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Selenazolidines as Novel Organoselenium Delivery Agents

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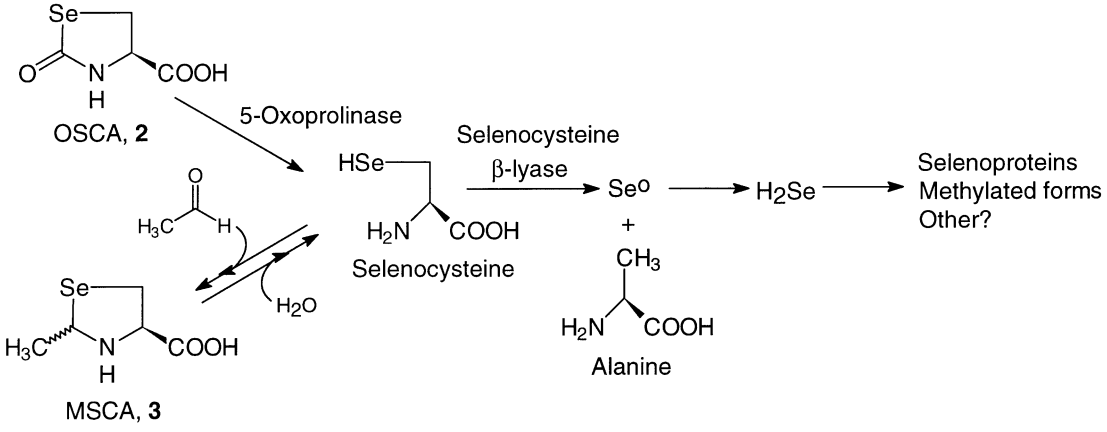
Abstract—Two new classes of selenazolidine-4(*R*)-carboxylic acids (2-oxo and 2-methyl-SCAs) were synthesized and characterized. Both were designed as latent forms of selenocysteine, intended to provide a chemically superior delivery form for selenium. The prodrugs may be clinically useful when selenium supplementation at supranutritional levels is indicated, such as in cancer chemoprevention. © 2001 Elsevier Science Ltd. All rights reserved.

Selenium supplementation has recently moved from the realm of correcting nutritional deficiencies to one of pharmacological intervention, especially in the clinical domain of cancer chemoprevention. While the mechanisms by which selenium is achieving this result remain unclear, the chemical form in which the selenium is provided consistently shows a marked influence on biological outcomes, largely due to differential metabolic processing.[1–4] Inorganic selenium salts, such as sodium selenite, represent popular supplements, but organic forms appear to possess superior properties from both a safety and efficacy standpoint.

Several interesting organoselenium compounds are under investigation as cancer chemopreventive agents.[5–8] The naturally-occurring selenium-containing amino acid, selenocysteine, is of particular interest to us because its metabolic release of selenium to the central pool (H_2Se) does not require a multistep, reductive process that consumes reducing equivalents in the cell and generates potentially toxic reactive intermediates, as is the case with other forms. Instead, cellular β-lyase activity cleaves selenocysteine to alanine and Se^0, which is then apparently spontaneously reduced to H_2Se (Scheme 1).[9,10] The use of selenocysteine in biological studies has been hampered by its chemical instability,



Scheme 1.

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especially its ease of oxidation to the diselenide.

We report here the synthesis of novel forms of selenocysteine that were designed to mask chemically reactive functional groups on the amino acid and serve as latent delivery forms (Scheme 2). Two prototype compounds were explored. 2-Oxoselenazolidine-4(*R*)-carboxylic acid (OSCA, **2**) was patterned after its sulfur-containing analogue, 2-oxothiazolidine-4(*R*)-carboxylic acid (OTCA),[11,12] and was designed to require the action of 5-oxoprolinase to release free selenocysteine (Scheme 1). 2(*R,S*)-Methylselenazolidine-4(*R*)-carboxylic acid (MSCA, **3**) was likewise patterned after the thiazolidine, 2(*R,S*)-methylthiazolidine-4(*R*)-carboxylic acid (MTCA)[13] and was designed to liberate selenocysteine after non-enzymatic ring opening and hydrolysis, in a concentration-dependent, equilibrium-controlled fashion (Scheme 1).

