it is not transferred directly to the liver [4]. Therefore, the consecutive reactions in the bloodstream, i.e., uptake by, reduction in and efflux from RBCs, followed by binding to albumin and transfer to the liver, are important processes for inorganic Se, selenite, from the nutrient viewpoint. Although Se bound to albumin is a minor form in plasma compared with the major Se in plasma, i.e., eGPx and selenoprotein P, it is important in the metabolism of Se taken up in the form of selenite.

These processes are important also from the toxicological viewpoint because it has been demonstrated that the reduced Se effluxed from RBCs forms a complex with mercuric ions [10], and then the complex forms a ternary complex with selenoprotein P [11], a plasma selenoprotein [12,13]. The detoxification of mercuric ions administered together with selenite has been explained by the formation of the ternary complex. However, it is not known how the reduced form of Se effluxed from RBCs forms a complex with mercuric ions.

The object of the present study was to reveal the roles of albumin in the process of efflux of Se from RBCs after its reduction in the RBCs and in the transfer of Se to the liver. The mechanism underlying the selective binding of the reduced form of Se to albumin was also examined. In the present study, Se in the serum was speciated efficiently by a hyphenated technique, HPLC–ICP-MS, with use of an enriched stable isotope.

2. Experimental

2.1. Reagents

^{82}Se-enriched sodium selenite was prepared by oxidation of the enriched metal (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 and subsequent neutralization with 1 *M* NaOH [8,14]. Sodium selenite (natural abundance), bovine serum albumin (BSA), and S-cysteinyl–bovine serum albumin (Cys–BSA) were purchased from Sigma (St. Louis, MO, USA). The reduced (GSH) and oxidized (GSSG) forms of glutathione of reagent grade were obtained from Wako (Osaka, Japan), the standard solution of Se (1000 ppm) from Kanto (Tokyo), pentobarbital from Takeda (Osaka) and heparin from Dainippon (Osaka).

2.2. Animal experiments

Male rats of the Wistar strain were purchased at 10 weeks of age from a breeder (Clea Japan, Tokyo), and Nagase analbuminemia rats (NAR) were purchased at 10 weeks of age from another breeder (Japan SLC, Hamamatsu, Japan). They were fed a commercial diet (CE-2; Clea Japan) with tap water ad libitum. Both strains, with mean body weights of 230 and 250 g, respectively, were used at 12 weeks of age.

Heparinized whole blood was obtained from a vein of a lower limb of cannulated male Wistar and NAR rats at 1, 3, 5, 10, 30, 60 and 90 min after a single i.v. injection of ^{82}Se-enriched sodium selenite (100 μg ^{82}Se/kg body weight), and plasma was separated by centrifugation at 8000 *g* for 10 s.

In in vitro experiments, RBCs were separated from heparinized blood of Wistar and NAR rats, centrifuged at 1060 *g* for 15 min to remove residual plasma, and then resuspended at 50% (v/v) in Dulbecco's phosphate-buffered saline (PBS). RBCs (50% suspension) incubated with ^{82}Se-enriched sodium selenite or sodium selenite (natural abundance) for 3 min at 37°C were centrifuged at 8000 *g* for 20 s. The Se-containing RBCs were separated from the supernatant, and then incubated with plasma proteins of Wistar or NAR rats, BSA, Cys-BSA, GSH or GSSG for 10 min at 37°C. The soluble fraction of the reaction mixture was separated by centrifugation at 8000 *g* for 1 min and then subjected to HPLC–ICP-MS analysis.

2.3. Analytical procedures

The concentrations of Se in whole blood and plasma were determined after wet-ashing with an acid mixture (HNO_3–$HClO_4$, 4:1, v/v) at *m*/*z* 78 for Se (natural abundance) and at 82 for enriched Se with an ICP-MS (HP 4500; Yokogawa Analytical Systems, Musashino, Japan).

A 0.1-ml aliquot of the soluble fraction of the reaction mixture was applied to a size-exclusion column (Asahipak GS 520 or GS 320, 500×7.6 mm; Showa Denko, Tokyo), and then eluted with 50 m*M* Tris–HCl buffer (pH 7.4), on an HPLC (Model 576; GL Sciences, Tokyo) at the flow-rate of 1.0 ml/min. The eluent was monitored with an ultraviolet (UV) detector, and then introduced directly into the nebulizer tube of the ICP-MS to detect Se (*m*/*z* 77, 78 and 82) [8,14–16].

