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Chromium(III) complexes as intermolecular probes ☆

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Abstract

Metal ion complexes provide flexible paramagnetic centers that may be used to define intermolecular contacts in a variety of solution phase environments because both the charge and electronic relaxation properties of the complex may be varied. For most complex ions, there are several proton equilibria that may change the effective charge on the complex as a function of pH which in turn affects the efficacy of application for defining the electrostatic surfaces of co-solute molecules. We report here spectrophotometric and nuclear spin relaxation studies on aqueous solutions of chromium(III) complexes of EDTA, DTPA, and bis-amides of both. The effective charges available from these paramagnetic centers range from −3 to +1 and we report the pH ranges over which the effective charge is defined with confidence for application in magnetic relaxation experiments.

Keywords: Chromium(III); Magnetic relaxation; Intermolecular probes; Relaxation dispersion

1. Introduction

Nuclear magnetic relaxation induced by magnetic dipole–dipole coupling between a nuclear spin and an electron spin has many important applications including contrast agents for magnetic imaging procedures, solvent suppression, and defining long-distance constraints in structural magnetic resonance spectroscopy. The strong distance dependence of the dipole–dipole coupling provides a powerful means for mapping experimentally the details of intermolecular interactions in solution [1–13]. The changes in the nuclear spin relaxation rate constant in a target molecule caused by a freely diffusing paramagnetic explorer are generally measured to define how the diffusing paramagnet accesses the target molecule in solution. The structural detail in defining the intermolecular contacts is limited by the number of resolved nuclear magnetic resonance peaks that may be separately observed and assigned. For macromolecules like proteins, there are many resonances that provide a detailed report of the intermolecular exploration. Often the diffusing paramagnetic molecule is a small organic radical such as a nitroxide or a simpler molecule such as oxygen.

Oxygen has the advantages of small size, no charge, and a short electron relaxation time, which makes the paramagnetic contribution to nuclear spin relaxation relatively simple to interpret in terms of effects of proximity [14,13]. Organic radicals like nitroxides are more convenient than oxygen because it is relatively easy to control the charge and concentration; however, quantitative interpretation of the relaxation rate contribution is complicated by large effects from transient binding interactions and the coupling between the relaxation rate and the translational mobility of the radical [15]. Metal ion complexes offer the opportunity to control the size and charge of the diffusing explorer molecule as well as the electronic relaxation properties of the paramagnetic center, which may, in principle, be adjusted to fit the problem.

Unlike the case of metal complexes designed as relaxation agents for use in clinical imaging applications, it is

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an advantage to have relaxation agents with very short electron spin relaxation times when using them to define intermolecular contacts. If the electron relaxation time is long, as in a nitroxide, then the correlation time for the electron–nuclear coupling between the diffusing relaxation agent and the solute of interest is a function of the relative diffusion constant, which may depend on the local environment, particularly in a macromolecule such as a protein. However, if the electron relaxation time is short compared with the translational correlation time, then the time dependence in the electron–nuclear coupling is modulated by the electron relaxation, not by the relative motion of the spins. In this case, the paramagnetic contribution to the nuclear relaxation rate is a linear function of the effective local concentration of the paramagnetic molecule, or a measure of effective proximity to the detected nuclear spin. Thus, it is useful to examine the chemistry and nuclear spin relaxation effectiveness of metal systems with short electron spin relaxation times such as nickel(II) or chromium(III) complexes. Chromium is attractive because the substitution chemistry is slow so that reactivity with co-solute functional groups within the acquisition time of the experiment is minimized. On the other hand, hexaaquachromium(III) ion does not have a particularly short electron spin–lattice relaxation time, which nickel does for example [17]. However, the electronic symmetry of the chromium complex is reduced considerably from the symmetric hexaaqua complex in the cases studied here, and like the manganese, iron, and gadolinium examples in the literature, the electron relaxation time is reduced in the complexes compared with the aquaions, although as we will show, not to an optimal value.

