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Albumin-Mediated Selenium Transfer by a Selenotrisulfide Relay Mechanism

Mamoru Haratake,* Masafumi Hongoh, Mihoko Miyauchi, Ritsuko Hirakawa, Masahiro Ono, and Morio Nakayama*

Graduate School of Biomedical Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521, Japan

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In this study, we demonstrated a human serum albumin (HSA)-mediated selenium transfer; the selenium exported from red blood cells (RBCs) was bound to HSA through the selenotrisulfide and then transferred into the hepatocyte. After the treatment of the RBCs with selenite, the selenium efflux from the RBCs occurred in an HSA concentration-dependent manner. Pretreatment of HSA with iodoacetamide almost completely inhibited the selenium efflux from the RBC to the HSA solution. The selenium efflux experiment was carried out in an HSA solution (45 mg/mL), and subsequently the HSA solution was subjected to gel permeation chromatographic separation. The peak fraction of the selenium content was consistent with that of the HSA. The selenium bound to HSA in this solution was completely eliminated by a treatment with penicillamine (Pen), which resulted in the generation of penicillamine selenotrisulfide, PenSSeSPen. The selenium efflux from the RBCs was also occurred in a Pen solution, and PenSSeSPen was observed in the resulting Pen solution. The selenium exported from the RBC was thought to bind to the HSA via a selenotrisulfide linkage with its single free thiol. A model of the selenium-bound HSA was prepared by the reaction of the HSA with PenSSeSPen. The selenium from PenSSeSPen can bind to HSA by a thiol exchange between Pen and the free thiol of HSA, which produces the selenotrisulfide-containing HSA (HSA−SSeSPen). When HSA−SSeSPen was incubated with isolated rat hepatocytes, the selenium content in the hepatocytes increased along with its decrease in the incubation medium. To verify the results from the model experiments using HSA−SSeSPen, we conducted the HSA-mediated selenium transfer experiment from RBC treated with selenite to the hepatocytes. The selenotrisulfide-containing HSA was able to transport the selenium into the hepatocyte. Overall, the selenium transfer from the RBC to the hepatocytes involves a relay mechanism of thiol exchange that occurs between the selenotrisulfide and thiol compounds (selenotrisulfide relay mechanism: R−SSeS−R + HSA−SH → HSA−SSeS−R + R′−SH → R−SSeS−R′).

Introduction

Human serum albumin (HSA) is the most abundant protein component of blood plasma. HSA, a 585-residue single protein, is monomeric, but contains three structurally similar α-helical domains; each domain can be further divided into subdomains A and B, which contain six and four α helices, respectively.[1−3] Another structural feature is that it contains intramolecular 17 disulfide linkages and the single free thiol at Cys34. This protein can bind a number of relatively insoluble endogenous compounds and thus facilitates their whole-body transport throughout the circulation system.[3] HSA is also capable of binding a wide variety of exogenous drugs,[3,4] and much of the interest in this protein is derived from its effects on drug delivery. In general, drug binding to the HSA can be an important determinant of pharmacokinetics, restricting the unbound concentration and affecting distribution and consequent elimination.[4] Therefore, information on the binding modes of endogenous ligands and drugs will be invaluable toward efforts to exploit the carrier properties of the HSA.

* To whom correspondence should be addressed: E-mail: haratake@nagasaki-u.ac.jp (M.H.), morio@nagasaki-u.ac.jp (M.N.)

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Selenium is an essential trace element in all higher forms of animal life, being important for the control of the intracellular redox states, the immune system, reproduction, and so on.[5] In humans, it has catalytic and/or structural roles in the 25 selenoproteins that were deduced from the selenoproteomes.[6] The family of selenium-dependent glutathione peroxidases (GPx-1, GPx-2, GPx-3, GPx-4, GPx-6) is the best-known example of the redox function, which catalyzes the reduction of hydrogen peroxide and phospholipid hydroperoxides in living systems. The selenium element enters our body from various food sources mostly in organic forms, such as selenomethionine and selenocysteine. During the systemic delivery of selenium, selenoprotein P (SelP) that is present in the plasma is thought to be the selenium supply protein for the biosynthesis of the other selenoproteins including GPxs.[7,8] Whereas inorganic selenite (SeO_3^{2-}) is rare as a chemical form of the food-source compounds, it is an effective source compound most frequently used in the selenium supplementation for medical treatments. Plant sources of selenium contain mostly selenomethionine, but selenium-enriched yeast (a common form of selenium supplement) is found to contain significant amounts of selenite,[9,10] and selenite is also used to supplement infant formula and other products.[11,12] Thus, a better understanding of the systemic delivery mechanisms of selenium from selenite is of significance from the viewpoints of medical treatments and toxicology. In the bloodstream, selenite is immediately taken up from the plasma into the red blood cell (RBC) through the band 3 protein (anion exchanger 1, AE1)[13] and then returned to the plasma after reductive metabolism in the RBC.[14–16] The reduced selenium metabolite exported to the plasma is thought to bind to albumin and is subsequently transferred to the peripheral organs and tissues. However, details of such a selenite metabolic pathway in the bloodstream, in which the interaction of selenium with serum albumin is followed by its systemic distribution, still remain unclear.