3. Results

3.1. Metabolic fate of Se in the bloodstream after an i.v. injection of selenite into Wistar and NAR rats

The role of albumin among plasma proteins in the metabolism of selenite in the bloodstream was examined by comparing the metabolic fate of Se injected as selenite into Wistar (normal albumin content) and NAR rats (completely deficient in albumin), as shown in Fig. 1. Se injected in the form of selenite was taken up by RBCs within 1 min in both strains, and disappeared in a similar manner. There were no apparent differences in the distributions and disappearance patterns between the strains, suggesting that albumin does not function, at least in the rate limiting step, in the metabolism of selenite in the bloodstream.

3.2. Distribution of Se after efflux from RBCs in the presence and absence of albumin

The role of albumin in the process of efflux of Se from RBCs was examined in vitro using two different combinations of rats and plasma of both strains, as shown in Fig. 2. A small amount of ^{82}Se-enriched selenite was incubated with RBCs prepared from Wistar rats, and then the effect of the presence of albumin in plasma on the distribution of Se effluxed from the RBCs was examined by HPLC–ICP-MS. Under the conditions employed, plasma proteins were separated into two fractions, i.e., globulins and albumins, which were eluted before and after the retention time of 14 min, respectively [17]. The distribution of sulfur, S, indicated that albumin is completely absent in the plasma of NAR rats (Fig. 2b) compared within that of Wistar rats (Fig. 2a). Although extracellular glutathione peroxidase (eGPx) and selenoprotein P were not separated into two

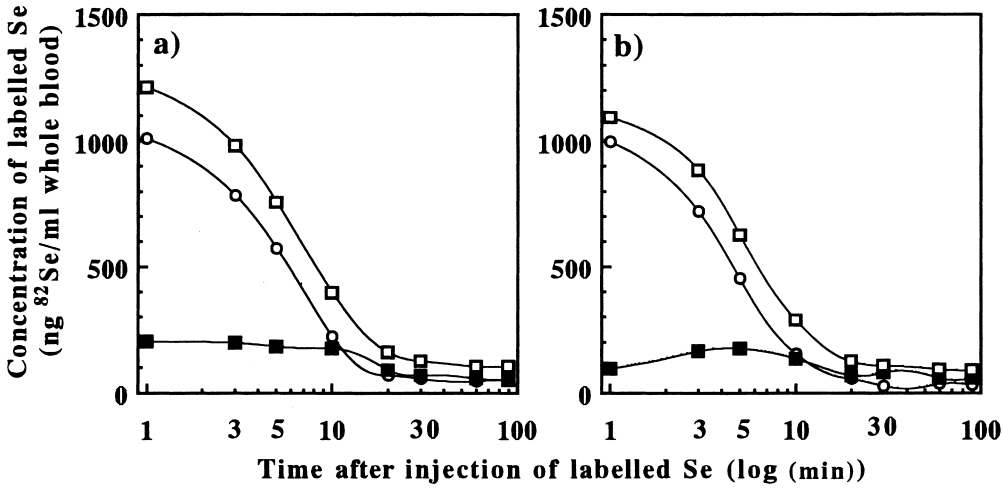

Fig. 1. Changes in the concentrations of labelled Se in whole blood and plasma after a single intravenous injection of ^{82}Se-enriched selenite into male Wistar (a) and NAR (b) rats. Rats were cannulated at an artery and vein of a lower limb under pentobarbital anesthesia, and then received a single i.v. injection of ^{82}Se-enriched selenite at a dose of 100 μg Se/kg body weight. Heparinized blood (approximately 0.5 ml each) was obtained at 1, 3, 5, 10, 30 and 60 min after the injection, and plasma was separated by centrifugation at 8000 *g* for 10 s. Concentrations of Se in plasma and RBCs were expressed as ng/ml of whole blood. The concentrations of Se in RBCs (○) were calculated by subtracting the concentrations in plasma (■) from those in whole blood (□).

