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Interactions of Cu(I) with Selenium-Containing Amino Acids Determined by NMR, XAS, and DFT Studies

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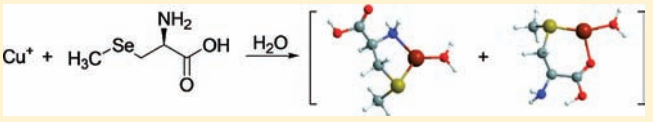
Supporting Information

ABSTRACT: Cu(I) coordination by organoselenium compounds was recently reported as a mechanism for their prevention of copper-mediated DNA damage. To establish whether direct Se−Cu coordination may be involved in selenium antioxidant activity, Cu(I) coordination of the selenoamino acids methyl-*Se*-cysteine (MeSeCys) and selenomethionine (SeMet) was investigated. NMR results in D_2O indicate that Cu(I) binds to the Se atom of both MeSeCys and SeMet as well as the carboxylic acid oxygen atom(s) or amine nitrogen atoms. X-ray absorption spectroscopy (XAS) and density functional theory (DFT) results confirm Se−Cu coordination, with the identification of a 2.4 Å Se−Cu vector in both the Se- and Cu-EXAFS data. XAS studies also show Cu(I) in an unusual three-coordinate environment with the additional two ligands arising from O/N (2.0 Å). DFT models of 1:1 Cu-selenoamino acid complexes suggest that both selenoamino acids coordinate Cu(I) through the selenium and amino groups, with the third ligand assumed to be water. These compounds represent the first structurally characterized copper(I) complexes with sulfur- or selenium-containing amino acids.



INTRODUCTION

Selenium is a trace element essential for cellular functioning and is incorporated into antioxidant proteins and other selenoenzymes, including thioredoxin reductase (TrxR) and glutathione peroxidase (GPx).[1−3] Major dietary sources of selenium are the naturally occurring amino acids selenomethionine (SeMet) and methyl-*Se*-selenocysteine (MeSeCys) found in grains and vegetables grown in selenium-rich soils, especially members of the onion and cabbage family.[4−6] SeMet is incorporated randomly into proteins in place of methionine (Met), and both SeMet and MeSeCys have been used as selenium supplements.[4,7−9]

SeMet has potent growth inhibition and apoptotic activities against multiple human cancer cell lines,[10−12] but also acts as an antioxidant to prevent cell death.[13−16] As a result, SeMet has been widely tested in clinical trials for cancer prevention.[12,17−21] However, high doses of SeMet cause acute and chronic toxicity in animal and avian models, although the mechanism of its biological effects of SeMet remains uncertain.[22,23] SeMet is believed to exert some of its antioxidant activity through a GPx-like mechanism,[24,25] and an oxidized intermediate has been characterized by ^{77}Se NMR and DFT calculations as a pH-dependent equilibrium of a selenoxide and a selenurane.[26] MeSeCys also can act as an antioxidant and a prooxidant[6,9,27,28] but may undergo selenoxide elimination to bioactive terminal selenium derivatives.[29,30]

Recently, MeSeCys and SeMet have been found to prevent copper-mediated oxidative DNA damage by copper coordination, a mechanism distinct from their GPx-like activities.[28,31] Because copper binding results in antioxidant activity, understanding how these selenoamino acids bind copper is required to understand and interpret this new antioxidant mechanism. In this work, copper(I) coordination by SeMet and MeSeCys (Scheme 1) was investigated using one-dimensional ^{1}H, two-dimensional ^{1}H-COSY, and ^{1}H−^{13}C HSQC NMR spectroscopy; X-ray absorption spectroscopy (XAS); and density functional theory (DFT) calculations of the 1:1 adducts. This work represents the first example of a Cu(I) complex with sulfur- or selenium-containing amino acids to be structurally characterized, and suggests that selenoamino acid coordination to the Cu(I) ion through Se is a likely first step in prevention of oxidative damage.

EXPERIMENTAL AND THEORETICAL METHODS

Materials and Methods. All NMR experiments were performed under argon or vacuum using standard air-sensitive techniques. Due to the air sensitivity of Cu(I)−amino acid complexes, D_2O or H_2O was carefully degassed three times using the freeze−pump−thaw method. ^{1}H NMR spectra were obtained on Bruker-AVANCE 300 and 500 MHz NMR spectrometers. ^{1}H COSY and ^{1}H−^{13}C HSQC spectra were obtained on Bruker-AVANCE 500 MHz NMR spectrometer. Chemical shifts are reported in δ relative to tetramethylsilane (TMS) and referenced to D_2O (δ 4.79).[32] pH measurements of D_2O solutions were performed on a standard pH meter and converted to pD using the formula pD = pH + 0.4.[33]

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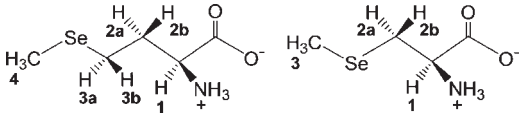
Scheme 1. Structures of Selenomethionine (SeMet, left) and Methylselenocysteine (MeSeCys, right) Showing Atom Numbering



