# EPR Spectroscopic Studies on the Formation of Chromium(V) Peroxo Complexes in the Reaction of Chromium(VI) with Hydrogen Peroxide

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The Cr(V) products of the reaction of Cr(VI) with $H_2O_2$ were studied by EPR spectroscopy. In addition to the well-characterized tetrakis($\eta^2$-peroxo)chromate(V) complex, $[Cr(O_2)_4]^{3-}$, with $g_{iso} = 1.9723$ ($A_{iso} = 18.4 \times 10^{-4}$ $cm^{-1}$), three new species were observed with isotropic EPR parameters, $g_{iso} = 1.9820$, $g_{iso} = 1.9798$ ($A_{iso} = 16.3 \times 10^{-4}$ $cm^{-1}$), and $g_{iso} = 1.9764$ ($A_{iso} = 18.1 \times 10^{-4}$ $cm^{-1}$). While $[Cr(O_2)_4]^{3-}$ is stable at high concentrations of $H_2O_2$ and in alkaline solution, the species with a signal at $g_{iso} = 1.9798$ is stabilized at low relative concentrations of $H_2O_2$ and in neutral solution. The signal at $g_{iso} = 1.9764$ is most prominent in weakly acidic (pH = 4−7) solutions and low relative concentrations of $H_2O_2$. Finally, the signal at 1.9820 is only minor and is apparent at low pH values and low $[H_2O_2]$. From the pH and $[H_2O_2]$ dependences, and by analogy with the V(V) chemistry, the species giving rise to the signals at $g_{iso} = 1.9820$, $g_{iso} = 1.9798$, and $g_{iso} = 1.9764$ are assigned as the oxo($\eta^2$-peroxo)chromium(V), $[Cr(O)(O_2)(OH_2)_n]^+$, aquaoxobis($\eta^2$-peroxo)chromate(V), $[Cr(O)(O_2)_2(OH_2)]^-$, and the hydroxotris($\eta^2$-peroxo)chromate(V), $[Cr(O_2)_3(OH)]^{2-}$, complexes, respectively. The implications of these Cr(V) peroxo species for understanding the in vitro DNA damage caused by Cr(VI) and $H_2O_2$ and the genotoxicity of carcinogenic Cr(VI) complexes are discussed.

## Introduction

Chromium(VI) is carcinogenic[1,2] due to its conversion by intracellular reductants to more active species that initiate DNA damage.[3] In this regard, $H_2O_2$ is of interest because it is a ubiquitous byproduct of oxidative cellular metabolism[4] and is formed during the autoxidation of the principal intracellular reductants, such as ascorbic acid[5] and glutathione.[6] Incubation of $H_2O_2$ with Cr(VI) and DNA in vitro results in intermediates that cause DNA cleavage,[7] but the damaging intermediates have not been characterized. It has been postulated[7−11] that $OH^•$ radicals formed during the decomposition of Cr(V)/peroxo complexes, or through Fenton-type reactions,[12−15] cause DNA damage; however, the results from competition kinetic studies are not consistent with this postulate.[16−18] In preliminary experiments, three Cr(V) EPR signals were detected in the reaction of $Na_2Cr_2O_7$ with $H_2O_2$.[19] This may be important in understanding in vitro damage to DNA caused by intermediates in the reduction of Cr(VI) by biologically relevant molecules, such as ascorbic acid,[20] glutathione,[8] cysteine,[21] and NAD(P)H,[11] where a role for $H_2O_2$ has been implicated. $K_3[Cr^V(O_2)_4]$ also causes higher levels of nephrotoxicity than chromate on the mouse kidney, while $Cr(NO_3)_3$ and a Cr(V)/GSH complex, Cr-$(GSH)_3$, did not exhibit any detrimental effect.[22]

The Cr(VI)/$H_2O_2$ reaction is a classic system that has been extensively studied during the last 100 years. There are several excellent reviews concerned with the Cr(VI)/$H_2O_2$ reaction and the products/intermediates in the reaction.[23−26] Several peroxo species, including the well-characterized tetraperoxochromate-(V) and oxodiperoxochromate(VI) complexes, have been reported in the reaction, depending on the solution pH and the reactant concentrations.[27] Here, the natures of the Cr(V) species generated in the reaction of Cr(VI) with $H_2O_2$ are investigated using EPR spectroscopy and their relevance to Cr(VI) genotoxicity is discussed.

