Ternary Complexes between Cationic Gd^{III} Chelates and Anionic Metabolites in Aqueous Solution: An NMR Relaxometric Study

Mauro Botta,*[a] Silvio Aime,*[b] Alessandro Barge,[b] Gabriella Bobba,[b, c] Rachel S. Dickins,[c] David Parker,[c] and Enzo Terreno[b]

Abstract: The ^{1}H and ^{17}O NMR relaxometric properties of two cationic complexes formed by Gd^{III} with a macrocyclic heptadentate triamide ligand, L^1, and its N-methylated analogue, L^2, have been investigated in aqueous media as a function of pH, temperature and magnetic field strength. The complexes possess two water molecules in their inner coordination sphere for which the rate of exchange has been found to be sensibly faster for the N-methylated derivative and explained in terms of electronic effects (decrease of the charge density at the metal center) and perturbation of the network of hydrogen-bonded water molecules in the outer hydration sphere. The proton relaxivity shows a marked dependence from pH and decreases of about six units in the pH range 6.5 to 9.0. This has been accounted for by the displacement of the two water molecules by dissolved carbonate which acts as a chelating anion. The formation of ternary complexes with lactate, malonate, citrate, acetate, fluoride and hydrogenphosphate has been monitored by ^{1}H NMR relaxometric titrations at 20 MHz and pH 6.3 and the value of the affinity constant, K, and of the relaxivity of the adducts could be obtained. Lactate, malonate and citrate interact strongly with the complexes ($\log K \geq 3.7$) and coordinate in a bidendate mode by displacing both water molecules. Larger affinity constants have been measured for GdL^2. Acetate, fluoride and hydrogenphosphate form monoaqua ternary complexes which were investigated in detail with regard to their relaxometric properties. The NMR dispersion (NMRD) profiles indicate a large contribution to the relaxivity of the adducts from water molecules belonging to the second hydration shell of the complexes and hydrogen-bonded to the anion. A VT ^{17}O NMR study has shown a marked increase of the rate of water exchange upon binding which is explained by coordination of the anion in an equatorial site, thus leaving the water molecule in an apical position, more accessible for interactions with the solvent molecules of the second hydration shell which facilitate the exchange process.

Keywords: gadolinium · imaging agents · macrocyclic ligands · solvation · water exchange

Introduction

The study of selective anion binding by synthetic receptors represents an important subject of current research which promises several applications to different areas of chemistry.[1] In a recent paper we have discussed the results of a luminescence study on the reversible binding of bioactive oxyanions at chiral Eu and Tb centers.[2] The tricationic complexes of these metal ions with heptadentate ligands have two coordinated water molecules that can be partially or completely displaced by anion binding. This process is revealed by changes in the lifetime of the excited states and in the form of the emission spectra. These macrocyclic complexes resemble those currently used as contrast agents in magnetic resonance imaging (MRI), for example [Gd(DOTA)]$^-$ (DOTA = 1,4,7,10-tetraazacyclodocecane-1,4,7,10-tetraacetic acid) and GdHPDO3A (HPDO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclodocecane-1,4,7-triacetic acid),[3] and can be regarded as good models for the preparation of sensory systems for anionic metabolites. The corresponding Gd complexes possess the ability to catalyze the water proton relaxation rate that is modulated by binding to a given anion.[4] The anion can interact at the metal center in a monodentate or bidentate mode thus displacing one or

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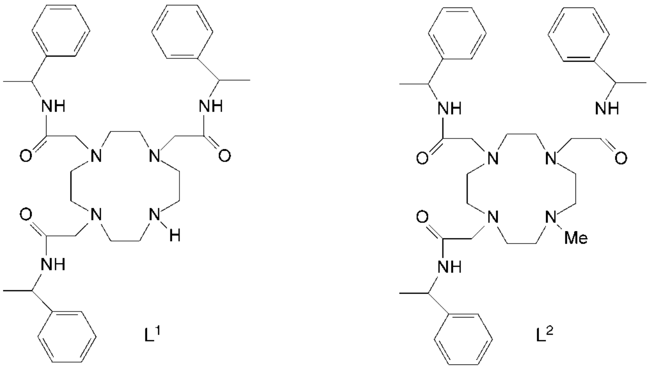
two of the coordinated water molecules.[2] The decrease of the hydration state of the paramagnetic complex is accompanied by a change in the water proton relaxation rate.[5] This is a function of the anion concentration and can be measured at a fixed magnetic field strength to yield the formation constant of the ternary complex.

Furthermore, in magnetic resonance spectroscopy (MRS) there is a growing interest in the possibility to monitor the concentration of metabolites (e.g. lactate, malonate) as a diagnostic tool for pathological states and tissue functionality.[6] The use of complexes of lanthanide cations (all but La, Gd, Lu) able to interact selectively with the metabolite of interest may allow the spectral discrimination of the intra- and extracellular concentrations, if the binding is strong enough and the ternary complexes are labile on the NMR timescale, thus enhancing the diagnostic information.[7] To reach this goal, a better understanding of the relationship between the chemical structure of the complexes and the binding affinity with the anionic substrates represents a preliminary step which can be conveniently tackled with NMR relaxometric techniques.