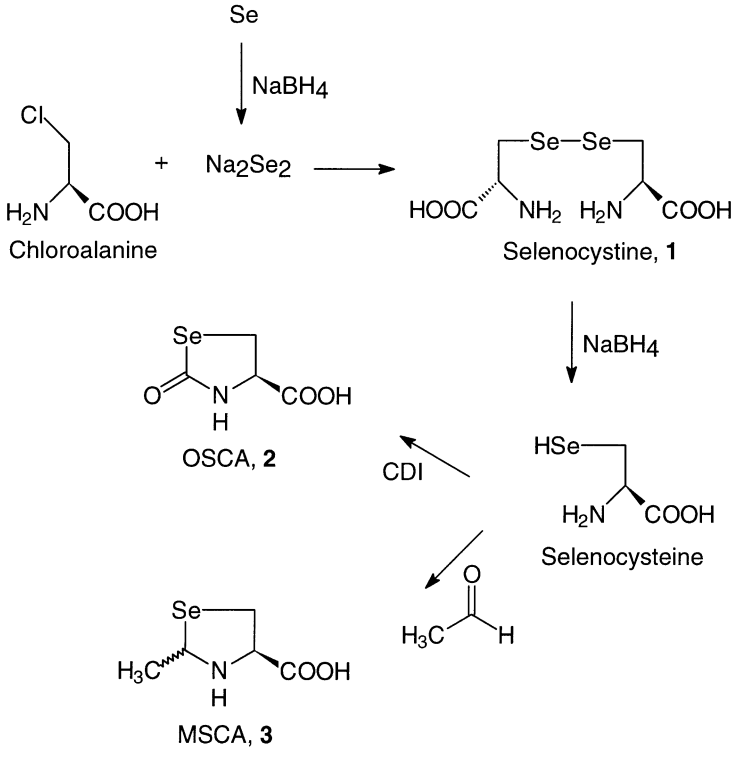
The chemical rationale for the selenazolidines relies, in large part, on the corresponding chemistry of the thiazolidines. While selenium and sulfur chemistries are certainly not identical, they are similar in many respects, which allowed the synthesis of these new classes of organoselenium compounds. The properties of sulfur-containing compounds are often relied upon to rationalize the properties of corresponding selenium-containing derivatives because the chemistry is similar, at least in a qualitative sense.[14] For example, the electronic configuration of the ground state species is similar (Se-$4p^4$ outer shell; S-$3p^4$ outer shell).[15] This fact alone produces many similar chemical entities from selenium and sulfur, including halides, oxides, and so on. The crystalline atomic radius is 1.98 Å for Se^{2-} and 1.84 Å for S^{2-},[15,16] providing similar steric considerations for ring formation. Pauling electronegativities of the two atoms are also similar: selenium = 2.55 and sulfur = 2.58.[15] The point is that selenazolidine production from selenocysteine makes chemical sense based on the corresponding thiazolidine production from cysteine. Interestingly, the biochemistry of the two elements can be quite dissimilar. For example, the standard reduction potential of selenate versus sulfate (below) shows that selenate is more readily reduced in aqueous solution compared to sulfate.[15,16] This exemplifies the general trend of reduction for selenium species in normal metabolism while sulfur species tend to be oxidized.

$$SeO_4^{2-} \xrightarrow{0.03} SeO_3^{2-} \xrightarrow{-0.36} Se^0 \xrightarrow{-0.67} Se^{2-}$$

$$SO_4^{2-} \xrightarrow{-0.94} SO_3^{2-} \xrightarrow{-0.66} S^0 \xrightarrow{-0.48} S^{2-}$$

The mechanism of thiazolidine ring formation (and likely selenazolidine ring formation as well) involves the attack of the nitrogen atom on a carbonyl donor, producing an intermediate Schiff's base.[17,18] The ring is then closed by attack of the nucleophilic thiol (selenol) on the intermediate double bond. Such reactions are common for thiols and are known to occur with selenols as well; in fact, selenide ion is a more potent nucleophile facilitating selenazolidine formation.[14,19]

