

Modulation of the water exchange rates in [Gd–DO3A] complex by formation of ternary complexes with carboxylate ligands

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Ternary complexes of formula [Gd–DO3A–L–(H₂O)] (where L is a carboxylate-containing ligand) display exchange lifetimes of the metal-coordinated water that can be modulated as a function of L.

Paramagnetic Gd(III) chelates are currently under intense scrutiny for their use as contrast agents (CAs) for magnetic resonance imaging (MRI).¹ Their efficiency is usually expressed in terms of relaxivity (r_1), which represents the relaxation enhancement of the water protons at mmol concentration of the paramagnetic agent. The relaxivity is the result of a complex interplay of several structural, dynamic and electronic parameters. Recently, it has been shown that the exchange lifetime of the coordinated water, τ_M , may have a key role in determining the relaxivity of a Gd(III) complex.² In fact, it has been found that τ_M covers a whole range of values ranging from few ns in the aqua-ion³ up to several μ s in complexes of octadentate neutral macrocyclic ligands.⁴ In systems containing one coordinated water only ($q = 1$) it has been shown that the exchange process occurs through a dissociative mechanism and the relative exchange rate is dependent upon the energy difference between the nona-coordinate ground state and the octa-coordinate transition intermediate.⁵ Thus, an increased encumbrance in the ground-state structure results in a smaller energy jump to reach the intermediate state with a consequent increase in the water exchange rate.⁶ Moreover, it has been found that in DOTA-like systems, the isomer possessing a twisted antiprismatic structure displays an exchange rate of the bound water that is *ca.* 50 times faster than that observed for the isomer endowed with a more compact square antiprismatic geometry.^{7,8} Thus, minor structural variations may have remarkable effect on the exchange lifetime of the coordinated water in lanthanide(III) chelates.

As far as the use as CA for MRI is concerned, optimal values of τ_M for the attainment of high relaxivities [once the Gd(III) is part of a slowly moving macromolecular substrate] fall in the range of few tenths of ns.⁹ Thus, it is relevant to gain more insight into the structural factors which are responsible of the exchange rate of the coordinated water. Recently, it has been shown that coordinatively unsaturated Gd(III) chelates with $q > 1$ are able to readily form ternary complexes with suitable ligands such as carboxylates. The formation of such adducts involves the replacement of one or more Gd-bound water molecules by the ligand.^{10,11} Herein, we show that the mixed complexes [Gd–L–L'–(H₂O)], where L is the heptadentate ligand DO3A¹² and L' is a carboxylate-containing ligand, display exchange lifetimes of the metal coordinated water which can be modulated as a function of the added ligand.

The formation of ternary adducts has been followed by analyzing the changes of relaxivity upon L' concentration. The [Gd–DO3A] complex has a relatively high r_1 value (6.0 mM⁻¹ s⁻¹ at 20 MHz and 25 °C¹⁴) as the largely dominant isomer¹³

displays two metal-bound water molecules (average q value = 1.8). Upon adding sodium propionate or L-alanine to a solution of [Gd–DO3A], a progressive decrease of r_1 was observed (Fig. 1). This effect is related to the replacement of one Gd-coordinated water molecule by the entering ligand. The fitting of these data according to the PRE (proton relaxation enhancement) theory¹⁵ allowed us to estimate either the relaxivities of the ternary complexes, which are consistent for Gd(III) chelates of the expected molecular size and $q = 1$, or the binding constant between the paramagnetic complex and the ligand (Table 1).

As a further check, we evaluated, by luminescence measurements, the metal complex–substrate affinity as well as the hydration state of the resulting mixed complexes. The comparison between the rate constants of the luminescence decay for [Tb–DO3A] adducts in H₂O and D₂O assures about the presence of a residual lanthanide bound water molecule, whereas by looking at the changes in the intensity of the emission spectra at 545 nm [corresponding to the $\Delta J = 1$ transition of Tb(III) ion] upon the addition of the substrates, K_A values in agreement with those measured by the PRE analysis were obtained (Table 1).

The exchange rate of the metal bound water molecule in this class of paramagnetic complexes may be conveniently deter-

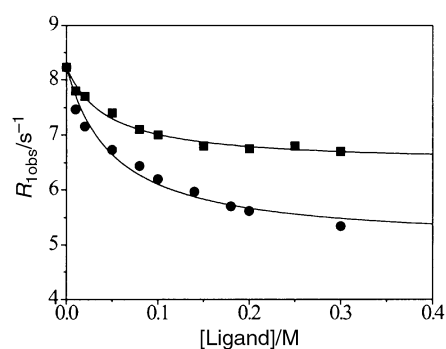


Fig. 1 PRE titration of a 1.15 mM solutions of [Gd–DO3A–(H₂O)₂] with L-alanine (■) and sodium propionate (●); 0.235 Tesla, pH 7, 25 °C.

