Relaxometric Determination of the Exchange Rate of the Coordinated Water Protons in a Neutral Gd^III Chelate

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Abstract: The exchange rate of the coordinated water molecule in the neutral complex [Gd(DTPA-BBA)(H$_2$O)] (DTPA-BBA = 1,7-bis[(N-benzylcarbamoyl)methyl]-1,4,7-triazaheptane-1,4,7-triacetate or diethylenetriaminopentaacetate N,N'-bis(benzylamide)) is slower than in the parent complex [Gd(DTPA)(H$_2$O)]$^{2-}$. From the analysis of the temperature dependence of the solvent ^{17}O NMR transverse relaxation time in an aqueous solution of the paramagnetic complex, a value of 4.5×10^5 s^{-1} (at 298 K) is obtained for the exchange rate of the coordinated water molecule. This rate constant does not vary in the pH range 7–12. Conversely, over the same pH range and at 298 K and 20 MHz, the longitudinal water proton relaxivity increases from 4.8 to 6.5 s^{-1}mM^{-1}. The analysis of the dependence of the longitudinal water proton relaxation rate on magnetic field and temperature at pH 7 and pH 12 shows that the increase in relaxivity at basic pH has to be assigned to the contribution of the prototropic exchange at the water molecule in the inner coordination sphere of the metal ion. This exchange process is catalyzed by OH$^-$ ions ($k_P = 1.7 \times 10^9$ M^{-1} s^{-1} at 298 K) and causes an increase in the observed relaxivity when it occurs at a rate larger than the exchange rate of the entire water molecule. At pH 12 the limiting effect of the slow exchange rate for the coordinated water molecule is removed, and the longitudinal water proton relaxivity measured at this pH then represents the maximum value attainable for this complex.

Introduction

The exchange of protons among water molecules is a slow process at pH values close to neutrality, but its rate markedly increases at both basic and acidic pH, as well as in buffered solutions. The evaluation by NMR spectroscopy of the prototropic exchange rates in pure water was reported by Meiboom et al., who showed that the exchange lowers the transverse relaxation time T_2 of the water protons following modulation of the ^{17}O–^{1}H coupling constant.[1] ^{1}H NMR spectroscopy is also the method of choice for investigating prototropic exchange processes involving the water molecules in the first hydration sphere of a metal ion. To obtain reliable values of the kinetic parameters involved in this dynamic process, the exchange of the whole water molecule has to be negligible compared to proton exchange. This condition is nicely met for trivalent transition metal ions such as Al^III, Cr^III, and Rh^III, in which the rate constants for the tightly bound inner-sphere water molecules are very small ($k_{ex} < 2$ s^{-1}).[2] Since the hydrated complexes of these metal ions are readily hydrolyzed, the prototropic exchange rate for these species was determined at acidic pH values.[3–5]

To date, no report dealing with the prototropic exchange of a water molecule coordinated to lanthanide(III) ions has appeared in the literature, in spite of the large number of studies devoted to their chelates due to their extensive use in biomedical applications.[6] In particular, Gd^III complexes are widely used in medical diagnosis as contrast agents (CA) for magnetic resonance imaging (MRI) because of their ability to increase the proton relaxation rates ($R_i = 1/T_i$, $i = 1,2$) of tissue fluid.[7] In all the complexes so far used in clinical practice, the nine coordination sites of the Gd^III ion are occupied by eight donor atoms of a polyaminocarboxylate ligand and one water molecule. Polyaminocarboxylate and related ligands have been found to be particularly suitable for ensuring a high thermodynamic (and possibly kinetic) stability of the resulting complexes, which reduces the possibility of the in vivo release of the toxic Gd^III ions. Furthermore, the high thermodynamic stability constant of these chelates prevents the occurrence of hydrolytic processes at the metal ion and thus allows the investigation of their properties even at high pH values.

