Synthesis of D- and L-Selenomethionine Double-Labeled with Deuterium and Selenium-82

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The synthesis of D- and L-selenomethionine labeled with ^{82}Se and three deuteriums at Se-methyl group (D- and L-[$^{2}H_3$, ^{82}Se]selenomethionine) was described. D- And L-[$^{2}H_3$, ^{82}Se]selenomethionine were prepared by condensation of (*R*)- and (*S*)-2-amino-4-bromobutylic acid with lithium [$^{2}H_3$, ^{82}Se]methaneselenolate, which was prepared from metal ^{82}Se and [$^{2}H_3$]methyl iodide. The optical purities of D- and L-[$^{2}H_3$, ^{82}Se]selenomethionine were determined by HPLC with a chiral stationary phase column and were found more than 99% ee. The chemical ionization mass spectra showed that the molecular related ion for *N*-isobutyloxycarbonyl ethyl ester derivatives of [$^{2}H_3$, ^{82}Se]selenomethionine did not overlap with the *m*/*z* values known from that of non-labeled selenomethionine.

Key words selenomethionine; D-amino acid; stable isotope; Selenium-82; deuterium; GC-MS

Selenium has been recognized as an essential element of human nutrition. Various forms of selenium, such as selenite, selenate, selenocysteine and selenomethionine, can be utilized as nutritional sources.[1—4] Since selenomethionine is more effective and less toxic than inorganic selenium, synthetic selenomethionine or its enriched food sources are appropriate supplemental forms of selenium. David *et al.*[5] reported that some formula contained racemic selenomethionine. McAdam and Levander[6] showed little difference in the acute toxicity and nutritional bioavailability between D- and L-selenomethionine in rats and suggested that D-selenomethionine might be converted into the L-enantiomer. L-Selenomethionine is transformed to L-selenohomocysteine, similarly to the de-methylation pathway for L-methionine to L-homocysteine. Then, L-selenohomocysteine is re-methylated to reform L-selenomethionine, or condensed with L-serine to form L-selenocystathionine, which is transformed to L-selenocysteine. However, little information is available on the metabolic fate of D-selenomethionine, especially conversion of D-selenomethionine into the L-enantiomer.

In our previous study, the use of stable isotope labeled D-methionine and the stereoselective gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) method[7] proved to be a powerful methodology for examining the pharmacokinetic behavior of exogenously administered D-methionine and for studying the conversion of D-methionine into the L-enantiomer. We have shown that almost all D-methionine exogenously administered were converted into the L-enantiomer in rats.[8]

We have initiated studies to characterize the pharmacokinetic behavior of selenomethionine enantiomers by the stable isotope methodology. Successful application of the methodology to the metabolic investigation is dependent upon the availability of compounds labeled at predesigned positions. Selenium have six naturally occurring isotopes [^{74}Se (0.89%), ^{76}Se (9.37%), ^{77}Se (7.63%), ^{78}Se (23.77%), ^{80}Se (49.61%) and ^{82}Se (8.73%)], which give rise to a cluster of isotope peaks in mass spectrometry. To avoid the interference of the isotope clusters, we have chosen to introduce three deuteriums and ^{82}Se into the Se-methyl group of selenomethionine. Moreover, it has become feasible to investigate the extent of conversion of D-selenomethionine into the L-enantiomer without considering the transmethylation cycle (de-methylation and re-methylation), because L-[$^{2}H_3$, ^{82}Se]selenomethionine and the reformed L-[^{82}Se]selenomethionine could be distinguished from each other by GC-MS-SIM.

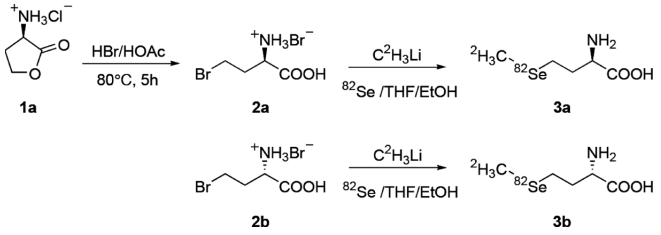

Chart 1. Synthesis of D- and L-[$^{2}H_3$, ^{82}Se]Selenomethionine

The present paper describes the preparation of optically pure D- and L-selenomethionine double-labeled with three deuteriums and ^{82}Se.

