Determination of the Prototropic Exchange Rate at the Water Molecule Coordinated to an Anionic Paramagnetic Gd^{III} Chelate

Silvio Aime*[a], Simonetta Geninatti Crich[a], Eliana Gianolio[a], Enzo Terreno[a], Andrea Beltrami[b], and Fulvio Uggeri[b]

Dipartimento di Chimica I.F.M.[a],
Via Pietro Giuria 7, I-10125 Torino

Bracco S.p.A.[b],
Via Egidio Folli 50, I-20134 Milano

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A thorough investigation of the proton and oxygen-17 relaxation rates of water nuclei has been carried out for a solution containing an amphiphilic, paramagnetic Gd^{III} chelate of potential interest as a contrast agent for MRI. It has been found that at pH = 7, 298 K and 20 MHz (proton observation frequency), the contribution to the overall relaxation enhancement from the water molecule in the inner coordination sphere is dominated by the relaxation time (T_{1M}^H) of its protons. However, upon formation of a slowly tumbling adduct with β-cyclodextrin, the observed relaxation enhancement is also affected by the exchange lifetime (τ_M^H) of the coordinated water and by the transfer rate of its protons. This situation has been exploited to assess the prototropic exchange rate from the coordinated water to the bulk, which is base-catalyzed. In fact, under these experimental conditions, at basic pH, the proton transfer is faster than the exchange of the whole water molecule, and it becomes the factor determining the observed relaxation enhancement. The effect is further enhanced at low temperature as a consequence of the concomitant lengthening of either τ_R (which causes a reduction of T_{1M}^H) and/or the exchange lifetime of the coordinated water molecule.

Introduction

Recently, it has been shown that the exchange rate of the coordinated water molecule in Gd^{III} complexes of octadentate ligands may be markedly slower than in chelates with ligands of lower denticity.[1] The observed behaviour has been accounted for in terms of the occurrence of a dissociative exchange mechanism, the rate-determining step of which is determined by the energy difference between the nonacoordinated ground state and the octacoordinated activated state.[2] The actual value of the exchange rate of the water molecule coordinated at the paramagnetic metal site may be accurately evaluated by measuring the temperature dependence of the transverse relaxation rate of the ^{17}O-NMR water resonance of an aqueous solution containing the Gd^{III} chelate.[3]

Currently, the main interest in water-soluble Gd^{III} complexes stems from their use as contrast agents for MRI, owing to their remarkable ability to enhance the relaxation rate of water protons in the tissues in which they are distributed.[4] The contribution to the overall relaxation rate arising from the metal-bound water molecule in a Gd^{III} chelate may result either from the exchange of the whole water molecule or from the exchange of just its protons. At physiological pH, the latter process is much slower than the former,[5] making the assessment of the prototropic exchange rate for the protons of the metal-bound water molecule rather difficult.

Recently, we showed that the determination of the exchange rate for the protons of the water molecule in the inner coordination sphere of a Gd^{III} chelate may be successfully pursued by quantitative analysis of the pH dependence of the water ^1H-NMR longitudinal relaxivity, provided that the exchange lifetime of the whole metal-bound water molecule (τ_M^O) is longer than, or at least of the same order of magnitude as, the longitudinal relaxation time of its protons (T_{1M}^H).[6][7]

Until now, this behaviour has been observed only for Gd^{III} chelates endowed with a cationic or neutral residual charge. In the most commonly encountered anionic Gd^{III} complexes, τ_M^O is shorter than T_{1M}^H at ambient temperature. Thus, in such systems, the assessment of the prototropic exchange rate first requires a reduction of T_{1M}^H.

In principle, one may envisage several ways of shortening T_{1M}^H, e.g. by increasing the molecular correlation time through the formation of reversible adducts between the Gd^{III} chelate and a slowly tumbling substrate, or by lowering the temperature. In the latter case, besides the reduction of T_{1M}^H, a further advantage may be obtained by the decrease of τ_M^O as well.

In this work, we deal with the assessment of the prototropic exchange rate at the metal-bound water molecule in a derivative of $Gd(DTPA)^{2-}$, in which one acetate proton is replaced by a cyclohexyloxymethyl residue [$Gd(COPTA)^{2-}$; COPTA = 4-carboxy-5,8,11-tris(carboxymethyl)-1-cyclohexyl-2-oxa-5,8,11-triazatridecan-13-oic acid, Chart 1]. On the basis of the data collected to date for polyaminocarboxylate Gd^{III} chelates, we would expect the exchange lifetime at room temperature of the metal-bound water molecule in

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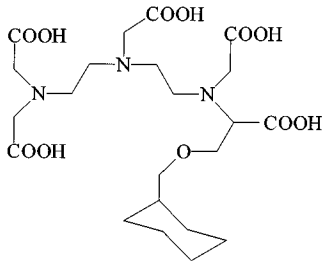
this anionic complex to be lower than T_{1M}^H. However, the presence of the hydrophobic tail should allow this chelate to reversibly form an adduct with a slowly tumbling substrate such as β-cyclodextrin (β-CD). The consequent lengthening of the reorientational correlation time, τ_R, of the complex should result in a decrease of T_{1M}^H, allowing the prototropic exchange rate to be determined through its effect on the overall water proton relaxation rate.



