Effects of inorganic selenium compounds on oxidative DNA damage

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Abstract

Exposure of Escherichia coli or mammalian cells to H₂O₂ results in cell death due to iron-mediated DNA damage. Since selenium compounds have been examined for their ability to act as antioxidants to neutralize radical species, and inorganic selenium compounds are used to supplement protein mixes, infant formula, and animal feed, determining the effect of these compounds on DNA damage under conditions of oxidative stress is crucial. In the presence of Fe(II) and H₂O₂, the effects of Na₂SeO₄, Na₂SeO₃, SeO₂ (0.5–5000 μM), and Na₂Se (0.5–200 μM) on DNA damage were quantified using gel electrophoresis. Both Na₂SeO₄ and Na₂Se have no effect on DNA damage, whereas SeO₂ inhibits DNA damage and Na₂SeO₃ shows antioxidant or pro-oxidant activity depending on H₂O₂ concentration. Similar electrophoresis experiments with [Fe(EDTA)]²⁻ (400 μM) and Na₂SeO₃ or SeO₂ show that metal coordination by the selenium compound is required for antioxidant activity. In light of these results, Na₂SeO₄ may be safer than Na₂SeO₃ for nutritional supplements.

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1. Introduction

Generation of reactive oxygen species (ROS) in cells can damage cellular components including nucleic acids, lipids, and proteins [1]. Oxidative damage in vivo is caused by ROS such as the hydroxyl radical (·OH) produced in the Fenton reaction [2,3].

Fe(II) + H₂O₂ → Fe(III) + ·OH + OH⁻

Iron-generated hydroxyl radical is the primary cause of oxidative DNA damage and cell death in both prokaryotes [4] and eukaryotes, including humans [4,5]. This DNA damage can lead to conditions such as aging [6], cancer [7], neurodegenerative [2], and cardiovascular diseases [8]. Since cellular reductants such as NADH reduce Fe(III) back to Fe(II), the production of hydroxyl radical is catalytic in vivo [9].

Two kinetically-distinct modes of cell killing exist when Escherichia coli or mammalian cells are exposed to H₂O₂. Mode I killing is faster than Mode II and occurs at low peroxide concentrations (1–5 mM), whereas Mode II killing occurs at peroxide concentrations greater than 10 mM and is independent of H₂O₂ concentration [9,10]. Similar kinetics are also observed for in vitro DNA damage with maximal damage under Mode I conditions at 50 μM H₂O₂ and under Mode II conditions at H₂O₂ concentrations greater than 10 mM [9–11]. Iron-mediated cleavage of the DNA backbone under Mode I and II conditions occurs selectively at different DNA sequences: RTGR (with cleavage at the thymidine deoxyribose) for Mode I damage and RGGG for Mode II (R = A or G) [11]. The difference in sequence-selectivity is attributed to differential localization of Fe(II) ions on DNA. Under Mode I conditions, hydroxyl radical is likely generated by Fe(II) bound to solvent-accessible sites on the deoxyribose-phosphate backbone of DNA, whereas DNA damage under Mode II conditions occurs when H₂O₂ reacts with more-sterically-hindered Fe(II) bound to DNA bases, particularly guanine [11].

Antioxidants can prevent or reduce oxidative DNA damage [12–14], and selenium has been widely studied for its antioxidant and anticancer effects [15–17]. Selenium is
also an essential micronutrient for both humans and animals with a RDA ranging from 55 to 350 μg/day for humans [18], and it is incorporated as selenocysteine in the active site of antioxidant proteins, including glutathione peroxidases (GPx) and thioredoxin reductases [15, 19, 20]. Selenium is found in most multivitamins and dietary supplements [18] as selenomethionine, selenite (SeO\textsubscript{3}\textsuperscript{2–}), or selenate (SeO\textsubscript{4}\textsuperscript{2–}) [21]. In addition, selenite and selenate are also incorporated into animal feed, protein mixes, and infant formula [18]. Selenium additives to animal feed, 16.8 tons annually for sheep and cows alone [22], improve animal performance and increase selenium dietary intake for people consuming meat products [23].