NMR Experiments. Selenomethionine (3.92 mg, 2.00 μmol) or *Se*-methylcysteine (3.64 mg, 2.00 μmol) was dissolved in phosphate buffer (1.0 mL in D_2O, 200 mM) at pD 7.5. $[Cu(NCCH_3)_4][BF_4]$[34] (6.40 mg, 2.00 μmol) was also dissolved in phosphate buffer (1.0 mL in D_2O, 200 mM) at pD 7.5. Half of each solution (0.5 mL) was then mixed and cannula transferred into an NMR tube under argon to make a sample with a final Cu(I) to selenoamino acid ratio of 1:1 and a final concentration of 1.0 mM for each species. A similar procedure was used to prepare NMR samples in pure D_2O (pD 7.5) or in D_2O with 10% glycerol (vol/vol, pD 7.5); no significant changes in chemical shifts or coupling were observed compared to similar spectra in D_2O alone (Figure S4). Attempts to prepare NMR samples with 1:5 Cu(I) to selenoamino acid ratios (1.0 mM and 5.0 mM concentrations, respectively) resulted in sample precipitation over time, preventing acquisition of useable spectra. Immediately after sample preparation, all NMR tubes were flame-sealed to prevent oxygen contamination.

X-ray Absorption Spectroscopy. Samples were generated in air just prior to collecting XAS by quickly mixing a $CuSO_4$ solution, ascorbic acid, and glycerol in MOPS buffer [pH 7.4, 10 mM; MOPS = 3-(*N*-morpholino)propanesulfonic acid] with a solution of MeSeCys, such that the final concentration was 1 mM Cu. Both 1:1 (Cu/amino acid) complexes and 1:5 complexes were prepared. Glycerol was added at 30% vol/vol to prevent ice crystal formation during the rapid freeze in liquid nitrogen. The solutions were passed through a syringe filter (0.2 μm) to ensure that no Cu precipitates were in the solution prior to loading the XAS Lucite cells with a Kapton tape cover. The spectrum of a sample of MeSeCys without copper was collected as a control. Spectra for Cu-SeMet complexes were also measured (500 μM final concentration), but these samples were more unstable in the X-ray beam, and more prone to precipitation.

X-ray absorption spectra were measured at SSRL (BL9-3, 100 mA) and NSLS (X3B, 240 mA) at cryogenic temperatures (<20 K) using a solid state Ge detector (Canberra 30 element at SSRL, 13 element at NSLS) to detect either the selenium or copper Kα fluorescence. At SSRL, a Lytle fan and 3 mil Ni filter were used to minimize background scatter. Both beamlines were run fully tuned by using a harmonic rejection mirror upstream from the monochromator. Count rates were kept well within the linear range of the detector. The spectrum of either a selenium or copper foil was measured simultaneously, and the edge inflection point of the calibration foils was set to 12 658 eV (Se) or 8980.3 eV (Cu).[35] Data were analyzed using EXAFSPAK, a free software program available from SSRL. EXAFSPAK was interfaced with FEFF7.0 for theoretical phase and amplitude functions.[36] The absorber−scatterer distance, R, and the Debye−Waller factor, σ^2, were freely varied in the fits, whereas coordination number (N) values were fixed but incrementally altered to refine the best value. Values of E_0 were fixed at −12 eV for initial fits and then were allowed to vary once the coordination environment was determined.

DFT Calculations. Geometry optimizations were performed with the BP86[37,38] exchange-correlation functional using PQS version 3.3.[39] Copper and selenium were represented by the Ermler−Christiansen relativistic effective core potential.[40] Nitrogen, oxygen, and hydrogen centers attached to noncarbon heavy atoms were represented by the Dunning split-valence triple-ζ plus polarization function basis set (TZVP).[41] Hydrocarbon fragments were double-ζ quality with polarization functions added to carbon.[42] Reported structures were characterized as minima on the potential energy surface by frequency calculations. Time-dependent DFT (TD-DFT) calculations were performed using Gaussian 03[43] and the mPW1PW91[44] exchange-correlation functional to generate the first 20 excited states of the Cu(I)−amino acid complex.

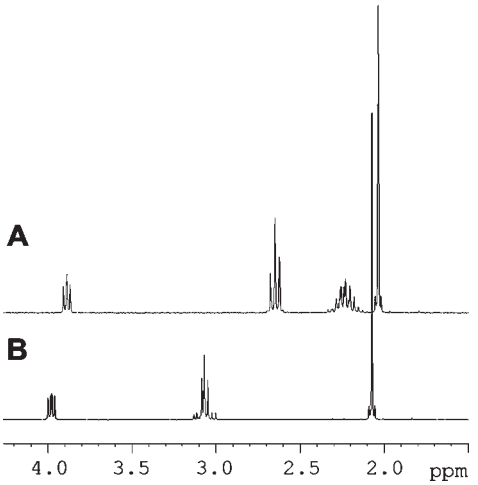


Figure 1. ^{1}H NMR spectra of (A) SeMet and (B) MeSeCys in D_2O/phosphate buffer (200 mM) at pD 7.5.