Selenazolidine rings have been synthesized previously. In 1972, a series of papers reported the production of selenazolidines from selenocysteamine, selenocysteine, and selenopenicillamine (β,β-dimethylselenocysteine).[20–23] This work focused on exploring the mechanism of selenazolidine formation starting from H_2Se and aziridine derivatives;[20–22] the only reaction involving selenocys-



Scheme 2.

teine that was attempted was its conjugation with acetone, which produced little or no selenazolidine product (2,2-dimethylselenazolidine-4-carboxylic acid).[23] Later work by Cavallini and colleagues outlined the synthesis of the 'parent' compound, selenazolidine-4-carboxylic acid (selenaproline), and its study as an inhibitor of protein synthesis.[24–26]

The synthesis of both of the novel selenazolidines reported here required L-selenocystine (**1**) as a key intermediate. It is commercially available, but is very expensive in stereochemically pure form. Therefore, we prepared the required intermediate from β-chloro-L-alanine[27] (Scheme 2). The reaction is accomplished by the in situ generation of Na_2Se_2 from elemental selenium, which, upon sodium borohydride reduction, displaces the chlorine atom, preserving the stereochemistry at the chiral carbon. Yields averaged ~90% for this reaction.

OSCA (**2**) was synthesized from **1** by reducing the diselenide with sodium borohydride and then condensing the L-selenocysteine with 1,1′-carbonyldiimidazole (CDI)[21] (Scheme 2); yields of ~40% were common. Initial synthetic attempts utilized phenylchloroformate[12] as the carbonyl source. Neither this donor, nor the related benzylchloroformate or ethylchloroformate produced measurable levels of product. It appeared by NMR and MS analysis that the chloroformates preferentially reacted with the amine functional group to form yellow carbamate products, rather than with both the selenol and amine to form the desired selenazolidine (data not shown). The ^{1}H NMR spectrum of **2** showed the α-proton at ~4.5 ppm and the diastereotopic β-protons at ~3.5–4.0 ppm. The spectrum is consistent with that of the sulfur-containing compound, OTCA.[12] The ^{77}Se NMR spectrum of **2** showed a single peak at ~1353 ppm using dimethyl selenide as an external reference. This points out the dramatic effect of the adjacent carbonyl group on the chemical shift of the selenium atom; the chemical shift of the selenium in **1** was ~288 ppm. The positive ion FABMS of **2** showed the characteristic six-line pattern of selenium,[15] with a molecular ion at m/z 195.9, based on ^{80}Se, the isotope with the highest natural abundance (49.6%).[15]

MSCA (**3**) was similarly prepared in about 60% overall yield by the reduction of **1**, followed by reaction with acetaldehyde[13] as the carbonyl donor (Scheme 2). The reaction produced a pair of diastereomers at C-2, as evidenced by the diagnostic pair of quartets at ~5.1 and 4.9 ppm in the proton NMR spectrum, consistent again with the sulfur-containing analogue, MTCA.[13] The ratio of diastereomers was usually ~4:5, with the isomer with the methyl and carboxylic acids group *cis* to each other less prevalent, presumably due to steric effects. The different spatial relationship of the methyl group in each diastereomer with respect to the selenium atom was observed as two closely spaced, but separate singlets in the ^{77}Se NMR (~320 and 317 ppm). The positive ion FABMS again demonstrated the characteristic six-line pattern of selenium, with a molecular ion at m/z 196.0 (^{80}Se).

MSCA was designed to dissociate via a non-enzymatic ring opening and hydrolysis mechanism, while OSCA requires enzymatic processing, making it stable in aqueous solution. Prodrug breakdown was estimated by monitoring the loss of selenazolidine peaks in the ^{1}H NMR spectra in D_2O at neutral pH. As shown in Figure 1, incubation of MSCA showed time- and temperature-dependent decomposition, with the concomitant formation of selenocystine, as determined by liquid chromatography–mass spectrometry LC–MS (EI–MS $[M^+ + 1]$ $m/z = 336.9$; ^{80}Se). On the other hand, OSCA showed no detectable breakdown by NMR or formation of selenocystine by LC–MS (data not shown).