The ability of the $\mathrm{Ln^{III}}$ complexes of L^1 and L^2 (Scheme 1) to bind reversibly to oxy-anions (acetate, carbonate, citrate, lactate, malonate phosphate) in aqueous media has been previously reported (Ln = Eu, Tb);[2] recently some ternary complexes have been characterised by luminescence techniques, ^{1}H NMR spectroscopy and X-ray crystallography (Ln = Eu, Yb).[8] In this work we have examined in detail the relaxation properties of the corresponding $[\mathrm{GdL^1}]^{3+}$ and $[\mathrm{GdL^2}]^{3+}$ complexes, with particular attention to the dependence on pH, temperature and magnetic field strength, and the changes induced in these properties by formation of ternary complexes with a variety of oxy-anions of biological relevance.



Scheme 1. Chemical structure of the ligands.

Results and Discussion

From luminescence lifetime measurements and ^{1}H NMR spectra it was recently established that the $\mathrm{Eu^{III}}$ complexes of L^1 and L^2 possess in aqueous solution two water molecules ($q = 2$) in their inner coordination sphere and adopt a dominant monocapped square antiprismatic geometry.[2] Since no changes in the degree of hydration were measured for the corresponding $\mathrm{Tb^{III}}$ derivatives, we may safely assume that this holds also for $[\mathrm{GdL^1}]^{3+}$ and $[\mathrm{GdL^2}]^{3+}$. Neutral or anionic diaqua complexes of $\mathrm{Gd^{III}}$ have relaxivity values of about $8-10\,\mathrm{mM^{-1}\,s^{-1}}$,[3a, b] at 25 °C and 20 MHz, primarily determined by their molecular weight and not influenced by the water exchange rate, k_{ex}.[9] On the contrary, the corresponding monoaqua ($q = 1$) tetraamide derivatives were found to be characterised by an exceedingly long mean residence lifetime ($\tau_M = 1/k_{ex} = 8-19\,\mu s$ at 25 °C) of the coordinated water molecule which markedly limits their relaxivity near neutral pH and allows a detailed description of the structure and dynamics of the hydration spheres of these ion-paired complexes.[10, 11]

Under similar experimental conditions (25 °C, 20 MHz and pH 6.5) $[\mathrm{GdL^1}](\mathrm{CF_3SO_3})_3$ and $[\mathrm{GdL^2}](\mathrm{CF_3SO_3})_3$ have relaxivities of 8.5 and $9.1\,\mathrm{mM^{-1}\,s^{-1}}$, respectively. These values confirm the presence of two water molecules coordinated to the Gd center and indicate that these inner sphere water molecules exchange with bulk solvent at a rate significantly faster than that found for the corresponding tetraamide complexes.

Variation of relaxivity with pH: The pH dependence of r_{1p} often provides useful information on the kinetic stability of the complex towards acid-catalyzed dissociation,[3b, 9, 12] the occurrence of coordination equilibria,[13] the formation of hydroxo species or ternary adducts with carbonate,[14] and base-catalyzed prototropic exchange processes.[10, 11] In the case of the related monoaqua tetraamide $\mathrm{Gd^{III}}$ complexes, the relaxivity shows a characteristic behaviour with pH: whereas in the range 4 to 9 r_{1p} assumes a constant low value, due to the slow rate of water exchange that markedly limits the contribution of the inner sphere term, it shows a steep increase in both the acid and basic regions as a result of the catalysis of the prototropic exchange. In the case of the triamide derivatives the presence of two inner sphere water molecules with shorter residence lifetimes modifies profoundly the profile of r_{1p} with pH, as shown in Figure 1. The relaxivity, measured at 20 MHz and 25 °C, shows a constant value in the pH range 2 to 7 and then it markedly decreases to assume at pH > 8.5, a value of about $3.2\,\mathrm{mM^{-1}\,s^{-1}}$, about 30 % higher than that typical of $\mathrm{Gd^{III}}$ complexes with $q = 0$.[3a] This behaviour is the result of the displacement of the coordinated water molecules by formation of a ternary complex with the carbonate ($[\mathrm{HCO_3}]^-$ or, more likely, $[\mathrm{CO_3}]^{2-}$) present in the

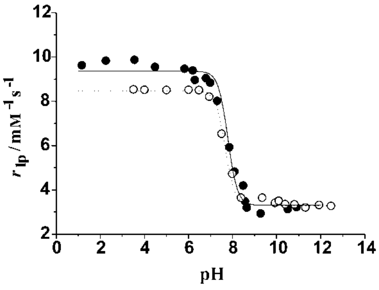


Figure 1. Plot of proton relaxivity r_{1p} at 20 MHz and 25 °C versus pH for $[\mathrm{GdL^1}]^{3+}$ (○) and $[\mathrm{GdL^2}]^{3+}$ (●).