Results and Discussion

Determination of the Water Proton Exchange Lifetime τ_M^H

Analysis of the temperature dependence of the transverse relaxation rate for the ^{17}O water nuclei is the most accurate method for evaluating the exchange lifetime of the water molecule/s directly coordinated to the metal in a paramagnetic Gd^{III} chelate.

This approach allows assessment of the exchange lifetime of the metal-bound water oxygen nucleus which, at neutral pH and in non-buffered solutions, corresponds to the exchange lifetime of the metal-bound water protons, i.e. $\tau_M^H = \tau_M^O$.

The paramagnetic contribution (R_{2p}^O) to the observed transverse relaxation rate of water ^{17}O nuclei (R_{2obs}^O) is given by equation (1)

$$R_{2p}^O = R_{2obs}^O - R_{2d}^O \tag{1}$$

where R_{2d}^O is the transverse relaxation rate measured in a solution containing a diamagnetic chelate of the corresponding ligand at the same concentration.

According to the theory first proposed by Swift and Connick[8], R_{2p}^O depends on several parameters [equation (2)]

$$R_{2p}^O = P_M(\tau_M^O)\frac{R_{2M}^{O2} + (\tau_M^O)^{-1}R_{2M}^O + \Delta\omega_M^{O2}}{R_{2M}^{O2} + (\tau_M^O)^{-1})^2 + \Delta\omega_M^{O2}} \tag{2}$$

such as the chemical shift difference between the metal-bound and bulk water ^{17}O nuclei $\Delta\omega_M^O$, the molar fraction of the metal-bound water molecules P_M, their intrinsic ^{17}O transverse relaxation rate R_{2M}^O, and the exchange rate τ_M^O.

For quickly tumbling Gd^{III} chelates and at relatively low magnetic field strength (as used in this work), R_{2M}^O is dominated by the nucleus-electron scalar relaxation mechanism, which may be evaluated using equation (3).

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

Here, 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) are the correlation times for the dynamic processes modulating the scalar interaction. Both the longitudinal and the transverse electronic relaxation times (T_{1E} and T_{2E}), as well as the exchange lifetime of the metal-bound water molecule, may modulate the scalar interaction. The correlation time for this process is given by equation (4).

$$\tau_{Ei}^{-1} = \tau_M^{O^{-1}} + T_{iE}^{-1} \tag{4}$$

The scalar coupling constant is related to the unpaired electron spin density at the ^{17}O nucleus and it mainly depends on the distance between the metal ion and the metal-bound ^{17}O nucleus. Since for polyaminocarboxylate Gd^{III} chelates with a single inner-sphere water molecule this distance does not seem to vary significantly, for the $Gd(COPTA)^{2-}$ complex we used the same value as that reported for the closely related $Gd(DTPA)^{2-}$ chelate (-3.8×10^6 rad s^{-1}).[1]

For Gd^{III} complexes, T_{iE} values are basically related to the modulation of the transient zero-field splitting (ZFS) of the electronic spin states arising from the dynamic distortions of the ligand field caused by the solvent collision and, according to the Bloembergen-Morgan and Rubinstein theory,[9][10] they are expressed by equations (5) and (6)

$$T_{1E}^{-1} = \frac{1}{25}\Delta^2[4S(S+1)-3]\left[\frac{\tau_v}{1+\omega_s^2\,\tau_v^2} + \frac{4\tau_v}{1+4\omega_s^2\,\tau_v^2}\right] \tag{5}$$

$$T_{2E}^{-1} = \frac{1}{50}\Delta^2[4S(S+1)-3]\left[3\tau_v + \frac{5\tau_v}{1+\omega_s^2\,\tau_v^2} + \frac{2\tau_v}{1+4\omega_s^2\,\tau_v^2}\right] \tag{6}$$

where Δ^2 is related to the square of the mean transient ZFS energy, τ_v is the correlation time for the collision-related modulation of the ZFS hamiltonian, and ω_s is the electronic Larmor frequency.

