The Water-Exchange Rate in Neutral Heptadentate DO3A-Like Gd^{III} Complexes: Effect of the Basicity at the Macrocyclic Nitrogen Site

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The rate of exchange of the two water molecules coordinated to a Gd^{III} centre in neutral complexes with heptadentate DO3A derivatives is modulated by the basicity of the macrocyclic nitrogen bearing the pendant group: a lower basicity results in a slower water-exchange rate.

Introduction

The exchange rate (k_{ex}) of the metal-coordinated water protons in lanthanide(III) complexes plays a fundamental role in determining their efficiency as contrast agents (CA) for Magnetic Resonance Imaging (MRI) applications.[1] In the case of Gd^{III}-based agents the attainment of high relaxivity values is strongly dependent on k_{ex}. In fact, the paramagnetic relaxation of the water protons for a given Gd^{III} chelate reaches the maximum efficiency when k_{ex} is significantly larger than the longitudinal relaxation rate of the water protons bound to the Gd^{III} ion, i.e. $k_{ex} >> R_{1M}$. To this regard, it has been calculated that a k_{ex} value of about $5 \times 10^7\ s^{-1}$ at 298 K allows the attainment of the highest relaxivity (at 0.47 T) for a large-sized Gd^{III}-based system.[2] Conversely, a novel class of paramagnetic CAs based on saturation transfer, the so-called CEST (Chemical Exchange Saturation Transfer) agents, require much lower k_{ex} values.[3,4] In these Ln^{III} chelates (with $Ln \neq Gd$) the contrast in the MR image arises from the decrease of the NMR signal intensity of the bulk water protons following the irradiation of the water protons coordinated to the Ln^{III} ion. Here, the condition $\Delta\omega > k_{ex}$ has to be fulfilled ($\Delta\omega$ is the chemical shift separation, in rad·Hz, between the free and metal-bound water protons) and, therefore, k_{ex} values of about two orders of magnitude smaller than the optimal values required for Gd^{III}-based agents are necessary for ensuring an efficient saturation transfer.

It is worth remembering that k_{ex} refers to the exchange of the water protons coordinated to the metal centre and not to the exchange of the whole water molecule ($k_{ex\text{-}w}$). Nevertheless, at pH values around neutrality the proton exchange is extremely slow (about $10^3\ s^{-1}$) when compared with the typical $k_{ex\text{-}w}$ values measured for Ln^{III} chelates. Thus, it is reasonable to assume that at physiological pH values k_{ex} corresponds to $k_{ex\text{-}w}$.

In the case of nine-coordinate Gd^{III} complexes, it has been established that the water-exchange process occurs through a dissociative pathway in which the transition state is represented by an eight-coordinate species.[5] Thus, the exchange rate is related to the free-energy difference, $\Delta G^{\#}$, between the transition and the ground states. Several factors contribute to the $\Delta G^{\#}$ value. $\Delta G^{\#}$ is usually dominated by the enthalpic term, $\Delta H^{\#}$, which mainly depends on the strength of the $Ln-O_{(water)}$ bond. The highly electrostatic nature of this bond accounts for the marked dependence of the water-exchange rate on the overall electric charge in isostructural Ln^{III} chelates. In fact, relatively fast and exceedingly slow exchange rates have been observed for anionic and cationic Gd^{III} complexes, respectively.[1] Moreover, the $\Delta H^{\#}$ term may be affected either by the steric crowding at the water coordination site or by the structural properties of the second hydration sphere of the metal ion.[6–8] The large number of parameters that contribute to determine the water-exchange process renders the assessment of the relative role played by each single contribution quite difficult. For this reason, the evaluation of the k_{ex} values for Gd^{III} complexes differing in only one main struc-

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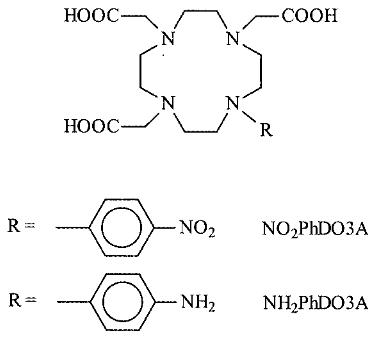
tural or electronic feature may be particularly useful. On this basis, it was deemed of interest to investigate the dependence of the water-exchange rate on the basicity of the macrocyclic nitrogen bearing the non-coordinating substituent in two neutral heptadentate DO3A-like Gd^{III} chelates. To this purpose, the ligands reported below, and their corresponding Eu^{III}, Gd^{III} and Yb^{III} complexes, have been synthesised. In spite of a very similar structure, the two Ln^{III} chelates have a noticeable difference in the electronic availability at the macrocyclic nitrogen bearing the phenyl group [$\sigma_{para}(NO_2) = 0.78$, $\sigma_{para}(NH_2) = -0.66$].



