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DNA oxidation by peroxo-chromium(v) species: oxidation of guanosine to guanidinohydantoin

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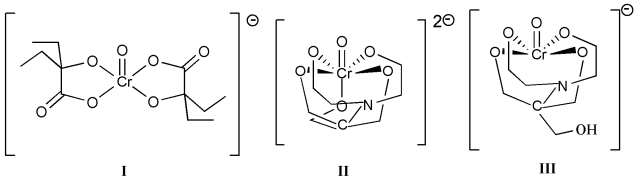
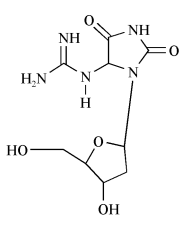
Reactions of peroxo-chromium(v) complexes with DNA afforded mainly guanine oxidation yielding, a four-electron oxidation product, guanidinohydantoin, and exhibited extensive base labile strand scission.

It is amply documented that chromium(VI) compounds are highly carcinogenic and mutagenic.[1,2] Workers exposed to chromium dust show a high percentage of lung and other bronchial cancers. It is commonly accepted that hypervalent chromium-(V) and -(IV) intermediates formed by cellular reducing agents[2–11] are primarily involved in oxidative DNA damage.[5,6,8–11] Although the exact coordination environments of these metastable chromium-(V) and -(IV) complexes are yet to be evaluated, these complexes are believed to be oxo- and peroxo-chromium species. In earlier reports, we have documented that oxo-chromium(V) complexes coordinating with carboxy and alkoxy ligands, initiate DNA damage primarily through the oxidation of ribosyl moiety.[12,13,14] In particular, oxidations of ribose at C1′ and C5′ positions were observed leading to the formation of 5-methylene-2-furanone and furfural, the latter appears to be the primary product for double stranded DNA. In addition, we observed 8-oxo-guanosine as a minor product. Although the participation of peroxo-chromium species in DNA damage has been speculated by many workers[11,15,16] the DNA oxidation by peroxo species is largely unknown, primarily due to the paucity of stable peroxo-chromium(V) complexes. In this communication, we report the formation of guanidinohydantoin due to guanine oxidation by a peroxo-chromium(V) complex. To the best of our knowledge, this is the first report that deals with the formation of this guanine oxidation product by a peroxo-chromium(V) complex.

Reactions† of bis(2-hydroxyethylbutanato)oxochromate(V) (Cr^V–HEBA; **I**) and bis(hydroxyethyl)amino-tris(hydroxymethyl)methaneoxochromate(V) (Cr^V–BT; **II**, **III**) complexes with



I **II** **III**



IV

hydrogen peroxide resulted in peroxo-chromium(V) complexes. These peroxo complexes, formed with compounds **I** and Cr^V–BT, exhibited an EPR signal at $g = 1.981$ which is distinctly different from the EPR signals of the two starting complexes at $g = 1.978$ and 1.965. In addition to the formation of the peroxo-complex, the reaction of peroxide with compound **I** also generated hydroxyl radicals, as evidenced by the formation of four-line EPR signals with 1:2:2:1 intensity distribution and 14.9 G hyperfine coupling constant for the hydroxy-DMPO adduct. The reaction of Cr^V–BT with hydrogen peroxide in the presence of a thirty-fold excess of the BT ligand did not yield appreciable hydroxyl radicals. First, the reaction of double stranded DNA with compound **II** in the presence of hydrogen peroxide and a large excess of BT ligand was investigated to minimize complications due to the participation of hydroxyl radicals in the DNA oxidation. Following the reaction, nuclease P1 and alkaline phosphatase were used to cleave the DNA at the 3′ and 5′-phosphate ends. The resulting cleavage products were subjected to high performance liquid chromatography (HPLC) separation and electrospray ionization–mass spectrometry (ESI–MS) characterization.

Fig. 1 shows a typical HPLC chromatogram of the products after enzymatic digestion by exonucleases. The four nucleosides resulting from the digestion were easily characterized from their retention times and UV spectra. Various fractions from the HPLC separations were collected and then lyophilized at room temperature. The dried samples were redissolved in water and analyzed using ESI–MS by direct infusion. Although many of the products revealed by chromatographic separations were common to those observed for the oxo-chromium(V) oxidation mentioned earlier, the main product, giving rise to peak A, is unique for the peroxo oxidation product and the primary focus of the present study. This product exhibits a retention time that falls between the dT and dA in our reversed phase HPLC separation. The same product was observed when the reaction was carried out with poly(GC), ruling out the possibility that a modified A or T was formed in the reaction. This main product peak with *m*/*z* of 274 corresponds to guanidinohydantoin (structure **IV**) and exhibits the fragmentation patterns for the authentic species. Reactions of compound

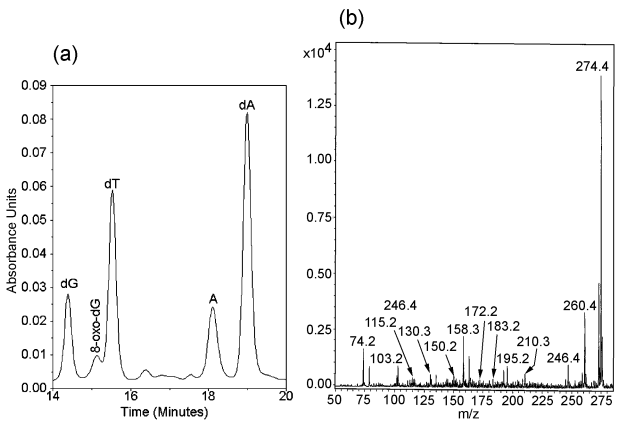


Fig. 1 (a) Part of the HPLC chromatogram for the reaction of ds-DNA with complex **II** in the presence of H_2O_2. Note that the retention time for dC is 4.8 min and therefore does not appear in the displayed chromatogram. (b) ESI–MS of fraction A.