In order to maximize the enhancement of the water proton relaxation rate by a Gd^III chelate, the exchange rate of the water molecule in the first hydration sphere of the metal ion has to be of the order of 10^8 s^{-1}. For Gd^III complexes the rate constants k_{ex} fall in a rather broad range of values, from 8.3×10^8 s^{-1} in the case of the octaaquo ion to 4.5×10^5 s^{-1} for the neutral complex [Gd(DTPA-BMA)(H$_2$O)].[8]

This large range can be rationalized in terms of the different exchange mechanisms proposed for the two Gd^III complexes. The exchange of the water molecule in [Gd(H$_2$O)$_8$]$^{3+}$ occurs by an associatively activated mechanism (A or I$_A$)[9] which involves

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the formation of a nine-coordinate activated state, whereas for the nine-coordinate Gd^{III} chelate, exchange takes place by formation of an eight-coordinate activated state (dissociatively activated interchange mechanism, I$_D$) following the loss of the metal-coordinated water molecule. Thus, k_{ex} is related to the energy difference between the ground state and the activated state.[10]

As the neutral Gd^{III} complexes so far investigated exhibit slow exchange of the whole inner-sphere water molecule,[8, 10–15] they are good candidates for the assessment of the prototropic exchange rate at the metal-coordinated water molecule. Furthermore, since a slow water exchange rate may reduce the potential efficacy of a Gd^{III} chelate in diagnostic applications, it is particularly interesting to evaluate whether or not the prototropic exchange rate affects the relaxivity of the paramagnetic CA.

Here we show that the prototropic exchange rate can be accurately determined by measuring the longitudinal water proton relaxation rate of aqueous solutions containing a hydrated neutral Gd^{III} complex, provided the exchange rate of the coordinated water molecule has been determined by analysis of the temperature dependence of the ^{17}O NMR transverse relaxation rate. To this purpose we have considered the Gd^{III} chelate of the ligand DTPA-BBA **1**,[15–18] for which slow exchange of the coordinated water molecule has already been suggested.[15] Like a number of related bisamide derivatives of DTPA,[13, 19–26] DTPA-BBA acts as an octadentate ligand towards the Gd^{III} ion, with a formation constant of about $10^{17}\,M^{-1}$ at 298 K and pH 7.0.[15]

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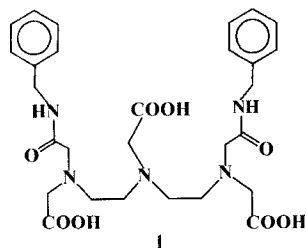
Results and Discussion

Determination of the exchange rate of the inner-sphere water molecule by ^{17}O NMR: The exchange rate of the coordinated water molecule in [Gd(DTPA-BBA)(H$_2$O)] has been determined by analyzing the temperature dependence of the ^{17}O transverse relaxation rate of the solvent water. According to Swift and Connick,[27] the paramagnetic contribution R^O_{2p} to the observed relaxation rate R^O_{2obs} ($R^O_{2p} = R^O_{2obs} - R^O_{2d}$, where R^O_{2d} is the diamagnetic term measured in a solution of the diamagnetic Lu^{III} complex) is given by Equation (1), where P_M is the molar frac-

$$R^O_{2p} = P_M k^O_{ex} \frac{R^{O\,2}_{2M} + k^O_{ex}R^O_{2M} + \Delta\omega^{O\,2}_M}{\left(R^O_{2M} + k^O_{ex}\right)^2 + \Delta\omega^{O\,2}_M} \tag{1}$$

tion of the coordinated water, R^O_{2M} represents its ^{17}O transverse relaxation rate, k^O_{ex} its exchange rate and $\Delta\omega^O_M$ the chemical shift difference between the ^{17}O NMR resonances of coordinated and bulk water.

In principle, R^O_{2M} consists of four contributions [Eq. (2)], where the superscripts *sc*, *dip*, *quad* and *Cur* refer to the nucleus–

$$R^O_{2M} = R^{O\ sc}_{2M} + R^{O\ dip}_{2M} + R^{O\ quad}_{2M} + R^{O\ Cur}_{2M} \tag{2}$$

electron scalar, nucleus–electron dipolar, nuclear quadrupolar and Curie relaxation mechanisms, respectively.[28]

At the magnetic field strength used here (2.1 T) and for rapidly reorienting Gd^{III} chelates such as [Gd(DTPA-BBA)(H$_2$O)], R^O_{2M} is dominated by the scalar term. In fact, we estimated that for this Gd^{III} complex the contribution arising from the other relaxation mechanisms is less than 1 % over the entire investigated temperature range (273–363 K).

