Competition between 1,2-Diol and 2-Hydroxy Acid Coordination in Cr(V)-Quinic Acid Complexes: Implications for Stabilization of Cr(V) Intermediates of Relevance to Cr(VI)-Induced Carcinogenesis

Rachel Codd and Peter A. Lay*

Contribution from the School of Chemistry, University of Sydney, New South Wales 2006, Australia

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Abstract: For the speciation of Cr(V) intermediates formed during the intracellular reduction of Cr(VI) to be understood, the intramolecular competition between 1,2-diol and 2-hydroxy acid coordination to Cr(V) as a function of pH has been studied in quinic acid complexes. The Cr(V)-2-hydroxy acid complex, [K{Cr(O)-(qaH)2}2]H2O (qaH = 1R,3R,4R,5R-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, I), has been isolated and characterized. In aqueous solutions at pH values < 4.0, K[Cr(O)(qaH)2]H2O gives two EPR signals (giso = 1.9787, Aiso = 17.2 × 10^-4 cm^-1; giso = 1.9791, Aiso = 16.4 × 10^-4 cm^-1). The relative intensities of the signals are independent of [qaH]/[Cr(V)], and of increasing [qaH] and [Cr(V)] at constant [qaH]/[Cr(V)] and pH values. These signals are consistent with those found with well-characterized Cr(V)-2-hydroxy acid complexes and are assigned to two geometric isomers of the [Cr(O)(O1,1,qaqH2)]^- linkage isomer. Both the 2-hydroxy acid (O1,0,O2) and vic-diol (cis-O1,0,O2; trans-O1,0,O2) groups of qaH are viable Cr(V) donors. In the reduction of Cr(VI) by GSH in the presence of an excess of qaH3, the EPR spectra are similar to that of K[Cr(O)(qaH)2]H2O at low pH values (<4.0). At intermediate pH values (pH 5.0–7.5) additional signals appear (giso = 1.9791, giso = 1.9794, giso = 1.9799), which have EPR spectral data consistent with the presence of Cr(V)-qa linkage isomers, featuring one of each donor type (1 × 2-hydroxy acid; 1 × diol). By using EPR spectral simulation, we deduced that the cis-diol linkage isomer, [Cr(O)(O1,1,qaqH2)(O1,1,qaqH2)]^-; an isomer of magnitude more thermodynamically stable to intramolecular ligand exchange compared to the trans-diol linkage isomer, [Cr(O)(O1,1,qaqH2)(O4,1,qaqH2)]^-; at pH values >7.5, the Cr(V)-qa EPR spectra reveal two triplets (giso = 1.9800, giso = 1.9802), which are ascribed to geometric isomers of a bis-diol Cr(V)-qa complex, [Cr(O)(O1,1,qaqH2)2]^-; the concentration of the trans-diol isomer, [Cr(O)(O4,1,qaqH2)2]^-; is predicted to be negligible. This assignment is supported by the similarity of the EPR spectral data with those formed in the (Cr(VI)) reduction by GSH in the presence of the related polyol (cis-O1,0,O2; trans-O1,0,O2) ligand, shikimic acid (3R,4R,5R-3,4,5-trihydroxycyclohexanecarboxylic acid, II), which does not possess a 2-hydroxy acid moiety. The relative intensities of the EPR signals of the Cr(V)-qa species (giso = 1.9800, giso = 1.9801), ascribed to geometric isomers of [Cr(O)(O1,1,qaqH2)2]^-; are independent of increasing pH and of [qaH2] at pH values > 4.0. The results show that 2-hydroxy acid ligands are favored with respect to 1,2-diols for stabilizing Cr(V) at low pH values relevant to phagocytosis of insoluble chromates (pH ~4), but the opposite is the case when soluble chromates are taken up by the cells at pH ~7.4. Both classes of ligands compete effectively for complexation of Cr(V) compared to glutathione at all pH values studied.

Introduction

Occupational exposure to Cr(VI) in industries such as stainless steel welding and electroplating is of great concern, due to its known carcinogenicity toward humans. While it is undisputed that Cr(VI) is carcinogenic, there exists a healthy debate regarding the species most likely to be responsible for cellular damage and the mechanism(s) involved in genotoxic damage. Chromium(VI) itself is unable to react with DNA in vitro, but or with isolated nuclei, but in the presence of reducing agents, it causes a wide variety of DNA lesions, including Cr-DNA adducts, DNA-DNA cross-links, DNA-protein cross-links, and oxidative damage to proteins.

damage. Selected genotoxic effects of Cr(VI) observed in vivo include chromosomal aberrations and the formation of micro-nuclei, sister-chromatid exchanges, DNA strand breaks, and unscheduled DNA synthesis. Following the discovery of a long-lived EPR-active Cr(V) species, formed upon the reduction of Cr(VI) by microsomes in the presence of NADPH, attention became focused on the possible role(s) played by Cr(V) species in Cr(VI)-induced carcinogenesis. This led to the formation of the uptake-reduction model of Cr(VI)-induced carcinogenesis, which postulates that Cr(VI) enters the cell via the nonspecific anion-transport channels and is then reduced intracellularly, yielding species that are reactive toward genetic material. The reactive intermediates implicated include the following: Cr(VI) esters, Cr(V) or Cr(IV) species, and radical species (hydroxyl and thyl). Chromium(VI) may be reduced enzymatically or by small molecular weight redox-active molecules, such as ascorbate, glutathione (GSH), hydroxides, or nucleotides.

Extensive studies on the readily synthesized, relatively stable Cr(V)-2-hydroxy acid complexes, [Cr(O)(ehba)₃]⁻ (ehba = 2-ethyl-2-hydroxybutanoato(2⁻)) and [Cr(O)(hmba)₂]⁻ (hmba = 2-hydroxy-2-methylbutanoato(2⁻)), have illustrated that [Cr(O)(ehba)₃]⁻ induces cleavage of negatively supercoiled plasmid DNA (pUC9) and is mutagenic with V79 Chinese hamster lung cells in the micronucleus assay. The Cr(V)-2-hydroxy acid complexes are useful models for understanding the chemistry in vivo between Cr(V) and naturally occurring intracellular 2-hydroxy acids, such as, lactate and citric acids, and the oxidized forms of sugars (aldonic, aldaric, and uronic acids).

Several studies have also examined Cr(V)-diol speciation, which is important because Cr(V) species are formed by the Cr(VI) oxidation of ribonucleotides but not deoxyribonucleotide.
are diol groups (e.g., ascorbic acid, ribose, d-glucose, and derivatives) and 2-hydroxy acids (e.g., citric, malic, and lactic acids). These different functional groups can be mimicked by different regions of qaH₅, which has a tert-2-hydroxy acid moiety in addition to a cis-diol (O⁻⁻O⁺) and a trans-diol (O⁺⁻O⁻) group. All of these functional groups are potential chelates for Cr(V). Therefore, this ligand enables intramolecular competition experiments to be conducted with regard to different functional groups (tert-2-hydroxy acid versus vic-diol) and different orientations within the same functional group (cis- versus trans-diol). A thorough understanding of the signature EPR spectra of the individual complexes formed between Cr(V) and these model ligands is important in terms of providing a basis for the interpretation of likely Cr(V) complexes formed in vivo and has important implications with respect to the better understanding of Cr(VI)-induced carcinogenesis.

