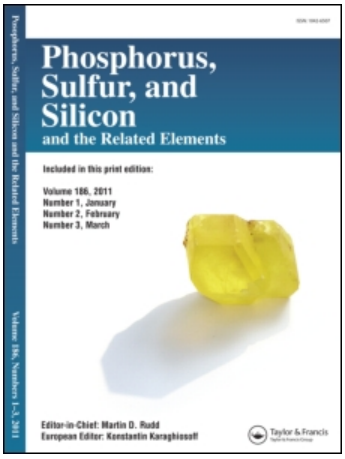

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Metabolism of Selenium and its Interaction with Mercury: Mechanisms by a Speciation Study

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To cite this Article Suzuki, Kazuo T. and Ogra, Yasijmitsu(2001) 'Metabolism of Selenium and its Interaction with Mercury: Mechanisms by a Speciation Study', Phosphorus, Sulfur, and Silicon and the Related Elements, 171: 1, 135 — 169
To link to this Article: DOI: 10.1080/10426500108046631
URL: http://dx.doi.org/10.1080/10426500108046631

Phosphorus, Sulfur, and Silicon, 2001, Vol. 171, pp. 135-169
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Metabolism of Selenium and its Interaction with Mercury: Mechanisms by a Speciation Study

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Metabolic, nutritional and toxicological aspects of selenium (Se) were studied using a hyphenated technique. Se in biological samples was separated by HPLC and Se in the eluate was detected in-line by mass spectrometry with ionization by inductively coupled argon plasma (HPLC-ICP MS). The distributions of Se in the soluble fractions of various organs and body fluids were determined after ingestion or injection of naturally occurring Se or Se enriched with a stable isotope in the form of selenite, selenate or selenomethionine. Metabolic pathways specific to each Se species were discussed based on the results of speciation of each Se metabolite.

Selenite in the bloodstream was taken up by red blood cells and reduced to selenide, and then the reduced form of Se (selenide) was transported to the plasma, where it was bound selectively to albumin and was then transported to the liver. On the other hand, intravenous selenate was taken up directly by the liver. Excess Se derived from any nutritional Se species is mainly excreted in the urine after being methylated in the liver.

The mechanisms underlying the interaction between Se and mercuric ions in the bloodstream were explained by the formation of a ternary complex, $\{(HgSe)_n\}_m$-selenoprotein P (n is the number of (HgSe) complexes and m is the number of the binding sites for the $(HgSe)_n$ complex on selenoprotein P). The complex between Hg and Se in the bloodstream was thus explained by the interaction between their specific chemical species in each metabolic pathway.

The sensitivity of the HPLC-ICP MS method was enhanced with the use of enriched stable isotopes by the simultaneous detection and speciation of both endogenous and exogenous Se.

Keywords: selenium; metabolism of selenium; speciation of selenium; mercury; interaction between selenium and metals; selenoprotein P

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1. INTRODUCTION

Selenium (Se) is an essential micronutrient for normal functioning of the body and constitutes the active center of selenoproteins in the form of the selenocysteinyl (SeCys) residue.[1-3] Se can be present in the form of a metal, salt and/or oxo acid in nature. However, Se expresses its biological activity as the essential element in the form of the selenol (-SH) group in SeCys residues, which is incorporated into selenoproteins through the UGA codon (named the opal codon and is stop codon in the normal translation process)[4-6] and the selenocysteine insertion sequence (SECIS).[7]

Selenoproteins are defined as proteins containing Se in the form of SeCys residues according to the UGA codon, proteins containing Se in the form of selenomethionyl (SeMet) residues not being classified as selenoproteins (FIGURE 1).

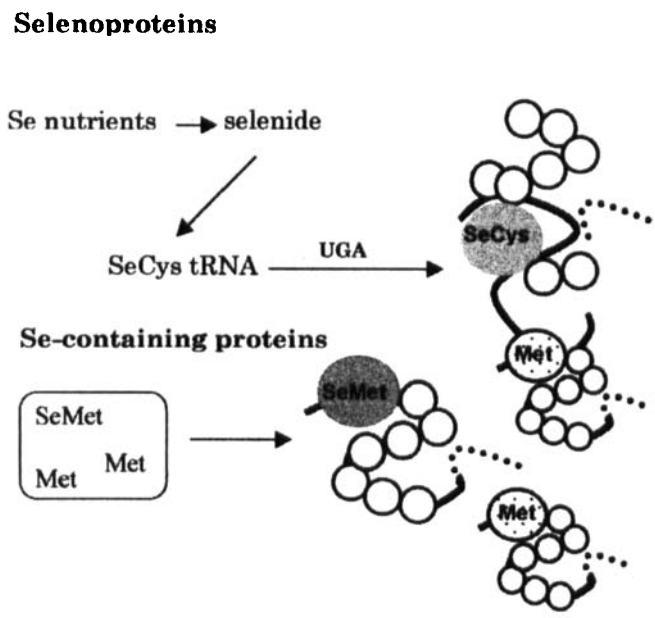

FIGURE 1. Definition of selenoproteins.

The unique metabolic pathways for Se leading to its utilization as selenoproteins and to its excretion as methylated metabolites certainly depend on the intrinsic chemical nature of Se. Se is known to interact with various metals/elements and these interactions seem to also depend on the chemical nature of the Se species in the metabolic pathway.

Se is similar to sulfur (S) in chemical nature although its redox potential is lower than that of S, which makes Se more readily reducible than S, and selenite is reduced to selenide by glutathione (GSH) in vivo and in vitro. The interactions between Se and other elements should be explained by the chemical reactions/interactions between the different

chemical species of Se and interacting metals/elements of specific chemical forms in specific biological environments. The chemical species of Se and metals/elements can be determined by means of hyphenated techniques.

The present paper presents the details of the metabolic pathway for Se based on the Se species and route of administration. The mechanism underlying the interaction between Se and Hg was also elucidated based on the results of our speciation study involving a hyphenated technique, i.e., the combination of separation by HPLC and detection by mass spectrometry with ionization by inductively coupled argon plasma (ICP MS).