RESULTS AND DISCUSSION

Previous work by Battin et al. demonstrated that selenoamino acid−copper binding is essential for copper-mediated DNA damage inhibition as the primary mechanism for antioxidant ability.[28,31] However, the coordination number and geometry of these complexes is unknown, as is whether copper binds through the selenium, carboxylic acid, the amine, or some combination of these groups. Since Cu(I) is diamagnetic and amenable to NMR spectroscopy, NMR studies of the copper binding to SeMet and MeSeCys were undertaken. The ^{1}H NMR spectra of SeMet and MeSeCys (10 mM) in D_2O with phosphate buffer (200 mM) at pD 7.5 are shown in Figure 1, and chemical shifts are provided in Tables 1 (SeMet) and 2 (MeSeCys) using the molecule numbering shown in Scheme 1. The ^{1}H chemical shifts and coupling for both compounds were consistent with previously published work.[45]

When 1 equiv of Cu(I) is added to MeSeCys in phosphate buffer (200 mM, pD 7.5; Figure S1) or in pure D_2O (pD 7.5; Figure 2), the ^{1}H NMR resonances are significantly shifted compared to MeSeCys alone. In phosphate buffer, the H2 and H3 resonances are shifted downfield by δ 0.1, indicating Cu(I) binding to the selenium atom. In D_2O, the H2 and H3 resonances are similarly shifted by δ 0.06 and 0.2, respectively. The generally larger change in chemical shifts without phosphate buffer suggests stronger copper−selenium binding without buffer competition for binding. A slight shift of δ 0.07 and 0.03 for H1 in phosphate buffer and D_2O, respectively, indicates that Cu(I) may bind weakly to the carboxylic acid oxygen atom(s) or the amine nitrogen. These observed NMR shifts are consistent with observations from the interactions of Ag(I) with methyl-*S*-cysteine (MeCys) and Met.[46] In both cases, the ^{1}H NMR resonances broaden significantly upon copper(I) addition, but no color change is observed, indicating that no Cu(II) is formed in the NMR sample. After 24 h, the ^{1}H NMR resonances of

Table 1. ^{1}H, ^{1}H-COSY, and ^{1}H−^{13}C HSQC NMR Data for Selenomethionine (SeMet; 1 mM) in D_2O (pD 7.5) with and without Addition of 1 equiv Cu(I)

		^{1}H resonance (δ, coupling in Hz)			^{1}H COSY[b]	^{13}C (δ)[c]
label	assignment	SeMet in buffer[a]	SeMet + Cu(I) in buffer[a]	SeMet + Cu(I) in D_2O	SeMet + Cu(I) in D_2O	
1	C*H*NH_3^+	3.84 (dd; $^3J_{CH}$ = 5.7, 6.8)	3.82 (br s)	3.84 (br s)	2	
3a, 3b	CH_2	2.63 (t; $^3J_{CH}$ = 7.8)	2.23 (br s)	2.79 (br s)	2	24.6
2a, 2b	CH_2	2.32−2.10 (m)	2.68 (br s)	2.28 (br s)	1,3	31.2
4	CH_3Se	2.04 (s; $^2J_{HSe}$ = 10.5)	2.09 (br s)	2.18 (br s)		9.5
CH_3CN			2.06 (br s)	2.07 (br s)		∼2

[a] In D_2O/phosphate buffer (200 mM; pD 7.5). [b] Hydrogen atom coupling as determined from ^{1}H COSY NMR spectrum. [c] ^{13}C NMR shifts as determined from ^{1}H−^{13}C HSQC spectrum.

Table 2. ^{1}H, ^{1}H-COSY, and ^{1}H−^{13}C HSQC NMR Data for Methyl-*Se*-selenocysteine (MeSeCys; 1 mM) in D_2O (pD 7.5) with and without Addition of 1 equiv Cu(I)

		^{1}H resonance (δ, coupling in Hz)			^{1}H COSY[b]	^{13}C (δ)[c]
label	assignment	MeSeCys in buffer[a]	MeSeCys+ Cu(I) in buffer[a]	MeSeCys + Cu(I) in D_2O	MeSeCys + Cu(I) in D_2O	
1	C*H*NH_3^+	3.95 (dd; $^3J_{CH}$ = 4.5, 6.9)	4.02 (br s)	3.98 (br s)	2	
2a (*trans*)	CH_2	3.06 (d; $^3J_{CH}$ = 4.5, $^2J_{HSe}$ < 11.7)	3.16 (br s)	3.11 (br t)	1	25.6
2b (*cis*)	CH_2	3.03 (d; $^3J_{CH}$ = 6.3, $^2J_{HSe}$ < 10.4)	3.16 (br s)	3.11 (br t)	1	25.6
3	CH_3Se	2.04 (s; $^2J_{HSe}$ = 10.5)	2.22 (br s)	2.22 (br s)		7.6
CH_3CN			2.07 (br s)	2.07 (br s)		∼2

[a] In D_2O/phosphate buffer (200 mM; pD 7.5). [b] Hydrogen atom coupling as determined from ^{1}H COSY NMR spectrum. [c] ^{13}C NMR shifts as determined from ^{1}H−^{13}C HSQC spectrum.

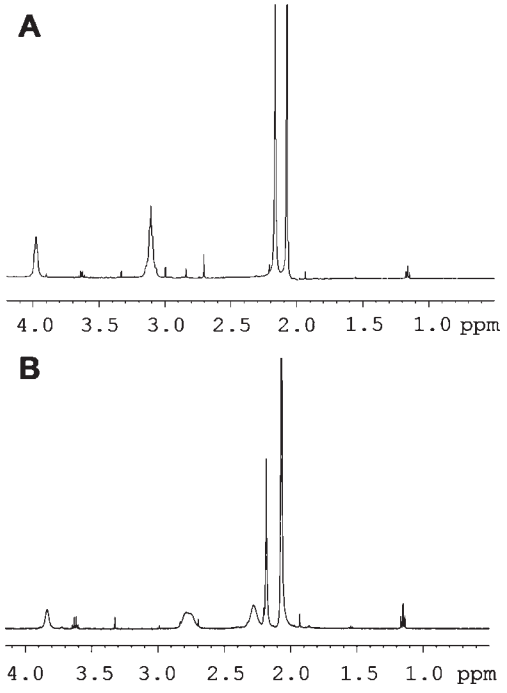


Figure 2. ^{1}H NMR spectra of (A) MeSeCys and (B) SeMet 5 min after addition of 1 equiv Cu(I) in D_2O at pD 7.5.

