Stability and Ligand Exchange Reactions of Chromium(IV) Carboxylato Complexes in Aqueous Solutions[1]

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The quantitative *in situ* generation of a range of Cr(IV) carboxylato complexes in aqueous media has been achieved by a combination of the newly-developed Cr(IV) ligand exchange chemistry together with the existing methods of reduction of Cr(VI) or Cr(V) complexes. The reactions Cr(VI) + As(III) and Cr(V) + V(IV) in buffer solutions of the corresponding ligands were used for generation of Cr(IV) complexes with 2-hydroxy-2-methylbutanoate (hmba), 2-ethyl-2-hydroxybutanoate (ehba), and (−)-quinate (qa) ligands. Addition of oxalate (ox), malonate (mal), or 2-picolinate (pic) to the generated Cr(IV) complexes led to the quantitative formation of the new Cr(IV) species. Spectral and chemical properties of Cr(IV) complexes with the mentioned ligands have been described for the first time (except for the known Cr(IV)−ehba complexes). In excess ligand, Cr(IV) appears to exist mainly as bis-chelated oxo complexes, on the basis of UV−visible and CD spectroscopic data. All of the studied Cr(IV) complexes exhibit bell-shaped pH dependences of their stabilities. The regions of maximum stabilities and maximal half-lives ($[Cr(IV)]_0 = 0.1$ mM; 25 °C) are as follows: pH ∼ 3 and ∼30 min for Cr(IV)−hmba and Cr(IV)−ehba; pH ∼ 5 and ∼1.5 h for Cr(IV)−ox; pH ∼ 5 and ∼1.5 min for Cr(IV)−mal; pH ∼ 5 and ∼20 min for Cr(IV)−pic; pH ∼ 6 and ∼1 h for Cr(IV)−qa. The stabilities of Cr(IV) complexes have been compared with those of the corresponding Cr(V) complexes (studied by EPR spectroscopy). The results are discussed in terms of the possible roles of Cr(IV) and Cr(V) complexes as the DNA-damaging agents in chromium-induced genotoxicities.

Introduction

It was claimed only a decade ago, that "Cr(IV) does not have any aqueous solution chemistry", since the known Cr(IV) compounds (such as alkoxo and porphyrinato complexes) are generally unstable in aqueous media.[2] One family of Cr(IV) compounds, which are relatively stable in water solutions (several hours at pH = 2−6), are the diperoxo triamines $[Cr^{IV}(O_2)_2(NR_3)_3]$.[3] However, the reactivity of such complexes is determined by the presence of the peroxo groups more than by the Cr(IV) center.[4]

Recently, Gould and co-workers[3] showed that Cr(IV)−ehba complexes can be stabilized in aqueous media by the one-electron reduction of the well-characterized $[Cr^{V}O(ehba)_2]^-$ complex[5,6] with reductants, such as V(IV).[7] However, the reactions of aqueous Cr(VI), in the presence of excess ehba, with two-electron reductants, such as As(III), are more convenient.[4] For both types of processes, slightly acidic media (pH = 2−4) are essential. The Cr(IV)−ehba complexes are relatively stable at these pH values but quickly disproportionate to Cr(V) and Cr(III) complexes at higher pH values.[8]

Two other types of Cr(IV) complexes were reported to form in aqueous media. The generation and reactivity of a Cr(IV) aqua complex (presumably $[Cr^{IV}O(OH_2)_5]^{2+}$) have been investigated in strongly acidic media ($\tau_{1/2} \sim 30$ s in 1 M $HClO_4$, 25 °C),[9] and a Cr(IV)−glutathione intermediate was postulated during the reduction of Cr(VI) by glutathione in slightly acidic (pH = 2−4) solutions.[10] Thus, the known aqueous Cr(IV) chemistry is limited to acidic media (pH ≤ 4). However, the growing interest in possible roles of Cr(IV) intermediates in the mutagenic and carcinogenic properties of Cr compounds[11−13] (the mutagenicity of the other "intermediate" oxidation state, Cr(V), is now well established)[14,15] requires studies over the physiological range of pH values (4.5−7.4).[16]

Previous studies of the reactivity of Cr(IV) complexes in aqueous solutions were devoted mainly to their redox reactions in acidic media.[3,9,17,18] However, very little is known about Cr(IV) ligand exchange chemistry. The existence of equilibria between Cr(IV) complexes with one, two, or three ehba ligands[17] was recently disputed.[18] The possibility of ligand exchange

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(1) The following abbreviations for the ligands were used in this work: hmba = 2-hydroxy-2-methylbutanoate(2−); edta = ethylenediaminetetraacetate(4−); ehba = 2-ethyl-2-hydroxybutanoate(2−); mal = malonate (propanedioate(2−)); ox = oxalate (ethanedioate(2−)); pic = picolinate (2-pyridinecarboxylate(−)); qa = (−)-quinate ((1*R*,3*R*,4*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylate(2−)); Lig = any of the above-mentioned ligands.

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equilibria involving the Cr(IV)−ehba complexes and oxalate has been briefly mentioned.[19]

This work is aimed primarily toward the investigation of Cr(IV) ligand exchange reactions in order to generate new types of relatively stable Cr(IV) complexes under biologically-relevant conditions. In addition, the Cr(IV)−ehba complexes were reinvestigated to clarify the conflicting reports on the natures of these species.[17,18]

Experimental Section

Caution. *As(III) and Cr(VI) compounds are human carcinogens,*[20] *and Cr(V) complexes are mutagenic and potentially carcinogenic.*[14,15] *Contact with skin and inhalation must be avoided.*

Reagents. The following were used in the preparations of the reaction solutions: acetic acid, D,L-atrolactic (2-hydroxy-2-methylbenzeneacetic) acid hemihydrate, arsenic(III) oxide, *cis*- and *trans*-(1*S*,2*S*)- and *trans*-(1*R*,2*R*)-1,2-cyclohexanediols, glycine, 2-ethyl-2-hydroxybutanoic acid, 2-hydroxy-2-methylbutanoic acid, malonic (propanedioic) acid, and salicylic (2-hydroxybenzoic) acid sodium salt (all Aldrich); picolinic (2-pyridinecarboxylic) acid and oxalic (ethanedioic) acid dihydrate (Fluka); (−)-quinic ((1*R*,3*R*,4*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylic) acid (ICN Biomedical); ethylenediaminetetraacetic acid disodium salt, D-glucose, D-gluconic acid sodium salt, pyruvic (2-oxopropanoic) acid sodium salt, $Na_2CrO_4 \cdot 4H_2O$, $VOSO_4 \cdot 5H_2O$, $NaVO_3$, $NaHSO_3$, $NaClO_4 \cdot H_2O$, NaOH, and $HClO_4$ (all Merck); d,L-lactic (2-hydroxypropanoic) acid lithium salt and L-proline (Sigma); and Milli-Q water. All commercial reagents were of analytical grade and were used without further purification.[21] $M[Cr^{V}O(Lig)_2] \cdot H_2O$ (M = Na, K; Lig = ehba, hmba, qa), $Na[Cr^{III}(Cys)_2] \cdot 2H_2O$ (Cys = L-cysteine), and $(NH_4)_2[V^{IV}O(OH_2)(ox)_2] \cdot H_2O$ complexes were synthesized by known procedures.[5,22−24] Solutions of As(III) were prepared by dissolving As_2O_3 in NaOH solutions (molar ratio As(III):NaOH = 1:3). Solutions of V(IV), prepared by dissolving $VOSO_4 \cdot 5H_2O$ in 0.1 M $HClO_4$, were standardized by permanganatometric titration[25] and stored under argon. Buffer solutions of the ligands, containing As(III) and 1 M $NaClO_4$, were passed through a column of Dowex A-1 chelating resin (Fluka) to ensure the absence of traces of heavy metals.