The single free thiol at Cys34 of HSA binds a range of drugs and metal ions[17] but is thought to be involved in disulfide linkages with endogenous thiols.[18–20] Stamler et al. also reported that the free thiol of HSA serves as the carrier in the systemic circulation for endogenous nitric oxide.[21] As selenite is metabolized into reduced chemical forms with low oxidation states, such as glutathione selenotrisulfide (GSSeSG) and hydrogen selenide (H_2Se), by the reaction with certain thiol-containing components in RBC, the free thiol of HSA is thought to be a potential binding site via a −SSe− or −SSeS− linkage for the selenium species that is exported from the RBC. Self et al. proposed that selenotrisulfides with thiol-containing lipoic acid and lipoamide formed in the cells are potential intermediates for the selenium delivery to selenophosphate synthetase.[22] Painter reported that the reaction of selenious acid with low-mass thiol compounds (RSH) produced selenotrisulfide (RSSeSR) in vitro ($H_2SeO_3 + 4\ RSH \rightarrow RSSeSR + RSSR + 3\ H_2O$).[23] Later, the stoichiometry of the reaction of selenious acid with reduced glutathione (GSH) and the reactivity of GSSeSG with free thiols of reduced pancreatic ribonuclease in an acidic medium was well characterized by Ganther.[24–26] GSSeSG was also actually identified in a yeast extract by mass spectrometric techniques.[27] On the basis of these facts, we attempted to elucidate the molecular events of the albumin-associated selenium transport pathway in the systemic circulation, with particular attention to selenotrisulfide in the present study.

Experimental Section

Materials. L-Penicillamine (Pen) and iodoacetamide were obtained from Tokyo Chemical Industry Co., Ltd. Rhodamine B isothiocyanate and human serum albumin [HSA; purity, 98.9%; the number of thiol groups determined by the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, from Sigma Co.) method, 0.31 ± 0.03 per HSA molecule] were from Sigma Co. Penicillamine selenotrisulfide (PenSSeSPen) was synthesized from Pen and selenious acid according to a previously described procedure:[28,29] Elemental analysis (%), Anal. Calcd for $C_{10}H_{20}N_2O_4S_2Se$: C 31.97, H 5.33, N 7.46, Se 21.04. Found: C 31.13, H 5.86, N 7.24, Se 21.89. $[\alpha]_D$, +7.07. λ_{max} in deionized water 266 nm (ϵ_{mM}: 1.47). All other chemicals were of commercial reagent or special grades and used as received.

Preparation of HSA−SSeSPen. PenSSeSPen (4 mM) was combined with HSA (40 mg/mL) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated for 10 min, and then the unreacted PenSSeSPen was removed by dialysis against 0.01 M phosphate buffer (pH 7.4) [Spectra/Por Membrane (molecular mass cutoff, 6−8 kDa), Spectrum Laboratory. Inc., CA, USA]. The resulting

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mixture was lyophilized, and a white solid was obtained. The purified HSA−SSeSPen contained 0.29 ± 0.08 selenium per HSA molecule. For the fluorescence labeling of HSA−SSeSPen, the HSA−SSeSPen (40 mg/mL) and an equimolar amount of rhodamine B isothiocyanate (0.6 mM) were combined in 0.01 M phosphate buffer (pH 8), then reacted for 2 h at ambient temperature. After dialysis against deionized water for 24 h, the content in the dialysis bag was subjected to lyophilization.

Measurement of PenSSeSPen by Reversed-Phase Liquid Chromatography (RPLC). The chromatographic conditions for measurement of PenSSeSPen were as follows: column, a COSMOSIL 5C18-AR-II (150 × 4.6 i.d. mm); column temperature, ambient; mobile phase, 0.05 M acetate buffer (pH 4) methanol (9:1 by volume); flow rate, 1 mL/min; detection, UV 270 nm. A linear relationship between the concentration and peak area of the standard PenSSeSPen was obtained over the range from 100 nM to 1 mM (relation coefficient, 0.999). PenSSeSPen to be generated from the reaction of Pen with HSA−SSeSPen was estimated from its peak area.

Determination of Selenium and Protein Concentrations. The selenium concentrations were fluorometrically determined using 2,3-diaminonaphtalene (DAN) after the digestion with a 1:5 mixture by volume of perchloric acid and nitric acid.[30] The selenium standard solution [1000 ppm as selenium (IV) dioxide in 0.5 M nitric acid] for the fluorometry was obtained from Kanto Chemical Co., Inc. The protein concentrations were measured by a BCA protein assay.[31]

Isolation of Red Blood Cells and Treatment with Selenite. Fresh human venous blood was collected in a heparinized VENOJECT II tube (TERUMO, Tokyo, Japan). Each sample was centrifuged at 1400 *g* for 10 min at room temperature, and the plasma, buffy coat, and upper 10% of the RBC layers were removed by aspiration. The precipitated RBCs were washed three times with isotonic phosphate buffer (pH 7.4). The purified RBCs were treated with selenite (8 μM) in isotonic phosphate buffer (pH 7.4) at 37 °C for 10 min [hematocrit 20% (v/v)].