MeSeCys with 1 equiv Cu(I) show no appreciable change, so this major product is stable for at least 1 day (Figure S2).

Similar ^{1}H NMR experiments were performed with SeMet by adding 1 equiv of Cu(I) in D_2O (Figure 2, Table 1). In the presence of Cu(I), H2a, H3, and H4 resonances are shifted downfield by δ 0.15, 0.16, and 0.18, respectively, compared to SeMet alone, indicating direct copper−selenium binding. ^{1}H NMR studies of Cu(I) coordination to the methionine derivative *N*-[*N*-((5-*H*-thieny1)methylidene)-methionyl]histamine show similar downfield NMR shifts for hydrogen atoms 2 ($\Delta\delta$ = 0.05), 3 (0.12), and 4 (0.14) upon Cu(I) binding to the methionine group.[47] Although an X-ray crystal structure of this methionine derivative with Cu(I) was not obtained, a structure of the analogous Ag(I) complex was found to coordinate through the S atom.[46] The slight shift of δ 0.01 for H1 suggests a very weak interaction, if any, between Cu(I) and the oxygen atoms of the carboxylic acid group or the nitrogen atom of the amine group. After 24 h, the major Cu-SeMet product shows identical ^{1}H NMR resonances (Figure S2), but additional minor products form compared to Cu-MeSeCys samples.

In contrast, no significant ^{1}H NMR chemical shift changes are observed when 1 equiv Cu(I) is added to SeMet in phosphate buffer (Figure S1, Table 1), although peak broadening is again observed. Thus, in buffered samples, copper−selenium binding is present, as determined from the ^{1}H NMR resonance broadening, but is much weaker for the SeMet complex with Cu(I). To eliminate the possibility that phosphate buffer might be competing for copper binding with the selenoamino acids, the phosphate buffer was removed in subsequent experiments.

^{1}H−^{13}C HSQC experiments of Cu-MeSeCys (Figure 3, Table 2) and Cu-SeMet (Figure S2, Table 1) samples provide ^{13}C NMR chemical shifts for the carbon atoms of the coordinated selenoamino acids (no correlation was seen for H1 in either sample), and ^{1}H COSY data show the expected correlations between adjacent protons of the amino acids (Tables 1 and 2). Minor products could not be fully assigned on the basis of the

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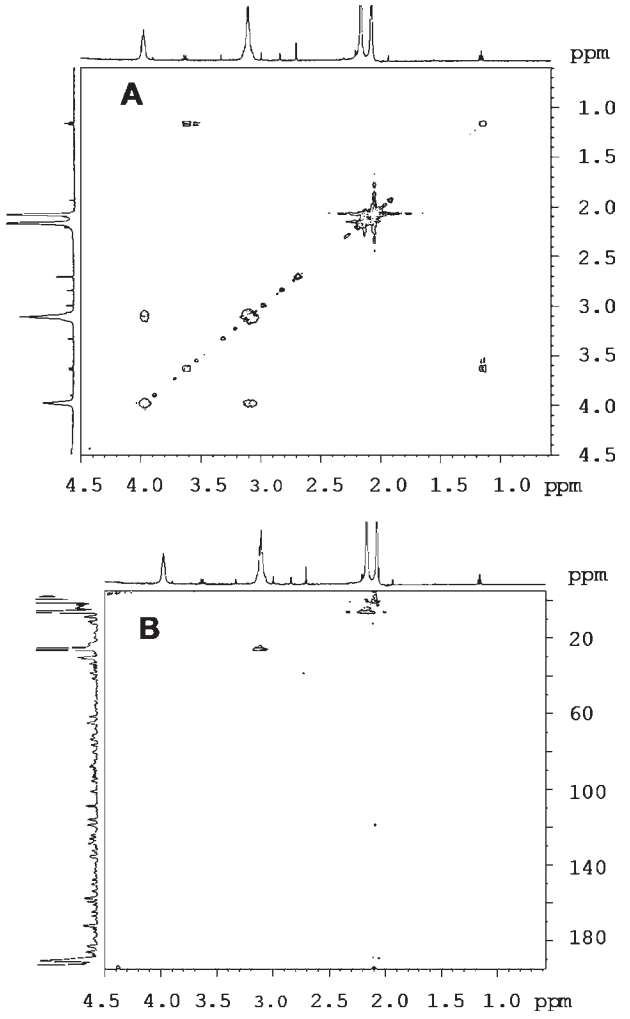

Figure 3. 2D NMR spectra of Cu-MeSeCys: (A) ^{1}H COSY and (B) ^{1}H−^{13}C HSQC after addition of 1 equiv Cu(I) to MeSeCys in D_2O at pD 7.5.

NMR results, but on the basis of work by Ritchey et al.,[26] the minor compounds may be small amounts of the respective oxidized selenoamino acids. Upon addition of Cu(I), the chemical shifts for these minor product ^{1}H NMR resonances are observed at δ 2.84 (J_{HSe} = 12 Hz) and 3.0 for Cu-SeMet and Cu-MeSeCys, respectively (Figures 2 and S2).