Experimental Section

Chemicals. Quinic acid (qaH₅, ICN Biomedicals), shikimic acid (saH₄, Sigma, 99%), glutathione (GSH, Aldrich, 96%), N,N'-dimethylformamide (DMF, Aldrich, 98%), ethylene glycol, K₂Cr₂O₇ (Merck, GR), Na₂Cr₂O₇·2H₂O (Merck, GR), Na(CH₃)₂AsO₂·H₂O (Aldrich, 98%), methanol (Ajax, AR Grade), acetic acid (BDH, AR grade), and dimethyl sulfoxide (DMSO, Sigma, 99.5%) were used as received. All aqueous solutions were prepared using distilled water.

Syntheses. Caution: Na₂Cr₂O₇ is carcinogenic, and Cr(V)-2-hydroxy acid complexes are mutagenic and potential carcinogens. These substances should be handled with due care, avoiding skin contact and inhalation of dust.

(A) K[Cr(O)(qaH₃)₂]·H₂O. Finely ground anhydrous K₂Cr₂O₇ (0.411 g, 1.40 mmol) was added to a solution of qaH₃ (1.609 g, 8.37 mmol) in methanol (500 mL), and the mixture was stirred for 2 h. The red-brown solution was filtered through a sintered-glass filter to remove unreacted K₂Cr₂O₇, and the volume of the filtrate was reduced to 85 mL via rotary evaporation (external bath <35 °C) at which point a finely divided red-brown powder appeared. The reaction solution was left at ~22 °C overnight. The red-brown product was filtered, and the solid was washed with diethyl ether (2 × 25 mL) under a nitrogen atmosphere. Yield: 0.241 g (18.3%). Anal. calc. for C₉H₉CrO₄: Cr, 33.27%; H, 4.39%; K, 10.29%; C, 7.74%. Found: C, 33.11%; H, 4.10%; Cr, 10.60%; K, 7.31%. FTIR (KBr matrix): 3300 (s, br), 2950 (w), 1684 (s), 1677 (s), 1444 (w), 1419 (w), 1336 (w), 1288 (m), 1263 (m), 1241 (m), 1157 (w), 1118 (m), 1070 (m), 1050 (m), 993 (s), 962 (w), 846 (m), 816 (m), 752 (m), 725 (w), 696 (m), 638 (m), 574 (s), 532 (w) cm⁻¹. 1H NMR (400 MHz, CD3OD): δ, ppm (J, Hz) 3.96 (m, 1 H). EPR Spectra of the Cr(VI)/GSH Reaction in the Presence of Excess qaH₅ or qaH₄, respectively. Solutions of Cr(VI) were prepared using an Activon pH meter (Model 210) with an Activon calomel pH probe (AEP 321). For small volumes (≤1 mL), the pH values were measured using a HANNA microcomputer pH meter (HI 9023) with a micro pH probe (HI 1083B).

EPR Spectra of K[Cr(O)(qaH₃)₂]·H₂O. Spectra were obtained from aqueous solutions of K[Cr(O)(qaH₃)₂]·H₂O, or from reaction solutions of Cr(V)–qa or –sa species generated by the reduction of Cr(VI) by GSH in the presence of excess qaH₅ or saH₄, respectively. Spectra were acquired at t = 4 min after mixing the solutions. Aqueous stock solutions (0.1, 0.2 M) of GSH were prepared at the start of each series of experiments and were kept on ice between use. For experiments requiring conditions of a constant pH value, the qaH₅ stock solution either was self-buffering (qaH₅–qaH₄, pH = 4.0) or was prepared in caducate buffer (pH 6.4), using NaOH for pH adjustment in both instances. The pH values of bulk solutions (10 mL) were measured using an Activon pH meter (Model 210) with an Activon calomel pH probe (AEP 321). For small volumes (≤1 mL), the pH values were measured using a HANNA microcomputer pH meter (HI 9023) with a micro pH probe (HI 1083B).

EPR Spectra of K[Cr(O)(qaH₃)₂]·H₂O. Spectra were obtained from aqueous solutions of K[Cr(O)(qaH₃)₂]·H₂O (pH 4.0) in the presence of excess qaH₅ (qaH₅–qaH₄/Cr(VI) = 2: 5, 20). Two series (pH 4.0 or 6.4) of EPR spectra were acquired from aqueous solutions of K[Cr(O)(qaH₃)₂]·H₂O in the presence of qaH₅, where the pH value and the [qaH₅]/[Cr(VI)] were kept constant: [qaH₅]/[Cr(VI)] = 2 (2: 1, 4: 2, 10: 5, 20: 10). The temperature dependence of the aqueous EPR spectra of K[Cr(O)(qaH₃)₂]·H₂O (2 mM) was examined at 12, 17, 23, 31, 40, or 46 °C.

EPR Spectra of the Cr(VI)/GSH Reaction in the Presence of Excess qaH₅ or saH₄. Spectra were obtained from solutions of Cr(VI), GSH, and qaH₅ or saH₄, where the final concentrations of reactants were 40, 2, and 100 mM, respectively. The pH values of the solutions were adjusted using stock NaOH solutions, prior to making the final reaction solution to volume. Although the system was buffered, the variation in the pH values was negligible during the aging of the solution or spectral acquisition. Spectra were obtained at the following pH values: 2.71, 4.40, 5.45, 6.84, 7.52, 8.35, or 9.92 (saH₄) and 2.45, 4.17, 5.08, 6.18, 7.28, 8.17, or 9.40 (qaH₅). EPR spectra were also obtained from aqueous solutions of Cr(VI), GSH, and saH₄ (pH = 3.0), where the final concentrations of reactants were 40, 2, and either 100, 250, 500, or 800 mM, respectively. Additional series of EPR spectra were acquired from solutions of Cr(VI), GSH and qaH₅ or qaH₄ where the [saH₄]/[Cr(VI)] or [qaH₅]/[Cr(VI)] was varied: [saH₄]/[Cr(VI)] = 2.5, 6.25, 12.5, 20 (pH 6.8) or [qaH₅]/[Cr(VI)] = 2.5, 10, 40, 80 (pH 6.9).

