EPR Evidence for Chromium(V) Binding to Phosphate and Pyrophosphate: Implications for Chromium(V)−DNA Interactions

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The +5 oxidation state of chromium, Cr(V), is a known intracellular reduction product of the carcinogen chromate, Cr(VI).[1−4] While there is much evidence that Cr(V) participates in the mechanism of Cr(VI) carcinogenesis,[5,6] little spectroscopic evidence for binding of Cr(V) to DNA and/or DNA substituents via a ligand exchange process has been reported. Previously, Cr(V)−phosphate complexes had been observed only during the reduction of Cr(VI) in concentrated ortho-, meta-, and pyrophosphoric acids.[7,8] In this paper, we demonstrate the ability of a model mutagenic Cr(V) complex to undergo ligand exchange in aqueous solutions and to bind in a monodentate fashion with phosphate and in a bidentate chelate with pyrophosphate. Our findings suggest a mechanism whereby Cr(V) formed through intracellular reduction of Cr(VI) can undergo ligand exchange and bind to the phosphate moieties present within both DNA and deoxyribonucleotides.

The Cr(V) complex bis(2-ethyl-2-hydroxybutyrato)oxochromate(V),[9] $[Cr(ehba)_2O]^-$ (**I**), was used in these experiments because of its known mutagenicity and DNA-cleaving ability.[5] The reaction of **I** with pyrophosphate ($H_2P_2O_7^{2-}$) was carried out as a model for bidentate phosphate linkages between Cr(V) and deoxyribonucleotides. Bidentate binding of pyrophosphate with Cr(V) is expected to generate a more stable complex than phosphate ($H_2PO_4^-$) itself. Ligand exchange with formation of a bidentate DNA−phosphate-based chelate has been postulated by Farrell *et al.* to be the intermediate of **I**−DNA binding motif.[5]

Two distinct Cr(V)−pyrophosphate species, **II** and **III**, were observed via EPR[10] in the reaction of **I** with pyrophosphate. Formation of **II** was observed at pH's ranging from 4.0 to 7.0, at either room temperature (RT) or 4 °C (Figure 1), with the greatest signal intensity at pH's of 5.0−6.0. The four-line EPR spectrum was assigned to a mixed-ligand species, $[Cr(ehba)(H_2P_2O_7)O]^-$ (**II**), with a g value of 1.977, $\Delta H = 0.70$ G, and normalized concentrations[11] of 69 μM at RT and 50 μM at 4 °C. The observed shift to lower g values for **II** versus that of **I**, $g = 1.980$, is consistent with greater spin−orbit coupling for the pyrophosphate ligands versus that of the ehba ligands.[12] Simulation of the EPR spectrum **(**Figure 1**)** demonstrated that the new species, **II**, has two inequivalent phosphorus atoms with superhyperfine coupling constants of $A_P = 4.7$ and 5.6 G, suggesting that **II** has the same distorted trigonal bipyramidal geometry as the parent complex, **I**.[13] On the basis of the known *trans*-labilizing effect of the oxo group,[14] the larger superhyperfine splitting, $A_P = 5.6$ G, was assigned to the quasi-axial phosphorus and the smaller superhyperfine splitting, $A_P = 4.7$ G, was assigned to the equatorial phosphorus, Scheme 1. Reaction of 0.50 mM **I** with 100 mM pyrophosphate, at 4 °C and pH = 5.0, also resulted in a second four-line EPR spectrum corresponding to **III** at a concentration of 11 μM,[11] which was not observable at RT at any pH. The four-line spectrum due to **II** partially obscured the 1:4:6:4:1 superhyperfine splitting pattern of the bis(pyrophosphate) species, $[Cr(H_2P_2O_7)_2O]^-$ (**III**). The simulated spectrum had a g value of 1.971 and $\Delta H = 0.80$ G for **III** and four equivalent phosphorus nuclei with superhyperfine splittings of $A_P = 3.8$ G, which is consistent with a square pyramidal geometry as shown in Scheme 1. Alternatively, a trigonal bipyramidal structure undergoing a series of fast intramolecular Berry twists, as seen with the bis(1,2-ethanediolato)oxochromate(V) complex,[13] would also account for the observation of four equivalent phosphorus nuclei in complex **III**. Reactions with D_2O were undertaken to ensure that the splitting was due solely to the $I = {}^1/_2$ phosphorus and not to an $I = {}^1/_2$ hydrogen arising from a possible hexacoordinate Cr(V) species with an aquo or hydroxo ligand. Further splittings of the peaks would be expected with coordination of the $I = 1$ deuterium. No change in the spectrum was observed in D_2O with respect to H_2O, implying that all superhyperfine splittings arise solely from phosphorus and that the complex remains five-coordinate. Due to low signal intensity, ^{53}Cr isotopic hyperfine splittings for species **II** and **III** could not be measured.