In conclusion, 2-oxo- and 2(*R,S*)-methylselenazolidine-4(*R*)-carboxylic acids were synthesized as novel prodrug forms of L-selenocysteine. The 2-oxo form (OSCA) was stable to incubation in aqueous solution, while the 2-methyl form (MSCA) demonstrated non-enzymatic breakdown. Both may represent valuable latent forms of L-selenocysteine, which may be useful in clinical situations where selenium supplementation at supranutritional levels is indicated.

The chemicals used for synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) or Sigma Chemical Co. (St. Louis, MO, USA). All other materials were acquired from Fisher Scientific (Pittsburgh, PA, USA). Nuclear magnetic resonance (NMR) spectra were obtained using either a Varian Unity 400 or 500 MHz FT-NMR spectrometer, as indicated. The NMR spectra were taken in D_2O and the water peak was set to δ 4.7 ppm in the ^{1}H spectra. When necessary, one drop of 30% NaOD in D_2O was used for solubility purposes. The ^{77}Se NMR spectra were recorded using dimethyl selenide (Aldrich) as an external standard (δ 0.0 ppm). Infrared (IR) spectra using KBr pellets were obtained using a Perkin-Elmer 1600 series FT-IR spectrophotometer. Fast atom bombardment mass spectrometry (FABMS) analysis was conducted at the University of Utah, Department of Chemistry using a Finnegan MAT-95. Liquid chromatography–mass spectrometry LC–MS analysis was carried out on a

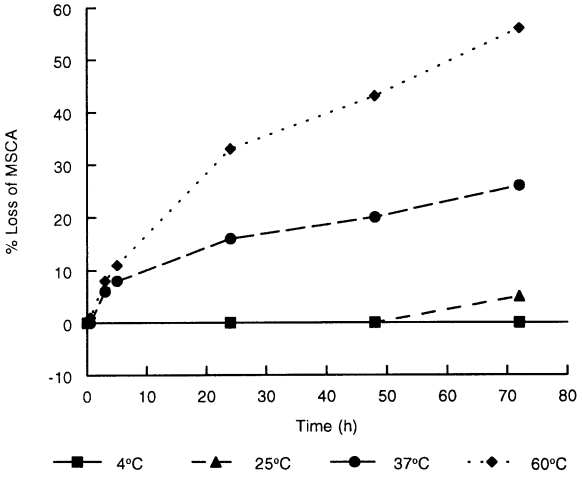


Figure 1. Percent loss of MSCA by ^{1}H NMR at various temperatures. OSCA showed no breakdown under the conditions tested.

Finnegan LCQ DECA quadrupole ion trap mass spectrometer interfaced with a HP1100 LC system at the University of Utah Mass Spectrometry Facility. Elemental analysis was performed at Galbraith Laboratories (Knoxville, TN, USA). Polarimetry data were collected using a JASCO model DIP-370 digital polarimeter. Melting points were determined using a Laboratory Devices USA Mel-Temp II melting point instrument and are uncorrected. Thin layer chromatography (TLC) was carried out using Whatman (Clifton, NJ, USA) flexible backed 60 Å silica gel plates (0.25 mm thickness) with visualization by iodine vapors. The syntheses were accomplished by degassing all solvents and constantly bubbling argon through solvents during reaction. pH adjustment was accomplished with either 6 M NaOH or 6 M HCl as appropriate.