## Experimental Section

**Materials.** Sodium dichromate dihydrate (Merck, 99.5%), sodium chromate tetrahydrate (Aldrich, 99%), sodium hydroxide (Fluka, >98%), aminotris(hydroxymethyl)methane hydrochloride (Sigma, >99%),

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aminotris(hydroxymethyl)methane (Amresco, >99.8%), and 1,1,1-tris-(hydroxymethyl)ethane (Aldrich) were used as supplied. Hydrogen peroxide (Pacific, 20.4% w/w) was standardized by the literature method.[28] ***Caution***! *Cr(VI) is carcinogenic and mutagenic*; *the intermediates generated in the reaction of Cr(VI) with $H_2O_2$ are capable of cleaving DNA.*[7,8] *Appropriate precautions should be taken to avoid inhaling Cr(VI) and to avoid skin contact while handling solutions of these chemicals.*

Equal volumes (5 mL) of $Na_2Cr_2O_7$ (or $[CrO_4]^{2-}$) and $H_2O_2$ solutions were mixed, and the solution pH was adjusted to the required value by the addition of 0.5 M NaOH solution. The pH was measured by an Activon model 210 pH meter fitted with an Activon BJ 321 calomel pH electrode. The concentration values given in the figure captions are the initial concentrations after mixing.

**Apparatus.** A Bruker ESP 300 spectrometer operating at ~9.7 GHz was used for recording EPR spectra from solutions contained in a Wilmad quartz flat cell. The magnetic fields were measured by a Bruker ER 035M NMR gaussmeter, and the microwave frequencies, by a Hewlett-Packard 5352B microwave frequency counter. Each spectrum was recorded 3 min after the solutions were mixed unless otherwise stated. EPR spectrometer settings were as follows: central field, 3480 G; sweep width, 100 G; microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 0.968 G; receiver gain, $2 \times 10^4$; conversion time, 5.12 ms; time constant, 1.28 ms; number of scans, 20; data collection time, ~140 s; temperature ~293 K.

## Results

Four EPR signals at $g_{iso} = 1.9820$, 1.9798, 1.9764, and 1.9724 were observed immediately after initiation of the reaction of Cr(VI) (10 mM) with $H_2O_2$ (100 mM) at pH = 7.0 (Figure 1). During the first 15 min, the intensities of these signals steadily increased and then decreased, such that the signal intensity at 30 min was only ~15% of the maximum value. All of these signals were detected at pH 4−8 within 30 min after initiating the reaction. While the minor $g_{iso} = 1.9820$ signal became more prominent in acidic solutions, it was only a shoulder on the $g_{iso} = 1.9798$ signal. The same signals were observed at lower concentrations of reactants (5 mM) and using a lower microwave power; however, signals of higher intensity and better resolution were obtained using [Cr(VI)] = 10 mM and $[H_2O_2] = 10-400$ mM reactant concentrations and 20−200 mW microwave power. The ratios of the signal intensities were independent of the microwave power, showing no selective saturation effects.

The relative signal intensities were dependent on the acidity of the reaction media and the ratio of the reactants. A decrease in the solution pH value resulted in a decrease in the intensity of the $g_{iso} = 1.9798$ signal but an increase in the intensity of the $g_{iso} = 1.9764$ signal. The latter becomes the major species over the pH range of 4−6 (Figure S1). The signal at $g_{iso} = 1.9820$ is more prominent at lower concentrations of $H_2O_2$. The overall EPR signal intensity due to these species increased with increasing the $[H_2O_2]$:[Cr(VI)] ratio from 2:1 to 6:1, and relatively, the signal intensity at $g_{iso} = 1.9724$ increased with respect to that at $g_{iso} = 1.9764$. However, a further increase in the ratio to 10:1 resulted in decreased signal intensities for all of the signals. In moderately acidic conditions (pH < 4), the reduction of Cr(VI) to Cr(III) proceeded very rapidly such that no Cr(V) signal was observed at pH values below 4.0.

The change in the relative intensities of these signals as a function of the $[H_2O_2]$:[Cr(VI)] ratio was studied at pH values around 8. The intensity of the $g_{iso} = 1.9724$ signal increased with increasing the $[H_2O_2]$:[Cr(VI)] ratio from 5:1 to 20:1, while the intensity for the signal at $g_{iso} = 1.9798$ decreased as the ratio increased (Figure 2).