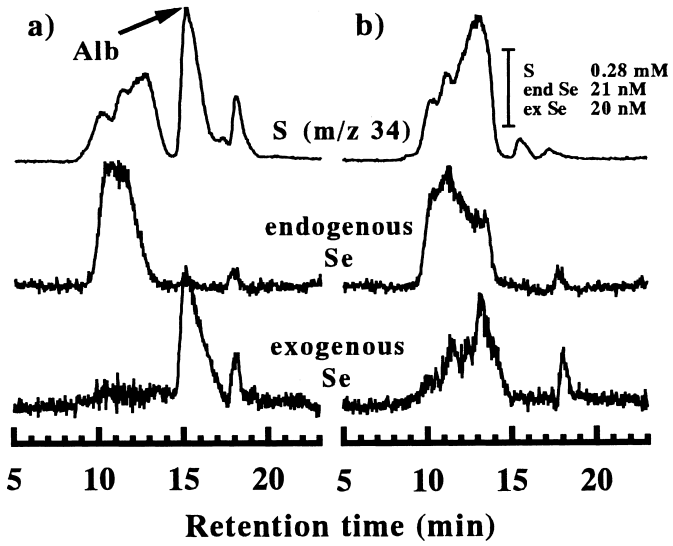

Fig. 2. Distributions of Se in plasma of Wistar (a) and NAR (b) rats after incubation with RBCs of Wistar rats preincubated with a low concentration of selenite. A 50% suspension of RBCs in saline prepared from blood of Wistar rats was incubated with ^{82}Se-enriched selenite at a final concentration of 30 ng ^{82}Se/ml (375 n*M*) at 37°C for 3 min, and then the RBCs were separated from plasma by centrifugation at 8000 *g* for 20 s. The RBCs were incubated with fresh plasma of both strains at 37°C for 1 min, and then the distributions of sulfur (S, *m*/*z* 34), and endogenous (*m*/*z* 78) and exogenous Se (*m*/*z* 82) in the plasma were determined, on a GS 520 column, by HPLC–ICP-MS.

distinct peaks in the distribution profile of the endogenous Se in the present serum samples owing to the heparin-binding property of selenoprotein P [8], the broad peak of endogenous Se was bigger in NAR (Fig. 2b) than in Wistar rats (Fig. 2a). The distribution of exogenous Se differed between the serum samples from Wistar and NAR rats, i.e., exogenous Se was bound to albumin in the Wistar serum, while it is distributed on globulins in the NAR serum. However, the amount of Se effluxed from RBCs was comparable in the two kinds of sera.

The effect of the presence of albumin on the distribution of Se effluxed from RBCs was examined by using a greater amount of selenite, as shown in Fig. 3. RBCs prepared from Wistar rats were incubated in the presence of 0, 10 or 50 μ*M* selenite (natural abundance), and a higher amount of Se was accumulated in the RBCs than in the experiment in Fig. 2. Then, the RBCs were incubated with plasma prepared from Wistar and NAR rats, and the distribution of Se was determined by HPLC–ICP-MS. Endogenous Se was detected in the globulin fraction, as shown in Fig. 3a and b. The exogenous Se in the globulin fraction increased with the concentration of selenite preincubated with RBCs in both animal groups. In addition to Se in the globulin fraction, exogenous Se was increased in the albumin fraction of the serum from Wistar rats (Fig. 3b). The amounts of Se bound to plasma proteins were 1.57 and 2.23 times higher in Wistar rats (Fig. 3b) than in NAR rats at concentrations of 10 and 50 μ*M* Se, respectively.

3.3. Capacity of albumin to bind Se after efflux from RBCs

Although Se can be bound selectively to albumin after efflux from RBCs in vivo and in vitro, the capacity of albumin to bind Se is limited, as estimated from the distributions of Se in Fig. 3b. Therefore, the capacity of albumin to bind the reduced form of Se effluxed from RBCs was determined. The reduced form of Se was obtained by reduction of selenite with excess GSH, and the amount of Se bound to albumin was estimated as the ratio of the peak intensity of Se bound to albumin to the S (*m*/*z* 34) peak intensity of albumin, as shown in Fig. 4. Se bound to only 4–4.5% of the albumin present in the BSA specimen.

