Prototropic and Water-Exchange Processes in Aqueous Solutions of Gd(III) Chelates

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1. Introduction

Since 1972, when Lauterbur first showed that, by superimposing linear field gradients to the static magnetic field in an NMR experiment, it is possible to obtain projections of an object,[1] magnetic resonance imaging (MRI) has developed impressively, becoming one of the most powerful tools "to look inside matter". A MR image is a topological representation of NMR parameters, and therefore the contrast is determined not only by the complex interplay of endogenous factors (T_1, T_2, and proton density) but also by a number of instrumental parameters. In MR images, the contrast may be further enhanced by the use of suitable contrast agents (CAs), which act by enhancing the proton relaxation rates in the tissues where they distribute.[2−7]

In the last 10 years, Gd(III) chelates have been under intense scrutiny for their potential use as CAs for MRI. In fact, the possible use of Gd(III) was recognized initially because of its high effective magnetic moment (seven unpaired electrons) and its relatively long electronic relaxation time. The high coordination number (8−10) displayed by the Gd(III) ion allows it to be chelated by ligands of high denticity, thereby limiting the toxicity associated with the release of the lanthanide ion, while maintaning one or more water molecules (q) directly coordinated to the paramagnetic center. The administration of Gd-based CAs is routinely performed in clinical diagnosis (ca. 35% of MRI examinations make use of CAs), and it is particularly useful in assessing organ perfusion and any abnormalities in the blood−brain barrier or in kidney clearance. Several other applications, primarily in the field of angiography and tumor targetting, will soon become available.[8,9]

The design of novel CAs requires a good understanding of the factors which determine the relaxation enhancement of the tissue protons.[10] In principle, in an aqueous solution of a paramagnetic complex, the relaxation enhancement of the solvent water protons is propagated to the bulk through the exchange of the water molecules dipolarly coupled to the metal ion. The water molecule may be directly coordinated to the paramagnetic center (inner sphere), it may belong to a second coordination sphere, or it may simply diffuse at the surface of the complex (outer sphere). The prototropic exchange may also be involved in the transmission of the magnetic interaction and this may deal with water protons in the first or second coordination spheres or with other exchangeable protons in the proximity (on the ligand or on substrates tightly associated with it) of the paramagnetic center (Figure 1).

It is customary to assess the ability of a paramagnetic complex to act as CA for MRI by determining its "in vitro" relaxivity (r_{1p}), which refers to the relaxation enhancement of solvent water protons promoted by a given complex at a 1 mM concentration and observation frequency of 20 MHz. Though commonly used, this procedure can be misleading, particularly for complexes with two or more coordinated waters that can be displaced by the anions (lactate, carbonate, citrate, etc.) present in a physiological medium, with consequent reduction of r_{1p}. However, it is worth noting that the hydration state q can be independently assessed by measuring the excited-state lifetime of the corresponding Eu(III) or Tb(III) complexes in H_2O and D_2O.[2]

In the past 5−6 years, we and other research teams have addressed the problem of getting an in-depth understanding of the structural, dynamic, and electronic

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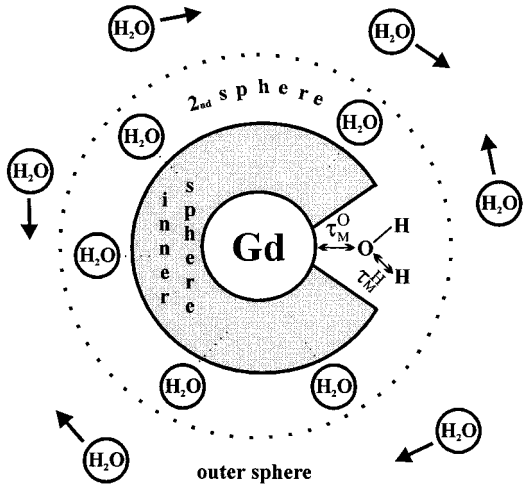


FIGURE 1. The relaxivity of a Gd(III) chelate is governed by the dipolar interaction between the metal center and the proximate water protons. The propagation of the paramagnetic effect may occur through (i) the exchange of the inner-sphere water (τ_M^{O-1}) protons and/or the exchange of its protons only (τ_M^{H-1}); (ii) the exchange of water molecules hydrogen bonded to polar groups on the complex surface and/or the exchange of mobile hydrogens on the ligand; or (iii) the diffusion around the chelate of the outer-sphere water molecules.

factors determining the relaxivity of a given Gd(III) complex.[7,11] On carrying out these studies, a number of different model structures have been considered, and the feedback from these investigations has allowed us to gain a detailed knowledge of the hydration sphere of these Gd(III) complexes that may be similarly extended to other coordination compounds in aqueous solution. Moreover, this has shown the great potential of the relaxometric measurements in investigating the hydration sphere of metal chelates.

2. Exchange Rates of the Coordinated Water Molecule

In the case of Gd(III) chelates characterized by $q \geq 1$, a large contribution to the observed relaxation enhancement of solvent water protons arises from the exchange of the bound water molecule(s) with the bulk solvent.