Initially, the ability of selenium to prevent hydroxyl radical formation by decomposition of H\textsubscript{2}O\textsubscript{2} to water via GPx enzymes was proposed as a mechanism for their antioxidant activity [15,24]. However, other studies indicate that while selenium is an effective antioxidant, this is not the sole basis for its anticancer activity [15,25,26]. Recently, a new mechanism involving selenometabolites was proposed to explain the anticancer effects of selenium [15,17]. Compounds such as selenite generate hydrogen selenide (H\textsubscript{2}Se), used to incorporate selenium into selenocysteine and selenomethionine. Thus, H\textsubscript{2}Se produced in high concentrations may react with oxygen to produce ROS toxic to cells [19,27,28]. Additionally, selenometabolites such as selenodiglutathione have been found to inhibit cell growth and induce apoptosis in tumor cells [15,17,29].

Although the mechanisms of antioxidant and anticancer activity for selenium compounds are unclear, organoselenium compounds such as selenomethionine have been shown to have antioxidant activity [25,26,30]. In addition, a plethora of anticancer studies have been conducted with both selenium compounds and organoselenium compounds showing conflicting results [15–17,29,31–33]. Few studies have been conducted with either sodium selenite (Na\textsubscript{2}SeO\textsubscript{3}) and selenium dioxide (SeO\textsubscript{2}) [34]. Takahashi and coworkers found that both Na\textsubscript{2}SeO\textsubscript{3} (10 μM) and SeO\textsubscript{2} (50–100 μM) induced apoptosis in HSC-3 cells, whereas Na\textsubscript{2}SeO\textsubscript{4} (100 μM) did not, suggesting that the former compounds are better anticancer agents [15]. In addition, SeO\textsubscript{2} (3–30 μM) was shown to inhibit the growth of lung cancer GLC-82 cells via apoptosis in a dose-dependent and time-dependent manner [35].

Despite the widespread use of inorganic selenium compounds in vitamins and animal feed, relatively little work has been done to determine their antioxidant properties. Thus, we have used gel electrophoresis to study the effects of Na\textsubscript{2}SeO\textsubscript{3}, Na\textsubscript{2}SeO\textsubscript{4}, SeO\textsubscript{2}, and Na\textsubscript{2}Se (Fig. 1) on DNA damage in the presence of H\textsubscript{2}O\textsubscript{2} under both Mode I and II conditions. For the selenium compounds that inhibited DNA damage, the mechanism by which these compounds prevented such damage was also investigated.

### 2. Materials and methods

#### 2.1. Materials

NaCl (99.999% to avoid trace metal contamination), Na\textsubscript{2}SeO\textsubscript{3}, SeO\textsubscript{2}, Na\textsubscript{2}SeO\textsubscript{4} · 10H\textsubscript{2}O, glacial acetic acid, NaOH, 30% H\textsubscript{2}O\textsubscript{2} solution, FeSO\textsubscript{4} · 7H\textsubscript{2}O, and bromophenol blue were from Alpha Aesar. Glucose, agarose, and ampicillin were from EMD Chemicals. TRIS hydrochloride and sodium EDTA were from J.T. Baker. HCl was from WWR Scientific; ethidium bromide from Lancaster Synthesis Inc. Xylene cyanol, peptone, and yeast extract came from EM Science. D\textsubscript{2}O and DCl were from Acros, and NaOD was from Cambridge Isotope Laboratories, Inc. Water was purified using the NANOpure Diamond water deionization system (Barnstead International, Dubuque, IA). Iron-free microcentrifuge tubes were prepared by washing the tubes in 1 M HCl prior to use and rinsing thoroughly. \textsuperscript{77}Se NMR data were obtained from a Bruker Avance 500 MHz NMR spectrometer.

#### 2.2. Purification of plasmid DNA

**DH1 E. coli** cells were transfected with pbSSK, plated on LB/amp plates, and incubated for 16 h at 37 °C. Cell cultures were grown in TB/amp medium inoculated with a single colony and incubated for 15 h at 37 °C. Plasmid DNA was purified from the cell pellets using a QIAprep Spin Miniprep kit, and Tris–EDTA (TE) buffer was used to elute the DNA. Dialysis of plasmid DNA was performed against 130 mM NaCl for 24 h at 4 °C. The resulting DNA concentration was found using UV–vis measurements at A\textsubscript{260} (1A\textsubscript{260} = 50 ng/μL). Purity of plasmid DNA was determined via gel electrophoresis of a digested sample, and all absorbance ratios were within acceptable limits (A\textsubscript{250/260} < 0.95, and A\textsubscript{250/280} > 1.8).