Cr(IV) Generation Studies. The reactions, Cr(VI) + As(III) and Cr(V) + V(IV), were followed using an Applied Photophysics SX-17 MV stopped-flow spectrophotometer with a photodiode-array (PDA) detector at λ = 350−750 nm; resolution ∼1 nm. Typically, 250 time-dependent spectra were collected during the reaction (logarithmic time base; integration time 2.56 ms). The reactions Cr(V) + V(IV) were also studied by observing absorbance changes at 560 nm (since V(V) products do not absorb at this wavelength; see Results), using the SX-17 MV with photomultiplier (PM) detector. For the single-wavelength studies, kinetic curves consisted of 1000 points in logarithmic time base. Typical conditions were as follows: (a) for Cr(VI) + As(III) reactions, $[Cr(VI)]_0$ = 0.1 mM, $[As(III)]_0$ = 5 mM, [Lig] = 100 mM, pH = 2.5−4.5, reaction time 1000 s; (b) for Cr(V) (excess) + V(IV) reactions, $[Cr(V)]_0$ = 1 mM, $[V(IV)]_0$ = 0.02−0.2 mM, [Lig] = 10−200 mM, pH = 3.5, reaction time 20 s; and (c) for Cr(V) + V(IV) (excess) reactions, $[Cr(V)]_0$ = 0.1 mM, $[V(IV)]_0$ = 2 mM, other conditions as for (b). The reactions (a)-(c) were carried out at 25 ± 0.1 °C in solutions containing 1 M $NaClO_4$. Since V(IV) is easily oxidized by aerial oxygen, all Cr(V) + V(IV) reactions were carried out under an Ar atmosphere. No such precautions were taken for the reactions Cr(VI) + As(III) because the kinetic parameters were independent of $[O_2]$. The time-dependent spectra, collected by the PDA detector, were processed using the global analysis (Glint)[26] software, as described previously.[27] The kinetic curves collected by the PM detector were processed using the nonlinear least-squares algorithm within the SX-17 MV software.

Equilibrium Studies. Typical conditions for studies of the ligand exchange equilibria were as follows: $[Cr(IV)]_0$ = 0.1 mM (generated by the reaction of 0.1 mM Cr(VI) with 5 mM As(III));[28] [Lig] = 0−200 mM; $[NaClO_4]$ = 1 M; pH = 2.5−6.0;[29] and 25 ± 0.1 °C. Spectral changes were observed using an HP 8452 A diode-array spectrophotometer (λ = 250−800 nm; resolution 2 nm; integration time 0.2 s). In most cases, the stabilities of the Cr(IV) complexes were sufficient for steady-state spectral measurements (no significant absorbance changes during 1 min). However, for the least stable complexes (like Cr(IV)−mal), time-dependent spectra were observed and the results were extrapolated to zero time using the Glint software. Processing of the resultant spectra and estimations of the equilibrium constants were carried out using Origin software.[30]

Decomposition Studies. The decomposition of Cr(IV) complexes in water (in the absence of excess Cr(VI)) proceeded by two parallel pathways: disproportionation to Cr(III) and Cr(V) (eq 1, second order with respect to [Cr(IV)]) and oxidation of the ligand (eq 2, first order with respect to [Cr(IV)]).[8] Decompositions of Cr(IV) complexes were

$$2\text{Cr(IV)}-\text{Lig} \rightarrow \text{Cr(V)}-\text{Lig} + \text{Cr(III)}-\text{Lig} \quad (1)$$

$$\text{Cr(IV)}-\text{Lig} + \text{Lig} \rightarrow \text{Cr(III)}-\text{Lig} + \text{oxidized Lig} \quad (2)$$

studied by recording the time-dependent spectra on the HP 8452 A spectrophotometer (λ = 250−800 nm; resolution 2 nm; time between measurements 15−60 s; integration time 0.2 s) at $[Cr(IV)]_0$ = 0.1 mM (generated by the reaction of 0.1 mM Cr(VI) with 5 mM As(III)),[28] [Lig] = 100 mM, pH = 1−8,[29] $[NaClO_4]$ = 1 M, and 25 ± 0.1 °C. Given the complex nature of the decomposition processes (eqs 1 and 2), the half-life ($\tau_{1/2}$) was chosen as the simplest empirical parameter to compare the stabilities of the complexes ($[Cr(IV)]_0$ and [Lig] were kept constant in all experiments; see the conditions above). Half-lives for all complexes were determined from the absorbance changes at 550 nm, where the absorbances of Cr(III) and Cr(V) complexes were negligible in comparison with those of Cr(IV) (see Results).

The Cr(V) complexes formed from Cr(IV) disproportionations (eq 1) themselves disproportionate slowly to Cr(III) and Cr(VI) (eq 3). This

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Table 1. UV−Visible and CD Spectral Characteristics of Cr(IV) Carboxylato Complexes

no.	complex[a,b]	spectral features (250−800 nm)[c]
1	$[Cr^{IV}O(ehbaH)_2]^0$	300 sh (3780); 370 sh (1710); 422 min (1280); 512 max (2380)
2	$[Cr^{IV}O(OH_2)_2(ehba)]^0$	270 sh (5500); 650 max (180)
3	$[Cr^{IV}O(hmbaH)_2]^0$	300 sh (3900); 370 sh (1860); 412 min (1530); 502 max (2940)
4	$[Cr^{IV}O(qaH)_2]^0$	UV−visible: 370 min (1390); 480 max (3940) CD: 415 max ($\Delta\epsilon = +1.25$ cm^2 mM^{-1})
5	$[Cr^{IV}O(pic)_2]^0$	422 min (990); 518 max (2240)
6	*cis*-$[Cr^{IV}O(OH_2)(ox)_2]^{2-}$	300 sh (3300); 364 min (1340); 410 sh (1670); 530 max (2460)
7	*cis*-$[Cr^{IV}O(OH_2)(mal)_2]^{2-}$	448 min (760); 562 max (1380)

[a] Numbers correspond to the structures in Scheme 1. [b] Complexes **1**, **3**, and **4** were generated by the reactions of 0.1 mM Cr(VI) with 5 mM As(III) in 100 mM buffer solutions of the corresponding ligands (pH = 3.5); [$NaClO_4$] = 1 M; 21 °C. The spectrum of complex **2** was estimated from the [Lig] and pH dependences for the spectra of Cr(IV)−ehba complexes (Figure 1, eq 5). Complexes **5**−**7** were obtained by additions of 100 mM of the corresponding ligand buffers to the solutions of Cr(IV)−hmba complexes (generated by the reaction of 0.1 mM Cr(VI) with 5 mM As(III) in 10 mM hmba buffer); pH = 3.5; [$NaClO_4$] = 1 M; 21 °C. [c] sh = shoulder; extinction coefficients (M^{-1} cm^{-1}) are given in parentheses.

process accelerates with an increase in pH values.[31] Thus, the full disproportionation of Cr(IV) is represented by eq 4. Decompositions

$$3Cr(V) \rightarrow 2Cr(VI) + Cr(III) \quad (3)$$

$$3Cr(IV) \rightarrow Cr(VI) + 2Cr(III) \quad (4)$$

of Cr(IV) under the studied conditions led to the formation of the mixtures of Cr(VI), Cr(V), and Cr(III) complexes. Concentrated NaOH solutions were added (to pH ∼ 13) to the reaction solutions after the practically complete (≥95%) decomposition of Cr(IV); the alkaline solutions were heated at 60 °C for 2−3 min (these conditions provide the fast disproportionation of Cr(V) by eq 3).[31] After the solutions were cooled to room temperature, the UV−visible spectra, showing the characteristic $[CrO_4]^{2-}$ peak (λ_{max} = 372 nm; $\epsilon_{max} = 4.81 \times 10^3$ M^{-1} cm^{-1})[32] were recorded. The amount of Cr(VI), thus determined, was used as the indicator for the ratio between disproportionation (eq 1) and ligand oxidation (eq 2) pathways in the decomposition of each Cr(IV) complex. In separate experiments, NaOH was added to the reaction solutions immediately after the generation of Cr(IV) and then Cr(VI) was determined as described above. The amounts of Cr(VI) found were one-third of $[Cr(IV)]_0$ (in accordance with eq 4), thus confirming the validity of the analytical procedure.