This contribution (r_{1p}^{is}) is determined by the relaxation time (T_{1M}) and the exchange lifetime (τ_M) of the protons of the water molecule(s) in the inner coordination sphere:[12]

$$r_{1p}^{is} = \frac{1.8 \times 10^{-5} q}{T_{1M} + \tau_M} \qquad (1)$$

Thus, r_{1p}^{is} is maximized when $T_{1M} > \tau_M$ (fast exchange conditions) and T_{1M} is as short as possible. An early combined NMR and EPR study indicated a short exchange lifetime of the bound water for the Gd(III) aquo ion (0.9 ns) and its complex with PDTA (3 ns) (Chart 1, **1**) and relatively little dependence on the coordination polyhedron.[11]

For more than 10 years, it has been assumed that τ_M for the polyaminocarboxylate complexes of Gd(III) was of

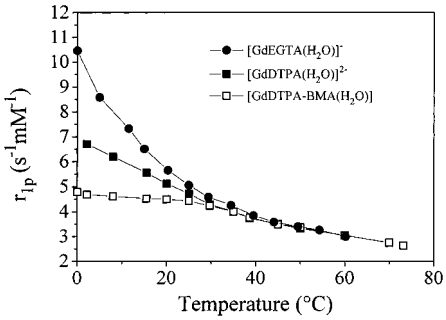
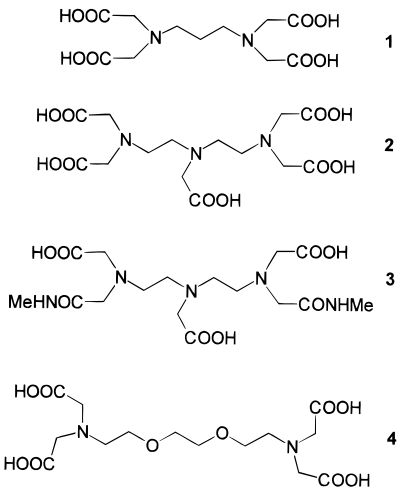


FIGURE 2. The temperature dependence of the relaxivity allows a qualitative assessment of the exchange lifetime of the metal-bound water protons: the shorter the τ_M, the higher the relaxivity at low temperatures (0.47 T, pH 7).

Chart 1



the same order of magnitude as that found for the octaaquo ion. Surprisingly enough, some years ago it was found that monohydrated Gd(III) chelates may display relatively long exchange lifetimes, i.e., they may be of the same order of magnitude or even longer than T_{1M} (intermediate/slow exchange).[13,14] The occurrence of this condition is easily verified by measuring the temperature dependence of r_{1p}. In Figure 2, r_{1p} vs T is reported for three complexes (Chart 1) in the range 273−340 K.

Over this temperature range, only $[GdEGTA(H_2O)]^-$ (Gd-**4**) displays relaxivity values which follow a single-exponential law as found typically in a system whose relaxivity is mainly controlled by T_{1M}, which, in turn, at proton Larmor frequency of 20 MHz, is determined by the molecular reorientational time τ_R. In fact, $1/T_{1M}$ is given by

$$1/T_{1M} = Df(\tau_R) \qquad (2)$$

where D is the dipolar coupling between the proton and electron magnetic moments.[12]

The complexes $[GdDTPA(H_2O)]^{2-}$ (Gd-**2**) and [Gd-DTPA-BMA(H_2O)] (Gd-**3**) are of similar size to $[GdEGTA(H_2O)]^-$, and, therefore, the observed differences cannot be ascribed to abnormalities in their molecular motions. Rather, these abnormalities are indicative of differences between T_{1M} and τ_M, which exhibit opposite temperature

dependences; i.e., on lowering the temperature, T_{1M} decreases and τ_M increases. The behavior shown by $[GdDTPA(H_2O)]^{2-}$ and [GdDTPA-BMA(H_2O)] is then typical of systems with relatively long τ_M values. In the low-temperature range, τ_M markedly "quenches" the relaxation enhancement expected for these systems.

In principle, the exchange lifetime for the protons on the coordinated water may result either from the exchange rate of the whole water molecule ($1/\tau_M^O$) or/and from the prototropic exchange ($1/\tau_M^H$):

$$(\tau_M)^{-1} = (\tau_M^O)^{-1} + (\tau_M^H)^{-1} \quad (3)$$

Fortunately, the two possibilities may be easily differentiated. In fact, the exchange rate of the metal-bound water may be accurately determined by measuring the paramagnetic contribution (R_{2p}^O) to the observed transverse relaxation time (R_{2obs}^O) of water ^{17}O nuclei at variable temperature:

$$R_{2p}^O = R_{2obs}^O - R_{2d}^O \quad (4)$$

where the diamagnetic term R_{2d}^O is evaluated from a solution containing a diamagnetic analogue of the chelate of interest.

R_{2p}^O is related to τ_M^O through the values of $\Delta\omega_M^O$ (the ^{17}O chemical shift difference between coordinated and bulk water) and R_{2M}^O (the transverse relaxation rate of the coordinated water oxygen):[11]

$$R_{2p}^O = \frac{[C]q}{55.6}(\tau_M^O)^{-1}\frac{R_{2M}^{O\,2} + (\tau_M^O)^{-1}R_{2M}^O + \Delta\omega_M^{O\,2}}{(R_{2M}^O + (\tau_M^O)^{-1})^2 + \Delta\omega_M^{O\,2}} \quad (5)$$

The temperature dependence of $\Delta\omega_M^O$ is described by the following equation:

$$\Delta\omega_M^O = \frac{g_e\mu_B S(S+1)B}{3k_BT}\frac{A}{\hbar} \quad (6)$$

where B is the applied magnetic field strength and $A/\hbar$ is the Gd-^{17}O scalar coupling constant.