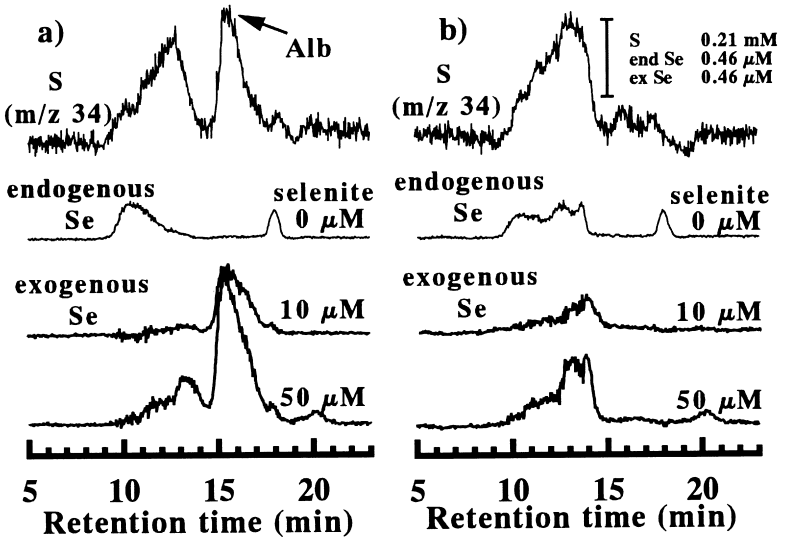

Fig. 3. Distributions of Se in plasma after incubation with RBCs preincubated with high concentrations of selenite. Selenite (natural abundance) was incubated with a 50% suspension of RBCs in saline prepared from blood of Wistar or NAR rats at a final concentration of 10 or 50 μ*M* at 37°C for 3 min, and then the RBCs were separated from plasma by centrifugation at 8000 *g* for 20 s. The RBCs of Wistar or NAR rats were incubated with fresh plasma of both strains at 37°C for 1 min, and then the distributions of S and Se in the plasma were determined, on a GS 520 column, by HPLC–ICP-MS; (a) Wistar RBCs and Wistar plasma; (b) NAR RBCs and NAR plasma. The distribution of Se was determined for a control (without addition of selenite; Se, 0 μ*M*), and two doses of selenite (with addition of selenite; Se, 10 and 50 μ*M*). The control profile shows the distribution of endogenous Se, while the latter two profiles show the distributions of exogenous Se after subtracting the endogenous Se profile from the respective profiles.

3.4. Binding site of albumin for Se after efflux from RBCs

The reduced form of Se effluxed from RBCs was shown to bind to only a percentage of albumin (Fig. 4), which suggested the participation of an intermolecular disulfide bond between one thiol group out of the 35 cysteinyl residues in albumin and a thiol group in GSH or cysteine because of the presence of a percentage of nonmercaptoalbumin in normal blood plasma [18]. The reduced form of Se was supplied by RBCs that had taken up selenite, and the RBCs were incubated with BSA and Cys-BSA to examine the participation of the thiol group in albumin and the intermolecular disulfide bond, as shown in Fig. 5. The reduced form of Se was bound, in a dose-dependent manner, to BSA that contained mercaptoalbumin and nonmercaptoalbumin. However, the Se was bound to only 4–5% of the albumin present in the medium. Likewise, the reduced form of Se was bound to Cys-BSA (nonmercaptalbumin) in a dose-dependent manner. However, again, the Se was bound to only 4–5% of the albumin present in the medium. The results indicate that although only a certain percentage of albumin is in a form that is able to bind the reduced form of Se, the intermolecular disulfide bond, which is present in only some percentage, is not able to bind the reduced form of Se.

Although the thiol group and the intermolecular disulfide bridge do not explain the active form of albumin that binds the reduced form of Se, the possible participation of thiol and disulfide groups in the binding of the reduced form of Se was examined, as shown in Fig. 6. The reduced form of Se was effluxed from RBCs to only a limited extent in the presence of GSH (Fig. 6a). On the other hand, the Se was effluxed much efficiently from RBCs in the presence of GSSG, and the Se effluxed from RBCs gave two distinct peaks at retention times of 13.2 and 15.1 min. The former peak was eluted at the same retention time as hemoglobin, while the latter peak was eluted slightly slower than GSSG but faster than GSH.