2. BACKGROUND OF THE SELENIUM STUDY INVOLVING A SPECIATION TECHNIQUE

2.1. Nutritional aspects of Se

Se is an essential micronutrient with a very narrow range between deficiency and excess, suggesting a small biological pool of it, i.e., the intake of diets containing higher than 0.2 and lower than 2.0 μg Se/g diet is thought to be adequate for experimental animals.[8] Se deficiency in humans is caused by the prolonged intake of regional products of low Se content owing to a geochemically low Se concentration in the soil, as in the case of Keshan disease patients,[9,10] and also by long term total parenteral nutrition (TPN) of low Se content.[11] On the other hand,

excess Se is extremely toxic, probably owing to the production of reactive oxygen species[12] (TABLE 1).

Both inorganic and organic forms of Se can be utilized as nutritional sources.[16] Selenite and selenate are inorganic forms, while SeCys and SeMet are organic forms (FIGURES 2 and 3).

One of the inorganic forms of Se, selenate, is reduced to the other inorganic form of Se, selenite, and then to the key intermediate, selenide. On the other hand, the organic forms of Se, i.e., SeMet, SeCys and their equivalents, are metabolized through the action of β-lyase to give the same key intermediate, selenide.[17,18] Thus, selenide is assumed to be the common key intermediate for both inorganic and organic Se in the metabolism of Se of any dietary source.

TABLE 1. Endemic diseases linked to a deficiency or excess of Se.

Disease	Area	Sympton	
[deficiency]			
Keshan disease	China (Keshan)	cardiac failure	[9]
White muscle disease	New Zealand, China	muscle pain, calcium deposition	[13]
Kashin-Beck disease	Tibet	osteoarthropathy	[14]
[excess]			
Enshi disease	China (Enshi)	loss of hair and nails	[15]

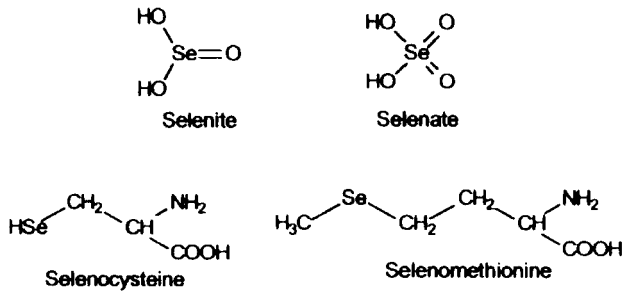

FIGURE 2. Chemical structures of Se compounds.

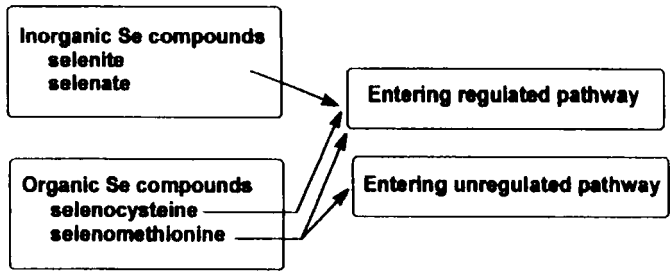

FIGURE 3. Dietary sources of Se.

Selenide is then either utilized for the synthesis of selenoproteins after being transformed to selenophosphate[19,20] and $SeCys^{SeCys}tRNA$[6] or excreted after being methylated,[21-24] as schematically shown in FIGURE 4. Therefore, selenide is the checkpoint compound for either further utilization or excretion in the urine of Se taken up by the body. As a result, selenide is assumed to be the common key intermediate in the metabolism of both organic and inorganic forms of Se, and at the same time it is the checkpoint compound as to the utilization and

excretion of Se, as schematically shown in the metabolic pathway for Se in FIGURE 4.

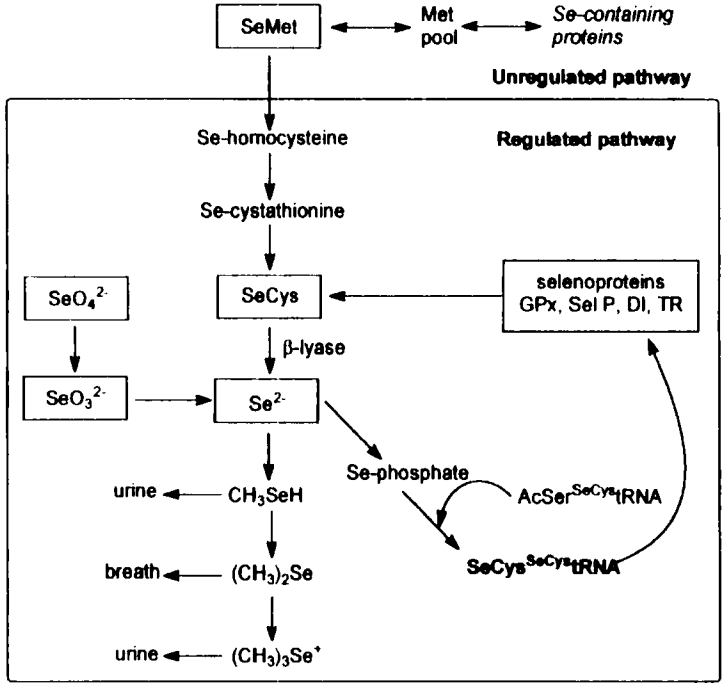

FIGURE 4. Schematic representation of the metabolic pathway for Se.

Apart from the well-regulated metabolic pathway for each Se species, both organic and inorganic Se in the body, as shown in FIGURES 3 and 4, there is an exceptional pathway for SeMet. Se in Se-containing compounds (selenite, selenate, SeCys and SeMet) is metabolized on the recognition of the Se-containing chemicals by the body and these compounds are metabolized to give the common

intermediate selenide in the body owing to their chemical nature as Se-containing compounds. Namely, Se of any nutritional source is utilized or excreted only after the nutritional Se species is transformed to selenide, indicating that only the Se in the nutritional Se species is utilized for the synthesis of selenoproteins.