We report here a series of studies on chromium(III) complexes with diethylenetriaminepentaacetic acid and ethylenediaminetetraacetic acid as well as the bis-2-methoxyethylamides of both ligands so that the net charge of the complex may range from -3 to $+1$. The combination of UV–visible spectroscopy with measurements of spin–lattice and transverse relaxation times of the solvent water protons, as well as magnetic relaxation dispersion profiles, provides a useful characterization of the aqueous chemistry of these metal complexes. We find that the structure and effective charge of these complex ions are quite sensitive to pH; however, the similarity of the first coordination sphere and similar sizes in this group of diffusing relaxation agents make them useful for exploring the electrostatic properties of macromolecules such as proteins.

2. Experimental

Diethylenetriaminepentaacetic acid chromium(III) disodium salt hexahydrate (97%), ethylenediaminetetraacetic dianhydride (98%), 2-methoxyethylamine (98%), potassium dichromate, ethylenediaminetetraacetic acid, and diethylenetriaminetetraacetic acid were purchased from Aldrich Chemical. Chromium chloride hexahydrate was purchased from EM Science, Cherry Hill, NJ; DTPA-bis(2-methoxyethylamide) was generously donated by Mallinckrodt, St. Louis, MO.

Deuterated water was purchased from Cambridge Isotope Laboratory, Cambridge, MA; glycerol was purchased from Mallinckrodt Baker, isopropyl alcohol, acetonitrile, and acetone were purchased from Fisher Scientific, ethyl alcohol was purchased from AAPER Alcohol and Chemical. Water was purified by a Barnstead Millipore filtration system (resistance >17.5 MΩ).

EDTA-bis(2-methoxyethylamide) was prepared by treating a stirred suspension of ethylenediaminetetraacetic dianhydride (10 g, 0.039 mol) in isopropyl alcohol (30 mL) with 2-methoxyethylamine (3.08 g, 0.041 mol). The reaction mixture was heated to 65 °C for 2 h, then cooled and filtered. White solid was precipitated from the filtrate with the addition of acetonitrile, filtered, washed with ethanol, and dried at ~40 °C. ^{1}H NMR in D_2O, δ (ppm): 3.85 (s, 4H), 3.74 (s, 4H), 3.60 (t, 4H), 3.49 (t, 4H), 3.38 (s, 6H), 3.34 (t, 4H). A similar procedure was used to prepare DTPA-bis(2-methoxyethylamide) which yielded the ^{1}H NMR in D_2O: δ (ppm): 3.90 (s, 4H), 3.77 (s, 4H), 3.74 (s, 2H), 3.59 (t, 4H), 3.47 (t, 4H), 3.40 (t, 4H), 3.38 (s, 6H), and 3.33 (t, 4H).

Chromium(III) EDTA-bis(methoxyethylamide) chloride, sodium chromium(III)-EDTA, and chromium(III)-DTPA-bis(2-methoxyethylamide) were prepared by heating a suspension of ligand (0.02 mol) and chromium(III) chloride (0.0195 mol) in water to 70 °C for several hours with stirring; pH was held between 3.5 and 6.5 by the addition of sodium hydroxide solution. As the reaction proceeded, the white solid dissolved and the color changed from green to deep purple, and the pH stabilized. The reaction mixture was filtered, the filtrate poured into acetone, and the mixture was stirred vigorously until the chromium complex precipitated. The solid was filtered, washed with acetone, ethanol, and dried in air.

Analysis for chromium content was performed by oxidizing known amount of chromium(III) complex with ammonium persulfate [18], then reacting the Cr(VI) with 1,5-diphenylcarbazide with formation of the red complex [19]. Absorbance was measured at 540 nm, and potassium dichromate volumetric standard (Aldrich) was used for constructing calibration curves.

UV–visible spectra were obtained using a Cary 4E spectrophotometer. NMR spectra were accumulated on a Varian Unity Plus spectrometer operating at 500 MHz for protons. The relaxation dispersion measurements were obtained on a field cycling NMR spectrometer constructed in this laboratory and described elsewhere [15,16]. The spectrometer achieves high

resolution and high sensitivity by pneumatically moving the sample between a 7 T polarization/detection field and a satellite field, which is variable. The sample is first polarized in the high field, it is then moved pneumatically to the satellite relaxation field for a variable relaxation period, then pneumatically returned to the high field where the magnetization is detected using standard Fourier transform spectroscopy. Spin–lattice relaxation rate constants in the satellite field are obtained by monitoring the signal amplitudes detected at high field as a function of the time that the sample spends in the relaxation field.