Ion-Exchange Studies. Sephadex A-25 and C-25 ion-exchange resins (Pharmacia LKB) were used for the studies of ion-exchange behavior of Cr(IV) complexes. The Cr(IV) complexes were generated as described above, except that the reaction solutions did not contain $NaClO_4$. Solutions of the Cr(IV) complexes were sorbed onto the column (cooled to ∼4 °C) and then were eluted sequentially with water and NaCl solutions of increasing concentrations. Crystallographically characterized[23,33] $Na[Cr^{III}(Cys)_2]$ and $(NH_4)_2[V^{IV}O(OH_2)(ox)_2]$ complexes were used as the reference sources of thermodynamically stable and highly colored 1− and 2− anions, respectively.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded on a Jasco 710 spectropolarimeter (λ = 300−700 nm; resolution 1 nm; scan rate 500 nm/min; response time 0.125 s; temperature 21 ± 1 °C). The CD spectra were processed using the Origin software. The Cr(IV) complexes, used for CD spectroscopic studies, were generated by the Cr(VI) + As(III) reactions, as described above, except that a higher $[Cr(VI)]_0$ was used. The latter was necessary to obtain optimal CD signals. However, the disproportionations of Cr(IV) complexes (eq 1) were faster due to the higher $[Cr(IV)]_0$ (typically 0.5 mM in comparison with 0.1 mM used in the UV−visible spectroscopic studies). Chromium(III) complexes (used for comparative spectroscopic studies of Cr(IV) and Cr(III)) were generated *in situ* by reductions of the corresponding Cr(IV) complexes with excess $NaHSO_3$.[3]

EPR Spectroscopy. X-band EPR spectra of Cr(V) complexes were recorded at 21 ± 1 °C, using flat quartz cell on a Bruker ESP 300 spectrometer, equipped with a HP 5352B frequency counter and a Bruker ER 035 M gaussmeter. The parameters for acquisition of EPR spectra were as follows: center field, 3500 G; sweep width, 100 G; resolution, 1024 points; microwave frequency, ∼9.66 GHz; microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 0.97 G; time constant, 1.28 ms; sweep time, 5.24 s; number of scans, 20; receiver gain, 1×10^4. Changes of Cr(V) concentrations with time were determined by measuring the areas of the EPR signals using the deconvolution procedure in WIN-EPR software.[34]

Results

Generation of Cr(IV) by Reactions of Cr(VI) with As(III) in Carboxylate Buffers. Gould and co-workers[4] have shown that the reaction of Cr(VI) with an excess of As(III) (a "pure" two-electron reductant) in ehba buffer (pH = 2−4) under ambient conditions leads to the relatively fast (reaction times 30−300 s dependent on the pH and the ligand concentration) formation of Cr(IV)−ehba complexes, which then undergo slow decomposition. We have tested the analogous reactions in a number of carboxylate buffers (Table S1 and Figure S1 in Supporting Information). In some of the buffers (ox, mal, edta, pyruvate, glycinate, prolinate, salicylate, and acetate + D-glucose), the reactions led to the formation of Cr(III), without any observable intermediates. In another group of buffers (pic, citrate, atrolactate, gluconate, and lactate) unstable intermediates were formed. Finally, in the cases of hmba, ehba, and qa buffers, practically quantitative yields of relatively stable intermediates were achieved (Figure S1b). Spectra of these intermediates possess strong absorbances at 400−600 nm, characteristic for Cr(IV) complexes[3] (Table 1 and Figure S1a). Thus, Cr(IV) complexes with the tertiary 2-hydroxy carboxylato ligands, hmba, ehba, and qa, exhibit considerable stability in slightly acidic aqueous media. Notably, the same ligands are also among the best for stabilizing the corresponding Cr(V) complexes, which have been isolated and characterized.[5−6,22]

Generation of Cr(IV) in the Reactions of Cr(V) Complexes with V(IV). The reaction of $[Cr^{V}O(ehba)_2]^-$ with the one-electron reductant V(IV), leading to the intermediate formation of Cr(IV)−ehba complexes, has been studied previously[7] by observation of the spectral changes at a single wavelength (490 nm). Application of the global analysis allowed us to study this and related reactions in more detail; typical results are shown in Figures S2 and S3, Supporting Information. The reaction of excess $[Cr^{V}O(ehba)_2]^-$ with V(IV) in ehba buffers led to a fast (time scale 1−10 s) growth of absorbance at 400−700 nm due to the formation of two colored species (Figure S2a): Cr(IV)−ehba (λ_{max} = 512 nm) and V(V)−ehba[35] (absorbing at 400−550 nm). The molar amounts of Cr(IV)−ehba complex formed correspond to the molar amounts of V(IV) introduced into the system (at $[Cr(V)]_0 \geq 10[V(IV)]_0$, Figure S2b). Analogous results were obtained for the reactions of an excess of either

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$[Cr^{V}O(hmba)_2]^-$ or $[Cr^{V}O(qa)_2]^-$ in the corresponding ligand buffers with V(IV). The estimated spectra of Cr(IV) complexes (after subtraction of the absorbances of V(V) complexes) correspond (within 5% experimental error) to those obtained from the Cr(VI) + As(III) reaction (Table 1, Figure S1a). The reactions of Cr(V) complexes with excess V(IV) in hmba, ehba, and qa buffers led to the formation of transient Cr(IV) intermediates, which were reduced rapidly to Cr(III) complexes (Figure S3a). Kinetics of these reactions were further complicated by transformations of the initially formed brightly yellow V(V) complexes to pale yellow products, presumably V(V) dimers[35] and/or V(IV,V) oligomers.[36] Nevertheless, taking into account the spectral changes due to V(V) reactions, the calculated spectra of the Cr(IV) intermediates were the same, within experimental error, as those obtained by the previous methods (an example for Cr(IV)−ehba is shown in Figure S3b).

Dependences of UV−Visible Spectra of Cr(IV) Complexes on pH and Ligand Concentrations. The data confirm previous observations[3,4] that the absorbances at 400−600 nm for the Cr(IV)−ehba complexes decreased with decreasing ligand concentrations. Furthermore, we observed the same phenomenon for the Cr(IV)−hmba and Cr(IV)−qa complexes (Figure S4 in Supporting Information). The dependences of the Cr(IV) spectra on the ligand concentration and pH were studied in more detail for the Cr(IV)−ehba complexes. The analogous trends have been found for Cr(IV)−ehba complexes obtained from both the Cr(V) + V(IV) and Cr(VI) + As(III) reactions (Figure 1a,b); *i.e.*, the decrease of absorbance values with decreasing ligand concentration becomes more pronounced at higher pH values. The experimental data (Figure 1c) are described by the equilibrium:

$$\mathbf{1} \overset{K}{\rightleftharpoons} \mathbf{2} + \mathrm{ehbaH_2} \qquad (5)$$

where **1** and **2** are the two different Cr(IV)−ehba complexes, $K = (5 \pm 2) \times 10^{-3}$ M (1 M $NaClO_4$, 25 °C), and $ehbaH_2$ is the protonated form of the ligand ($pK_a = 3.30$).[29]

From the data of Table 1, the absorbance of complex **2** at 560 nm is negligible in comparison with that of complex **1**. This allowed an estimation of the spectrum for complex **2** (Table 1, Figure S5 in Supporting Information). Within the experimental error (±5%), the spectrum of **2** was independent of pH and ligand concentration, thus confirming the validity of eq 5. The spectra of Cr(IV) complexes were independent (error ±5%) of both [Lig] and pH at high concentrations of the ligands ([Lig] ≥ 150 mM and pH = 2−4 for Cr(IV)−ehba and Cr(IV)−hmba; [Lig] ≥ 50 mM and pH = 3−7 for Cr(IV)−qa).