For Gd(III) chelates, R_{2M}^O is dominated by the electron−nucleus scalar interaction, which is modulated by the electronic relaxation times T_{iE} ($i = 1, 2$) or by exchange lifetime τ_M^O:[12]

$$R_{2M}^O = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_s^2\,\tau_{E2}^2}\right) \quad (7)$$

$$\tau_{Ei}^{-1} = \tau_M^{O-1} + T_{iE}^{-1} \quad (8)$$

Thus, it follows that the maximum observed in the temperature dependence of R_{2p}^O (Figure 3) corresponds to the changeover from the slow kinetic region at low temperatures (R_{2p}^O is determined by the exchange rate τ_M^{O-1}) and to the fast exchange region at high temperatures (R_{2p}^O is determined by τ_{Ei}^{-1}).

Finally, the dependence of R_{2p}^O upon the temperature is expressed in terms of the Eyring relationships, which account for the thermal activation of the exchange process and of the modulation of the electronic relaxation.

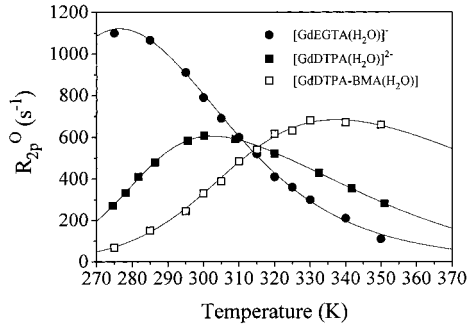


FIGURE 3. The quantitative analysis of the temperature dependence of the water, ^{17}O -R_{2p}, is the most useful technique for determining the exchange lifetime of the whole metal-bound water molecule, τ_M^O ([GdL] = 50 mM, 2.1 T, pH 7).

The calculated values of τ_M^O (at 298 K) for the three complexes shown in Chart 1 are the following: 32 ns for $[GdEGTA(H_2O)]^-$,[15] 300 ns for $[GdDTPA(H_2O)]^{2-}$,[13] and 2200 ns for [GdDTPA-BMA(H_2O)].[11]

This finding clearly indicates that the differences in the behavior of r_{1p} vs T shown by these complexes are directly related to the differences in the exchange lifetime of the coordinated water.

3. Determinants of Water Exchange Rate

The τ_M^O values found for $[GdDTPA(H_2O)]^{2-}$ and [GdDTPA-BMA(H_2O)] are 1 and 2 orders of magnitude lower, respectively, compared to that reported early on for $[GdPDTA(H_2O)_2]^-$ ($q = 2$).[16]

Variable-pressure NMR measurements carried out by the Merbach group in Lausanne have clearly shown that this increase in the mean residence lifetime is accompanied by a change of sign for the activation volume $\Delta V^{\#}$.[17] A negative value of $\Delta V^{\#}$ is indicative of a water-exchange associative mechanism where the bond formation of the entering water molecule precedes the bond breaking of the leaving water molecule. This mechanism implies the expansion of the coordination cage of the metal in the intermediate. Conversely, a positive value of $\Delta V^{\#}$ is consistent with a transition state where the dissociation of the bound water has occurred without participation of the incoming water molecule. In the PDTA complex, Gd^{3+} can easily accommodate another water molecule and expands the coordination number (CN) from 8 to 9. Both $[GdDTPA(H_2O)]^{2-}$ and [GdDTPA-BMA(H_2O)] are enneacoordinate complexes and cannot increase their CN further. In the latter two cases, the exchange of the bound water is not facilitated by the participation of the entering water molecule; i.e., the rate-determining step is represented by the formation of an octacoordinate activated state with $q = 0$ (Figure 4), resulting in a lower exchange rate.[11]

An illustrative example of the strong dependence of the water-exchange rate on the CN of the ground state of the complex is represented by the two Gd^{3+} complexes with the heptadentate macrocyclic ligands PCTA (**5**) and PCTP (**6**) (Chart 2).[18]

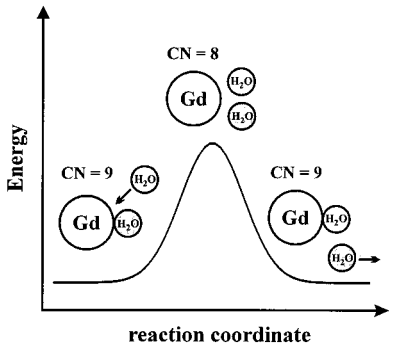
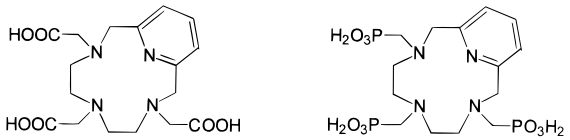


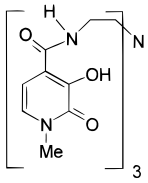
FIGURE 4. The water-exchange process for an enneacoordinate Gd(III) complex follows an idealized dissociative pathway. This may be represented by a simple diagram involving a transition state where the metal ion has reduced its coordination number from 9 to 8.