L-Selenocystine (1). L-Selenocystine (**1**) was synthesized by a modification of known procedures.[27] At room temperature, selenium powder (3.0 g, 38 mmol) was suspended in water (10 mL). Sodium borohydride (3.0 g, 79 mmol) was dissolved in water (19 mL) and slowly added to the selenium suspension with stirring. Another equivalent of selenium powder (3.0 g, 38 mmol) was added to the reaction and the mixture was stirred for 15 min. The reaction mixture was placed briefly on a steam bath (1–2 min) to drive the reaction to completion. β-Chloro-L-alanine HCl (3.2 g, 20 mmol) was dissolved in water (20 mL) at pH 9 and added dropwise to the selenium solution over 2 h; stirring was continued overnight at 37 °C. The reaction mixture was acidified to pH 2 and hydroxylamine hydrochloride (218 mg, 3.1 mmol) was added. CAUTION: Added safety precautions were implemented during the work up of this reaction due to the production of hydrogen selenide gas. The reaction vessel was sealed tightly to prevent release of H_2Se into the air, and the exhaust was forced through two lead acetate traps, each containing 25 g lead acetate in 300 mL water, for 2 h. As an added precaution, a respirator rated for H_2S was routinely used. Vacuum filtration was performed to remove elemental selenium, and the filtrate was adjusted to pH 6–6.5 and left to crystallize at 4 °C for 3–5 days. The yellowish-orange crystals were collected by vacuum filtration and redissolved in 1 M HCl (20 mL). Any remaining solids were removed by vacuum filtration. The filtrate was adjusted to pH 6–6.5 with and left to crystallize again for 3–5 days. The yellow crystals were collected by vacuum filtration and dried by vacuum overnight: 3.0 g, 9.0 mmol (90%). Mp 174–176 °C (d) (reported, lit.[27] 184–185 °C). TLC *n*-BuOH/H_2O/acetic acid (3:2:1), R_f 0.32. ^{1}H NMR (D_2O/NaOD, 500 MHz) δ 3.6 (dd, J=5, 7 Hz, 1H, H-α), 3.3 (dd, J=5, 12 Hz, 1H, H-β1), 3.2 (dd, J=7, 12 Hz, 1H, H-β2); ^{13}C NMR (D_2O/NaOD, 125 MHz) δ 181.0 (COOH), 56.3 (C-α), 35.8 (C-β); ^{77}Se NMR (D_2O/NaOD, 95.3 MHz) δ 288.1. IR (KBr) ν_{max} 3500, 3000 cm^{-1}. FABMS [M$^+$+1] m/z 336.9 (^{80}Se). $[\alpha]_D^{25}$ −29.0° (*c* 0.5, 0.1 N NaOH). Anal. calcd for $C_6H_{12}N_2O_4Se_2$: C, 21.6; H, 3.63; N, 8.38. Found: C, 21.2; H, 3.57; N, 8.29.

2-Oxoselenazolidine-4(*R*)-carboxylic acid (OSCA, 2). L-Selenocystine (**1**) (0.25 g, 0.75 mmol) was suspended in an evacuated flask containing 0.05 N NaOH (10 mL) and ethanol (3 mL). To the solution, sodium borohydride (0.1 g, 2.6 mmol) was added slowly over about 10 min. The yellow solution was stirred for an additional 20 min until it became colorless and then was placed in an ice bath. The pH was adjusted to 5–6. 1,1′-Carbonyldiimidazole[28] (0.2 g, 1.2 mmol) was added over 30 min, and the reaction mixture was stirred for 1 h. If the reaction mixture became yellow again, the above reduction and carbonylation steps were repeated. The reaction mixture was acidified to pH 2 and extracted with ethyl acetate (3×15 mL). The combined ethyl acetate layers were washed with saturated NaCl solution (2×15 mL), dried over $MgSO_4$, then concentrated and dried under vacuum: 0.12 g, 0.62 mmol (41%). Mp 144–146 °C (d). TLC *n*-BuOH/H_2O/acetic acid (3:2:1), R_f 0.65. ^{1}H NMR (D_2O, 500 MHz) δ 4.5 (dd, J=6, 8 Hz, 1H, H-4), 3.8 (dd, J=8, 10 Hz, 1H, H-5a), 3.6 (dd, J=6, 10 Hz, 1H, H-5b); ^{13}C NMR (D_2O, 125 MHz) δ 178.7, 178.5 (COOH, C-2), 61.0 (C-4), 34.3 (C-5); ^{77}Se NMR (D_2O, 95.3 MHz) δ 1352.8. IR (KBr) ν_{max} 3300, 3000, 1700 cm^{-1}. FABMS [M$^+$+1] m/z 195.9 (^{80}Se). $[\alpha]_D^{25}$ −67.9° (*c* 0.5, water). Anal. calcd for $C_4H_5NO_3Se$: C, 24.8; H, 2.60; N, 7.22. Found: C, 24.4; H, 2.72; N, 6.93.

