ARTICLE

OBC
www.rsc.org/obc

Experimental and theoretical evidence for cyclic selenurane formation during selenomethionine oxidation†

Joshua A. Ritchey, Bonnie M. Davis, Patricia A. Pleban* and Craig A. Bayse*

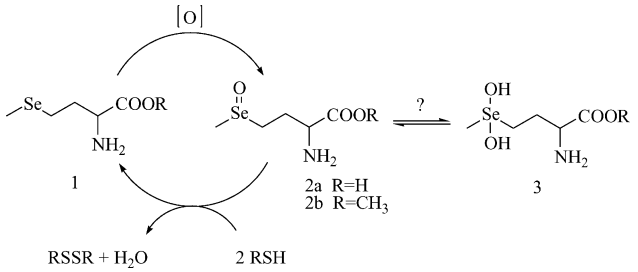
Department of Chemistry and Biochemistry, Old Dominion University, Hampton Boulevard, Norfolk, VA, 23529. E-mail: cbayse@odu.edu, ppleban@odu.edu

Received (Pittsburgh, PA) 20th September 2005, Accepted 19th October 2005
First published as an Advance Article on the web 15th November 2005

The oxidation products of selenomethionine (SeMet) have been studied *via* experimental ^{77}Se NMR and theoretical ^{77}Se chemical shifts. Four signals are observed: a diastereomeric pair of selenoxides at 840 ppm and two unidentified resonances at 703 and 716 ppm. Theoretical ΔG and chemical shifts suggest the 703 and 716 ppm resonances correspond to hypervalent selenium heterocycles, called selenuranes, formed by reaction with the amine or acid group of the amino acid and the selenoxide. To identify which of these selenuranes is formed, the amine and acid groups were individually protected. The *N*-formyl SeMet formed only the selenoxide pair at 840 ppm. The oxidized SeMet methyl ester produced signals at 703 and 716 ppm which are assigned as the Se–N selenurane.

Introduction

Selenomethionine (SeMet), a major dietary source of Se,[1] is a naturally occurring amino acid found primarily in grains, yeast, and some vegetables.[2] It randomly replaces methionine[2] in tissue proteins and can accumulate[3] due to its long residence time. SeMet is not incorporated directly into protein active sites, but provides Se for the biosynthesis of selenocysteine (SeCys) found in glutathione peroxidase and other selenoproteins. SeMet can also act as an antioxidant[4] and has been shown to follow a catalytic cycle[5] similar to glutathione peroxidase (GPx)[6] (Scheme 1). The major oxidation product, SeMet selenoxide **2a**, can be reduced back to SeMet with the addition of two equivalents of thiol.[5] Concentrations of Se above that required for optimal enzyme activity have been shown to prevent cancer.[7] SeMet has potential as a natural chemopreventative[6] and is currently in Phase III clinical trials.[8]



Scheme 1

Various groups have reported evidence for *Se*-dihydroxyselenomethionine **3** in analogy to an intermediate suggested in Oki and Iwamura's mechanism for racemization of selenoxides.[9] Zainal *et al.* assign a 1018 cm^{-1} IR frequency to the O–Se–O stretch of **3**,[10] and Block *et al.* report **3** in an oxidized sample of SeMet due to a m/z = 232 peak[11] corresponding to the mass of **2a** and a molecule of water. Shimizu *et al.* have presented evidence in favor of the formation of a dihydroxyselenide intermediate, although this intermediate appears to be shortlived.[12] Additional work by Paetzold *et al.* have shown *via* IR and Raman spectroscopy that the reported dimethyl and diethyl dihydroxyselenides are actually selenoxides hydrogen-bonded to a molecule of water.[13] These conflicting reports warrant further investigation by other methods.

^{77}Se NMR is a powerful analytical tool for direct observation of Se speciation due to its ability to display small changes in the Se environment over a large chemical shift range.[14] Theoretical ^{77}Se NMR has also been shown to be a potential tool for the identification of species in spectra of biological selenoproteins,[15] in addition to its application to other organoselenium compounds.[16] Subsequently, a study of the theoretical ^{77}Se chemical shift using a series of basis sets and methods showed that limited basis sets and either GIAO-MP2 or GIAO-DFT(mPW1PW91) chemical shifts had an overall error of 13.4 and 11.4%, respectively.[17]

In this study, theoretical and experimental methods are used jointly to identify the by-product in SeMet oxidation. The experimental NMR analyses, in addition to ^{77}Se and ^{1}H NMR, include COSY and HETCOR analysis of the oxidized SeMet. We give details indicating the commonly reported dihydroxyselenomethionine formation is actually unfavorable. Instead of this dihydroxy compound, we present evidence for the formation of a selenium heterocycle during the oxidation of SeMet.

Results

^{1}H NMR of SeMet shows a singlet at 2.0 ppm. The ^{1}H NMR spectrum of the oxidized SeMet reaction mixture shows multiple products (Fig. 1). ^{77}Se NMR was chosen as the initial means of identification since its spectrum shows only four Se-containing species, thus simplifying identification of the products of oxidation. While the signals are slightly pH dependent, four signals appear at pH 6: 839, 838, 716, and 703 ppm (Fig. 2).

