

Metabolism of Selenoamino Acids and Contribution of Selenium Methylation to Their Toxicity

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Selenium (Se) is an essential trace element and a toxicant for animals. Selenocystine (CySeSeCy) and selenomethionine (SeMet), selenoamino acids, are one of the chemical forms in which selenium exists in foods. This review summarized recent studies on the relation of toxicity and metabolism of selenite, CySeSeCy and SeMet in experimental animals. Hepatotoxicity is caused by repeated oral administration of CySeSeCy. CySeSeCy is metabolized by reduced glutathione (GSH) and/or glutathione reductase to hydrogen selenide (H₂Se) via selenocysteine-glutathione selenenyl sulfide (CySeSG). The H₂Se is a key intermediate in the methylation process of inorganic and organic selenium compounds. Accumulation of H₂Se resulting from inhibition of the Se-methylation metabolism, the detoxification pathway of selenium, is found in animals following repeated oral administration of a toxic dose of CySeSeCy. The Se-methylation inhibition is caused by a reduction in the S-adenosylmethionine (SAM) level due to the repression of methionine adenosyltransferase activity. The excess of H₂Se produced by inhibition of methionine adenosyltransferase contributes to the hepatotoxicity caused by CySeSeCy. Moreover, SeMet is now known to be directly metabolized to monomethylselenol (MMSe) as selenide by γ -elimination enzyme in mouse liver. The disturbances in detoxification pathway of Se compounds such as methylation process may be involved in the development of selenosis.

Key words — selenite, selenocystine, selenomethionine, toxicity, hydrogen selenide, Se-methylation

INTRODUCTION

Selenium (Se) is an essential dietary element for health because selenoproteins such as glutathione peroxidase contain a selenocysteine (CySeH) moiety in their active site. Selenium is also toxic at relatively low levels, however, this toxicity means that selenium compounds must be used with caution because of the narrowness between the physiologically required dose and toxic dosage. Therefore, it is important to elucidate the metabolic pathway of selenomethionine (SeMet) in mammals, considering mechanisms of toxicity, detoxification and physiological availability.

A wide variety of selenium compounds exists,

both in organic forms: CySeH, selenocystine (CySeSeCy) and SeMet in agricultural foods and meats, and in inorganic forms: selenite and selenate in drinking water. In daily life, we ingest these selenicals with our ordinary diet. Little is known, however, about the systemic toxicity of CySeSeCy in animals, since reports of such studies have been concerned mainly with the toxicity of selenite and selenate, inorganic selenium compounds.

Selenite is metabolized by glutathione (GSH) or glutathione reductase to hydrogen selenide (H₂Se) via selenodiglutathione and glutathionylselenol intermediates.¹⁾ H₂Se is subject to sequential enzymatical methylation by S-adenosylmethionine (SAM) as methyl donor, resulting in the formation of mono-, di- and tri-methylated derivatives.²⁻⁵⁾ The trimethylselenonium ion (TMSe) is excreted in urine, whereas dimethyl selenide (DMSe) is a volatile product and is exhaled via the lung.⁶⁾ These methylated

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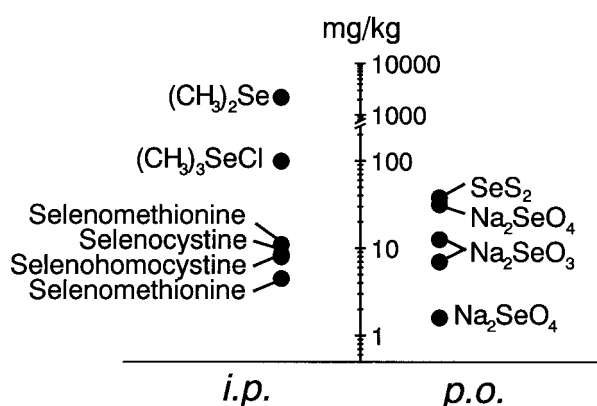


Fig. 1. Oral and Intraperitoneal LD₅₀ Values of Various Selenium Compounds to Rat

selenium metabolites are generally considered much less toxic than the parent compound;⁷⁾ thus the methylation process has been regarded largely as a detoxification mechanism of selenium.

In this review, we describe recent studies on the relation of toxicity and Se–methylation metabolism of selenium compounds, basing upon our research on the toxicity and Se-methylation of selenite and CySeSeCy, and the metabolism of SeMet.