Selenium Binding Experiments. The selenium compounds (PenSSeSPen and HSA−SSeSPen) and HSA were separately dissolved in the indicated medium and then both solutions were combined. The mixture was first incubated in a water bath with shaking at 120 min^{-1}, and then an aliquot was withdrawn from the mixture at appropriate time intervals. After centrifugation at 5800 *g* using an Ultrafree-MC (nominal molecular mass cutoff: 30 000) to remove the HSA, and the PenSSeSPen and selenium concentrations in the filtrate were determined by RPLC and the DAN method subsequent to wet digestion, respectively.

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry. A matrix solution was prepared by dissolving an excess amount of 2,5-dihydroxybenzoic acid (Fluka, USA) in 20% (v/v) ethanol. Sample solutions were mixed with the matrix solution in a 3:1 mixture by volume, and an aliquot was applied to an AnchorChip target (Bruker Daltonics Inc., CA, USA) that was loaded with a 2,5-dihydroxybenzoic acid matrix thin layer. The mass spectra were acquired in the linear positive ion mode using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker Daltonics Inc., CA, USA). Each spectrum was produced by accumulating data from 200 consecutive laser shots. The molecular mass calibration was carried out using the #206195 Peptide Calibration Standard (~ 1−4 kDa) (Bruker Daltonics Inc., USA).

Isolation of Rat Hepatocytes and Their Incubation with Selenium Compounds. A male Wister rat (3−4 weeks old) was sacrificed under ether anesthesia, and its liver was removed after a portal vein injection of 0.05% collagenase (3 mL). The isolated liver was thoroughly rinsed with isotonic phosphate buffer (pH 7.4). The liver tissue was chopped into pieces about 1 mm in diameter and digested in 0.05% collagenase solution at 37 °C until the tissue had almost completely disaggregated. The sample was passed through sterilized cotton-made gauze to remove any undigested tissue. Subsequently, the hepatocyte suspension was centrifuged at 1400 *g* for 5 min at room temperature, and the supernatant and upper 10% of the hepatocytes were removed by aspiration. After washing three times with isotonic phosphate buffer (pH 7.4), the purified hepatocytes (5 × 10^8 cell/mL) were incubated with HSA−SSeSPen (selenium concentration, 8 μM) in the same buffer. An aliquot was withdrawn from the suspension at appropriate time intervals, and then the selenium content of the samples was determined by the DAN method subsequent to wet digestion. Fluorescent labeling of HSA−SSeSPen was carried out using Rhodamine B isothiocyanate according to the conventional procedure.[32]

Statistical Analysis. All data were presented as the mean ± standard error of the mean ($n \geq 5$). Statistical analyses were performed using a PRISM 4 (GraphPad Software, Inc.). Multiple mean values were compared by a one or two-way analysis of variance with a Bonferroni posthoc test. Comparisons were considered statistically significant at $P < 0.05$.

Results and Discussion

Suzuki et al. reported that selenium from selenite in red blood cells (RBCs) reappeared in the plasma and was bound to serum albumin.[15] The selenium transferred to the serum albumin is thought to be subsequently distributed to the peripheral organs and tissues. First, we examined the binding mode of selenium from RBCs to human serum albumin (HSA), and, second, the transfer of the selenium bound to HSA to the isolated rat hepatocytes and renal cells.

After treatment of RBCs with selenite, the selenium efflux from the RBCs to the HSA solutions was examined. Aliquots of the RBC suspensions were withdrawn at the indicated time intervals, followed by centrifugation, and the selenium contents in HSA solutions and RBCs were determined by the DAN method subsequent to the wet digestion (part A of Figure 1). The selenium efflux from the RBC occurred in an HSA concentration-dependent manner. The selenium content in the RBCs gradually decreased for about 60 min, whereas those in the HSA solutions increased in an inverse manner. Such kinetic behavior of the selenium efflux was similar to that in the blood plasma in vitro.[14–16] The HSA molecule has the single free thiol at Cys34 that is the most likely to interact with the selenium species.[3] Pretreatment of HSA with iodoacetamide, a thiol-alkylating agent to form $R-SCH_2CONH_2$, almost completely inhibited the selenium efflux from the RBC to the HSA (45 mg/mL) (part B of

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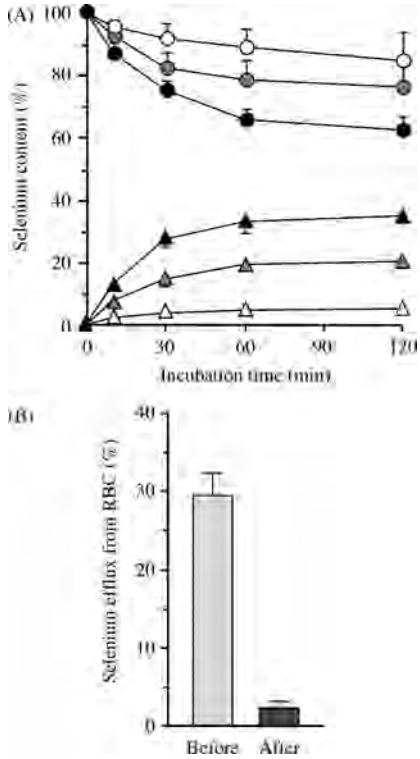