Generation of Cr(IV) Complexes by Ligand Exchange Reactions. The ligands used for this purpose should be (i) stable to oxidation (Cr(IV) is a very powerful oxidant)[18] and (ii) highly soluble in water. The ability of ox to exchange with Cr(IV)−ehba complexes was noticed previously,[19] although no details were supplied. The mal and pic ligands are also suitable for this reaction. The addition of Lig2 (where Lig2 = ox, mal, or pic) to the solutions of Cr(IV)−Lig1 complexes (Lig1 = ehba, hmba, or qa), generated by the reactions Cr(VI) + As(III) in the corresponding buffers, led to changes in the UV−visible spectra. The final spectra were dependent on the nature of Lig2 (the corresponding spectral features are given in Table 1) but independent of the nature and concentration of Lig1. It is essential for the formation of Cr(IV)−Lig2 complexes that Lig2 be added after the generation of the Cr(IV)−Lig1 complex.

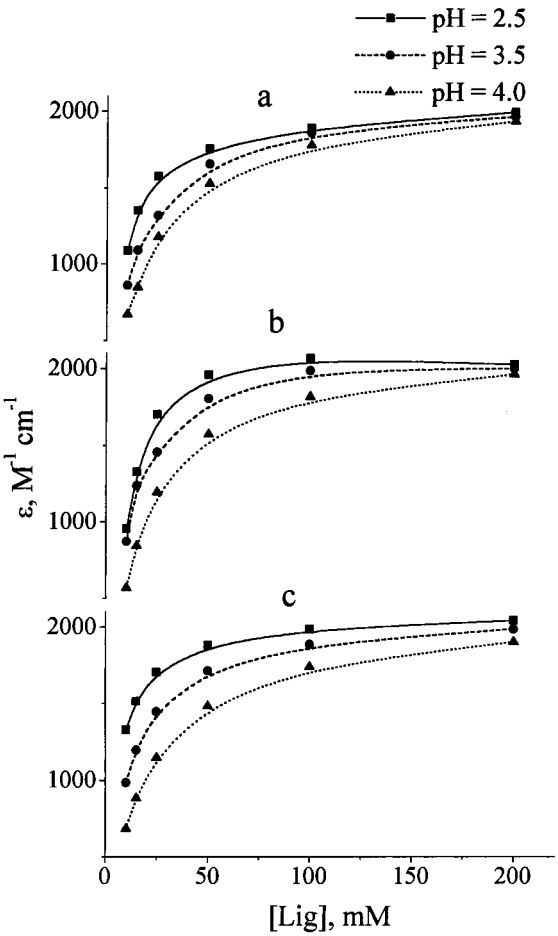


Figure 1. Changes of extinction coefficients (560 nm) with changing [Lig] and pH for Cr(IV)−ehba complexes: (a) experimental data, Cr(IV)−ehba complexes generated in the reaction of 1 mM $Na[Cr^{V}O(ehba)_2]$ with 0.05 mM V(IV), $[NaClO_4] = 1$ M, 25 °C; (b) experimental data, Cr(IV)−ehba complexes generated in the reaction of 0.1 mM Cr(VI) with 5 mM As(III), $[NaClO_4] = 1$ M, 25 °C; (c) the points are estimated from eq 5, using $\epsilon(\mathbf{1}) = 2100$ M^{-1} cm^{-1}, $\epsilon(\mathbf{2}) = 0$, $K = 5\times10^{-3}$ M, and $pK_a(ehbaH_2) = 3.30$.

Attempts to generate Cr(IV)−Lig2 by carrying out the reaction Cr(VI) + As(III) in the buffer systems Lig1 + Lig2 led to the formation of the mixtures of Cr(IV) and Cr(III) complexes (Figure S6 in Supporting Information).

Decomposition of Cr(IV) Complexes. Kinetics of the decomposition reactions of the Cr(IV)−ehba complexes at pH 2−4 were studied in detail by Gould and co-workers.[8] In the current work, the stabilities of different Cr(IV) complexes were compared over a broad range of pH values. Under the conditions used for the decomposition studies (see Experimental Section), the initial spectra of the Cr(IV) complexes were practically independent of pH. The results (Figure 2) show that the bell-shaped pH dependences of stabilities are characteristic for all of the complexes. The Cr(IV)−pic, Cr(IV)−ox, and Cr(IV)−qa complexes are significantly more stable at pH ≥ 4.5 than the Cr(IV)−hmba and Cr(IV)−ehba complexes. However, only the Cr(IV)−qa complex is reasonably stable at pH ∼ 7 ($\tau_{1/2} \sim 500$ s; see Figure 2). The Cr(IV)−mal complex is generally less stable than the other Cr(IV) complexes (inset in Figure 2). The results of analyses of the reaction mixtures after the practically complete (≥95%) decomposition of Cr(IV) (Table S2, Supporting Information) show that, in the pH regions corresponding to the maximum stability of each complex, the Cr(IV)−mal, Cr(IV)−ehba, and Cr(IV)−hmba complexes are decomposing mainly by disproportionation (eq 1). While disproportionation reactions are also prevalent in the decomposi-

(36) (a) Chasteen, N. D. *Struct. Bonding* (*Berlin*) **1983**, *53*, 105−138. (b) Codd, R.; Hambley, T. W.; Lay, P. A. *Inorg. Chem.* **1995**, *34*, 877−882.

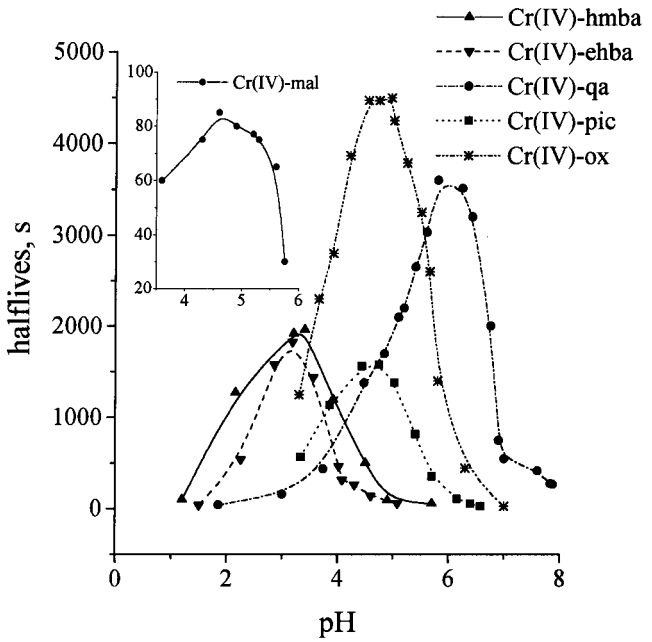


Figure 2. pH dependences of the half-lives of decompositions of Cr(IV) complexes (25 °C). See Experimental Section for the conditions of the decomposition studies.

