Cancer Prevention

Inorganic Oxo Compounds React with Alkylating Agents: Implications for DNA Damage**

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The cancer-preventing properties of selenium have been long known and are more potent than any other normal component of the human diet.[1–4] For example, patients taking selenium-supplemented brewer's yeast experienced significant reductions of colorectal, lung, and prostate cancer incidences as well as cancer related mortality.[5] Rat model studies have shown that the toxicity of food-borne alkylating toxins, such as diethylnitrosamine or methylnitrosourea, can be minimized by addition of various forms of selenium (e.g., Na_2SeO_3, Na_2SeO_4) to the diet.[6–8] Similar work with the vanadium compounds $VOSO_4$, $NaVO_3$, and Na_3VO_4 has also demonstrated the ability to prevent cancer induced by alkylating carcinogens.[9–11] The potential for dietary inorganic species to have significant impact on cancer prevention is illustrated by the "Selenium and Vitamin E Chemoprevention Trial" (SELECT).[12,13] In this ongoing trial sponsored by the National Cancer Institute, over 30000 males at risk for development of prostate cancer are having their diets supplemented with selenium alone, vitamin E alone, both selenium and vitamin E, or a placebo.[12,13] Despite this great promise of preventing cancer with inorganic species, no significant mechanistic data are available to explain the activity.[1–4] Indeed the scope of which inorganic compounds prevent cancer has not been explored fully.

The toxicity of food- and tobacco-borne carcinogens is often derived from activation into DNA-damaging alkylating agents. Diethylnitrosamine, for example, is enzymatically oxidized, undergoes subsequent decomposition, and yields the potent alkylating agent ethyl diazonium ($[C_2H_5N_2]^+$).[14] This alkylating toxin then reacts with nucleophilic sites of DNA (e.g., guanine N^7, thymine O^4) to give ethylated DNA. Unable to base-pair properly, this damaged DNA yields genomic mutations after replication. We wonder if the presence of alternate nucleophiles may allow for mitigation of this DNA alkylation. Under aqueous conditions, both selenium[15] and vanadium[16–18] exhibit tendencies to form anionic oxo species (e.g., $[SeO_4]^{2-}$, $[VO_4]^{3-}$). Perhaps these oxo compounds can consume electrophilic alkylating toxins, thereby minimizing DNA damage. By testing a hypothesis of inhibiting DNA damage, we present results to indicate that select inorganic compounds can both prevent alkylation of DNA and detoxify alkylating agents.

A modified assay for the observation of DNA alkylation[19] was used in which supercoiled pUC19 DNA was treated with the common alkylating toxin diethyl sulfate ($(C_2H_5O)_2SO_2$).[20] Base alkylation renders the sugar–base glycosidic bond susceptible to hydrolysis.[19] After alkylation, the DNA was hydrolyzed to release ethylated bases (e.g., N^7-ethylguanine) and create abasic sites. Endonuclease cleavage of DNA specifically at abasic sites then transformed the supercoiled DNA into nicked (one cleavage event) and linear (two or more cleavages) forms. Gel electrophoresis was used to separate and identify each form of the DNA (supercoiled, nicked, linear) thereby providing observation of DNA alkylation. Figure 1a shows the gel of a typical experiment (pH 7) containing DNA (lane 1) and various controls of DNA + enzyme (lane 2), DNA + Na_2SeO_4 (lane 3), DNA + Na_2SeO_4 + enzyme (lane 4), and DNA + $(C_2H_5O)_2SO_2$ (lane 5). In lane 6 is a reaction of DNA + $(C_2H_5O)_2SO_2$ + enzyme in which the supercoiled DNA form was consumed by alkylation and the subsequent digest. Lanes 7–12 show similar alkylation reactions with increasing concentrations of Na_2SeO_4 added to DNA + $(C_2H_5O)_2SO_2$ + enzyme. The Na_2SeO_4:$(C_2H_5O)_2SO_2$ ratios, ranging from 0.01 up to 10, are provided in Figure 1a. As can be seen, especially in lanes 11 and 12, addition of Na_2SeO_4 preserved the unalkylated, supercoiled DNA. Analogous experiments were performed in which the effects of the vanadium salt Na_3VO_4 upon DNA alkylation were examined (Figure 1b). Similar to that seen with the selenium compound, Na_3VO_4 maintained a portion of DNA in the unalkylated, supercoiled form. Control experiments demonstrated that neither Na_2SeO_4 nor Na_3VO_4 inhibited activity of the enzyme (data not shown). From these results, we conclude that both Na_2SeO_4 and Na_3VO_4 prevent DNA-alkylation damage in a concentration-dependent manner.