However, there is an additional metabolic pathway for SeMet. SeMet can be utilized for the synthesis of proteins without being discriminated from methionine (Met), i.e., intact SeMet is incorporated into general proteins according to the same codon and tRNA as those for Met.[25-27] As a result, SeMet in diets can be utilized either as a Se source for selenoproteins after being metabolized to selenide or as a source of intact SeMet instead of Met for general proteins (FIGURE 1). Se incorporated into general proteins in the form of SeMet also can be utilized as the Se source for selenoproteins during the metabolic turnover of general proteins. Therefore, Se that is incorporated into general proteins in the form of SeMet can also act as a biological pool of Se. In other words, only excess dietary Se of SeMet origin can cause elevation of the Se concentration in the body to more than the adequate (homeostatic) level. Thus, Se is essential for normal functioning of the body through its biological action in the form of selenoenzymes.

Se in a nutritional source can be utilized for the synthesis of selenoproteins (selenoenzymes) only after it is transformed to selenide (or its equivalent). Se in selenoproteins is present in the form of SeCys residues and is well regulated by the body. Se present in the form of SeMet in diets can be utilized as intact SeMet residues instead of Met residues (unregulated Se).

2.2. Metabolic aspects of Se

In animals, both inorganic (selenite and selenate) and organic forms (SeCys and SeMet) of Se can be utilized as nutritional sources. Although the Se source for animals including humans is mostly organic forms in usual diets, inorganic forms can also be utilized efficiently.

In plants, the inorganic forms of Se (selenite and selenate) are transformed to the organic forms (SeCys, SeMet and their methylated derivatives). In the case of excessive Se in soil or in accumulator plants, Se accumulates in the form of low molecular weight Se compounds with the masked forms of selenol groups such as SeMet, Se-methylseleno cysteine and selenocystine (CysSe-SeCys).[28, 29]

In animals, the inorganic forms of Se (selenite and selenate) in diets are reduced to the assumed common intermediate selenide, and the organic forms of Se (SeCys and SeMet residues in diets) are transformed by β-lyase to give the same intermediate selenide (FIGURE 4). Therefore, in animals, inorganic and organic forms of Se are transformed to the same key intermediate (selenide) through metabolic pathways specific to each chemical species of Se and route of uptake. Se is, therefore, controlled by the same mechanism once each Se species is transformed to selenide. Selenide is not only the common key intermediate for the metabolism of both inorganic and organic sources in diets, but also the checkpoint compound for the utilization and excretion of Se.

Selenide is transformed to selenophosphate,[19,20] and then the Se is transferred to O-acetylserine^{SeCys}tRNA to give SeCys^{SeCys}tRNA.[4,5] Se is

then incorporated into selenoproteins in the form of SeCys residues according to the corresponding codon to SeCys, UGA and the selenocysteine insertion sequence (SECIS)[7] (FIGURE 5).

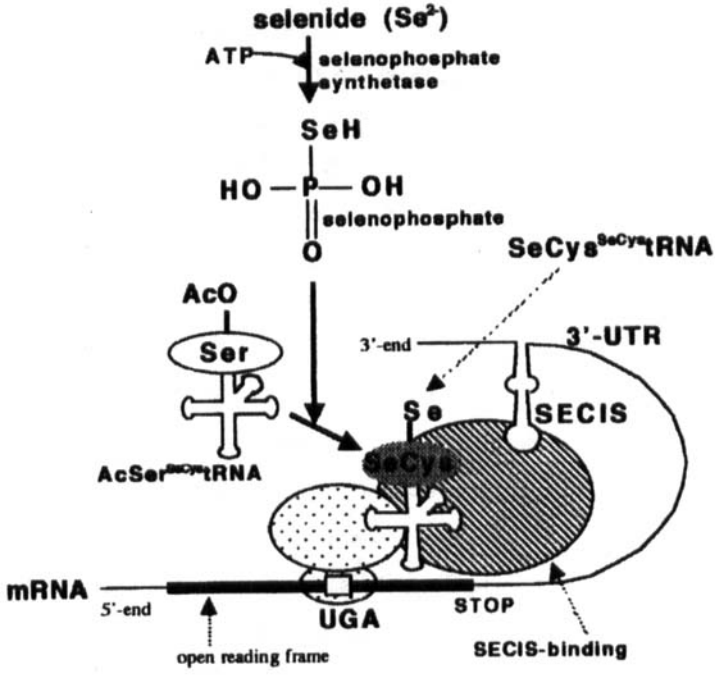

FIGURE 5. Scheme for the incorporation of Se into selenoproteins.

Se is usually excreted in the urine in the form of monomethylated Se. When excess Se is administered, Se is excreted more in the form of trimethylated Se in the urine.[30] However, highly excessive administration of Se leads to exhalation of Se in the form of

dimethylselenide in the breath.[31] The donor of a methyl group for methylated Se is S-adenosylmethionine (SAM), and methyltransferase is assumed to catalyze the transfer of a methyl group at each step to give monomethylselenol (MMSe), dimethylselenide (DMSe), and trimethylselenonium ions (TMSe) [23] (FIGURE 6). Similar stepwise methylation is known for the metabolism of arsenic and methyl transferases specific to each methylation step have been proposed.[32-34] However, in the stepwise methylation of Se, it is not known whether each methylation step is catalyzed by the different methyltransferases or by the same one.

MMSe and TMSe are excreted in the urine, while DMSe is exhaled in the breath. Se is excreted in the breath only when excess Se is loaded. Hence, the metabolic pathway and excretion of Se into the urine and breath have to be explained reasonably. The major urinary form of Se has been reported to be MMSe,[23] and we have also proposed it to be MMSe.[30] However, our recent experiment raised a question as to the chemical structure of MMSe.[35]

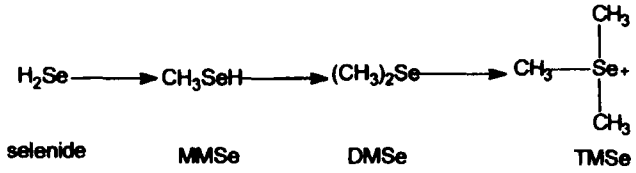

FIGURE 6. Stepwise methylation of Se.

The precise mechanism underlying the excretion of Se in the urine has been studied by means of a speciation method involving HPLC-ICP MS, as explained in FIGURE 11 (Chapter 3.5.).