3. Results

Spectrophotometric data for four chromium(III) complexes are summarized in Fig. 1. In all cases the pH dependence shows isosbestic points that strongly support an equilibrium between two species. The absorbance is shown at fixed wavelengths as a function of pH in Fig. 2. The solid lines are computed from a fit to a single proton ionization with equilibrium constants summarized in Table 1. Within experimental error, the equilibrium constants deduced from absorbance measurements at different wavelengths are in agreement. Also shown are equilibrium constants from previous work for CrEDTA and CrDTPA, which are in reasonably good agreement with the present values.

The magnetic field dependence of the water-proton-spin–lattice-relaxation rate constants is shown as a function of magnetic field strength in Fig. 3. We observe the water protons that may diffuse in the vicinity of the paramagnetic metal complex, exchange with the hydrogen atoms on amino, amide, or carboxy groups of the ligand, and with the protons of water in the first coordination sphere, all of which enhance proton relaxation. The relaxivity, i.e., the relaxation rate per millimolar concentration of metal complex, is small and in the range expected for outer sphere contributions [20]; however, quantitative interpretation is difficult because the

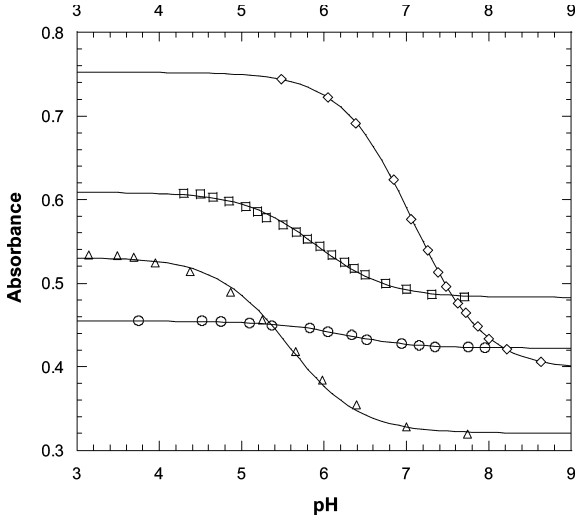

Fig. 2. Optical absorbance as a function of pH for $[Cr(III)EDTA]^-$ (◇); $[Cr(III)EDTA\text{-bis-(2-methoxyethylamide)}]^+$ (△); $[Cr(III)DTPA]^{2-}$ (○); and [Cr(III)DTPA-bis-(2-methoxyethylamide)] (□) in water at 21 °C. The solid lines are computed from least squares fits to a single proton ionization model with the equilibrium constants summarized in Table 1.