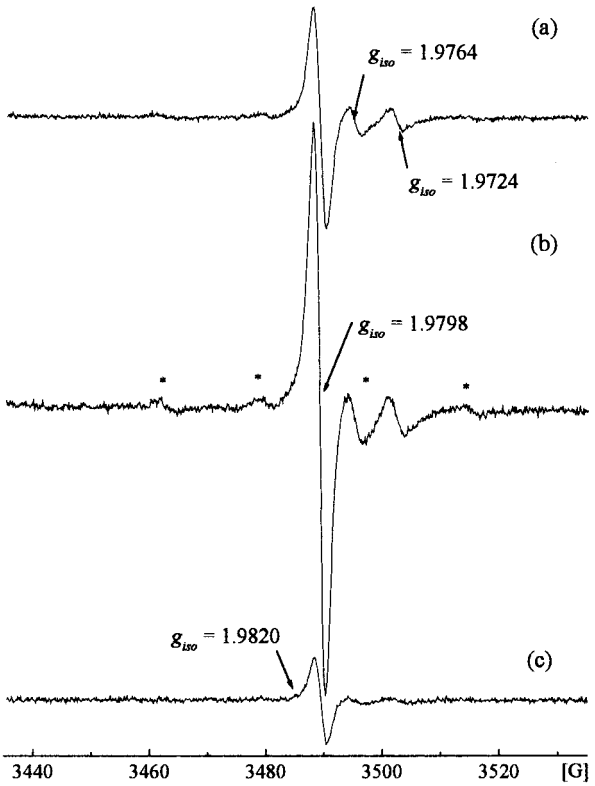


**Figure 1.** X-band EPR spectra of the Cr(V) intermediates formed in the reaction of Cr(VI) (10 mM) with $H_2O_2$ (100 mM). The reaction conditions and operating frequencies were as follows: (a) $t = 3$ min, pH = 7.00, $\nu = 9.6681$ GHz; (b) $t = 10$ min, pH = 6.99, $\nu = 9.6682$ GHz; (c) $t = 32$ min, pH = 6.97, $\nu = 9.6691$ GHz. The hyperfine coupling signals for the $g_{iso} = 1.9798$ signal are marked by asterisks.

At a fixed reactant ratio $[H_2O_2]$:[Cr(VI)] of 10:1, an increase in the pH value of the solution from 6 to 10 resulted in an increased intensity for the signal at $g_{iso} = 1.9724$ and a reduced intensity for the signal at $g_{iso} = 1.9798$ (Figure 3). The signal at $g_{iso} = 1.9798$ was best observed at pH ~ 7, while the signal at $g_{iso} = 1.9724$ was longer-lived in alkaline solution. At pH = 10, the $g_{iso} = 1.9723$ signal dominated the spectrum and decreasing the reactant ratio $[H_2O_2]$:[Cr(VI)] from 10:1 to 5:1 led to an increased intensity for the $g_{iso} = 1.9798$ signal and a decreased intensity for the $g_{iso} = 1.9723$ signal. The signal at $g_{iso} = 1.9764$ was observed only at pH values ≤ 8.0.

In Tris-HCl buffer (50 mM), the signal at $g_{iso} = 1.9722$ was dominant at pH 8.5 with a minor signal at $g_{iso} = 1.9798$, 3 min after initiation of the reaction (Figure 4). At lower pH values, higher [Tris-HCl] values, and/or longer reaction times, a new signal at $g_{iso} = 1.9781$ became a major signal. It had the same characteristics as the new signal produced in unbuffered solutions of Cr(VI)/$H_2O_2$ in the presence of $CH_3C(CHOH)_3$ (Figure 4).

## Discussion

The Cr(VI)/$H_2O_2$ reaction exhibits exceptionally complicated and fascinating chemistry, which leads to a large number of species, depending on the reaction conditions. Beside the formation of the Cr(VI) complexes, viz. the blue peroxochromic acid in highly acidic solution (pH below 4)[29] and a violet deprotonated form of peroxochromic acid in weakly acidic solution (pH between 4 and 7),[30] the present EPR results show the presence of at least four Cr(V)/peroxo complexes. Their