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aerated aqueous solution. An analogous decrease of r_{1p} at high pH values is typically observed in Gd^{III} complexes with $q=2$, but the effect is significantly smaller.[4, 14, 15] The strength of the binding interaction depends on the overall charge of the complexes: negligible for anionic systems and increasingly strong for neutral and cationic complexes. In a related derivative with alaninate side arms the overall charge of the Gd^{III} complex is zero and the complete displacement of the inner sphere water molecules was observed only when an excess of $NaHCO_3$ was added to the solution.[16] Clearly, the presence of pendant carboxylate groups reduces the ease of access of the carbonate anion to the metal center by electrostatic forces. The Gd^{III} complexes of L^1 and L^2 possess a tripositive charge and show the highest affinity for carbonate binding reported so far. The experimental data in Figure 1 can be quantitatively analysed to obtain the apparent formation constants, K, defining the stability of the ternary complexes. Under the reasonable assumption that the binding involves primarily the CO_3^{2-} anion, the overall behaviour is the results of the presence in solution of the following two equilibria, characterized by the equilibrium constants K and β_2, respectively:

$$[GdL(H_2O)_2]^{3+} + CO_3^{2-} \overset{K}{\leftrightarrow} [GdL(CO_3)]^{+} + 2H_2O \qquad (1)$$

$$H_2CO_3 + 2H_2O \overset{\beta_2}{\leftrightarrow} CO_3^{2-} + 2H_3O^{+} \qquad (2)$$

In Equation (2) β_2 represents the product of K_1 and K_2, the dissociation constants of the carbonic acid. If we assume a 1:1 binding model, at each pH value the measured paramagnetic contribution to the relaxation rate, R_{1p}, is given by the sum of the relaxivities of the free, r_{1p}^{F}, and "bound", r_{1p}^{B}, complexes multiplied by their molar concentrations C_F and C_B:

$$R_{1p} = ([C_F]r_{1p}^{F} + [C_B]r_{1p}^{B}) \cdot 1000 \qquad (3)$$

Whereas r_{1p}^{F} is known and r_{1p}^{B} is the limiting value of the relaxivity at high pH, the concencentrations of the the free and "bound" complexes are pH dependent and can be expressed in terms of Equation (1) and (2), where C_T and L_T are the total concentrations of the paramagnetic complex (typically 0.4–1.0 mM) and carbonated species in solution, respectively:

$$[C_B] = \frac{(K\beta_2 L_T + K\beta_2 C_T + [H^+]^2 + \beta^2) - \sqrt{(K\beta_2 L_T + K\beta_2 C_T + [H^+]^2 + \beta^2)^2 - 4K\beta_2^2 L_T C_T}}{2K\beta_2} \qquad (4)$$

From the known values of the solubility of CO_2 in water at 25 °C L_T can be estimated to be of the order of 1×10^{-3} M. However, due to the difficult evaluation of this parameter under the experimental conditions used its actual value was calculated by the fitting procedure. In Equations (3)–(4) β_2 (2.07×10^{-17}), C_T and r_{1p}^{F} are known and thus the fitting procedure yields the K and r_{1p}^{B} values. For $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ the calculated values of K are 3.2 (±0.2) × 10^4 and 3.0 (±0.2) × 10^4 M, respectively. These constants are quite high, as expected for the interaction of a small, highly charged anion with tripositive complexes.

The relaxivity of the ternary complexes, about 2.4 mM^{-1} s^{-1}, is of the same order of magnitude as that typically measured for structurally similar, anionic or neutral Gd^{III} complexes with $q=0$ or calculated from the analysis of the NMRD profiles, ≈2.5 mM^{-1} s^{-1}.[17, 18] However, in the case of the related tetraamide complexes the outer sphere relaxivity is only 1.9 mM^{-1} s^{-1}.[11] In the case of these cationic complexes the diffusing water molecules approach the complex to a minimum distance which is longer than for anionic or neutral complexes. This increase (ca. 25% at 20 MHz and 25 °C) is probably the result of an additional contribution from water molecules localised sufficiently close the paramagnetic center through hydrogen-bonding interaction with the carbonate anion (see below).

Variable temperature ^{17}O NMR spectroscopy: A qualitative analysis of the relaxivity of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ suggested mean residence lifetimes for the inner sphere water molecules that are shorter than those typical of the related tetraamide complexes. A precise assessment of τ_M has been obtained from the measurement of the water ^{17}O NMR transverse relaxation rate, R_2, as a function of temperature, at 9.4 T and pH 6.3. The experimental profiles, measured for a 15 mM solution of the complexes, are shown in Figure 2 and reveal significant differences between the two complexes.

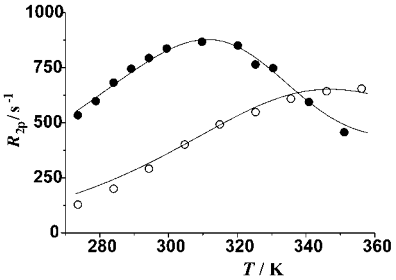

Figure 2. Plots of the paramagnetic contribution to the ^{17}O transverse relaxation rate of water, R_{2p}, versus temperature for 15 mM aqueous solutions of $[GdL^1]^{3+}$ (○) and $[GdL^2]^{3+}$ (●) at pH 6.3 and 9.4 T.