2.3. Toxicological aspects of Se

Se is an essential micronutrient because of its biological function through the formation of the active center as the selenol group (-SeH) of SeCys residues in selenoenzymes such as glutathione peroxidase (GPx), thioredoxine reductase (TR), and 5'-iodothyronine deiodinase (DI). Only one selenol group (SeCys residue) is present in these selenoenzymes. Se in the body does not seem to be present in the form of free selenol groups except for in these enzymes and selenoprotein P (Sel P), the latter being a plasma selenoprotein containing exceptionally as many as 10 SeCys residues.[36-39] The presence of free selenol groups is harmful because of their high reactivity. In fact, Se in accumulator plants is present in non-reactive forms such as SeMet, Se-methylselenocysteine and selenocystine,[28,29] although these masked forms of Se are not present at detectable levels in mammals.

The assumed common key intermediate selenide is the most reactive form among Se species in the metabolism of Se. The toxicity caused by excess Se seems to have two origins; one is reactive selenol groups, typically in the form of selenide or its equivalent, which are detected in the liver in acid-labile forms or bound non-selectively to proteins when excess Se is administered.[40-42] Selenide may attack disulfide bridges in proteins and convert the disulfide reductively to thiol groups, resulting in conformational changes of proteins. Selenide also forms stable metal selenides similar to metal sulfides, and may remove essential metals as precipitated forms in the body. A reduced form of Se such as selenide and selenol groups is produced easily by thiol groups in

the body such as GSH, and then it can reduce oxygen to give reactive oxygen species (ROS). As a result, excess Se is easily metabolized through the redox reaction in the presence of thiol groups in the body and the unregulated presence of Se produces toxicity.

The toxicity of Se caused by a deficient supply of it is obviously due to the defective synthesis of selenoproteins because of the defective supply of Se for the synthesis of selenoenzymes.

Apart from the toxicity caused by Se itself, it is well known that Se interacts with various metals/elements in the body, and reduces the toxicity of both Se and the interacting metals/elements.[43-45] However, the mechanisms underlying the interactions are not known in most cases. The interaction between Se and mercury (Hg) is the most well-known among the interactions between Se and metals/elements.[46-50] The ternary complex formed between Se of selenite origin, mercuric ions and a plasma protein has been explained by our recent study, the precise mechanism being explained in the present review in Chapter 4.

2.4. Speciation study involving a hyphenated technique, HPLC-ICP MS

Speciation is the technical term for the characterization and quantification of the chemical species of metals/elements. Chemical species in biological samples are separated according to certain principles, and are then detected with a tool specific to the species. Therefore, combined techniques consisting of separation and detection

methods, which are generally named hyphenated techniques, have been recognized as promising speciation methods.[51-53]

Metals/elements can be detected specifically by spectrophotometry such as atomic absorption (AAS) and atomic emission spectrophotometry (AES) or by mass spectrometry (MS) with ionization by inductively coupled argon plasma (ICP MS).[54-58] As these detection tools can be used for samples in aqueous solution without any attachments, gel filtration, ion exchange or affinity HPLC are appropriate as separation tools, and they can be connected in-line with these detection tools, namely, HPLC-AAS, -ICP AES and -ICP MS (FIGURE 7).

Among these element-specific detectors for an HPLC, ICP MS has attracted much attention because of its high sensitivity and the simultaneous detection of multi-elements. In addition, as MS detects elements at the isotope level, tracer experiments can be carried out with the use of enriched stable isotopes as tracers.[59,60] This makes it possible to detect not only endogenous multi-elements simultaneously, but also to detect endogenous and exogenous elements simultaneously with high sensitivity. Furthermore, the use of an ICP MS as the detector for an HPLC allows the speciation of both endogenous and exogenous multi-elements in a single experiment, i.e., simultaneous speciation of endogenous and exogenous multi-elements is possible under identical conditions. Hence, the HPLC-ICP MS method with the use of enriched stable isotopes opens a new chapter in the study of trace elements.

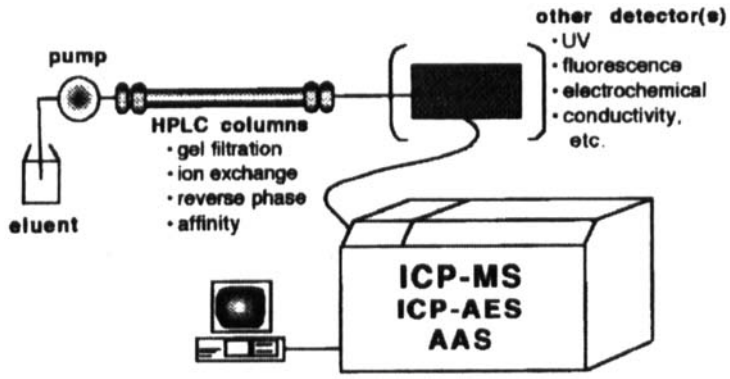

FIGURE 7. Hyphenated techniques for speciation of metals/elements.

2.5. Se research with the speciation technique involving enriched stable isotopes

Se is an ultra micronutrient, and is present in the plasma and liver of rats at concentrations of around 0.5 μg/ml and 1 μg/g wet weight, respectively. Owing to the low concentrations, Se in biological materials is difficult to detect by means of conventional spectrophotometric methods. Se is less detectable than other elements by the mass spectrometric method because of its high ionization potential. As a result, Se is a difficult element in the speciation of chemical forms in biological samples compared with other metals such as copper in terms of the sensitivity in its detection.

Although Se is not detectable enough even with MS, ICP MS is still much more sensitive than the conventional methods employed so far for measuring Se. Chemical forms (species) of Se in biological samples can be determined by detecting Se after separating Se-containing

compounds on an appropriate HPLC column (HPLC-ICP MS method), and this is the most convenient and sensitive method currently available.[61-63]

Enriched stable isotopes are used not only for tracer experiments but also to enhance the detection of the corresponding isotopes. Examples of these merits are described in Chapters 3 and 4.