#### 2.3. DNA nicking experiments under Mode I conditions

The indicated concentrations of Na\textsubscript{2}SeO\textsubscript{3}, Na\textsubscript{2}SeO\textsubscript{4}, SeO\textsubscript{2} and Na\textsubscript{2}Se, 0.1 pmol plasmid DNA, 130 mM NaCl, 10 mM ethanol, 2 μM FeSO\textsubscript{4} · 7H\textsubscript{2}O at pH 6 were combined and allowed to stand for 5 min at room temperature. H\textsubscript{2}O\textsubscript{2} (50 μM) [11] was then added and incubated for 30 min. EDTA (50 μM) was added after this time and a total volume of 10 μL was maintained with ddH\textsubscript{2}O. DNA was separated on 1% agarose gels via electrophoresis, stained with ethidium bromide for 30 min, and imaged on an UVIproDBT-8000 gel imager (UVITec, Cambridge,
UK). Quantification of closed-circular and nicked DNA was performed using the UVIpro software and results were shown in a bar graph. Ethidium stains supercoiled DNA less efficiently than nicked DNA, so supercoiled DNA band intensities were multiplied by 1.24 prior to comparison. For gels run with [Fe(EDTA)]$^{2-}$ (400 µM) as the iron source, a similar procedure was used, substituting [Fe(EDTA)]$^{2-}$ (400 µM) for FeSO$_4$·7H$_2$O.

2.4. DNA nicking experiments under Mode II conditions

Similar procedures to Mode I experiments were followed with increases in ethanol (100 mM) and H$_2$O$_2$ (50 mM) [11]. Experiments with Na$_2$SeO$_3$ were performed at pH 7 without iron.

2.5. Data analysis

Percent DNA damage inhibition was determined using the formula $1 - [\%N/\%B] \times 100$, where $\%N = \%$ nicked DNA in the selenium-containing lanes and $\%B = \%$ nicked DNA in the Fe(II)/H$_2$O$_2$ lane. Percentages are corrected for residual nicked DNA prior to calculation. Results are the average of three trials, and standard deviations are indicated by error bars. Percent DNA damage was calculated using the formula $[\%N/\%B] \times 100$, where $\%B = \%$ of nicked DNA in the H$_2$O$_2$ only lane. Statistical significance was determined by calculating $p$ values at 95% confidence ($p < 0.05$ indicates significance) as described by Perkowski and Perkowski [36]. A complete listing of these values can be found in supporting information.

2.6. $^{77}$Se NMR experiments

$^{77}$Se NMR samples (0.75 mL) were prepared by adding SeO$_2$ (2 M) or Na$_2$SeO$_3$ (0.6 M) to solutions of NaCl (2 equiv.) in D$_2$O. Appropriate amounts of NaOD or DCl were added to achieve the desired pD (6.4 or 7.4). Conversion of pH into pD was performed using the formula $p[D^+] = 0.4 + p[H^+]$ [37,38].

3. Results

The selenium compounds in Fig. 1 differ in both oxidation state of the selenium atom and in charge. Selenide, selenite, and selenate are charged species with $-2$, $+4$ and $+6$ selenium oxidation states, respectively. Selenium dioxide, however, is neutral with a $+4$ selenium oxidation state. Our DNA damage studies examine both the ability of these compounds to inhibit iron-mediated DNA damage, and the effects of selenium oxidation state and charge on this activity.

3.1. Mode I experiments with inorganic selenium compounds

A gel electrophoresis experiment testing selenite for its ability to inhibit DNA damage under Mode I conditions (50 µM H$_2$O$_2$, 10 mM ethanol) is shown in Fig. 2a. At this H$_2$O$_2$ concentration, the rate of Mode I DNA damage is maximal; ethanol was added to emulate organic molecules in cells that scavenge hydroxyl radical [11]. From the gel in Fig. 2a, it is clear that both H$_2$O$_2$ alone (lane 3) and Na$_2$SeO$_3$ with H$_2$O$_2$ alone (lane 4) had no effect on DNA damage as compared to the plasmid-only lane (lane 2). Addition of both Fe(II) (2 µM) and H$_2$O$_2$ (lane 5) produced 94% damaged (nicked) DNA. Addition of increasing concentrations of Na$_2$SeO$_3$ (0.5–5000 µM, all $p < 0.02$) resulted in increased undamaged (closed, circular) DNA (Fig. 2a, lanes 6–10), indicating that Na$_2$SeO$_3$ inhibited oxidative DNA damage. From quantification of these gel bands, we determined that the highest Na$_2$SeO$_3$ concentration (5000 µM) inhibited 91% of iron-mediated DNA damage.

