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# Evaluation of the metabolic chiral inversion of D-selenomethionine in rats by stable isotope dilution gas chromatography–mass spectrometry

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ABSTRACT

The stereoselective pharmacokinetics of selenomethionine enantiomers in rats has been studied to evaluate the chiral inversion of D-selenomethionine to the L-enantiomer. After bolus intravenous administration of D- or L-selenomethionine to rats, the plasma concentrations of these two enantiomers were determined by stereoselective gas chromatography–mass spectrometry with selected ion monitoring. This method involved derivatization of selenomethionine enantiomers with HCl in methanol to form methyl ester followed by *N*-acylation with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride to form the diastereomeric amide, and separation of the diastereomer on GC with an achiral column. Plasma concentrations of administered D- and L-selenomethionine declined with terminal half-lives of 96 ± 17 min and 91 ± 6 min, respectively. L-Selenomethionine appeared rapidly in plasma after administration of D-selenomethionine, whereas D-selenomethionine was not detected in plasma after administration of L-selenomethionine. The fraction of conversion of D-selenomethionine to L-selenomethionine was estimated to be 61.3 ± 14.5%. The present method evaluates the stereoselective pharmacokinetics of selenomethionine enantiomers, including the estimation of the metabolic chiral inversion.



## 1. Introduction

Selenium is recognized as an essential element for humans. However, it is also known to be highly toxic, and the boundary between innocuous and toxic levels is extremely narrow [1]. Various forms of selenium, such as selenite, selenate, selenocysteine and selenomethionine can be used as nutritional sources [2–4]. Since selenomethionine is more effective and less toxic than inorganic selenium [5], synthetic selenomethionine or its enriched food sources are appropriate supplemental forms of selenium. Selenomethionine also serves as a pharmacological agent against methylmercury induced neurotoxicity [6–8] and cisplatin induced nephrotoxicity [9,10].

Selenomethionine exists as its D- or L-enantiomer, but the potential pharmacological differences of selenomethionine enantiomers to mitigate the toxicity of hazardous agents have not been thoroughly investigated. L-Selenomethionine is more easily assimilated into the body, although it may also be more toxic than the D-form [11]. Rao et al. [9] revealed in rats that pretreatment with D-selenomethionine reduced the nephrotoxicity of cisplatin without reducing its antitumor activity compared with pretreatment with L-selenomethionine. Recently, Moreno et al. [12] reported that D- and L-selenomethionine counteract the toxicity of methylmercury in *Chlorella sorokiniama*. They suggested that D-selenomethionine was not directly involved in detoxification against methylmercury toxicity, but could be transformed into L-selenomethionine to be active against methylmercury toxicity. Some nutritional studies in human have indicated there is negligible difference in the bioavailability of D- and L-selenomethionine, and these studies also suggested that D-selenomethionine is converted to the L-enantiomer in human and rats [11,13,14].

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However, minimal information is available on the metabolic fate of D-selenomethionine, in particular, its conversion into the L-enantiomer.

We have initiated studies to characterize the stereoselective pharmacokinetic behaviors of selenomethionine enantiomers. In a previous paper, we reported a procedure for determining the concentrations of selenomethionine enantiomers in plasma [15]. This method involved derivatization of selenomethionine enantiomers with HCl in methanol to form methyl ester followed by *N*-acylation with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) to form diastereomeric amide (MTPA-OMe) derivative, and separation of the diastereomeric amide on GC with an achiral column. We have also synthesized DL-[$^{2}H_3$, $^{82}$Se]selenomethionine for use as an analytical internal standard [15,16].

The aim of this study was to evaluate the pharmacokinetic behaviors of selenomethionine enantiomers and determine the fraction of metabolic inversion of D-selenomethionine to L-selenomethionine in rats after intravenous administration of D-selenomethionine.

## 2. Materials and methods

### 2.1. Reagents

DL-Selenomethionine was purchased from Wako Pure Chemical Industries (Osaka, Japan). (*S*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) and 10% HCl/methanol were purchased from Tokyo Kasei (Tokyo, Japan). A strong cation-exchange solid-phase extraction column BondElut SCX ($H^+$ form, size 1 mL/100 mg) was purchased from Agilent (Santa Clara, CA, USA). Chloroform stabilized with amylene was purchased from Kanto Chemicals (Tokyo, Japan). DL-[$^{2}H_3$,$^{82}$Se]Selenomethionine was synthesized in our laboratory according to previously described methods [15,16]. All other chemicals and solvents were of analytical-reagent grade and were used without further purification.

### 2.2. Resolution of DL-selenomethionine

A solution of DL-selenomethionine (1.03 g, 5.3 mmol) in 20 mL of acetic acid was refluxed for 5 min, followed by the addition of acetic anhydride (3 mL, 31.5 mmol). The reaction mixture was allowed to reflux for 5 min and then left at room temperature for 2 h. After removal of the solvent under reduced pressure, the residual syrup was treated with water (5 mL × 2) followed each time by evaporation to yield *N*-acetyl-DL-selenomethionine (1.01 g, 87.6%) as a colorless solid.