Chart 2



5 **6**

Chart 3



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$[GdPCTA(H_2O)_2]$ is enneacoordinated and has two water molecules in the inner sphere, with a mean residence lifetime at 298 K of 70 ns. On the other hand, $[GdPCTP(H_2O)]^{3-}$ presents an octacoordinate structure with $q = 1$, and in fact the water-exchange rate is about 1 order of magnitude faster than that of its carboxylate analogue.

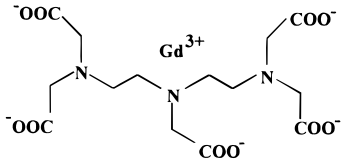
Pursuing an analogous approach, Raymond and coworkers proposed the use of a novel class of Gd(III) complexes with hydroxypyridinone-containing ligands.[19] The first member of this class (TREN-Me-3,2-HOPO, **7**) is shown in Chart 3.

The corresponding neutral Gd(III) complex is eight-coordinate and contains two water molecules in the inner coordination sphere. The coordination cage around the metal ion contains a hole that is expected to activate the water exchange by the intervention of an associative exchange mechanism. It follows that the water-exchange rate intimately reflects the coordination mode of the complexes in solution.

An important aspect that has been elucidated in recent years is the increase of the water-exchange rate across the Ln series for isostructural complexes. This behavior has been rationalized on the basis of the stabilization of the eight-coordinate transition state and/or destabilization of the nine-coordinate ground state following the reduction of the ionic radius, which in turn increases the steric constraint at the water binding site.[20]

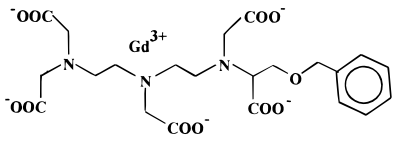
The key role played by the steric crowding at the water binding site in determining the exchange rate of the bound water in enneacoordinate complexes is nicely evidenced by comparing the τ_M^O values for $[GdDTPA(H_2O)]^{2-}$ with those measured for the derivatives containing one, two, and three benzyloxymethylenic substituents (Figure 5). The steric constraint of the coordination cage caused by the presence of such bulky substituents results in an increase in the exchange rate on going from the parent $[GdDTPA(H_2O)]^{2-}$ to the trisubstituted derivative.[21]

Analogous considerations can be made to account for the very small τ_M^O value reported above for $[GdEGTA(H_2O)]^{-}$. In this complex, two coordinating oxygen atoms are linked by an ethylenic group, which induces a steric compression of the atoms around the site occupied by the water molecule, thus favoring the exchange process.[15]



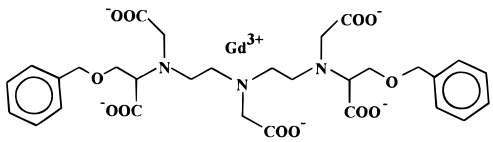
GdDTPA

$\tau_M^O = 340$ ns



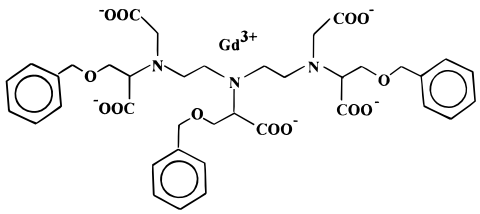
GdBOPTA

$\tau_M^O = 290$ ns



GdDTPA (BOM)$_2$

$\tau_M^O = 260$ ns



GdDTPA (BOM)$_3$

$\tau_M^O = 180$ ns

FIGURE 5. The increase of the number of the substituents on the DTPA skeleton causes a reduction in the exchange lifetime of the metal-bound water molecule. τ_O^M values refer to exchange lifetimes at 298 K.

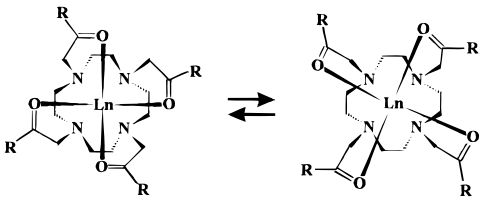


FIGURE 6. Schematic representation of the two diastereoisomers of the Ln(III) macrocyclic complexes based on the tetraazacyclododecane ring. The two isomers differ either in the conformation of the 12-membered ring ($\delta\delta\delta\delta$ or $\lambda\lambda\lambda\lambda$) or in the arrangement of the pendant arms (clockwise, $\triangle$, or anticlockwise, Λ).