Identification of selenomethionine selenoxide

The pair of signals at 839 and 838 ppm have been reported as SeMet selenoxide.[18] The typical range for selenoxides has also been reported as 812–941 ppm.[19] Therefore, these downfield signals are assigned as the diasteromeric selenoxide pair. When the pD of this sample is increased to above 11, the diastereomer peaks coalesce. The existence of the diastereomers is confirmed in the ^{13}C spectra. By performing an attached proton test (APT), the diastereotopic methyl carbons are clearly observed at about 30 ppm.

† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C, and ^{77}Se NMR of SeMet derivatives, ^{77}NMR of SeMet oxidations at pD 6 and 12, APT of oxidized selenomethionine at pD 11, COSY spectra of SeMet and derivatives and HETCOR of oxidized selenomethionine. See DOI: 10.1039/b513238j.

DOI:10.1039/b513238j

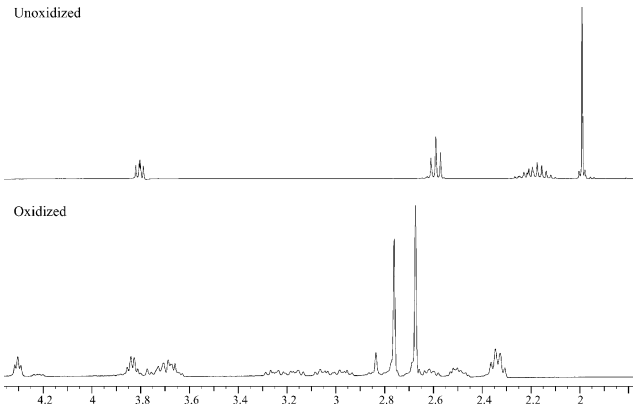


Fig. 1 ^{1}H NMR spectra of oxidized and unoxidized SeMet at pD 5.

^{1}H NMR data agrees with the observation of the selenoxide. The singlet at 2.0 ppm, which represents the unoxidized methyl group, has Se satellites with $^{2}J(CH_3,Se) = 10$ Hz (Fig. 1). After oxidation, one of the newly formed signals appears at 2.65 ppm with $^{2}J(CH_3,Se) = 12$ Hz. This coupling constant is consistent with methyl selenoxides (11.2–14.4 Hz).[19]

SeMet has been shown to have pH-dependent ^{1}H signals.[20] This behavior is also observed in the ^{77}Se spectra at a pD range from 4 to 10. The unoxidized SeMet signal shifts from 75 ppm at pD 7 down to 69 ppm at pD 11. Similarly, the oxidized SeMet diastereomer signals at 840 and 839 at pD 7 shift upfield by 4 ppm at pD 11. The shift can be attributed to the loss of hydrogen bonding in the deprotonated species. When oxidized at pDs below 4, SeMet slowly decomposes by β-elimination, giving methylseleninic acid and other oxidation products.

Identification of unknown oxidation products

In addition to the selenoxide signals in the ^{77}Se NMR spectra, there are two signals at 703 and 716 ppm (Fig. 2a) which do not correspond to any elimination product. Similarly, the ^{1}H shows two singlets at 2.74 and 2.81 ppm. The singlet at 2.74 ppm shows Se satellites with $^{2}J(CH_3,Se) = 10.4$ Hz. Previous reports have assigned this singlet at 2.74 ppm to the dihydroxyselenomethionine.[20,21]

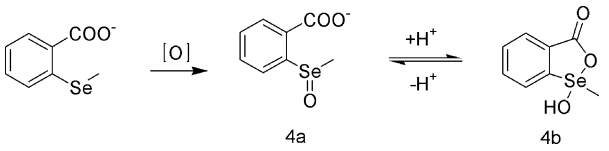
The relative amounts of these species and the selenoxide are pD dependent. From ^{1}H integrations at pD 7, the ratio of selenoxide to the unidentified compounds is approximately 4 : 5.5, but at pD 11 this ratio becomes 4 : 1.3. At higher pDs, the signals at 2.74 and 2.81 are no longer visible and only the selenoxide singlet appears at 2.65 ppm. Similar behavior is observed in the ^{77}Se spectra. At pD 7, the 703 and 716 ppm signals are present with the selenoxide pair at 840 ppm. Above pD 11, the selenoxide pair coalesces at 840 ppm and the unknown signals disappear. When the same sample is acidified and a new spectra collected, the selenoxide peaks separate, the 703 and 716 ppm resonances reform and the singlets at 2.74 and 2.81 increase in intensity.

COSY and HETCOR analysis

A COSY spectra of oxidized SeMet at pD 11 shows a single methyl signal at 2.65 ppm, indicating the sole presence of **2a**. The only correlations observed at this pH are the β protons around 2.0 ppm correlated to both the α and γ protons at about 3.3 and 3.1 ppm respectively. Observation of only the selenoxide supports the ^{77}Se NMR observations at high pH, whereas multiple products are observed at pD 8. The COSY spectra at pD 8 shows two different β protons coupled to different γ protons that appear to be enantiotopic. HETCOR analyses show three different methyl groups, as well as allowing the assignment of the γ and α protons from the previously assigned ^{13}C spectra.

Discussion

Nakanishi *et al.* have reported a cyclic selenium heterocycle known as a selenurane in oxidized samples of selenoanisoles.[19] The selenurane (**4b**) was favored at low pH; however, the selenoxide (**4a**) was more stable at high pH (Scheme 2). Kurose *et al.* also observed an acid–base equilibrium between bornyl selenoxides and selenuranes.[22] They found that by controlling the pH of the reaction solution, they could preferentially form the selenurane or selenoxide, and that this process was reversible.



