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The Central Role of Metal Coordination in Selenium Antioxidant Activity

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Oxidative DNA damage occurs in vivo by hydroxyl radical generated in metal-mediated Fenton-type reactions. Cell death and mutation caused by this DNA damage are implicated in neurodegenerative and cardiovascular diseases, cancer, and aging. Treating these conditions with antioxidants, including highly potent selenium antioxidants, is of growing interest. Gel electrophoresis was used to directly quantify DNA damage inhibition by selenium compounds with copper and H_2O_2. Selenocystine inhibited all DNA damage at low micromolar concentrations, whereas selenomethionine showed similar inhibition at 40 times these concentrations, and 2-aminophenyl diselenide showed no effect. DNA damage inhibition by these selenium compounds does not correspond to their glutathione peroxidase activities, and UV–vis and gel electrophoresis results indicate that selenium–copper coordination is essential for DNA damage inhibition. Understanding this novel metal-coordination mechanism for selenium antioxidant activity will aid in the design of more potent antioxidants to treat and prevent diseases caused by oxidative stress.

Metal-mediated formation of hydroxyl radical (•OH; shown below) from hydrogen peroxide (H_2O_2) is the primary cause of DNA damage and cell death under oxidative stress conditions in both prokaryotes[1] and eukaryotes, including humans.[2,3] The reduced metal ions are regenerated by cellular reductants, making •OH radical formation catalytic in vivo.

$$Fe^{2+} \text{ or } Cu^{+} + H_2O_2 \rightarrow Fe^{3+} \text{ or } Cu^{2+} + OH^{-} + {}^{\bullet}OH$$

Oxidative DNA damage leads to a wide variety of conditions, including cardiovascular disease,[4] cancer,[5] neurodegenerative diseases,[6] and aging.[7] Antioxidants can prevent or ameliorate this oxidative damage,[8−10] and antioxidant supplementation is of great interest for the treatment and prevention of these diseases.[11−14] Selenium in particular is widely studied for its antioxidant properties: the selenium antioxidant drug ebselen reduces tissue damage during stroke,[15] and in a 10-year clinical trial, patients taking selenium supplements had 50% fewer incidences of cancer.[16] Selenium, as selenocysteine, is required for the activity of antioxidant enzymes such as glutathione peroxidases and thioredoxin reductases.[17] Selenocysteine also coordinates to metal ions in the active sites of formate dehydrogenases[18,19] and [NiFeSe] hydrogenases.[20] In addition to selenoproteins, small-molecule selenium metabolites are essential to this element's biological antioxidant activity.[21]

Traditionally, quantification of the antioxidant abilities of selenium compounds has focused on their ability to decompose H_2O_2 in a manner similar to that of glutathione peroxidase (GPx).[22] Quantifying selenium GPx activity involves spectroscopic measurement of the H_2O_2 decomposition in nonaqueous solvents.[23] The focus on the GPx activity confines understanding of the selenium antioxidant behavior to neutralization of H_2O_2, a relatively inert oxidative species compared to •OH.[24] For example, *Escherichia coli* strains lacking catalase to neutralize H_2O_2 show no significant increase in cell death when exposed to H_2O_2 compared to

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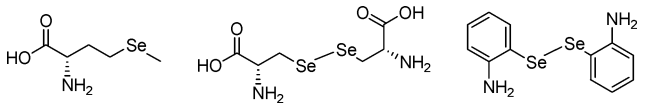

Figure 1. Selenium compounds tested: selenomethionine (left), selenocystine (center), and 2-aminophenyl diselenide (right).

wild-type cells, suggesting that high H_2O_2 levels alone do not contribute to increased cell death.[1] Despite the differences between GPx measurements and biological conditions, De Silva and co-workers reported that selenium compounds with high GPx activity prevent oxidative damage to DNA, although peroxynitrite was used instead of metal-generated •OH to promote DNA damage.[25] The GPx measure of selenium antioxidant activity, however, is likely oversimplified. In fact, the antioxidant activity of ebselen has also been attributed to its faster reduction by thioredoxin reductase instead of reduction by glutathione.[26]

The focus on H_2O_2 neutralization by the GPx activity neglects the fact that selenium can coordinate to metal ions and potentially prevent the formation or release of the more damaging •OH radical. In light of the importance of metal-mediated •OH radical generation and subsequent DNA damage, our efforts have focused on directly quantifying the inhibition of metal-mediated oxidative DNA damage by the selenium compounds selenomethionine, selenocystine, and 2-aminophenyl diselenide (Figure 1). Our results confirm metal ion coordination as a novel mechanism for selenium antioxidant activity.