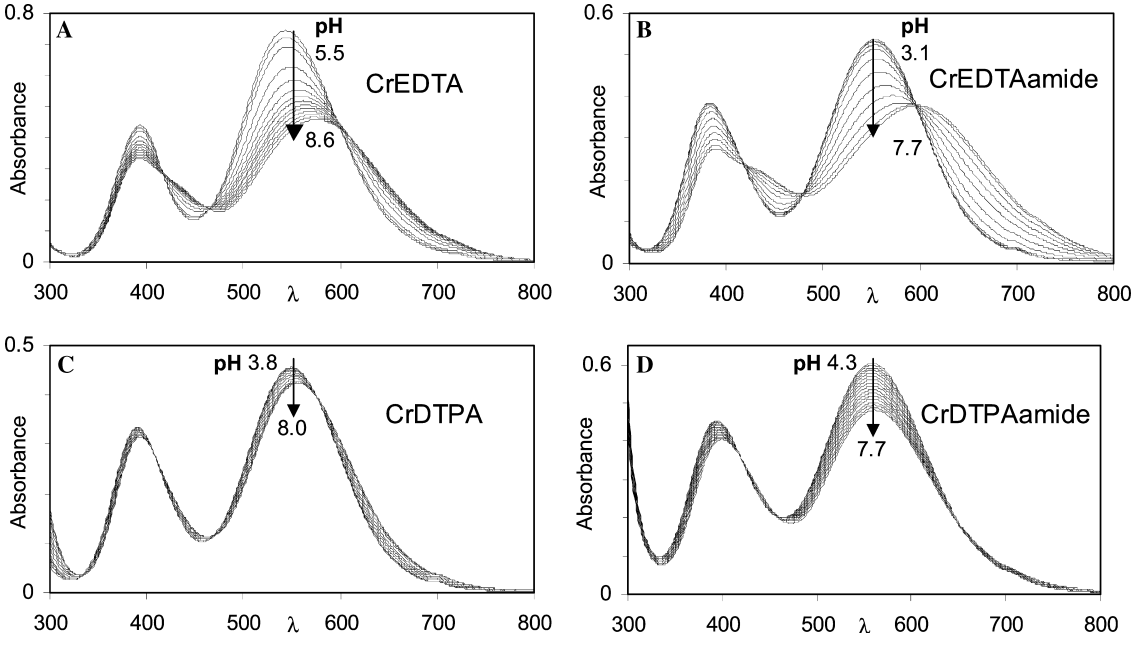

Fig. 1. UV–visible spectra at various pH values for: (A) $[Cr(III)EDTA]^-$; (B) $[Cr(III)EDTA\text{-bis-(2-methoxyehtylamide)}]^+$; (C) $[Cr(III)DTPA]^{2-}$; and (D) [Cr(III)DTPA-bis-(2-methoxyethylamide)] in water at 21 °C. Specific pH values are shown in Fig. 2.

Table 1
pK_a values for chromium(III) complexes at ambient temperature

Method	CrDTPA	CrEDTA	CrDTPAam	CrEDTAam	Reference
Spectrophotometric ~551 nm	6.2 ± 0.1	7.1 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	This work
Spectrophotometric ~390 nm	6.2 ± 0.1	7.2 ± 0.1	6.1 ± 0.1	5.6 ± 0.1	This work
Spectrophotometric ~440 nm	7.6 ± 0.1				This work
Water-proton relaxation	7.7 ± 0.1	7.3 ± 0.2	5.9 ± 0.1	5.5 ± 0.1	This work
Potentiometric titration	1.45; 2.85; 6.13; 7.65	1.8; 7.39			[27,47]
Spectrophotometric	6.17; 7.64				[27]

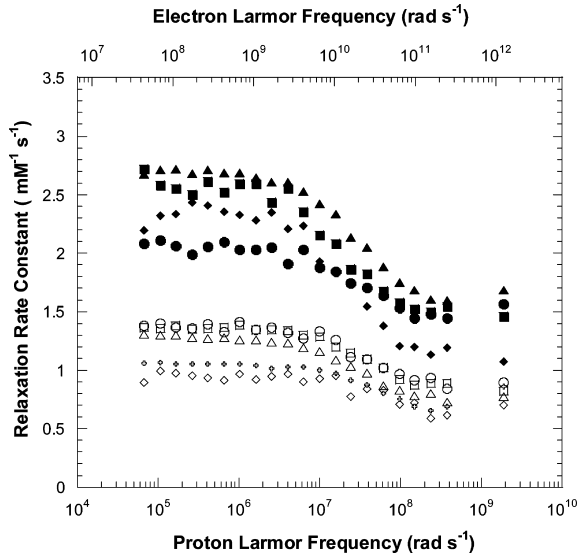

Fig. 3. The water-proton-spin–lattice-relaxation rate constant as a function of magnetic field strength represented as the nuclear and electron Larmor frequencies for $[Cr(III)EDTA]^-$ at pH 3.5 (♦), 4.5 (✣), and 8.2 (◇), $[Cr(III)EDTA\text{-bis(2-methoxyethylamide)}]^+$ at pH 4.0 (▲) and 7.0 (△), $[Cr(III)DTPA]^{2-}$ at pH 4.0 (●) and 7.8 (○), and [Cr(III)DTPA-bis(2-methoxyethylamide)] at pH 4.4 (■) and 7.0 (□).