The scalar relaxation, related to the Gd^{III} unpaired electron spin density at the ^{17}O nucleus, is given by Equation (3), where

$$R^{O\ sc}_{2M} = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_s^2\tau_{E2}^2}\right) \tag{3}$$

S is the electronic spin quantum number ($^7/_2$ for Gd^{III}), $A/\hbar$ is the Gd ^{17}O scalar coupling constant and τ_{Ei} ($i = 1,2$) represents the correlation time of the processes modulating the scalar interaction. This modulation may occur through both the longitudinal and the transverse electronic relaxation times (T_{1E} and T_{2E}) and the mean residence lifetime τ^O_M of the water molecule at the paramagnetic site, that is, $\tau_{Ei}^{-1} = (\tau^O_M)^{-1} + T_{Ei}^{-1}$.

The scalar coupling constant is a measure of the spin density of the Gd^{III} unpaired electron at the ^{17}O nucleus. This constant is related to the distance r_O between the metal ion and the coordinated water molecule. Since r_O does not vary significantly for polyaminocarboxylate Gd^{III} chelates with a single inner-sphere water molecule, we used the value of -3.8×10^6 rad s^{-1} for $A/\hbar$, as previously reported for the closely related [Gd(DTPA-BMA)(H$_2$O)].[8]

For Gd^{III} chelates T_{iE} are mainly related to the modulation of the zero-field splitting (ZFS) of the electronic spin states due to the dynamic distortions of the ligand-field interaction, and according to Bloembergen–Morgan theory[29] are expressed by the Equations (4) and (5), where Δ^2 is the trace of the square of

$$T_{1E}^{-1} = \frac{1}{25}\Delta^2[4S(S+1)-3]\left[\frac{\tau_v}{1+\omega_s^2\tau_v^2} + \frac{4\tau_v}{1+4\omega_s^2\tau_v^2}\right] \tag{4}$$

$$T_{2E}^{-1} = \frac{1}{50}\Delta^2[4S(S+1)-3]\left[3\tau_v + \frac{5\tau_v}{1+\omega_s^2\tau_v^2} + \frac{2\tau_v}{1+4\omega_s^2\tau_v^2}\right] \tag{5}$$

the transient ZFS tensor **D** ($\Delta^2 = D^2_{xx} + D^2_{yy} + D^2_{zz}$), τ_v is the correlation time for collision-related modulation of the ZFS Hamiltonian and ω_s is the electronic Larmor frequency. Therefore, the temperature dependence of R^O_{2M} is expressed in terms of the temperature dependence of τ^O_M, τ_v, and $\Delta\omega^O_M$ by Equations (6) and (7), where the subscripts j refers to the different correlation

$$\tau_j^{-1} = \frac{\left(\tau_j^{-1}\right)^{298.15} T}{298.15} exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{6}$$

$$\Delta\omega^O_M = \frac{g_e\mu_B S(S+1)B}{3k_B T}\frac{A}{\hbar} \tag{7}$$

times ($j = M, v$), ΔH_j is the activation enthalpy for the corresponding dynamic processes, B is the magnetic field strength, and k_B is the Boltzmann constant.

Figure 1 shows the temperature dependence of R_{2p}^O for [Gd(DTPA-BBA)(H$_2$O)] at pH 7. The bell-shaped profile has

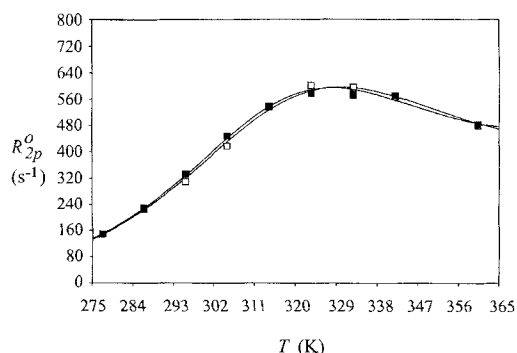Figure 1. Temperature dependence of the water ^{17}O NMR transverse relaxation rate for [Gd(DTPA-BBA)(H$_2$O)] (0.048 M) at 2.1 T, pH 7 (filled squares) and 12 (open squares).

been fitted on the basis of Equations (1)–(7), and the calculated parameters are listed in Table 1. The values obtained are quite similar to those reported in the literature for other Gd^{III} com-

Table 1. Calculated relaxation parameters for [Gd(DTPA-BBA)(H$_2$O)] [a].

