Supporting Information

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

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Agarose Gel Electrophoresis Assay for Monitoring DNA Alkylation

An enzyme cleavage assay^[1] was adapted to observe and quantify alkylation damage of DNA. Alkylation followed by hydrolysis releases alkylated bases (e.g., *N*⁷-ethylguanine) and yields abasic sites^[1]. The AP endonuclease enzymes cleave DNA specifically at abasic sites^[2]. The commercially available enzyme exonuclease III (Exo III) was used in our studies. Although usually considered an *exonuclease* (i.e., cleavage of DNA with asymmetric ends), Exo III exhibits appreciable *endonuclease* activity under specific conditions^[2-5]. For example, changing the reaction buffer salt from MgCl₂ to CaCl₂ with NaCl maximizes *endonuclease* activity of this enzyme while minimizing *exonuclease* cleavage^[2-5].

To visualize DNA alkylation damage in the presence and absence of inorganics, we use supercoiled (“form I”) plasmid DNA. Cleaving one of the duplex DNA strands yields a circular (“nicked”, “relaxed”, or “form II”) form of the DNA. Subsequent cleavage of the second strand results in a linear form (“form III”) of the DNA. Each of these three forms can be easily distinguished by agarose gel electrophoresis. Taken together, transformation of supercoiled DNA into nicked, linear, or smaller linear fragments (a product of multiple cleavage events) is brought about by using plasmid DNA, alkylating agents, hydrolysis, and an endonuclease.

This work was carried out using the commercially available pUC19 plasmid. The plasmid was transformed into *Escherichia coli* INV110 cells for production of large quantities by standard methods^[4] and extracted using a commercially available kit (BioRad Quantum Prep Plasmid Kit). The INV110 bacterial line is deficient in the *dam* and *dcm* genes, thereby avoiding potential complication from cellular methylases. Alkylation experiments were run with a pUC19 concentration of 150 μM in nucleotides, determined by absorption at 260 nm. Reaction concentration of diethyl sulfate ((H₅C₂O)₂SO₂), the alkylating agent used in these studies, was 1.5 mM. Owing to solubility limitations, the diethyl sulfate used was from a stock solution in dimethyl sulfoxide (DMSO). The final DMSO portion of the reaction solution was 15%. In control reactions without an alkylating agent, DMSO was added. Unless stated otherwise, the final selenium (Na₂SeO₄) or

vanadium (Na_3VO_4) concentrations were 1.5 mM. Thus, the ratio of nucleotide:alkylating agent:inorganic was 1:10:10. All stock solutions were adjusted to pH = 7.0 with HCl or NaOH, as appropriate. Dilutions of these solutions with distilled, deionized water to concentrations used in the reaction solutions showed that pH = 7.0 was maintained. No buffers were used in order to avoid potential complicating effects of additional ions. Experiments performed using pH = 7.0 TRIS and sodium acetate buffered solutions yielded similar results, although higher concentrations of alkylating agent were needed (data not shown).

Upon combining the DNA, alkylating agent, and where applicable Na_2SeO_4 or Na_3VO_4 , the reaction was run for 20 minutes at room temperature. Ethanol precipitation of DNA (two volumes cold ethanol with NaCl added) was used to stop the reactions and separate the alkylating agent and inorganic salt from the DNA. The plasmid DNA was then resuspended in distilled, deionized water, to which was added the Exo III enzyme (33 units) and a buffer to maximize endonuclease activity (66 mM TRIS, pH = 8.0, 125 mM NaCl, 5 mM CaCl_2 , 10 mM dithiothreitol). After incubation at 37 °C for 30 minutes, the enzyme digest was stopped by addition of 75 mM ethylenediaminetetraacetic acid (EDTA) in 40% glycerol. Samples were then analyzed by gel electrophoresis. The gel was 1% agarose, prestained with 20 µg ethidium bromide, 20.4 cm long, and run at 75 volts for 90 minutes. Gels were imaged by placement on an ultraviolet light box and recorded with a digital camera.

References

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Quantification of Gel Bands

Bands on gels were compared quantitatively by use of a digital camera and image analysis software (NIH Image). Figure S1 shows intensity profiles of lanes 6 and 11 from Figure 1A (left) and Figure 1B (right). These plots provide quantitative data for each band within a given lane on a gel. For example, lane 6 of the Na_2SeO_4 gel contains DNA with the alkylating agent after the enzyme digest, but no Na_2SeO_4 . All of the supercoiled form of this plasmid was consumed. Lane 11 shows the same reaction, but one equivalent of Na_2SeO_4 relative to the alkylating agent has been added to the mix. A small band for the supercoiled plasmid form is now visible. By integrating peak areas, we find that one equivalent of Na_2SeO_4 preserves 6.7% of total DNA in the supercoiled form (Figure S1). A similar analysis performed for one equivalent of Na_3VO_4 per equivalent of alkylating agent is shown in Figure S1, lower right. This one equivalent of Na_3VO_4 preserved 40% of the total DNA in the supercoiled form.

Figure S1

Figure S1. Digital image analysis of select lanes from Figure 1. The upper profiles (a and b) show reactions of DNA and alkylating agent after enzyme digest. The lower left profile (c) demonstrates that one equivalent of Na_2SeO_4 preserves 6.7% of the supercoiled plasmid. For analogous experiments with Na_3VO_4 , 40% of the supercoiled form is preserved (d) relative to a control (b).

Prevention of DNA Alkylation by Inorganic Salts without Controlled pH

Owing to solubility limitations, solutions of certain inorganic salts could not be prepared at pH = 7.0. Consequently, solutions of these compounds were prepared at pH values allowing sufficient solubility. When a given salt is listed, the pH value of the solution follows in parentheses. The following salts afforded protection of DNA from alkylation: VCl_3 (2.0) and VO_2SO_4 (2.4). Salts that did not influence DNA alkylation: $\text{Al}(\text{NO}_3)_3$ (2.9), $\text{Ga}(\text{NO}_3)_3$ (2.4), $\text{Cr}(\text{NO}_3)_3$ (2.6), FeCl_2 (3.1), FeCl_3 (1.6), $\text{Fe}(\text{NO}_3)_3$ (1.3), ZnCl_2 (6.6), $\text{Zn}(\text{NO}_3)_2$ (5.2).

Figure S2

Figure S2. ^{51}V NMR spectrum of a 1:1 reaction (50 mM each) between $(\text{C}_2\text{H}_5\text{O})_2\text{SO}_2$ and $[(\text{C}_4\text{H}_9)_4\text{N}]_3(\text{V}_3\text{O}_9) \cdot 0.5\text{H}_2\text{O}$ in distilled CD_3CN after 315 minutes.

Figure S3

Figure S3. ^{51}V NMR spectrum of a 3:1 reaction (150 and 50 mM) between $(\text{C}_2\text{H}_5\text{O})_2\text{SO}_2$ and $[(\text{C}_4\text{H}_9)_4\text{N}]_3(\text{V}_3\text{O}_9)\cdot 0.5\text{H}_2\text{O}$ in distilled CD_3CN after 50 minutes.

Figure S4

Figure S4. ^{51}V NMR spectrum of a 3:1 reaction (150 and 50 mM) between $(\text{C}_2\text{H}_5\text{O})_2\text{SO}_2$ and $[(\text{C}_4\text{H}_9)_4\text{N}]_3(\text{V}_3\text{O}_9)\cdot 0.5\text{H}_2\text{O}$ in distilled CD_3CN after 42 hours.