3. METABOLISM OF Se

3.1. Uptake and distribution of Se in organs of rats depending on age

Se of any chemical species in the nutritional source can be utilized for the synthesis of selenoproteins only through the common key intermediate selenide, as shown in FIGURE 4. However, the metabolism of Se leading to selenide is dependent on the chemical species and the route of intake/uptake.

When rats were fed a diet containing Se solely in the form of ^{82}Se-enriched selenite as the nutritional source of Se, endogenous Se was replaced with exogenous ^{82}Se proportionally with the time of feeding, as shown in the livers of the rats in FIGURE 8.[64] However, the extent of the exchange depended on the age of the rats, and differed from organ to organ. The rate of exchange is higher in younger rats, and becomes lower when rats start to be fed ^{82}Se at older than 8 weeks of age (unpublished data). The rate is different for each organ; the liver

showing the highest and the testes the lowest rate among the organs examined.[64] Close examination of the data indicated that a high exchange rate is not only dependent on the growth rate of an organ but also on the intrinsic properties of the organ. Namely, fast growing organs such as the liver take up more Se than resting organs. However, although the brain weight does not increase or increases only slightly, the endogenous Se was exchanged efficiently with exogenous Se in the brain.[64] It was also indicated that growing rats are more susceptible to a deficiency of Se.

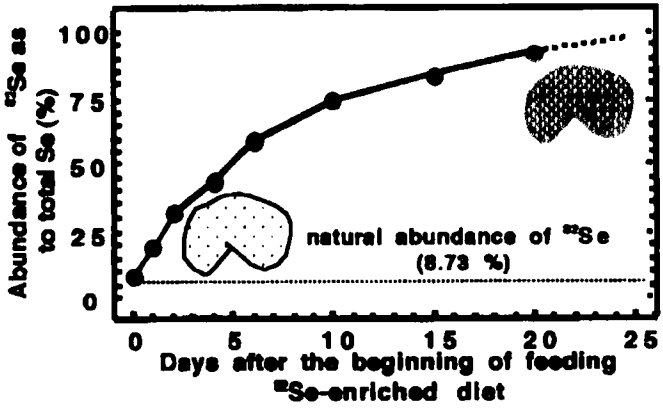

FIGURE 8. Exchange of endogenous Se with exogenous Se of ^{82}Se-enriched selenite in the livers of rats (schematic presentation).

3. 2. Uptake and distribution of Se of selenomethionine origin in diets

When selenite is absorbed in rats, Se of selenite origin is either utilized for the synthesis of selenoproteins or excreted in the urine after

being metabolized into the common key intermediate (selenide) (FIGURE 4). Selenite is not excreted directly in the urine in its intact form. Se of selenite origin remaining in the body is present mostly in the form of selenoproteins. On the other hand, SeMet absorbed in rats is either utilized for the synthesis of general proteins instead of Met in the form of intact SeMet or utilized for that of selenoproteins after being metabolized to the common intermediate (selenide) (FIGURE 3). Se of SeMet residues in general proteins can also be utilized for the synthesis of selenoproteins after being metabolized to selenide, as shown in FIGURE 4.

As Se of selenate origin is utilized after being reduced to selenite,[65] it can be retained in the body only in the form of selenoproteins, as in the case of Se of selenite origin. SeCys absorbed in the body can be utilized for the synthesis of selenoproteins or excreted in the urine only after being metabolized to selenide as well. Intact SeCys is not utilized for the synthesis of proteins. As a result, the concentration of Se in the body is assumed to be that of selenoproteins when the nutritional source of Se is selenite, selenate or SeCys. On the other hand, the concentration of Se in the body is assumed to increase depending on the concentration of SeMet owing to the unregulated incorporation of the intact SeMet into general proteins.[27,66-68]

As to the concentrations of Se, organs/tissues/body fluids can be divided into two groups; in one group they increase depending on the Se concentration in the diet of the SeMet species, while in the other one they do not increase irrespective of the species or concentration of Se in the diet.[27,66] Red blood cells (RBCs) and hair, in which these proteins

do not undergo metabolism, were shown to belong to the former group.[27] On the other hand, liver, kidneys and plasma, in which these proteins are metabolized constantly, were shown to belong to the latter group.

The dietary Se is proportionally excreted in the urine irrespective of the Se species in the diet. On the other hand, the amount of Se in hair depends on the amount and species of the dietary Se, i.e., the concentration of Se in hair increases only with excessive dietary SeMet, as shown in FIGURE 9.[66] These data confirmed that Se of SeMet origin increases more than that of the other three origins because the uptake of intact SeMet is not controlled by Se homeostasis.

Se of SeMet origin is excreted in the form of SeMet residues in general proteins in RBCs and hair, as mentioned above. Hair is known to contain Hg because it is one route for its excretion.[69] It is also well documented that Hg and Se co-exist in the body.[46,47] Efficient excretion of Se of SeMet origin into the hair in our case was further examined as to whether or not there is any interaction between SeMet residues and Hg in the hair. It was concluded that there was no obvious interaction between SeMet residues and Hg (unpublished data).

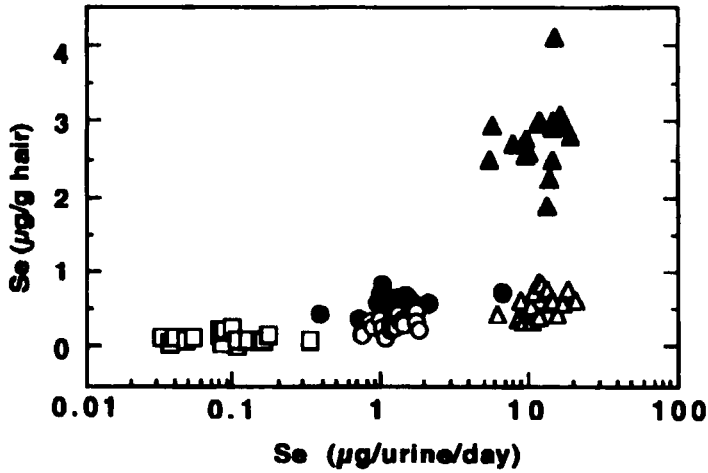

FIGURE 9. The relationship between the amount of Se in urine and the concentration of Se in hair after feeding diets containing different concentrations of Se of selenite and selenomethionine origin.