Scheme 2

These previous studies suggest that selenurane formation may occur in SeMet oxide through the amine or the carboxylic acid (Scheme 3). An amine-based selenurane **5** could exist over a wide pH range, while a carboxylic acid-based compound **6** would be restricted to low pH due to the pK_a values for the amine and acid group: 9.28 and 2.13, respectively. When the pH of the oxidized SeMet sample is adjusted to above 11, the pair of signals ~840 ppm coalesce and the pair at ~700 ppm disappear completely. When the pD is then decreased to 7, the selenoxide pair separates again, while the signal at 703 ppm reforms. The formation of only the 703 ppm peak indicates the presence of one species. This acid–base equilibrium process appears identical

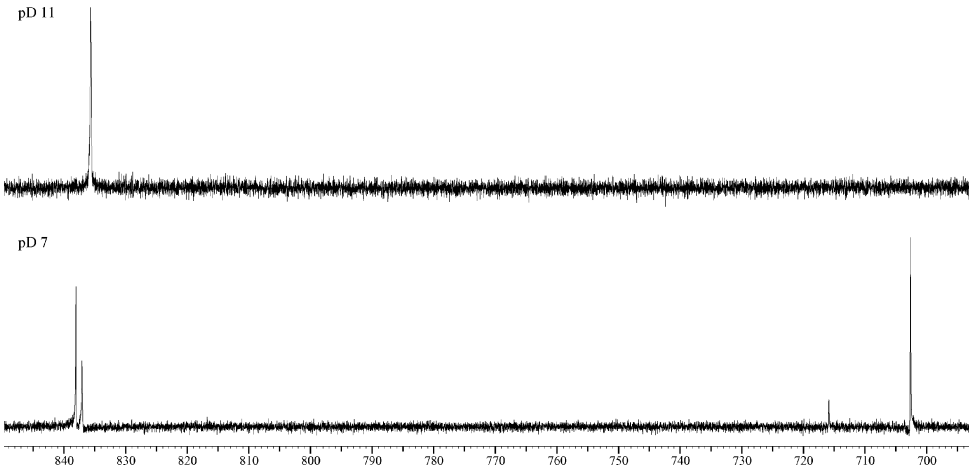
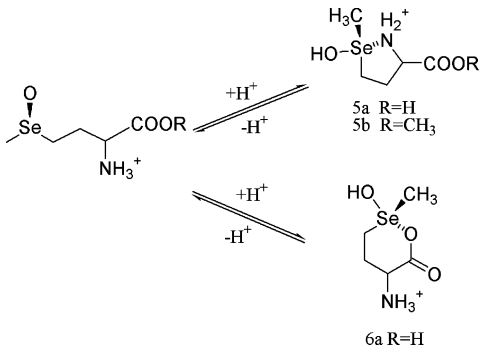


Fig. 2 ^{77}Se NMR of oxidized SeMet at (a) pD 7 and (b) pD 11.



Scheme 3 Acid–base-dependent formation of possible selenuranes.

to that observed by Kurose *et al.*[22] The measured coupling constants $^{2}J(\mathrm{Se,C}H_3)$ of these selenuranes range from 9.5–10.9 Hz, while those for selenoxides are from 11.2–14.4 Hz. The observed coupling constants for oxidized SeMet are 12.0 Hz at 2.65 ppm and 10.4 Hz at 2.74, indicative of a selenoxide and selenurane respectively. This selenurane is consistent with a Se–N radical species reported by Assmann *et al.* in one-electron reductions of **2a**.[23] Additionally, the ^{77}Se NMR shows the selenurane signal upfield relative to the selenoxide due to increased shielding of the Se nucleus in its hypervalent configuration; behavior that is identical to the oxidation of selenoanisole.

Bayse has shown that theoretical ^{77}Se chemical shifts of small model compounds correlate well to those of selenoproteins and selenoamino acids.[15] Gauge-invariant atomic orbital (GIAO) calculations were performed on models of selenuranes **3**, **5** and **6** constructed from Me_2SeO and water, formic acid, ammonia and ammonium (Table 1 and Fig. 3). The hydrate discussed above as an intermediate in selenoxide exchange is a minimum on the PES and is exothermic with respect to Me_2SeO and water, but the chemical shift is too low to be the 700 ppm shift observed in SeMet oxidation. This difference does not appear to be due to deficiencies in the model or basis set. Our recent study[17] of the reliability of ^{77}Se chemical shifts calculated theoretical shifts in several basis sets for the model compound $Me_2Se(OH)_2$. The consistency of those results precludes significant change in the calculated values with larger basis sets. The amine and formic acid derivatives are also exothermic hypervalent compounds,

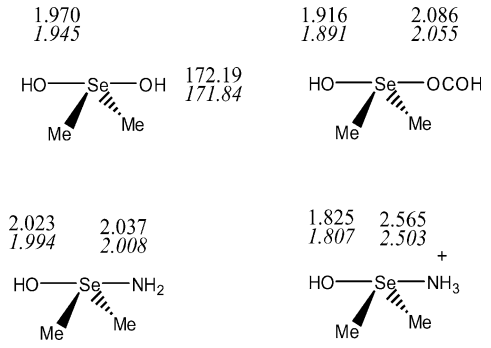


Fig. 3 Bond distances (Å) and angles (°) for model selenuranes calculated at the B3LYP and mPW1PW91 (italics) levels.

but neither has a chemical shift in the proper range. Addition of ammonium across the selenoxide bond gives a very stable complex and, more importantly, a chemical shift very similar to the unindentified resonance. The structure is similar to that of the ammonia species but with a shorter Se–O and longer Se–N distance.