Whereas for $[GdL^2]^{3+}$ the data points define a bell-shaped curve which includes a maximum centred at 310 K, for $[GdL^1]^{3+}$ R_2 increases over the entire range of temperatures and shows an apparent maximum around 350 K. This shift towards higher T of about 40 K of the maximum of the curve is consistent with a slower rate of exchange of the coordinated water molecules. This qualitative information has been confirmed by fitting the experimental profiles to the Swift and Connick equations following a well established method that yields reliable estimates of the activation parameters for the water exchange and the electronic relaxation times of the Gd^{III} ion (Table 1).[9, 19] Furthermore, it must be noted that we implicitly assumed in the analysis that the two water molecules exchange with the bulk at the same rate. This assumption is corroborated by a recent crystallographic work in which Eu–water distances of 2.418 and 2.421 Å are reported for the Eu-diaqua complex of a cyclen-triamide of CH2CONMe$_2$ (triflate salt).[20]

Table 1. Best-fit parameters obtained from the analysis of the ^{17}O-R_{2p} versus T profile of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ at 9.4 T, 25 °C and pH 6.3.

	$[GdL^1]^{3+}$	$[GdL^2]^{3+}$	GdDO3A[a]
k_{ex}^{298} [$\times 10^6\,s^{-1}$]	0.66 ± 0.1	1.7 ± 0.2	6.3
ΔH_M [$kJ\,mol^{-1}$]	17 ± 0.4	11 ± 0.3	44
Δ^2 [$s^{-2} \times 10^{19}$]	3.8 ± 0.5	3.8 ± 0.4	3.9
τ_V [ps]	20 ± 2	20 ± 3	16
ΔH_V [$kJ\,mol^{-1}$]	33 ± 2	51 ± 3	1.8
ΔH_R [$kJ\,mol^{-1}$]	20[b]	20[b]	18

[a] Data from ref. [14]. [b] Fixed in the analysis.

As expected, the calculated τ_M values are sensibly different: 0.59 (± 0.06) and 1.5 (± 0.2) μs for $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$, respectively. Such a difference, which also accounts for the slightly different relaxivities of the complexes (see below), could arise from their different solution structures. It has been clearly shown that in the case of the tetraamide derivatives, as well as in other Gd^{III} complexes with DOTA-like ligands, the rate of water exchange k_{ex} is a function of the relative population of the two conformational isomers present in their aqueous solutions, characterised by square antiprismatic (SAP) and twisted square antiprismatic (TSAP) geometries.[21] In the present case, this explanation does not find support in the ^1H NMR spectra of the corresponding Eu^{III} complexes which appear rather similar and indicative of the presence in solution of a major species, consistent with the SAP isomeric form.[2] Moreover, the electronic relaxation parameters of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ are nearly identical (Table 1) and this represents a further indication of very similar coordination geometries. Alternatively, the faster water exchange rate of the N-alkylated complex might be attributed to:

i) an electronic effect of the methyl group, resulting in an increased basicity of the N atom and a decreased charge density at the metal center, which should labilize the bound water molecules;
ii) a perturbation of the network of the second hydration sphere water molecules. The tricationic mono- and di-aqua complexes form ion-pairing interactions with the triflate anions that favour the formation of a strongly hydrogen-bonded array of water molecules that involves also the inner sphere water(s), as clearly indicated in the solid state by X-ray structural studies.[22] It follows that the rate of exchange will be dependent on the structure and influenced by any perturbation of these second hydration sphere water molecules. The presence of the hydrophobic methyl group in $[GdL^2]^{3+}$ may perturb the ordered arrangement of hydrogen-bonded water molecules near the paramagnetic center and may not inhibit either the departure of the leaving and/or the approach of the incoming water(s).

Support for this latter interpretation comes from the observation of a marked dependence of k_{ex} on the nature of the counteranion in related tetraamide complexes.[23] On the other hand, higher values of k_{ex} have been measured for N-alkylated derivatives of the neutral complex GdDO3A where only inductive effects of the substituents can play a significant role.[24] It is quite possible that both these effects are operative in the case of the cationic triamide complexes.

NMRD profiles: The $1/T_1$ water proton NMRD profiles of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ have been measured, at 25 °C and pH 6.3, over the interval of proton Larmor frequencies from 0.01 to 20 MHz (Figure 3). As shown in Figure 3, $[GdL^2]^{3+}$ has a relaxivity higher than $[GdL^1]^{3+}$ over the entire range of frequencies. The experimental data have been analysed with an iterative least-square fitting procedure by assuming contributions to the relaxivity of inner- and outer hydration spheres water molecules and using the standard set of equations.[3b, 9] In the analysis the following parameters were kept fixed: the hydration number $q = 2$, the distance of closest approach of the outer sphere solvent proton nuclei to the Gd^{III} ion $a = 3.8$ Å, the relative diffusion coefficent $D = 2.24 \times 10^5\,cm^2\,s^{-1}$ and τ_M (the latter values were taken from the VT ^{17}O NMR study). The other parameters were considered as adjustable within a range of values typical of this class of macrocyclic Gd^{III} complexes: $r \approx 2.96-3.16$ Å ($r \mathrel{\hat{=}}$ Gd–H distance of the inner sphere water molecule(s), $\tau_R \approx 70-140$ ps ($\tau_R \mathrel{\hat{=}}$ reorientational correlation time), $\tau_V \approx 1-25$ ps ($\tau_V \mathrel{\hat{=}}$ correlation time characterizing the electron spin relaxation) and $\Delta^2 \approx 0.5-50 \times 10^{19}\,s^{-2}$ (Δ^2 $<M\varepsilon>$ trace of the square of the transient zero-field splitting tensor).[3a, 11]