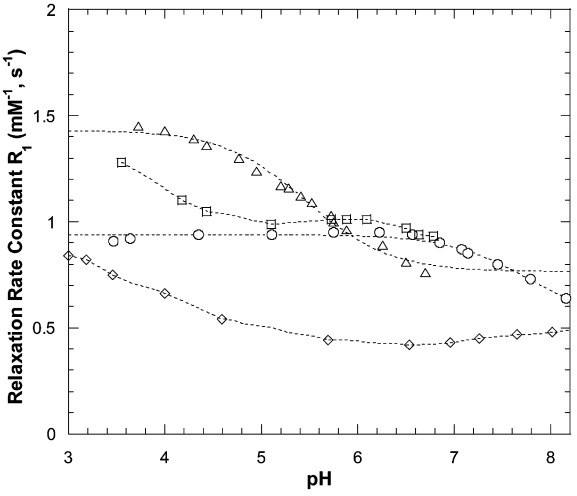

Fig. 4. The water-proton-spin–lattice relaxation rate constant at 500 MHz as a function of pH for aqueous solutions of $[Cr(III)EDTA]^-$ (◇), $[Cr(III)EDTA\text{-bis(2-methoxyethylamide)}]^+$ (△), $[Cr(III)DTPA]^{2-}$ (○), and [Cr(III)DTPA-bis(2-methoxyethylamide)] (□) at 25 °C.

zero-field splitting in the chromium(III) electron system complicates the description of the electron–nuclear coupling. Thus, for first coordination sphere interactions the Solomon, Bloembergen, and Morgan equations are insufficient, as are outer sphere theories that do not include the magnetic field dependence of the electron quantization [21–25]. The inflection points in the MRD profiles lie in the frequency range corresponding to effective correlation times of tens of picoseconds for all complexes shown. These values are close to correlation times expected for the relative translation of the water and metal complex, or for rotational reorientation of the metal complex. However, the increase in relaxivity with decreasing pH at constant chromium(III) complex concentration implies additional contributions to the water-proton-spin-relaxation-rate constant that are not from changes in the relative diffusion of the interacting spins.

Fig. 4 shows the water-proton-spin–lattice-relaxation-rate constants at 500 MHz as a function of pH for the set of chromium complexes. The pH dependence is not strong in any case; for each compound the amplitude in relaxivity change is less than 0.5 $mM^{-1}\,s^{-1}$. There are two inflection points as a function of pH for the water proton relaxivity of CrEDTA and CrDTPA-bis-amide solutions. The relaxation rate for the chromium(III) DTPA is constant in the pH range from 3.5 to 6.5, but decreases at pH values above 7. For the bis-amide complexes the relaxivity increases with decreasing pH. Data acquisition for the bis-amide complexes was complicated by time dependence of the pH after adjusting it to the values above 6.8. We attribute this change to slow ligand substitution by hydroxide ion, and did not collect data above pH 6.8 because the time dependence would compromise the application of the metal complexes as intermolecular probes. By contrast, no time dependence was observed for the pH in solutions of the DTPA and EDTA complexes. However, in both cases the relaxation rate constant is a weak function of pH at values over 6.5. Changes in this pH range were also observed spectrophotometrically. In addition, the CrEDTA complex has a weak pH dependence of the water proton relaxivity at pH <5 where first coordination sphere proton exchange is likely. However, similar pH dependence of the CrDTPA complex was not found in this pH range.

Fig. 5 shows the complimentary transverse relaxation rate constants for water protons as a function of pH for the same chromium(III) complexes. In all cases, the transverse relaxation rate constants are larger than the longitudinal rate constants. For both of the bis-amide complexes, the relaxivity decreases with increasing pH over a broad range. For the EDTA complex, there is a peak in the transverse relaxation rate near pH 4, and a decline to lower values by pH 6.5, but for the DTPA complex the transverse relaxation rate rises to a maximum at pH 6.9. In neither case is the change in the relaxation rate with increasing pH proportional to the hydroxide or hydrogen ion concentration.