Table 2. Equilibrium Constants for Ligand Exchange in Cr(IV) Complexes at pH = 3.8[a]

Lig1	Lig2	*K*	Lig1	Lig2	*K*
hmba	ox	$(6 \pm 3) \times 10^5$	hmba	qa	1.2 ± 0.5
ehba	ox	$(2 \pm 1) \times 10^4$	hmba	ehba	$(2 \pm 1) \times 10^{-1}$
hmba	pic	$(3 \pm 1) \times 10^3$	hmba	mal	$(2 \pm 1) \times 10^{-1}$
ehba	pic	$(2 \pm 1) \times 10^2$	qa	ox	10 ± 3[b]

[a] It was assumed that, under the conditions [Cr(IV)] = 0.1 mM, [Lig] = 10−100 mM, 1 M $NaClO_4$, and 25 °C, Cr(IV) exists mainly in the form of bis-chelated oxo complexes (see Discussion). Equilibrium constants $K = ([Cr^{IV}O(Lig2)_2][Lig1]^2)/([Cr^{IV}O(Lig1)_2][Lig2]^2)$ were determined by UV−visible spectrophotometry. The low stabilities of the Cr(IV) complexes and the relatively small differences in absorbance spectra of the complexes (see Table 1) are the sources of large experimental errors. [b] At pH = 4.4.

tions of the Cr(IV)−ox and Cr(IV)−qa complexes, oxidations of the ligands (eq 2) are significant in these cases. Finally, the decomposition of the Cr(IV)−pic complex under the studied conditions occurs mainly by oxidation of the ligand (Table S2). The results of experiments with variable As(III) concentrations (2−50 mM) showed that As(III) does not take a significant part in the decomposition of Cr(IV). For all of the Cr(IV) complexes, a decrease in the pH values led to the relative increase in the contribution of the ligand oxidation pathway (eq 2).

Ligand Exchange Equilibria of Cr(IV) Complexes. The ligand exchange reactions of Cr(IV) complexes were studied at pH = 3.8, *i.e.*, under the conditions where all of the complexes had reasonable stabilities (Figure 2). The results (Table 2) allowed the ligands to be placed in the following order of increasing stability of the corresponding Cr(IV) complexes toward ligand exchange reactions: hmba < ehba ∼ mal < qa < pic < ox. At low concentrations of hmba buffer (10−20 mM), 0.2 mM ox was sufficient for complete ligand exchange with 0.1 mM Cr(IV)−hmba, independent of the buffer concentration. This suggests the formation of a bis(oxalato)chromium(IV) complex. The ligand exchange reactions were studied in more detail for the system Cr(IV)−qa−ox, since these two ligands form the most long-lived (Figure 2) and significantly spectrally different (Table 1) Cr(IV) complexes. The results

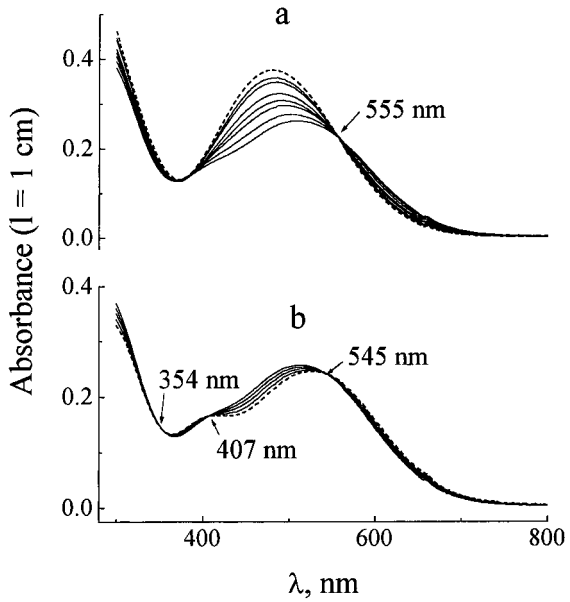


Figure 3. Spectral changes in the system Cr(IV)−qa−ox with changing [ox]/[qa] ratio at pH 4.4 ($[Cr(IV)]_0$ = 0.1 mM; 25 °C): (a) spectrum of the Cr(IV)−qa complex at [qa] = 100 mM (dashed line) and spectra of the Cr(IV)−qa−ox complex at [ox]/[qa] = 0.01−0.33 (solid lines); (b) spectrum of the Cr(IV)−ox complex at [ox] = 100 mM (dashed line) and spectra of the Cr(IV)−qa−ox complex at [ox]/[qa] = 0.72−10.0 (solid lines). Isosbestic points are shown by arrows.

(Figure 3) are consistent with the sequential ligand exchange reactions

$$Cr^{IV}(qa)_2 + ox \overset{K_1}{\rightleftharpoons} Cr^{IV}(ox)(qa) + qa \qquad (6)$$

$$Cr^{IV}(ox)(qa) + ox \overset{K_2}{\rightleftharpoons} Cr^{IV}(ox)_2 + qa \qquad (7)$$

where $K_1 = 26 \pm 5$ and $K_2 = 0.4 \pm 0.1$ (pH = 4.4, 1 M $NaClO_4$, 25 °C); ox and qa are the sums of all protolytic forms of the corresponding ligands (the protolytic equilibria were not taken into account, since the studies were performed at a constant pH value).

Interactions of Cr(IV) Complexes with Ion-Exchange Resins. The Cr(IV)−qa and Cr(IV)−ox complexes were sorbed onto a column of Sephadex A-25 anion-exchange resin (pH ∼ 5; 4 °C) and were eluted by 0.15 and 1.5 M NaCl solutions, respectively. Such a behavior corresponds to monoanionic (for Cr(IV)−qa) and dianionic (for Cr(IV)−ox) complexes (as it was shown in ion-exchange experiments with the reference complexes $[Cr(Cys)_2]^-$ and $[VO(OH_2)(ox)_2]^{2-}$, performed under the same conditions). All of the other Cr(IV) complexes quickly decomposed on the ion-exchange column; thus their charges could not be determined by this method. Decompositions of the Cr(IV)−hmba, Cr(IV)−ehba, and Cr(IV)−mal complexes led to the formation of chromate(VI) (detected as the yellow zone on Sephadex A-25), while Cr(IV)−pic decomposed entirely to Cr(III) (visually observed as a decoloration). These data are in agreement with the results of the Cr(IV) decomposition studies in solutions (Table S2). None of the complexes were retained by a Sephadex C-25 cation-exchange resin.

CD Spectroscopic Studies of Optically-Active Cr(IV) Complexes. Since the Cr(IV)−qa complexes are optically-active, their properties were also studied by CD spectroscopy (Figures S7 and S8 in Supporting Information). However, the interpretation of CD spectroscopic data was complicated by two main factors. First, the CD signals are relatively weak and the noise level is high (Figure S7a). Slow scan rates or multiple

scanning could not be used for increasing the signal:noise ratio because of the instability of the complexes. Second, Cr(III) and Cr(V) are inevitably present in the reaction mixture due to the decomposition of Cr(IV) and the CD signals of Cr(III), Cr(IV), and Cr(V) complexes with qa ligands are of comparable intensity at all wavelengths in the range 300−700 nm (Figure S7a). By contrast, the absorbances in UV−visible spectra for the Cr(III) and Cr(V) complexes at $\lambda = 500-600$ nm are negligible compared to those of Cr(IV) (Figure S7b). Within the experimental error (±10%), the CD spectra of Cr(IV)−qa complexes (Table 1) were independent of pH and [Lig] at high ligand concentrations ([Lig] ≥ 50 mM; pH = 3−7; typical spectra are shown at Figure S8a). By contrast, the CD spectra of Cr(V)−qa complexes were strongly pH-dependent (Figure S8b). This difference in behaviors of the Cr(IV)−qa and Cr(V)−qa complexes was observed also by UV−visible spectroscopy (Figure S9 in Supporting Information).