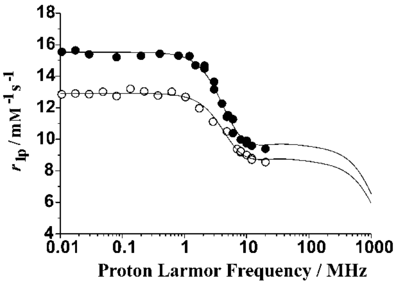

Figure 3. $1/T_1$ NMRD profiles of $[GdL^1]^{3+}$ (○) and $[GdL^2]^{3+}$ (●) at pH 6.3 and 25 °C. The solid curves through the data points are calculated with the parameters reported in Table 2.

The best-fit parameters are reported in Table 2, and are compared with the corresponding values of GdDO3A, a structurally related neutral complex. The results unambigously indicate that the difference in the NMRD profiles of the two complexes primarily derive from their different values of τ_M, thus confirming the previous qualitative conclusions. In

Table 2. Best-fit parameters obtained from the analysis of the $1/T_1$ NMRD profiles of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ at 25 °C and pH 6.3.

	$[GdL^1]^{3+}$	$[GdL^2]^{3+}$	GdDO3A[a]
Δ^2 [$s^{-2} \times 10^{19}$]	3.8 ± 0.1	3.9 ± 0.3	3.9
τ_V [ps]	20 ± 2	19 ± 0.5	16
τ_R [ps]	125 ± 5	120 ± 7	66
r [Å]	3.02 ± 0.06	3.02 ± 0.04	3.15[b]
q	2[c]	2[c]	2[b]
a [Å]	3.8[c]	3.8[c]	3.8
D ($cm^2\,s^{-1} \times 10^5$)	2.24[c]	2.24[c]	2.24

[a] Data from ref. [14]. [b] In solution the complex has been reported to exist as a mixture of a dominant species with $q = 2$ and a minor species with $q = 1$.[31] Therefore, the mean hydration number may be slightly less than two. Since we obtained the value of the ratio q/r^6 from the fitting procedure, by imposing an integer q value of two, the derived apparent distance is somewhat longer than usual. [c] Fixed in the analysis.

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$[GdL^1]^{3+}$ the exchange correlation time is sufficiently long ($\tau_M = 1.5\,\mu s$) so as to limit the inner sphere component of the relaxivity, as previously observed for the Gd^{III} complexes with bisamide derivatives of DTPA.[25] For $[GdL^2]^{3+}$ τ_M has a value about three times lower and this effect is negligible.

Formation of ternary complexes: The stepwise displacement of water molecules from the inner coordination sphere of $[GdL^1]^{3+}$ and $[GdL^2]^{3+}$ results in a significant decrease in the relaxivity of the complexes. Thus, the binding of oxy-anions of biological relevance is easily revealed and can be conveniently investigated by monitoring the variation of the longitudinal water proton relaxation rate R_1, at a fixed temperature and pH, following the addition of increasing amount of the substrate to a solution of the complex. The titration curve thus obtained can be analyzed according to the proton relaxation enhancement (PRE) method that allows the assessment of the apparent affinity constant K and of the relaxivity of the ternary complex, r_{1p}^{b}.[26] Typically, a 0.2 mM solution of the complex (corresponding to an observed relaxation rate of $\approx 2.2\,s^{-1}$) is titrated with the appropriate anion at pH 6.3, to avoid any interference from competitive binding of dissolved carbonate, and the changes of R_1 are measured at 20 MHz and 25 °C. Representative data for the interaction of $[GdL^1]^{3+}$ with lactate and malonate and the corresponding best-fit curves are shown in Figure 4 which evidences a different binding affinity for the two anions but a very similar relaxivity values of the ternary complexes. Analysis of the data indicates that malonate ($\log K = 4.30 \pm 0.03$) interacts more strongly than lactate ($\log K = 3.70 \pm 0.04$) but the resulting ternary complexes are characterized by identical relaxivity ($2.8 \pm 0.1\,mM^{-1}s^{-1}$). This is not surprising if we consider that both anions coordinate in a bidentate mode by displacing the two inner sphere water molecules and then give rise to complexes with $q = 0$. Such behaviour follows that illustrated above for carbonate and has been observed also for citrate ($\log K = 4.36 \pm 0.2$; $r_{1p}^{b} = 3.0 \pm 0.3\,mM^{-1}s^{-1}$).