Results and Discussion

Convenient synthetic routes to selenomethionine labeled on selenium[9—11] or methyl group[12] have been published, but the synthesis of deuterium and ^{82}Se double-labeled selenomethionine has not been reported. With few exceptions, the synthesis of Se-labeled selenomethionine had been achieved by treating 2-amino-4-bromobutanoic acid with a labeled lithium methaneselenolate.

The synthetic route to D- and L-[$^{2}H_3$, ^{82}Se]selenomethionine is illustrated in Chart 1. 2-Amino-4-bromobutanoic acid, a key intermediate in this synthesis, was obtained by either ring opening of 2-amino-4-butyrolactone (homoserine lactone) with HBr[13] or bromination of 2-amino-4-hydroxybutanoic acid (homoserine) with HBr in AcOH.[14] We have synthesized (*R*)-2-amino-4-hydroxybutanoic acid (**2a**) from commercially available D-homoserine lactone (**1a**) yielding

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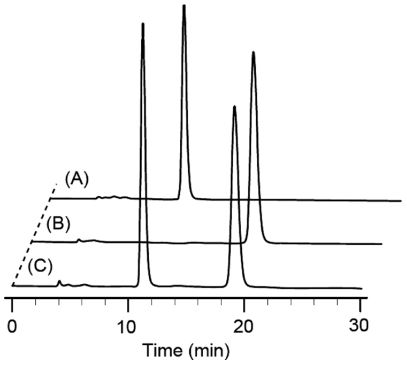

Fig. 1. HPLC Tracings of D-[$^2H_3$, ^{82}Se]Selenomethionine (A), L-[$^2H_3$, ^{82}Se]Selenomethionine (B) and Non-labeled DL-Selenomethionine (C)

Column: Crownpak CR (150×4 mm i.d.), mobile phase: 0.06% $HClO_4$, flow rate: 0.3 ml/min, temperature: 25 °C, UV detector: 200 nm.

91% using the ring opening method with minor modification. Selenomethylation of compound (**2a**) with lithium [$^2H_3$, ^{82}Se]methaneselenolate, which was prepared from ^{82}Se metal and [$^2H_3$]methyl iodide, gave D-[$^2H_3$, ^{82}Se]selenomethionine (**3a**) in 47% yield. Similarly, L-[$^2H_3$, ^{82}Se]selenomethionine (**3b**) was prepared by selenomethylation of commercially available (*S*)-2-amino-4-bromobutylic acid (**2b**) with lithium [$^2H_3$, ^{82}Se]methaneselenolate in 54% yield.

^{1}H-NMR data for D- and L-[$^2H_3$, ^{82}Se]selenomethionine (**3a** and **3b**) were identical to their corresponding non-labeled selenomethionine, except for the absence of signals of Se-methyl protons. The enantiomeric purity of D- and L-[$^2H_3$, ^{82}Se]selenomethionine were determined by HPLC with a chiral stationary column (Crownpak CR) eluted with 0.06% $HClO_4$. Under these conditions, HPLC analysis of DL-selenomethionine provided baseline separation at the retention time of 11.2 min (D-form) and 19.8 min (L-form) as shown in Fig. 1. Both enantiomers were found to be >99% (ee).