Results and Discussion

The synthesis of the ligands (see Exp. Sect.) was carried out by reacting 1,4,7,10-tetraazacyclododecane (TAZA) with *p*-nitrofluorobenzene (molar ratio 3:1) in acetonitrile. The purified mono-alkylated TAZA derivative was then exhaustively carboxymethylated with bromoacetic acid at pH 10. The NH$_2$PhDO3A ligand (pale brown) was obtained upon hydrogenation (Pd/C) of the nitro derivative. The synthesis of the Ln^{III} complexes was carried out in water at pH 7 by mixing equimolar amounts of the ligand and the corresponding Ln^{III} chloride.

Figure 1 shows the pH dependence of the relaxivity, r_1, for the two Gd^{III} complexes at 0.235 *T* (corresponding to a proton Larmor frequency of 10 MHz) and 298 K.

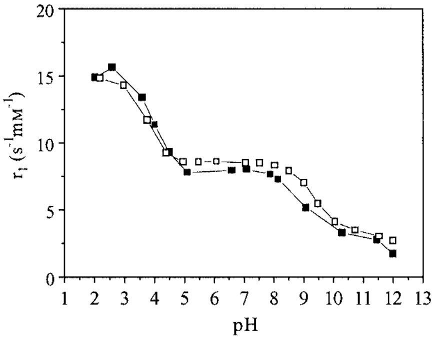


Figure 1. pH dependence of relaxivity for [Gd(NO$_2$PhDO3A] (■) and [Gd(NH$_2$PhDO3A] (□) at 298 K and 10 MHz

Qualitatively, both chelates behave similarly to other neutral heptadentate DO3A-like complexes.[9] r_1 is constant in the pH interval 5 and 8; at lower pH values r_1 increases owing to the acid-catalysed demetallation process, whereas at higher pH values the relaxivity decreases owing to the formation of ternary adducts with anionic species like carbonate (from dissolved CO$_2$) and hydroxide anions which displace the metal-bound water molecule(s). This behaviour, coupled with the relatively high r_1 values {8.0 and 8.5 $s^{-1}\cdot mM^{-1}$ at 298 *K* and pH = 7 for [Gd(NO$_2$PhDO3A)] and [Gd(NH$_2$PhDO3A)], respectively} provides a strong indication that the hydration sphere of the two complexes is closely analogous to that of the parent [Gd(DO3A)] complex,[10] i.e. characterised by the presence of two water molecules coordinated to the metal ion. Nevertheless, it is likely that the complexes are present in solution as a mixture of species which may differ in the conformation of the donor atoms wrapped around the metal centre (Δ/Λ and $\delta\delta\delta\delta/\lambda\lambda\lambda\lambda$ isomerism)[11] or in the hydration state of the metal ion (equilibrium between nine- and eight-coordinate isomers).[10,12]

In principle, each species may have a different k_{ex} value[13] and thus the isomeric population of the two complexes has to be checked.

Figure 2 shows a comparison of the ^{1}H NMR high resolution spectra of the Eu^{III} chelates. In spite of the complexity of the spectra, it is evident that they are very similar in terms of chemical shift range and number and relative intensity of the resonances. Analogous conclusions can be drawn by analysing the ^{1}H NMR spectra of the corresponding Yb^{III} complexes.

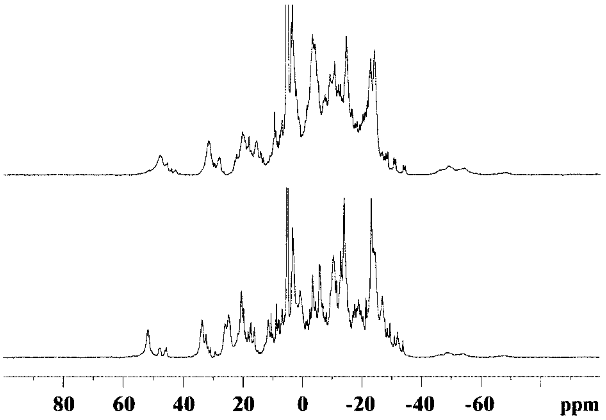


Figure 2. ^{1}H NMR spectra at 7.05 T, pH 7 and 275 K in D$_2$O of [Eu(NH$_2$PhDO3A)] (top) and [Eu(NO$_2$PhDO3A)] (bottom)