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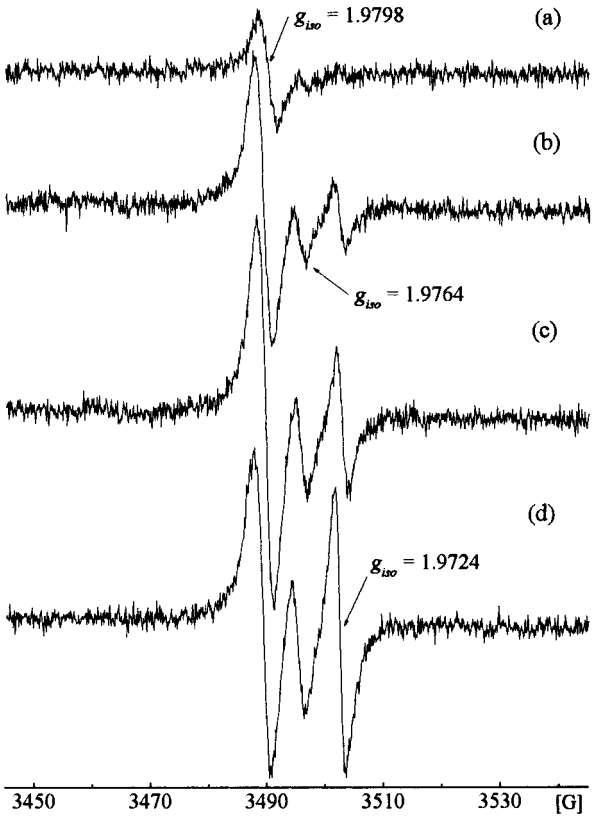



**Figure 2.** X-band EPR spectra of the Cr(V) intermediates formed in the reaction of Cr(VI) (10 mM) with $H_2O_2$ of different concentrations. All spectra were taken 10 min after the reaction. The reaction conditions and operating frequencies were as follows: (a) $[H_2O_2] = 50$ mM, pH = 8.17, $\nu = 9.6706$ GHz; (b) $[H_2O_2] = 100$ mM, pH = 8.05, $\nu = 9.6686$ GHz; (c) $[H_2O_2] = 150$ mM, pH = 7.98, $\nu = 9.6698$ GHz; (d) $[H_2O_2] = 200$ mM, pH = 7.85, $\nu = 9.6688$ GHz.

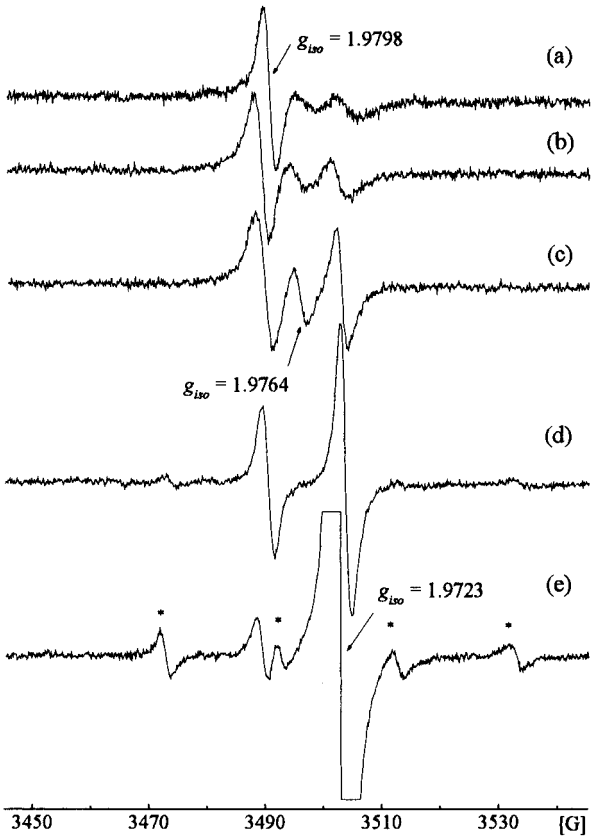
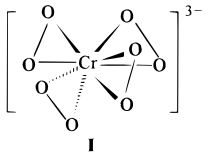



**Figure 3.** X-band EPR spectra of the Cr(V) intermediates generated in the reaction of Cr(VI) (10 mM) with $H_2O_2$ (200 mM) at different pH values. All spectra were collected 10 min after the reaction. The reaction conditions and operating frequencies were as follows: (a) pH = 6.09, $\nu = 9.6718$ GHz; (b) pH = 7.03, $\nu = 9.6681$ GHz; (c) pH = 7.85, $\nu = 9.6688$ GHz; (d) pH = 9.10, $\nu = 9.6724$ GHz; (e) pH = 9.92, $\nu = 9.6691$ GHz. The $^{53}$Cr hyperfine coupling signals for the $g_{iso} = 1.9723$ signal are marked by asterisks.

