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Spiroiminodihydantoin as an Oxo-Atom Transfer Product of 8-Oxo-2′-deoxyguanosine Oxidation by Chromium(V)

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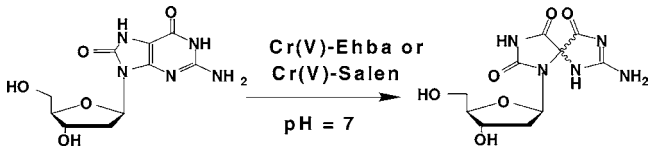
ABSTRACT



Oxidation of the DNA lesion 8-oxo-2′-deoxyguanosine by the two electron oxidants *N*,*N*′-ethylenebis(salicylideneanimato)oxochromium(V) (Cr(V)-salen) and bis(2-ethyl-2-hydroxybutyrato)oxochromium(V) (Cr(V)-ehba) at neutral pH forms spiroiminodihydantoin by an oxo-atom transfer mechanism. The chromium complexes are models of a DNA oxidation pathway caused by the carcinogen chromate.

The two-electron oxidation product of guanine, 8-oxo-2′-deoxyguanosine (8-oxoG), has been considered a primary product of cellular oxidative DNA damage.[1−3] Generation of 8-oxoG within cellular DNA, as well as its subsequent oxidation products, leads to mutation and cancer. The 8-oxoG has a lower reduction potential than guanine (8-oxoG = 0.74 V; dG = 1.29 V), making it prone to further oxidative degradation to compounds such as spiroiminodihydantoin (Sp).[4−8] The presence of Sp in DNA exposed to oxidants has been shown in vitro, but its in vivo relevance has yet to be firmly established. The mechanism by which oxidants generate such lesions, leading to mutation, is therefore of great interest.[9−13] We have taken a classical approach to elucidating the mechanism of Sp formation, following the incorporation of stable isotope labels during oxidation of DNA with model Cr(V) complexes.

Our work has focused on the oxidation of DNA by the carcinogen chromium(VI). At physiological pH, Cr(VI) lacks the ability to directly oxidize DNA. Once Cr(VI) has entered a cell, it is reduced to Cr(III) by endogenous reductants such as ascorbic acid or glutathione.[14] During the Cr reduction process, intermediate high-valent oxidation states of chromium, such as Cr(V) and Cr(IV), are known to form both in vitro and in vivo.[14−16] Both Cr(V) and Cr(IV) are highly

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reactive and may be the primary cause for oxidative DNA damage by chromium.[17] Our past work has shown that, when Cr(VI) is reduced by ascorbic acid in the presence of duplex DNA, preferential oxidation of guanine will occur to generate 8-oxoG and Sp.[18] Extension of this work with Cr(VI)-treated *Escherichia coli* has identified the formation of Sp and 8-oxoG in cellular DNA.[19] Our studies have shown Sp to be the dominant lesion formed, and we propose that the cellular toxicity of chromium is partially due to the ability of high-valent Cr complexes to form the Sp lesion from the further oxidation of 8-oxoG.

N,*N*′-Ethylenebis(salicylideneanimato)oxochromium(V) (Cr(V)-salen) **1** and bis(2-ethyl-2-hydroxybutyrato)oxochromium(V) (Cr(V)-ehba) **2** (Figure 1) are two models of

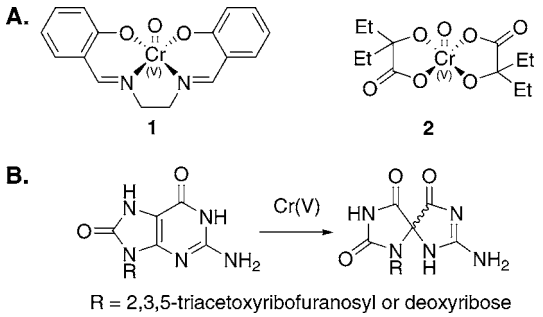

Figure 1. (A) Cr(V) complexes used to model cellular high-valent Cr complexes. (B) Oxidation of 8-oxoG by Cr(V).