Further theoretical calculations were performed on SeMet oxide, the hydrate and the Se–O and Se–N selenuranes. Fully protonated derivatives were used because the unidentified resonances are observed primarily at low pH. Geometric parameters for the lowest energy conformation of these derivatives are shown in Fig. 4. Four isomers of the selenoxide were constructed to reflect the chirality of the selenoxide group (*R* or *S*) and the orientation of the carboxylic acid with respect to the amine (interaction with OH or CO), and optimized at the B3LYP and mPW1PW91 levels. In each case, a hydrogen bond forms between the amine protons and the selenoxide oxygen, which results in a bond length slightly longer (1.70 Å) than that calculated for Me_2SeO (1.67 Å). This hydrogen-bonded interaction is an artifact of the gas-phase calculations and will be replaced in aqueous solution by solvation of the two groups. B3LYP shows the *S*-$\mathbf{2a}_{OH}$ enantiomer (Fig. 4) to be the lowest energy conformer (mPW1PW91 shows a slight preference for *R*-$\mathbf{2a}_{OH}$), and this is chosen as the reference energy for all conformations and derivatives. GIAO-MP2/B3LYP and GIAO-DFT(mPW1PW91) show the *S*-enantiomers downfield of the *R*-enantiomers by 20–25 ppm, a larger split than observed experimentally. The GIAO-MP2//B3LYP chemical shifts of the pair of enantiomers match very well with the experimental observations of the selenoxide (2–3% error). The error for the mPW1PW91 shifts is larger (5–6%), but our study of ^{77}Se chemical shifts[17] showed that mPW1PW91 and other DFT methods tend to overestimate the shielding of selenoxides.

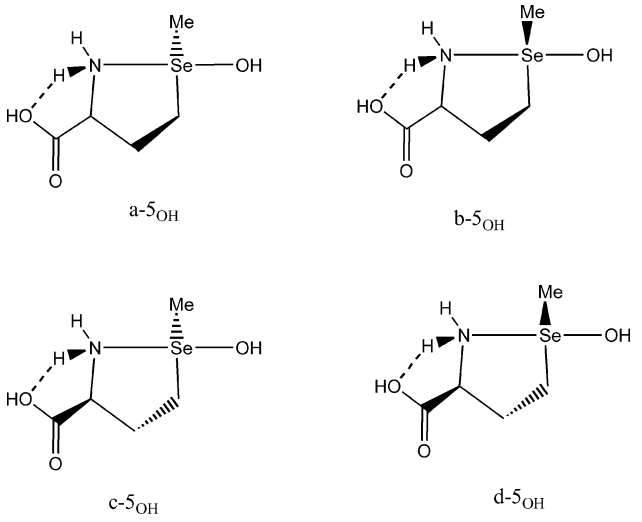
The theoretical ^{77}Se chemical shifts of the hydrate **3** are roughly 150 ppm upfield of the observed resonance (GIAO-MP2//B3LYP 530 ppm; GIAO-DFT(mPW1PW91) 498 ppm). The theoretical ΔG_f of the hydrate from water and the selenoxide is also unfavorable (28 kcal mol^{-1}), but the magnitude is likely due to the loss of the hydrogen bonding in **2a**. The local geometry around Se indicates hypervalency as for model **7** with a slight asymmetry in the Se–O bond distances (Fig. 4). The charge on Se is more positive than **2a**, suggesting that **3** should be found upfield of the selenoxide. GIAO calculations suggest that the hydrate should appear in the range 500–550 ppm, but no proposed hydrate has been reported in this region.

The stereochemistry of the selenoxide and the pucker of the five-membered ring allow for four different conformations (labels a–d) of the Se–N selenurane **5a** (Scheme 4). An additional four conformations can be generated from the orientation of the carboxylic acid with respect to the amine (interaction with OH or CO). The optimized geometries of **5a** (Fig. 4) compare favorably with the model compound **10**, each showing the linear N–Se–O bond angle of hypervalent selenium. The Se–N distances are ~0.08 Å shorter than **10** due to intramolecular hydrogen bonding to the carboxylic acid group and the steric restraints of the ring. Of the two possible orientations of -COOH, that with OH interacting with the amine hydrogens is most stable; all four $\mathbf{5}_{OH}$ comformers lie within 1.0 kcal mol^{-1} of *S*-$\mathbf{2a}_{OH}$, whereas

Table 1 Theoretical ^{77}Se chemical shifts (ppm) and reaction energies (kcal mol^{-1}) for model selenuranes $Me_2Se(X)(OH)$

	XH	ΔE^a B3LYP	δ_{Se} GIAO-MP2/B3LYP[b]	ΔE^a mPW1PW91	δ_{Se} GIAO-DFT mPW1PW91[b]
7	HOH	−4.71	476	−5.55	507
8	HCOOH	−8.34	579	−8.98	552
9	NH_3	−9.76	374	−7.90	351
10	NH_4^+	−34.2	740	−33.9	696

[a] ΔE for the reaction $Me_2SeO + XH \rightarrow Me_2Se(X)(OH)$. [b] Calculated relative to Me_2Se at the same level and basis set.



