Mechanism of DNA damage by chromium carcinogens

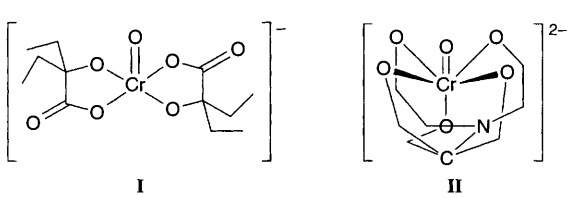
Rathindra N. Bose* and B. Stephen Fonkeng

Department of Chemistry, Kent State University, Kent, OH 44242, USA

Oxochromium(v) centres initiate DNA cleavage through a chromium(v)–phosphate diester intermediate by hydrogen abstraction from the C$^{1'}$ position of the ribose sugar, leading to the formation of 5-methylene-2-furanone, 3′-phosphate terminus and DNA bases; by contrast a plasmid DNA (pUC19) is only nicked.

The carcinogenic and mutagenic activities of chromium(VI) are documented *in vivo* and in cultured cells.[1–3] In fact, bronchial carcinomas among workers producing chromate pigments are widespread.[4] Since Cr^{VI} and Cr^{III} do not damage DNA, metastable hypervalent chromium-(V) and -(IV) species are suspected to be the putative damaging agents.[5] These transient atypical oxidation states have been observed during the reduction of Cr^{VI} by biological reducing agents in the presence and absence of hydrogen peroxide.[6–10] Recently, an acid-stable chromium(V) complex has been shown to nick pUC9 DNA in acidic solution (pH = 3).[11] However, DNA damage by Cr^{VI} in biological reducing agents has been observed only in the presence of peroxide and other biological reducing agents.[5,6,10b] Therefore, the coexistence of hydroxyl and other organic radicals in these reactions[6–10] and susceptibility of acid-catalysed glycoside bond cleavage of DNA[11] have cast doubt on the role of hypervalent chromium species in cleaving DNA. Recently, we have stabilized a chromium(V) species[12] at physiological pH values, allowing us an opportunity to examine DNA damage by this rare oxidation state.

The reaction of bis(2-ethyl-2-hydroxybutanoato)oxochromate(V) **I** (Fig. 1) with plasmid pUC19 and single-stranded calf-thymus DNA (ss-CTDNA)† in bis-tris (BT) buffer solution (pH = 7.0, T = 37 °C) resulted in cleavage of the nucleic acids. The plasmid DNA was nicked as shown by the appearance of a slow moving band (compared to the plasmid DNA on a 0.8% agarose gel), characteristic of a more relaxed linear DNA. On the other hand, ss-CTDNA suffered extensive damage by releasing four bases, nucleotide and sugar oxidized products which were characterized by HPLC, proton and ^{31}P NMR, and mass spectrometry. For example, HPLC chromatograms exhibited several peaks whose retention times match‡ with adenine, guanine, cytosine, thymine, 3′-GMP, 5-methylene-2-furanone (5-MF) and chromium(III) species. The proton NMR spectrum of the thymine fraction exhibited a singlet at δ 1.83, whereas the 3′-GMP fraction showed a ^{31}P NMR peak with characteristic chemical shifts over the pH range 2–10. Secondary mass spectrometry§ verifies the presence of the molecular ion at m/z 96 for 5-MF. In addition, the UV spectrum of authentic 5-MF (absorption maximum, at 267 nm) is in accord with that recorded with a diode array detector during the separation. In another experiment, the products of ss-CTDNA reaction were incubated with H_4edta for 1 h to displace coordinated phosphate from Cr^{III}, and ^{31}P NMR spectra were recorded in the pH range 2–8. The spectra exhibited two resonances in addition to that for the unreacted CTDNA. The chemical shift data at pH 7.0, along with the variation of chemical shift as a function of pH, indicate that these two peaks correspond to orthophosphate and 3′-dGMP. The same products were obtained when the reaction was followed under a nitrogen atmosphere.

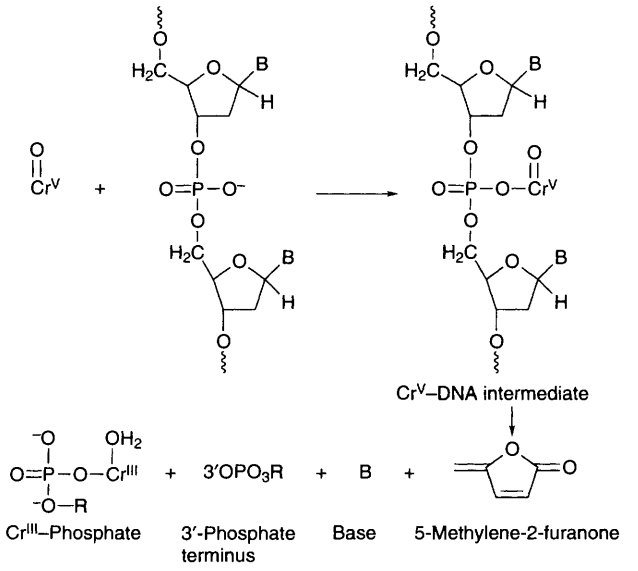


I **II**

Fig. 1 Structures of {bis(2-ethyl-2-hydroxybutanoato)-oxochromate(v) **I** and {bis(hydroxyethyl)aminotris[(hydroxymethyl)-methane]}oxochromate(v) **II**