EPR signals were observed over a wide range of conditions and the distribution of the four species (mono-, di-, tri-, and tetraperoxo) is a function of the pH and the relative concentration of the reactants. It is possible that some Cr(V) peroxo dimer species are present, but these are likely to be EPR silent at room temperature. Chromium(IV) and Cr(III) peroxo complexes are also probable intermediates, although they will not be observed in the room-temperature EPR spectra.[26,31] There is also some evidence for the formation of polynuclear peroxo species of Cr(III), $[Cr(O_2)Cr]^{4+}$ and $[Cr(O_2)Cr(O_2)Cr]^{5+}$.[32] A detailed understanding of the Cr(VI)/$H_2O_2$ reaction is complicated by the catalytic decomposition of $H_2O_2$, temperature and reactant concentration variance, and the reaction media (e.g. buffer, solvent), etc., that were employed in various studies reported in the literature. The mechanisms are complicated further by the regeneration of the Cr(VI) reactants by dissociation of Cr(VI) peroxo complexes and/or the disproportionation of Cr(V) or Cr(IV) peroxo intermediates. The following discussion is restricted to the Cr(V) species, only one of which has been characterized previously, and is organized according to the species that predominate under certain conditions.

**Alkaline Solution, the $g_{iso} = 1.9723$ Signal.** In basic solution, in the presence of excess $H_2O_2$, the EPR signal at $g_{iso} = 1.9723$, with a $^{53}$Cr hyperfine value ($A_{iso}$) of $18.4 \times 10^{-4}$ $cm^{-1}$, is due to the well-characterized $[Cr(O_2)_4]^{3-}$ complex (**I**).[33]




**I**

Its presence at neutral pH has also been deduced from visible spectroscopy.[34] First crystallized by Riesenfeld et al.,[35,36] **I** has $\eta^2$-peroxo ligands arranged in an eight-coordinate dodecahedron.[37,38] Although its EPR signal is most stable in strong alkaline solution, it is formed over a wide range of pH values and an increase in the $[H_2O_2]$ favored this signal over those of the other species. Even though the complex is well-characterized, different workers have reported different $g_{iso}$ values for **I**.[14] Aiyar *et al.*[8] attributed a $g_{iso}$ value of 1.974 to **I** at 77 K, which was consistent with the value of Kawanishi *et al.*[7] ($g_{iso} = 1.9735$) obtained using higher concentrations of $Na_2CrO_4$ (40 mM) with 400 mM $H_2O_2$ in Tris-HCl buffer (pH 8.0). By

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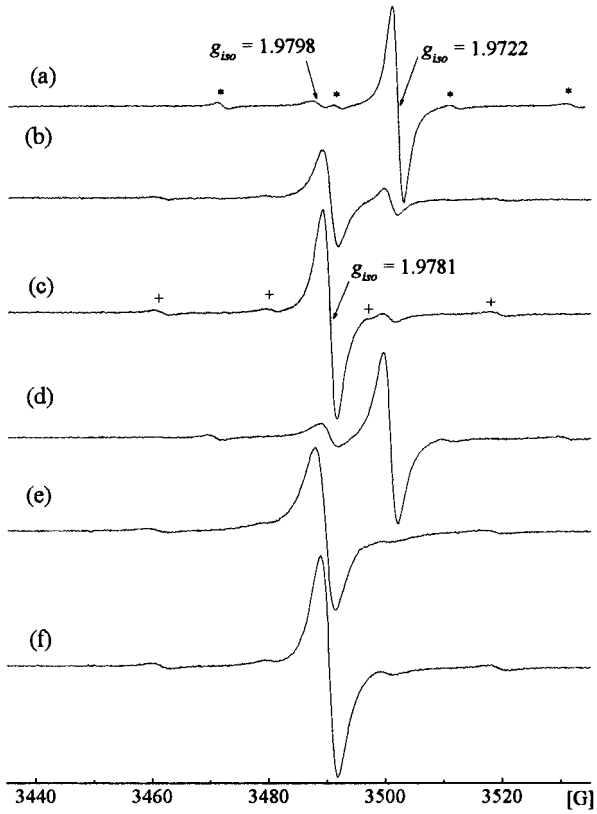