Scheme 4

$\Delta G = 5$–8 kcal mol^{-1} for the $\mathbf{5}_{CO}$ structures. Donation of electron density from the carboxylic acid group decreases the acidity of the amine protons, allowing for a more basic nitrogen and a stronger Se–N bond. The reduced acidity of these protons also makes them less acidic than the Se–OH proton, the Mulliken charge of which is +0.7e greater than the amine protons. A rough estimate of the acidity of the Se–OH proton based upon H_2O, H_3O^+, NH_3 and NH_4^+ gives a pK_a of 11.4, implying that **5a** may be observable even at high pH. Therefore, at high pH the OH proton will be lost first, causing the selenurane ring to collapse back to the selenoxide. Alternatively, this species may be formulated as an intramolecular interaction between the lone pair of the amine and a protonated selenoxide.

The theoretical ^{77}Se chemical shifts of $\mathbf{5a}_{OH}$ are upfield of **2a**, as expected from the hypervalent structure and the increased charge on Se. The GIAO-MP2/B3LYP shifts in Table 2 range from 748–763 ppm, only slightly upfield of the selenoxides. However, the GIAO-DFT(mPW1PW91) shifts are an almost perfect match to the experimentally observed resonances (710–729 ppm). The lowest energy conformation a-$\mathbf{5a}_{OH}$ (Figure 5) is upfield of the other three OH conformations by 10–20 ppm, so these results may indicate that the 703/716 pair consists of two stable conformations of the Se–N selenurane.

Four conformations were calculated for the Se–O selenurane **6**: boat *vs.* chair and axial *vs.* equatorial orientation of the Se methyl group. Despite the data collected on the model compounds, the Se–O selenurane also has theoretical shifts in the range of the experimentally observed resonances. These structures (*e.g.*, ax-chair-**6** in Fig. 4) show a shorter Se–OH bond than the hydrate due to asymmetry of the 3c4e bond, and an acidic SeOH proton that would be lost in basic pH to reform the selenoxide. However, these species are endergonic relative to the selenoxides. The Se–O bond to the carboxylic acid is slightly shorter than that in the crystal structure of **4b** (2.378 Å).[24]

The theoretical results suggest that the resonances at 703 and 716 ppm are Se–N selenuranes, but cannot conclusively eliminate the Se–O species. Experimental confirmation of the theoretical results was sought by derivatizing SeMet to block interactions between Se and the amino acid groups. Esterification was used to prevent formation of **6** whereas conversion of SeMet to a formyl amide was used to prevent formation of **5a** by reducing the Lewis basicity of the nitrogen.

Upon oxidation of the *N*-formyl derivative at pH 12, the ^{77}Se spectra showed only two signals at 840 and 839 ppm. Proton NMR shows a single methyl group at 2.62 ppm. Although the Se satellites are not observed through the baseline noise, the chemical shift is similar to that of the SeMet selenoxide. When the pH of the solution was decreased, only the selenoxide pair in the Se NMR and the single methyl signal in the proton spectra remained. The oxidized methyl ester derivative **5b** at low pH shows signals at 703 and 723 ppm, the exact signals observed in the ^{77}Se NMR SeMet oxidation spectra. The proton spectra show methyl signals at 3.77 ppm, corresponding to the ester

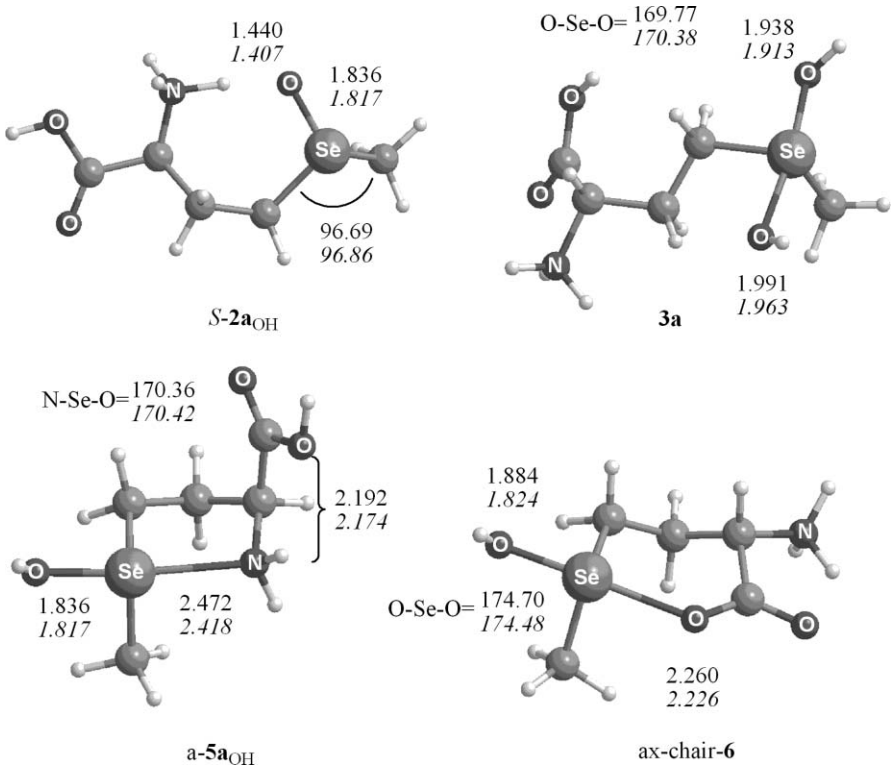


Fig. 4 Bond distances (Å) and angles (°) for the lowest energy conformations of **2a**, **3a**, **5a** and **6** calculated at the B3LYP and mPW1PW91 (italics) levels.