Similar electrophoresis experiments were conducted for Na$_2$SeO$_4$, SeO$_2$ and Na$_2$Se under identical Mode I conditions. In this case, Na$_2$SeO$_4$ (Fig. 2b) and Na$_2$Se (Fig. 2c), showed no inhibition of DNA damage with increasing concentration. In contrast, adding SeO$_2$ in the presence of Fe(II) and H$_2$O$_2$ showed a slight increase in DNA damage (Fig. 2D, lanes 6–8) at concentrations of 0.5–50 µM compared to the Fe(II)/H$_2$O$_2$ lane (lane 5). This
corresponds to a 20% increase in DNA damage at 50 μM SeO2 (p = 0.02). At higher concentrations (500 and 5000 μM, lanes 9 and 10), however, SeO2 inhibited DNA damage by 17% (p = 0.01) and 100% (p < 0.0001), respectively.

Fig. 3 shows a graph of percent DNA damage inhibition vs. concentration of selenium compound for the four inorganic selenium compounds tested. Inhibition of DNA damage is clearly greatest for Na2SeO3 and high concentrations of SeO2, whereas SeO2 shows increased DNA damage at lower concentrations, and both Na2SeO4 and Na2Se show no effect on DNA damage.

3.2. [Fe(EDTA)]2− experiments under Mode I conditions

Since our studies indicated that both Na2SeO3 and SeO2 inhibit DNA damage at high concentrations (500–5000 μM) under Mode I conditions, we investigated metal coordination as a possible mechanism for the inhibitory effect of these compounds. Iron binding to the selenium compound could prevent the generation or release of the hydroxyl radical upon exposure to H2O2. To test this metal coordination hypothesis, we performed gel electrophoresis experiments with the [Fe(EDTA)]2− complex as the iron source. The chelating ligand EDTA (ethylenediaminetetraacetic acid) completely coordinates Fe(II), preventing potential coordination between the selenium compound and iron. Although completely coordinated, [Fe(EDTA)]2− does generate DNA-damaging hydroxyl radical in the presence of H2O2.

Gel electrophoresis experiments showed that addition of [Fe(EDTA)]2− (400 μM) and H2O2 under Mode I conditions produced approximately 63% nicked DNA (Fig. 4, lane 5). As the concentration of Na2SeO3 was increased, no effect on oxidative DNA damage was observed (lanes 6–10). Similarly, increasing concentrations of SeO2 had no effect on DNA damage under the same conditions (Fig. 4b). The lack of DNA damage inhibition by both Na2SeO3 and SeO2 with [Fe(EDTA)]2− instead of FeSO4 as the iron source suggests that metal coordination is required for the effects of both compounds on DNA damage under Mode I conditions.

3.3. Mode II experiments with inorganic selenium compounds

At higher peroxide concentrations (>10 mM), in vitro iron-mediated DNA damage was shown to occur independently of H2O2 concentration [11]. This Mode II DNA damage likely differs from Mode I damage in that the generated hydroxyl radical results from the reaction of H2O2 with Fe(II) bound to the bases of DNA [11]. Because Mode I and II DNA damage result from hydroxyl radical being produced at different sites of iron localization on DNA, we also determined the effects of Na2SeO3, Na2SeO4, SeO2 and Na2Se on oxidative DNA damage under Mode II conditions.

Experiments performed under Mode II conditions were identical to those for Mode I except that the concentrations of H2O2 (50 mM) and ethanol (100 mM) were increased to ensure that DNA damage was caused only by Mode II radicals [10,11]. Initial experiments with Na2SeO3 indicated that this compound increases DNA damage in the presence of H2O2 without addition of Fe(II). Therefore, further experiments with selenite under Mode II conditions were conducted at pH 7 in the absence of iron.