The temperature dependence of R_{2M}^O is, therefore, expressed by the temperature effect on τ_M^O, τ_v, and $\Delta\omega_M^O$ according to equations (7) and (8)

$$\tau_j^{-1} = \frac{(\tau_j^{-1})^{298.15}\,T}{298.15}\exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{7}$$

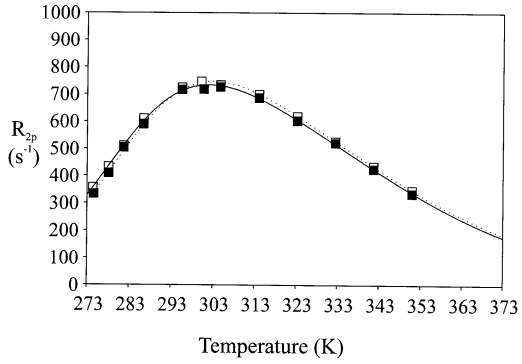
$$\Delta\omega_M^O = \frac{g_e\mu_B S(S+1)B}{3k_B T}\frac{A}{\hbar} \tag{8}$$

where the subscripts j refer to the two dynamic processes (j = M, v), ΔH_j is their activation enthalpy, B is the magnetic field strength, k_B is the Boltzmann constant, g_e is the free-electron Landè factor (2.0023), and μ_B is the Bohr magneton.

In Figure 1, we present a comparison between the temperature dependence of ^{17}O-R_{2p}^O for $Gd(COPTA)^{2-}$ and for the

parent Gd(DTPA)$^{2-}$, both recorded at 2.1 T, pH = 7 and at a concentration of 50 mM.

Figure 1. Temperature dependence of the transverse water ^{17}O relaxation rate at 2.1 T and pH = 7 for 50 mM solutions of Gd(COPTA)$^{2-}$(■) and Gd(DTPA)$^{2-}$ (□)



The two profiles appear to be superimposable, both being characterized by a relaxivity maximum centered at about 300 K. This means that the introduction of the hydrophobic substituent on one acetate arm of the Gd(DTPA)$^{2-}$ chelate does not significantly affect the exchange process of the metal-bound water molecule. An analogous observation has been recently reported by Muller et al. for the related Gd(EOB-DTPA)$^{2-}$ complex [EOB-DTPA = 3,6,9-triaza-3,6,9-tris(carboxymethyl)-4-(4-ethoxybenzyl)undecanedicarboxylic acid].[11] The fitting of the data afforded a τ_M^O value at 298 K for Gd(COPTA)$^{2-}$ of 290 ns, a value very close to those obtained for Gd(DTPA)$^{2-}$ (320 ns) and Gd(EOB-DTPA)$^{2-}$ (201 ns)[11] chelates.

Determination of the Longitudinal Water Proton Relaxation Time of the Coordinated Water Molecule τ_{1M}^H

In principle, the observed longitudinal relaxation rate of the water protons (R_{1obs}^H) of an aqueous solution containing a paramagnetic Gd^{III} chelate is given by the sum of three terms [equation (9)]

$$R_{1obs}^H = R_{1p}^{His} + R_{1p}^{Hos} + R_{1d}^H \quad (9)$$

where R_{1p}^{His} represents the paramagnetic contribution from the metal-bound water molecule, R_{1p}^{Hos} is the contribution from the water molecules that diffuse around the complex, and R_{1d}^H corresponds, in analogy to the ^{17}O approach, to the diamagnetic contribution measured in the presence of an equimolar amount of a diamagnetic analogue of the chelate.

The paramagnetic contribution to the observed relaxation rate ($R_{1p}^{His} + R_{1p}^{Hos}$) for a 1 mM solution of the paramagentic complex is called "relaxivity" and it is commonly indicated as r_{1p}.

According to the established theory,[12] R_{1p}^{His} for a 1 mM concentration of a Gd^{III} chelate with one exchanging metal-bound water molecule is given by equation (10)

$$R_{1p}^{His} = \frac{1.8 \cdot 10^{-5}}{T_{1M}^H + \tau_M^H} \quad (10)$$

where T_{1M}^H is the longitudinal relaxation time of the exchangeable metal-bound water protons.

This parameter is related to the dipolar interaction between the unpaired electrons of the metal and the inner-sphere water proton nuclei through equation (11)

$$(T_{1M}^H)^{-1} = \frac{2}{15}\frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r_H^6}\left[\frac{3\tau_{C1}}{1+\omega_I^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2}\right] \quad (11)$$

where γ_I is the proton gyromagnetic ratio, r_H is the mean distance between the metal and the inner-sphere water protons, and ω_I and ω_S are the nuclear and electronic Larmor frequencies, respectively.

The two correlation times (τ_{ci}; i = 1,2) associated with the modulation of the proton-electron dipolar coupling are given by equation (12)

$$\tau_{ci}^{-1} = \tau_R^{-1} + (\tau_M^H)^{-1} + T_{iE}^{-1} \quad (12)$$

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

On the contrary, the outer-sphere contribution, R_{1p}^{Hos}, is not affected by the exchange process occurring at the metal site, since it basically depends, according to the model developed by Hwang and Freed,[13] on the distance of minimum approach between the metal and the diffusing water molecules a, the relative diffusion coefficient D, and the electronic relaxation times (T_{iE}) [equation (13)]

$$R_{1p}^{Hos} = C^{os}\left(\frac{1}{aD}\right)[7J(\omega_s) + 3J(\omega_I)] \quad (13)$$

where C^{os} is equal to 5.8×10^{-13} M^{-1} s^{-2} for a 1 mM concentration of the paramagnetic chelate and $J(\omega_i)$ represents the non-Lorentzian spectral density functions, including the dependence of T_{iE}, a and D.