Parameter	^{17}O	^{1}H (pH 7)	^{1}H (pH 12)
Δ^2 [10^{19} s^{-2}]	2.5(0.1)	4.1(0.2)	4.8(0.2)
τ_V^{298} [ps]	38.9(1.5)	23.1(1.0)	23.1(1.0)
k_{ex}^{298} [10^5 s^{-1}]	4.5(0.2)	4.0(0.2)	170.0(8.5)
τ_R^{298} [ps]	–	90.1(2.2)	91.8(2.2)
ΔH_v [kJ mol^{-1}]	22.5(1.1)	–	–
ΔH_M [kJ mol^{-1}]	28.5(1.2)	..	–

[a] Numbers in parentheses represent standard deviations in mean parameter estimates for 1000 simulated relaxation rate data sets obtained by repeatedly introducing a random error of 1% into the experimental data set and estimating best parameters.

plexes;[8, 30] in particular, the exchange rate of the coordinated water at 298 K ($k_{ex}^O = 4.5 \times 10^5$ s^{-1}) is identical to the value found for [Gd(DTPA-BMA)(H$_2$O)].[10]

Field and temperature dependence of longitudinal water proton relaxivity: R_{1p}^H represents the paramagnetic contribution to the observed water proton relaxation rate ($R_{1p}^H = R_{1obs}^H - R_{1d}^H$) and is given by the sum of two terms, namely the inner-sphere (R_{1p}^{His}) and the outer-sphere relaxivity (R_{1p}^{Hos}) [Eq. (8)].[31] The former

$$R_{1p}^H = R_{1p}^{His} + R_{1p}^{Hos} \tag{8}$$

represents the contribution of the exchanging water protons from the first coordination sphere of the metal ion to the bulk and is given by Equation (9), where T_{1M}^H is the longitudinal

$$R_{1p}^{His} = \frac{P_M}{T_{1M}^H + \tau_M} \tag{9}$$

relaxation rate of the inner-sphere water protons and τ_M is their mean residence lifetime. As already mentioned, at neutral pH the prototropic exchange rate is expected to be rather long (ca. 10^3 s^{-1} for pure water[11]) and thus τ_M basically coincides with τ_M^O ($\tau_M^O = 1/k_{ex}^O$).

According to the well-established Solomon–Bloembergen–Morgan theory, the magnetic-field dependence of T_{1M}^H is described by Equation (10), where γ_I is the proton gyromagnetic

$$(T_{1M}^H)^{-1} = \frac{2}{15} \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r_H^6} \left[\frac{3\tau_{C1}}{1+\omega_I^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2} \right] \tag{10}$$

ratio, g_e is the free-electron Landé factor (2.0023), μ_B is the Bohr magneton, r_H is the mean Gd^{III}–H(water) distance, and ω_I and ω_S are the nuclear and electronic Larmor frequencies, respectively.[29, 32, 33] Since the distance between the water oxygen atom and the lanthanide ion in the X-ray structure of [Lu(DTPA-BBA)(H$_2$O)] is 2.39 Å,[14] we used a reasonable r_H value of 3.0 Å in our calculations.

The two correlation times τ_{c1} and τ_{c2} associated with the modulation of the proton–electron dipolar coupling are given by the relationship in Equation (11), where τ_R represents the

$$\tau_{ci}^{-1} = \tau_R^{-1} + \tau_M^{H^{-1}} + T_{iE}^{-1} \tag{11}$$

reorientational correlation time of the Gd^{III} chelate. Since the rotational motion of the whole complex is assumed to be isotropic, τ_R is simply related to its molecular size, and for small Gd^{III} chelates it usually dominates τ_{ci} at the magnetic field strengths routinely employed in MRI applications (0.2–1.5 T).

The outer-sphere term R_{1p}^{Hos} describes the contribution of the nonbonded water molecules surrounding the Gd^{III} complex. According to the approach developed by Hwang and Freed,[34, 35] this mechanism depends mainly on the diffusion-controlled dipolar constant C^{os} (5.8×10^{-10} s^{-2}), the molar concentration M of the paramagnetic solute, the distance of closest approach between the paramagnetic center and the water molecules a, the sum of the solute and solvent diffusion coefficients D, the electronic relaxation times T_{iE} (which are included in the non-Lorentzian spectral density functions $J(\omega_i)$[36] related to the translational diffusion motion), and the magnetic field strength [Eq. (12)].

$$R_{1p}^{Hos} = C^{os}\left(\frac{M}{aD}\right)\left[7J(\omega_S) + 3J(\omega_I)\right] \tag{12}$$

For low molecular weight Gd^{III} chelates, the outer-sphere contribution accounts for about 50% of the overall observed proton relaxivity at imaging field strengths, and the analysis of the ^{1}H NMRD (nuclear magnetic relaxation dispersion) profiles collected over the years allowed us to obtain good estimates for a and D,[31, 37] which are currently assumed to be 3.8 Å and 2.2×10^{-5} cm^{2}s^{-1} (at 298 K).