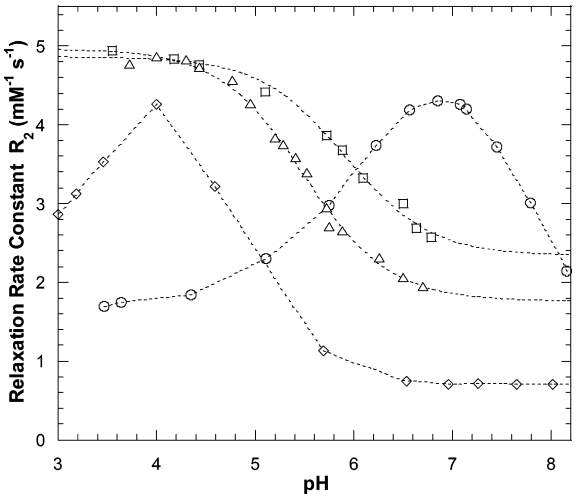

Fig. 5. The water-proton transverse relaxation rate constant at 500 MHz as a function of pH for aqueous solutions of $[Cr(III)EDTA]^-$ (◇), $[Cr(III)EDTA\text{-}bis(2\text{-}methoxyethylamide)]^+$ (△), $[Cr(III)DTPA]^{2-}$ (○), and [Cr(III)DTPA-bis(2-methoxyethylamide)] (□) at 25 °C.

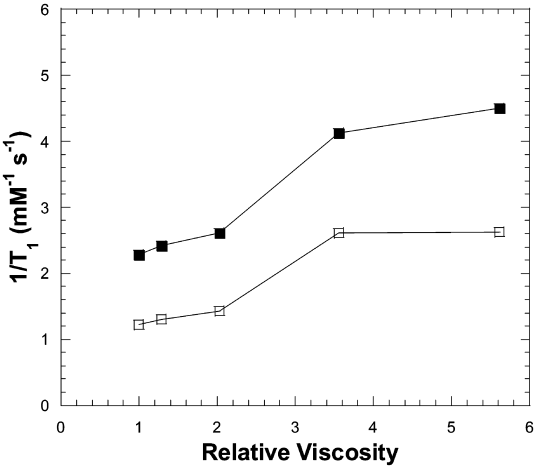

Fig. 6. The water-proton-spin–lattice-relaxation rate constant for [Cr(III)DTPA-bis(2-methoxyethylamide)] as a function of the relative viscosity in aqueous glycerol solutions (0, 10, 25, 40, and 50% by weight) at a Larmor frequency of 10.6 kHz (■) and 300 MHz (□).

To test the sensitivity of the paramagnetic contributions to the changes in translational and rotational reorientation correlation times, the spin–lattice relaxation rate constant was measured as a function of viscosity as shown in Fig. 6. Viscosity was varied by changing glycerol content from 0 to 50% by weight at constant metal-complex concentration. Measurements were made at a Larmor frequency of 10.6 kHz and at 300 MHz; the response is similar. At low values of viscosity, the relaxation rate increases with viscosity, but becomes less dependent on viscosity at relative values greater than 3.

4. Discussion

The data in Fig. 1, which shows three isosbestic points for each complex as the pH is varied between 3 and 8, demonstrate that the aqueous solutions of these chromium complexes are well represented as an equilibrium between two species differing by the addition of a proton. All spectra exhibit two absorption maxima, one near 550 nm, and one near 390 nm, which shift to longer wavelengths with increasing pH. The representative data in Fig. 2 shows that the UV–visible data are well described by single equilibrium constants that are summarized in Table 1. Similar results have been reported for $[CrEDTA]^-$ [26] and $[CrDTPA]^{2-}$ [27]. This earlier work suggested that the violet complex at low pH contains one water molecule in the first coordination sphere. The blue complex, which appears at high pH, was ascribed to the formation of the hydroxo complex by loss of a proton from a coordinated water molecule. The pH dependence of the water-proton-spin–lattice-relaxation-rate constants is consistent with this conclusion because the relaxation rate increases with decreasing pH to values larger than expected for a completely outer sphere or diffusional process when compared with other chromium(III) complexes [20].