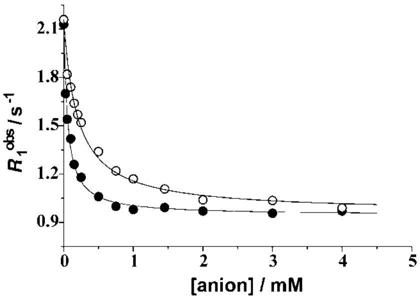


Figure 4. Plot of the observed proton relaxation rate of a 0.2 mM solution of $[GdL^1]^{3+}$ as a function of the concentration of added lactate (○) and malonate (●) at 20 MHz, 25 °C and pH 6.3, showing the fit to the experimental data points.

Analogous experiments were carried out in the case of $[GdL^2]^{3+}$ and stronger interaction with malonate ($\log K = 4.62 \pm 0.10$) and, in particular, with lactate ($\log K = 4.41 \pm 0.04$) was observed. The general tendency of the binding constants to increase following N-methylation was also observed in the case of the Eu^{III} and Tb^{III} derivatives. The ternary complexes of $[GdL^2]^{3+}$ present relaxivity values lower than those of the corresponding complexes of $[GdL^1]^{3+}$ ($r_{1p}^{b} = 2.0-2.2\,mM^{-1}s^{-1}$). The differences (20–30%) are not negligeable and, as in the case of the water exchange rate, may be related to a different structure (i.e., number, distance and residence lifetime of H-bonded water molecules) of the second hydration sphere of the ternary complexes.

Unlike the examples discussed so far, other anions can displace a single water molecule and bind the complexes in a monodentate manner, as clearly evidenced by a measurement of the hydration state of the Eu^{III} and Tb^{III} derivatives by luminescence.[2] This appears to be the case for acetate, phosphate and fluoride. In fact, from a titration experiment of a solution of $[GdL^1]^{3+}$ with sodium acetate (20 MHz, 25 °C, pH 6.3) we have calculated a much weaker binding constant ($\log K = 2.26 \pm 0.12$) and a markedly higher limiting relaxivity for the ternary complex ($r_{1p}^{b} = 4.7 \pm 0.1\,mM^{-1}s^{-1}$). This value of relaxivity is consistent with the presence of one coordinated water molecule and is comparable to the r_{1p} values usually measured for other Gd^{III} polyaminopolycarboxylate complexes with $q = 1$.[3a,b] On the other hand, upon addition of a large excess of fluoride and phosphate to a solution of $[GdL^1](CF_3SO_3)_3$, under identical experimental conditions, the relaxivity decreases from 8.5 to 6.4 and $8.2\,mM^{-1}s^{-1}$, respectively. These r_{1p}^{b} values are much higher (36 and 74% for F^- and HPO_4^{2-} (or $H_2PO_4^-$), respectively) than the value measured for acetate and could hardly be associated with a $q = 1$ systems, without the support of the independent determination from luminescence data for the isostructural complexes of Eu^{III} and Tb^{III}. Clearly, the characterization of the hydration state by ^1H relaxometric data alone can lead to erroneous results, as different mechanisms contribute to r_{1p} whose relative importance is often difficult to evaluate quantitatively. Whereas the typical contributions to the relaxivity arising from the modulation of the electron–nucleus dipolar interaction and involving the water molecule(s) coordinated to (inner sphere term) and diffusing by (outer sphere term) the paramagnetic metal ion are quite adequate to account for the r_{1p}^{b} value of the ternary complex with acetate, these fail in the case of the isostructural complexes $[Gd(L^1)(F)(H_2O)]^{2+}$ and $[Gd(L^1)(HPO_4)(H_2O)]^{+}$. For these latter, a further contribution is present which arises from the well recognized ability of fluoride and phosphate anions to promote strong hydrogen-bonding interactions with water molecules. If the lifetime of these hydrogen bonds is longer than the diffusional correlation time of the outer sphere solvent molecules, then the contribution to the relaxivity of the second sphere water molecules can be considerable and comparable to that arising from the inner sphere water(s).[27] Relevant examples include the increase of relaxivity observed when the iron-bound water molecule is replaced by a fluoride ion in methemoglobin[28] and the case of GdDOTP, a macrocyclic complex with four coordinated phosphonate groups, that has *no bound water molecules* but a relaxivity quite similar to that of the mono-aqua carboxylate analogue, GdDOTA.[27]

The NMRD profiles of $[Gd(L^1)(F)(H_2O)]^{2+}$ and $[Gd(L^1)(HPO_4)(H_2O)]^{+}$ have been measured at 25 °C and are shown in Figure 5 compared with the corresponding

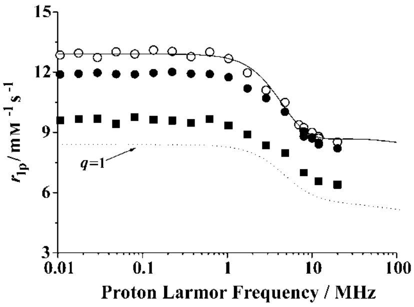


Figure 5. $1/T_1$ NMRD profiles of $[Gd L^1]^{3+}$ (○) and its ternary complexes with phosphate (●) and fluoride (■) at pH 6.3 and 25 °C. The lower dotted curve represents a simulation, with the parameters of Table 2, of the NMRD profile for the hypothetical complex of $[GdL^1]^{3+}$ with $q = 1$.

profile of the di-aqua complex and the calculated profile of the hypothetical species $[Gd(L^1)(H_2O)]^{3+}$. In spite of the loss of one coordinated water molecule, the relaxivity of the ternary complex with phosphate is quite similar to that of the free complex over the entire range of magnetic field strengths. In the case of $[Gd(L^1)(F)(H_2O)]^{2+}$ the effects are much less pronounced and the experimental data are intermediate between those of $[Gd(L^1)(HPO_4)(H_2O)]^+$ and the simulated profile of the free complex with $q = 1$.