Chart 4

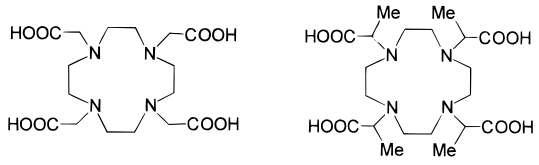
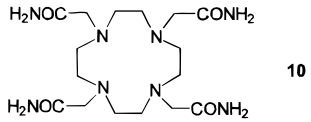


Chart 5



Also in the case of cyclic DOTA-like chelates, several interesting observations can be made. At 298 K, the parent $[GdDOTA(H_2O)]^-$ (Chart 4, Gd-**8**) displays a τ_M^O value of 244 ns,[11] whereas the closely related $[GdDOTMA(H_2O)]^-$ (Gd-**9**) has a τ_M^O value of 68 ns only.[7]

From high-resolution NMR work on the corresponding Eu(III) complexes, it is known that the macrocyclic ligands wrap around the lanthanide(III) ion to yield two structural isomers, namely AP and TAP. The coordination polyhedron of the AP isomer corresponds closely to the regular square antiprismatic geometry, whereas a twisted square-antiprismatic coordination cage with a smaller tilt angle between the two square planes is assigned to the TAP isomer. The interconversion between the two isomers (Figure 6) is slow on the NMR time scale.[22]

The results for the Eu(III) complexes indicate a large dominance of the AP isomer for the DOTA chelate[23] and an almost exclusive presence of the TAP isomer in the case of DOTMA chelate. Thus, on the basis of the likely expectation that such isomeric compositions are maintained for Gd(III) chelates, one might conclude that the observed large difference in τ_M^O observed could be related to the structural differences between the two isomeric species.

This hypothesis has been supported by a ^{1}H NMR study on a related Eu(III) complex with a DOTA-like ligand containing four N-carboxamide groups (DOTAM (**10**), Chart 5).[24]

At low temperature in acetonitrile, in addition to the expected set of ligand resonances, an extra peak for each isomer has been observed and assigned to the water

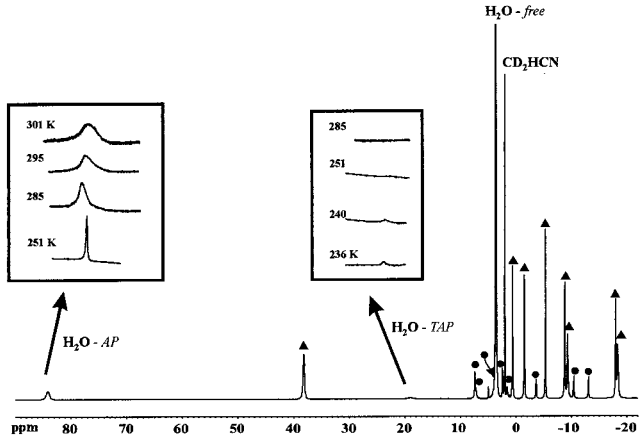


FIGURE 7. 400-MHz ^{1}H NMR spectrum of $[EuDOTAM(H_2O)](SO_3CF_3)_3$ at 232 K in CD_3CN containing a small amount of H_2O. The resonances of the two isomeric forms of the complex are labeled with ▲ (AP) and ● (TAP). The insets show the expanded spectral regions of the bound water peaks for the two isomers recorded at different temperatures. The exchange broadening of the two peaks occurs in a rather different temperature range as a result of the different water-exchange rates in the two isomers.

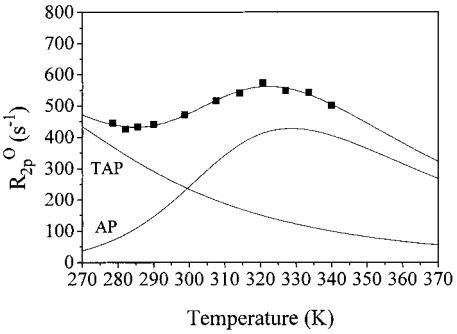
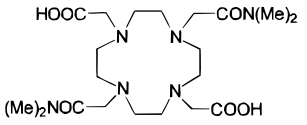


FIGURE 8. Temperature dependence of the water, ^{17}O-R_{2p}, for Gd-**11** ([Gd-**11**] = 46 mM, 2.1 T, pH 7). The fitting of the data was performed by considering the equilibrium between AP and TAP isomeric forms. The AP/TAP ratio changes significantly with temperature, the values found being 1.87 at 273 K and 1.42 at 323 K. At any temperature, the observed ^{17}O-R_{2p} results from the molar ratio and the exchange rate of the two isomers. At 298 K, τ_M^O is 1.8 μs and 20 ns for AP and TAP, respectively.

Chart 6



molecule coordinated to the Eu(III) ion (Figure 7). The chemical exchange between free and bound water takes place at a rather different rate in the two isomers, being much faster in the TAP isomer.

A similar result has been obtained from ^{17}O NMR measurements. In collaboration with A. D. Sherry (University of Texas at Dallas), we have recently investigated the relaxometric properties of a DOTA-like Gd(III) complex, where only two carboxylic moieties have been transformed into *N*-bis-methylcarboxamide functionalities (Chart 6, **11**). ^{17}O-R_{2p} data (Figure 8) had to be analyzed in terms of the sum of contributions arising from two isomeric species characterized by a large difference in

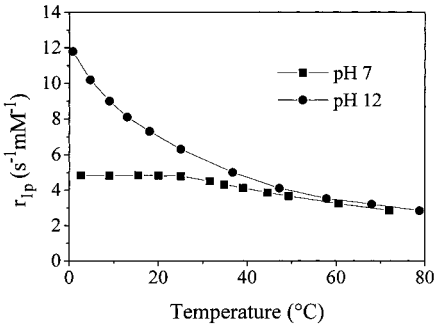
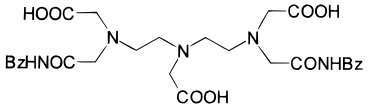


FIGURE 9. Temperature dependence of the relaxivity for Gd-**12** (0.47 T). At basic pH, the base-catalyzed prototropic exchange reduces τ_M and, thus, removes the quenching effect of the long τ_M^O value observed at neutral pH.