**Figure 4.** X-band EPR spectra of the Cr(V) intermediates formed in the reaction of Cr(VI) with $H_2O_2$ in Tris-HCl buffer: (a) [Cr(VI)] = 10 mM, [$H_2O_2$] = 200 mM, [Tris-HCl] = 50 mM, pH = 8.5, $t$ = 3 min, $\nu$ = 9.6685 GHz; (b) [Cr(VI)] = 20 mM, [$H_2O_2$] = 200 mM, [Tris-HCl] = 200 mM, pH = 7.0, $t$ = 3 min, $\nu$ = 9.6630 GHz; (c) same as (b), $t$ = 10 min, $\nu$ = 9.6630 GHz; (d) [Cr(VI)] = 20 mM, [$H_2O_2$] = 400 mM, [Tris-HCl] = 200 mM, pH = 7.0, $t$ = 3 min, $\nu$ = 9.6624 GHz; (e) [Cr(VI)] = 20 mM, [$H_2O_2$] = 400 mM, [Tris-HCl] = 200 mM, pH = 5.0, $t$ = 3 min, $\nu$ = 9.6612 GHz; (f) [Cr(VI)] = 20 mM, [$H_2O_2$] = 200 mM, [$CH_3C(CH_2OH)_3$] = 200 mM, pH = 6.5, $t$ = 3 min, $\nu$ = 9.6629 GHz. The $^{53}$Cr hyperfine coupling signals for the $g_{iso}$ = 1.9722 signal are marked by asterisks, and those for the $g_{iso}$ = 1.9781 signal, by plus signs.

**Table 1.** pH Dependence of the $g_{iso}$ Values for the Tetraperoxochromate(V) Complex

| pH | $g_{iso}$ | pH | $g_{iso}$ |
|---|---|---|---|
| 6−8 | 1.9724 | 10 | 1.9722 |
| 9 | 1.9723 | 11 | 1.9721 |

employment of the same concentrations of Cr(VI) and $H_2O_2$ in unbuffered aqueous solution, **I** was shown to coexist with at least two Cr(V) other species with signals at $g_{iso}$ = 1.9798 and 1.9764. Our reported $g_{iso}$ value for **I** is quite close to that reported in a recent publication ($g_{iso}$ = 1.9720).[12] The $g_{iso}$ value of this species shows little variation with changing pH values of the solution (Table 1). Parallel chemistry is observed with vanadium(V) reactions with $H_2O_2$ where $[V(O_2)_4]^{3-}$ is generated, although the mono-, bis-, and tris($\eta^2$-complexes) have been more extensively characterized.[39] It is this parallel between the V(V) and Cr(V) peroxo chemistry that will be used as an aid in characterizing the new Cr(V) species identified here (Table 2).

**Cr(V) Species in Acidic Solutions.** In acidic solutions, pH ≤ 2.0, addition of $H_2O_2$ to $VO_2^+$(aq) results in the formation of the red $VO(O_2)^+$(aq) and the yellow $V(O)(O_2)_2^-$(aq) complexes according to the equilibria in eqs 1 and 2. Thus, the

**Table 2.** Assignment of Likely Structures for the Cr(V) Complexes Formed in the Reaction of Cr(VI) with $H_2O_2$

| complex | $g_{iso}$ | $A_{iso}$ ($10^{-4}$ cm$^{-1}$) | complex | $g_{iso}$ | $A_{iso}$ ($10^{-4}$ cm$^{-1}$) |
|---|---|---|---|---|---|
| $[Cr(O)(O_2)(OH_2)_x]^+$ | 1.9820 | NA[a] | $[Cr(O_2)_3(OH)]^{2-}$ | 1.9764 | 18.1 |
| $[Cr(O)(O_2)_2(OH_2)]^-$ | 1.9798 | 16.3 | $[Cr(O_2)_4]^{3-}$ | 1.9723 | 18.4 |
| $[Cr(O)(O_2)(Tris)]^{2-}$ [b] | 1.9781 | 17.7 | | | |

[a] Not observed due to the low intensity of this signal. [b] A signal with the same parameters is observed in unbuffered solutions containing $CH_3C(CH_2OH)_3$.