Male rats of 5 weeks of age were fed a Se-deficient diet for 3 weeks, and then they were divided into 5 groups and fed different diets, i.e. deficient in Se (Se < 0.03 μg/g; open square), or adequate (open symbols) or excess (closed symbols) Se of selenite (circles) or selenomethionine (triangles) origin for 12 weeks. 24 hr-urine was collected every two weeks and hair was cut every two weeks. Each point denotes the amount of Se in 24 hr-urine (μg/day) and the concentration of Se in hair (μg/g).[52]

3. 3. Metabolism of ^{82}Se-selenite in the bloodstream

The metabolic pathway for Se of selenite origin in the bloodstream was elucidated based on the results of a speciation study involving ^{82}Se-enriched selenite and the HPLC-ICP MS method. FIGURE 10 shows the schematic metabolic pathway and mechanism based on the results of in vivo and in vitro experiments, as explained below; ^{82}Se of selenite injected intravenously into rats was taken up by RBCs and disappeared from the plasma within several minutes [70, 71]. However, the ^{82}Se taken up by the RBCs disappeared from the RBCs within 10-20 min without the appearance of ^{82}Se in the plasma. After the disappearance of ^{82}Se from RBCs and plasma in several ten minutes, ^{82}Se started to appear again in the plasma after 30 min and increased with time for the following 24 hr.[35] However, the ^{82}Se was detected at a retention time different from that of selenite, i.e., at the retention time of selenoprotein P (Sel P). Following Sel P, ^{82}Se started to be detected at the retention of eGPx at 3 hr after the injection of ^{82}Se-selenite.[35]

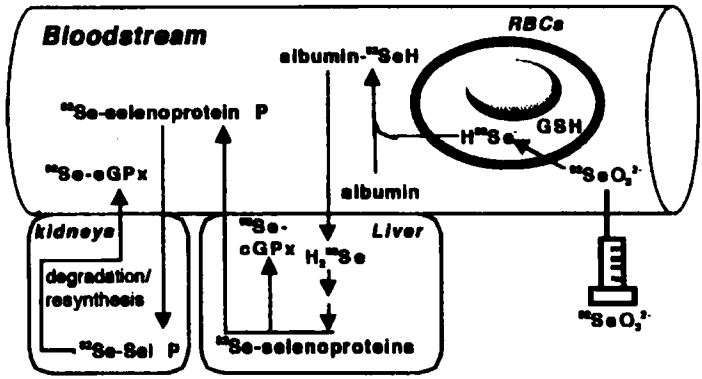

FIGURE 10. Schematic diagram of the metabolism of Se of selenite origin in the bloodstream. cellular glutathione peroxidase (cGPx), extracellular glutathione peroxidase (eGPx), red blood cell (RBC).

The in vivo study mentioned above did not explain how selenide in RBCs is transported and transformed to Sel P and eGPx in the plasma. An in vitro experiment on whole blood revealed that selenite is taken up rapidly by RBCs and then transported to the plasma after being reduced to selenide in the RBCs.[71] The selenide effluxed from RBCs binds selectively to albumin.[42] Then, the ^{82}Se is assumed to be transferred to the liver, where the ^{82}Se is utilized for the synthesis of Sel P, which is excreted into the bloodstream.[35,61,70] eGPx is known to be synthesized in the kidneys and then excreted into the bloodstream.[35,72] The increase in the ^{82}Se peak of eGPx following Sel P in the in vivo study may be explained by the uptake by and degradation of Sel P in the kidneys, followed by the use of the Se for the synthesis of eGPx and excretion into the bloodstream, as shown schematically in FIGURE 10.[35]

Selenate was also injected intravenously into rats.[65] Apart from selenite, selenate is either taken up intact by the liver or excreted directly in the urine without being metabolized. Therefore, it was decided that only selenite is taken up by RBCs and then transformed to selenide before being transferred to the liver. Selenate is not transformed in the bloodstream. The difference may be explained by the high reduction potential for selenate to selenite compared with that for selenite to selenide.

3. 4. Metabolism of Se taken up by the liver

^{82}Se of selenite origin is transferred from the plasma to the liver in the form of selenide or its equivalent.[42] Although the mechanism underlying this transfer is not known yet, the ^{82}Se taken up by the liver is either utilized for the synthesis of selenoproteins or excreted in the urine, as schematically shown in FIGURE 4. Two ^{82}Se peaks were detected for the liver supernatant after an intravenous injection of ^{82}Se-selenite, which were named tentatively peaks A and B according to their order of elution from an HPLC gel filtration column.[65] The faster eluting peak A material was transformed with time into the peak B one. The peak A material was converted in vitro to the peak B one in the presence of a methylation agent (methyl iodide). These in vivo and in vitro results suggest that the peak A material is methylated metabolically to the peak B one. Furthermore, it was found that the peak B material is identical with the major urinary metabolite of Se, suggesting that the peak A

material is methylated to the peak B one in the liver and then the peak B material is excreted in the urine.[65] However, the chemical structures of the two materials, A and B, have not been determined yet.

The ^{82}Se of selenate origin in the liver supernatant was similar to that of selenite origin in the distribution profile on a gel filtration column with the HPLC-ICP MS method.[65] ^{82}Se peaks other than the two major ones, A and B, were not detectable for the liver supernatant even when ^{82}Se-selenate was administered, suggesting that selenate is rapidly reduced to selenite in the liver and then transformed into the peak A and B materials.[73]

The peak A and B materials are assumed to be an intermediate and the final metabolite, respectively, of Se of selenite and selenate origin, leading to the excretion of excessive Se. Contrary to the intermediate leading to excretion, no assumed intermediates leading to the synthesis of selenoproteins have been detected on HPLC-ICP MS chromatography. Sel P is the selenoprotein that is produced most efficiently on the administration of selenite and selenate.