The large number of parameters responsible for the water proton relaxation enhancement in the presence of a paramagnetic chelate makes their simultaneous and precise evaluation rather difficult. Nevertheless, this goal may be achieved by measuring the water proton relaxation rate over an extended range of magnetic field strengths.[14] The resulting plot of R_{1obs}^H vs. the proton Larmor frequency, called the NMRD profile (Nuclear Magnetic Relaxation Dispersion), is then fitted through eqs. 9−13 to obtain reliable values for the relaxation parameters. The possibility of fixing the values of some of the parameters involved makes the determination of others more accurate. The analysis of the NMRD profiles collected in recent years for a series of structurally similar Gd^{III} polyaminopolycarboxylate chelates provided us with reliable estimates for some relaxation parameters. In particular, values of 2.96 Å, 3.6 Å and 2.6×10^{-5} cm^2 s^{-1} (at 298 K) were obtained for $r_H a$ and D, respectively, from the fitting of the NMRD data for the strictly analogous Gd(BOPTA)$^{2-}$ chelate.[15] On this basis, we have assumed that these parameters do not change for the two complexes and, with these constraints, along with the values of q (1) and τ_M^H (290 ns as obtained from ^{17}O data), we carried out the fitting of the NMRD data for Gd(COPTA)$^{2-}$.

In Figure 2, the NMRD profile (25°C and pH = 7) of $Gd(COPTA)^{2-}$ is presented, along with the outer-sphere contribution to the relaxivity, while in Table 1 the best-fitting parameters are compared with those reported for $Gd(DTPA)^{2-}$.[15]

Figure 2. $1/T_1$ NMRD profile of a 1 mM aqueous solution of $Gd(COPTA)^{2-}$ at pH = 7 and 298 K. The dotted curve represents the outer-sphere contribution to the overall relaxivity

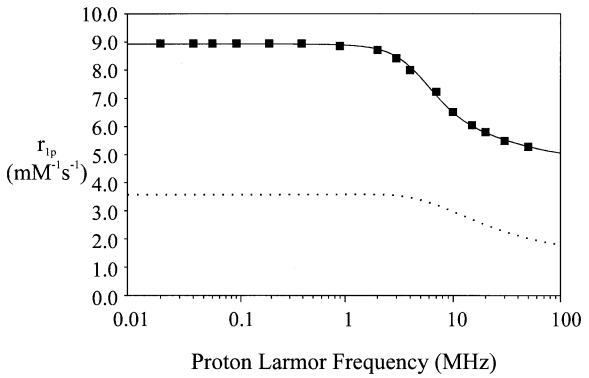


Table 1. Relaxation parameters calculated from NMRD data for $Gd(COPTA)^{2-}$ and $Gd(DTPA)^{2-}$ complexes at pH = 7 and 298 K[a]

Parameter	$Gd(DTPA)^{2-}$[b]	$Gd(COPTA)^{2-}$
Δ^2 ($/10^{19}$ s^{-2})	5.3 (0.3)	4.0 (0.2)
τ_V^{298} (ps)	19.0 (1.0)	25.0 (1.4)
τ_M^H (ns)	320[c]	290[c]
τ_V^{298} (ps)	73.0 (2.0)	80.1 (4.1)

[a] Numbers in parentheses represent standard deviations in mean parameter estimates on 1000 simulated relaxation rate data sets obtained by repeatedly introducing a random error of 1% into the experimental data set and estimating best parameters. – [b] From ref.[15]. – [c] The value was obtained in this work from the ^{17}O data and it has been kept fixed in the fitting of the NMRD profiles.

The main differences between the two sets of parameters lie in the higher τ_R value and the smaller r value obtained for $Gd(COPTA)^{2-}$. A longer reorientational correlation time may be easily accounted for in terms of a larger molecular size for this chelate and very similar τ_R values at 298 K have been reported for both $Gd(BOPTA)^{2-}$ (88.0 ps)[15] and Gd(EOB-DTPA)$^{2-}$ (84.0 ps)[11] complexes.

Once the relaxation parameters for the metal-bound water protons are known, it is possible to calculate their T_{1M}^H at 25°C. We obtained a value (5.3 μs) an order of magnitude larger than the water exchange lifetime (0.29 μs), indicating that for the $Gd(COPTA)^{2-}$ complex at 0.47 T, 298 K and pH = 7, the condition $T_{1M}^H > \tau_M^H$ is undoubtedly fulfilled.