$$VO_2^+(aq) + H_2O_2 \rightleftharpoons VO(O_2)^+(aq) + H_2O \qquad K_1 = 3.5 \times 10^4 \quad (1)$$

$$VO(O_2)^+(aq) + H_2O_2 \rightleftharpoons V(O)(O_2)_2^-(aq) + 2H^+ \qquad K_2 = 1.3 \quad (2)$$

diperoxo complex becomes favored in excess $H_2O_2$ at pH values of ≥2, which are the conditions studied in the Cr reactions. By analogy with the V system, $[Cr(O)(O_2)(OH_2)_n]^+$ is expected to be a minor species that should be more apparent at the lowest pH values and the lowest $H_2O_2$/Cr(V) ratios studied, whereas $[Cr(O)(O_2)_2(OH_2)]^-$ is expected to be the major species under the conditions used here at low pH values. The two EPR signals that match these criteria are those observed at $g_{iso}$ values of 1.9820 and 1.9798, which are assigned to $[Cr(O)(O_2)(OH_2)_n]^+$ and $[Cr(O)(O_2)_2(OH_2)]^-$, respectively. They also have pH and [$H_2O_2$] dependences that are consistent with eq 3; however, quantitative data are difficult to obtain because of the consumption of $H_2O_2$ in the reactions.

$$[Cr(O)(O_2)(OH_2)_n]^+ + H_2O_2 \rightleftharpoons [Cr(O)(O_2)_2(OH_2)]^- + 2H^+ \quad (3)$$

In acidic solution, the reaction is complicated by the condensation of $[CrO_4]^{2-}$ to form $[Cr_2O_7]^{2-}$ and the protonation of chromate to form hydrogen chromate, $[HCrO_4]^-$. The reaction of Cr(VI) with $H_2O_2$ in strongly acidified solution results in the rapid formation of blue perchromic acid. The latter quickly undergoes decomposition to Cr(III) in acidic solution (pH < 4) on standing, with the evolution of dioxygen.[40] It is accepted that the perchromic acid has a formula of $CrO_5 \cdot H_2O$ or $[Cr(O)(O_2)_2(OH_2)]$. This blue peroxo complex is unstable in aqueous solution and has not been isolated, but it can be stabilized in nonaqueous solvents. There are several complexes, e.g., $[CrO(O_2)_2(pyridine)]$[41] and $[CrO(O_2)_2(2,2'$-bipyridine)],[42] that have been characterized by X-ray diffraction. They are prepared by the addition of bases, such as pyridine or 2,2′-bipyridine, into a diethyl ether solution of $[CrO(O_2)_2]$. The $CrO_5 \cdot$pyridine complex exhibits a pentagonal pyramidal structure with pyridine occupying the equatorial position in the same plane of the two peroxo ligands.[43] This structure provides additional evidence for the formation of aquaoxobis($\eta^2$-peroxo)-chromium(VI).

When Cr(VI) is added to $H_2O_2$ solution in the pH range 4−7, a violet species is formed.[7,44] This Cr(VI)/peroxo species is thought to be the deprotonated form of peroxochromic acid, i.e., $[Cr^{VI}O(O_2)_2(OH)]^-$,[30] but is also unstable. Since Cr(V)

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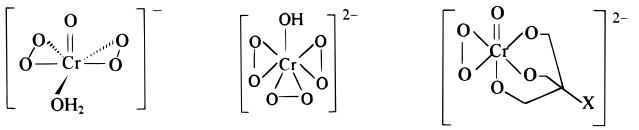
signals were observed under the same conditions, the species giving rise to the $g_{iso} = 1.9798$ signal possibly originated from the one-electron reduction of the peroxochromic acid, with the formation of the corresponding Cr(V) species, $[CrO(O_2)_2(OH_2)]^-$ (**II**). It is expected that the Cr(V) species at $g_{iso} = 1.9798$ may



**II** **III** **IVa, X = NH$_2$, IVb, X = CH$_3$**

have a coordination geometry similar to that of $[Cr(O)(O_2)_2$·pyridine].[43]

Because the remaining signal at $g_{iso} = 1.9764$ becomes more prominent with respect to the signal due to **II** but decreases in intensity with respect to that due to **I** as the $[H_2O_2]$ or pH increases, the results are consistent with equilibria outlined in eqs 4 and 5. The signal at $g_{iso} = 1.9764$ is assigned as being due to $[Cr(O_2)_3(OH)]^{2-}$ (**III**). This is expected to have a structure similar to that of $[V(O_2)_3(F)]^{2-}$, which has been characterized by X-ray crystallography.[45] Again, the consumption of $H_2O_2$ during the reactions makes quantitative determination of equilibrium constants difficult, but the dependences of the signals on pH and $[H_2O_2]$ are in semiquantitative agreement with eqs 4 and 5.