Reactions of **I** with free phosphate were studied by EPR for possible monodentate binding modes. Reaction of 0.50 mM **I** with 100−500 mM phosphate demonstrated no detectable binding at RT and pH's of 4.0−7.0. However, when experi-

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(10) EPR spectra were recorded at RT or 4 °C using a Bruker ESP-300 spectrometer equipped with a liquid-nitrogen-cooled variable-temperature apparatus. Spectral parameters at 4 °C were 100-kHz field modulation, 1.0 G modulation amplitude, 5.12 ms time constant, 9.429−9.433 GHz microwave frequency, 1×10^5 receiver gain, 2 mW microwave power attenuated at 20 dB, 3350−3450 G sweep width, and a 21 s scan time. RT parameters were identical except for a 9.769−9.772 GHz microwave frequency, and 3480−3580 G sweep width. All signals were averaged over nine scans. Measurements were done on *ca.* 100 μL volume samples drawn into a capillary tube that was sealed on one end with Dow-Corning high-vacuum grease. The g values were determined with respect to the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), $g = 2.0036$.
(11) Concentrations were determined from a standard curve of $[Cr(ehba)_2O]^-$ in 100 mM aqueous 2-ethyl-2-hydroxybutryric acid. Results were normalized between runs against the ^{53}Cr hyperfine signal of $[Cr(ehba)_2O]^-$.
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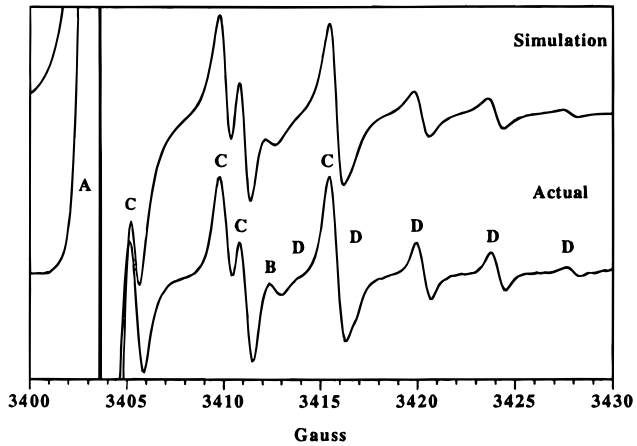


Figure 1. EPR spectra for the reaction of 0.50 mM **I** and 100 mM pyrophosphate in aqueous pH = 5.0 solution at 4 °C, both simulated and actual spectra. Peaks were assigned as follows: (A) $[Cr(ehba)_2O]^-$ starting material with $g = 1.980$; (B) ^{53}Cr, $I = {}^3/_2$ hyperfine splitting from the $[Cr(ehba)_2O]^-$ starting material with $g = 1.980$; (C) $[Cr(ehba)(H_2P_2O_7)O]^-$ (**II**) with $g = 1.977$ and phosphorus superhyperfine splittings of $A_P = 4.7$ and 5.6 G; (D) $[Cr(H_2P_2O_7)_2O]^-$ (**III**) with $g = 1.971$ and phosphorus superhyperfine splittings of $A_P = 3.8$ G.