For $[Cr(III)DTPA]^{2-}$, the small changes in absorbance associated with the proton equilibrium at pH ~6.2 (Fig. 2) are consistent with the data of Bucci et al. [27] and deprotonation of the ligand terminal backbone nitrogen [28] that is not involved directly in coordination to the chromium ion. There are no significant changes in the water-proton spin–lattice relaxation rate in this pH range, which supports the conclusion that the ionization event is not directly involved with the metal ion first coordination sphere. However, there is an increase in the transverse relaxation rate, which is consistent with chemical shift changes that may attend opening of hydrogen bond rings on the free ligand arm formed by each protonated outer nitrogen and the two attached carboxylic acid groups as well as pH dependent changes in the exchange rates. For the remaining three complexes, the optical density changes are significantly larger, which is consistent with changes in the first coor-

dination sphere that attend ionization of a coordinated water molecule. For [Cr(III)DTPA] there are changes in the water-proton spin–lattice and transverse relaxation rates above pH 7, as well as continuing changes in the absorbance, which have been identified with ionization of the coordinated water molecule with a pKa of 7.65.

For this set of complexes, the position of the proton equilibrium corresponding to the ionization of the first sphere water is sensitive to the net charge of the complex ion. The change in pK_a from 7.65 for the dianionic DTPA complex to 5.56 for the positively charged EDTA–bis-amide complex is consistent with the increased positive charge facilitating loss of a proton. For this set of complexes, the pK_a is a function of the charge on a complex ion as a proton donor, and there is a good linear relationship between the free energy obtained from the equilibrium constants and the net charge as shown in Fig. 7. This relationship supports the identification of net charge in the complexes at different pH values. Table 2 summarizes the pH ranges where the complexes are at least 90% in the charge form

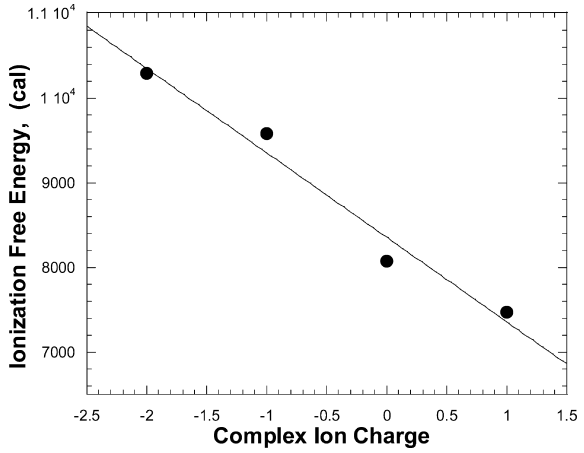

Fig. 7. The free energy for the first sphere water ionization process vs the net charge on the Cr(III) complex of EDTA, DTPA, and the bis-amides of both.

Table 2
pH values for specific complex

Complex ion	pH range	Charge
$[Cr(H_2O)(EDTA)]^-$	2.9–3.1	−1
$[Cr(EDTA)]^-$	5.0–6.2	−1
$[Cr(OH)(EDTA)]^{2-}$	>8	−2
$[Cr(H_2O)(HDTPA)]^-$	3.8–5.2	−1
$[Cr(H_2O)(DTPA)]^{2-}$	is not present at 90%	−2
$[Cr(OH)(DTPA)]^{3-}$	>8.6	−3
$[Cr(H_2O)(DTPA\text{-}bis\text{-}MEA)]^0$	3.0–5.0	0
$[Cr(H_2O)(DTPA\text{-}bis\text{-}MEA)]^-$	>7.0	−1
$[Cr(H_2O)(EDTA\text{-}bis\text{-}MEA)]^+$	3.0–4.7	+1
$[Cr(H_2O)(EDTA\text{-}bis\text{-}MEA)]^0$	>6.5	0

listed based on the values of the dissociation constants summarized in Table 1.

Although the EDTA and DTPA complexes were readily studied over a wide pH range, the bis-amide complexes of both parent ligands were not. Attempts to adjust the pH to values above ~6.8 led to time dependent spectrophotometric changes with time constants of several hours with the pH slowly dropping back to approximately 6.8. The slow rate is consistent with substitution at the metal center, perhaps by hydroxide, or catalyzed by hydroxide ion. It is not likely that the amides hydrolyze near neutral pH [28]. We did not study this change further because it makes application of bis-amide complexes as intermolecular probes in the alkaline pH range significantly problematic.