cellular high-valent Cr.[16,20] Due to the mixed nitrogen/oxygen ligand chelation, the Cr(V)−salen complex is proposed to mimic chromium−peptide interactions, while the Cr(V)−ehba complex mimics the chromium−ascorbic acid complex. Both complexes have been shown to preferentially oxidize duplex DNA at guanine and preferentially oxidize 8-oxoG in DNA containing an 8-oxoG lesion.[6,20] Here, we report on the mechanism of formation of the major product of 2′,3′,5′-triacetoxy-8-oxo-2′-deoxyguanosine **3** (acyl-8-oxoG) oxidation by Cr(V)−salen **1** or Cr(V)−ehba **2**.

The oxidation of a 1.5 mM solution of **3** in aqueous phosphate buffer (75 mM, pH 7) at 37 °C by 0.75 mM Cr(V)−salen **1** or Cr(V)−ehba **2** leads to the formation of only one stable oxidation product, 2′,3′,5′-triacetoxyspiroiminodihydantoin **4** (acyl-Sp). Acyl-Sp was identified by HPLC (Figure 2) eluting at 10 min and was observed as a double peak from the mixture of epimers formed on generation of

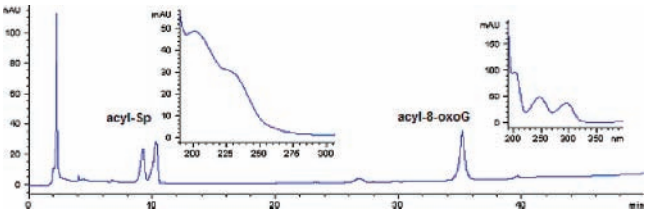

Figure 2. Typical reverse phase chromatogram showing the separation of products of the oxidation of acyl-8-oxoG by Cr(V)−salen at pH 7.

the spiro ring system.[21] It was unequivocally identified by its characteristic UV absorption spectrum, showing the 230 nm shoulder (Figure 2), and by its signature mass spectrum, showing the M + H peak at 442 amu, the M + Na peak at 464 amu, and a fragment B + H_2 at 184 amu generated by cleavage of the glycosidic bond.

The oxidation of 8-oxoG to Sp is a two-electron process. The Cr(V)−salen and Cr(V)−ehba complexes are thought to be two-electron oxidants,[22] where the axial oxygen on the Cr(V) complex is directly transferred to 8-oxoG, resulting in an immediate two-electron oxidation species that forms Sp while reducing Cr(V) to Cr(III). This mechanism implies that a 1:1 stoichiometry of Cr(V) to 8-oxoG is required in the oxidation reaction.

To determine if the oxidation mechanism involved an oxo-atom transfer mechanism, acyl-8-oxoG was oxidized with various stoichiometric equivalents of Cr(V)−salen or Cr(V)−ehba. The data obtained were compared to the oxidation of acyl-8-oxoG with $IrCl_6^{2-}$, a reaction shown to occur by sequential one-electron transfer processes.[12] The increase in product **4** and decrease in reactant **3** was monitored by HPLC (Figure 3A). One equivalent of Cr(V)−ehba or Cr(V)−salen was sufficient to oxidize nearly all the acyl-8-oxoG to acyl-Sp, whereas two equivalents of $IrCl_6^{2-}$ was required for a complete reaction. These data imply that the Cr(V) complexes do indeed undergo a two-electron transfer process under these conditions.