Figure 2 shows the chemical ionization mass spectra for *N*-isobutyloxycarbonyl ethyl ester derivatives of non-labeled selenomethionine, D-[$^2H_3$, ^{82}Se]selenomethionine and L-[$^2H_3$, ^{82}Se]selenomethionine. The molecular related ion $[M+H]^+$ clusters for the derivative of non-labeled selenomethionine appeared in the range of *m/z* 320 to 330, which corresponded to the isotopic isomer with natural abundances of C, H, O, N and Se isotopes. The relative intensities of the ion clusters were close to the theoretical values. The ion clusters derived from selenium isotopomers was disappeared on the mass spectrum for the derivatives of D- and L-[$^2H_3$, ^{82}Se]selenomethionine. The mass peak at *m/z* 331 of [$^2H_3$, ^{82}Se]selenomethionine did not overlap with the *m/z* peaks known from non-labeled selenomethionine.

The present procedure is a simple but effective for the synthesis of optically active selenomethionine double-labeled with deuterium and ^{82}Se. The stable isotope labeled selenomethionine should be useful for pharmacokinetic study.

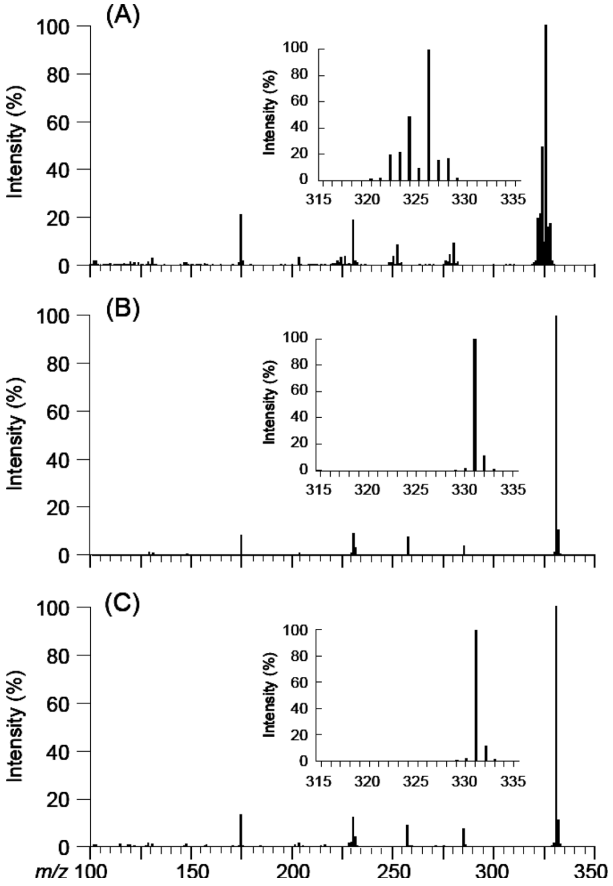

Fig. 2. Chemical-Ionization Mass Spectra for *N*-Isobutyloxycarbonyl Ethyl Ester Derivatives of Non-labeled Selenomethionine (A), D-[$^2H_3$, ^{82}Se]Selenomethionine (B) and L-[$^2H_3$, ^{82}Se]Selenomethionine (C)

Experimental

Materials and Methods DL-Selenomethionine, 45% HBr in acetic acid and isobutyl chloroformate were purchased from Wako Pure Chemicals (Osaka, Japan). (*R*)-2-Amino-4-butyrolactone hydrochloride and (*S*)-2-amino-4-bromobutyric acid hydrobromide were purchased from Aldrich (Milwaukee, U.S.A.). [$^2H_3$]Methyl iodide (>99.5% atom ^{2}H) was purchased from ISOTEC (Tokyo, Japan). ^{82}Se metal powder (>99.72% enriched) was purchased from Eurisotop (Gif-Sur-Yvette, France). All other chemicals and solvents were of analytical-reagent grade and were used without further purification. ^{1}H-NMR (400 MHz) and ^{13}C-NMR spectra (100 MHz) were recorded on a Bruker (Rheinstetten, Germany) DPX400 spectrometer. The samples were dissolved in deuterium oxide (0.5 ml) containing [$^2H_4$]-methanol as a reference for ^{13}C-NMR. Chemical shifts were expressed in δ (ppm) downfield from H^2HO (δ_H 4.80) for ^{1}H-NMR and [$^2H_4$]methanol (δ_C 49.0) for ^{13}C-NMR. *J*-Values were given in Hz. IR spectra were recorded on a Jasco (Tokyo, Japan) FT/IR-620 spectrometer. Optical rotations were measured on a Jasco P-1030 polarimeter. Mass spectra were obtained on a Micromass (Manchester, U.K.) Q-Tof Ultra mass spectrometer by electrospray ionization. Gas Chromatography-mass spectrometry (GC-MS) analysis was conducted on a Perkin-Elmer GC-MS system (GC AutoSystem XL with TurboMass Gold mass spectrometer, Norwalk, CT, U.S.A.). A methylsilicone bonded phase fused-silica capillary column Inertcep-1MS (15 m×0.25 mm i.d.) with a 0.25 μm film thickness (GL Science, Tokyo, Japan) was connected directly to the ion source. The initial column temperature was set at 120 °C. After the sample injection, it was maintained for 2 min and was increased at 40 °C/min to 250 °C. The temperature of the injector was 280 °C. The mass spectrometer was operated in chemical ionization mode with isobutane as the reagent gas.