Mass spectrometry data indicate that SeMet and MeSeCys form complexes with Cu(I) in a 1:1 ratio,[28] but these data do not provide an indication of the copper binding site(s) on the selenoamino acids. Attempts to grow crystals of the Cu-selenoamino acid adducts suitable for X-ray crystallography were unsuccessful. Therefore, results from XAS and DFT studies of MeSeCys and SeMet with Cu(I) are presented to establish their copper coordination modes.

X-ray Absorption Spectroscopy. XAS solution spectra of the Cu(I)/selenoamino acid complexes in both 1:1 and 1:5 ratios were collected, the former to minimize excess selenium and the latter to maximize complex formation. Data were collected at both the selenium edge (Figure S5) and the copper edge for the 1:1 complexes. For both selenoamino acid samples, the copper edge energy and shape are consistent with three-coordinate Cu(I) (Figure S6).[48]

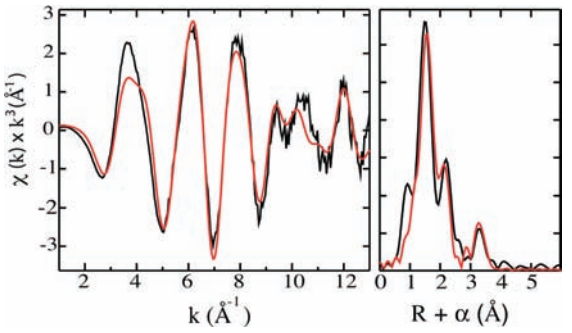

Figure 4. Best fit to the Cu-EXAFS of the 1:5 Cu/MeSeCys complex. Unfiltered experimental data is in black; the best 3 shell fit from Table 3 is in red.

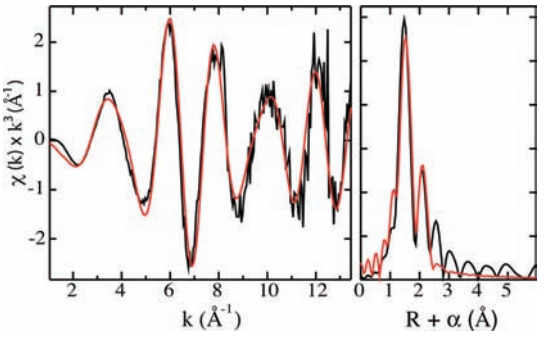

Figure 5. Best fit to the Se-EXAFS of the 1:1 Cu/MeSeCys complex. Unfiltered experimental data is in black; the best 2 shell fit from Table 4 is in red.

EXAFS analysis of the Cu−MeSeCys complex also indicates a coordination number of three, with a single selenium and two oxygen or nitrogen groups providing the optimal fit to the data. The Fourier transforms (FT) of the Cu-EXAFS data for the Cu−MeSeCys 1:5 complex (Figure 4) show a peak at 2.1 Å that corresponds to a heavy ligand at 2.39 Å in the fits. The Se-EXAFS of the 1:1 complex (Figure 5) likewise shows a heavy scatterer at the same distance in addition to the expected carbon scatterers at 1.95 Å, demonstrating that the Cu sees the Se and the Se sees the Cu at the same distance. Furthermore, the data in Figure 6 indicate that the intensity of this feature diminishes in the Se-EXAFS FT as excess Se is added (1:5 complex) and disappears in the control spectra of MeSeCys without copper. The FT of the Cu−SeMet samples also indicate a heavy scatterer in the first coordination sphere at 2.3−2.4 Å (data not shown). However, the SeMet samples were more prone to precipitation and X-ray-induced changes in the spectrum, as evidenced by changes in the edge as multiple scans were collected and large Cu−Cu peaks if the samples were not filtered.

The fits to the 1:1 Cu−MeSeCys samples are shown in Tables 3 (Cu-EXAFS) and 4 (Se-EXAFS). The Debye−Waller factor is high for the Cu−Se interaction, but is consistent with literature values for Cu−Se bonds.[49,50] Both the size of the scatterer and the selenoether nature of the ligand would be expected to yield a long, disordered bond.

The XAS results are fully consistent with the NMR results, demonstrating that MeSeCys is binding Cu(I) with selenium as a ligand at a distance of 2.4 Å. The selenium edge spectrum of the control MeSeCys sample changes in shape upon Cu binding,

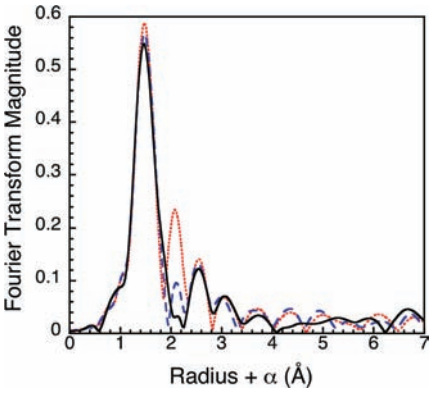

Figure 6. Comparison of EXAFS Fourier transforms at the selenium edge for samples at different Cu/ligand ratios for 1:1 Cu/MeSeCys (red, small dashes), 1:5 Cu/MeSeCys (blue, long dashes), and 0:1 Cu/MeSeCys (black, solid line). The peak at 2.1, corresponding to the 2.4 Å Se−Cu interaction, is less apparent when excess selenium is present and is not observed in the sample without copper.