The previous mechanism for the formation of Sp using one-electron oxidants via an electron abstraction mechanism[12] identified 5-hydroxy-8-oxoG, **5**, as an intermediate. At pH 7, intermediate **5** undergoes an acyl shift to form the Sp derivative **4**. We considered, therefore, the possibility that the Cr(V) oxidants were directly forming **4** by two-electron oxidation through the transfer of the axial oxygen, but the possibility of two sequential single electron abstractions could not be ruled out. To resolve this, we oxidized **3** in $H_2{}^{18}O$ using the high-valent Cr(V) species **1** and **2** or Ir(IV). If oxo-atom transfer were occurring, a ^{16}O-Sp-containing product, m/z = 300 for the nonacylated species, would form since the donated oxygen would be supplied directly from the axial ^{16}O oxygen of the Cr complex. As previously demonstrated,[12,18] oxidation occurring by two single electron

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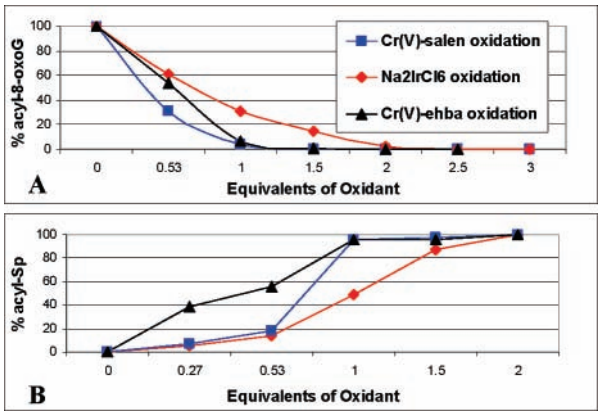

Figure 3. Product/reactant distribution experiments. The relative concentration of product (Sp) and reactant (8-oxoG) was monitored as the amount of oxidant (Cr(V)−salen, Cr(V)−ehba, or Na_2IrCl_6) was increased. (A) Relative decrease in reactant acyl-8-oxoG. (B) Relative increase in formation of the Sp product.

abstractions such as that shown for Ir(IV) results in almost 100% formation of the ^{18}O-Sp product, m/z = 302, due to nucleophilic attack by ^{18}O water. The oxo-atom **II** and electron abstraction **I** oxidation pathways are represented in Figure 4.

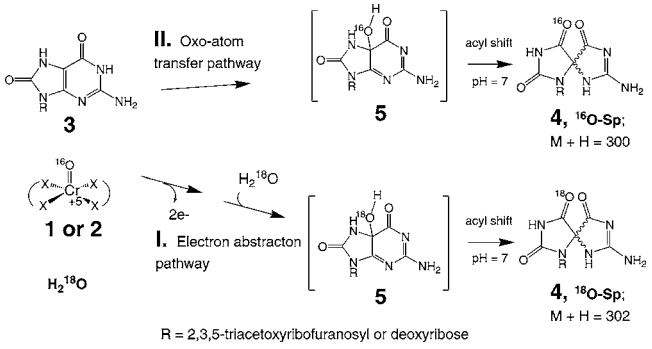

Figure 4. Theoretical oxidation products in ^{18}O-labeled water through the single electron abstraction mechanism and the two-electron oxo-atom transfer mechanism.

We reacted a 1.5 mM solution of either **1** or **2** with unacylated 8-oxoG (1.5 mM) in $H_2{}^{18}O$ (pH 7, 37 °C). Excess chromium was removed either by microanion or cation chromatography, and Sp was purified as previously described.[18] LC-ESI-MS analysis of the reaction product showed a significant introduction of ^{16}O (Figure 5A). These data demonstrate that oxidation occurs through a mechanism other than two single electron abstractions and likely by oxo-atom transfer.

The ^{16}O:^{18}O isotope ratios for Cr(V)−ehba and Cr(V)−salen oxidation products showed about 50% ^{18}O-Sp/50% ^{16}O-Sp formation and about 30% ^{16}O-Sp/70% ^{18}O-Sp formation, respectively (Figure 5 and Supporting Information). We had