References:

(29) WINEPR; Version 921201; Bruker-Franzen Analytic GmbH: Bremen, 1996.
(50) WinSIM EPR Calculations for MS—Windows; Version 0.96: National Institute of Environmental Health Sciences, 1995.
Results

$\text{K[Cr(O)(qaH$_3$)$_2$]H$_2$O}$. The complex was isolated as a finely divided red-brown powder with a magnetic moment ($\mu_{\text{eff}} = 2.10 \mu_B$) indicating the presence of a single unpaired electron as for the Cr(V) ion ($d^1$). Consistent with Na[Cr(O)(hmba)$_2$] and Na[Cr(O)(ehba)$_2$] ($\mu_{\text{eff}} = 2.05 \mu_B$), the magnetic moment of K[Cr(O)(qaH$_3$)$_2$]H$_2$O, is slightly higher than the spin-only value. The FTIR spectrum of K[Cr(O)(qaH$_3$)$_2$]H$_2$O shows a strong, sharp peak at 993 cm$^{-1}$, characteristic of $\nu_{\text{Cr-O}}$ of Cr(V)-2-hydroxy acid complexes.$^{51}$ The electronic absorption spectrum of K[Cr(O)(qaH$_3$)$_2$]H$_2$O in DMSO is similar to that of Na[Cr(O)(hmba)$_2$]$_2$H$_2$O ($\lambda_{\text{max}} \sim 550$ nm) and also shows the definitive signature for Cr(V)-2-hydroxy acid complexes at $\lambda \sim 800$ nm ($\epsilon \sim 20$ M$^{-1}$ cm$^{-1}$).$^{51}$ Despite many attempts, crystals of K[Cr(O)(qaH$_3$)$_2$]H$_2$O suitable for X-ray structural analysis have not been obtained as yet. It is possible that the complex may exist in the solid state as more than one geometric isomer as has been observed for similar complexes in solution.$^{52,53}$ The XAFS structure of [Cr(O)(qaH$_3$)$_2$]$^-$ is also consistent with a bis(2-hydroxy acid) coordination mode.$^{30,54}$ The solid-state EPR spectra of the Cr(V)-2-hydroxy acid complexes, K[Cr(O)(qaH$_3$)$_2$]H$_2$O, Na[Cr(O)(hmba)$_2$]$_2$H$_2$O and Na[Cr(O)(ehba)$_2$]$_2$H$_2$O, exhibit a single broad signal (Figure 1, upper graphic) with comparable $g_{\text{iso}}$ values (Table 1). The signal line widths vary among the spectra, in the following order of highest to lowest: K[Cr(O)(qaH$_3$)$_2$]H$_2$O $>$ Na[Cr(O)(hmba)$_2$]$_2$H$_2$O $>$ Na[Cr(O)(ehba)$_2$]$_2$H$_2$O.

Solution EPR Spectra of [Cr(O)(L)$_2$]$^-$ (L = qaH$_3$, hmba, ehba) in Water. The central Cr signal of the EPR spectrum of an aqueous solution of K[Cr(O)(qaH$_3$)$_2$]H$_2$O shows two symmetrical signals with $g_{\text{iso}} = 1.9787$ and $g_{\text{iso}} = 1.9791$ (Figure 1, middle graphic). The EPR spectra of the structurally analogous complexes, Na[Cr(O)(hmba)$_2$]H$_2$O and Na[Cr(O)(ehba)$_2$]H$_2$O, show singlets ($g_{\text{iso}} = 1.9785$ and $g_{\text{iso}} = 1.9784$, respectively), which are unsymmetric in the second-derivative plots. The presence of more than one species in the aqueous EPR spectra of all the complexes is unambiguously established from the $^{53}$Cr-hyperfine satellite region, where two resolvable sets of signals are observed (Figure 1, lower graphic). The $A_{\text{iso}}$ values of the two resolved species are very similar among K[Cr(O)(qaH$_3$)$_2$]H$_2$O, Na[Cr(O)(hmba)$_2$]$_2$H$_2$O, and Na[Cr(O)(ehba)$_2$]$_2$H$_2$O (Table 1). The possibility of the multiple species in the case of Na[Cr(O)(hmba)$_2$]$_2$H$_2$O (hmba has a chiral carbon) arising from chiral species, Na[Cr(O)(R-hmba)$_2$]$_2$H$_2$O or Na[Cr(O)(S-hmba)$_2$]$_2$H$_2$O, is discounted, since very similar spectra are obtained from solutions of Na[Cr(O)(hmba)$_2$]$_2$H$_2$O and the achiral complex, Na[Cr(O)(ehba)$_2$]$_2$H$_2$O. The two signals observed in the aqueous solution EPR spectra of Na[Cr(O)(L)$_2$]$_2$H$_2$O (L = hmba, ehba) have been assigned as being due to geometric isomers,$^{52,53}$ and it is expected that geometric isomers are similarly observed for K[Cr(O)(qaH$_3$)$_2$]H$_2$O.

$\text{[qaH}_3]/[\text{Cr(V)}]$ Dependence on K[Cr(O)(qaH$_3$)$_2$]H$_2$O Speciation at pH 4.0. The possibility of the two signals in the aqueous EPR spectra of K[Cr(O)(qaH$_3$)$_2$]H$_2$O being due to mono-chelate and bis-chelate Cr(V)-qa species was addressed by examining the effect of [qaH$_3$] at constant pH (4.0 $\pm$ 0.1, Figure S1, Supporting Information). The independence of the

were due to mononuclear \([\text{Cr(O)(qaH}_3]^2^-\) and polymeric \([\text{Cr(O)(qaH}_3]^m\) complexes was eliminated.

Temperature Dependence of \(\text{K[Cr(O)(qaH}_3]^2\text{-H}_2\text{O Speciation in Water.}\) The intensity of the signal at \(g_{\text{iso}} = 1.9787\) increased relative to that at \(g_{\text{iso}} = 1.9791,\) with increasing temperature (Figure 2). The linear plot of \(\ln([g_{\text{iso}}(1.9787)]/[g_{\text{iso}}(1.9791)]) \text{ versus } 1/T\) (Figure S4) gave \(\Delta H^o\) and \(\Delta S^o\) values of 5.4 kJ mol\(^{-1}\) and 11.0 J K\(^{-1}\) mol\(^{-1}\), respectively. The small values of \(\Delta H^o\) and \(\Delta S^o\) suggest that the coordination groups of the two species are similar.

pH Dependence of Cr(V)-sa Speciation. The EPR spectra obtained upon the reduction of Cr(VI) by GSH in the presence of excess saH4 exhibited (where the pH value > 5.5) a triplet with \(g_{\text{iso}} = 1.9801\) and \(1H\) \(a_{\text{iso}} = 0.95 \times 10^{-4} \text{ cm}^{-1}\) (Figure 3). There is very little change in the spectra upon increasing the pH values from 6.84 to 9.92. The \(1^1\)Cr-hyperfine satellites are well resolved (Figure S5) with \(A_{\text{iso}} = 16.6 \times 10^{-4} \text{ cm}^{-1}\). The \(g_{\text{iso}}\) and \(A_{\text{iso}}\) values agree closely to the expected values (\(g_{\text{iso}} = 1.9800,\) \(A_{\text{iso}} = 16.5 \times 10^{-4} \text{ cm}^{-1}\)) for a five-coordinate oxide-Cr(V) species with four alcoholato donors.\(^{41}\) At pH values < 4.5, an additional broad signal is observed at \(g_{\text{iso}} \approx 1.9792.\) As the pH increases, the relative concentration of this signal decreases, compared to the signals at higher \(g_{\text{iso}}\) values. The second-derivative plots of the spectra of both the central Cr signal (Figure 3) and the \(1^3\)Cr-hyperfine region (Figure S5) show that the triplet signal (at pH values > 5.5) is slightly unsymmetric, which suggests the presence of more than one species. It is possible, for example, that the species giving rise to the triplet exists as more than one geometric isomer. The \(1^1\)H \(a_{\text{iso}}\) value (0.95 \times 10^{-4} \text{ cm}^{-1}) suggests that the dominant Cr(V)-sa species involves coordination to the \(\text{cis-diol (3,4-)}\) group of the ligand, by analogy with EPR spectral simulation of Cr(V)-cis-1,2-cyclohexanedion complexes (\(1^1\)H \(a_{\text{iso}} \approx -0.9 \times 10^{-4} \text{ cm}^{-1}\)).\(^{37}\) Since saH4 has two pairs of \(\text{vic-diol groups (3,4- and 4,5-)},\) the possibility of the presence of other linkage isomers cannot be discounted, although a somewhat more complicated EPR spectrum would be expected if alternative linkage isomers were present in significant concentrations.