Figure 2 (lower curve) shows the relaxometric profile for a 1 mM solution of [Gd(DTPA-BBA)(H$_2$O)] recorded at 25 °C and pH 7. The values of the relaxation parameters obtained from the

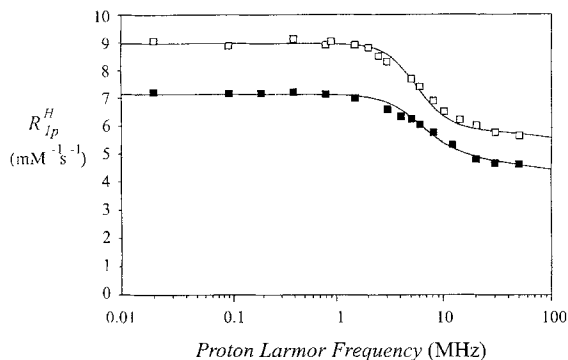Figure 2. Magnetic field dependence of the water proton longitudinal relaxivity (^{1}H NMRD) of [Gd(DTPA-BBA)(H$_2$O)] at 25°C, pH 7 (filled squares) and 12 (open squares).

fitting procedure [Eqs. (8–12)] are listed in Table 1. The good correspondence between the ^{1}H and [17]O exchange rate constants corroborates the hypothesis that the prototropic exchange rate at neutral pH is remarkably slow and therefore does not affect R_{1p}^H. Furthermore, this result confirms the occurrence of a slow exchange rate of the inner-sphere water molecule for this neutral Gd[III]–DTPA bisamide complex, as already inferred from the qualitative analysis of the temperature dependence of R_{1p}^H at pH 7 and 20 MHz (Figure 3).[15]

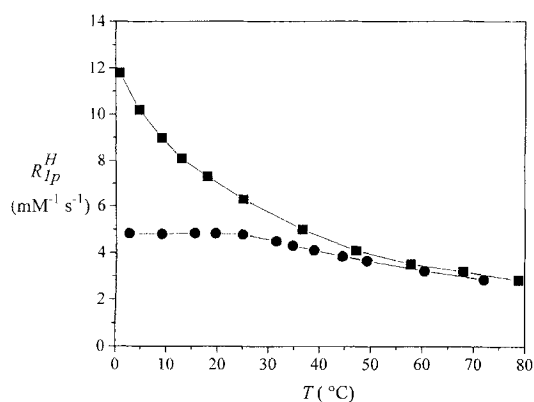Figure 3. Temperature dependence of the water proton longitudinal relaxivity for [Gd(DTPA-BBA)(H$_2$O)] at 0.47 T, pH 7 (circles) and 12 (squares).

At this magnetic field strength, the temperature dependence of the outer-sphere contribution corresponds essentially to the temperature dependence of D, and R_{1p}^{Hos} decreases with increasing temperature. The same behavior is expected for the inner-sphere component of the relaxivity when the fast-exchange condition holds ($\tau_M < T_{1M}^H$); this appears to be the case at temperatures above 30°C. In contrast, the flat profile measured at lower temperatures indicates the progressive approach to the slow exchange condition ($\tau_M > T_{1M}^H$), which results in a marked quenching of the relaxivity. The large τ_M value may also explain the small (compared to the value expected on the basis of the molecular size) longitudinal relaxivity measured at room temperature (4.8 mM^{-1}s^{-1} at 0.47 T).

Assessment of the contribution to R_{1p}^H arising from prototropic exchange: The observation of the highest possible relaxivity for this complex may be possible in the conditions of a fast pro-

totropic exchange superimposed on the slow exchange of the whole water molecule. Although the prototropic exchange is catalyzed by both H$_3$O$^+$ and OH$^-$ ions, we decided to carry out the measurements only at the basic limb, in order to avoid the complications associated with the fast dissociation of the complex that occurs at low pH.