Table 1 Relaxivities, 1:1 association constants and hydration numbers (q) for [Ln–DO3A–L'–(H₂O)] (Ln = Gd or Tb) ternary complexes (20 MHz, 25 °C)

L'	[Gd–DO3A–L']		[Tb–DO3A–L']	
	$r_1/\text{mM}^{-1} \text{ s}^{-1}$	K_A/M^{-1}	K_A/M^{-1}	q
Propionate	3.7	17	25	1.0
L-Alanine	4.7	19	< 50	1.35

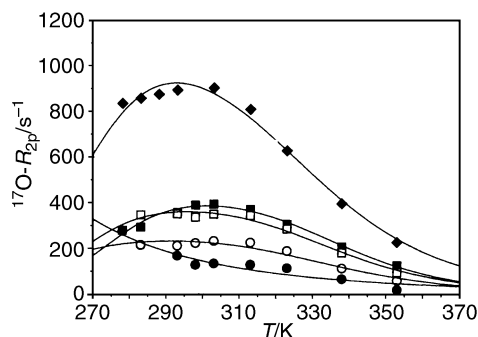


Fig. 2 $^{17}\text{O}-R_{2p}$ vs. T of 27.3 mM solutions of $[\text{Gd}-\text{DO3A}-(\text{H}_2\text{O})_2]$ (◆), $[\text{Gd}-\text{DO3A}-\text{propionate}-(\text{H}_2\text{O})]^-$ (●), $[\text{Gd}-\text{DO3A}-\text{alanine}-(\text{H}_2\text{O})]$ (■), $[\text{Gd}-\text{DO3A}-\alpha\text{-aminobutyrate}-(\text{H}_2\text{O})]$ (□) and $[\text{Gd}-\text{DO3A}-\beta\text{-aminobutyrate}-(\text{H}_2\text{O})]$ (○); 7.1 Tesla, pH 7.

mined by measuring the temperature dependence of water $^{17}\text{O}-R_{2p}$.

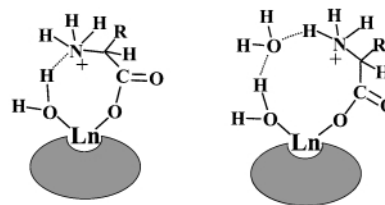
In fact, the R_{2p} values are basically a function of q , τ_M and the electronic relaxation times of the metal ion T_{ie} ($i = 1, 2$). Two different regimes can be met, one occurring at low temperatures, in which the changes in R_{2p} are mainly determined by τ_M (slow exchange region) and one at higher temperatures, essentially dominated by T_{ie} (fast exchange region). For many systems displaying τ_M in the range of few hundreds of nanoseconds R_{2p} displays a bell-shaped curve (as shown in Fig. 2 for $[\text{Gd}-\text{DO3A}-(\text{H}_2\text{O})_2]$ at 7.05 T, $\tau_M^{298} = 80$ ns). The $^{17}\text{O}-R_{2p}$ values for the ternary complexes $[\text{Gd}-\text{DO3A}-\text{propionate}-(\text{H}_2\text{O})]^-$ and $[\text{Gd}-\text{DO3A}-\text{alanine}-(\text{H}_2\text{O})]$ are significantly smaller than those of the parent chelate owing to the reduction of q in the ternary adduct. Interestingly, the observed behaviour is consistent with the occurrence of a fast exchange of the metal bound water molecule in the case of $[\text{Gd}-\text{DO3A}-\text{propionate}-(\text{H}_2\text{O})]^-$ ($\tau_M^{298} = 8$ ns). Thus on going from $[\text{Gd}-\text{DO3A}-(\text{H}_2\text{O})_2]$ to $[\text{Gd}-\text{DO3A}-\text{propionate}-(\text{H}_2\text{O})]^-$ there is a considerable shortening of the residence lifetime of the inner sphere water molecule. Furthermore, the observed τ_M is much shorter than that of $[\text{Gd}-\text{DOTA}-(\text{H}_2\text{O})]^-$ and, interestingly, it is in the range of optimal values for providing the highest relaxivities for MRI applications.⁹

Surprisingly, the profile for the $[\text{Gd}-\text{DO3A}-\text{alanine}-(\text{H}_2\text{O})]$ adduct displays a different shape, indicative of a longer τ_M value ($\tau_M^{298} = 180$ ns). This may reflect the occurrence of a hydrogen-bonding interaction between the metal bound water molecule and the positively charged α -amino group of the amino-acid, which could also be mediated by a solvent water molecule (Scheme 1).

In order to further check this hypothesis $^{17}\text{O}-R_{2p}$ vs. T profiles of the adducts between $[\text{Gd}-\text{DO3A}-(\text{H}_2\text{O})_2]$ and aminobutyrate anions differing in the position of the protonated amino group were recorded (Fig. 2).

The $^{17}\text{O}-R_{2p}$ values for the adduct with α -aminobutyrate are similar to those of the L-alanine adduct ($\tau_M^{298} = 120$ ns). However, when the $-\text{NH}_3^+$ group is shifted to the β -position, the exchange lifetime decreases significantly ($\tau_M^{298} = 80$ ns).

Therefore, these results clearly suggest that the position of the protonated amino group plays a key role for controlling the water exchange rate of the metal bound water molecule.



Scheme 1 Proposed representations of the ternary $[\text{Ln}-\text{DO3A}-\text{alanine}-(\text{H}_2\text{O})]$ adduct. The elongation of the residence lifetime of the metal bound water molecule may result from the occurrence of the hydrogen bonding network.

In summary, though it is difficult to foresee a MRI application for the Gd(III) based ternary complexes investigated in this work, the results here reported indicate a novel route to the modulation of the exchange rate of the coordinated water in Gd(III) complexes. Moreover, one may seek for the formation of ternary complexes between suitably functionalized Gd(III) chelates and endogenous substrates containing carboxylate functionalities.

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