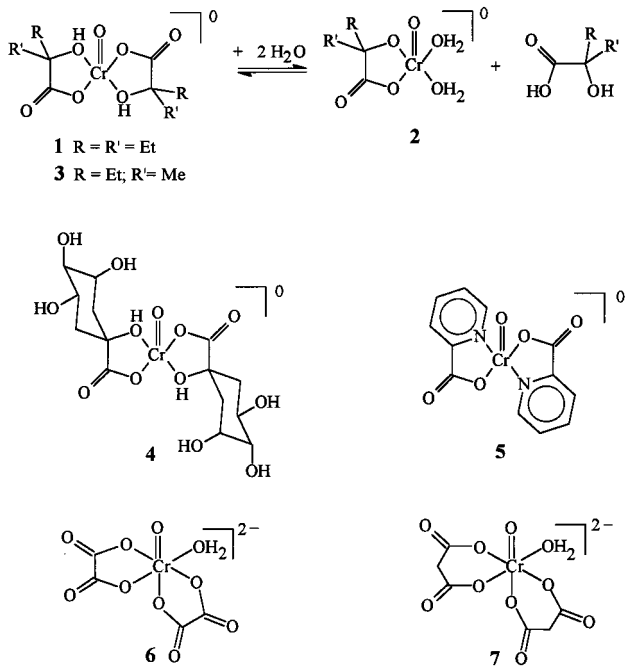
Comparison of Stabilities of the Cr(IV) and Cr(V) Complexes. Chromium(V) complexes formed during the disproportionation of Cr(IV) (eq 1) are easily detected by EPR spectroscopy at room temperature.[37] Kinetic curves for decay of the Cr(IV)−hmba, Cr(IV)−ehba, and Cr(IV)−qa complexes (UV−visible spectroscopy) were compared with those for the accumulation and decay of the corresponding Cr(V) complexes (EPR spectroscopy). The results (*e.g.*, Figure S10, Supporting Information) show that, under the reaction conditions ([Lig] = 10−200 mM; pH = 2−8; 21 °C), Cr(IV) complexes with hmba, ehba, and qa ligands are significantly less stable than the corresponding Cr(V) complexes.

The rates of Cr(V) decay (measured by EPR spectroscopy), under the above mentioned conditions, did not significantly change with variations in [As(III)] (2−50 mM). Thus, the rates of the reactions Cr(V) + As(III) under these conditions are negligible, compared to the relatively fast reactions Cr(VI) + As(III).

EPR spectra of the Cr(IV)−Lig1−Lig2 systems (Lig1 = hmba, ehba, or qa; Lig2 = pic, ox, or mal) under the conditions corresponding to the full replacement of Lig1 by Lig2 in Cr(IV) complexes (Table 2; confirmed by UV−visible spectroscopy) show only the signals of the corresponding Cr(V)−Lig1 complex. Furthermore, only Cr(V)−Lig1 signals were observed in solutions containing 0.1 mM $[Cr^{V}O(Lig1)_2]^-$ and 100 mM Lig2 buffer (pH 3.8). These data lead to the conclusion that Cr(V) complexes with pic, ox, and mal ligands are apparently at very low concentrations under the studied conditions.[38] This is probably the result of the unfavorable equilibria for ligand exchange with Lig2 rather than of fast decomposition of Cr(V)−Lig2, since the EPR signals of Cr(V)−Lig1 are stable for several hours in the presence of excess Lig2.

Relatively stable Cr(V)/1,2-diol (including D-glucose) complexes are found at physiological pH values.[37,39] By contrast, no evidence was found for the formation of Cr(IV) complexes with *trans*-(1*S*,2*S*)-, *trans*-(1*R*,2*R*)-, or *cis*-1,2-cyclohexanediol or D-glucose. No observable intermediates were formed in the reductions of Cr(VI) to Cr(III) by As(III) in the presence of

Scheme 1. Assigned Structures of Cr(IV) Carboxylato Complexes



D-glucose (Table S1). The additions of Lig2 (Lig2 = 1,2-cyclohexanediols or D-glucose) to the solutions of Cr(IV)−Lig1 complexes (Lig1 = ehba, hmba, or qa) at [Lig1] = 10−50 mM, [Lig2] = 50−250 mM, and pH = 4−9 did not lead to any significant UV−visible or CD spectral changes that could correspond to the formation of new Cr(IV) species. In addition, the presence of Lig2 did not significantly change the rates of decomposition of Cr(IV)−Lig1 complexes under the above-mentioned conditions.

Chromium(V)−edta complexes are formed during the reaction of Cr(VI) with the Co(II)−edta complex at pH = 3−4 in the presence of excess edta, and the possible formation of Cr(IV)−edta complexes in this system was also postulated.[40] However, when the edta buffers (pH = 3−4) were added in excess to the solutions of Cr(IV)−ehba complexes (generated by the Cr(VI) + As(III) + ehba reactions), no significant changes in UV−visible spectra were observed. Similarly, no evidence was found for the formation of Cr(IV) complexes with other 2-amino carboxylates (glycinate and L-prolinate).

Discussion

Solution Structures of Cr(IV) Complexes. The assigned structures of Cr(IV) complexes with ehba (**1**, **2**), hmba (**3**), qa (**4**), pic (**5**), ox (**6**), and mal (**7**) ligands are given in Scheme 1 (only protonated forms of complexes **1**, **3**, and **4** are shown). The UV−visible and CD spectral features of the corresponding complexes are summarized in Table 1.

The Cr(IV) oxidation state in complexes **1**−**4** is confirmed by the same spectral characteristics of the species obtained either by one-electron reductions of Cr(V) with V(IV) or by two-electron reductions of Cr(VI) with As(III) in buffer solutions of the corresponding ligands. However, the Cr(V) + V(IV) reaction is much less useful than the Cr(VI) + As(III) reaction, for three reasons. First, although the reactions of excess Cr(V) with V(IV) lead to quantitative yields (with respect to V(IV)) of the relatively stable Cr(IV) complexes, the spectral changes

(37) EPR spectral data on the Cr(V) complexes have been published: Barr-David, G.; Bramley, R.; Brumby, S.; Charara, M.; Codd, R.; Farrell, R. P.; Hanson, G. R.; Irwin, J. A.; Ji, J.-Y.; Lay, P. A. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1207−1216.

(38) However, Cr(V)−ox complexes are formed by the ligand exchange reactions Cr(V)−ehba + oxH_2 in more acidic media (pH = 0−1.5). See: (a) Farrell, R. P.; Judd, R. J.; Lay, P. A.; Bramley, R.; Ji, J.-Y. *Inorg. Chem.* **1989**, *28*, 3403−3410. (b) Farrell, R. P. Ph.D. Thesis, University of Sydney, 1993.

(39) (a) Branca, M.; Dessí, A.; Kozlowski, H.; Micera, G.; Swiatek, J. *J. Inorg. Biochem.* **1990**, *39*, 217−226. (b) Irwin, J. A. Unpublished results.

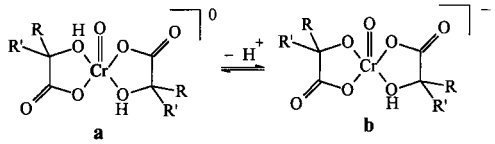
(40) Ohashi, K.; Aramaki, M.; Kaise, M.; Yamamoto, K. *Anal. Sci.* **1989**, *5*, 73−77.

due to the formation of Cr(IV) are small in comparison with the absorbances of excess Cr(V). In the reactions of Cr(V) with excess V(IV), Cr(IV) is formed only as a transient intermediate en route to further reduction to Cr(III). Second, the analysis of the absorbance changes due to the formation of Cr(IV) is complicated by the formation of colored V(IV) and V(V) complexes.[41] On the other hand, As(III) and As(V) compounds are colorless in the wavelength region of interest. Finally, V(IV) is easily oxidized by aerial oxygen under the reaction conditions. As a consequence, to obtain accurate results in reactions involving small concentrations of V(IV), O_2 must be rigorously excluded, but it does not affect the Cr(VI) + As(III) reactions.