As Fig. 5a illustrates, combining increasing concentrations of Na2SeO3 with H2O2 (50 mM, lanes 5–9) resulted in increasing oxidative DNA damage. At the highest Na2SeO3 concentration tested (5000 μM, lane 10), 90% more nicked DNA was produced when compared to the H2O2 only lane (lane 3; p = 0.0004). This increase in DNA damage with increasing Na2SeO3 concentration indicated that under Mode II conditions, Na2SeO3 promotes DNA damage in the presence of H2O2 alone (Fig. 6a).

Sodium selenate (0.5–5000 μM) was also tested under Mode II conditions with Fe(II) (2 μM) at pH 6. As seen from the gel in Fig. 5b, Na2SeO4 had no effect on DNA damage, regardless of concentration (lanes 6–10). Under
similar conditions, addition of Na$_2$Se also had no effect on DNA damage (Fig. 5c). In contrast, adding increasing concentrations of SeO$_2$ (5–5000 μM) under the same Mode II conditions showed inhibition of DNA damage (Fig. 5d, lanes 6–10, all p < 0.05).

Unlike selenite, adding only SeO$_2$ and H$_2$O$_2$ (lane 4) had no effect on DNA damage compared to the DNA control (lane 2). Increasing the concentration of SeO$_2$ to 5000 μM resulted in inhibition of 81% DNA damage (lane 10; p = 0.003). Thus, under Mode II conditions, SeO$_2$ had an inhibitory effect on DNA damage that is concentration-dependent. A comparison of DNA damage inhibition by Na$_2$SeO$_4$, Na$_2$Se, and SeO$_2$ under Mode II conditions is shown in Fig. 6b.

3.4. [Fe(EDTA)]$^{2-}$ experiments under Mode II conditions

Under Mode II conditions, SeO$_2$ was the only selenium compound to inhibit DNA damage. Experiments similar to those under Mode I conditions, using [Fe(EDTA)]$^{2-}$ as the iron source, were therefore conducted to determine whether metal coordination is required for SeO$_2$ inhibition of DNA damage under Mode II conditions. The gel in Fig. 7 shows that [Fe(EDTA)]$^{2-}$ combined with H$_2$O$_2$ (lane 5) resulted in 100% nicked DNA. Upon addition of increasing concentrations of SeO$_2$ (lanes 6–10), the percentage of DNA damage did not change. This suggests that iron coordination is required for SeO$_2$ to prevent DNA damage under Mode II conditions in much the same manner as was found for SeO$_2$ and Na$_2$SeO$_3$ inhibition of DNA damage under Mode I conditions.
3.5. \textsuperscript{77}Se NMR experiments

Several studies indicate that in aqueous solution at pH 6, \textit{SeO}_2 exists as HSeO\textsubscript{3} (SeO\textsubscript{2} + H\textsubscript{2}O \rightarrow HSeO\textsubscript{4} + H\textsuperscript{+}) \cite{39–41}, a species similar to Na\textsubscript{2}SeO\textsubscript{3} in aqueous solution. Since our gel results with Na\textsubscript{2}SeO\textsubscript{3} and Se\textsubscript{O}_2 showed differing effects of these compounds on DNA damage, \textsuperscript{77}Se NMR experiments were performed to elucidate the specification of Se\textsubscript{O}_2. Aqueous solutions of Se\textsubscript{O}_2 showed singlets at \( \delta 1317 \) and \( 1299 \) at pH 6 and 7, respectively, which did not change over a period of three days. Under the same conditions, Na\textsubscript{2}SeO\textsubscript{3} showed a singlet at \( \delta 1274 \) at both pH 6 and 7. Thus, our results indicate that Na\textsubscript{2}SeO\textsubscript{3} and Se\textsubscript{O}_2 do not form the same compound under these conditions.