Table 2 Theoretical ^{77}Se chemical shifts (ppm) and relative Gibbs free energies (kcal mol^{-1}) for SeMet derivatives

	ΔG^a B3LYP	δ_{Se} GIAO-MP2/B3LYP^b	ΔG^a B3LYP	δ_{Se} GIAO-DFT (mPW1PW91)^b
S-**2a**$_{OH}$	0.00	832	0.00	787
S-**2a**$_{CO}$	1.69	836	1.81	795
R-**2a**$_{OH}$	5.13	820	−0.13	776
R-**2a**$_{CO}$	1.69	828	1.71	786
3a	28.05	530	28.01	498
a-**5a**$_{OH}$	0.11	748	0.81	710
b-**5a**$_{OH}$	−0.09	763	0.03	729
c-**5a**$_{OH}$	0.24	753	0.22	719
d-**5a**$_{OH}$	0.50	754	0.61	716
a-**5a**$_{CO}$	7.71	739	7.78	697
b-**5a**$_{CO}$	7.05	754	6.93	716
c-**5a**$_{CO}$	5.68	775	6.16	738
d-**5a**$_{CO}$	6.27	759	6.23	683
eq-chair-**6**	10.30	775	10.39	675
ax-chair-**6**	7.62	720	7.67	698
ax-boat-**6**	12.12	722	12.21	743
eq-boat-**6**	9.07	732	8.85	684

^{a} ΔG is calculated relative to *S*-**2a**$_{OH}$. ^{b} Calculated relative to Me_2Se at the same level and basis set.

Table 3 Theoretical ^{77}Se chemical shifts (ppm) and relative Gibbs free energies (kcal mol^{-1}) for derivatives of SeMet methyl ester

	ΔG^a B3LYP	δ_{Se} GIAO-MP2/B3LYP^b	ΔG^a B3LYP	δ_{Se} GIAO-DFT (mPW1PW91)^b
S-**2b**$_{OH}$	0.00	828	0.00	774
S-**2b**$_{CO}$	0.16	835	0.06	804
R-**2b**$_{OH}$	−1.92	821	−1.95	776
R-**2b**$_{CO}$	−0.08	829	−0.05	784
a-**5b**$_{OH}$	−0.92	741	−1.36	705
b-**5b**$_{OH}$	−0.24	753	−1.16	717
c-**5b**$_{OH}$	−1.00	747	−1.34	713
d-**5b**$_{OH}$	2.11	721	1.76	673
a-**5b**$_{CO}$	−2.69	731	−3.05	690
b-**5b**$_{CO}$	−3.55	748	−3.66	711
c-**5b**$_{CO}$	−2.83	748	−4.40	713
d-**5b**$_{CO}$	−3.54	745	−1.13	706

^{a} ΔG is calculated relative to *S*-**2a**$_{OH}$. ^{b} Calculated relative to Me_2Se at the same level and basis set.

methyl, and two signals at 2.83 and 2.87 ppm, which represent the Se methyls of two enantiomers of the selenurane. The α-H appears at 4.6 ppm, slightly further downfield due to the increased electron density from the Se now interacting with the amine nitrogen. At pH values above 7, the ester undergoes base-catalyzed hydrolysis and the resultant spectrum is that of the oxidized SeMet. Geometry optimizations of the SeMet methyl ester derivatives **5b** give similar structures and theoretical shifts to the SeMet selenuranes **5a** (Table 3), but the **5b**$_{CO}$ conformations are lower in energy. Notably, formation of the Se–N selenuranes is exergonic, which reflects the sole formation of the 700 ppm resonances in the experiment.

Conclusions

The oxidation of selenomethionine using hydrogen peroxide in aqueous solutions generates the expected selenomethionine oxide as well as a Se–N selenurane as detected by ^{1}H and ^{77}Se NMR. The selenoxide **2** and Se–N selenurane **5** are in an acid–base equilibrium with one another, and the zwitterionic character of SeMet aids in stabilization of the selenurane. Theoretically obtained ^{77}Se chemical shifts are in good agreement with experimental results for both **2a** and **5a**. The ΔG for the Se–N selenurane is zero, explaining the observation of both **2a** and **5a** in the oxidation of SeMet. The exergonic ΔG for **5a** accounts for the sole formation of the selenurane during the oxidation of the methyl ester derivative.