Determination of the Prototropic Exchange Lifetime for the Metal-Bound Water Proton τ_M^{Hp}

The exchange lifetime of the protons of water molecules is strongly pH-dependent, being very long at neutral pH (of the order of ms) and significantly shorter (down to ns values) as the pH is adjusted to values either side of 7. The prototropic exchange process may be adequately treated as a pseudo-first-order mechanism, characterized by a rate constant k_p^i, the values of which for pure water at 301 K were reported to be in the range $4.1-7.1 \times 10^9$ M^{-1} s^{-1} and $2.2-4.3 \times 10^9$ M^{-1} s^{-1} for the acidic (k_p^a) and basic (k_p^b) catalysis, respectively.[5]

The prototropic exchange lifetime for the protons of a water molecule coordinated to a paramagnetic Gd^{III} chelate may be determined by analyzing the pH dependence of the water proton relaxivity. As it is well-known that the Gd^{III} chelates derived from the DTPA ligand are not sufficiently stable under very acidic conditions, we are forced, in the case of $Gd(COPTA)^{2-}$, to deal only with the base-catalysis of the prototropic exchange.

In order to account for the contribution arising from the prototropic exchange rate 1, the relaxation data are fitted to a modified version of equation 10, where τ_M^H is replaced by [equation (14)]

$$\tau_M^H = [(\tau_M^O)^{-1} + (\tau_M^{Hp})^{-1}]^{-1} \quad (14)$$

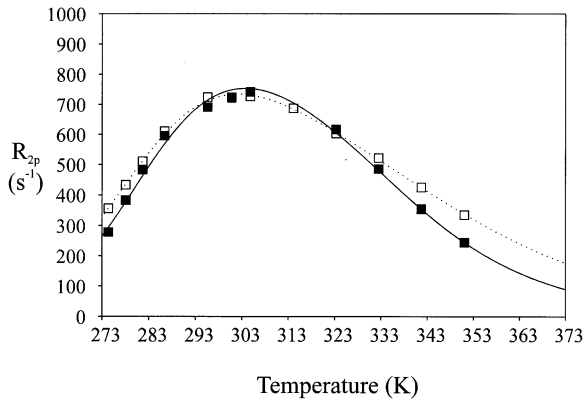
and [equation (15)]

$$\tau_M^{Hp} = \left(k^b \frac{Kw}{[H_3O^+]}\right)^{-1} \quad (15)$$

As it is reasonable to assume that the outer-sphere contribution to the observed relaxivity is unaffected by the pH of the solution, the reliability of the method is based on two main assumptions: (i) T_{1M}^H does not change over the investigated pH range, and (ii) at neutral pH the observed relaxivity has to be affected by the water exchange lifetime, i.e the condition $T_{1M}^H \approx \tau_M^H$ has to be fulfilled.

The temperature dependence of the water ^{17}O-R_{2p} is particularly sensitive to changes of the coordination cage of the chelate, thus allowing assessment of whether the pH of the solution causes either structural changes or variations in the exchange lifetime of the whole water molecule (τ_M^O). In Figure 3, the temperature dependence ^{17}O-R_{2p} profiles for $Gd(COPTA)^{2-}$ at pH = 7 and pH = 12 are shown.

Figure 3. Temperature dependence of the transverse water ^{17}O relaxation rate at 2.1 T and pH = 7 for 50 mM solution of $Gd(COPTA)^{2-}$ at pH = 7 (□) and pH = 12 (■)

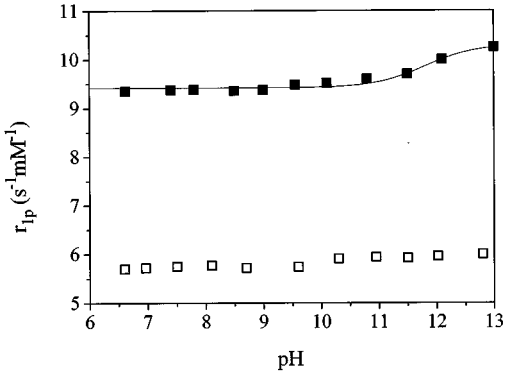


The two profiles are rather similar, being the differences observed in the best fitting parameters within experimental error. These findings support the view that the structure of this Gd^{III}

chelate, and consequently its T_{1M}^H value, is not affected by the pH of the solution.

On the contrary, as emphasized in the previous paragraph, the second condition is not valid for $Gd(COPTA)^{2-}$ at 298 K and 0.47 T and, therefore, the water proton relaxivity is not affected by the pH of the solution (Figure 4, bottom). Thus, this behaviour prevents the determination of the prototropic exchange rate for this chelate under these experimental conditions.