HPLC was performed on a Jasco PU-980 instrument equipped with a UV detector operated at 200 nm, a 3-line degasser and a Rheodyne injector with a 20-μl loop. Separation was carried out on a Crownpak CR column (150×4 mm i.d., Daicel Chemical, Tokyo, Japan) coupled with a guard column containing the same stationary phase (10×4 mm i.d.) using 0.06% $HClO_4$ as mobile phase. The column temperature and flow rate were optimized to 25 °C and 0.3 ml/min, respectively.

(*R*)-2-Amino-4-bromobutanoic Acid 2a: A solution of (*R*)-2-amino-4-butyrolactone hydrochloride **1a** (306 mg, 2.2 mmol) in 45% HBr in acetic acid (10 ml) was refluxed for 5 h. The reaction mixture was allowed to stand for 12 h at room temperature to precipitate a colorless solid. The precipitate was collected and washed with diethyl ether. Recrystalization of the product

from ethanol–diethyl ether obtained (*R*)-2-amino-4-bromobutanoic acid hydrobromide **2a** (536 mg, 91%). mp 187—188 °C (dec.) [lit[13], S-form 187—188 °C (dec.)]. ^{1}H-NMR (^2H_2O) δ: 2.41 (1H, m, 3-H), 2.59 (1H, m, 3-H), 3.66 (2H, m, 4-H), 4.47 (1H, m, 2-H). ^{13}C-NMR (^2H_2O) δ: 28.9 (4-C), 33.7 (3-C), 52.4 (2-C), 172.3 (1-C). IR (KBr) cm^{-1}: 2972, 1719, 1600, 1485, 1433, 1360, 1330, 1210, 1163, 1131, 1110, 1074, 1022, 970, 921, 865, 800, 763. High resolution-electrospray ionization (HR-ESI)-MS *m/z* 181.9809 $[M+H]^+$ (Calcd for $C_4H_9NO_2Br$: 181.9817). $[\alpha]_D$ +7.6 (c=0.1, H_2O). *Anal.* Calcd for $C_4H_9NO_2Br_2$: C, 18.27; H, 3.45; N, 5.33; O, 12.17; Br, 60.78. Found: C, 18.30; H, 3.47; N, 5.32.