Because Cu^+ is a soft metal ion and selenium is a soft ligand, selenium compounds should coordinate better to Cu^+ than the harder Fe^{2+} ion.[27] Furthermore, copper generates damaging •OH radical 60 times faster than Fe^{2+}.[28] Thus, we tested antioxidant effects of selenium compounds on copper-mediated DNA damage with gel electrophoresis. A H_2O_2 concentration of 50 μM was used because metal-mediated oxidative DNA damage is fastest at this concentration.[1]

For these experiments, metal-free plasmid DNA is combined with Cu^{2+} (4 μM), ascorbic acid (5 μM), and H_2O_2 at pH 7. Given the limited stability of Cu^+, Cu^{2+} is reduced in situ with ascorbic acid. Ethanol (10 mM) is also added to simulate organic molecules present in a cell that could react with •OH.[1] The •OH radical generated by Cu^+/H_2O_2 cleaves (or "nicks") one DNA strand, causing the normally supercoiled plasmid DNA to unwind. The degree of DNA damage is assessed using electrophoresis to separate the damaged and undamaged forms.[29] Adding a selenium antioxidant inhibits DNA damage, so the antioxidant potential of these compounds can be quantified and directly compared. Reduction potentials (vs NHE) of selenocystine (−488 mV[30]) and 2-aminophenyl diselenide (−654 mV[31]) are much lower than

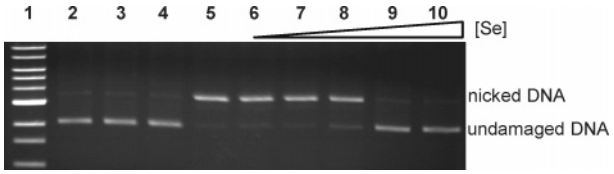

Figure 2. Agarose gel showing a reduction in oxidative DNA damage with the addition of Cu^{2+}/ascorbic acid, H_2O_2, and selenocystine (Se). Lanes: (1) 1-kb ladder; (2) plasmid DNA; (3) DNA + H_2O_2; (4) DNA + Se + H_2O_2; (5) DNA + Cu^{2+}/ascorbic acid + H_2O_2; (6−10) same as lane 5 with increasing [Se] of 0.001, 0.1, 1, 10, and 25 μM, respectively.

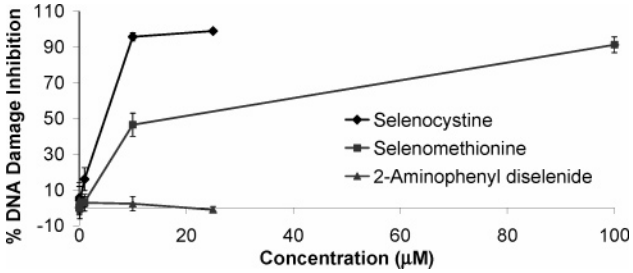

Figure 3. Graph showing the inhibition of oxidative DNA damage by selenium antioxidant compounds. $[Cu^{2+}] = 4$ μM, [ascorbic acid] = 5 μM, $[H_2O_2] = 50$ μM, and [EtOH] = 10 mM. Results shown are averages of at least three trials, and standard deviations are indicated by error bars.

those for $Cu^{2+/+}$ (153 mV[32]) or ascorbic acid (58 mV[33]), so neither Cu^+ nor ascorbic acid will reduce these selenium compounds.

For the gel shown in Figure 2, selenocystine was combined with Cu^{2+} and ascorbic acid for 5 min prior to DNA addition. Once DNA was added, the reaction was again allowed to stand for 5 min before adding H_2O_2. The oxidative damage reactions continued for 30 min and were then quenched by the addition of ethylenediaminetetraacetic acid to chelate the copper. Agarose gels were run, visualized with ethidium, and the DNA band intensities were quantified.[34] As seen in Figure 2, lanes 2 and 3, H_2O_2 alone does not damage DNA in the absence of Cu^+, although such H_2O_2 concentrations readily kill *E. coli*.[1] In this experiment, 25 μM selenocystine completely inhibits oxidative DNA damage (Figure 2, lane 10) caused by copper-generated •OH radicals.

A comparison of DNA damage inhibition for the three selenium compounds is shown in Figure 3. Selenocystine and selenomethionine inhibit 99% of the DNA damage at 25 and 1000 μM, respectively, whereas 2-aminophenyl diselenide does not inhibit DNA damage under these conditions. More dramatically, 10 μM selenocystine inhibits 96% of the DNA damage, compared to only 46% damage inhibition observed for 10 μM selenomethionine.

Most importantly, these results demonstrate that selenium antioxidants significantly inhibit DNA damage at biologically relevant (low micromolar[35]) selenium concentrations. Sele-

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(34) Complete experimental details are provided in the Supporting Information.

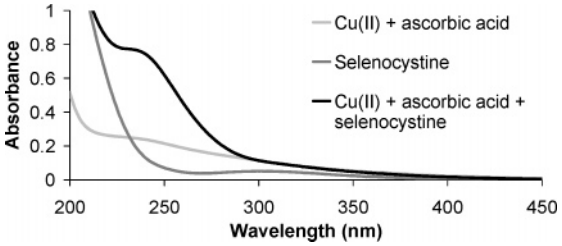

Figure 4. UV−vis spectra of selenocystine (117 μM), Cu^{2+}/ascorbic acid (58 and 73 μM, respectively), and Cu^{2+}/ascorbic acid + selenocystine in water (pH 7). The addition of selenocystine to ascorbic acid results in no change in the UV−vis absorbance (not shown).