<table>
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\(^a LW = \text{line width. } ^b A_{\text{iso}} = 10^{-4} \text{ cm}^{-1.} ^c \text{The } g_{\text{iso}} \text{ values and the line widths of the individual species are unable to be distinguished.}\)
Figure 4. Room temperature (~20 °C) X-band EPR spectra of the Cr(V) intermediates in the reaction of Cr(VI) (40 mM) with GSH (2 mM) in the presence of saH4 (100, 250, 500, or 800 mM), presented as (a) first- (expt and sim) and (b) second-derivative plots; ratio of [saH4]/[Cr(VI)] (pH) = 2.5 (2.94), 6.25 (2.97), 12.5 (2.97), or 20 (3.01).

Figure 5. Room temperature (~20 °C) X-band EPR spectra of the Cr(V) intermediates in the reaction of Cr(VI) (40 mM) with GSH (2 mM) in the presence of qaH (100 mM) at pH 2.45, 4.17, 5.08, 6.18, 6.91, 7.12, 8.17, or 9.40; presented as (a) first- (expt and sim) and (b) second-derivative plots.

The Cr(V)-sa spectra, centered at $g_{iso} = 1.9792$ (Figure 3), which was further investigated by varying [saH4]/[Cr(VI)] at constant pH (Figure 4). In this case, the intensity of the signal at the lower $g_{iso}$ value increases, relative to that of the signals at $g_{iso} = 1.9800$ and $g_{iso} = 1.9801$. Since the relative concentration of the signal at $g_{iso} = 1.9792$ increases with increasing [saH4] compared to the bis-chelate Cr(V)-sa species, the former signal must be due to species which have a ligand-to-metal ratio > 2.

Equilibrium Constants for Speciation of Cr(V)-sa Complexes. The spectra were simulated as two triplets, representing the purported geometric isomers of $[Cr(O)(O^2-O^4-saH_2)]^2$ and one doublet ($g_{iso} = 1.9792$) [H $g_{iso} = 0.82 \times 10^{-4}$ (cm$^{-1}$)]. The pH and [saH4] dependence of the Cr(V)-sa system fit the equilibrium shown in eq 1, where A = bis-chelate Cr(V)-sa species (trianionic) and B = tris-sa Cr(V) species (dianionic). The ligand concentration dependence study was conducted at pH values < pK$_a$ of saH4 (4.01),[5] and therefore, only a first-order dependence on [H$^+$] is expected.

$$A + saH_4 + H^+ \rightleftharpoons B; \quad K_1 = \frac{[B]}{[A][saH_4][H^+]}$$

A plot of [B]/([A][H$^+$]) versus [saH4] is linear with a slope of $K_1$ and an intercept close to the origin (Figure S7). The values of $K_1$ for the formation of the putative tris-sa Cr(V) species ($g_{iso} = 1.9792$) are on the order of 6 × 10$^3$ M$^{-2}$ (Table S1). Values for $K_1$ calculated at pH 2.71 from eq 1 were of a magnitude similar to that obtained above. It is possible that the putative tris-sa Cr(V) species exists as more than one geometric isomer, although for the simulation, the species was treated as a single isomer.

pH Dependence of Cr(V)-qa Speciation. At low pH values (2.45, 4.17), the EPR spectra of the Cr(VI)/GSH reaction in the presence of qaH3 (Figure 5, central signal, and Figure S8, $^{51}$Cr-hyperfine satellites) are the same as those observed from an aqueous solution of K[Cr(O)(qaH3)$_2$]$\cdot$H$_2$O. As the pH value increases, additional signals are observed at $g_{iso}$ values ~1.9794. At pH 9.40, a broad triplet appears at $g_{iso} = 1.9801$, similar to that observed for the Cr(V)-sa species. Concomitant with the appearance of new signals with increasing pH values, the relative intensity of the signal at $g_{iso} = 1.9787$ decreases. The signals appearing at intermediate pH values (between pH 5.1 and 7.3) may be due to bis-chelate Cr(V)-qa species with donation occurring via one 2-hydroxy acid group and one diol group. A donor set of this type would be expected to yield a Cr(V) species with a higher $g_{iso}$ value, compared to a bis(2-hydroxy acid) Cr(V)-species.[41] Additional minor signals appeared when the pH value was > 6 (Figure 5). These signals ($g_{iso} = 1.9751, 1.9762, 1.9768, and 1.9776$) are just perceptible at pH 6.18 and increase in intensity at pH 6.91. The minor signals are more clearly observed in the second-derivative plot of the $^{51}$Cr-hyperfine region of the EPR spectrum at pH 6.18 (Figure S8).

Ligand Exchange and Geometric Isomerization Equilibrium Constants in the Cr(VI)-qaH$_{1n}$ System. The spectra obtained at pH values between 2.45 and 7.28 were simulated as the linkage isomers $[Cr(O)(O^2,O^2-qaH_2)]^{-}, [Cr(O)(O^2-O^4-qaH_3)(O^2-O^4-qaH_2)]^2-$, and $[Cr(O)(O^2,O^2-qaH_3)(O^2,O^2-qaH_3)]^2-$ and two geometric isomers of each linkage isomer. At pH 8.17, these species were assumed to be present together with the bis-diol linkage isomer, $[Cr(O)(O^2,O^2-qaH_2)]^2-$ (as two geometric isomers). At pH 9.40 the spectrum was simulated as two triplets, representing the two geometric isomers of $[Cr(O)(O^2,O^2-qaH_2)]^2-$.
predominance of the \([\text{Cr}(O)(O^1, O^2\text{-qaH})_3]^-\) linkages isomer found in the Cr(V)-sa system. The EPR parameters of the species proposed for the Cr(V)-qa system are given in Table 2, and the relative concentrations of the species as a function of pH are shown in Figure 6. The equilibrium constants for the formation of the 2-hydroxy acid-diol Cr(V)-qa mixed-linkage isomers can be deduced from a plot of \([D]/[C]\) versus \([1/\text{H}^+]\) according to eq 2, where \(C = [\text{Cr}(O)(O^1, O^2\text{-qaH})_3]^-\) and \(D = [\text{Cr}(O)(O^1, O^2\text{-qaH})(O^4, O^5\text{-qaH})_2]^{1-}\) (two geometric isomers: \(D_1, D_2\)) or

\[
\begin{align*}
K_1 &= \frac{[E]/[D]}{[\text{H}^+]}, \\
K_2 &= \frac{[\text{H}^+]/[C]}{[\text{D}]}.
\end{align*}
\]