Chart 7



their τ_M^O values. The isomeric ratio is temperature dependent and is parallel to the AP/TAP ratio measured in the ^{1}H NMR spectra of the corresponding Eu(III) complex.

4. Prototropic Exchange Rates

The exchange of protons between the coordinated water and the bulk solvent may be evaluated by means of ^{1}H relaxometric measurements once the exchange of the whole water molecule has been independently assessed by means of ^{17}O-R_{2p} measurements. To obtain reliable values for the kinetic parameters involved in this dynamic process, the exchange of the coordinated water must be as slow as possible with respect to the proton exchange. Thus, Gd(III) complexes which exhibit slow exchange of the whole inner-sphere water molecule are good candidates for the assessment of the prototropic exchange rate of the metal-coordinated water molecule.

The prototropic exchange is catalyzed by both [H_3O^+] and [OH^-] ions, and, therefore, measurements of r_{1p} at different pH values may reveal the occurrence of a fast prototropic exchange superimposed on the slow exchange of the whole water molecule.

For instance, the measurement of r_{1p} vs T at pH 12 for [GdDTPA-BBA(H_2O)] (Figure 9; Chart 7, Gd-**12**) shows that the "quenching" effect of τ_M^O observed on the analogous profile at pH 7 is removed.[25]

The profile of r_{1p} vs T at pH 12 approaches the single-exponential behavior expected for systems characterized by the condition $T_{1M} > \tau_M$. The pH dependence of r_{1p} for [GdDTPA-BBA(H_2O)] at room temperature is reported in Figure 10.

To avoid the dissociation of the complex occurring at low pH, the measurements were carried out only in the basic region. The relaxation enhancement in the pH range 8.0−12.5 is entirely attributable to the contribution arising from the prototropic exchange, that at pH > 8.5 is faster than the exchange of the whole water molecule. This conclusion is supported by the observation that the

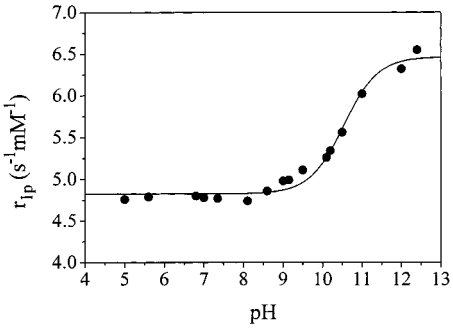


FIGURE 10. pH dependence of the relaxivity for Gd-**12** (0.47 T, 298K). The relaxation enhancement observed at basic pH reflects the reduction of τ_M due to the base catalysis of the water protons' transfer.

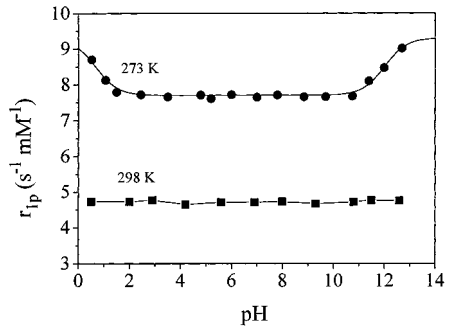


FIGURE 11. Relaxivity versus pH for [GdDOTA(H_2O)]$^-$ (0.47 T). At ambient temperature, τ_M is sufficiently short to prevent the relaxivity enhancement caused by the fast prototropic exchange, but at low temperature, this process, acid- and base-catalyzed, promotes an increase of the relaxivity.

exchange rate of the inner-sphere water molecule remains unchanged over the investigated pH range.[25]

The evaluation of the prototropic exchange over an extended range of pH values has been possible in the case of [GdDOTA(H_2O)]$^-$. At ambient temperature, the relaxivity of [GdDOTA(H_2O)]$^-$ is independent of the pH of the solution (Figure 11, bottom), thereby showing the occurrence of the fast exchange condition at any pH. The prototropic exchange may significantly contribute to the observed relaxivity at low temperature. In fact, a decrease in temperature causes (i) a shortening of T_{1M}, through its dependence on the molecular reorientational time, and (ii) an elongation of the exchange lifetime of the coordinated water molecule.

As shown in Figure 11 (top), at 273 K the water-exchange rate is slow enough to allow the prototropic exchange to affect the relaxivity at both the acidic and basic extremes of the pH scale. This curve can be adequately fitted by using a τ_M value which accounts for the various operating contributions:

$$\tau_M = \frac{1}{(\tau_M^O)^{-1} + k_p^A[H_3O^+] + k_p^B(K_W/[H_3O^+])} \qquad (9)$$

where k_p^i (i = A, B) are the rate constants for the acid- and base-catalyzed prototropic exchange, respectively.