It is reasonable to assume that the coordinated fluoride ion interacts with a single water molecule, whereas the phosphate anion could promote multiple hydrogen-bonding interactions that give a stronger relaxivity enhancement. Under the hypothesis that the electronic parameters do not change significantly upon binding to F^- and that the increase of r_{1p}^b over the value found for the acetate adduct is fully attributable to one second hydration sphere water molecule, then from the NMRD parameters of Table 2 we can estimate its average distance from the Gd ion: $r = 3.5–3.8$ Å. By considering a Gd–F distance of about 2.3 Å, as typical of a coordinated carboxylate group, this result gives for the F–H bond length a rough estimation of about 1.2–1.5 Å, for a linear geometry.

Finally, the presence of a coordinated water molecule in the ternary complexes with monodentate anions prompted us to measure its rate of exchange with the goal of assessing if changes of k_{ex} were detectable following the interaction with the anions and the variation of q from 2 to 1. An increase of k_{ex} is expected to be promoted by the decreased overall positive charge of the complexes and by the increased steric interaction at the water coordination site that could destabilize the nine-coordinate ground state thus diminishing the activation energy for the exchange (dissociative) process. A VT ^{17}O NMR study was undertaken for the three ternary complexes at pH 6.3 and 9.4 T (2.1 T for $[Gd(L^1)(F)(H_2O)]^{2+}$) and the experimental data, fitted to $q = 1$ species, gave for τ_M the following values (at 25 °C): 0.74 ± 0.05, 0.56 ± 0.04 and 0.16 ± 0.02 μs for the adducts with F^-, HPO_4^{2-} and CH_3COO^-, respectively.[29] The experimental profiles of the ternary complexes with phosphate and acetate are compared in Figure 6 with that of $[GdL^1]^{3+}$ (triflate salt) and clearly show the decrease in R_2 occurring on passing from the $q = 2$ to the $q = 1$ species and the concomitant shift to low temperatures of the maximum of the curve.

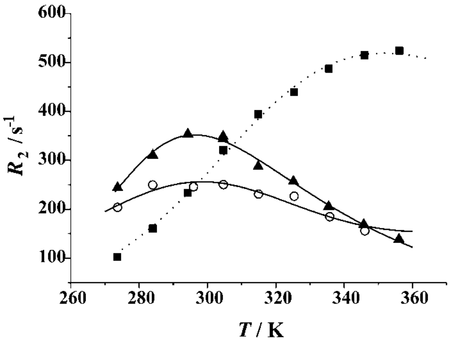
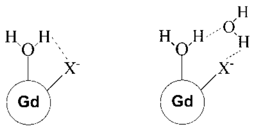


Figure 6. Plots of the paramagnetic contribution to the ^{17}O transverse relaxation rate of water, R_{2p}, versus temperature for 12 mM solutions of $[GdL^1]^{3+}$ (■) and its ternary complexes with acetate (▲) and phosphate (○) at pH 6.3 and 9.4 T.

The increase of k_{ex} is remarkable and particularly evident in the case of the adduct with acetate. An increase of the rate of water exchange of one order of magnitude may be too large to be accounted for simply by the decrease of the charge of the complex from +3 to +2. A possible explanation could be a different rate of exchange for the coordinated water molecules (axial and equatorial), being $k_{ex}(\text{ax}) > k_{ex}(\text{eq})$. If this is the case, then, when the formation of a ternary complex with a monodentate anion involves the displacement of the water molecule in the equatorial plane, a markedly higher rate of exchange of the remaining axial water molecule will be measured in the adduct. Conversely, similar k_{ex} values for the free complex and the adduct would suggest that the binding of the anion is in the axial position. The X-ray crystal structure of a related compound suggests that in the free complexes the Ln–O_w distances are quite similar and thus the enthalpic contribution due to the Gd–O_w bond breaking in the $\Delta G^\#$ for the exchange process is similar for both water molecules.[20] However, the axial water is located in a more open site with more hydrogen bonded water molecules to assist in the exchange process, whereas the equatorial position is more hydrophobic, less open and therefore water exchange is presumably less easy. In other words, a different rate of exchange could arise from the different accessibility of the axial and equatorial water molecules to water molecules of the second hydration sphere.[30] Then, the large increase of k_{ex} measured for the ternary adduct of acetate (and, to a lesser extent, of fluoride and phosphate) could be the indication of the coordination of the anion in the equatorial plane. There is not direct X-ray evidence of this but some support comes from a recent structural, luminesce and NMR study on the ternary complexes formed by several anions and cationic di-aqua Eu^{III}, Gd^{III} and Yb^{III} complexes. By the analysis the paramagnetic dipolar shifts (Yb and Eu), which are predominantly determined by the polarisabiliy of the axial ligand, it is suggested that the monoaqua ternary complexes with hydrogenphosphate and fluoride present an apical water molecule.[8] Strictly analogous considerations hold for the ternary adduct with acetate and thus we may conclude that the present results point to suggest a common solution structure, where the anion occupies a site in the equatorial plane and the water molecule a capping position above this plane.