Further confirmation that the isostructurality between the two Ln^{III} chelates is retained for the Gd^{III} complexes was gained by the analysis of their NMRD (Nuclear Magnetic Relaxation Dispersion) profiles (Figure 3). Again, the two complexes display a very similar magnetic-field dependence of their r_1 values (measured at pH 7 and 298 K) even if the relaxivity of the *p*-nitro derivative is slightly lower over the entire range of magnetic-field strengths investigated.

Finally, the exchange rate of the water molecules coordinated to Gd^{III} has been determined through the well-established NMR method based on the analysis of the tempera-

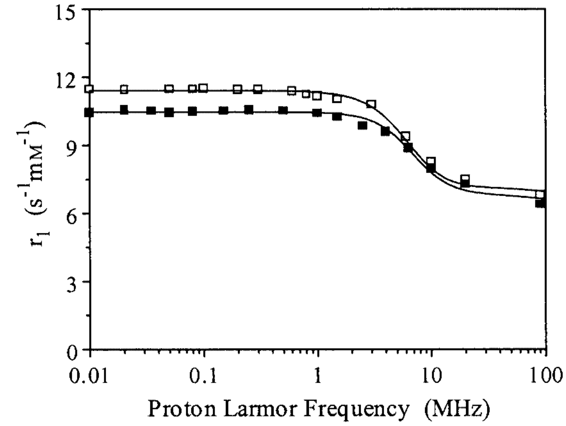


Figure 3. ^1H NMRD profiles for [Gd(NO_2PhDO3A)] (■) and [Gd(NH_2PhDO3A)] (□) at pH 7 and 298 K

ture dependence of the paramagnetic contribution to the transverse relaxation rate of the ^{17}O-water nuclei. Figure 4 reports the data obtained for the two complexes.

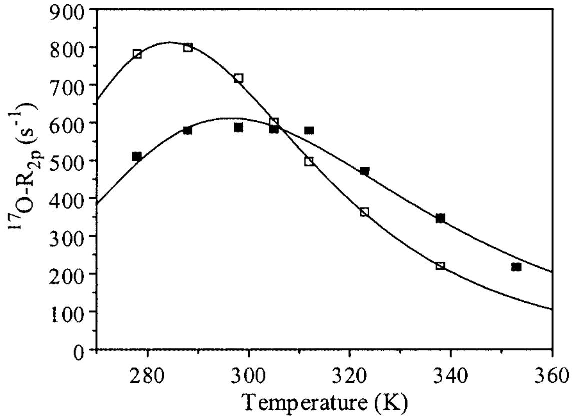


Figure 4. Temperature dependence of $^{17}O-R_{2p}$ for [Gd(NO_2PhDO3A)] (■) and [Gd(NH_2PhDO3A)] (□) at pH 7 and 7.05 T ([GdL] = 10 mM)

The profiles appear rather different, with the top of the bell-shaped curve shifted to lower temperature for the *p*-amino derivative. This behaviour suggests that k_{ex} is significantly influenced by the nature of the substituent at the macrocyclic nitrogen.

A quantitative analysis of the ^{17}O NMR spectroscopic data, according to the available theory,[5] confirms this effect. The result of the best-fitting procedure, on the assumption that the two labile water molecules have the same exchange rate, is reported in Table 1, along with the results obtained from the analysis of the NMRD profiles carried out according to the usual inner- and outer-sphere model.[1]

No relevant differences were obtained, within the same experimental data set, for the parameters controlling the electronic relaxation times Δ^2 and τ_V, which represent the mean energy of the transient zero-field splitting (ZFS) and its time modulation, respectively. Nevertheless, different Δ^2 and τ_V values were obtained depending on the experimental technique. It is likely that this discrepancy is due to the limitations of the currently available theory for describing adequately the magnetic-field dependence of the electronic relaxation times, but, fortunately, this concern has only a small effect on the estimation of k_{ex}. Therefore, the relaxometric analysis indicates that the water exchange for the *p*-amino derivative occurs faster than the *p*-nitro complex.