(*R*)-[$^2H_3$, ^{82}Se]2-Amino-4-methylselenylbutanoic Acid (D-[$^2H_3$, ^{82}Se]Selenomethionine) 3a: [$^2H_3$]Methyl iodide (10 g, 69.0 mmol) was added with stirring to lithium (1.1 g, 158 mmol) in dry diethyl ether (50 ml) under nitrogen atmosphere at a rate adequate to maintain gentle reflux of diethyl ether. The concentration of [$^2H_3$]methyllithium was estimated by hydrolysis of an aliquot (0.2 ml) and titration with 0.1 M HCl and was found to 1.1 mol/l. To 30 ml of ^{82}Se (metal powder, 96 mg, 1.2 mmol) in dry THF was added 6 ml of [$^2H_3$]methyllithium solution, and the resulting solution was stirred at room temperature until all ^{82}Se was dissolved. A solution of (*R*)-2-amino-4-bromobutanoic acid hydrobromide **2a** (360 mg, 1.4 mmol) in dry ethanol (10 ml) was gradually added and stirred for 1 h. After evaporating the solvent under reduced pressure, the residue was dissolved in 1 M HCl (50 ml) and washed with diethyl ether (2×30 ml). The aqueous layer was neutralized with 1 M NaOH. The solution was applied to a cation-exchange Dowex 50W X8 column (130×20 mm i.d., H^+ form), washed with water (200 ml) and eluted with 1 M ammonia (300 ml). After evaporating the solvent under reduced pressure, the crude product was obtained as a colorless solid, which was recrystallized with water–ethanol to yield D-[$^2H_3$, ^{82}Se]selenomethionine **3a** (142 mg, 47%) as colorless crystalline solid. mp 228 °C (dec.). ^{1}H-NMR (^2H_2O) δ: 2.23 (2H, m, 4-H), 2.65 (2H, t, J=7.7 Hz, 3-H), 3.87 (1H, dd, J=5.4, 7.0 Hz, 2-H). ^{13}C-NMR (^2H_2O) δ: 20.0 (4-C), 31.9 (3-C), 55.7 (2-C), 175.1 (1-C). IR (KBr) cm^{-1}: 2933, 1582, 1510, 1445, 1407, 1347, 1317, 1221, 1152, 972, 866. HR-ESI-MS *m/z* 203.0221 $[M+H]^+$ (Calcd for $C_5H_9{}^2H_3NO_2{}^{82}Se$: 203.0223). $[\alpha]_D$ +5.8 (c=0.1, H_2O). *Anal.* Calcd for $C_5H_8{}^2H_3NO_2{}^{82}Se$: C, 29.71; H(^{2}H), 5.49; N, 6.93; O, 15.84; ^{82}Se, 40.58. Found: C, 29.56; H, 5.41; N, 7.05.

(*S*)-[$^2H_3$, ^{82}Se]2-Amino-4-methylselenylbutanoic Acid (L-[$^2H_3$,^{82}Se]Selenomethionine) 3b: L-[$^2H_3$, ^{82}Se]Selenomethionine **3b** was synthesized from 8 ml of 1.2 M [$^2H_3$]methyllithium in diethyl ether solution, 95.1 mg of ^{82}Se and 400 mg of (*S*)-2-amino-4-bromobutylic acid **2b** by the same manner as described above. The purified product (133 mg, 54%) was obtained as a colorless solid. mp 228 °C (dec.). ^{1}H-NMR (^2H_2O) δ: 2.25 (2H, m, 4-H), 2.67 (2H, t, J=7.9 Hz, 3-H), 3.88 (1H, dd, J=5.4, 7.0 Hz, 2-H). HR-ESI-MS *m/z* 203.0214 $[M+H]^+$ (Calcd for $C_5H_9{}^2H_3NO_2{}^{82}Se$: 203.0223). $[\alpha]_D$ −6.8 (c=0.1, H_2O). *Anal.* Calcd for $C_5H_8{}^2H_3NO_2{}^{82}Se$: C, 29.71; H(^{2}H), 5.49; N, 6.93; O, 15.84; ^{82}Se, 40.58. Found: C, 29.68; H, 5.46; N, 7.03.

Derivatization for GC-MS Derivatization was carried out by the similar manner by our previous work.[15] Briefly, to a solution of selenomethionine (2 mg/ml) in H_2O–ethanol–pyridine (30 : 16 : 4, v/v/v) were added 0.05 ml of isobutyl chloroformate. After stirring for 1 min, the sample was extracted with 1 ml of chloroform. After removal of the solvent under a stream of nitrogen, the residue was dissolved in 2 ml of ethyl acetate and 0.5—1 μl of the solution was subject to GC-MS.

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