Figure 1. (A) Selenium efflux from red blood cells (RBCs) to human serum albumin (HSA) isotonic phosphate buffer solutions. HSA concentration: 4.5 (white), 22.5 (gray), 45 (black) mg/mL, Triangle: outside RBC, circle: inside RBC. RBCs were preincubated with selenite (8 μM) in isotonic phosphate buffer (pH 7.4) for 10 min to a hematocrit of 20% (v/v). (B) Effect of thiol blockade of HSA on selenium efflux from RBCs to HSA solution. HSA was pretreated with iodoacetamide (5 mM) at 37 °C for 10 min and then thoroughly dialyzed against isotonic phosphate buffer (pH 7.4) to remove residual iodoacetamide [Spectra/Por Membrane (molecular mass cutoff, 6−8 kDa), Spectrum Laboratory. Inc., CA, USA]. The number of thiol groups of HSA before and after iodoacetamide treatment were 0.31 ± 0.03 and 0.03 ± 0.00 SH per HSA molecule, respectively. Iodoacetamide treated-HSA (45 mg/mL) was combined with RBCs pretreated according to the procedure in (A).

Figure 1). Therefore, the thiol group of HSA plays a critical role in the selenium efflux event from the RBC.

The HSA solution after the selenium efflux experiment was subjected to gel permeation chromatographic separation to confirm whether or not the selenium from RBC was bound to the HSA. If the selenium species exported from RBC is a low-molecular-mass material, it is thought to easily separate by this chromatographic separation mode. The peak fraction of the selenium content (fraction #2) was consistent with that of the HSA that was estimated from the absorbance at 254 nm (Figure 2), which demonstrates that the selenium from the RBC is bound to the HSA. This observation was in good agreement with previous reports.[14,16] We tried to directly elucidate the chemical form of the selenium-bound HSA by MALDI-TOF mass spectrometry, but could not clearly detect it. It seems to be due to the small difference in molecular mass between the HSA and the selenium-bound HSA, compared to the molecular mass of the HSA (66.5 kDa).

To obtain information on the binding manner of the selenium from the RBC, the selenium efflux experiment was carried out in a HSA solution (45 mg/mL), and the HSA solution was subsequently treated with penicillamine (Pen).

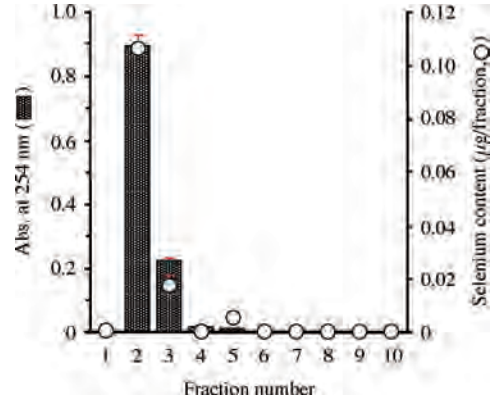

Figure 2. Gel permeation chromatographic analysis of human serum albumin (HSA) solution after the selenium efflux from RBCs. Column: Sephadex G-50 (fine), 20 × 0.7 i.d. cm; mobile phase: isotonic phosphate buffer (pH 7.4); flow rate: 0.3 mL/min; fraction volume: 3 mL.

The chemical structure of Pen only differs by two methyl groups at the β carbon atom from Cys. The Pen molecule can cleave the selenotrisulfide linkage by a thiol exchange reaction, and allows the formation of the chemically stable selenotrisulfide, PenSSeSPen, even at physiological pH.[29,33] When the HSA solution was treated with 1 mM Pen, other selenium-containing species appeared in a separate fraction (fraction #5) that differs from the HSA-containing fractions (fractions #2 and #3) (part A of Figure 3). When treated with a higher Pen concentration (5 mM), the selenium content of this fraction increased, whereas those of the HSA-containing fractions mostly decreased (part B of Figure 3). In addition, when the selenium species generated by the treatment with 1 mM Pen was analyzed by reversed-phase liquid chromatography (RPLC) with UV 270 nm detection, its retention behavior was identical to that of the authentic PenSSeSPen sample (Figure S1 in the Supporting Information). Thus, the selenium exported from RBC was thought to bind to HSA via a selenotrisulfide linkage with its thiol. If so, an unknown material, probably a thiol compound from the RBC, would be released from the HSA together with the selenium by the Pen treatment and would displace Pen due to its higher concentration. We attempted to identify the free unknown material or selenotrisulfide that is only displaced Pen on either side of the selenium by RPLC equipped with a thiol-sensitive electrochemical detector and MALDI-TOF mass spectrometry, but we could not detect them. If the selenodisulfide ($RSSe^-$) is involved in the selenium-bound HSA, it can easily decompose to produce red elemental selenium as the terminal product. However, the selenium-bound HSA solution was fairly stable without developing the red color even when it was kept for two months at 4 °C.