Table 3. Copper EXAFS Fits to the 1:5 Complex of MeSeCys[a]

1 Cu/5 MeSeCys				copper edge		
no. shells	scatterer	CN	R (Å)	σ^2 (Å^2)	F'	ΔE_0
2	N or O	2	1.98	0.0050	0.200	−12
	Se	1	2.39	0.0102		
1	N or O	3	1.99	0.0077	0.366	−12
2	N or O	2	1.99	0.0049	0.279	−12
	N or O	1	2.45	0.0015		
3[b]	N or O	2	1.98	0.0049	0.147	−12
	Se	1	2.39	0.0099		
	N or O	2	3.77	0.0043		

[a] 1−13 k, fits to unfiltered data. [b] Shown in Figure 4.

analogous to the changes in chemical shift seen with Cu binding in the NMR data (Figure S5). Both the Cu edge and the Cu-EXAFS fits indicate three-coordinate Cu(I), with the additional two ligands fitting with O/N at 2.0 Å. The final confirmation of complex formation comes from analysis of the selenium EXAFS data that indicates Se−Cu binding at 2.4 Å, with the feature diminishing as excess selenium is added or disappearing in the absence of Cu. The NMR data confirm that a single species predominates in solution, an important consideration in XAS experiments. Both the NMR and XAS data indicate that the Cu−SeMet complex is less stable than the Cu−MeSeCys complex.

DFT Calculations. For the modeling studies, bidentate coordination of the amino acids was assumed through the selenium center and either the amine or carboxylate group (Scheme 2). The remainder of the Cu(I) coordination sphere was assumed to be completed by one water molecule for 3-coordination at the copper center, yielding $[Cu(MeSeCys)(OH_2)]^+$ ($\mathbf{1_{Se}}$) and $[Cu(SeMet)(OH_2)]^+$ ($\mathbf{2_{Se}}$). Attempts to optimize structures with two water molecules bonded to Cu resulted in dissociation of one of the water molecules. For the Se/N linkage isomers, the neutral amino acid (NH_2/COOH) was maintained for an overall +1 charge of the complex ion.

Geometry optimizations of the Se/O isomers were started with the amino acid in the zwitterionic form (NH_3^+/COO^-),

Table 4. Selenium EXAFS Fits to the 1:1 Complex of MeSeCys[a]

1 Cu/1 MeSeCys				selenium edge		
no. shells	scatterer	CN	R (Å)	σ^2 (Å^2)	F'	ΔE_0
2	C	2	1.95	0.0023	0.251	−12
	Cu	1	2.40	0.0122		
1	C	2	1.95	0.0024	0.369	−12
2	C	2	1.95	0.0024	0.248	−12
	C	2	2.42	0.0035		
2[b]	C	2	1.95	0.0023	0.159	−12
	Cu	0.7	2.39	0.0087		

[a] 1−13.5 k, fits to unfiltered data. [b] Shown in Figure 5.

but the proton migrated to the carboxylate in the fully optimized structures. Se/O structures with axial conformations of the NH_2 group were not calculated because these geometries required breaking the resulting $COOH\cdots NH_2$ hydrogen bonding interaction. Formation of complexes calculated from the amino acids and $[Cu(OH_2)_2]^+$ were favorable by ∼35 kcal/mol (ΔE + ZPE). The diaquocopper(I) ion was used rather than $[Cu(OH_2)_4]^+$, because the latter is unstable in the gas phase.[51]

For the MeSeCys complex $\mathbf{1_{Se}}$, four conformations of the five-membered ring formed through bonding of the amine to the Cu(I) center of the Se/N isomer and two conformations of the six-membered cyclic Se/O isomers were considered on the basis of the position (axial vs equatorial) of the Me and NH_2 or COOH groups (Scheme 2 and Table 5). Bond distances around the Cu(I) center for the Se/N isomers (∼2.35 Å for Cu−Se and 2.06−2.11 Å for Cu−N, respectively) are in better agreement with EXAFS data than the Se/O isomers which have slightly shorter Cu−Se distances (∼2.25 Å). The isomers with Cu−Se/N binding were generally favored over the Cu−Se/O isomers by 2−3 kcal/mol. The preference for Se/N coordination to Cu(I) agrees with conclusions about the Ag(I)−Met interaction (S/N coordination) determined from ^{1}H NMR chemical shifts.[46] The relative energies of the Se/N conformers (Table 5) are generally less than 1 kcal/mol, and rapid interconversion of these species is consistent with the broad resonances observed in the ^{1}H NMR spectra for the Cu(I) complexes (Figures 1 and 2).

The lowest energy conformation $\mathbf{1_{SeN}}^{\mathbf{eqax}}$ (Figure 7) is stabilized by the hydrogen bonding interaction between the carboxylic acid and the amine proton and limited steric interactions with the methyl group. The coordination about the metal in these three-coordinate species is roughly planar, with distortions from the optimal trigonal planar angles due to the constraints of the bidentate MeSeCys ligand. The Se/O isomers are slightly less stable than the Se/N isomers and may be present in small concentrations in solution. These Cu−Se/O species are best described as two-coordinate about the Cu(I) center due to the linear arrangement of Se and the aquo ligand (e.g., 163° in $\mathbf{1_{SeO}}^{\mathbf{eqeq}}$). The carboxylic acid forms a weak $Cu\cdots O$ interaction at roughly a 90° angle to the selenium and aquo ligands (i.e., approximately T-shaped).

