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Methylselenocysteine Treatment Leads to Diselenide Formation in Human Cancer Cells: Evidence from X-ray Absorption Spectroscopy Studies

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Supporting Information

ABSTRACT: The selenoamino acids methylselenocysteine (MeSeCys) and selenomethionine (SeMet) have disparate efficacies as anticancer agents. Herein, we use X-ray absorption spectroscopy to determine the chemical form of selenium in human neuroblastoma cells. Cells treated with MeSeCys contain a significant diselenide component, which is absent from SeMet-treated cells and suggests that metabolites of MeSeCys are capable of altering the redox status of the cells. The differences in the speciation of Se in the selenoamino acid-treated cells may provide insight into the differing anticancer activities of MeSeCys and SeMet.

The anticancer properties associated with supranutritional doses of selenium have resulted in the increasing popularity of Se as a dietary supplement. Organic Se compounds are commonly used in dietary selenium supplements as they are considered less toxic than their inorganic counterparts. However, it is widely recognized that the anticancer properties of Se are dependent on the form of the Se compound. The dependence on form is exemplified in the contradictory results of two clinical trials of 200 μg of Se supplementation per day in the prevention of cancer. In the Nutritional Prevention of Cancer (NPC) Trial, Se supplementation was shown to reduce the incidence of a number of cancers,[1] including prostate cancer,[2] whereas the Selenium and Vitamin E Cancer Prevention Trial (SELECT) ceased early as there was no reduction in the incidence of prostate cancer.[3] In the NPC Trial, a number of organic Se compounds, including SeMet and MeSeCys, were present in the selenized yeast tablets in which the form of Se in the tablet was poorly controlled.[4] Conversely, SeMet was the form chosen to deliver all of the supranutritional Se during the SELECT trial.[5] The choice of SeMet as the preventative agent in the SELECT trial over other organic Se compounds is thought to be a significant factor in the failure of the SELECT trial to demonstrate a reduced incidence of cancer with Se supplementation.[6]

SeMet and MeSeCys are selenoamino acids that have been investigated for their anticancer properties. MeSeCys is generally more toxic than SeMet, presumably due to its metabolism to methylselenol (MeSeH), an antitumorigenic compound [7,8] and putative superoxide generator,[9] whereas SeMet is adventitiously incorporated into selenoproteins in place of methionine.

Previously, we have shown that Se in SeMet-treated human lung cancer cells is found exclusively as carbon-bound species, whereas in MeSeCys-treated cells a significant proportion of Se is present as diselenide species.[10]

Herein, we have used X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopies to show that SeMet and MeSeCys are selectively metabolized by SH-SY5Y human neuroblastoma cells to similar species to those found in lung cancer cells. Moreover, we provide further evidence for the presence of a diselenide species in MeSeCys-treated cells.

SH-SY5Y cells were treated with selenoamino acids at concentrations below their respective IC_{50} concentrations (210 $\pm$ 20 μM for MeSeCys and 460 $\pm$ 70 μM for SeMet). Selenium K-edge XANES spectra of MeSeCys, SeMet, and selenoamino acid-treated cells are shown in Figure 1. Within 4 h of treatment, both MeSeCys and SeMet have undergone significant metabolism. After 24 h, the spectra of the SeMet- and MeSeCys-treated cell spectra differ by 0.5 eV in the position of the second peak (12 665.8 and 12 666.3 eV, respectively) and in the ratio of the intensities of the high- to low-energy peaks (0.89 versus 0.84, respectively). These differences are represented in the difference plot in Figure 1, along with the differences between the spectra of SeMet and MeSeCys model compounds. The differences between the selenoamino acid-treated cells are borne out in linear combination fitting of model compound spectra (see ref 10 and Figures S1−S3 of the Supporting Information for details of model compounds) to the experimental spectra (Table 1). Good fits (with residuals $<0.6 \times 10^{-3}$) to the spectra of MeSeCys-treated cells were achieved and they revealed that the majority of MeSeCys had been metabolized within 24 h to selenocysteine (SeCys) and a diselenide, modeled using diselenocystine (CysSeSeCys). There was little change in speciation between 4 and 24 h. Similarly, the spectra of SeMet-treated cells 4 and 24 h after treatment showed little difference. Unfortunately fits to the SeMet-treated cell spectra were poor (with residuals $>0.75 \times 10^{-3}$), which indicates that the library of