$$[Cr(O)(O_2)_2(OH_2)]^- + H_2O_2 \rightleftharpoons [Cr(O_2)_3(OH)]^{2-} + H_3O^+ \quad (4)$$

$$[Cr(O_2)_3(OH)]^{2-} + H_2O_2 \rightleftharpoons [Cr(O_2)_4]^{3-} + H_3O^+ \quad (5)$$

The $g_{iso} = 1.9781$ signal is not observed in the Cr(VI)/$H_2O_2$ reaction in unbuffered solution but is observed in the Tris-HCl buffer. In solution, it is in equilibrium with the tetrakis($\eta^2$-peroxo)chromate(V) complex. Since no Cr(V) EPR signals were observed when Cr(VI) was mixed with Tris-HCl alone in aqueous solution, this species was assigned to a mixed-ligand Cr(V)/peroxo/Tris complex (**IVa**) in which the three hydroxylate groups of Tris are coordinated to Cr(V). The less negative charge of **IVa** compared to **I** is consistent with the pH dependence of their signals. Neither the $-NH_2$ group nor $Cl^-$ present in Tris-HCl buffer is involved in the coordination, since a similar species with identical $g_{iso}$ and $A_{iso}$ values (**IVb**) was also formed in the Cr(VI)/$H_2O_2$ reaction in an unbuffered solution containing $CH_3C(CH_2OH)_3$.

**Genotoxic Effects.** The intermediates generated in the reaction of Cr(VI) with $H_2O_2$ are capable of inducing DNA cleavage.[7,8] Since $H_2O_2$ is also formed during the autoxidation of GSH in aerated solution, which is catalyzed by trace metal contaminants (including in the presence of Cr),[5] Cr/peroxo species generated in such Cr/GSH reactions have the potential to damage DNA in vitro. Kortenkamp *et al.*[46,47] found that the intermediates formed during the reduction of Cr(VI) by GSH caused DNA damage. However, by removing contaminating metal ions from all solutions, Aiyar *et al.*[8] could not observe DNA strand breaks in such reactions. They observed DNA strand breaks only for the reaction in the presence of $H_2O_2$.[9] Later, Kortenkamp *et al.*[48] found that the DNA strand breaks were mediated by reactive oxygen species. Either the exclusion of dioxygen or the addition of catalase into the reaction mixture minimizes the DNA strand breaks.[48,49] Recently, the Cr(V)/peroxo species were observed in the reaction of Cr(VI) with GSH and $H_2O_2$, but their presence in the reaction in the absence of added $H_2O_2$ is not certain.[50] On the basis of these observations, it is proposed that Cr(V)/peroxo complexes are also partly responsible for the in vitro DNA damage in the reaction of Cr(VI) with GSH in the presence of $H_2O_2$. By analogy with the Cr(V)/ascorbate system,[19] it is likely that the damage results directly from the Cr(V) peroxo species (and/or peroxo complexes in other oxidation states) rather than hydroxyl radicals. Hydroxy radicals are very high energy species and are unlikely to be produced in such reactions when two-electron pathways for the consumption of $H_2O_2$ are readily available in Cr redox chemistry.

It has also been found that the nature of the buffering agent can influence the extent of in vitro DNA strand breaks in such systems;[48,51] however, the reason for this has not been fully explored. The present study shows that the use of buffer can selectively favor and even stabilize the formation of certain species. At pH = 8.5, in unbuffered solutions, three species coexisted in the reaction of Cr(VI) with $H_2O_2$, while in Tris-HCl buffer, at pH = 8.5, the species at $g_{iso} = 1.9723$ predominated. Mixed peroxo/Tris complexes were also observed under some conditions. Chromium(V) complexes with Tris have been observed previously in other systems when this buffer has been used[19,52] or with the related 2,2-bis(hydroxymethyl)-2-[bis(2-hydroxyethyl)amino]ethanol buffer.[53]

The question remains as to the relevance of this in vitro chemistry to in vivo Cr genotoxicity. The concentration of $[H_2O_2]$ in cells is very low (typically nanomolar)[54] in cells due to the scavenging of $H_2O_2$ by enzymes. Therefore, the high reactivity of Cr(V)/peroxo species in causing in vitro DNA damage is tempered by the exceedingly low concentrations of such species that are expected to be present in vivo. Under these conditions, it is likely that other Cr(V) or Cr(IV) species may be responsible for genotoxic effects that ultimately lead to cancer.

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**Supporting Information Available:** Figure S1, showing the $[H_2O_2]$ dependences of the EPR spectra at pH 5 (1 page). Ordering information is given on any current masthead page.

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