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I with double stranded DNA also resulted in the four-electron oxidation product indicated above. However, these reactions generated other products including furfural and base propenals, primarily due to the participation of hydroxyl radical.

The nature of the Cr^{V}–peroxo complexes and other reactive intermediates formed in these reactions, and the species responsible for the oxidation of guanosine to guanidinohydantoin need to be addressed. In aqueous solution, Cr^{V}–HEBA slowly distributes to mono- and bis-ligated forms,[17] while the Cr^{V}–BT compound establishes a rapid equilibrium between five- and six-coordinated species.[18,19] In the presence of extra HEBA ligand, compound **I** almost exclusively exists as the bis-ligated form. Since the reactions of hydrogen peroxide with Cr^{V}–HEBA and Cr^{V}–BT are faster than the formation of the mono-ligated form of **I**, and that the same peroxo species is formed in the presence of added HEBA and BT ligand (as much as thirty-fold excess over the Cr(v) complexes), it is highly likely that the penta-coordinated forms, for both HEBA and BT-complexes, bind peroxide through their open basal sites. In fact, the *g*-value of our peroxo complex, Cr(v)(HEBA)O_2 (1.981), is very close to the *g*-value, 1.982, of a mono-peroxo chromium(v) complex[20] generated by direct reaction between Cr(vi) and H_2O_2. Secondly, in the presence of excess BT ligand, both for compound **I** and **II**, hydroxyl radicals were barely detected due to the highly radical scavenging ability of the poly-alcoholic ligand, yet substantial guanidinohydantoin was formed. Thirdly, in the absence of hydrogen peroxide, the initial chromium(v) complexes did not afford guanidinohydantoin. Collectively, these data indicate that the Cr^{V}–peroxo species is largly responsible for the formation of guanidinohydantoin.

The formation of guanidinohydantoin, the four-electron oxidation product of guanine, is different from those observed for the oxo-chromium(v) complexes in which primary oxidation processes were confined within the ribosyl moiety. Since the peroxo–Cr(v) was reduced to an aqua–Cr(iii), the chromium(v) complex renders four-electron oxidation. The four-electron oxidation product may be formed *via* an oxidation of 8-oxo-dG, an intermediate two-electron oxidation product of guanine residue. This intermediate is known to undergo further oxidation due to its lower reduction potential compared to that of the starting substrate. In patricular, 8-oxo-dG has at least 0.5 V potential advantage over its parent reduced form, dG.[21,22] The proposal of invoking 8-oxo-dG as an intermediate finds support from recently reported oxidation by $IrCl_6^{2-}$. In fact, Borrows and coworkers[23] have observed the same product due to oxidation of 8-oxo-guanosine by hexachloroiridate(iv) anion, an one-electron oxidant. These workers postulated a mechanism that involves the oxidation at C5, site of the purine ring *via* the formation of 5-hydroxy-8-oxo-7,8-dihydroguanine intermediate. To validate such a mechanism, an oxidation reaction of 8-oxo-dG with peroxo-chromium(v) was initiated. In this reaction the 8-oxo-purine was further oxidized to guanidinohydantion. Since Dalal and co-workers[24–26] observed the formation of 8-oxo-G by hydroxyl radicals, which did not undergo further oxidation, we rule out the possibility that the further oxidation of 8-oxo-dG in our system was accomplished by hydroxyl radicals. Meunier and coworkers,[27] on the other hand, have postulated a different mechanism for the formation of guanidinohydantoin by an oxomanganese(v)–porphyrin complex without invoking 8-oxo-dG. These workers proposed the formation of a guanine cationic radical formation at the C5 site *via* two-electron oxidation by Mn(v), followed by hydroxylation at the C5 and C8 sites and subsequent two-electron oxidation by a second manganese unit. The latter mechanism differs from that proposed by Burrows and coworkers in that the oxidation did not involve 8-oxo-dG. Our data cannot rule out the mechanism proposed by Meunier and coworkers. The oxidation of DNA has also created extensive base labile scissions predominately at G-sites (data not shown), which is consistent with the observation that 8-oxo-dG alone does not induce alkali-labile DNA cleavage[28] and support the notion that such cleavages require further oxidation of 8-oxo-dG[27,28]

Notes and references

† Reaction of DNA with peroxo–Cr(v) was initiated by incubating calf thymus ds-DNA (3000 μg mL^{-1}) with bis(hydroxyethyl)amino-tris(hydroxymethyl)methaneoxochromate(v) (2 mM) in the presence of H_2O_2 (200 mM) at 37 °C and pH 7.2 buffered by the bis-tris ligand (30 mM) for 3–18 h. At the end of the reactions, EDTA (50 mM, pH 8.0) was added to replace bound ligand onto chromium(iii) by chelating with EDTA. The reaction of 8-oxo-dG with the peroxo–Cr(v) complex was also studied in exactly the same manner and products were characerized by HPLC and LC–MS techniques.

Gradient HPLC separation was performed on a Water system (Model 515 HPLC pump) equipped with a photodiode array detector (Model 996) and programmable gradient pump (ISCO Model 2360.) as deteiled elsewhere.[14] Electron paramagnetic resonance experiments were performed on an X-band instrument (IBM 200D-SCR) in the presence of the radical capturing agent DMPO as described before[8] The electrospray mass spectrometry experiments were performed on a Bruker ESQUIRE-LC 00145 with data analysis software version 2.0. The direct infusion was performed in the positive mode, and the parameters were set as follows: drying gas at 7 L min^{-1}, nebulizer at 10 psi, dry temperature at 120 °C, cap exit at 50 V, skimmer at 10 V, and trap drive at 48.1.

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