Besides lowering the temperature, the slow exchange condition ($T_{1M} \leq \tau_M$) can also be met by lengthening the

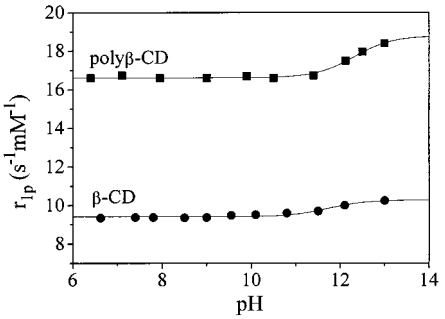
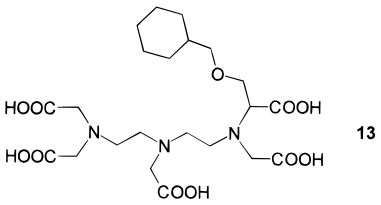


FIGURE 12. pH dependence of the relaxivity for Gd-**13**-βCD and Gd-**13**-poly-βCD adducts (0.47T, 298K). The reduction of T_{1M} allows the slow exchange condition to be approached, and the relaxivity enhancement at basic pH is more evident for the adduct with the larger molecular size and the lower T_{1M} value.

Chart 8



13

molecular reorientational time through the formation of reversible adducts between the Gd(III) chelates and a slowly tumbling substrate.[3,7] For instance, we considered $[GdCOPTA(H_2O)]^{2-}$ (Chart 8, Gd-**13**), which forms a tight inclusion compound with the β-cyclodextrin cavity ($K_A = 1.5 \times 10^3$ M^{-1} at 298 K).[26]

A value of 2.4 μs was calculated for T_{1M} of the metal-bound water protons in the inclusion compound. This value is only 2.2 times lower than that for the unbound chelate, and the condition $T_{1M} \approx \tau_M$ is not yet met due to the exchange rate of the coordinated water being equal to 0.29 μs, as determined by ^{17}O NMR spectroscopy. Neverthless, the pH dependence of the longitudinal water proton relaxivity for the GdCOPTA/β-CD adduct measured at 298 K (Figure 12, bottom) is characterized by a slight increase of relaxivity at pH > 11, assignable to the occurrence of a fast base-catalyzed prototropic exchange.

A higher increase in relaxivity may be obtained through a further reduction of T_{1M}. In fact, on passing from β-CD to its polymeric derivative poly-β-CD (MW ~6000), the relaxivity enhancement observed at basic pH for $[Gd-COPTA(H_2O)]^{2-}$ is more pronounced (Figure 12, top).

5. Exchangeable Protons on the Coordinated Ligand

Exchangeable protons on the ligand of Gd(III) chelates can contribute to the relaxation enhancement of solvent water protons if their distance from the paramagnetic ion does not exceed 4 Å. This contribution may be assessed by measuring the relaxivity as a function of pH and temperature. As an illustrative example, we report the case of $[GdHPDO3A(H_2O)]$ (Chart 9, Gd-**14**), which is one of the four CAs currently used for MRI in medical diagnosis. At 298 K, r_{1p} of $[GdHPDO3A(H_2O)]$ is 4.2 s^{-1} mM^{-1}, and τ_M^O

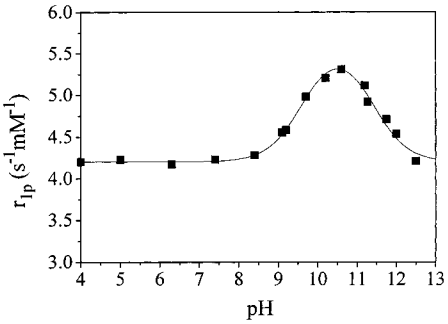
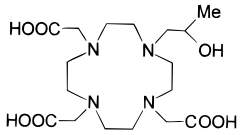


FIGURE 13. Relaxivity versus pH for Gd-**14** (0.47 T, 298 K). The relatively high relaxivity observed at pH 12.5 is a contribution of water molecules in the second coordination sphere and hydrogen-bonded to the negative alkoxide group.

Chart 9



14

is equal to 350 ns.[8] Such a measurement indicates that the water-exchange rate is fast enough to preclude the detection of any effect on the relaxivity from the prototropic exchange process at this temperature. However, as shown in Figure 13, the r_{1p} of $[GdHPDO3A(H_2O)]$ is markedly pH dependent.

The relaxivity gain observed as the pH of the solution increases to 10.5 has then to be assigned to the base-catalyzed exchange of the hydroxyl proton. When the effect is at the maximum, this contribution to the overall r_{1p} reaches the value of 1.2 s^{-1} mM^{-1}, which is consistent with the exchange of one proton at a distance of ca. 3 Å from the paramagnetic center. Beyond pH 10.5, a decrease in r_{1p} takes place which completely cancels, at pH 13, the relaxivity enhancement described above. From potentiometric measurements, it has been established that the pK_A of the alcoholic OH group is 11.36.[27] Thus, on going from pH 10.4 to 12.4, a progressive formation of the deprotonated anionic form $[Gd(HPDO3A-H)]^{-1}$ occurs. ^{17}O-R_{2p} measurements over the 0−60 °C temperature range strongly suggest that the deprotonated species lacks any coordinated water. Which contribution, then, determines the observed r_{1p} of $[Gd(HPDO3A-H)]^{-1}$?