2(*R*,*S*)-Methylselenazolidine-4(*R*)-carboxylic acid (MSCA, 3). L-Selenocystine (**1**) (0.13 g, 0.39 mmol) was suspended in an evacuated flask containing 0.05 N NaOH (5 mL) and ethanol (1.5 mL). Sodium borohydride (0.05 g, 1.3 mmol) was added slowly over about 10 min. The reaction mixture was stirred for an additional 20 min until it changed to colorless and was then placed in an ice bath. The pH was adjusted to 5–6. Acetaldehyde (0.24 mL, 4.3 mmol) was added in 80 μL aliquots over 30 min, and the reaction mixture was stirred for 3 h. At that point, ethanol (20 mL) was added and the mixture was stored in the refrigerator overnight. The yellow precipitate (selenocystine by MS and NMR; data not shown) was filtered off, and the filtrate was dried under vacuum: 0.09 g, 0.47 mmol (60%). Mp 136–138 °C (d). ^{1}H NMR (D_2O, 400 MHz) δ diastereomer A 5.1 (q, J=7 Hz, 1H, H-2), 4.6 (t, J=7 Hz, 1H, H-4), 3.5–3.3 (m, 2H, H-5), 1.7 (d, J=7 Hz, 3H, CH_3); diastereomer B 4.9 (q, J=7 Hz, 1H, H-2), 4.3 (dd, J=7, 11 Hz, 1H, H-4), 3.5–3.3 (m, 2H, H-5), 1.7 (d, J=7 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 100 MHz) δ diastereomer A 171.7 (COOH), 66.4 (C-4), 50.7 (C-2), 24.2 (C-5), 18.8 (CH_3); diastereomer B 171.5 (COOH), 64.9 (C-4), 50.7 (C-2), 24.2 (C-5), 21.1 (CH_3); ^{77}Se NMR (D_2O, 76.2 MHz) δ 320.2, 315.7. IR (KBr) ν_{max} 3250, 2900, 1690 cm^{-1}. FABMS [M$^+$+1] m/z 196.0 (^{80}Se). $[\alpha]_D^{25}$ −83.9 (*c* 0.5, water). Anal. calcd for $C_5H_9NO_2Se \cdot 1/2\ H_2O$: C, 29.6; H, 4.96; N, 6.90. Found: C, 29.4; H, 4.45; N, 6.81.

Breakdown of selenazolidine prodrugs

Solutions of MSCA and OSCA were prepared in D_2O (pH ~ 4.0) and incubated for various times at various temperatures. ^{1}H NMR (400 MHz) spectra were then obtained. The breakdown of the prodrugs was estimated as the percent reduction in the most downfield

peak compared to the water peak at 4.7 ppm. The yellow precipitate that formed during the incubations was redissolved by adding one drop of 30% NaOD in D_2O. LC–MS analysis[29] of the samples confirmed the formation of selenocystine from MSCA. OSCA was stable to the incubation conditions.

Acknowledgements

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29. Sample (20 μL) separation was achieved using a C18 column (1×250 mm) at room temperature. The mobile phase consisted of solvents A (0.1% HCOOH in H_2O) and B (90% acetonitrile, 10% *iso*-propanol, 0.08% HCOOH). Elution was carried out at a flow rate of 0.05 mL/min with 5 min A, a 25 min steady gradient to 60% A and 40% B, and a 20 min steady gradient to 100% B, monitoring at 214 and 280 nm. MS was performed in the positive ion mode, at a voltage ~4.5 kV.