A conformational analysis of $\mathbf{2_{Se}}$ showed that the six-membered cyclic $\mathbf{2_{SeN}}$ isomers were 2−5 kcal/mol more stable than the seven-membered cyclic $\mathbf{2_{SeO}}$ isomers (Table 5). On the Gibbs free energy surface, $\mathbf{2_{SeN}}^{\mathbf{eqax}}$ is the lowest energy conformation and is stabilized by the hydrogen-bonding interaction between the carboxylate carbonyl oxygen and an amine proton. If

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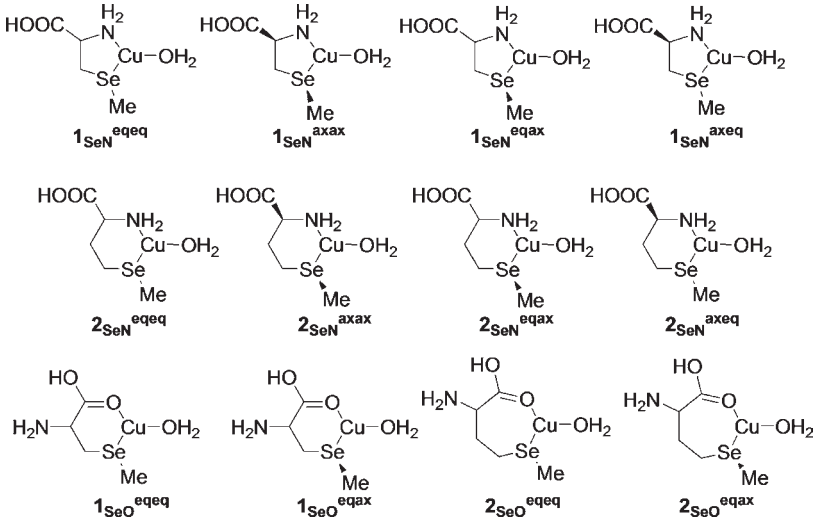
Scheme 2. Conformations of Cu(I)−Selenoamino Acid Aquo Complexes Used for DFT Modeling[a]



[a] All species carry a net positive charge (+1).

Table 5. Selected DFT(BP86) Structural Parameters and Relative Energies for the Cu(I)−MeSeCys and −SeMet Complexes 1 and 2

	d_{Cu-Se}, Å	$d_{Cu-N/O}$, Å	$d_{Cu-Owat}$, Å	∠Se−Cu−N/O, deg	∠Se−Cu−O_{wat}, deg	∠O_{wat}−Cu−N/O, deg	Δ*E* + ZPE, kcal/mol	Δ*G*, kcal/mol
$\mathbf{1}_{SeN}^{axax}$	2.343	2.114	2.011	93.2	145.5	121.1	0.5	0.4
$\mathbf{1}_{SeN}^{axeq}$	2.346	2.082	2.007	95.5	141.1	123.4	1.1	0.9
$\mathbf{1}_{SeN}^{eqax}$	2.363	2.064	2.004	95.3	138.0	126.7	0.0	0.0
$\mathbf{1}_{SeN}^{eqeq}$	2.355	2.065	2.003	94.8	139.1	126.1	0.7	0.5
$\mathbf{1}_{SeO}^{eqax}$	2.280	2.084	1.985	106.5	159.6	93.8	3.1	3.3
$\mathbf{1}_{SeO}^{eqeq}$	2.277	2.103	1.981	104.4	163.0	92.6	3.0	3.2
$\mathbf{2}_{SeN}^{axax}$	2.314	2.094 (2.604)[a]	2.065	109.6	141.6	108.7	0.4	1.7
$\mathbf{2}_{SeN}^{axeq}$	2.299	2.104 (2.636)[a]	2.051	103.2	148.6	108.2	−0.6	0.8
$\mathbf{2}_{SeN}^{eqax}$	2.318	2.024	2.065	115.6	129.9	114.5	0.0	0.0
$\mathbf{2}_{SeN}^{eqeq}$	2.311	2.029	2.066	113.3	132.5	114.2	0.4	0.7
$\mathbf{2}_{SeO}^{eqax}$	2.285	2.059	2.032	127.9	146.8	85.2	2.5	3.3
$\mathbf{2}_{SeO}^{eqeq}$	2.276	2.140	1.976	106.1	168.0	85.8	3.2	5.1

[a] Values in parentheses are for the Cu···O distance.

thermal and entropy corrections are not included, the $\mathbf{2}_{SeN}^{axeq}$ conformer is −0.6 kcal/mol more stable. Each of the COOH axial Se/N conformers has a weak Cu···O interaction, and one may consider the coordination sphere to be a distorted tetrahedron. This interaction counterbalances steric interactions involving the equatorial methyl group. In the Se/O complexes, the coordination sphere about copper is more strained due to the limitations of the 7-membered ring, and the ligands are coordinated with a linear arrangement of Se and the aquo ligand for an overall T-shaped geometry similar to the $\mathbf{1}_{SeO}$ isomers.

The experimental UV−vis spectra for mixtures of amino acid, $CuSO_4$, and ascorbic acid (to reduce the cuprous ion) show a small shoulder at ∼240 nm.[28,31] TD-DFT(mPW1PW91) calculations on the DFT(BP86)-optimized structures of $\mathbf{1}_{SeN}^{eqax}$ and $\mathbf{2}_{SeN}^{eqax}$ predict a transition around 245 nm for excitation from a Cu d-type MO (HOMO − 1) to the lowest unoccupied MO (LUMO) characterized by antibonding character between the Cu−OH_2 bonding fragment and the amino acid (Figure 8). These transitions are the longest wavelength excitations with a significant oscillator strength (f = 0.015−0.028), a measure of the intensity, relative to the set of calculated excitations. Note that these are not the HOMO−LUMO excitations which occur at longer wavelengths with oscillator strengths 2 orders of magnitude lower than the HOMO − 1−LUMO transitions.