3. 5. Urinary excretion of Se after methylation

Se is mostly excreted in the urine with a normal nutritional diet as to Se and it is exhaled in the breath only when Se is administered in a large excess. The major urinary metabolite of Se with a normal Se diet was once identified as monomethylselenol (MMSe), which is identical with the peak B material in the liver.[65] The deficient, adequate and

excessive Se levels in diets are assumed to be less than 0.02, around 0.2 and around 2.0 μg/g diet for laboratory animals.[27] Se is excreted in the urine in the form of the peak B material (once identified mistakenly as MMSe. But its chemical structure has not been determined yet), and then trimethylselenonium ions (TMSe) increase in the urine after the amount of the peak B material has plateaued.[30] The Se species in the breath is dimethylselenide (DMSe), and it is exhaled in the breath only when a large excess of Se is administered.[31] The proposed scheme for the excretion of methylated Se in the urine and breath is shown in FIGURE 11.[30]

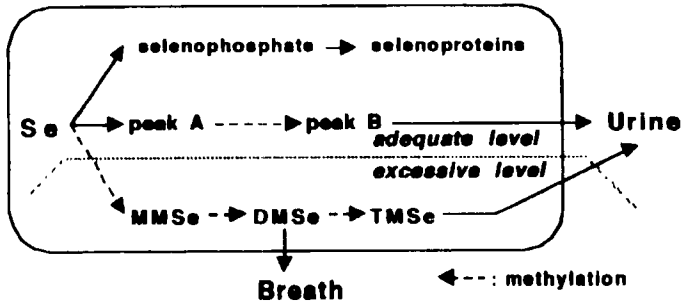

FIGURE 11. Proposed scheme for the stepwise excretion of methylated Se metabolites.

4. BIOLOGICAL INTERACTION OF Se WITH Hg INJECTED INTRAVENOUSLY INTO THE BLOODSTREAM

4. 1. Interaction of Se with Hg injected intravenously into the bloodstream

Se is known to interact with various metals/elements in the body and the resulting interaction alters the toxicity of not only the interacting metals/elements but also Se.[74] The interaction with Hg is the most often mentioned phenomenon. Many types of interaction are possible between Se and Hg depending on their chemical species, i.e., endogenous or exogenous Se, and inorganic or organic Hg. It is also well documented that Hg accumulating in marine mammals is accompanied by Se in the same molar ratio.[46] Hg accumulating in the Hg mine workers is also accompanied by the same molar amount of Se.[47] These observations have called attention to the mechanisms underlying the interaction.

The interaction between Se and Hg in the bloodstream is the most well defined experimentally. Namely, exogenous Se of inorganic Se origin (selenite) and exogenous Hg of inorganic Hg origin (mercuric ions) form a ternary complex of Hg, Se and a plasma protein in the bloodstream when both Hg and Se are injected intravenously into animals at the same time.[75] However, further mechanisms underlying the interaction such as the plasma protein and their stoichiometry have not been elucidated. A new approach has been made with the speciation technique involving the HPLC-ICP MS method on this mechanism.

4.2. Formation of a ternary complex between Se, Hg and selenoprotein P

^{82}Se-Enriched (97 % enriched) selenite and mercuric ions (naturally occurring ^{202}Hg) were injected intravenously into rats in equimolar amounts, and then the distributions of ^{82}Se and ^{202}Hg were determined on a gel filtration column by the HPLC-ICP MS method. The two elements were detected at the same retention time and in the same molar ratio in the chromatogram. This retention time was different from those of Se and Hg injected singly and separately, suggesting that the Se and Hg were different from those injected separately and bound to the same protein in the same molar ratio.[76]

Although these two constituents of the ternary complex were detected simultaneously as ^{82}Se and ^{202}Hg with the HPLC-ICP MS method, the third constituent (the plasma protein) was not detectable. This is because the plasma protein was hardly removed from the ternary complex. As a result, the plasma protein was hard to detect with conventional analytical methods.

Fortunately the plasma protein was determined by chance with the HPLC-ICP MS method. Se was detected not only as the enriched tracer form (exogenous ^{82}Se) but also as the naturally occurring one (endogenous ^{78}Se or ^{77}Se). The latter procedure gave two endogenous Se peaks, i.e., those of the major two plasma selenoproteins (extracellular glutathione peroxidase, eGPx, and selenoprotein P, Sel P), in every chromatogram, as shown in FIGURE 12.[77] It was observed that one of the two major peaks, that of Sel P, shifted to a retention time

higher than the original one when the ternary complex was formed in vivo by administering selenite and mercuric ions intravenously to rats. The possible participation of Sel P in the formation of the ternary complex was confirmed not only in vivo but also in vitro. Furthermore, the mechanisms underlying the interaction were elucidated in detail using the speciation technique.

The three constituents of the ternary complex were assumed to consist of exogenous mercuric ions and selenite, and endogenous Sel P. Therefore, all three constituents can be traced simultaneously with the HPLC-ICP MS method.[52,77] Namely, Hg is not present at a detectable level in normal growing experimental animals and Hg of mercuric ions administered experimentally to rats can be detected easily as a naturally occurring isotope, ^{202}Hg. Se of selenite origin can be traced as ^{82}Se-enriched selenite. Furthermore, Se of Sel P origin can be detected as naturally occurring ^{78}Se or ^{77}Se of Sel P, which contains as much as ten Se atoms (SeCys residues) per molecule, as deduced from the genomic DNA sequence.[38]

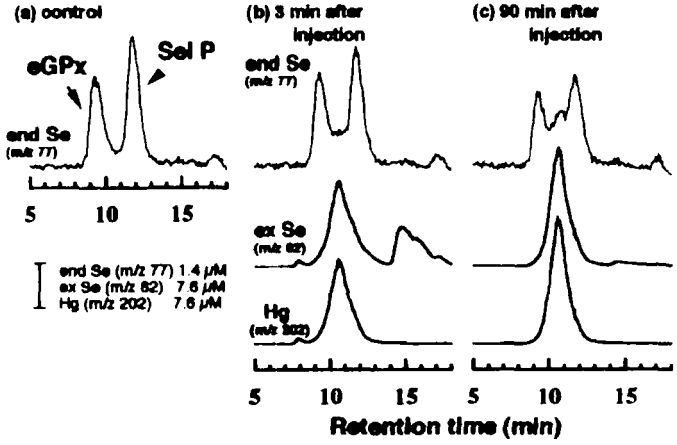

FIGURE 12. Participation of selenoprotein P in the formation of the ternary complex with Se of selenite origin and Hg of mercuric ions in vivo in the bloodstream.