Table 1. Best-fit parameters (at 298 K) obtained from the analysis of $^{17}O-R_{2p}$ versus T profiles and NMRD profiles of [Gd(NO_2PhDO3A)] and [Gd(NH_2PhDO3A)] at pH 7

	[Gd(NO_2PhDO3A)]		[Gd(NH_2PhDO3A)]	
	NMRD[a]	$^{17}O-R_{2p}$ [b]	NMRD	$^{17}O-R_{2p}$
k_{ex} (× $10^6 s^{-1}$)	[c]	7.4 ± 1.2	[c]	17.6 ± 3.8
ΔH_M (kJ·mol^{-1})	[d]	33.8 ± 1.6	[d]	36.2 ± 2.8
Δ^2 (× 10^{19} s^{-2})	5.8 ± 0.5	11.6 ± 0.8	5.7 ± 0.4	9.8 ± 0.4
τ_V (× 10^{-12} s)	22.0 ± 0.3	13.0 ± 0.6	20.3 ± 0.7	15.0 ± 0.7
τ_R (× 10^{-12} s)	77.0 ± 0.4	[d]	81.1 ± 0.3	[d]

[a] The fitting of the NMRD profiles has been carried out by keeping fixed the following parameters: r = 3.1 Å, q = 2, a = 3.8 Å, D = 2.25 × 10^{-5} cm^2·s^{-1}. [b] The fitting of the temperature dependence of $^{17}O-R_{2p}$ has been performed by keeping fixed the following parameters: ΔH_v = 5.0 kJ·mol^{-1}, (A/ħ) = −3.8 × 10^6, q = 2. [c] This parameter has been fixed to the value obtained by the fitting of the data obtained with the other experimental technique. [d] This parameter cannot be obtained from the fitting.

It seems reasonable that such a difference could be accounted for in terms of the different electronic properties of the two *p*-substituents on the aromatic ring. The strong electron-withdrawing character of the *p*-nitro group makes the Gd−N bond much more polarised, thus increasing the positive charge at the metal centre. This effect results in a stabilisation of the nine-coordinate ground state of the complex, thus slowing down the rate of exchange of the coordinated water molecules. The opposite electronic effect of the *p*-amino group leads to a destabilisation of the $Gd^{III}-O_{(water)}$ bond and to a faster exchange rate.

Interestingly, the water-exchange regime reported for [Gd(DO3A)] (11.0 × 10^6 s^{-1})[10] is intermediate as expected on the ground of the basicity of the secondary amino group.

The results reported here might also help to rationalise the fast water-exchange rates (ca. 5.0 × 10^7 s^{-1} at 298 K) reported in the literature for several *N*-benzyl-DO3A derivatives[14] and support the hypothesis forwarded for explaining the relatively fast exchange rate observed in a cationic *N*-methyl DO3A derivative.[15] In fact, in these complexes a destabilisation of the $Gd^{III}-O_{(water)}$ bond is expected on the basis of the inductive effect (−I) of the alkyl group on the macrocyclic nitrogen.

Conclusion

In conclusion, the experimental evidence reported in this communication supports the view that the exchange rate of the water molecules coordinated to the Gd^{III} ion in a nine-coordinate DO3A-like arrangement may be successfully modulated through a proper choice of the electronic properties of the substituent on the macrocyclic nitrogen.

However, whether the two water molecules exchange at the same rate and/or the substituent effect described herein affects the dissociation rate at the two coordination sites to a different extent still remain undefined.

Experimental Section

Synthesis of NO$_2$PhDO3A: This ligand was synthesised in two steps. First, a suspension of 1,4,7,10-tetraazacyclododecane, TAZA, (35.05 g, 0.2035 mol) in acetonitrile (500 mL) was dissolved whilst stirring at 60 °C by adding 50 mL of water. To this solution was then added dropwise 50 mL of a solution of acetonitrile containing 1-fluoro-4-nitrobenzene (9.57 g, 0.0678 mol). After 8 h at 60 °C the suspension was cooled down and filtered. The crude solid was dissolved in 1 L of boiling water and the resulting solution (obtained by filtration) was first diluted 1:1 with water, loaded onto a chromatographic column (Amberlite® XAD 1600) and then eluted with water in order to separate the unchanged TAZA. The 1-(4-nitrophenyl)-1,4,7,10-tetraazacyclododecane ligand (14.08 g; 0.048 mol, 70.8% yield) was obtained by eluting the column with methanol and subsequent evaporation of the solvent.