Inspection of the gels in Figure 1 reveals that the abilities of Na_2SeO_4 and Na_3VO_4 to prevent DNA alkylation are not identical. From digital-imaging quantification of the bands in lanes 6 and 11 we find that, with 1:1 ratios of inorganic:alkylating agent, Na_2SeO_4 preserved 6.7% of DNA unalkylated whereas Na_3VO_4 protected 40%.[20] Perhaps higher charge density of the $[VO_4]^{3-}$ ion, relative to the $[SeO_4]^{2-}$ ion, affords greater nucleophilicity and consequently an enhanced ability to react with electrophilic alkylating agents. Interestingly, cancer-prevention trials in rats are often performed by administering Na_2SeO_4 at 4 ppm in drinking water[6] whereas Na_3VO_4 is dosed lower at 0.5 ppm.[9,10]

Such differences in preventing DNA alkylation by these two compounds led us to examine an array of inorganic salts, all at pH 7 and in a background of 150 mM $MgCl_2$ to prevent simple cationic shielding of DNA nucleophilicity.[20–22] The following compounds prevented DNA alkylation at inorganic:alkylating agent ratios of 10 or less: $NaHCO_3$, Na_2CO_3, $NaNO_2$, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, $Na(OOCCH_3)$, $NaIO_3$, Na_2MoO_4, $Na_2S_2O_4$, Na_2SeO_3, Na_2SeO_4, $NaVO_3$, Na_3VO_4, and $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$.[23] No protection of DNA was found from NaCl, NaBr, NaI, $NaNO_3$, KCl, KBr,

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[**] This work was funded by the Cancer Research and Prevention Foundation. Additional support was provided to J.J.W. by an Alfred P. Sloan Foundation Research Fellowship.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/ange.200353363