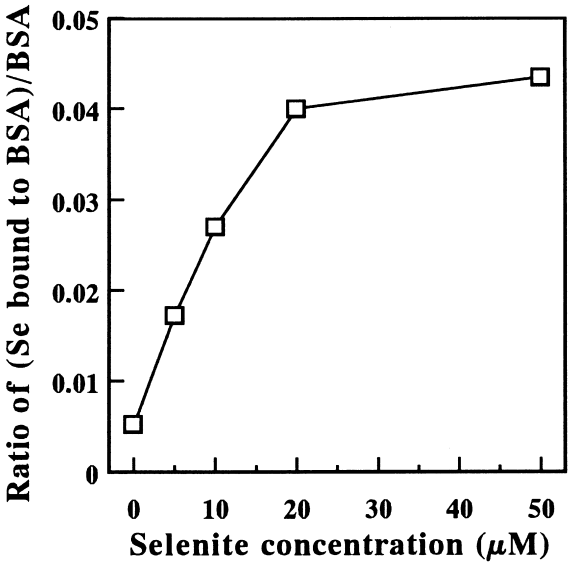

Fig. 4. Estimation of the capacity of albumin to bind Se. Sodium selenite (final concentration, 0–50 μ*M*) was incubated with reduced glutathione (GSH; final concentration, 5 m*M*) and bovine serum albumin (BSA; final concentration, 0.5 m*M*) in PBS at 37°C for 10 min, and then the distribution of Se was determined, on a GS 520 column, by HPLC–ICP-MS. The relative ratios (arbitrary units) of the peak intensities of Se bound to albumin to those of S bound to albumin were plotted against the concentrations of selenite.

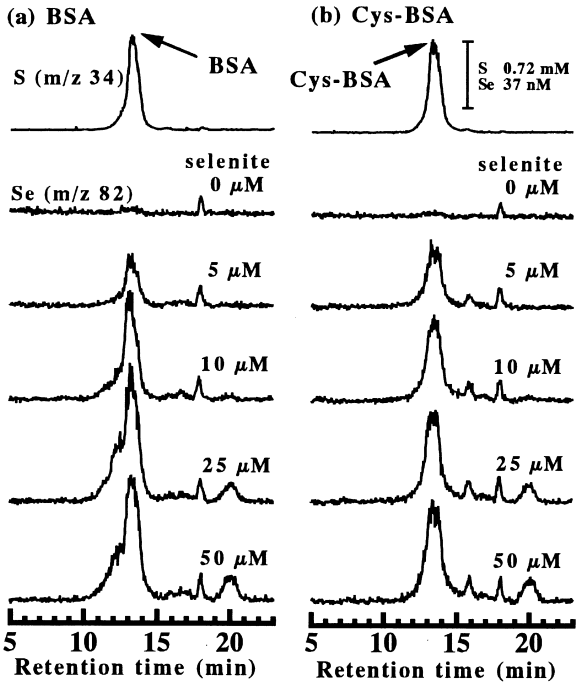

Fig. 5. Binding of Se effluxed from red blood cells to albumin. A 50% suspension of RBCs in saline prepared from blood of Wistar rats was incubated with selenite (natural abundance) at final concentrations of 0, 5, 10, 25 and 50 μ*M* at 37°C for 3 min, and then the RBCs were separated by centrifugation at 8000 *g* for 20 s. The RBCs were incubated with 0.5 m*M* bovine serum albumin (BSA) (a) or S-cysteinyl-bovine serum albumin (Cys-BSA) (b) at 37°C for 10 min. The distributions of Se in the incubation solution were determined, on a GS 520 column, by HPLC–ICP-MS.

4. Discussion

The participation of albumin in the metabolism of inorganic Se, selenite, was pointed out by Sandholm [19], and our recent study also indicated its specific role as transporter of Se from RBCs to the liver [8]. Although albumin is a specific transporter of Se in the bloodstream, the present experiments involving NAR rats (Fig. 1) indicated the minimal role of albumin in the metabolism of Se in the bloodstream in vivo. However, the in vitro experiments suggested that globulins can compensate for the transport by albumin of only a small amount of Se (Fig. 2), but not a high amount of Se (Fig. 3). The results also suggest that the rate limiting step for the disappearance of Se injected i.v. into rats is the step of efflux from RBCs into the plasma, and the Se bound to albumin or globulins in the absence of albumin is transferred efficiently to the liver and other organs. Therefore, inorganic Se, selenite, can be metabolized normally in the bloodstream even in the absence of albumin when the dose is low.