Toxicity of Selenium Compounds

The toxicity of selenium has been known from the early twentieth century when “blind stagger” of livestock was recognized as acute toxicity and “alkali disease” as chronic toxicity following their ingestion of seleniferous grains, grasses and weeds.⁸⁾ “Blind stagger” is an acute disorder and is characterized by signs of central nervous system impairment. Alkali disease, which is characterized by retarded growth, emaciation, deformed hoofs, loss of hair, arthritis, and eventual death, is a chronic disorder.⁸⁾

In animal experiments with rats and mice, chronic symptoms of hepatic damage such as yellow liver atrophy and anemia are recognized significantly following repeated oral administration of selenate or selenite.^{9,10)}

Figure 1 shows the oral and intraperitoneal LD₅₀ values of various selenium compounds to rat. Among selenium compounds, selenoamino acids like CySeSeCy and SeMet are less toxic than inorganic selenium compounds like selenite and selenate. DMSe and TMSe are much less toxic, suggesting the role of methylating pathway in detoxifying Se compounds.

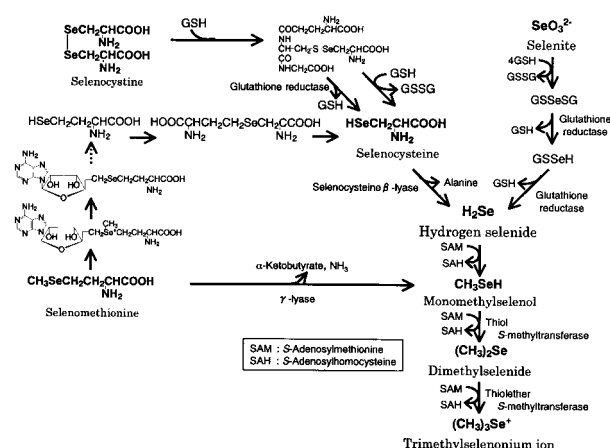


Fig. 2. Metabolic Pathway of Various Selenium Compounds

Possible Metabolic Pathway of Selenium Compounds

The metabolic pathways of selenite, CySeSeCy and SeMet are shown in Fig. 2. It is known that selenite is metabolized in the body by GSH to H₂Se through selenodiglutathione and glutathionylselenol intermediates;^{1,11)} selenate is also metabolized.¹²⁾ DMSe is formed by the methylation of H₂Se, and is subsequently methylated to form TMSe.²⁻⁵⁾ TMSe, which is an end product of inorganic selenium metabolism, was also found in urine from rats treated with CySeSeCy.¹³⁾

The metabolic pathway of CySeSeCy to the Se-methylation process has been elucidated by the authors.^{14,15)} After oral administration of CySeSeCy, selenocysteine–glutathione selenenyl sulfide (CySeSG), selenocysteine–containing metabolite, is first produced by the reaction between CySeSeCy and GSH in the small intestine. In the second step, CySeSG is nonenzymatically reduced to CySeH by excess GSH in the liver. It was also recognized that CySeSG was enzymatically reduced to CySeH by glutathione reductase in the presence of NADPH. In the third step, CySeH is decomposed by selenocysteine β-lyase¹⁶⁾ to H₂Se. These facts suggested that CySeSG may be a stable precursor of H₂Se in animals. The H₂Se is transformed enzymatically to methylated selenium metabolites through the same methylation pathway as selenite.

We report another possible metabolism of SeMet to selenide. SeMet is probably cleaved by γ-lyase in the liver, although the SeMet may be metabolized to H₂Se through a methionine-like metabolism via selenohomocystine and CySeH.¹⁷⁾ Finally, we found that SeMet is enzymatically metabolized by γ-lyase

to form monomethylselenol (MMSe). The MMSe is also enzymatically methylated to form DMSe and TMSe. The detailed metabolisms of CySeSeCy and SeMet are described as follows.

Metabolic Pathway of Selenocystine and Selenomethionine to Methylation Process

Selenocystine^{14,15,18}: The chemical form of selenium-containing metabolites in the small intestine following a single oral administration of CySeSeCy was investigated with ICR male mice. Selenium content in the small intestine of animals treated with 50 mg/kg CySeSeCy significantly increased 15 min, 1 h and 6 h after treatment. In contrast, CySeSeCy significantly depressed the intestinal GSH level 1 h after administration. A significant negative correlation between the selenium level and the level of GSH in the small intestine was observed ($r=-0.83$, $p < 0.001$). Chromatographic analysis of the intestinal metabolites of CySeSeCy by a Sephadex G-25 column showed that selenium-containing metabolites were eluted into two fractions: the low-molecular-weight fraction contained the CySeSeCy, while the high-molecular-weight fraction contained CySeH-containing metabolite. When CySeSeCy or CySeH was reacted directly with excess GSH in the presence of intestinal cytosol, the CySeH-containing metabolite was eluted in the high-molecular-weight fraction in the Sephadex G-25 chromatogram. This metabolite was detected also in plasma and liver cytosol of mice after oral administration of CySeSeCy. These results suggested that CySeH-containing metabolite is produced by the reaction of CySeSeCy with excess GSH in the small intestine, and that this metabolite is then transported to the liver through plasma.