Figure 4. pH dependence of the longitudinal water proton relaxivity at 20 MHz and 298 K of $Gd(COPTA)^{2-}$ free (□) and bound to β-CD (■)



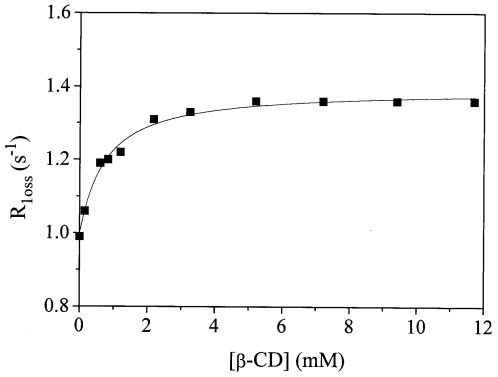
In order to meet the conditions under which the prototropic contribution to the overall relaxation rate can be expressed, we followed two different approaches: (i) formation of a paramagnetic adduct with a slowly tumbling substrate, such as β-cyclodextrin, in order to reduce T_{1M}^H, and (ii) decrease of the experimental temperature in order to either reduce T_{1M}^H or increase τ_M^H.

Reduction of T_{1M}^H Through Non-Covalent Interaction with β-Cyclodextrin

The increase of the relaxivity of a Gd^{III} chelate caused by the lengthening of its reorientational correlation time τ_R is a well-known phenomenon. In fact, it represents the most useful approach to enhance the effectiveness of a CA for MRI applications.[16] Usually, the increase of τ_R is pursued through the formation of adducts between the paramagnetic chelate and slowly tumbling substrates. For this purpose, it is necessary to have a complex endowed with a suitable substituent able to interact with the macromolecular substrate. The benzyloxymethyl residue has been reported to be an excellent recognition synthon that is able to interact with many substrates such as β-cyclodextrin, albumin and micelles.[17][18][19] As the interaction of this group is basically hydrophobic in origin, we expect that the replacement of the phenyl group by cyclohexyl should not significantly modify the interaction properties of the former substituent. Furthermore, in the context of this work, we have to choose a macromolecular substrate, the chelate adduct of which affects the exchange mechanism of the metal-coordinated water molecule as little as possible. For this reason, β-cyclodextrin is the best candidate, since in this case the non-covalent interaction should involve only the hydrophobic tail of the amphiphilic complex and its coordination cage should remain unchanged.

The increase of relaxivity resulting from this interaction may be also exploited for evaluating the affinity of the chelate for β-cyclodextrin. In fact, by measuring the longitudinal water proton relaxation rates of a solution of $Gd(COPTA)^{2-}$ in the presence of increasing amounts of β-CD, we obtained the saturation profile shown in Figure 5, fitting of which allows derivation of an association constant of $(1.5 \pm 0.1) \times 10^3\ M^{-1}$ and a relaxivity for the bound complex of $9.2 \pm 0.1\ s^{-1}mM^{-1}$ (25°C and pH = 7), i.e. 1.6 times larger compared to the unbound chelate.

Figure 5. Longitudinal water proton relaxation enhancement of a 0.11 mM solution of $Gd(COPTA)^{2-}$ upon addition of β-CD (20 MHz, pH = 7, 298 K)

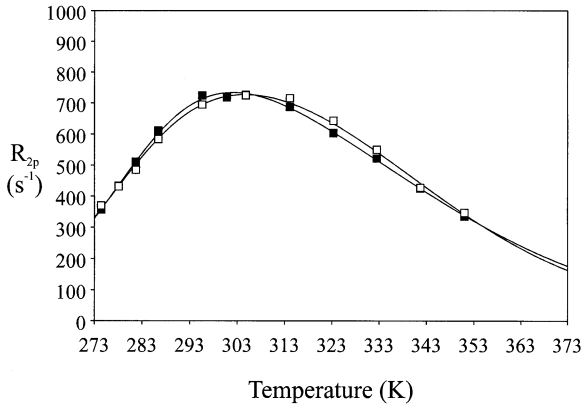


As the interaction with the β-CD essentially causes a lengthening of the reorientational correlation time of the Gd^{III} chelate, the outer-sphere contribution to the overall relaxivity of the bound complex is not expected to be significantly different compared to that of the unbound chelate. From the quantitative analysis of the NMRD profile of $Gd(COPTA)^{2-}$ shown in Figure 2, an R_{1p}^{Hos} value of $2.45\ s^{-1}mM^{-1}$ at 20 MHz and 25°C was obtained. Therefore, the inner-sphere contribution for the $Gd(COPTA)^{2-}$/β-CD adduct is $6.75\ s^{-1}mM^{-1}$ under the same experimental conditions.

In order to calculate the value of T_{1M}^H for the metal-bound water protons in the paramagnetic inclusion compound, it is also necessary to evaluate the value of their exchange lifetimes τ_M^H (equal to τ_M^O at neutral pH). The temperature dependence of the ^{17}O water transverse relaxation rate for the $Gd(COPTA)^{2-}$ chelate bound to β-CD is almost superimposable on the profile recorded for the unbound complex (Figure 6), confirming that τ_M^H does not vary appreciably upon interaction.