The three isotopes (^{202}Hg, ^{77}Se and ^{82}Se) were detected at their respective retention times on a gel filtration HPLC column with the HPLC-ICP MS method when mercuric ions and ^{82}Se-enriched selenite were injected separately into rats.[78] However, on the simultaneous injection of both mercuric ions and ^{82}Se-enriched selenite, the three constituents appeared at the same retention time in serum (FIGURE 12), indicating that a ternary complex of three constituents was formed. The stoichiometric relationship between the three constituents can be determined readily from their molar ratios.[52,78] The molar ratio of unity between ^{82}Se and ^{202}Hg indicates that Se of selenite origin and Hg of mercuric ions are bound to the ternary complex in the same molar ratio. The molar ratio between ^{82}Se (^{202}Hg) and ^{77}Se suggests the number of

binding sites on Sel P for ^{82}Se and ^{202}Hg. Thus, the stoichiometric relationship between the three constituents can be determined quantitatively and simply by calculating the ratios between the three isotopes with the HPLC-ICP MS method.

In a separate experiment as to the metabolic pathway for selenite in the bloodstream, selenite was shown to be taken rapidly by red blood cells (RBCs),[71] and then reduced to selenide in the presence of glutathione (GSH). The reduced form of Se (selenide) is transported to the plasma, and then binds selectively to albumin on reductive cleavage of one of the disulfide bonds.[42] The Se bound to albumin is then transferred to and taken up by the liver (FIGURE 10).

When mercuric ions are administered to rats simultaneously with selenite, selenite is reduced in RBCs and the reduced form (selenide) is transported to the plasma, where, in the presence of Hg^{2+}, selenide forms a complex with mercuric ions, i.e., the $(HgSe)_n$ complex. The $(HgSe)_n$ complex binds selectively to Sel P in the plasma. However, in the absence of Sel P, the $(HgSe)_n$ complex can bind to other plasma proteins.[76,77]

Selenite is readily reduced to selenide in the presence of GSH, and the $(HgSe)_n$ complex can be formed in vitro in the presence of RBCs, in which GSH - GSSG is recycled in the presence of an energy source. Likewise, the addition of selenite and mercuric ions to plasma produces the same ternary complex, $(HgSe)_n$-Sel P, in the presence of GSH instead of RBCs. In the absence of Sel P or other proteins, the $(HgSe)_n$ complex is precipitated. These observations suggest that the $(HgSe)_n$ complex is formed with a molar ratio of 1:1 and a small molecular size,

and the small $(HgSe)_n$ complex binds to the binding sites on Sel P, suggesting the formation of $\{(HgSe)_n\}_m$-Sel P, where n is the number of unit complexes and m is the number of the binding sites on Sel P.[71] The number of n was calculated to be 100, while m was at maximum 30. The stoichiometric relationship can thus be determined simply by calculating the ratio of isotopes.

4.3. Interaction of Se, transition metals and selenoprotein P to form a ternary complex

Hg is a typical transition metal that forms a stable metal selenide complex, such as mercuric selenide (HgSe), similar to those between transition metals and sulfide. This relationship indicates that transition metals other than Cd^{2+} and Ag^{+} may form a ternary complex in the presence of selenide and Sel P. Likewise, sulfide instead of selenide may participate in the formation of such a ternary complex.[79]

In fact, the production of the (CdSe)-Sel P complex was detected on HPLC-ICP MS in the incubation mixture of selenite/GSH and Cd^{2+} in the plasma.[79] Incubation of selenite/GSH in the plasma with Ag^{+} produced the (AgSe)-Sel P complex. However, the ratio of Ag/Se in the ternary complex differed depending on the ratio in the mixed solution. Namely, ratios of 1 and 2 in the ternary complex were obtained with ratios of Ag/Se = 1 and 2 in the plasma solution: the ternary complexes of either (AgSe)-Sel P and (Ag_2Se)-Sel P, respectively. The addition of Hg^{2+} and sodium sulfide (Na_2S) to the plasma produced the ternary

complex (HgS)-Sel P. Thus, a formation of the ternary complex between transition metals, selenide/sulfide and Sel P is now recognized as a new type of biological interaction for Se.[79]

Summarizing our observations in the present review, the metabolism of Se in the bloodstream and liver, and the mechanisms underlying the interactions between Se and transition metals are to be as shown in FIGURE 13.

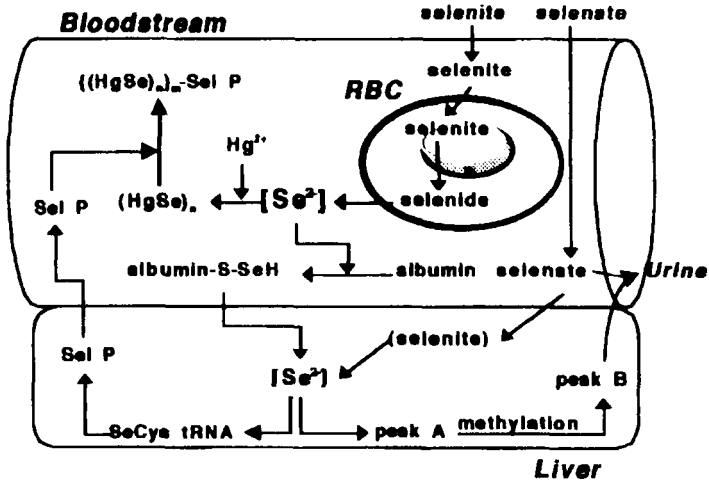

FIGURE 13. Schematic diagram for the metabolism of Se and its interaction with Hg in the bloodstream and liver.

ACKNOWLEDGEMENTS

The present authors wish to thank the co-workers in our references cited in this communication as co-authors for their enthusiastic work in the respective experiments. We acknowledge the

financial support of the Ministry of Education, Science, Sports and Culture, and Takeda Science Foundation.

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