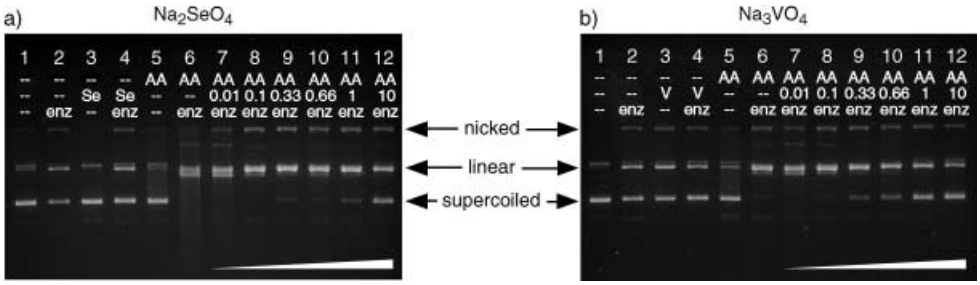

Figure 1. Agarose gels showing the ability of a) Na_2SeO_4 and b) Na_3VO_4 to prevent DNA alkylation damage. As described in the text and Supporting Information, an endonuclease enzyme-based assay was used to visualize alkylation. The presence of supercoiled pUC19 DNA (e.g., lane 1) indicates a lack of alkylation, whereas loss of this fast moving band demonstrates alkylation (e.g., lane 6). Where applicable, presence of the alkylating agent $(C_2H_5O)_2SO_2$ ("AA"), enzyme ("enz"), and inorganic compounds Na_2SeO_4 ("Se") or Na_3VO_4 ("V") are indicated atop each lane. All reactions were run at pH 7 with a DNA concentration of 150 μM in nucleotides. The $(C_2H_5O)_2SO_2$ concentration was 1.5 mM and the salts Na_2SeO_4 and Na_3VO_4 were at 1.5 mM unless stated otherwise. The numbers provided in lanes 7–12 (e.g., 0.1, 10) are ratios of concentrations for inorganic salts:alkylating agent. The ramps correspond to increasing concentrations of Na_2SeO_4 or Na_3VO_4. In lanes 7–12, higher concentrations of Na_2SeO_4 or Na_3VO_4 can be seen to maintain greater amounts of unalkylated DNA.[20]

KI, KNO_3, $MgCl_2$, $Mg(NO_3)_2$, $CaCl_2$, $Ca(NO_3)_2$, $CoCl_2$, $Co(NO_3)_2$, $MnCl_2$, $NiCl_2$, and $Ni(NO_3)_2$. Many other salts examined (e.g., $VOSO_4$, $FeCl_3$) were not soluble at pH 7, thereby yielding data not directly comparable with those described above.[20] The trend arising from these results appears to be that nucleophiles, in the form of anionic oxo species (e.g., $[CO_3]^{2-}$, $[MoO_4]^{2-}$, $[NO_2]^-$, $[VO_4]^{3-}$), are needed to prevent alkylation of DNA. By contrast, non-oxo compounds (e.g., NaCl, $NiCl_2$) provide no protection against DNA damage.

To understand the nature of these detoxification processes, we turn to model reactions between alkylating agents and inorganic compounds. The high activity of $[VO_4]^{3-}$ for preventing DNA alkylation brought our focus to vanadate chemistry. Although aqueous speciation of vanadates is exceedingly complex, with multiple species often coexisting in one solution (e.g., $[H_2VO_4]^-$, $[H_2V_2O_7]^{2-}$, $[V_4O_{12}]^{4-}$, $[V_5O_{15}]^{5-}$),[16–18] use of acetonitrile as a solvent simplifies the system, thereby permitting chemical insights on reactivity.[23] Detailed speciation data are available for the $[V_3O_9]^{3-}$ ion, prepared as the $[(C_4H_9)_4N]^+$ salt, and provide a suitable starting point for model reactions.[23] Figure 2 shows the ^{1}H NMR spectrum of a 1:1 reaction between $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$ and $(C_2H_5O)_2SO_2$ in which this vanadate transformed the alkylating toxin into relatively harmless ethanol. Spectral identification of the ethanol reaction product was confirmed by spiking the ^{1}H NMR reaction solution with a genuine sample as well as gas chromatography–mass spectrometry. Control experiments with $(C_2H_5O)_2SO_2$ alone did not produce ethanol. The proton source for ethanol formation may be adventitious water in the solvent or from the crystal lattice of $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$.[23] The predominant vanadium-containing product of this detoxification reaction was identified by ^{51}V NMR to be $[V_5O_{14}]^{3-}$.[20,24] A 3:1 reaction of $(C_2H_5O)_2SO_2$:$[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$ yielded $[V_5O_{14}]^{3-}$ initially, which then transformed to $[V_{12}O_{32}]^{4-}$.[20,25] When viewed as oligomers of metavanadate ($[VO_3]^-$), the starting trimeric $[V_3O_9]^{3-}$ ion (i.e., $([VO_3]^-)_3$) yielded products deficient in oxygen (i.e., $[V_5O_{14}]^{3-}$ versus $[V_5O_{15}]^{5-}$ and $[V_{12}O_{32}]^{4-}$ versus $[V_{12}O_{36}]^{12-}$) along with oxygen-containing ethanol. Thus the detoxifying transformation of an alkylating agent into an alcohol requires an inorganic oxide source.