Assignment of the 703/716 pair of resonances to the Se–N selenuranes was confirmed by partially protecting the amino acid group. Blocking the amine group not only prevents the formation of the Se–N selenurane, but also shows that the dihydroxyselenide **3** does not exist as a stable intermediate. These results also demonstrate that when SeMet incorporated into proteins is oxidized, it will be in the selenoxide form rather than form a cyclic heterocycle as has been shown for selenocysteine.[25]

Experimental

L-(+)-Selenomethionine **1** 99% ee and 35% wt% H_2O_2 were purchased from Acros. Deuterium oxide 99.0% was purchased from Aldrich Chemical Company. All reagents were used without further purification. NMR spectra were collected on a Varian 400 MHz Unityplus NMR spectrometer (^{1}H NMR at 399.88 MHz; ^{13}C NMR at 100.55 MHz; ^{77}Se NMR at 76.26 MHz) using 3-(trimethylsilyl)-1-propane sulfonic acid sodium salt (TPS) and Me_2Se (DMSe) as external standards. All reported shifts are relative to DMSe or TPS (0 ppm). ^{13}C and ^{77}Se spectra were proton-decoupled continuously using the WALTZ-16 decoupling scheme. FIDs were imported and transformed using BioRad KnowItAll Informatics v4.0 software for clarity. FT-IR spectra were taken on a Nicolet Magna-IR 560 spectrometer (KBr). Melting points were obtained on a HaakeBuchler melting point apparatus and are uncorrected. pH was measured with an Accumet micropH probe and pH meter with an expanded scale.

Oxidations were carried out by preparing a 0.10 M solution of **1** or its derivative in D_2O and adjusting its pH with NaOD or DCl. A crystal of TPS was added to the solution. The ^{1}H spectra of the unoxidized sample was collected and a microbulb (Wilmad #529-A) of DMSe was placed in the NMR tube. After

^{1}H and ^{77}Se spectra of the unoxidized sample were collected, an equimolar portion of 30% H_2O_2 was added to the tube. ^{1}H and ^{77}Se spectra were then collected for the oxidation reaction mixture.

Selenomethionine methyl ester hydrochloride

Using a modification of Rachele's procedure,[26] L-(+)-selenomethionine (0.1961 g, 1 mmol), 2,2-dimethoxypropane (15 mL), and conc. HCl (1 mL) were added to a 50 mL Erlenmeyer flask. The mixture was left unstirred for 18 h, resulting in a dark orange-brown solution. The solvent and resulting MeOH were removed *in vacuo*, yielding a dark brown solid. The solid was dissolved in a minimum amount of dry methanol and precipitated out of solution with anhydrous ether. The ester hydrochloride was recrystallized to yield 0.1755 g (83%) of a yellow-white flaky solid. Mp: 137–140 °C. IR: ν 1746, 1485, 1238, 1216, 1182, 1147 cm^{-1}. ^{1}H NMR: δ 1.95 (s, 3H), 2.14–2.37 (m, 1H), 2.60 (t, 1H), 3.58 (s, 3H), 4.22 (t, 2H) ppm. ^{13}C NMR: δ 5.8, 21.3, 32.3, 55.0, 56.1, 172.9 ppm. ^{77}Se NMR: δ 69.7 ppm (pD = 2).

N-Formyl selenomethionine

Using a modification of Sheehan and Yang's procedure,[27] L-(+)-selenomethionine (0.1991 g, 1 mmol) and formic acid (2.5 mL, 88%) were added to a 5 mL round-bottomed flask with a magnetic stir bar. Acetic anhydride (0.83 mL) was added dropwise, and the mixture stirred for 1 h. 0.2013 g (88%) of a white waxy precipitate was separated by filtration and deemed pure by TLC and NMR. Mp: 86–87 °C. IR (neat): ν 3353, 1705, 1612, 1357, 1227 cm^{-1}. ^{1}H NMR: (400 MHz, D_2O) δ 1.9 (s, 3H), 2.04–2.26 (m, 1H), 2.48–2.72 (t, 2H), 4.6 (q, 2H), 8.15 (s, 1H), 15.00 (s, 1H) ppm. ^{13}C NMR: δ 6.0, 23.2, 35.0, 56.4, 166.4, 180.6 ppm. ^{77}Se NMR: δ 63.2 ppm (pD = 4).

Theoretical methods

Geometry optimizations were performed at the DFT/B3LYP[28] and DFT/mPW1PW91[29] level in Gaussian 98.[30] Selenium was represented by the Hurley *et al.*[31] relativistic effective core potential double-ζ basis set augmented with even-tempered s, p, and d diffuse functions. Nitrogen and oxygen were represented by Dunning's split-valence triple-ζ plus polarization function basis set.[32] Carbon basis sets were double-ζ plus polarization quality.[33] Hydrogens attached to non-carbon heavy atoms were triple-ζ in quality[32] while those attached to carbon were double-ζ.[33] Vibrational frequencies were calculated to confirm structures as stationary points on the potential energy surface. Chemical shifts were calculated by GIAO[34]-MP2 from the DFT/B3LYP optimized geometry and GIAO-DFT(mPW1PW91) at the DFT/mPW1PW91 optimized geometry. The selenium ECP basis set was replaced in the GIAO calculations with the all-electron basis of Schafer *et al.*[35]

Acknowledgements

C.A.B. thanks the Thomas F. Jeffress and Kate Miller Jeffress Memorial Trust for generous support.