The selenium efflux experiment from the selenite-treated RBC was further conducted using Pen instead of HSA because the free thiol group in the HSA molecule seems to be one of the key elements that result in the selenium efflux from the RBC. Pen brought about the selenium efflux from the RBC, whereas no selenium efflux was observed when

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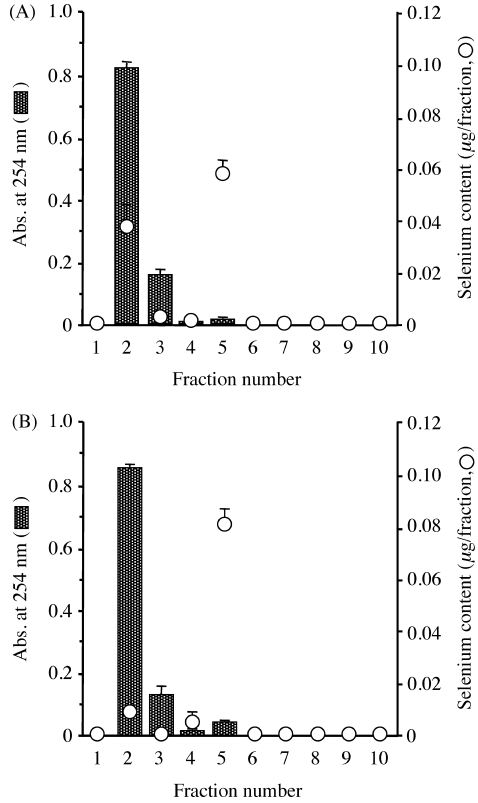

Figure 3. Selenium elimination from the selenium-bound human serum albumin (HSA) by penicillamine (Pen) treatment. HSA solution (45 mg/mL) was incubated with RBC suspension pretreated with selenite (8 μM) for 1 h and then separated from RBCs. The HSA solution was further incubated with Pen [1 (A) and 5 (B) mM] for another 20 min. Chromatographic conditions were the same as employed in Figure 2.

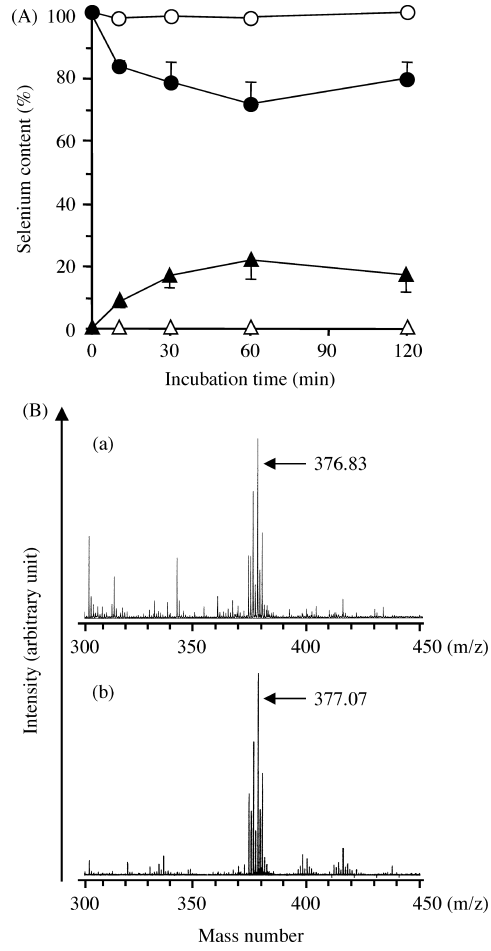

Figure 4. (A) Selenium efflux from red blood cells (RBCs) to penicillamine (Pen, closed) and oxidized Pen (PenSSPen, open) isotonic phosphate buffer solutions. Pen and PenSSPen concns: 1 mM; Triangle: outside RBC; circle: inside RBC. RBCs were preincubated with selenite (8 μM) in isotonic phosphate buffer (pH 7.4) for 10 min to a hematocrit of 20% (v/v). (B) MALDI-TOF mass spectra of selenium-containing species exported from RBC to Pen solution (a) and PenSSeSPen (b). Molecular mass of PenSSeSPen in panel (b) was calculated for $C_{10}H_{20}N_2O_4S_2{}^{80}Se$: 376,24.

the oxidized Pen (PenSSPen) was used (part A of Figure 4). In addition, formation of a selenotrisulfide-containing species (PenSSeSPen) was detected in the Pen solution after the selenium efflux experiment by MALDI-TOF mass spectrometry (part B of Figure 4). These data suggest that the selenium-bound HSA involves a selenotrisulfide-containing species with the free thiol of HSA.