The pH dependence of the proton longitudinal relaxivity R_{1p}^H for [Gd(DTPA-BBA)(H$_2$O)] at room temperature (Figure 4) indicates a marked relaxation enhancement in the pH range

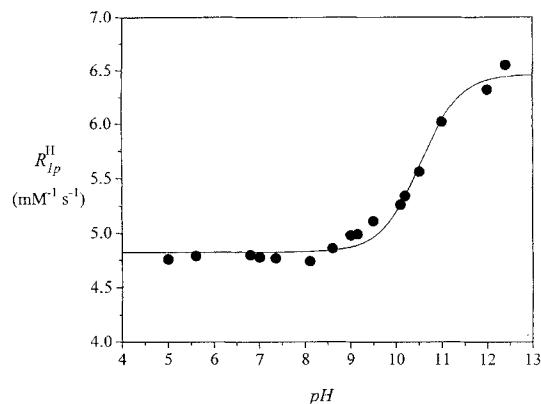Figure 4. pH dependence of the water proton longitudinal relaxivity for [Gd(DTPA-BBA)(H$_2$O)] at 0.47 T and 25°C.

8.0–12.5. The outer-sphere contribution is pH independent, since it only depends on the diffusion of the solvent molecules in the neighborhood of the paramagnetic center, and thus the relaxivity increase has to be ascribed exclusively to changes in the R_{1p}^{His} component. On the basis of Equation (9), this might arise either from a reduction of T_{1M}^H and/or τ_M^H, or from an increase in P_M (e.g., due to an increase in the number of inner-sphere water molecules). In order to discriminate between these possibilities it is useful to analyze the temperature dependence of R_{1p}^H at pH 12 and 20 MHz. The profile (upper curve in Figure 3) is fully consistent with the occurrence of the fast-exchange condition over the entire temperature range. Furthermore, the convergence of the two profiles to the same relaxivity value at high temperatures excludes an increase of P_M at basic pH. To assess whether the shortening of τ_M^H is due to an increase in the exchange rate of the whole water molecule or to a prototropic exchange effect, we measured the temperature dependence of the [17]O water transverse relaxation rate at pH 12 (Figure 1). The experimental data at the two pH values are perfectly superimposable; thus the exchange rate of the inner-sphere water molecule remains unchanged over the investigated pH range. Therefore, the most likely explanation for the observed behavior is the occurrence, at pH > 8.5, of a prototropic exchange that is faster than the exchange of the whole water molecule, that is, $k_{ex}^H > k_{ex}^O$. It follows that the data of Figure 4 can be fitted by means of Equation (13), a modified version of Equations (8) and (9) in which τ_M is represented explicitly by the sum of the two contributions.

$$R_{1p}^H = \frac{P_M}{T_{1M}^H + \left(k_{ex}^O + k_P \frac{K_W}{[H_3O^+]}\right)^{-1}} + R_{1p}^{Hos} \tag{13}$$

The value obtained for the pseudo-first-order prototropic exchange rate constant k_p at 25 °C is $1.7 \times 10^9 \, \text{M}^{-1} \text{s}^{-1}$, about half of the value found in pure water.[1] The coordination to the lanthanide(III) ion thus appears to affect the prototropic exchange process only to a very limited extent; at pH 12 this has a rate almost two orders of magnitude larger than that of the whole water molecule.

It is noteworthy that the values obtained for the outer-sphere contribution ($2.3 \, \text{mM}^{-1} \text{s}^{-1}$) and for T_{1M}^{H} (4.3×10^{-6} s) are fully consistent with those derived from the analysis of the NMRD profile, and this represents a further check of the accuracy of the procedure and of the reliability of the results.

As discussed above, the occurrence of a fast prototropic exchange removes the limiting effect on the relaxivity due to a low exchange rate of the coordinated water molecule. This is particularly clear from the analysis of the ^{1}H NMRD profile recorded at pH 12 (upper curve in Figure 2), which, over the entire magnetic field range, displays an amplitude substantially higher than that found at pH 7. A good fit between calculated and experimental data has been obtained by using the τ_M value calculated from Equation (13) (5.9×10^{-8} s), whereas the other parameters (Table 1) are basically independent of pH.