The slope of the line represents \(K_2\) and is on the order of \(10^{-7}\) and \(10^{-8}\) M for \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^4, O^5\text{-qaH})_2]^{1-}\) and \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^3, O^4\text{-qaH})_2]^{1-}\), respectively (Table 3). In both instances, the intercept is 0 within experimental error, as expected from such simple equilibria. The equilibrium constants, \(K_2\), for the formation of isomers of \([\text{Cr}(O)(O^1, O^2\text{-qaH})_2]^{1-}\) (eq 3) are on the order of \(10^{-8}\) M. These values, however, should be treated with caution, since the plot of \([E]/[D]\) versus \([1/\text{H}^+]\) only has two data points.

**Table 3. Values of the Equilibrium Constants, \(K_2\) for the Formation of Cr(V)-qa Linkage Isomers (Two Geometric Isomers per Linkage Isomer) from the Cr(VI)/GSH/qaH$_5$ Reaction**

<table>
<thead>
<tr>
<th>Species</th>
<th>(g_{iso})</th>
<th>(K_2) (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Cr}(O)(O^1, O^2\text{-qaH})_3]^-)</td>
<td>1.9787$^b$</td>
<td>1.2 \times 10^{-7}</td>
</tr>
<tr>
<td>([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^4, O^5\text{-qaH})_2]^{1-})</td>
<td>1.9791</td>
<td>2.1 \times 10^{-7}</td>
</tr>
<tr>
<td>([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^3, O^4\text{-qaH})_2]^{1-})</td>
<td>1.9794</td>
<td>4.6 \times 10^{-8}</td>
</tr>
</tbody>
</table>

**Figure 6.** Relative concentrations of Cr(V)-qa linkage isomers as determined from EPR spectral simulation,$^{36}$ generated from reactions of Cr(VI) with GSH in the presence of qaH$_5$ as a function of pH. Two geometric isomers have been included per linkage isomer. Legend: $\times$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})_3]^-\) \((g_{iso} = 1.9787)\); $\circ$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^4, O^5\text{-qaH})_2]^{1-}\) \((g_{iso} = 1.9791)\); $\Box$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^3, O^4\text{-qaH})_2]^{1-}\) \((g_{iso} = 1.9794)\); $\bullet$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^4, O^5\text{-qaH})_2]^{1-}\) \((g_{iso} = 1.9799)\); $\blacklozenge$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^3, O^4\text{-qaH})_2]^{1-}\) \((g_{iso} = 1.9800)\); $\square$ \([\text{Cr}(O)(O^1, O^2\text{-qaH})(O^3, O^4\text{-qaH})_2]^{1-}\) \((g_{iso} = 1.9802)\).

**Discussion**

Quinic acid features a tert-2-hydroxy acid group, and two pairs of vic-diol groups oriented in a cis-(3,4-) and trans-(4,5-) fashion. The energetically favored conformation of qaH$_5$ is the chair conformation (as shown), where the 1-OH group is in the axial position.$^{36}$ The conformation of shikimic acid is similar to that of qaH$_5$, with the difference being a single point of

unsaturation [C(1)−C(2)] in the cyclohexane ring. Stabilization of Cr(V) by saH₂ is most likely to involve the 3,4- and 4,5-diol regions of the molecule, although chelation via the carboxylato group is also possible.

K[Cr(O)(qaH₃)₂]·H₂O. The isolation of this complex and its aqueous chemistry has wide reaching implications for understanding competition between the coordination of cyclic diol (e.g., carbohydrates) and 2-hydroxy acid groups to transition metal ions in vivo. It has been proposed, on the basis of EPR spectra of Cr(V)-sugar complexes that, due to the bulk of carbohydrates, only mono-chelate Cr(V)-carbohydrate complexes can be formed.41,46,47 The isolation of K[Cr(O)(qaH₃)₂]·H₂O with the carbohydrate-like ligand, qaH₃, illustrates that this is not necessarily the case. Also, extensive EPR spectroscopic experiments of the species formed between Cr(V) and D-glucose have shown that bis-chelate Cr(V)-D-glucose complexes are readily formed in the presence of excess ligand.37,41 In addition to the 2-hydroxy acid coordination mode exhibited by qaH₅ in [Cr(O)(qaH₅)₂]⁻, the vic-diol groups of the ligand are also potential chelates, giving rise to six potential Cr(V)-qa linkage isomers (III–VIII). The mixed-valence trinuclear vanadium complex, (NH₄)₂[V₅(O)₂][VIV(O)](μ-qaH₃)·H₂O, for example, features I coordinated via both the 2-hydroxy acid moiety and the 3-OH group.57

[Cr(O)(qaH₃)₂]⁺ Speciation. The two EPR signals of K[Cr(O)(qaH₃)₂]·H₂O at giso = 1.9787 and giso = 1.9791 are due to different species of the bis-(2-hydroxy acid) linkage isomer. This is established by the pH, [Cr(V)] and [Cr(V)]/[qaH₅] dependencies, and the distinct Aiso values (Aiso (IIIa) = 17.2 × 10⁻⁴ cm⁻¹; Aiso (IIIb) = 16.4 × 10⁻⁴ cm⁻¹). The Aiso values are similar to those observed in the EPR spectra from aqueous solutions of Na[Cr(O)(ehba)₂]·H₂O (Aiso = 17.2 × 10⁻⁴ cm⁻¹; Aiso = 16.1 × 10⁻⁴ cm⁻¹) and Na[Cr(O)(hmba)₂]·H₂O (Aiso = 17.4 × 10⁻⁴ cm⁻¹; Aiso = 16.3 × 10⁻⁴ cm⁻¹), which have been assigned previously to the presence of more than one geometric isomer per Cr(V)-2-hydroxy acid complex.52,53 The presence of a maximum of three geometric isomers for Cr(V)-2-hydroxy acid complexes arises from the interchange of the alcoholato and/or the carboxylato donors about the distorted trigonal bipyramid.52 The possibility of an equilibrium existing between mononuclear and polymeric species, where coordination features qaH bridging two Cr(V) ions via the 2-hydroxy acid group (O₁, O₂) and either the 4,5- (O₁, O₂) (IX) or 3,4- (O₁, O₂) diol groups (X) were eliminated due to the EPR spectral invariance upon changing the concentrations of qaH and Cr(V) at a constant [qaH₃]/[Cr(V)] ratio and a constant pH value.