In order to characterize the chromium(v) intermediates in this reaction, EPR spectra of the reaction mixtures were recorded at regular time intervals for 1–5 h. The starting chromium(V) complex exhibits a signal at g = 1.978 and suffers rapid disproportionation at neutral pH to form Cr^{III} and Cr^{VI} species. The same complex is converted to a new Cr^{V}–BT complex, {bis(hydroxyethyl)aminotris[(hydroxymethyl)-methane]}oxochromate(V) **2**[12] through a ligand-exchange reaction, and the EPR signal for this chromium(V) species appears at g = 1.965. The reaction mixture exhibits a doublet centred at g = 1.974. The hyperfine coupling constant, A = 3.7 G, and the g-value are comparable to those of a pyrophosphato–chromium(V) complex (g = 1.974, A = 5.2 G) formed by the reaction between the chromium(V) complex and pyrophosphate anion (data not shown). The EPR data show that the phosphate diester of DNA is coordinated to chromium(V), and two lines are due to coupling with ^{31}P nuclei. All the EPR signals slowly decayed (1 h at room temp.) with the formation of oxidized DNA products as discussed above. The intermediate and products characterized in the DNA oxidation are shown in Scheme 1.

Oxidative DNA cleavage mechanisms based on the formation of specific products have been elucidated.[13] The formation of 5-MF is taken to be indicative of an oxidation process



Scheme 1 Intermediate and products which have been characterized in the reaction between ss-DNA and Cr^{V}

initiated at the $C^{1'}$ position of the ribose sugar. Although hydrogen abstraction pathways are widely accepted for metal and non-metal DNA cleavers including bleomycins[14,15] and enediyne[16] antitumour drugs, hydride transfer remains a possible alternative for oxochromate mediated oxidation since the latter route has been elucidated for organic oxidation using oxochromate centres.[17,18]

Extensive damage of a single-stranded, but not double-stranded, DNA, may be related to a number of factors including (*a*) the structural differences between these two biopolymers, (*b*) the ease with which Cr^{V} is coordinated to the phosphate diester moiety and (*c*) the susceptibility of chromium(V)–phosphate complexes toward disproportionation.

A comparison between oxochromium(V) and other metal complexes which can serve as artificial nucleases is in order. Hydrogen abstraction mechanisms[13–15,19–26] are invariably cited for oxidative damage of DNA by a variety of metal ions including photoactivated rhodium(III) complexes. Most of these metal ions, including Fe^{II}, Cu^{I} and Mn^{II}, are activated by either molecular oxygen, hydrogen peroxide, or other powerful oxidizing agents. The nature of these activated complexes is still a matter of debate. High-valent oxometalates and hydroxyl/peroxy radicals generated by Fenton type reactions are believed to be the activated species.[13] Nevertheless, these activated metal centres abstract hydrogens from $C^{1'}$, $C^{3'}$, $C^{4'}$ or $C^{5'}$ positions yielding a variety of products. In many cases, for a given metal centre [*e.g.* copper(I)–bipyridyl, manganese(II) porphyrins], hydrogen abstraction from more than one carbon site has been inferred.[13] DNA oxidations by ruthenium(IV) and osmium(IV) oxo complexes are thought to proceed through two major pathways: hydrogen abstraction at the $C^{1'}$ position of the sugar and oxidation of guanine base, producing 8-oxoguanine.[26]

The present study offers several key features toward understanding oxidative DNA damage at the molecular level. First, the identity of the oxidizing agent is well established, *i.e.* the reaction centre is an oxochromium(V) species. Since Cr^{VI} exclusively exists as an oxochromate centre, its one-electron reduction in cellular milieu at physiological pH is also expected to generate an oxochromium(V) species. Therefore, the use of an authentic oxochromium(V) species in cleaving DNA is relevant to understanding the role played by chromium carcinogens in biological systems. Second, mechanistic ambiguities due to the participation of hydroxyl and peroxy radicals can be eliminated since the cleavage takes place in the absence of oxygen. Third, the reaction proceeds through a chromium(V)–phosphate diester intermediate in which the phosphate is covalently bound to the metal ion. This particular pathway is unique. Although the $C^{1'}$ site in DNA is susceptible to attack by activated metal ions,[13] direct coordination through the phosphate diester has not been invoked in oxidative cleavages. For ruthenium and osmium oxo complexes, a weak outer sphere precursor complex, formed by an electrostatic attraction between the negatively charged phosphate diester and positively charged metal complex, has been proposed.[26] Metal-promoted hydrolytic cleavage of DNA by enzymes (nucleases), on the other hand, is believed to proceed through coordination to the phosphate moiety, followed by hydroxyl transfer to the electrophilic phosphorus atom.[27] The present study adds new mechanistic diversity in that phosphate coordination, followed by redox, has led to cleavage. Fourth, the oxidation is centred predominately at $C^{1'}$. Finally, unequal concentrations of released bases in the oxidized products point to a sequence and a structure-specific (but not a random) reaction. Studies are in progress to pinpoint sequence specificity, if any, and the reactive structural motif of DNA. To the best of our knowledge, this is the first study in which a chromium(V)–DNA intermediate and DNA cleavage products oxidized by Cr^{V} have been characterized.

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Footnotes

† The plasmid DNA was prepared and purified following the literature method.[28] The chromium(V) complex **I** was prepared by the method of Krumpolc and Rocek.[29]

‡ Retention times for various fractions along with authentic samples in s (in parentheses): cytosine, 169 (174 ± 5); guanine, 192 (186 ± 5); thymine, 236 (230 ± 8); adenine, 286 (291 ± 8); 5-MF, 459 (455 ± 10).

§ Tandem mass spectrum of 5-MF exhibited major peaks at *m/z* 96, 68, 42 and 26. The furanone derivative was prepared following the literature method.[30]

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