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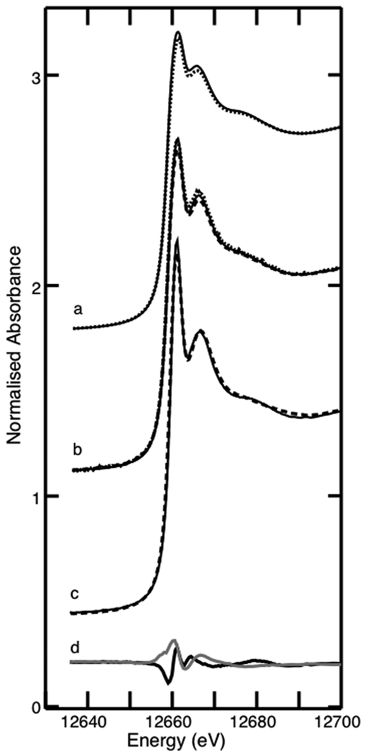

Figure 1. Se K-edge X-ray absorption near-edge spectra of (a) SH-SY5Y cells treated with 100 μM SeMet for 4 h (dotted line) and 24 h (solid line), (b) SH-SY5Y cells treated with 50 μM MeSeCys for 4 h (dotted line) and 24 h (solid line) and 100 μM MeSeCys for 24 h (dashed line), and (c) the model compounds SeMet (solid line) and MeSeCys (dashed line). Difference plots (d) of SeMet and MeSeCys model compound spectra (black) and spectra of SH-SY5Y cells treated with 100 μM SeMet or MeSeCys for 24 h (gray).

model compounds needs to be extended, most likely with spectra of SeMet and SeCys residues in proteins. However, it is clear from the XANES spectra that MeSeCys and SeMet follow different metabolic pathways, with a clear difference being the production of a significant proportion of diselenide species in MeSeCys-treated cells, which is not present in SeMet-treated cells.

The EXAFS spectra of the cells strongly support the results of the linear combination analysis of the XANES spectra. The Se K-edge EXAFS spectrum of MeSeCys-treated cells is shown in Figure 2. Carbon scatterers alone do not fully account for the observed EXAFS spectrum (Figure S4 in the Supporting Information). Notably, the change in the amplitude envelope at

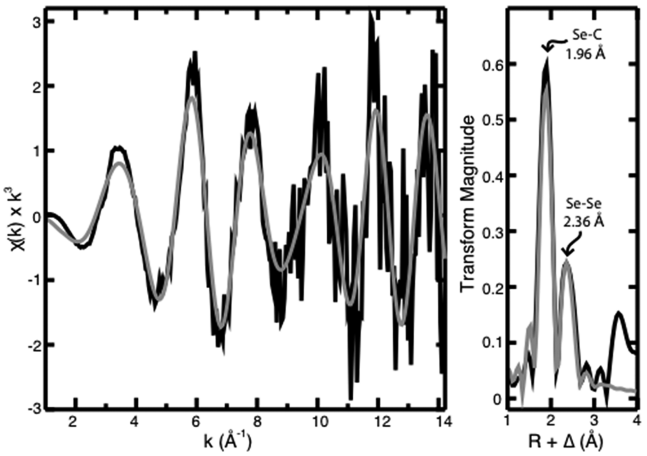

Figure 2. Se K-edge EXAFS spectrum (left) and corresponding Fourier transform (right) of SH-SY5Y cells treated for 24 h with 100 μM MeSeCys showing the experimental (black) and calculated (gray) spectra. Fit parameters are listed in Table S1 in the Supporting Information.