The CySeH-containing metabolite in intestinal cytosol of mice treated with CySeSeCy (50 mg/kg) was identified as CySeSG by high performance liquid chromatography using a gel filtration and reversed phase column. H₂Se formation was caused by anaerobic reaction between the CySeSG and liver cytosol containing selenocysteine β -lyase, which specifically acts on CySeH.¹⁶ The CySeSG was non-enzymatically reduced to CySeH by excess GSH in the liver cytosol, or enzymatically reduced to CySeH by glutathione reductase in the presence of NADPH. Thus, CySeSeCy is metabolized to CySeSG, which has a molecular weight of 473; and the CySeSG is then reduced by excess GSH and/or glutathione reductase to CySeH, which is decomposed by selenocysteine β -lyase to H₂Se.

*Selenomethionine*¹⁹: SeMet will be metabolized to CySeH in a similar pathway like methionine,¹⁷ and CySeH is decomposed to H₂Se by selenocysteine β -lyase. H₂Se is further methylated to MMSe, DMSe and TMSe. It was found that TMSe is produced quickly in mouse liver after oral administration of SeMet, in spite of many metabolic steps from SeMet to CySeH in the methionine-like metabolism. A hypothesis was then put forward that an enzyme which catalyzes the γ -elimination of SeMet, such as L-methionine γ -lyase (EC.4.4.1.11)²⁰ from *Pseudomonas putida*, exists in mouse liver, and an investigation was conducted to identify the metabolic pathway. Periodate-oxidized adenosine (PAD) and propargylglycine (PPG), the inhibitors of the enzymes involved in SeMet metabolism, were used. If γ -elimination enzyme exists in mouse liver, α -ketobutyric acid and MMSe might be produced from SeMet. When the S9 fraction from liver of PAD-pretreated mice in which only the metabolic pathway *via* CySeH was inhibited was incubated with SeMet, the formation of α -ketobutyric acid was observed. On the other hand, the amount of α -ketobutyric acid generated significantly decreased when SeMet was reacted with the S9 fraction from liver of PPG-pretreated mice in which both pathways were inhibited. In mice pretreated with PAD or PPG, oral administration of SeMet did not cause formation of TMSe in the liver. The amount of SeMet in liver from PAD-pretreated mice decreased, and the amount of acid-volatile Se derived from MMSe increased gradually. Such phenomena were not observed in the PPG-pretreated mouse group. These results indicate that SeMet is directly metabolized to MMSe by γ -elimination enzyme (γ -lyase) in mouse liver, and this may contribute to the quick metabolism of SeMet to form TMSe.

Contribution of Selenium-methylation Inhibition to Toxicity of Selenium Compounds^{13,21}

Relation of Se-methylation and toxicity of selenium compounds was investigated using ICR male mice treated with CySeSeCy as selenoamino acid. To elucidate the Se-methylation mechanism, animals received a single oral administration of CySeSeCy (5, 10, 20, 30, 40 or 50 mg/kg). Both the accumulation of total selenium and the production of TMSe as the end-product of methylation were increased in the liver by the dose of CySeSeCy. A negative correlation was found between production of TMSe and level of SAM as methyl donor. The relationship between CySeSeCy toxicity and Se-methylation was

determined by giving mice repeated oral administrations of CySeSeCy (10 or 20 mg/kg) for 10 days. Animals exposed only to a high dose incurred hepatic damage, showing a significant rise of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma. Urinary total selenium increased with CySeSeCy dose. TMSe content in urine represented 85% of total selenium at the low dose and 25% at the high dose. The potential of Se-methylation, the activity of methionine adenosyltransferase as the enzyme responsible for SAM synthesis, and the level of SAM in the liver were determined. The high dose resulted in inactivation of Se-methylation and a decrease in SAM level due to the inhibition of methionine adenosyltransferase activity. To elucidate whether hepatic toxicity of CySeSeCy is potentiated by depressed Se-methylation ability, mice were injected intraperitoneally with PAD (100 μ mol/kg), a known potent inhibitor of the SAM-dependent methyltransferase, 30 min before oral treatment of CySeSeCy (10, 20, or 50 mg/kg). Hepatic toxicity induced by CySeSeCy was enhanced by inhibition of Se-methylation. These results suggest that TMSe was produced in mouse liver following oral administration of CySeSeCy by SAM-dependent methyltransferase, which is also true of those involved in the methylation of inorganic selenium compounds such as selenite, in the liver of mice orally administered CySeSeCy. Depression of Se-methylation ability resulting from inactivation of methionine adenosyltransferase and enzymatical Se-methylation was also found in mice following repeated oral administration of a toxic dose of CySeSeCy. The excess selenide accumulating during the depression of Se-methylation ability may be involved in the hepatic toxicity caused by CySeSeCy.

These facts suggest that the excess selenide produced by the depression of Se-methylation in the liver also contribute to the hepatic damage in animals exposed to selenate, selenite, SeMet or CySeSeCy.

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