Linkage Isomerism in [Cr(O)(qaH₃)₂]⁺. The six different linkage isomers of [Cr(O)(qaH₃)₂]⁺, involving 2-hydroxy acid (O₁, O₂) and/or diol (O₁, O₂, O₃, O₄) chelation (III–VIII), would give rise to EPR signals with distinct giso and ¹H aiso values. The complexes featuring coordination to one 2-hydroxy acid and one diol group, or to two diol groups, are most likely to be dianionic and trianionic, respectively, since the uncoordinated carboxylic acid group would be deprotonated at the pH values studied. The Cr(V) and the isoelectronic V(IV) ions show a marked preference for binding to cis- rather than trans-diol groups of sugars and 1,2-cyclohexanediol,57,58,59 Also, the EPR spectral multiplicity of bis-chelate complexes formed between Cr(V) and either cis- or trans-cyclic-diol ligands would exhibit a triplet and singlet, respectively, arising from the orientation of the ring protons of the coordinated diol groups with respect to the basal plane of the complex.37 On the basis of this, the EPR spectra of [Cr(O)(O₁, O₂-qaH₃)₂]⁻ (III), [Cr(O)(O₁, O₂-qaH₃)-(O₁, O₄-qaH₃)]²⁻ (IV), and [Cr(O)(O₁, O₂-qaH₃)(O₃, O₄-qaH₃)]³⁻ (V) would exhibit a singlet, a doublet, and a singlet, respectively. Also, the concentration of V relative to IV would be expected to be small. As an alternative to the simulation of the EPR signals of K[Cr(O)(qaH₃)₂]·H₂O in water as two geometric isomers, a singlet (giso = 1.9787, rel. concn = 24.4%), a doublet (giso = 1.9788, ¹H aiso = 0.79 × 10⁻⁴ cm⁻¹, rel. concn = 59.8%) and a singlet (giso = 1.9790, rel. concn = 15.8%) could be used. In such an analysis, the giso values for the putative IV and V isomers are higher than that for III, which is to be expected on the basis of an empirically derived set of EPR parameters for specific donors.41 The trend in the giso values is also consistent with the higher giso value observed for [Cr(O)(ehba)(ed)]⁻ (ed = 1,2-ethanediolato(2−)) compared to [Cr(O)(ehba)₂]⁻.36 How-

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ever, such linkage isomerism for K\[Cr(O)(qaH 3 ) 2 \]âH 2 O at pH < 5.0 is not consistent with the experimental results, which would result in a pH dependence of the ratio of signals due to different degrees of ligand deprotonation in the linkage isomers. Therefore, the two signals at these low pH values must be due to geometric isomers. The relative concentrations of the geometric isomers of Na[Cr(O)(hmba)2 ]âH 2 O were estimated from the relative ratios of the high field 53 Cr-hyperfine satellite signals as 70:30 (A\_iso \((17.4 \times 10^{-4} \text{ cm}^{-1})\)
A\_iso \((16.3 \times 10^{-4} \text{ cm}^{-1})\)). The simulation of the central signal of the EPR spectrum of Na[Cr(O)(hmba)2 ]âH 2 O, using this ratio as a guide, “resolves” the broad central signal into two species with g\_iso \(1.9784\) (A\_iso \((17.4 \times 10^{-4} \text{ cm}^{-1})\)) and g\_iso \(1.9786\) (A\_iso \((16.3 \times 10^{-4} \text{ cm}^{-1})\)). Therefore, in the case of K[Cr(O)(qaH 3 ) 2 \]âH 2 O, the g\_iso values are separated by 0.0004 units compared to 0.0002 units for Na[Cr(O)(hmba)2 ]âH 2 O. In the latter case, the separation of the g\_iso values of the species is insufficient to effect an unambiguous resolution of the central EPR signal. This may be due to exchange broadening, and detailed studies at higher frequency (Q-band) would be required to establish whether the differences in g\_iso values have a kinetic or thermodynamic origin. The similarity in results obtained by the reduction of Cr(VI) by GSH in the presence of qaH 3 and the spectra of [Cr(O)-(qaH 3 ) 2 ] at low pH values shows that qaH 3 is an effective competitor for Cr(V), since Cr(V)-GSH complexes give distinct EPR signals (g\_iso \(1.996\), g\_iso \(1.986\)). The temperature dependence of the geometric isomers of [Cr(O)(qaH 3 ) 2 ] is similar to those of V(IV)-lactic or glycolic acid complexes. At near neutral pH values, two signals were observed in the EPR spectrum of an aqueous solution of V(IV) and lactic acid, which were found to be independent of the pH value and both the ligand and V(IV) concentrations. The ratio was found to vary, however, as a function of temperature. The study concluded that the most likely explanation for the two species was the presence of two geometric isomers. There are parallels between the V(IV)-lactic acid study and the current work in that the species with the higher g\_iso value has the lower A\_iso value. The similar trends in temperature dependence that exist between two systems, where in one case ((V(IV)-lactic acid) no linkage isomerism is possible, are consistent with other evidence for geometric isomerization.

At higher pH values (>5), new signals appear with 1H a\_iso values ranging between 0.80 \(\times\) \(10^{-4}\) and 0.95 \(\times\) \(10^{-4}\) cm\(^{-1}\), which is in good agreement with the 1H a\_iso values observed in the EPR spectra of complexes formed between Cr(V) and adenosine (0.75 \(\times\) \(10^{-4}\) cm\(^{-1}\)) or rhamnose (0.84 \(\times\) \(10^{-4}\) cm\(^{-1}\)), where complexation occurs via the cis-diol group of the sugar. Additional evidence in support of the linkage isomerism processes is the pH dependence of the equilibria and the similarity of the spectra of the high g\_iso value signals for geometric isomerization.
qaHs and the species generated for saH₄, which can only form 1,2-diolato complexes.

**Linkage Isomerism in [Cr(O)(saH₄)]³⁻.** The gᵢso and Aᵢso values of the saH₄ complexes are typical of five-coordinate oxo-Cr(V) species with four alcoholato donors.⁴¹ This ligand can form three linkage isomers with Cr(V), by virtue of the two pairs of vis-diol groups (XI–XIII). Since the ¹H Aᵢso value is similar to that observed for the bis-chelate species formed between Cr(V) and cis-1,2-cyclohexanediol,⁴⁷ the dominant Cr(V)-sa linkage isomer is also likely to feature cis-diol (3,4-) coordination (XI). The dominance of [Cr(O)(O⁺²-,saH₄)]⁵⁻ is an excellent example of the preference for Cr(V) to bind to cis-rather than trans-diol groups. This has been illustrated previously on several occasions for Cr(V),⁴³,⁴⁶ and also for V(IV)-diol binding.⁵⁹ Binding via the 4,5-diol group is evident in Cr(V)-qa species, which indicates that the donor properties of the 3,4-diol in saH₄ may be either sterically or electronically enhanced, compared to qaHs.