8 Å^{-1} in *k*-space and the peak at 2.36 Å in the Fourier transform are only modeled with the addition of a Se scatterer to the fit. Se and C scatterers were fit to the EXAFS with a Se−Se bond length of 2.36 Å and a Se−C bond length of 1.96 Å (Table S1 in the Supporting Information). Searches of the Cambridge Structural Database indicate that these bond lengths are at or near the median Se−Se and Se−C bond lengths of 2.36 Å and 1.93 Å, respectively.[11] Adding 100 μM of MeSeCys to complete cell culture media under the same conditions as the cell treatments but in the absence of cells does not produce a diselenide component (see EXAFS spectrum in Figure S5 in the Supporting Information). Clearly the diselenide is a product of intracellular metabolism of MeSeCys.

In contrast, the EXAFS spectrum of SeMet-treated cells is readily fit by only two C scatterers with a Se−C bond length of 1.95 Å (Figure S6 and Table S1 in the Supporting Information). Therefore, the comparison of the EXAFS spectra of MeSeCys- and SeMet-treated cells provides further evidence of the presence of a diselenide in the MeSeCys-treated cells, which is absent in SeMet-treated cells. An EXAFS spectrum of MeSeCys-treated cells has not been previously obtained due to the low levels of Se found in MeSeCys-treated A549 human lung cancer cells at treatment concentrations below the IC_{50} concentration.[10] The identification of a diselenide species in the XANES and EXAFS spectra of MeSeCys-treated cells in this

Table 1. Percent Se Species in SH-SY5Y Cells Treated with Selenoamino Acids, As Estimated by a Linear Combination of XANES Model Compound Spectra[a]

			percentage component fitted					
treatment compound	concentration (μM)	time (h)	CysSeSeCys	SeCys	SeMet	MeSeCys	N_{tot}[b]	residual ($\times 10^{-3}$)
MeSeCys	50	4	15(2)	51(5)		33(4)	0.99	0.55
MeSeCys	50	24	11(1)	51(4)		37(3)	0.99	0.33
MeSeCys	100	24	16(1)	65(4)		18(4)	0.99	0.41
SeMet	100	4		96(2)			0.96	0.91
SeMet	100	24		97(4)			0.97	0.78

[a]Values in parentheses are the estimated standard deviations derived from the diagonal elements of the covariance matrix and are a measure of precision. [b]N_{tot} is the sum of the fractions.

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work supports the identification of a diselenide species in the XANES spectra of A549 cells studied previously.

The presence of diselenides in the MeSeCys-treated cells suggests that either small selenol-containing metabolites of MeSeCys or protein-bound selenols have undergone oxidation. Selenols can be oxidized to diselenides under physiological conditions,[12] but the presence of selenols in SeMet-treated A549 cells without any detectable diselenide component[10] indicates that the level of oxidation under normal physiological conditions is not significant. The presence of a substantial level of diselenides in MeSeCys-treated cells is therefore indicative of a change in the redox environment of the cells brought about by MeSeCys and its metabolites, which supports the hypothesis that the MeSeCys metabolite MeSeH is a superoxide generator capable of altering the redox status of the cells.[9] However, this work does not provide direct evidence for the metabolism of MeSeCys to MeSeH as MeSeH is not contained in the model compound library used in the analysis of the XANES spectra.

The difference in the speciation of Se in cells treated with MeSeCys or SeMet, in particular the potential redox-altering capabilities of the metabolites of MeSeCys as evidenced by the presence of diselenides, may account for the observed differences in the toxicity of the selenoamino acids toward the SH-SY5Y cells. In order to better understand the implications of Se speciation in the prevention of cancer by supranutritional Se supplements, we are pursuing these speciation studies in rat models.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental section and additional XANES and EXAFS spectra and acknowledgment of synchrotron radiation facilities used. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

SeMet, selenomethionine; MeSeCys, Se-methylselenocysteine; NPC, Nutritional Prevention of Cancer (Trial); SELECT, Selenium and Vitamin E Cancer Prevention Trial; MeSeH, methylselenol; XANES, X-ray absorption near edge structure; EXAFS, extended X-ray absorption fine structure; SeCys, selenocysteine; CysSeSeCys, diselenocystine

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