The *N*-acetyl derivative (1.01 g, 4.2 mmol) was suspended in 20 mL of water, followed by the addition of 1 M aqueous ammonia until the *N*-acetyl derivative was dissolved and the pH of the solution reached 7.6. Porcine kidney acylase was added in two portions (10 and 5 mg) at 24 h intervals, and then incubated at 37 °C for 48 h. The enzymic hydrolysis was terminated by acidification with concentrated HCl to pH 4. After the protein was filtered with the aid of charcoal, the filtrate was acidified to pH 2 with concentrated HCl. The solution was subjected to cation-exchange column chromatography (Dowex 50 W X8, $H^+$ form, 100 mm × 10 mm i.d.). The column was washed with water until the washings were neutral pH and then eluted with 200 mL of 1 M aqueous ammonia. The eluate was evaporated under reduced pressure. A small portion of water was added to the residue and the pH was adjusted to 5 with acetic acid. The solution was evaporated under reduced pressure and the residue was recrystallized with water–ethanol to yield L-selenomethionine (364 mg, 86.8%) as a colorless solid. Anal. Calcd. for $C_5H_{11}NO_2Se$: C, 30.62; H, 5.65; N, 7.14. Found: C, 30.53; H, 5.57; N, 7.00.

The washings described above were evaporated under reduced pressure, followed by evaporation to yield *N*-acetyl-D-selenomethionine (502.6 mg) as a colorless solid. The compound (496.0 mg, 2.1 mmol) was dissolved in 10 mL of 2 M HCl and the solution was refluxed for 3 h. The solution was evaporated under reduced pressure, the residue was dissolved in water and the pH of the solution was adjusted to 5 with 1 M aqueous ammonia. The solution was evaporated under reduced pressure and the residue was recrystallized with water-ethanol to yield D-selenomethionine (201 mg, 48.2%) as a colorless solid. Anal. Calcd. for $C_5H_{11}NO_2Se$: C, 30.62; H, 5.65; N, 7.14. Found: C, 30.42; H, 5.72; N, 6.99. The enantiomeric purity of D- and L-selenomethionine was determined by derivatization to the MTPA-OMe derivative followed by GC–MS according to our previously published method [15]. Both enantiomers were found to be >99.9% (e.e.).

### 2.3. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at Juntendo University School of Medicine (No. 250142) and Tokyo University of Pharmacy and Life Sciences (P12-04). Male Sprague-Dawley rats aged 7 weeks old were obtained from Japan SLC (Hamamatsu, Japan) and were maintained at the Tokyo University of Pharmacy and Life Sciences. They were kept under a 12-h light:12-h dark cycle at 22–24 °C. All rats were acclimatized for at least 7 days, during which time they had free access to water and food (CE-2, Clea Japan, Tokyo, Japan).

### 2.4. Dose experiments

After an overnight fast, rats were anesthetized with sodium pentobarbital (50 mg/kg weight, intraperitoneally). D-Selenomethionine (20 μmol/kg body weight) or L-selenomethionine (20 μmol/kg body weight) dissolved in saline (0.5 mL dosing solution/kg body weight) was administered into the femoral vein. Heparinized blood samples (150 μL) were obtained 0.5, 1, 3, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240, 300 and 360 min after dosing. The blood was centrifuged to separate plasma at 1000 × g for 10 min. The plasma was stored at −20 °C until analysis.

### 2.5. Sample preparation

To 50 μL of rat plasma in a polypropylene microtube (1.5 mL) were added DL-[$^{2}H_3$, $^{82}$Se]selenomethionine (10.3 nmol in 100 μL) as an analytical internal standard. The plasma sample was deproteinized and extracted with ethanol (1 mL) with vigorous mixing for 30 s. After centrifugation at 1000 × *g* for 10 min, the supernatant was transferred into another polypropylene microtube and evaporated at 40 °C under a stream of nitrogen. The residue was dissolved in 1 mL of 40 mmol/L HCl and then applied to a BondElut SCX cartridge. The cartridge was prewashed and activated with 3 mL of methanol, 3 mL of a mixture of methanol and 0.1 mol/L HCl (1:1, v:v), and 3 mL of 0.1 mol/L HCl. The cartridge was washed with 1 mL of water and 1 mL of methanol, and then eluted with 0.5 mL of 10% HCl in methanol into a PTFE-lined screw-cap conical centrifuge tube (100 mm × 16 mm i.d.). The eluent was heated directly at 60 °C for 1 h. After removal of the solvent under a stream of nitrogen, the residue was reconstituted in 100 μL of 2% triethylamine in chloroform and 100 μL of 2% MTPA-Cl in chloroform, shaken for 30 s on a vortex mixer and left at room temperature for 1 h. After the reaction mixture was diluted with 1 mL of chloroform and washed with water (1 mL × 2), the solvent was evaporated at room temperature