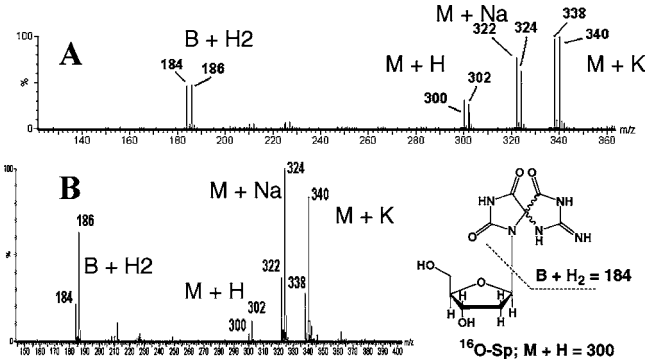

Figure 5. ^{18}O Labeling experiments. (A) Typical mass spectrum of Sp from 8-oxoG oxidation by Cr(V)−ehba in $H_2{}^{18}O$. (B) Typical mass spectrum of Sp from 8-oxoG oxidation by Cr(V)−salen in $H_2{}^{18}O$.

assumed that an oxo-atom transfer from the Cr(V) complexes to 8-oxoG would result in 100% ^{16}O-Sp formation; this was not seen. One possible explanation for the observed ^{16}O/^{18}O product ratios was the potential exchange of the ^{16}O axial oxygen in the Cr(V) complexes with the ^{18}O from water. If this exchange was fast, then some of the oxo-atom being transferred would therefore be an ^{18}O species. Infrared spectra of Cr(V) complexes were taken after $H_2{}^{18}O$ was added to them in dry acetonitrile (final Cr(V) concentration of ∼165 mM). The absorption of the axial oxygen stretch was monitored for an isotope shift. The Cr═O stretch for the Cr(V)−ehba complex was seen to shift, as previously reported,[17] from 993 to 964 cm^{-1}. The Cr═O stretch for Cr(V)−salen was also seen to shift from 1075 to 1035 cm^{-1} as ^{18}O was incorporated.

Although exchange of the axial oxygen with $H_2{}^{18}O$ was observed, this could not account for all the ^{18}O-Sp formation observed (Figure 5). We therefore proposed a mechanism of oxidation by these Cr(V) complexes in which the oxo-atom transfer occurred through the formation of an epoxide intermediate (Scheme 1). A similar oxidation mechanism has previously been reported for the oxidation of alkenes by Cr(V)−salen, via formation of an epoxide intermediate.[23,24] The most likely site of epoxide formation, based upon the known structure of the product, is the C4−C5 double bond. The C4−C5 epoxide product is susceptible to nucleophilic attack by water and, therefore, ring opening with the introduction of oxygen from the solvent. Loss of OH$^-$ at C4 would produce the 5-hydroxy intermediate **5**. An acyl migration then leads to the final product **4**. The ratio of ^{16}O retention to ^{18}O incorporation depends upon the propensity of the epoxide to open via attack at C4 (^{18}O incorporation) or C5 (^{16}O loss). Oxidation of 8-oxoG by Cr(V)−ehba resulted in an ^{18}O:^{16}O product distribution of approximately 50:50; Cr(V)−salen gave about a 30:70 ratio. The Cr(V)−

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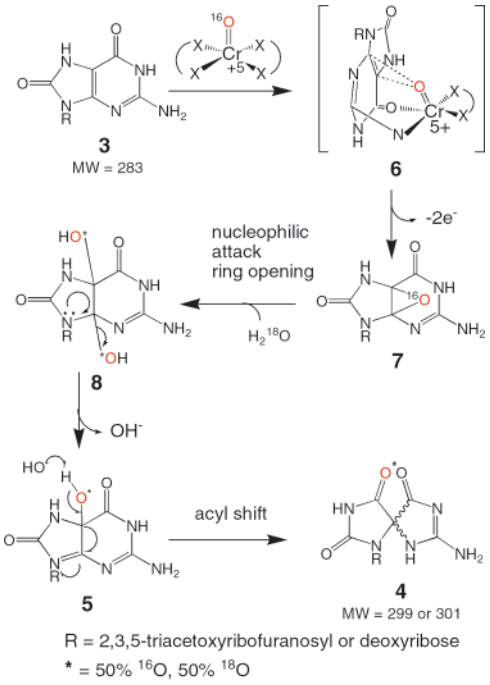
Scheme 1. Proposed Mechanism for the Formation of **4**



ehba oxidant was likely the more efficient oxidant; that is, the rate of solvent exchange with the complex was low compared to the rate of 8-oxoG oxidation. The bidentate ehba ligand allowed Cr(V) more flexibility to form a relatively stable coordination complex **6** with 8-oxoG facilitating the inner-sphere oxo-atom transfer. The more tightly bound tetradentate salen ligand may obstruct the formation of a coordination complex, allowing time for ^{18}O exchange to occur and increasing the amount of ^{18}O-Sp formed.