Most previous reports as well as the present spectrophotometric and magnetic relaxation studies indicate that there is a water molecule in the first coordination sphere of Cr(III) ion in complexes with EDTA, DTPA, and the bis-(amide) derivatives. However, there are several reports based on Raman spectroscopy, infrared spectroscopy, deuterium NMR, and circular dichroism studies, which suggest that the chromium(III) EDTA complex is coordinatively saturated by ligand donor atoms in slightly acidic media [26,29–31]. X-ray studies of the $K[Cr(III)EDTA]\cdot 2H_2O$ synthesized at pH 5 show octahedral coordination of the metal by the ligand [32]; however, studies of the complex prepared by the method of Hamm [33,34] showed a monoclinic crystal system with a quinquedentate ligand with a protonated carboxylate and one water molecule in the first coordination sphere. The water-proton spin–lattice-relaxation rate for solutions of this complex increases with decreasing pH to values above those expected for completely outer sphere or diffusional contributions to the relaxation rate. Thus, the magnetic relaxation data are consistent with increasing proton exchange with a first coordination sphere site on the metal complex. The presence or absence of a first coordination sphere water molecule makes no difference when the metal ion complex is used as a paramagnetic relaxation agent for molecules other than the solvent water. However, it is important to recognize the potential contribution of first coordination sphere water to the water proton relaxivity in cases where it may be convenient to normalize solute relaxation rate constants to the water proton relaxation rate constant [35,13].

The MRD profiles for these complexes are deceptively simple as shown in Fig. 3. We use the relaxation profile to aid in defining the chemistry, but do not attempt a quantitative parameterization of the data. We note that extensive work has been reported for the hexaaquachromium(III) complex, which is much more symmetrical than the present chelate complexes, and while far from simple, is less complicated than the present cases [17,36–41]. The data at higher pH values may be

fit using the relaxation equation of Freed [42]. Although the parameters of these fits are reasonable in the sense that the distances of closest approach and the diffusion constants are close to those found for more symmetrical chromium complexes studied as examples of outer sphere relaxation agents [20], we attribute no significance to the fit because the high field limit of the fitted line had to be adjusted by nearly a factor of two as if only part of the paramagnetic contribution disperses over the magnetic field range studied. More complete approaches have been proposed [23,24,43–45]; however, the parameters characterizing the electron spin relaxation rates for the several transitions in these complexes of effectively low symmetry are not known well. Thus, we do not attempt a quantitative analysis of the MRD data except to note that the water protons are not relaxed with high efficiency, which is consistent with a dominant contribution from translational or outer sphere effects. At low pH, the relaxation rates increase, which cannot be caused by changes in the outer sphere relaxation contributions because the effective relative translational diffusion constant should not change significantly. The increase in the relaxation rate constant with decreasing pH may reflect changes in the electron spin relaxation rates that characterize the complex electron spin manifold, which is also a function of magnetic field. As noted above, the increase is also consistent with increased proton exchange from first coordination sphere ligands. We note that the water-proton-spin–lattice-relaxation rates are all small at pH values above 7 although a coordinated hydroxide ion is usually proposed for this pH range. These magnetic relaxation data then require that the proton exchange from the species is negligible.

An interesting question is how the magnetic relaxation induced by these chromium complexes responds to their translational and rotational dynamics. When applied to interrogate protein charge distributions, for example, the translational dynamics of the metal complexes in the vicinity of the protein surface may be slower than in the bulk. The data on Fig. 6 for the bis-(amide) of [Cr(III)DTPA] show how the relaxation sensed in the water protons changes as the solution viscosity increases by a factor of 5 due to the addition of the glycerol up to 50% by weight. Although it is known that the rotational correlation time of simple metal complexes in aqueous glycerol solutions may not respond linearly with the viscosity increase [46] these data show that the paramagnetic contribution to the water-proton relaxation increases with increasing viscosity. This result is different from the dioxygen case [14], where the paramagnetic contribution to the water-proton relaxation rate is independent of viscosity over this range. We conclude that, although the electron spin relaxation rate constants may not be precisely defined in this system, the rotational and translational dynamics of the metal complex relative to the water or other co-solute contribute to the relaxation efficiency of the complex. Therefore, one may not assume that the paramagnetic relaxation is decoupled from the relaxation agent dynamics, particularly when applied to examination of protein or macromolecule systems.

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