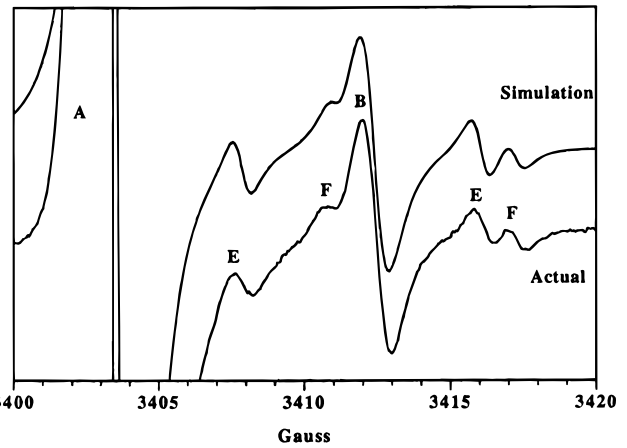
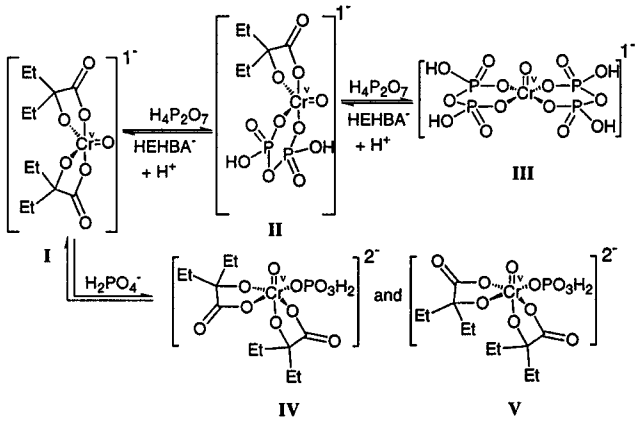


Figure 2. EPR spectra for the reaction of 0.50 mM **I** and 100 mM phosphate in aqueous pH = 5.0 solution at 4 °C, including both simulated and actual spectra. Peaks were assigned as follows: (A) $[Cr(ehba)_2O]^-$ starting material with $g = 1.980$; (B) ^{53}Cr, $I = {}^3/_2$ hyperfine splittings from the $[Cr(ehba)_2O]^-$ starting material, $g = 1.980$; (E) *fac*-$[Cr(ehba)_2(H_2PO_4)O]^{2-}$ (**V**) with $g = 1.976$ and $A_{Pfac} = 8.2$ G; (F) *mer*-$[Cr(ehba)_2(H_2PO_4)O]^{2-}$ (**IV**) with $g = 1.974$ and $A_{Pmer} = 6.2$ G.

Scheme 1



ments were carried out at 4 °C and at a pH of 5.0 to retard disproportionation and ligand dissociation, a weak (0.1 μM)[11] four-line EPR spectrum was observed (Figure 2). An overlapping ^{53}Cr hyperfine splitting from **I** complicated the spectrum but still allowed simulation (Figure 2). The four-line spectrum was shown by simulation to be consistent with formation of two separate monophosphate−Cr(V) species. These two phosphate species were considered to have octahedral geometry and were assigned as *fac* and *mer* isomers of *cis*-$[Cr(ehba)_2(H_2PO_4)O]^{2-}$, (**IV** and **V**), Scheme 1. The assignment of the two phosphate species as *cis* isomers was based both on previous work with the analogous *cis*-aquobis(oxalato)oxochromate(V) species[15] and on the large associated superhyperfine splittings. These two species, **IV**-*mer* and **V**-*fac*, had *g* values of 1.974, ($\Delta H = 0.70$ G) and 1.976 (0.70 G), respectively, with phosphorus superhyperfine splittings of $A_{Pmer} = 6.2$ G and $A_{Pfac} = 8.2$ G. The significantly lower signal intensity observed with the phosphate complexes versus the pyrophosphate complexes is consistent with the lower stability expected for monodentate versus bidentate binding. Assignment of complexes **IV** and **V** as octahedral is counter to the generally accepted inflexibility of the five-coordinate sterically-hindered parent complex **I**.[16,17] However, at the lower temperatures in this experiment, we postulate the trapping of a fluxional five- to six-coordinate intermediate with phosphate inserting either *fac* or *mer* to the carboxylate groups on the ehba ligands. No spectrum assignable to a bis-substituted Cr(V)−phosphate complex was observed.

These results show that phosphate oxygens of DNA and deoxyribonucleotides may allow tethering of Cr(V) for oxidation of DNA or deoxyribonucleotides.[18] The Cr(V)−phosphate binding motif has been previously suggested,[5] but not directly observed, as an intermediate in oxidative DNA cleavage and mutagenicity. The monodentate binding modes observed in this study may be relevant to Cr(V)-induced DNA damage. However, bidentate motifs of Cr(V) chelation with deoxyribonucleotide di- and triphosphates would be expected, and oxidation would deplete the intracellular pool of nucleotides needed for DNA synthesis, as has been observed in a human EUE heteroploid cell line treated with Cr(VI).[19]

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Supporting Information Available: A figure showing actual and simulated EPR spectra for the room-temperature reaction of Cr(V) with pyrophosphate at pH = 5.0 (1 page). Ordering information is given on any current masthead page.

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