On this basis, a value of T_{1M}^H of 2.4 μs for the metal-bound water protons in the inclusion compound may be calculated through eqs. 9–10. This value is only 2.2 times lower than that for the unbound chelate and the condition $T_{1M}^H \approx \tau_M^H$ is not yet met. Nevertheless, the pH dependence of the longitudinal water proton relaxivity for the $Gd(COPTA)^{2-}$/β-CD adduct measured at 298 K (Figure 4, top) is characterized by a slight increase of relaxivity at pH > 11. As the binding with β-CD basically occurs through van der Waals' forces,[20] the affinity of the Gd^{III} chelate for the cyclic oligosaccharide should not

Figure 6. Temperature dependence of the transverse water ^{17}O relaxation rate at 2.1 T and pH = 7 for 50 mM solution of $Gd(COPTA)^{2-}$ free (■) and bound to β-CD (□)



depend on the pH of the solution. Moreover, from the close similarity between the ^{17}O data for the free chelate and those of the adduct with β-CD (either at neutral or basic pH), we can safely rule out the possibility that the relaxation enhancement observed at basic pH is due to structural changes of the Gd^{III} complex. Therefore, the increase of relaxivity may be ascribed to the occurrence of a fast base-catalyzed prototropic exchange of the metal-bound water protons, which results in a reduction of τ_M^H at pH > 11. This hypothesis was further confirmed through a quantitative analysis of the data using equations 10, 14−15. The best fitting parameters (T_{1M}^H, τ_M^O and k_p^b) were in good agreement with the expected values at 298 K. In fact, values of 2.3 ± 0.02 μs and 0.29 μs were obtained for T_{1M}^H and τ_M^O, respectively. As far as the base-catalyzed prototropic exchange rate constant is concerned, we obtained a value of (7.3 ± 2.6) × 10^8 s^{-1} M^{-1}, which is lower than the value measured in pure water and similar to the value determined for the Gd(DTPA-BBA) chelate.[7]

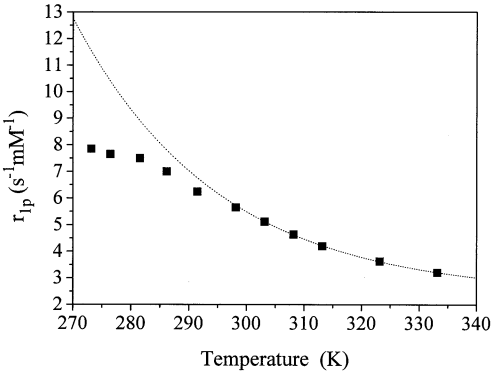
In conclusion, the reduction of T_{1M}^H caused by the formation of an inclusion compound with β-CD allows detection of the effect on the relaxivity arising from the prototropic exchange at the metal-bound water molecule of the Gd^{III} chelate. Unfortunately, the effect of the interaction on R_{1M}^H is not particularly marked owing to the small increase of τ_R for the chelate bound to the β-CD. For this reason, the associated relaxivity enhancement observed at basic pH is relatively small and the error in the estimation of k^b is consequently rather large (ca. 36%).

Reduction of T_{1M}^H by Lowering the Temperature

Another possible approach to achieve the condition $T_{1M}^H \leq \tau_M^H$ is to exploit the opposite temperature dependence of these two parameters. In particular, at low temperature, one can expect a decrease of T_{1M}^H (due to the increase of τ_R) and a simultaneous lengthening of τ_M^H. The temperature dependence of the longitudinal water proton relaxivity for $Gd(COPTA)^{2-}$ complex is depicted in Figure 7.

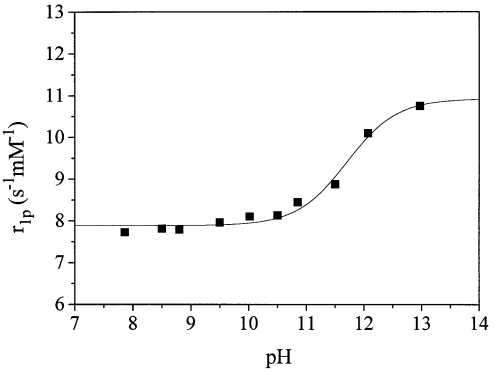
The profile may be split in two regions: at temperatures higher than 288 K, the relaxivity decreases pseudo-exponentially owing to the increase of T_{1M}^H, whereas at lower temperature, the elongation of τ_M^H and the simultaneous decrease of R_{1p}^{Hos} causes a flattening of the profile. On the basis of these data, at a temperature lower than 288 K, the condition $R_{1M}^H \approx \tau_M^H$ can be expected to hold.