Although Se is bound selectively to albumin after efflux from RBCs and albumin is a specific transporter of Se in the bloodstream, it was demonstrated that only some percentage of albumin is in the reactive form that is able to bind the reduced form of Se (Fig. 4). Furthermore, it was revealed that the thiol group at the 34th cysteinyl residue in the amino acid sequence of albumin (mercaptoalbumin) and its disulfide group with an intermolecular disulfide bridge (nonmercaptoalbumin) do not participate in the binding of Se (Fig. 5). These results indicate that another reasonable explanation is needed to explain the reactive form of albumin. As albumin is known to be present in several conformationally different

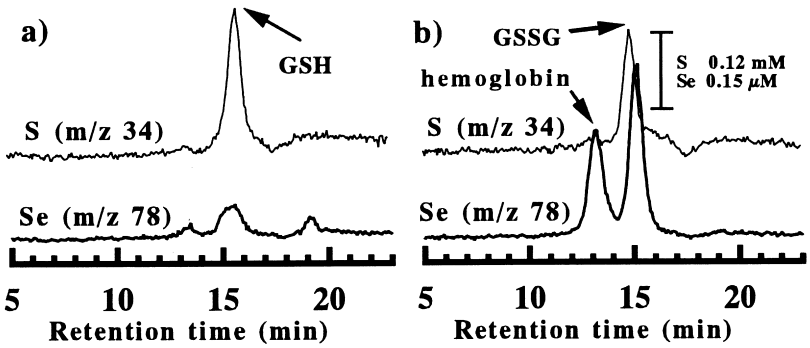

Fig. 6. Binding of Se effluxed from RBCs to glutathione. Selenite (natural abundance) (final concentration, 20 μ*M*) was incubated with a 50% suspension of RBCs in saline prepared from blood of Wistar rats at 37°C for 3 min, and then the RBCs were separated by centrifugation at 8000 *g* for 20 s. The RBCs were incubated with the reduced (GSH; final concentration, 5 m*M*) (a) and oxidized (GSSG; final concentration, 2.5 m*M*) (b) forms of glutathione at 37°C for 20 min, and then the distributions of S and Se were determined, on a GS 520 column, by HPLC–ICP-MS.

forms [20], it can be assumed that one of the conformations of albumin is able to bind the reduced form of Se.

Albumin was assumed to be altered conformationally when Cu is bound to it in vivo or in vitro in the presence of equimolar amounts of Cu and Ca, and a two-molar amount of cysteine [17]. The reduced form of Se was bound to the albumin that had been altered conformationally under the conditions mentioned above, and the effect of the conformational change on the capacity of albumin to bind the Se was examined. However, this conformationally changed albumin was not able to bind the Se (data not shown), suggesting that different conformational changes are responsible.

Although the thiol group and disulfide group at the 34th cysteinyl residue of albumin were indicated not to participate in the binding of the Se, the results shown in Fig. 6 suggest that the disulfide group rather than the thiol group is the binding site for the Se. Therefore, it can be concluded that one of the 17 intramolecular disulfide bonds is responsible for binding the reduced form of Se when albumin is altered conformationally from one of its major forms.

The efflux of Se from RBCs was enhanced in the presence of GSSG, and the Se was detected as the two distinct peaks, as shown in Fig. 6. The two Se peaks were tentatively assigned as that bound to hemoglobin and GSH (GSSeH) from their retention times. The latter Se peak is eluted slightly slower than the GSSG peak but faster than the GSH peak.

Hemoglobin is assumed to leak out due to hemolysis during the experimental procedures. As the same RBCs were used in both experiments and Se is bound only in the presence of GSH (Fig. 6b), Se bound to hemoglobin is not transferred directly from the reduced form of Se, that is effluxed from RBCs, but through GSSeH.

The reduced form of Se can only be trapped in the presence of albumin, GSSG or globulins as shown in the present study, but the chemical form has not been determined yet. Se can also be produced simply in the presence of selenite and excess GSH. The present study, Fig. 6, indicated that the reduced form of Se can bind to a disulfide bond but not to a thiol group, and GSSeH is produced in the presence of GSSG. These results indicate that the reduced form of Se, that is effluxed from RBCs, is selenide (H_2Se). It is assumed that selenide is the key intermediate in the metabolism of not only inorganic but also organic Se, and that it is utilized for the synthesis of selenoproteins or excreted after stepwise methylation [21–23].

Summarizing the present findings, selenite taken up by and reduced in RBCs is effluxed in the form of selenide into the plasma and then bound selectively to albumin, through one of the 17 intramolecular disulfide bonds, that is conformationally different from its major forms and present at only a certain

percentage. Globulins can compensate for albumin in its absence when the dose of Se is low.

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