In the second step, 2-bromoacetic acid (22.23 g, 0.16 mol) was added whilst stirring to an aqueous solution of 1-(4-nitrophenyl)-1,4,7,10-tetraazaciclododecane (11.73 g, 0.04 mol in 50 mL of water). The pH of the solution was brought to 10 by adding 2 N NaOH. The solution was heated at 50 °C for 6 h by keeping the pH constant. The reaction mixture was cooled down, filtered and the filtrate was acidified at pH 3 with HCl. This solution was loaded onto a chromatographic column (Amberlite® XAD 1600) and then eluted first with water, in order to separate the bromide ions, and then with methanol. After evaporation of the solvent the NO$_2$PhDO3A ligand was obtained in the acid form as a yellow solid (16.38 g, 0.035 mol, 87.6% yield). ^{1}H NMR (D$_2$O, *t*BuOH as internal reference, 298 K): δ = 8.0 (d, 2 H), 6.65 (d, 2 H), 3.7 (s, 4 H), 3.45 (s, 4 H), 3.35 (s, 2 H), 3−3.2 (m, 12 H) ppm. ^{13}C{^{1}H} NMR (D$_2$O, *t*BuOH as internal reference, 298 K): δ = 173.2, 169.2, 153.9, 142.7, 124.2, 122.3, 55.1, 51.6, 50.9, 50.0, 47.7, 46.9 ppm. MS (MALDI-TOF): calcd. for $C_{20}H_{29}N_5O_8$ 467.47 uma; found 468.4 [MH$^+$].

Synthesis of NH$_2$PhDO3A: Ammonia solution (26%) was added to a suspension of NO$_2$PhDO3A in water (7.70 g; 0.02 mol in 50 mL of water) until the ligand dissolution was complete. Then, 0.7 g of 10% Pd/C, previously treated with 5 mL of water, was added to the solution and the catalytic hydrogenation was carried out at room temperature and pressure with hydrogen (1180 mL, 0.05 mol) produced electrochemically (Hydrogen generator HG 600; CLAIND). After 4 h the suspension was filtered in order to remove the catalyst. The filtrate (brown colour) was lyophilised and then redissolved in water acidified (pH 2.3) with HCl. 0.5 g of Carbopuron was then added in order to partially decolourise and stabilise the product. After filtration, the solution was re-lyophilised and the NH$_2$PhDO3A ligand was obtained in the acid form as a dark-brown solid (8.16 g; 0.016 mol, 80% yield). ^{1}H NMR (D$_2$O, *t*BuOH as internal reference, 298 K): δ = 7.15 (d, 2 H), 6.7 (d, 2 H), 3.6 (s, 4 H), 3.4 (s, 4 H), 3.3 (s, 2 H), 3.27 (s, 8 H), 3.1 (s, 4 H) ppm. ^{13}C{^{1}H} NMR (D$_2$O, *t*BuOH as internal reference, 298 K): δ = 176.8, 169.1, 143.6, 138.9, 124.8, 116.0, 55.7, 54.2, 51.0, 50.2, 49.5, 47.9 ppm. MS (MALDI-TOF): calcd. for $C_{20}H_{31}N_5O_6$ 437.5 uma; found 438.3 [MH$^+$].

Synthesis of the Ln^{III} Complexes: The Ln^{III} complexes (Ln = Gd, Eu and Yb) were synthesised in water at room temperature by adding an equimolar amount of LnCl$_3$ to the selected ligand. The pH was monitored during the synthesis in order to keep it in the 6−8 range. The final concentration of the metal chelates was checked by Evans' method.[16]

NMR Measurements: The high resolution NMR spectra and the ^{17}O-R$_{2p}$ measurements were performed on a Bruker Avance 300 spectrometer (Karlsruhe, Germany) operating at 7.05 T (proton Larmor frequency of 300 MHz).

The water-proton longitudinal relaxation rates at 0.235 T (proton Larmor frequency of 10 MHz) as well as the NMRD profiles in the 0.000235−0.235 T range (0.01−10 MHz) were obtained from a Stelar Fast Field Cycling Relaxometer (Mede (Pv), Italy). The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty of ±0.1 °C). The NMRD profile was completed with data obtained at 20 MHz and 90 MHz by using a Stelar Spinmaster and a JEOL EX-90 NMR spectrometer, respectively.

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