Figure 7. Temperature dependence of the water proton relaxivity of $Gd(COPTA)^{2-}$ at 20 MHz and pH = 7



The pH dependence of the relaxivity of the $Gd(COPTA)^{2-}$ complex recorded at 278 K is shown in Figure 8.

Figure 8. pH dependence of the longitudinal water proton relaxivity at 20 MHz and 278 K of $Gd(COPTA)^{2-}$



At this temperature, the relaxivity enhancement promoted by the activation of the prototropic exchange appears to be much more pronounced compared to the effect observed at room temperature for the same system. Before carrying out a quantitative analysis of the data reported in Figure 8, it was thought useful to gain more information about the relaxation parameters of the chelate at 278 K. In particular, by fixing a value of D of 1.1 × 10^{-5} cm^2 s^{-1},[21] the fitting of the NMRD data recorded at this temperature and at pH = 7 afforded a value of 4.4 s^{-1} mM^{-1} for the outer-sphere contribution to the relaxivity and values of 3.2 ± 0.2 μs and 2.4 ± 0.1 μs for T_{1M}^H and τ_M^H (equal to τ_M^O at neutral pH), respectively. Interestingly, we obtained very similar T_{1M}^H and τ_M^H values (2.8 ± 0.1 μs and 2.4 ± 0.1 μs) from the fitting of the data shown in Figure 8. Furthermore, it is noteworthy that the values obtained for the water proton exchange lifetime at pH = 7 ($\tau_M^H = \tau_M^O$) from the analysis of both magnetic field and pH dependence of the ^1H relaxivity are in good agreement with the value calculated at 278 K from the ^{17}O data (1.3 ± 0.1 μs). As expected, the value of the rate constant for the base-catalyzed prototropic ex-

change process is lower [$(1.5 \pm 0.3) \times 10^8$ s^{-1} M^{-1}], but more accurate (standard deviation ca. 20%), compared to the value obtained at room temperature in the case of the Gd^{III} complex incorporated into β-CD.

Conclusion

In this work, we have shown that NMR (^1H and ^{17}O) relaxometric methods provide a powerful tool for assessing the exchange rate of the coordinated water molecule, as well as the proton transfer rate, in a paramagnetic Gd^{III} complex.

This has been possible through the decrease of the relaxation time of the protons of the coordinated water (T_{1M}^H) upon formation of a paramagnetic adduct with the slowly tumbling β-cyclodextrin substrate.

When T_{1M}^H becomes smaller than the exchange lifetime of the coordinated water, the prototropic contribution to the overall proton relaxation enhancement may be evaluated, since, at basic pH, the proton transfer rate becomes faster than the exchange of the whole water molecule.

Support from *MURST* CNR is gratefully acknowledged.

Experimental Section

Synthesis: The COPTA ligand and its Gd^{III} chelate were prepared using a procedure analogous to that previously reported for the BOPTA ligand[15] starting from diethylenetriamine and 2-bromo-3-(cyclohexylmethoxy)propanoic acid, followed by peralkylation of the monoalkylated intermediate with sodium bromoacetate in water at pH = 10. 2-Bromo-3-(cyclohexylmethoxy)propanoic acid was obtained by alkaline hydrolysis of the corresponding methyl ester, which was prepared according to a reported procedure.[22]

^1H-NMR Measurements: Longitudinal water proton relaxation rates were measured at 25°C and 0.47 T with a Stelar Spinmaster spectrometer [Stelar, Mede (PV), Italy] by using the inversion recovery (180° − τ − 90°) pulse sequence. 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 4 scans and the values of the magnetization were obtained in the time domain by averaging the first 128 points of the free induction decay. The reproducibility in T_1 measurements was ±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 52 k/j digital thermometer.

The ^1H-NMRD profiles were recorded on a Koenig-Brown field-cycling relaxometer by measuring water proton longitudinal relaxation rates at magnetic field strengths in the range from 2.4×10^{-4} to 1.5 T (corresponding to 0.01−50 MHz proton Larmor frequencies) with a T_1 uncertainty of ±1%.

^{17}O-NMR Measurements: The measurement of the ^{17}O transverse relaxation rates was carried out with a JEOL EX-90 spectrometer operating at 2.1 T (corresponding to a ^{17}O Larmor frequency of 12.2 MHz) by using an external D_2O lock. The temperature calibration was performed following the same procedure described for the Stelar SpinMaster spectrometer.

The value of the transverse relaxation rate was obtained by evaluating the linewidth at half-height ($\Delta\nu_{1/2}$) of the water ^{17}O signal ($R_2 = \pi\Delta\nu_{1/2}$). Solutions containing 2.6% of the ^{17}O isotope were used. ^{17}O-enriched (10.4%) water was purchased from Yeda (R. & D. Co., Rehovot, Israel).

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