**Cis vs Trans Binding in Cr(V)-Diol Complexes and Deconvolution of EPR Spectra.** In the Cr(V)-qa system, the concentration of IV would be expected to be greater than that of V, based on the intramolecular competition experiments using saH₄, which established a clear preference for cis-rather than trans-diol binding to Cr(V). This is borne out by the equilibrium constant for the formation of IV being 10 times the value for V. The equilibrium constant for the formation of the bis-chelate complex formed between V and cis-1,2-cyclohexanediol is also 10 times greater than that for the trans-analogue.⁶¹ The marked dominance of the binding of Cr(V) to cis-rather than trans-diol groups is easily rationalized, since the torsion angle of the chelate ring (O–C–C–O) in a cis-geometry can accommodate a reduction in the angle size (≤60°), which facilitates coordination. The same angle in a trans-diol arrangement cannot be less than 60°. This affect has been described in relation to the coordination of the isoelectronic V(IV) ion with sugars.⁵⁹ The linkage isomers, IV, V, and VI, would be predicted to yield a doublet, a singlet, and a triplet, respectively, where the EPR spectral multiplicity is a function of the number and orientation (cis- or trans-) of the ring protons of the coordinated diol group. In accordance with the preference shown by Cr(V) toward cis-rather than trans-diol binding, the concentration of the alternative bis-diol linkage isomers, VII and VIII, would be expected to be small, compared to VI. The presence of VII or VIII is not required to obtain a good fit between the observed and simulated EPR spectra.

At pH values between 5.0 and 8.0, the predominant Cr(V)-qa linkage isomers feature mixed-binding modes, with 2-hydroxy acid and diol donors. The Cr(V)-qa EPR spectra have been deconvoluted into the spectra predicted for the individual linkage isomers (Figure 7). Notably, the gᵢso values of the purported geometric isomers of XI differ only by 0.0001 units. The differences in the gᵢso values (Δgᵢso) of the purported geometric isomers for each Cr(V)-qa linkage isomer were 0.0004 for III, 0.0003 for IV, and 0.0005 for V. These differences in gᵢso values are more significant than that noted for the geometric isomers of VI (0.0002). This indicates that the changes to the electronic structure of geometric isomers of Cr(V) complexes with mixed-donor types (i.e., bis-(2-hydroxy acid), 2-hydroxy acid-diol) are greater than for the case where the donor types are similar (i.e., bis-diol).

**Minor Species at Low gᵢso Values.** The formation of the additional signal at gᵢso ~1.9792 in the Cr(VI)/GSH/saH₄ reaction is pH-dependent, as opposed to the formation of the bis-diol Cr(V)-sa species, which is invariant of pH (at pH values >5.5). The gᵢso value is too high to support the presence of six-coordinate oxo-Cr(V) complexes.⁵¹ The signal is broad, with a discernible shoulder, which was simulated as having ¹H-superhyperfine coupling arising from diol coordination. An oxo-

![Figure 7](image-url)
Cr(V) complex featuring one diol group and two monodentate carboxylato donors, \([\text{Cr(OO)}(\text{OH}-\text{saH}_4)_2(O^2-\text{saH})]^2-\) (XIV), is consistent with the dependence (relative to the bis complexes) of the signal on both pH (at pH values \(<4.01\)) and ligand concentration (at pH values \(\sim3.8\)). Monodentate carboxylato coordination has been observed previously in species formed between Cr(V) and acetic acid.\(^{62,63}\) It should be noted that the formation of XIV from XI is dependent upon the \(pK_a\) value of saH\(_4\). If saH\(_4\) was deprotonated (i.e., at pH values \(>4.01\)), an alternative structure XV would also be consistent with the ligand concentration dependence data. The latter structure gives good agreement between the observed and calculated\(^{41}\) \(g_{\text{iso}}\) values (1.9792 and 1.9791, respectively). The formation of XV, however, would be independent of pH (at pH values \(<pK_a\) value of saH\(_4\), which is not the case here.

The minor signals produced upon the reduction of Cr(VI) by GSH in the presence of excess qaH\(_2\)s at high pH values (pH 6.18 and 6.91) are not observed in the Cr(VI)/GSH/saH\(_4\) reaction. Therefore, it is unlikely that the species solely feature ancillary donors, such as aqua or hydroxo groups. Similar signals were observed in Cr(V)-d-glucose studies and were attributed to Cr(V) species formed with glucose oxidation products.\(^{37}\) It is possible that the minor Cr(V)-qa species contain oxidized ligand, since the same oxidation of saH\(_4\) at C(1) is not possible due to the unsaturation in the cyclohexane ring. Coordination to oxidized ligand, however, is inconsistent with the fact that Cr(VI) is a stronger oxidant at acidic pH values.\(^{24}\) The relative concentrations of these minor signals are independent of ligand concentration, which is consistent with the responsible species being a bis-chelate, but the low \(g_{\text{iso}}\) values of the species suggest the presence of six-coordinate oxo-Cr(V) species.\(^{51}\) Possible six-coordinate species may feature triol binding via either the 1,3,4- (XVI), 1,3,5- (XVII), or 1,4,5- (XVIII) triol groups. These binding modes would not be observed between Cr(V) and saH\(_4\), since the ligand does not have a 1-OH group. The calculated \(g_{\text{iso}}\) value for a six-coordinate oxo-Cr(V) species with five alcoholato donors (\(g_{\text{iso}} = 1.9759\)) is in reasonable agreement with the observed \(g_{\text{iso}}\) values. The high negative charges of these species are also consistent with their appearance at high pH values.

The possibility of 3,4,5-triol binding is unlikely because the model of this structure is quite strained.\(^{64}\) Also, the signals at low \(g_{\text{iso}}\) values are not observed in the saH\(_4\) system, in which the 3,4,5-triol coordination mode is also possible. On the basis of molecular models, the 1,3,4-triol bound Cr(V)-qa complex (XVI) is the least strained.\(^{64}\) The ligand in this structure is in a half-boat conformation and has one five-membered and one six-membered chelate ring. Although in the 1,3,5-triol Cr(V) complex (XVII) the ligand remains in the energetically favored conformation, the structure is somewhat strained, most probably due to the presence of two six-membered chelate rings. Of the three structures, XVIII is the most strained and is quite unlikely to be formed in significant amounts.

1\(^H\)-Superhyperfine Coupling. The lack of structural data for Cr(V)-dil complexes can be addressed by examining analogous structures formed between V(V) and cyclic diols. Recently, the structures of two V(V) complexes with sec-cis-diol ligands have been solved by X-ray crystallography.\(^{65,66}\) The complexes formed between V(V) and either methyl-0-4,6-benzylidene-α-d-mannopyranoside (MBMP)\(^{65}\) or adenosine\(^{66}\) are dinuclear, with the V(V) ions bridged by an alcoholato donor from the sugar ring. Complexes between V(V) and the 2-hydroxy acids, hmbaH\(_2\) and ehbaH\(_2\), also have this structural motif.\(^{67,68}\) The distorted-TBP coordination sphere of V(V)-ehba and -hmba dinuclear complexes is similar to that of the mononuclear Cr(V) complexes with the same ligands.\(^{28,69}\) Therefore, it is reasonable to assume that complexes formed between either V(V) or Cr(V) and cyclic diols would have similar structures. Two analyses have been used to assess the spatial relationship between the ring protons of the coordinated diol groups and the V(V) nuclei. First, the distance between the proton and the ligand plane has been determined, where the ligand plane is defined as the plane containing the donating O atoms (2), the second-shell C atoms (2), and the V(V) atom. Second, the dihedral angle, H–C–O–V, has been calculated. These results (Table S2; refer to V(V) and Cu(II) structures for clarification of the atom labeling schemes) show that, in complexes formed between V(V) and cis-cyclical diols, one proton essentially lies in the ligand plane, while the other proton lies perpendicular to the ligand plane. Therefore, the two protons of cis-cyclical diols are oriented quite differently with respect to the metal ion and would experience different degrees of orbital overlap with the metal orbital containing the unpaired electron. In the case of trans-diol binding, the ring-protons lie above and below the ligand plane, resulting in limited overlap. This scenario is well illustrated by the X-ray crystal structure of the polymeric complex formed between Cu(H) and qaH, in which the ligand is coordinated via the 2-hydroxy acid (\(O^1, O^3\)).