The UV−visible spectra of the species generated by addition of excess Lig2 (Lig2 = pic, mal, or ox) to the solutions of Cr(IV)−Lig1 complexes (Lig1 = ehba, hmba, or qa) are close to those of the initial complexes (all possess strong absorbances in 400−600 nm region; see Table 1) and are very different from the known UV−visible spectra of Cr(III)−Lig2 complexes (possessing weak absorbances at 400−450 and 550−600 nm).[2] These observations confirm the oxidation state of Cr in the Cr(IV)−Lig2 complexes. The complete replacement of Lig1 by Lig2 in such complexes is confirmed by the lack of dependence of the resulting spectra on the nature and concentration of Lig1. An additional argument for the formation of the new Cr(IV)−Lig2 complexes is the significant changes of the pH profiles of their stabilities in comparison with those of the parent Cr(IV)−Lig1 complexes (Figure 2). The question then can be asked, Why could Cr(IV)−Lig2 complexes not be obtained by the reactions Cr(VI) + As(III) in the presence of Lig2 (Table S1)? Furthermore, it was found that small additions of Lig2 prevent the quantitative formation of Cr(IV) in Cr(VI) + As(III) + Lig1 reactions (Figure S6). The possible answer can be found in the mechanism of these reactions, where the formations of Cr(VI)−As(III)−Lig1 adducts are involved in the rate-determining step.[42] The presence of the ligands of other types may lead to the formation of different adducts that are not suitable for two-electron transfer from As(III) to Cr(VI). The other possible reason is that the Cr(IV)−Lig2 complexes, formed during the the reaction Cr(VI) + As(III) + Lig2, rapidly react with Cr(VI) (by contrast, the reaction Cr(IV) + Cr(VI) is relatively slow for the Cr(IV)−ehba complexes).[3,4]

The structures of the Cr(IV) complexes (**1** and **3**−**5** in Scheme 1) were assumed to be similar to those of the crystallographically-characterized[5,6] $[Cr^{V}O(ehba)_2]^-$ and $[Cr^{V}O(hmba)_2]^-$ (five-coordinated oxo complexes, with a distorted trigonal-bipyramidal geometry), as opposed to the six-coordinated, octahedral geometry of the corresponding Cr(III) complexes.[2] Electrochemical studies[18] have shown (in the case of the ehba complexes) that the major structural rearrangement follows the electron transfer in Cr(IV)/Cr(III), not in the Cr(V)/Cr(IV) couple. An additional argument for the structural similarity of Cr(IV) and Cr(V), compared to Cr(III) complexes, is that the reactions Cr(V) + As(III) → Cr(III) are several orders of magnitude slower than the Cr(VI) + As(III) → Cr(IV) reactions,[43] although the former reactions are more thermodynamically favorable.[44]

Scheme 2. Possible Protolytic Equilibrium of Cr(IV) Complexes with 2-Hydroxy Carboxylato Ligands



Our results are in agreement with the conclusion[3,18] that Cr(IV) under the studied conditions exists mainly in the forms of bis-chelated complexes (Scheme 1). The main evidence previously used in support of the bis-chelated structure for the Cr(IV)−ehba complex was[3,4,7] (i) Cr(IV)−ehba reacts with Cr(VI) to form a Cr(V) bis-chelate, $[Cr^{V}O(ehba)_2]^-$, and (ii) the reactions of Cr(IV)−ehba with various reductants seem to yield Cr(III) complexes, containing two ehba ligands. Additional arguments found here are (i) 2 mol of ox is required for the formation of 1 mol of the Cr(IV)−ox complex and (ii) the ligand exchange in the Cr(IV)−qa−ox system proceeds in two sequential steps (eqs 6 and 7).

The existence of different protolytic forms for the Cr(IV) complexes with 2-hydroxy carboxylato ligands (Scheme 2) was suggested[18] by analogy with the known chemistry of V(IV)−ehba complexes.[11] In previous studies,[3,4] the uncharged form **a** (Scheme 2) of the Cr(IV)−ehba complexes was assumed to exist as the main one at pH 2−4, since the rates of its redox reactions were independent of ionic strength. The equilibrium studies (Table 2) showed that ehba, hmba, and qa are poorer ligands than pic and ox for Cr(IV) at pH = 3.8. This is an additional argument for the existence of **1**, **3**, and **4** mainly in the protonated forms **a** at this pH value, since the reverse order of stability was observed for the corresponding Cr(V) complexes, where the ligands are fully deprotonated.[5,6,22] On the other hand, the ion-exchange behavior of **4** at pH ∼ 5 shows the presence of a partially deprotonated form **b** (Scheme 2). Unfortunately, more detailed studies of the protonation of Cr(IV) complexes were difficult because (i) these reactions do not lead to appreciable changes in UV−visible and CD spectra and (ii) the Cr(IV) complexes are relatively stable only over a narrow pH region (Figure 2).

Dominance of the six-coordinate forms for Cr(IV) complexes with ox and mal ligands (**6** and **7** in Scheme 1) was suggested on the basis of (i) crystallographic data[33] for the V(IV) oxalato complex, *cis*-$[V^{IV}O(OH_2)(ox)_2]^{2-}$, and (ii) EPR studies of Cr(V) oxalato complexes, showing the prevalence of the six-coordinate form, similar to **6**.[37,38] The appearance of six-coordinated forms is much less likely for complexes with the more bulky ligands[18] (**1**, **3**, **4**, and **5** in Scheme 1). The results of ion-exchange studies suggesting a dianionic form for the Cr(IV)−ox complex are in agreement with structure **6**. The decomposition studies (Figure 2) showed that **6** is the most stable Cr(IV) complex under the studied conditions, while **7** is the least stable one. It suggests that five-membered chelates of Cr(IV) are much more stable than the six-membered ones, as found for the Cr(V) analogs.[45]

The dependences of UV−visible spectra on the ligand concentrations for Cr(IV)−ehba complexes were explained[3,4,17] in terms of the addition of a third ehba ligand (in monodentate fashion) to complex **1**. The arguments against this explanation

(41) The formation and further reactions of V(V)−ehba were not taken into account in a previous study[7] of the system Cr(V) + V(IV) + ehba. This led to some misinterpretations of the kinetic data. Thus, the formation and decomposition of Cr(IV) (monitored at 490 nm) were assigned to three sequential steps. In fact, there are only two steps corresponding to the formation and decomposition of Cr(IV), respectively (as shown from absorbance changes at 560 nm, where the influence of [V(V)] is negligible). The third step observed at 490 nm corresponds to further transformations of V(V)−ehba.

(42) This was established from kinetic studies of the reaction Cr(VI) + As(III) in ehba buffer at pH = 2−4.[4]

(43) This refers to the reactions in ehba, hmba, and qa buffers at pH = 2.5−4.5.

(44) Electrochemical studies (Eckert, J. M.; Judd, R. J.; Lay, P. A. *Inorg. Chem.* **1987**, *26*, 2191−2192) show that Cr(V) complexes are more powerful oxidants than Cr(VI) compounds.

(45) (a) Mitewa, M.; Bontchev, P. R. *Coord. Chem. Rev.* **1985**, *61*, 241−272. (b) Farrell, R. P.; Lay, P. A. *Comments Inorg. Chem.* **1992**, *13*, 133−175.

have been discussed in detail[18] and will not be repeated here. The alternative approach[18] is to assume the existence of an equilibrium between the mono- and bis-ligated Cr(IV)−ehba complexes. The detailed studies performed in the current work (Figure 1, eq 5) allowed us to assign structure **2** (Scheme 1) to the mono-ligated form. The estimated UV−visible spectrum of **2** (Table 1, Figure S5) is close to that of the Cr(IV) aqua complex.[46] Studies of the equilibrium between mono- and bis-ligated Cr(IV)−ehba, performed with Cr(IV) complexes generated by both the reactions Cr(V) + V(IV) and Cr(VI) + As(III), give similar [Lig] and pH dependences (Figure 1). Notably, the former reaction is accelerated with increasing pH, while the rate of the latter is decreased with increasing pH. Thus, the observed absorbance changes (Figure 1b) cannot be explained by the incomplete conversion of Cr(VI) to Cr(IV) at higher pH values.