4. Discussion

Selenium compounds are effective at preventing cancer, either due to their antioxidant ability to neutralize ROS \cite{15,31}, or through induction of apoptosis in cancer cells \cite{17,29,31}. Evidence for both prevention of cell death under oxidative stress and promotion of apoptosis has been found for Na\textsubscript{2}SeO\textsubscript{3} administered in similar concentrations: Na\textsubscript{2}SeO\textsubscript{3} (1 \textmu M) prevented cell death from ROS in hepatoma cells \cite{42} and stimulated benign mesothelial cell growth (7.5 \textmu M) \cite{29}, whereas in prostate cancer cells, Na\textsubscript{2}SeO\textsubscript{3} (0.5–5 \textmu M) inhibited cell growth via apoptosis \cite{17}. Our results help explain these contradictory chemopreventative (antioxidant) and anticancer effects of selenite, since we have found that for selenite and selenium dioxide, inhibition of oxidative DNA damage by hydroxyl radicals is dependent on both the concentration of selenium compounds and H\textsubscript{2}O\textsubscript{2}.

4.1. Mode I experiments with inorganic selenium compounds

Experiments conducted with Na\textsubscript{2}SeO\textsubscript{3} and Se\textsubscript{O}_2 show that both compounds inhibit DNA damage under Mode I conditions in a concentration-dependent manner with maximal DNA damage inhibition at 5000 \textmu M. Under the same conditions, Na\textsubscript{2}SeO\textsubscript{4} and Na\textsubscript{2}Se have no effect on DNA damage (Table 1). Although selenium concentrations of 5000 \textmu M are much larger than would be found in cells, plasma selenium concentrations in humans can reach 1.1–1.3 \textmu M after consuming 40 \textmu g of selenium supplement a day \cite{43}. At 0.5 \textmu M, Na\textsubscript{2}SeO\textsubscript{3} inhibited 37% of DNA damage \((p = 0.02)\), whereas Se\textsubscript{O}_2 at this concentration acted as a pro-oxidant, increasing DNA damage by 20% \((p = 0.02)\). These results indicate that Na\textsubscript{2}SeO\textsubscript{3} may be a more effective antioxidant \textit{in vivo} than Na\textsubscript{2}SeO\textsubscript{4}, Na\textsubscript{2}Se, or Se\textsubscript{O}_2.

It is not clear why Se\textsubscript{O}_2 acts as a pro-oxidant and an antioxidant depending upon concentration, but the pro-oxidant effect of Se\textsubscript{O}_2 at low concentrations may be due to its ability to generate radical species. Se\textsubscript{O}_2 is proposed to react with aqueous H\textsubscript{2}O\textsubscript{2} to form peroxyselenenic acid (Se\textsubscript{O}_2 + H\textsubscript{2}O \rightarrow HOS(\text{O})\text{O}OH), that then may decompose to yield OH \cite{44}. In addition, it has been reported that Se\textsubscript{O}_2 in aqueous solution at pH 6 exists as HSeO\textsubscript{3} \cite{39–41}, which can then be reduced to Se\textsubscript{O}_3 radical (Se\textsubscript{O}_2 + H\textsubscript{2}O \rightarrow HSeO\textsubscript{3} + H\textsuperscript{+} \rightarrow Se\textsubscript{O}_3 + OH\textsuperscript{-}) \cite{39}. However, if aqueous Se\textsubscript{O}_2 is present as HSeO\textsubscript{3}, it is surprising that we see significantly different effects with Se\textsubscript{O}_2 as compared to Na\textsubscript{2}SeO\textsubscript{3} in our gel electrophoresis studies. Our \textsuperscript{77}Se NMR studies of Se\textsubscript{O}_2 and Na\textsubscript{2}SeO\textsubscript{3} show different resonance frequencies for both compounds, indicating that they do not form the same species in aqueous solution. This result correlates well with the different effects of these two compounds on DNA damage. A separate study showed that Na\textsubscript{2}SeO\textsubscript{3} was more effective at inducing apoptosis than Se\textsubscript{O}_2 \cite{15}, suggesting that these compounds also have different properties \textit{in vivo}.