The selenotrisulfide-containing species (PenSSeSPen) was intended to provide a model system for the study of the subsequent selenium transfer from the HSA to the peripherals. A model of the selenium-bound HSA was prepared by the reaction of the HSA with PenSSeSPen. Previously, we reported that PenSSeSPen can react with the reactive thiol at Cys93 of the hemoglobin β chain through a thiol exchange reaction.[29] Incubation of PenSSeSPen with HSA at pH 7.4 resulted in the immediate binding of selenium to the HSA (Figure S2 in the Supporting Information). The binding rate of selenium to the HSA increased with an increase in the PenSSeSPen concentration and reached a plateau (part A of Figure 5), which is consistent with the amount of the thiol group of the HSA used in this study (0.31 SH group per HSA molecule). In addition, the selenium transfer from PenSSeSPen to the HSA was almost completely blocked when this transfer experiment was carried out using the iodoacetamide-pretreated HSA (part B of Figure 5). This result supports the fact that the free thiol of HSA is implicated in the selenium transfer mechanism between the HSA and PenSSeSPen. The content of the thiol group in the reaction mixture was further checked by the DTNB method. The thiol exchange is a reversible reaction and proceeds without changing the content of the thiol group in the reaction mixture [RSSeSR + 2 R′SH ↔ (RSSeSR′ + RSH + R′SH) ↔ R′SSeSR′ + 2 RSH]. The content of the thiol group after the reaction of PenSSeSPen with the HSA was 95.5 ± 1.9% of that from the added HSA. This result also supports that the selenium from PenSSeSPen is bound to the HSA in the form of selenotrisulfide via the thiol of HSA by thiol exchange reaction (HSA−SSeSPen). This selenium-bound HSA was fairly stable without any selenium elimination from the HSA after it was stored in the solution state at 4 °C for three months. Furthermore, the reactivity of PenSSeSPen was also examined in the human blood plasma. After PenSSeSPen was incubated in the plasma for 10 min, the plasma components were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then the selenium contents of the respective bands were fluorometrically determined by the DAN method subsequent to wet digestion. Almost 90% of the selenium from PenSSeSPen was found in the band assigned to the HSA (Figure S3 in the Supporting Information). Such reactivity to

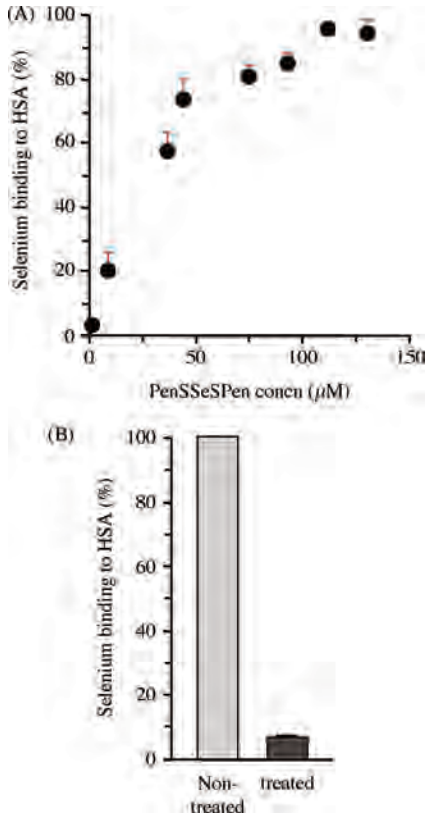

Figure 5. (A) Selenium transfer from penicillamine−selenotrisulfide (PenSSeSPen) to human serum albumin (HSA). HSA (0.45 mg/mL) was incubated with increasing concentration of PenSSeSPen (2−131 μM) for 10 min in 0.01 M phosphate buffer (pH 7.4). The reaction mixture of HSA and PenSSeSPen was ultrafiltered using an Ultrafree-MC (nominal molecular mass cutoff, 5000). The selenium content of the filtrates was determined by the DAN method subsequent to wet digestion. (B) Effect of thio1 blockade on selenium transfer from PenSSeSPen to HSA. The thiol group of HSA was blocked by the same procedure in Figure 1 (the number of thiol groups of HSA, 0.03 ± 0.00 SH per HSA molecule). HSA concn, 0.45 mg/mL; PenSSeSPen concn, 131 μM.

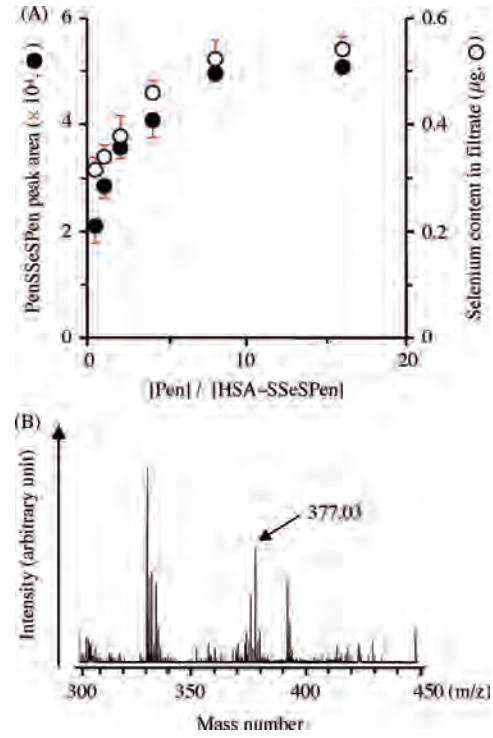

Figure 6. (A) Reaction of the selenium-bound human serum albumin (HSA−SSeSPen) with penicillamine (Pen). HSA−SSeSPen (30 mg/mL as HSA concentration, 0.13 mM as selenium concn) was incubated with various concentrations of Pen (0.25−3 mM) for 10 min in 0.01 M phosphate buffer (pH 7.4). (B) MALDI-TOF mass spectrum of reactant from HSA−SSeSPen and Pen.

PenSSeSPen is probably dependent on the abundance of the HSA in the plasma rather than the specificity of the thiol of HSA for PenSSeSPen because PenSSeSPen allowed the reaction with other thiol compounds, such as GSH, depending on their concentrations.