and the \textit{trans}-diol ($O^4, O^5$) region. As predicted, the ring protons lie well above [H(5i)] and below [H(4i)] the ligand plane, which is similarly reflected in the dihedral $H-C-O-Cu$ angles (Table S2). Recent studies of the EPR spectra of species formed between Cr(V) and \textit{cis}- or \textit{trans}-1,2-cyclohexanediol have shown that the proton lying in the ligand plane couples to a greater extent with the unpaired electron on the Cr(V) ion, compared to the proton lying perpendicular to the ligand plane. This seems reasonable since the overlap between the proton $s$ orbital and the Cr(V) orbitals containing the unpaired electron density will be maximized when the $H-C-O-Cr$ dihedral angle is closer to 0°, as in \textit{cis}-cyclic diols, compared to \textit{trans}-cyclic diols, where the same angle approaches 90° (Table S2). It is for these reasons that the EPR spectrum of the species formed between Cr(V) and saH$_4$ yields a triplet rather than a quintet, since only one proton per ligand is in the plane of the unpaired electron density of the Cr(V) ion. In contrast to cyclic-diol ligands, the protons of linear diols are magnetically equivalent due to rapid Berry twists, and therefore, the spectral multiplicity is a function of the number of protons and the rate of the fluxional behavior, as revealed from ENDOR spectroscopic studies at low temperature.

\textbf{Conclusions}

There is no doubt that small-molecule reductants, such as ascorbate and GSH, play an important role in intracellular Cr(VI) metabolism. What has been over-looked, perhaps due to the unfavorable kinetics of Cr(VI) reduction by alkoxide-containing molecules, is the importance of Cr(V)-diol species with respect to Cr(VI) metabolism. The results obtained here clearly establish that the reduction of Cr(VI) by GSH, in the presence of an excess of diol-containing ligands, yield long-lived EPR-active Cr(V)-diol species at physiological pH values. The nature of the Cr(V)-diol species found here is also consistent with those observed in the ligand-exchange reaction between Na[Cr(O)(ehba)$_2$]$\cdot$H$_2$O and D-glucose, where there is a marked


preference for coordination to donors disposed in a cis rather than a trans fashion. At pH values <4, the 2-hydroxy acid mode of binding between Cr(V) and qaH\textsubscript{5} predominates, which has relevance in terms of the local acidic cellular environment following phagocytosis. During phagocytosis, the vesicles experience a drastic decrease in pH. Over a 20 min period, for example, the pH value of a phagocytic cell dropped from 6.70 (t = 2 min) to <5 (t = 22.5 min).\textsuperscript{58} This has important implications with respect to the cellular uptake of water-insoluble carcinogens and has particular relevance to intracellular Cr(VI) metabolism on two accounts.\textsuperscript{73} First, the redox potential of Cr(VI) is higher at acidic pH values than at neutral pH. Second, Cr(V)-2-hydroxy acid complexes, which have been implicated as possible intermediates in Cr(VI)-induced carcinogenesis, are considerably more stable in this pH region, compared to near neutral pH values.\textsuperscript{51} By contrast, the diol-binding mode is preferred at normal physiological pH values (\textsim\textnum{7.4}) relevant to the intra- and intercellular environments encountered by soluble Cr(VI) compounds. The Cr(V)-diol species are remarkably stable, with increases in the EPR signal (at pH 6.91) observed at 1 h after the initiation of the reaction (data not shown). The relevance of Cr(V)-diol complexes with respect to Cr(VI) metabolism is highlighted by the results of a recent in vivo EPR study of the Cr(V) species formed in rats,\textsuperscript{40} where the EPR signals have $g_{\text{iso}}$ and $^{1}{H}_{\text{iso}}$ values very similar to those of the species described in the current work and are likely to be bis-chelate Cr(V)-diol complexes. It is becoming more apparent that diol ligands play an important role in the stabilization of Cr(V) species. It may be more appropriate to think of the small-molecule Cr(VI) reducing agents, such as GSH and ascorbate, as detoxifying agents, where the ultimate genotoxic agents are species formed between Cr(V) and sugars or sugar-like molecules. Indeed, ascorbate has been used as a Cr(VI)-detoxifying agent in skin preparations (1% ascorbic acid in poly(ethylene glycol)) for sufferers of Cr(VI)-induced dermatitis and in the protective masks of workers exposed to chromic acid mists.\textsuperscript{24}

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**Supporting Information Available:** Values of $K_{1}$, for the Cr(VI)/GSH/qaH\textsubscript{5} reaction and of selected molecular geometry parameters of V(V)- or Cu(II)-cyclic diol complexes (with structures) are presented in Table S1 and Table S2, respectively. The EPR spectra of the Cr(VI)/GSH/qaH\textsubscript{5} reaction as a function of [qaH\textsubscript{5}] at pH 4.0 (Figure S1), at pH 6.4 (Figure S3), and at pH 4.0, where [qaH\textsubscript{5}]/[Cr(VI)] is constant (Figure S2), are included. The plot of ln $K$ vs 1/$T$ for the aqueous speciation of $[\text{Cr(O)(qaH}_{3})_{2}^{-}]$ is given in Figure S4. The $^{53}$Cr hyperfine signals in the Cr(VI)/GSH/qaH\textsubscript{5} reaction (pH 6.84) or the Cr(VI)/GSH/qaH\textsubscript{5} reaction (pH 4.17 and 6.18) are given in Figures S5 and S8, respectively. The EPR spectra of the [saH\textsubscript{4}] dependence of the Cr(VI)/GSH/qaH\textsubscript{5} reaction (pH 6.80) and the plot of [D]/[C] vs [saH\textsubscript{4}] (pH 3.0) are given in Figures S6 and S7, respectively. The plot of [D]/[C] vs 1/[H\textsuperscript{+}] for the Cr(VI)/GSH/qaH\textsubscript{5} reaction is given in Figure S9. The EPR spectra of the Cr(V) intermediates in the Cr(VI)/GSH/qaH\textsubscript{5} reaction with increasing [qaH\textsubscript{5}]/[Cr(VI)] at pH 6.90 are presented in Figure S10. This material is available free of charge via the Internet at http://pubs.acs.org.

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