References

1 G. F. Combs and S. B. Combs, *Annu. Rev. Nutr.*, 1984, **4**, 257.
2 *J. Cell. Biochem.*, 1996, **S26**, 202.
3 M. L. Scott, 'Nutritional Importance of Selenium', in *Organic Selenium Compounds: Their Chemistry and Biology*, ed. D. Klayman and W. Günther, The Chemistry of Organometallic Compounds Series, Wiley & Sons, Inc., New York, 1973.
4 R. Walter and J. Roy, *J. Org. Chem.*, 1971, **36**, 2561.
5 A. Assmann, K. Briviba and H. Sies, *Arch. Biochem. Biophys.*, 1997, **349**, 201.
6 K. A. Caldwell and A. L. Tappel, *Biochemistry*, 1964, **3**, 1643.
7 H. E. Ganther and J. R. Lawrence, *Tetrahedron*, 1997, **53**, 12299.
8 E. A. Klein, S. M. Lippman, I. M. Thompson, P. J. Goodman, D. Albanes, P. R. Taylor and C. Coltman, *World J. Urol.*, 2003, **21**, 21.
9 M. Oki and H. Iwamura, *Tetrahedron Lett.*, 1966, **25**, 2917.
10 H. A. Zainal, D. E. LaCroix and W. R. Wolf, *Fresenius' J. Anal. Chem.*, 1996, **356**, 311.
11 E. Block, M. Birringer, W. Jiang, T. Nakahodo, H. J. Thompson, P. J. Toscano, H. Uzar, X. Zhang and Z. Zhu, *J. Agric. Food Chem.*, 2001, **49**, 458.
12 (*a*) T. Shimizu and M. Kobayashi, *J. Org. Chem.*, 1987, **52**, 3399; (*b*) T. Shimizu, M. Kobayashi and N. Kamigata, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 3761; (*c*) T. Shimizu, M. Enomoto, H. Taka and N. Kamigata, *J. Org. Chem.*, 1999, **64**, 8242.
13 V. R. Paetzold, U. Linder, G. Bochmann and P. Reich, *Z. Anorg. Allg. Chem.*, 1967, **352**, 295.
14 H. Duddeck, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1995, **27**, 1.
15 C. A. Bayse, *Inorg. Chem.*, 2004, **19**, 65.
16 (*a*) S. Hayashi and W. Nakanishi, *J. Org. Chem.*, 1999, **64**, 6688; (*b*) E. Block, M. Birringer, R. DeOrazio, J. Fabian, R. S. Glass, C. Guo, C. He, E. Lorance, Q. Qian, T. B. Schroeder, Z. Shan, M. Thiruvashi, G. S. Wilson and X. Zhang, *J. Am. Chem. Soc.*, 2000, **122**, 5052; (*c*) W. Nakanishi and S. Hayashi, *J. Phys. Chem. A*, 1999, **103**, 6074; (*d*) H. Fleischer, S. Glang, D. Schollmeyer, N. W. Mitzel and M. Bühl, *Dalton Trans.*, 2004, 3764.
17 C. A. Bayse, *J. Chem. Theory Comput.*, 2005, **1**, 1119.
18 B. Gammelgaard, C. Cornett, J. Olsen, L. Bendahl and S. Hansen, S., *Talanta*, 2003, **59**, 1165.
19 W. Nakanishi, Y. Ikeda and H. Iwamura, *Org. Magn. Reson.*, 1982, **20**, 117.
20 H. A. Zainal, W. R. Wolf and R. M. Waters, *J. Chem. Technol. Biotechnol.*, 1998, **72**, 38.
21 S. Padmaja, G. L. Squadrito, J.-N. Lemercier, R. Cueto and W. A. Pryor, *Free Radical Biol. Med.*, 1996, **21**, 317.
22 N. Kurose, T. Takahashi and T. Koizumi, *Tetrahedron*, 1997, **53**, 12115.
23 A. Assmann, M. Bonifačić, K. Briviba, H. Sies and K.-D. Asmus, *Free Radical Res.*, 2000, **32**, 371.
24 B. Dahlén, *Acta Crystallogr.*, 1973, **B29**, 595.
25 (*a*) H. J. Reich and C. P. Jasperse, *J. Am. Chem. Soc.*, 1987, **109**, 5549; (*b*) P. Chan, P. Cotelle, N. Cotelle, J. L. Bernier and J. P. Hénichart, *Bioorg. Med. Chem. Lett.*, 1991, **1**, 277.
26 J. R. Rachele, *J. Org. Chem.*, 1963, **28**, 2898.
27 J. C. Sheehan and D.-D. H. Yang, *J. Am. Chem. Soc.*, 1958, **80**, 1154.
28 (*a*) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648; (*b*) C. Lee, W. Yang and R. G. Parr, *Phys. Rev.*, 1988, **B37**, 785; (*c*) R. Colle and O. Salvetti, *Theor. Chim. Acta*, 1975, **37**, 329.
29 C. Adamo and V. Barone, *J. Chem. Phys.*, 1998, **108**, 664.
30 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle and J. A. Pople, *GAUSSIAN 98 (Revision A.9)*, Gaussian, Inc., Pittsburgh, PA, 1998.
31 M. M. Hurley, L. F. Pacios, P. A. Christiansen, R. B. Ross and W. C. Ermler, *J. Chem. Phys.*, 1986, **84**, 6840.
32 T. H. Dunning, *J. Chem. Phys.*, 1971, **55**, 716.
33 T. H. Dunning, *J. Chem. Phys.*, 1970, **53**, 2823.
34 (*a*) R. Ditchfield, *Mol. Phys.*, 1974, **27**, 789; (*b*) K. Wolinski, J. F. Hinton and P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251.
35 A. Schafer, H. Horn and R. Ahlrichs, *J. Chem. Phys.*, 1992, **97**, 2571.