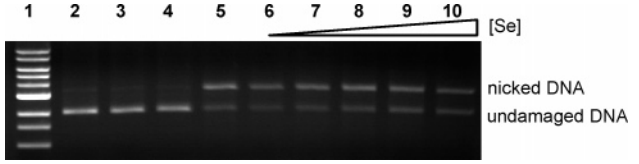

Figure 5. Agarose gel with $Cu(bipy)_2^{2+}$ (bipy = 2,2′-bipyridine) and ascorbic acid showing no decrease in oxidative DNA damage upon the addition of selenocystine (Se). Lanes: (1) 1-kb ladder; (2) plasmid DNA; (3) DNA + H_2O_2; (4) DNA + H_2O_2 + Se; (5) DNA + H_2O_2 + $Cu(bipy)_2^{2+}$/ascorbic acid; (6−10) same as lane 5 with increasing [Se] of 0.001, 0.1, 1, 10, and 25 μM, respectively.

nomethionine, which is used as a selenium supplement[36] but is not capable of strong metal binding, shows less ability to inhibit DNA damage than the potentially metal binding diselenide, selenocystine. The different behavior of selenocystine and 2-aminophenyl diselenide demonstrates that substituents on the selenium substantially alter the antioxidant activity. Studies to determine the effect of various substituents on the ability of selenium compounds to inhibit oxidative DNA damage are currently underway.

To compare these DNA damage inhibition results with GPx activities, we measured the GPx activity for the three selenium compounds in Figure 1.[34] 2-Aminophenyl diselenide and selenocystine had GPx activities 7.5 and 2.2 times that of selenomethionine, respectively. Based on GPx activity measurements, 2-aminophenyl diselenide should be the most potent antioxidant, a result clearly not supported by DNA damage inhibition studies. Thus, oxidative DNA damage inhibition by selenium compounds is significantly different from the GPx antioxidant mechanism and should be considered a novel alternate mechanism for selenium antioxidant activity.

Importance of Metal Coordination on the Selenium Antioxidant Activity. Because selenocystine greatly inhibits oxidative DNA damage by copper and H_2O_2, we examined the reactivity of selenocystine with Cu^+ by UV−vis spectroscopy. The UV−vis spectra of both Cu^{2+}/ascorbic acid and selenocystine in water are shown in Figure 4. Upon the addition of a Cu^{2+}/ascorbic acid solution to selenocystine, a large absorption band is observed (λ_{max} = 235 nm). Similar absorption bands are not observed when Cu^{2+}/ascorbic acid solutions are combined with 2-aminophenyl diselenide or selenomethionine (Supporting Information). The lack of change in the UV−vis spectra for the selenomethionine/Cu^+ solutions suggests that copper coordination by the amine and carboxylate groups of the amino acid is not significant and that the observed change in the UV−vis spectrum for selenocystine/Cu^+ is due to selenium−copper coordination. Similar absorption bands are observed (λ_{max} = 241−257 nm) for reported Cu^+/selenolate complexes.[37]

To further validate this novel mechanism, gel electrophoresis experiments were conducted using $Cu(bipy)_2^{2+}$ (bipy = 2,2′-bipyridine) as the copper source. $Cu(bipy)_2^{2+}$ is reduced by ascorbic acid to $Cu(bipy)_2^+$, and this Cu^+ complex causes DNA damage in the presence of H_2O_2 (Figure 5). The bidentate bipy ligands, however, completely coordinate the copper ion, leaving no possible site for a selenium compound to bind. Indeed, the UV−vis spectrum of $Cu(bipy)_2^{2+}$ with ascorbic acid does not show any significant change upon addition of selenocystine (data not shown), indicating that selenocystine does not coordinate to $Cu(bipy)_2^+$. Figure 5 shows the gel electrophoresis experiment under the same conditions as those used for the gel shown in Figure 2 but with 50 μM $Cu(bipy)_2^{2+}$ as the copper source and 63 μM ascorbic acid.

As can be seen in Figure 5, lane 4, the addition of $Cu(bipy)_2^{2+}$/ascorbic acid, H_2O_2 (50 μM), and ethanol (10 mM) results in largely nicked DNA. Upon the addition of selenocystine (lanes 6−10), no inhibition of DNA damage was observed. The sharp contrast between selenocystine antioxidant behavior with Cu^+ and the fully chelated $Cu(bipy)_2^+$ demonstrates that metal coordination is required for the antioxidant effects of selenocystine.

These experiments establish that selenium compounds prevent oxidative DNA damage from copper-generated •OH radical at biologically relevant concentrations and that the observed oxidative DNA damage inhibition requires selenium−metal coordination. In addition, the observed DNA damage inhibition by these selenium compounds contrasts with traditional GPx measurements for selenium antioxidant activity. Understanding this novel metal-coordination mechanism for the selenium antioxidant activity will aid in the development of more effective selenium antioxidants to prevent and treat diseases caused by oxidative stress.

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Supporting Information Available: Experimental details, gel electrophoresis, and UV−vis experiments for selenomethionine and 2-aminophenyl diselenide, tabular data of gel electrophoresis quantitation, and calculated percent DNA damage inhibition. This material is available free of charge via the Internet at http://pubs.acs.org.

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