The four inorganic selenium compounds studied in this work differ by both oxidation state and charge. Both Na\textsubscript{2}SeO\textsubscript{3} and Se\textsubscript{O}_2, with a selenium oxidation state of +4, inhibit DNA damage at high concentrations, whereas Na\textsubscript{2}SeO\textsubscript{4} with an oxidation state of +6 and Na\textsubscript{2}Se (−2) have no effect on DNA damage. This indicates that inorganic selenium compounds with selenium in the +4 oxidation state are better at preventing DNA damage under Mode I conditions than those in either the highest or lowest oxidation states. The lack of DNA damage inhibition with Na\textsubscript{2}SeO\textsubscript{4} can be explained if the selenium compound reacts with and neutralizes generated OH. Since the selenium atom in Na\textsubscript{2}SeO\textsubscript{4} is in its highest oxidation state, it cannot be oxidized by OH. The lack of DNA damage inhibition by Na\textsubscript{2}Se is more difficult to explain using charge arguments, since the oxidation state of the selenium is low. In addition, organic selenides (R–Se–R, selenium oxidation state of −2) are well-known for their antioxidant properties \cite{20,30}. However, based on their respective reduction potentials, selenide (0.924 V \cite{45}) cannot be oxidized by H\textsubscript{2}O\textsubscript{2} (0.38 V \cite{10}).

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA damage behavior</th>
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<tbody>
<tr>
<td>Na\textsubscript{2}SeO\textsubscript{3}</td>
<td>No antioxidant or pro-oxidant behavior under any conditions</td>
</tr>
<tr>
<td>Na\textsubscript{2}SeO\textsubscript{4}</td>
<td>Mode I: Antioxidant at all concentrations (0.5–5000 \textmu M)</td>
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<tr>
<td>Se\textsubscript{O}_2</td>
<td>Mode I: Pro-oxidant behavior with or without Fe(II) at all concentrations (0.5–5000 \textmu M)</td>
</tr>
<tr>
<td>Na\textsubscript{2}Se</td>
<td>Mode II: Antioxidant at all concentrations</td>
</tr>
</tbody>
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All antioxidant behavior requires Fe(II) coordination.
From our results, it is clear that charge on the inorganic selenium compounds does not play a significant role in preventing DNA damage. $\text{Na}_2\text{SeO}_3$, $\text{Na}_2\text{SeO}_4$ and $\text{Na}_2\text{Se}$ have the same $-2$ charge, however only $\text{Na}_2\text{SeO}_3$ was able to inhibit DNA damage under Mode I conditions. $\text{SeO}_2$, a neutral compound, also inhibits DNA damage at high concentrations, suggesting that oxidation state plays a much more significant role than overall charge in DNA damage inhibition.

4.2. Mode II experiments with inorganic selenium compounds

Similar to results observed under Mode I conditions, $\text{Na}_2\text{SeO}_4$ and $\text{Na}_2\text{Se}$ have no effect on DNA damage under Mode II conditions. In contrast to Mode I conditions, however, $\text{SeO}_2$ under Mode II conditions inhibited DNA damage at all concentrations tested, reducing DNA damage by a maximum of 81% at 5000 $\mu$M (Table 1). At more biologically-relevant concentrations of selenium (0.5 $\mu$M), $\text{SeO}_2$ showed weak antioxidant activity under Mode II conditions, in direct opposition to its pro-oxidant activity at the same concentration under Mode I conditions.

Interestingly, when $\text{Na}_2\text{SeO}_3$ is combined with $\text{H}_2\text{O}_2$ without Fe(II) under Mode II conditions, DNA damage is promoted in a concentration-dependent manner. This is very different behavior compared to its inhibitory effect at all concentrations under Mode I conditions. A possible reason for the pro-oxidant activity seen under Mode II conditions is that the $\text{Na}_2\text{SeO}_3$ is oxidized by $\text{H}_2\text{O}_2$ from $+4$ to $+6$ states, producing OH in the process. Considering the reduction potentials of selenite ($-0.05$ V [45]) and $\text{H}_2\text{O}_2$ (0.38 V [10]), selenite is capable of reducing $\text{H}_2\text{O}_2$. Additionally, $\text{SeO}_2^-$ formed from the one electron oxidation of $\text{Na}_2\text{SeO}_3$ by OH could also damage DNA [46]. It is unclear, however, why the antioxidant or pro-oxidant behavior of $\text{Na}_2\text{SeO}_3$ would be entirely dependent upon $\text{H}_2\text{O}_2$ concentration. Calculations indicate that the steady-state $\text{H}_2\text{O}_2$ concentration in unstressed $\text{E. coli}$ is $\sim 20$ nM, but with a high generation rate of $9-22$ $\mu$M/s [47]. Thus, $\text{H}_2\text{O}_2$ concentrations may increase quickly if an imbalance between generation and decomposition exists. If the antioxidant or pro-oxidant behavior of $\text{Na}_2\text{SeO}_3$ depends on cellular $\text{H}_2\text{O}_2$ concentrations, this highlights the need to accurately measure concentrations of $\text{H}_2\text{O}_2$ in both unstressed and oxidatively-stressed cells.