The Cr(IV) decomposition studies have shown that, at certain pH values (see Figure 2), the Cr(IV)−ox and Cr(IV)−qa complexes are remarkably stable in dilute aqueous solutions ($\tau_{1/2}$ = 1−1.5 h at $[Cr(IV)]_0$ = 0.1 mM and 25 °C). However, the second-order disproportionation reactions (eq 1) will make the isolation of these complexes from more concentrated aqueous solutions very difficult.

The most remarkable result of the Cr(IV) decomposition studies is the shift of the relative stability region for Cr(IV)−qa to higher pH values in comparison with Cr(IV)−ehba and Cr(IV)−hmba (Figure 2). Initially, it was thought that at higher pH values a linkage isomerization occurred to bind the 1,2-diol moieties of qa to Cr(IV), by analogy with the Cr(V)−qa complexes.[22,47] However, this explanation is unlikely because (i) no significant changes were observed in the UV−visible and CD spectra of the Cr(IV)−qa complexes with changing pH, in contrast with the Cr(V)−qa complexes (Figures S8 and S9), and (ii) no evidence was obtained for Cr(IV) complexation with 1,2-cyclohexanediols and D-glucose. The increased stability of Cr(IV)−qa at pH ≥ 4.5 may be caused by steric hindrance for the Cr(IV) disproportionation reaction due to the bulky qa ligands (see structure **4** in Scheme 1). At lower pH values, the decomposition of the Cr(IV)−qa complexes probably occurs mainly by intramolecular oxidation of the secondary alcohol groups with Cr(IV).[48]

The important difference in the chemical properties of Cr(IV) and Cr(V) is that 2-hydroxy carboxylates (ehba, hmba, and qa) and especially polyols (1,2-cyclohexanediols and D-glucose) are much poorer ligands for Cr(IV) than for Cr(V). This is probably the result of the lower acidity of the ROH groups bound to Cr(IV). Thus, the ROH groups of the Cr(IV) complexes with ehba, hmba, and qa ligands remain mainly protonated at pH = 2−4 (Scheme 1), while those of the corresponding Cr(V) complexes are fully deprotonated under these conditions.[5,6,22] An analogous reason probably causes the instability of the Cr(IV) complexes with polyol ligands in contrast to the corresponding Cr(V) complexes.[39] The other difference in Cr(IV) and Cr(V) chemistry is the formation of Cr(IV) complexes with ox and pic ligands, which are relatively stable at pH = 4−6 (Figure 2). The formation of Cr(V) complexes with ox and pic ligands at these pH values was not observed. On the other hand, oxalato and picolinato complexes are well-known for Cr(III).[2]

Implications for Cr(IV) and Cr(V) Genotoxicities. Both Cr(IV) and Cr(V) can be formed intracellularly by reduction of Cr(VI),[10,12] as well as by oxidation of Cr(III) with the activated oxygen formed in enzymatic reactions.[49] Recent EPR spectroscopic studies[50] show that relatively stable Cr(V) compounds exist *in vivo* bound to carbohydrates or carbohydrate residues of nucleotides. In this respect, the observation that Cr(IV) does not form complexes of any significant stability with 1,2-diol moieties of carbohydrates in neutral aqueous media can be an argument against a significant role for Cr(IV) in genotoxicity. The other argument is that the Cr(V)−carbohydrate complexes are stable for hours at physiological pH (7.4),[39] but the lifetimes of the known Cr(IV) complexes under these conditions are measured in minutes or seconds (Figure 2). On the other hand, a slightly acidic medium (pH = 4.5−5.5), which is applicable for the cellular uptake of insoluble chromates by phagocytosis,[16] is favorable for the existence of Cr(IV) complexes with different types of ligands (ox, pic, or qa; Figure 2). The shorter lifetimes of the Cr(IV) complexes in comparison with those of Cr(V) can be compensated by the higher reactivity of Cr(IV) in oxidation reactions due to the higher redox potential.[11,18] Furthermore, the intracellular concentrations of Cr(IV) complexes, causing DNA damage, are likely to be on the micromolar or nanomolar level (analogous to the case of Cr(V) complexes).[14,15] Therefore, the second-order decomposition reactions (eq 1) of Cr(IV) complexes in intracellular media are expected to be much slower than in the *in vitro* studies.

Perhaps an important advantage of Cr(IV) (in comparison with Cr(V)) in biological systems is its ability to form complexes with a wider variety of ligands. Thus, mainly Cr(V) complexes with 2-hydroxy carboxylate and 1,2-diol ligands can be expected to exist under physiological conditions, but Cr(IV) can additionally form complexes with bis(carboxylate) (like ox and mal) and heterocyclic (like pic) ligands. All of these types of ligands are likely to exist in intracellular media. Finally, Cr(IV) 2-hydroxy carboxylato complexes have a higher propensity to lose one ligand in comparison with their Cr(V) analogs. Thus, no significant UV−visible spectral changes were observed for the solutions of Cr(V)−ehba complex with increasing ligand concentrations ([Lig] = 10−200 mM; pH = 3.5; Figure S2a), while the spectra of the Cr(IV)−ehba complexes were strongly dependent on [Lig] under these conditions (Figures 1, S2a, and S4). The loss of one ligand from $[Cr^{V}O(ehba)_2]^-$ and the following complexation of the mono-ligated species with DNA have been postulated to be key steps in DNA cleavage by this Cr(V) complex.[14] In agreement with this mechanism, an excess of ehba effectively inhibits the cleavage process.[14] As intracellular reactions of Cr complexes are likely to proceed in the presence of ligands in great excess, the ability of Cr(IV) complexes to lose ligands more easily can give them a preference in reactions with DNA in comparison with Cr(V) complexes.

Thus, the chemical properties of Cr(IV) complexes in aqueous solutions make them likely candidates for the active species in Cr-induced genotoxicities. The published data[11−13] provide some evidence that Cr(IV) is a more potent DNA-damaging agent than Cr(V). However, these results are not definitive, since no data on the structure and stability of Cr(IV) species under the reaction conditions were obtained. The results of the

(46) For $[CrO(OH_2)_5]^{2+}$: λ_{max} = 260 nm; $\epsilon_{max} = (5 \pm 1) \times 10^3\ M^{-1}\ cm^{-1}$.[9]

(47) The effects of pH on the structures of Cr(V)−qa complexes in aqueous solutions have been studied in detail by EPR spectroscopy (Codd, R. Unpublished results).

(48) The analysis of the reaction mixtures show that only Cr(III), not Cr(V) or Cr(VI), is present in the reaction mixtures after the decomposition of the Cr(IV)−qa complex at pH < 3. Thus, the ligand oxidation is the only significant pathway of Cr(IV)−qa decomposition under these conditions.

(49) Dillon, C. T. Ph.D. Thesis, University of Sydney, 1995.

(50) Liu, K. J.; Shi, X.; Jiang, J.; Goda, F.; Dalal, N.; Swartz, H. M. *Ann. Clin. Lab. Sci.* **1996**, *26*, 176−184.

current work can be used as the basis for more systematic studies of Cr(IV) reactions with DNA.

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Supporting Information Available: Tables giving the results of global kinetic analysis for Cr(IV) generation reactions and results of [Cr(VI)] determinations in reaction mixtures after the decomposition of Cr(IV), typical UV−visible and CD spectra of the Cr(IV), Cr(V), and Cr(III) complexes with the studied ligands, and typical kinetic curves for the decomposition of Cr(IV) and Cr(V) complexes (12 pages). Ordering information is given on any current masthead page.

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