Because of the reversibility of the thiol exchange reaction,[33] PenSSeSPen was expected to generate from the reaction of the HSA−SSeSPen with Pen, when increasing the molar ratio of Pen to the HSA−SSeSPen. The resulting material of this reaction was analyzed by RPLC and the DAN method after filtration of the reaction mixture. The resultant in the filtrate was chromatographically identical to the authentic PenSSeSPen sample (Figure S4 in the Supporting Information). Its chromatographic peak area increased with the increasing molar ratio of Pen to HSA−SSeSPen. The selenium content in the filtrate also increased in a quite similar fashion. The selenium bound to HSA was almost completely eliminated from HSA−SSeSPen by the incubation with a nearly 10-fold molar concentration of Pen (part A of Figure 6). In addition, the MALDI-TOF mass spectrometric analysis independently revealed the generation of PenSSeSPen in the reaction mixture of HSA−SSeSPen and Pen (part B of Figure 6). Consequently, the selenium from PenSSeSPen can bind to HSA by a thiol exchange between Pen and the thiol of HSA, which produces the selenotrisulfide-containing HSA (HSA−SSeSPen). These data suggest the possibility that the selenium element supplied from selenite can be transferred from RBC to the peripheral organs and tissues through HSA.

The thiol exchange reactions between the selenotrisulfide-containing HSA and thiol compounds on the surface of the cell membranes are quite likely to subsequently occur after the efflux event from the RBC. Thus, the selenium transfer from the HSA−SSeSPen to the peripheral cells was investigated. When HSA−SSeSPen was incubated with isolated rat hepatocytes in Hank's balanced salt solution, the selenium contents in the hepatocyte increased along with a decrease in the selenium content in the incubation medium (part A of Figure 7). A similar selenium transfer from HSA−SSeSPen was also observed for isolated rat renal cells (part B of Figure 7). The possibility of direct selenium transfer from the RBC to the isolated rat hepatocyte not via HSA was further checked: when the selenite-treated RBCs were incubated with the hepatocytes without HSA, no selenium transfer from RBC to the hepatocyte was observed. In addition, no selenium was released from the RBCs in the HSA-free isotonic buffer solution. These facts demonstrate that the selenotrisulfide-containing HSA can transport the selenium to the peripherals.

To examine the mechanism of the selenium transfer to the hepatocytes, the HSA-SSeSPen was fluorescently labeled with rhodamine B (RhoB) through lysine residues in the HSA molecule. After incubation with the RhoB-labeled HSA-

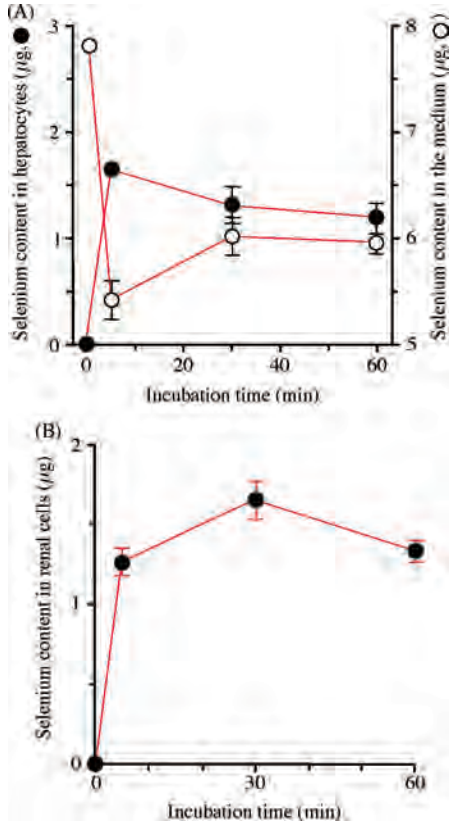

Figure 7. Changes in selenium content of isolated rat hepatocytes (A) and renal cells (B) during the incubation with HSA−SSeSPen. Incubation medium: Hank's balanced salt solution, initial selenium and HSA concentrations in the medium were 10 and 33 μM, respectively.

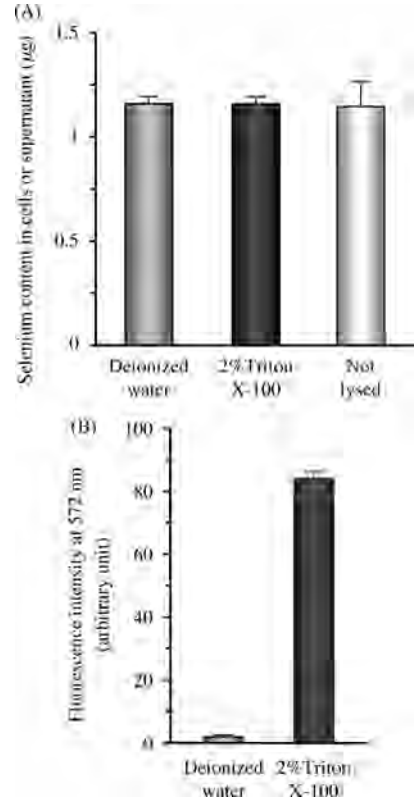

Figure 8. Selenium contents (A) and fluorescence intensity (B) of supernatant of lysate from isolated rat hepatocytes incubated with RhoB-labeled HSA−SSeSPen. Incubation conditions were the same as employed in Figure 7. Excitation wavelength, 543 nm.