As would be expected, MeSeCys and SeMet binding to Cu(I) is considerably different from reported SeMet, Met, and MeCys coordination to Cu(II). Reported Cu(II) structures show bidentate coordination through the amine nitrogen and carboxylate oxygen atoms of SeMet, Met, and MeCys rather than the Se or S atoms.[52−56] In contrast, the Cu(I) complexes of MeSeCys and SeMet coordinate in a trigonal planar geometry through the Se atom and likely the amine nitrogen; the third coordination site is likely occupied by an aquo ligand. Since MeSeCys and SeMet complexes with soft Pt^{2+} show Pt−Se coordination,[57−60] it is not surprising that the soft Cu(I) would also bind soft Se atoms. In proteins, Cu(I)−Se binding to incorporated selenocysteine residues has been well characterized.[49,61−63]

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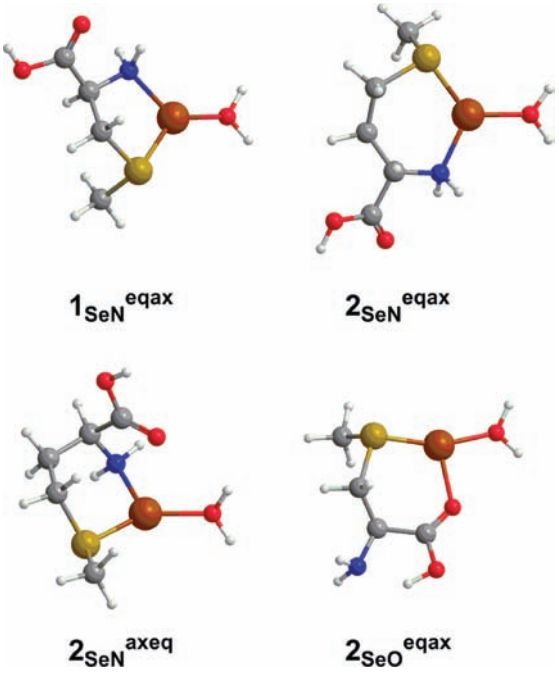

Figure 7. Minimum energy structures (DFT(BP86)) for the Se/N and Se/O isomers of **1** and **2**. Heavy atoms are indicated by orange (Cu), yellow (Se), red (O), blue (N), and gray (C) spheres.

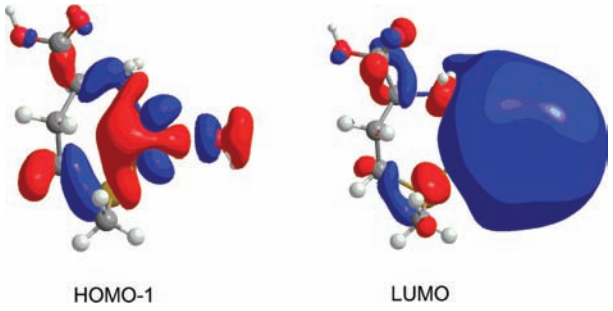

Figure 8. Molecular orbitals for the 245 nm transition of $\mathbf{2}_{SeN}^{eqax}$.

Mononuclear trigonal planar copper complexes with small-molecule selenium ligands are rare, with only $Cu(dmise)_2X$ (dmise = *N*,*N*′-dimethylimidazole selone; X = Cl, Br, I),[64] $[Cu(SeS_2CNC_4H_8)(S_2CN_2C_4H_8)]^-$,[65] and $Cu(PPh_3)(Se_2P_2N)$ structurally characterized.[66] A few trigonal planar Cu(I) complexes formed in aqueous solution with Cu−N or Cu−S bonds have been reported, including $[Cu_2(\mu\text{-cnge})_2(cnge)_2]^{2+}$ (cgne = 2-cyanoguanidine),[67] Cu(thiamine)Br_2,[68] and the thiourea complexes $Cu[SC(NH_2)]_2X$ (X = Cl, I).[69−71] Since Cu(I)−Se interactions occur in aqueous solution immediately upon combining Cu(I) and MeSeCys or SeMet in a 1:1 ratio, this direct Cu−Se binding is likely to be at least partly responsible for the observed inhibition of copper-mediated DNA damage by these selenoamino acids.

CONCLUSIONS

In contrast to Cu(II) coordination, NMR experimental results indicate that Cu(I) binds to the Se atom of both MeSeCys and SeMet, in addition to carboxylic acid oxygen atom(s) or amine nitrogen atoms. DFT and XAS results also confirm direct Se−Cu coordination, with a 2.4 Å Se−Cu vector observed in both the Cu- and Se-EXAFS data. The EXAFS data also indicate a three-coordinate Cu(I) complex, with the additional two ligands arising from O or N atoms at 2.0 Å from copper. DFT models of the 1:1 $Cu^I(OH_2)$−selenoamino acid complexes suggest that these species coordinate through the selenium and amino groups. These MeSeCys and SeMet complexes represent the first structurally characterized selenium- or sulfur-containing amino acid complexes of Cu(I). Since both selenoamino acids prevent Cu(I)-mediated DNA damage by metal binding, the observed Cu−Se interaction may be responsible for this antioxidant behavior.

ASSOCIATED CONTENT

Supporting Information. Listings of NMR data for Cu-SeMet and Cu-MeSeCys and spectra of the Se and Cu edges. This material is available free of charge via the Internet at http://pubs.acs.org.

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