Finally, to confirm that nucleophilic attack of an epoxide was indeed occurring and that isotope ratios were not just the result of simultaneous oxidation via the two pathways **I** and **II** (Figure 4), oxidation reactions were carried out in the presence of lysine, a much stronger nucleophile than water and a putative cellular product of chromate oxidation in DNA. If oxidation were occurring through pathways **I** and **II**, the presence of lysine during oxidation would eliminate or reduce ^{18}O-Sp formed by the single electron abstraction pathway **I** leaving only ^{16}O-Sp formed from oxo-atom transfer **II**. If Sp was forming by the proposed epoxidation mechanism (Scheme 1), then the presence of lysine would decrease Sp formation. Also, the inclusion of lysine would be expected to lead to the formation of a lysine-bound oxidation product. Such a result would indicate the potential for Cr oxidation to result in DNA−protein cross-linking. A 1.0 mM solution of acyl-8-oxoG was incubated with either 1.0 mM Cr(V)−salen or Cr(V)−ehba in the presence of 100 mM lysine. HPLC analysis of the reaction mixtures revealed none of the previously seen Sp oxidation product **4** (data not shown), indicating that oxo-atom transfer was occurring via epoxidation followed by nucleophilic attack (Scheme 1) at the C5 position. HPLC-ESI-MS identified one major product which was identified as a lysine-bound oxidation product (Figure 6). Further characterization

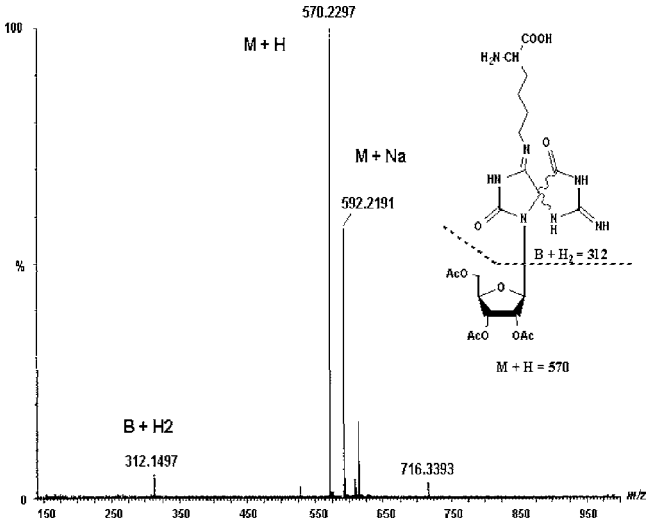


Figure 6. Mass spectrum of product from acyl-8-oxoG oxidized by Cr(V)−salen in the presence of lysine.

of this product is needed to verify its structure, but the masses are consistent with the structure shown. This clearly demonstrates that cross-links may be formed by oxidatively Cr-damaged DNA and protein.

In conclusion, the formation of Sp from the oxidation of 8-oxoG has previously been shown to occur by a single electron abstraction mechanism when Ir(IV) is used as the oxidant.[12] When suitable model complexes of the biologically relevant Cr species are used, an alternate mechanism for oxidation, a two-electron oxo-atom transfer process operates.

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Supporting Information Available: Experimental procedures, MS/MS of B+H2 of **4**, and example SIM chromatograms for isotope labeling experiments of **4** from oxidation of **3** by Cr(V)−salen. This material is available free of charge via the Internet at http://pubs.acs.org.

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