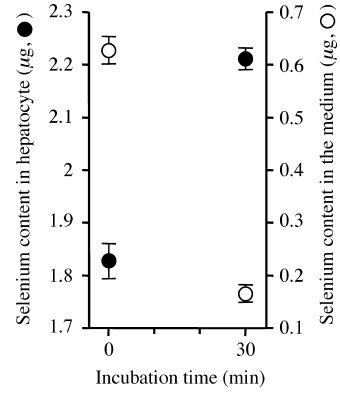

Figure 9. Selenium contents of isolated rat hepatocytes incubated with the selenium-bound HSA from RBC treated with selenite. RBCs were preincubated with selenite (8 μM) in isotonic phosphate buffer (pH 7.4) for 10 min to a hematocrit of 20% (v/v). The RBCs were subsequently incubated in an HSA solution (45 mg/mL) for 1 h. The HSA solution containing selenium exported from RBC was further incubated with a isolated rat hepatocyte suspension for another 30 min. Incubation medium, isotonic phosphate buffer (pH 7.4) throughout the experiments.

SSeSPen for 1 h, the hepatocytes were lysed by the addition of deionized water or Triton-X 100, a nonionic surfactant, plus sonication treatment, and were then centrifuged at 20 000 rpm and 4 °C for 1 h. The Triton-X 100 lysis rendered the hepatocyte suspension completely clear solution (complete cell lysis), whereas deionized water allowed the sedimentation of the plasma membrane portion and some organelles (osmotic cell lysis). The selenium content in the supernatant treated with Triton-X 100 was almost the same as that with deionized water. The supernatants from both the lysed cell samples also contained almost the same selenium amounts as the nonlysed hepatocyte that was incubated with the RhoB-labeled HSA−SSeSPen (part A of Figure 8). Therefore, most of the selenium from RhoB-labeled HSA−SSeSPen was thought to be present in the cytosol. The RhoB fluorescence was observed in the supernatant from hepatocytes lysed with Triton-X 100 but not with deionized water (part B of Figure 8), which means that HSA molecules are not internalized by the hepatocyte. On the basis of all these results, the selenium from HSA−SSeSPen appeared to be taken up into the cytosol after the cleavage from HSA−SSeSPen. Such a selenium transfer event also seems to involve the thiol exchange between the selenotrisulfide in HSA−SSePen and thiol-containing compounds on the membrane surface of the hepatocyte.

To verify these results from our model experiments using HSA−SSeSPen, we conducted the HSA-mediated selenium transfer experiment from RBC treated with selenite to the hepatocytes; the HSA solution was incubated with the selenite-treated RBCs for the selenium efflux, followed by incubation with isolated rat hepatocytes after removal of the RBCs, and the subsequent selenium transfer from the HSA to the hepatocyte was tested. After a 30 min incubation of the hepatocyte in the HSA solution, the selenium bound to the HSA was evidently transferred to the hepatocytes (Figure 9), as shown in Figure 7. The selenotrisulfide-containing HSA was able to transport the selenium element into the hepatocyte.

In the present study, we demonstrated an HSA-mediated selenium transfer; selenium exported from RBC was bound to the thiol of HSA, and then transferable from the HSA into the hepatocyte and the renal cell. This selenium transfer involves a relay mechanism of thiol exchange that occurs between the selenotrisulfide and thiol compounds [selenotrisulfide relay mechanism: RSSeSR + HSA−SH → HSA−SSeSR + R′SH → RSSeSR′]. The reactivity to the HSA is probably dependent on its high concentration in the plasma, not the specificity of the thiol of HSA for selenotrisulfide, because selenotrisulfide allows a reaction with other thiol compounds, such as Pen, depending on their concentrations. Recent evidence in Sel P knockout mice has shown that the distribution of selenium from the liver to testis and brain is mediated by Sel P.[34,35] It is also found that, in the absence of Sel P, selenium from selenite in the diet can be distributed from the liver to the peripherals by a yet unknown mechanism.[36] Taking the transport functions of HSA into consideration, this relay mechanism may play a role in the distribution of selenite metabolites to the peripheral organs. In the course of the HSA-mediated selenium transfer from RBC to the hepatocyte, the selenium element passes through the plasma membranes at least twice; however, its membrane-transport mechanism is not known yet. Recently, we reported that the selenium bound to the hemoglobin in RBC is transferred to the cytoplasmic thiol group of the band 3 protein on the basis of the intrinsic interaction between the two proteins.[37] We are now planning to reveal the mechanism underlying the transport of the selenium in the RBC plasma membrane. The selenotrisulfide relay mechanism may also be extendable to such a membrane transport pathway.

Supporting Information Available: Chromatographic analysis of the reactant from the interaction between HSA−SSeSPen and Pen, interaction of PenSSeSPen with HSA in isotonic phosphate buffer (pH 7.4) at 37 °C, electrophoregram of the blood plasma and selenium content of bands after the incubation of PenSSeSPen in human blood plasma, and chromatographic analysis of the reactant from interaction between HSA-SSeSPen and Pen. This material is available free of charge via the Internet at http://pubs.acs.org.

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