This transformation appears to be a general phenomenon given that we found a variety of alkylating agents (e.g., C_2H_5Br, C_2H_5I, $C_2H_5OSO_2CF_3$, $(C_2H_5O)_2SO_2$) all reacted with an assortment of vanadates (e.g., $[V_3O_9]^{3-}$,[23] $[HV_4O_{12}]^{3-}$,[26] $[V_5O_{14}]^{3-}$,[24] $[V_{10}O_{26}]^{4-}$,[27] $[H_3V_{10}O_{28}]^{3-}$[28]) to yield ethanol. When the selenium compound Ag_2SeO_4 was treated with the alkylating agents C_2H_5Br or C_2H_5I, ethanol was produced. Note that the phosphate $[(C_4H_9)_4N](H_2PO_4)$ did react with various alkylating agents, but an alcohol product was never released. Facile interconversion between different oxo species (e.g., $[V_5O_{14}]^{3-} \rightleftharpoons [V_{12}O_{32}]^{4-}$) may be the key to producing ethanol and preventing cancer. For aqueous phosphate, by contrast, mononuclear species predominate.[29] The 1:1 reaction between $(C_2H_5O)_2SO_2$ and $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$ (50 mM each) was approximately 75% complete after

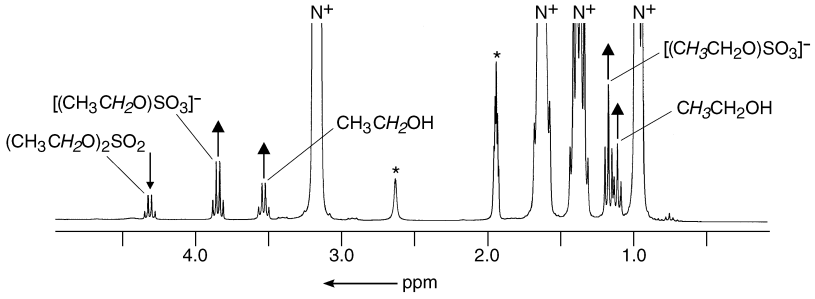

Figure 2. The ^{1}H NMR spectrum of a 1:1 detoxification reaction, in progress, between the alkylating agent $(C_2H_5O)_2SO_2$ and vanadate $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5\,H_2O$ in distilled CD_3CN. Arrows indicate the changes associated with the consumption of the starting material $(C_2H_5O)_2SO_2$ and formation of the products $[(C_2H_5O)SO_3]^-$ and ethanol. Water and CHD_2CN are marked with a "*". The $[(C_4H_9)_4N]^+$ counterion is designated "N^+". The methyl resonance of $(C_2H_5O)_2SO_2$ at $\delta = 1.36$ ppm is obscured by those of $[(C_4H_9)_4N]^+$.

20 minutes. Analogous reactions showed the following kinetic trend of reactivity: $[V_3O_9]^{3-} > [HV_4O_{12}]^{3-} > [V_5O_{14}]^{3-} \gg [V_{10}O_{26}]^{4-} \gg [H_3V_{10}O_{28}]^{3-}$. Again, charge density seems to dictate the ability to consume alkylating toxins.

Herein we have shown that inorganic oxo compounds can prevent DNA alkylation damage and that oxo compounds detoxify alkylating agents by oxide transfer. Although these reactions do not necessarily demonstrate processes occurring in cells, they do serve to illustrate interactions that are possible. These reactions raise the intriguing possibility that the inorganic compounds known to prevent cancer do so by a "carcinogen interception" mechanism in which oxo species consume the toxin, thereby preventing DNA damage. We present these results, propose a mechanism for minimizing DNA damage with inorganic compounds, and suggest that further work be done to understand this promising avenue of preventing cancer.

Received: November 19, 2003
Revised: March 8, 2004 [Z53363]

Keywords: antitumor agents · bioinorganic chemistry · DNA alkylation · DNA damage · vanadium

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