4.3. Mode I and II [Fe(EDTA)]$^{2-}$ experiments with inorganic selenium compounds

Experiments with [Fe(EDTA)]$^{2-}$ as the iron source were performed to determine whether DNA damage inhibition by inorganic selenium compounds is due to iron coordination. Increasing concentrations of $\text{Na}_2\text{SeO}_3$ and $\text{SeO}_2$ using the completely-coordinated [Fe(EDTA)]$^{2-}$ resulted in no change in DNA damage. These experiments indicate that iron coordination by $\text{Na}_2\text{SeO}_3$ and $\text{SeO}_2$ is required for inhibition of (or increase in, for lower concentrations of $\text{SeO}_2$) DNA damage under Mode I conditions. Similarly, under Mode II conditions, iron binding is also important for the inhibitory effect of $\text{SeO}_2$ on DNA damage. In a similar study performed with organic selenium compounds using copper and $\text{H}_2\text{O}_2$ to generate OH, metal coordination was also the proposed mechanism for DNA damage inhibition [30].

Coordination between iron and SeO$_2^{2-}$ or $\text{SeO}_2$ is likely: both $\text{Na}_2\text{SeO}_3$ and $\text{SeO}_2$ contain hard oxygen ligands, which can coordinate to Fe(II), a borderline hard Lewis acid. Because selenium is much softer than oxygen, it less likely that Fe(II) would bind to selenium, especially given its positive oxidation state in both $\text{Na}_2\text{SeO}_3$ and $\text{SeO}_2$. In fact, characterization of several iron-selenite complexes show that iron indeed binds selenite through the negatively-charged oxygen atoms [48,49].

Based on our results, $\text{Na}_2\text{SeO}_3$, and to a lesser extent $\text{SeO}_2$, exhibit both antioxidant and pro-oxidant behaviors dependent on $\text{H}_2\text{O}_2$ concentration. Understanding this seemingly contradictory behavior may help explain conflicting results seen in anticancer studies using $\text{Na}_2\text{SeO}_3$ [19,32,33,50,51]. We have clearly demonstrated the ability of inorganic selenium compounds both to prevent and generate DNA damage using a biologically-relevant DNA damage assay, but the complex behaviors of these compounds with regard to their concentration and concentration of $\text{H}_2\text{O}_2$ in vivo merit further study.

5. Conclusions

The essential micronutrient selenium is widely used in over-the-counter supplements, infant formulas, protein mixes, and animal feed, often in the form of its inorganic compounds. Despite having received much attention for their antioxidant and chemopreventative properties, uncertainties remain with regard to the concentration dependence and mechanism for these behaviors [15,17,29]. We have shown that $\text{Na}_2\text{SeO}_4$ and $\text{Na}_2\text{Se}$ are unable to inhibit iron-mediated DNA damage, a surprising result given the ability of organic selenides to inhibit DNA damage under similar conditions [20,30]. Additionally, $\text{SeO}_2$ and $\text{Na}_2\text{SeO}_3$ were found to behave as both pro-oxidants and antioxidants depending on the concentrations of selenium compound and $\text{H}_2\text{O}_2$. Identification of these complex antioxidant and pro-oxidant behaviors help to reconcile seemingly conflicting results obtained in cell studies with these compounds [15,17,31], and also suggest that the more damage-neutral $\text{Na}_2\text{SeO}_3$ may be more suitable for use in selenium supplementation. Our results also demonstrate that the formal oxidation state of the selenium atom is the primary determinant of antioxidant behavior for these inorganic selenium compounds, and not their overall charge. In addition, the antioxidant effects of both inorganic and organic selenium compounds have now been attributed to their coordination of the metal ions responsible for the production of reactive radicals [30], indicating
that this novel metal-coordination mechanism for antioxidant behavior may be relevant to antioxidant function in vivo.

6. Abbreviations

GLC-82 human lung adenocarcinoma cell
GPx glutathione peroxidase
HSC-3 human oral squamous carcinoma
RDA recommended daily allowance
ROS reactive oxygen species

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2007.03.016.

References