This finding provides further support for the view that no change either in the stoichiometry or in the structure occurs on going from pH 7 to pH 12. The occurrence of a hydroxyl species at the basic limb would be expected to cause significant changes in several parameters and, therefore, differences in the observed NMRD profile that cannot be accounted for by a variation in τ_M alone. Furthermore, the observation of identical ^{17}O transverse relaxation rates at pH 7 and pH 12 definetely rules out this possibility.

Conclusions

From the observations reported in this work we conclude that for neutral complexes of the size of [Gd(DTPA-BBA)(H$_2$O)] the highest relaxivity cannot be obtained at neutral pH because of the low values of both the water and prototropic exchange rates. Furthermore, this quenching effect of k_{ex}^O on the observed relaxivity would be even stronger if the Gd^{III} chelate were bound to a macromolecular substrate.[38] Under these conditions, the increase in τ_R leads to a reduction in T_{1M}^{H}, but this cannot be exploited for relaxation enhancement if τ_M is not short enough. In principle τ_M can be shortened by increasing the exchange rate of the coordinated water molecule or by catalysis of the prototropic exchange. At physiological pH values, the latter route appears rather difficult to realize, although the design of ligands containing groups with exchangeable protons involved in hydrogen bonding interactions with the coordinated water and endowed with suitable pK_a values could provide novel catalytic pathways for enhancing this process. As far as the exchange of the whole water molecule is concerned, one must remember that in these nine-coordinate complexes this occurs through a dissociative interchange mechanism that implies the exchange rate to be basically determined by the energy difference between the nine-coordinate ground state ($q = 1$) and the octacoordinate activated state ($q = 0$).

It follows that complexes with a destabilized ground state structure should have a lower activation energy for the exchange

process and an accelerated water exchange rate. Complexes endowed with such characteristics are under intense scrutiny[39] as they may provide a significant improvement in the attainable relaxivity upon interaction with macromolecular systems compared to currently available contrast agents.

Experimental Section

The DTPA-BBA ligand and its Gd^{III} and Lu^{III} complexes were synthethized and characterized by the literature procedure[9] from chemicals purchased from Sigma (St. Louis, MO, USA).

The longitudinal water proton relaxation rates were measured on a Stelar SpinMaster spectrometer (Stelar, Mede (PV), Italy) operating at a magnetic field strength of 0.47 T (corresponding to a proton Larmor frequency of 20 MHz) by means of the usual inversion–recovery pulse sequence (180°–τ–90°). A phase cycle ($+x$, $-x$, $-x$, $+x$) was applied on the 90° observation pulse in order to cut off the y-scale receiver offset. Each measurement consisted of four scans, and the values of the magnetization on the y axes were obtained in the time domain by averaging the first 128 points of the free induction decay. The reproducibility in T_1 measurements was $\pm 0.4\%$. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper–constantan thermocouple; the actual temperature was measured inside the probehead (uncertainty of ± 0.1 °C) by using a Fluka 52k/j digital thermometer.

The ^{1}H NMRD profiles were recorded on the Koenig–Brown field cycling relaxometer installed at the Department of Chemistry, University of Florence (Italy). This instrument measures water proton longitudinal relaxation rate in the range of magnetic field strength from 2.4×10^{-4} to 1.5 T (corresponding to 0.01–50 MHz proton Larmor frequencies) with a uncertainty in T_1 of $\pm 1\%$. The temperature was controlled by a circulating bath of 1,1,2-trichloroethylene.

The ^{17}O transverse relaxation rates were measured on a JEOL EX-90 spectrometer operating at 2.1 T (corresponding to a ^{17}O Larmor frequency of 12.2 MHz) with an external D$_2$O lock. The temperature calibration was performed by the same procedure described for the Stelar SpinMaster spectrometer. The value of the transverse relaxation rate was obtained by evaluating the line width at half height $\Delta v_{1/2}$ of the water ^{17}O signal ($R_2 = \pi \Delta v_{1/2}$). The diamagnetic contribution was obtained by measuring the temperature dependence of the ^{17}O transverse relaxation rate of a 0.048 M solution of [Lu(DTPA-BBA)(H$_2$O)]. These values were subtracted from those obtained in the presence of the same amount of the paramagnetic Gd^{III} complex.

For ^{17}O NMR measurements, solutions containing 2.6% of ^{17}O isotope were used. ^{17}O-enriched (10.4%) water was purchased from Yeda (R & D Co., Rehovot, Israel).

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