An insight into answering this question came from the observation that r_{1p} displays a monoexponential dependence as a function of temperature (0−60 °C range), indicating the occurrence of fast exchange conditions between paramagnetically relaxed protons (in the proximity of the metallic center) and the bulk solvent. We concluded that such behavior, in a species lacking water in the inner coordination sphere, has to be ascribed to water molecules in the second coordination sphere.

Thus, the r_{1p} value of 4.2, identical to the value found at pH 7, can be justified by the presence of a second coordination sphere water molecule tightly interacting with the negatively charged alkoxide functional group. It has been estimated by applying eq 2 that the protons of this H-bonded water are located at a distance of ca. 3.2 Å

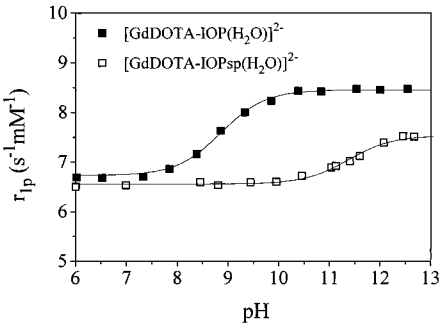
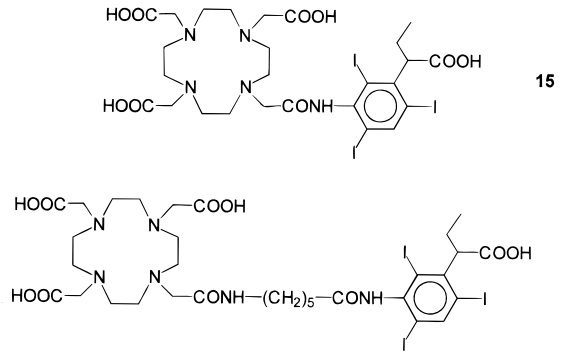


FIGURE 14. pH dependence of the relaxivity for $[GdDOTA\text{-}IOP(H_2O)]^{2-}$ and $[GdDOTA\text{-}IOPsp(H_2O)]^{2-}$ (20 MHz and 298 K).

Chart 10



from the Gd(III) ion. This additional contribution to the relaxivity when a negative charge is located in close proximity of the coordination cage appears to be a rather general phenomenon, as shown in the following example.

Let us compare the behavior of r_{1p} vs pH of the Gd(III) complexes of the ligands DOTA-IOP and DOTA-IOPsp (**15** and **16**, Chart 10), which were designed for targeting the hepatobiliary system through the recognition of the basolateral membrane receptors of liver cells.[28] Despite the similarity between the two complexes, the pH dependence of their r_{1p} appears significantly different (Figure 14). Potentiometric studies showed that the amidic N−H moiety in $[GdDOTA\text{-}IOP(H_2O)]^{2-}$ is rather acidic ($pK_A \sim 9$), and then, at pH > 10, the deprotonated species is largely dominant. In this case, $^{17}O\text{-}R_{2p}$ measurements indicate that the deprotonated species still maintains a water molecule in fast exchange with the bulk solvent. Thus, the high r_{1p} value (8.5 $mM^{-1}\ s^{-1}$) found at pH > 10 can be accounted for in terms of contributions arising from the exchange of the coordinated water and from a water molecule in the second coordination sphere H-bonded to the negatively charged nitrogen in addition to the outer-sphere component.

In the related $[GdDOTA\text{-}IOPsp(H_2O)]^{2-}$, the amidic moiety deprotonates only at much higher pH values, and then no relaxation enhancement is detected at intermediate basic pH values.

It is worth noting that the formation of the deprotonated $[GdDOTA\text{-}IOP(H_2O)\text{-}H]^{3-}$ promotes a significant increase in the exchange rate of the coordinated water (from $1.2 \times 10^6\ s^{-1}$ at pH 7 to $5 \times 10^6\ s^{-1}$ at pH 12), as determined from ^{17}O-VT R_{2p} measurements.

Such behavior agrees with previous findings that complexes endowed with a residual negative charge display shorter τ_M^O than neutral complexes.[7,11]

8. Conclusions

Although the relaxometric investigations on Gd(III) chelates were undertaken with the aim of obtaining a better understanding of the determinants of the proton relaxivity and improving their efficiency as CA for MRI, they yielded an important contribution to our understanding of the water and prototropic exchange processes. First, we showed that the combined use of ^1H and ^{17}O NMR spectroscopy allows the discrimination of the whole water exchange from the exchange of protons only, which is acid and base catalyzed. Second, we found that the pH dependence of the proton relaxivity can yield additional information on the second hydration sphere of the complex, on the exchange rate of mobile protons on the surface of the ligand, and on the pK_A of the coordinated water capable of forming hydroxo-containing species. Although these properties may eventually be assessed by other techniques, it is worth noting that, by using the relaxometric approach, one collects all relevant information on the different processes at once!

The relaxation rates of solvent water nuclei (^1H and ^{17}O) appear to act as very sensitive reporters of the minor changes occurring on the dissolved metal chelate. The paramagnetism of these species provides a strong amplification of the remote interactions which occur at the surface of the complex, which affect the relaxation properties of the strong ^1H and ^{17}O NMR resonance of the water solvent.

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