Finally, the relatively slower water exchange rate of the ternary complexes with fluoride and phosphate with respect to acetate also suggests a possible hydrogen-bonding interaction between the coordinated water molecule and the anion in the adducts, either directly or involving an intermediate water molecule (Scheme 2).



Scheme 2. Schematic representation of the possible interaction modes between the coordinated water molecule and a monodentate anion in the ternary complexes.

Conclusion

The di-aqua cationic complexes interact strongly with a variety of oxy-anions and the formation of ternary complexes is accompanied by large variations of the relaxometric properties of the complexes. The anions that bind in a bidentate mode (carbonate, lactate, malonate, citrate) displace two water molecules and form adducts with $q = 0$, characterized by relaxivity values about 60% lower than for the free complex. Other anions interact in a monodentate manner (fluoride, phosphate, acetate) and the corresponding ternary complexes maintain one coordinated water molecule. In these latter cases the changes of the relaxivity are modulated by the ability of the anion to promote hydrogen-bonding interactions with water molecules outside the inner coordination sphere of the Gd^{III} ion. The hydrogen-bonding interactions may involve also the coordinated water molecule and markedly influence its rate of exchange. Furthermore, evidence has been gained of a selective coordination of these anions in the equatorial plane of the complex rather than in the axial position.

The N-methylation of the ligand ring nitrogen has little influence on the relaxometric properties of the complex but exerts a profound effect on the affinity constant for the anions. In general, the K values for $[GdL^2]^{3+}$ are notably higher than for $[GdL^1]^{3+}$. This result, which may be associated with differential complex hydration, highlights the important fact that minor structural variation of the ligand may modulate in a significant manner the affinity of the complex for the oxy-anions. These data suggest the need of a systematic study to investigate in further detail the nature of the interaction and its dependence from the structural properties of the complexes. The final goal is the preparation of paramagnetic probes for oxy-anions of biological relevance endowed with high selectivity for diagnostic applications.

Experimental Section

All reagents and inorganic salts were purchased from Sigma-Aldrich and Fluka and used without further purification. The ligands L^1 and L^2 were synthesized by following procedures to previously reported,[2] and the corresponding Gd^{III} complexes were prepared according to the method described in the same paper for the analogous derivatives of Eu^{III} and Tb^{III}.

Water proton relaxivity measurements: The water proton $1/T_1$ longitudinal relaxation rates (20 MHz, 25 °C) were measured with a Stelar Spinmaster Spectrometer (Mede, Pv, Italy) on 0.1–1.5 mM aqueous solutions of the complexes. ^1H spin-lattice relaxation times T_1 were acquired by the standard inversion-recovery method with typical 90° pulse width of 3.5 ms, 16 experiments of 4 scans. The reproducibility of the T_1 data was ±1%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty of 0.1 ± °C). In a typical titration experiment several (8–12) aqueous solutions at pH 6.3 of the paramagnetic complex (0.2–0–3 mM) were prepared containing different concentrations of the anionic species (0–0.2 M) and the water proton relaxation rate of each solution was measured at 25 °C. The starting pH was adjusted by either HCl or KOH. Moreover, the pH of the solutions was controlled before and after the measurement. The $1/T_1$ nuclear magnetic relaxation dispersion profiles of water protons were measured over a continuum of magnetic field strength from 0.00024–0.28 T (corresponding to 0.01–12 MHz proton Larmor frequency) on the fast field-cycling Stelar Spinmaster FFC relaxometer installed at the "Laboratorio Integrato di Metodologie Avanzate", Bioindustry Park del Canavese (Colleretto Giacosa, To, Italy). The relaxometer operates under complete computer control with an absolute uncertainty in the $1/T_1$ values of ±1%. Additional data points at 20 and 90 MHz were recorded on the Stelar Spinmaster and on a JEOL EX-90 spectrometers, respectively. The concentration of the aqueous solutions of the complexes utilized for the measurements was in the range 0.5–1.0 mM.

VT ^{17}O relaxation measurements: Variable-temperature ^{17}O NMR measurements were recorded on a JEOL EX-90 (2.1 T) spectrometer, equipped with a 5 mm probe, by using a D_2O external lock. Experimental settings were: spectral width 10000 Hz, pulse width 7 μs (90°), acquisition time 10 ms, 1000 scans and no sample spinning. Aqueous solutions of the paramagnetic complexes (pH 6.3) containing 2.6% of ^{17}O isotope (Yeda, Israel) were used. The observed transverse relaxation rates (R_2) were calculated from the signal width at half height.

Theory: The theory behind the analysis of the water ^1H and ^{17}O relaxation properties can be found in several recent publications.[3a, 9, 14, 19]

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