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NMR relaxometric studies of Gd(III) complexes with heptadentate macrocyclic ligands

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ABSTRACT: The water ^{1}H and ^{17}O NMR relaxation properties of solutions containing Gd(III) chelates of the heptadentate DO3A, PCTA[12] and PCTP[12] ligands were thoroughly investigated and the results obtained are compared with those previously reported for other Gd(III) complexes with octadentate ligands {H_3DO3A = 1,4,7,10-tetraazacyclododecane 1,4,7-triacetic acid; H_3PCTA[12] = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid; H_6PCTP[12] = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-tris(methanephosphonic) acid}. The observed behaviour is consistent with a hydration number $q = 2$ in the case of GdDO3A and GdPCTA[12] and $q = 1$ in the case of PCTP[12]. The high relaxivity of the latter complex is accounted for in terms of the occurrence of an additional contribution arising from water molecules tightly bound to the phosphonate moieties on the surface of the paramagnetic chelate. Furthermore, it was found that the decreased relaxation rates observed at basic pH in the case of GdDO3A and GdPCTA[12] can probably be ascribed to a partial decrease in their hydration. The measurement of ^{17}O NMR transverse relaxation rates, in the temperature range 273–342 K, allowed the assessment of the water exchange rate between the coordination site and the bulk solvent. A particularly short exchange lifetime was measured for the octacoordinate GdPCTP[12], which suggests the occurrence of an associative exchange mechanism. Further insights into the understanding of the structural properties of the three complexes were gained by measuring the magnetic field dependence (NMRD profiles) of the proton relaxivity on a Koenig–Brown field cycling relaxometer. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: ^{1}H NMR; ^{17}O NMR; relaxation; field dependence; Gd(III) complexes; heptadentate ligands

INTRODUCTION

In recent years, water-soluble lanthanide(III) chelates of macrocyclic and linear polyaminopolycarboxylic ligands have found wide utilization in different applications in the biomedical field.[1–7] Among them, the use of Gd(III) complexes to add physiological information to magnetic resonance imaging (MRI) images represents a very important area of research.[8] These contrast agents (CA) are thermodynamically (and possibly kinetically) stable compounds able to catalyse the nuclear magnetic relaxation rates of water in the tissues where they distribute, thus improving the image contrast. The large majority of the gadolinium chelates so far considered as possible CA for MRI are represented by complexes of octadentate ligands. Such highly stable chelates are expected to minimize the toxic effects associated with the release of both the free metal ion and the ligand. In the case of polyaminopolycarboxylic ligands such as DOTA, DTPA, EGTA [H_4DOTA = 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetrazacyclododecane; H_5DTPA = 1,1,4,7,7-pentakis(carboxymethyl)-1,4,7-triazaheptane; H_4EGTA = 3,12-bis(carboxymethyl)-6,9-dioxa-3,12-diazatetradecanedioate] and related derivatives, the coordination cage is completed by one water molecule in the inner coordination sphere of the Gd(III) ion ($q = 1$).[9,10]

The efficacy of a paramagnetic complex to catalyse the nuclear magnetic relaxation of the solvent protons is routinely expressed in terms of relaxivity r_{1p}^{H}, i.e. the increase in the water proton longitudinal relaxation rate in a 1 mM solution of the CA. Typically, for the Gd(III)-polyaminopolycarboxylate complexes, r_{1p}^{H} is between 4 and 6 $mM^{-1}\ s^{-1}$ (at 20 MHz and 25 °C). The search for systems of improved relaxivity is usually based upon linking the paramagnetic metal chelate (in either a covalent or a non-covalent mode) to slowly moving substrates.[11] However, to exploit fully the relaxation enhancement promoted by a long molecular reorientational time, it is necessary to deal with complexes characterized by a fast exchange of the coordinated water. On the basis of the available data on Gd(III) complexes with $q = 1$, the residence lifetime of the coordinated water varies over more than one order of magnitude, depending on the overall electric charge and the structural features of the complex.[12]

On the other hand, it has recently been found that in aqueous solution Gd(III) is able to form octacoordinate complexes with macrocyclic ligands bearing phosphonic or phosphinic groups. In the case of the highly anionic complex with the macrocyclic tetraazatetraphosphonate ligand DOTP [H_8DOTP = 1,4,7,10-tetrakis(methylene-

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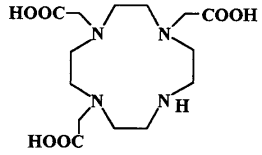
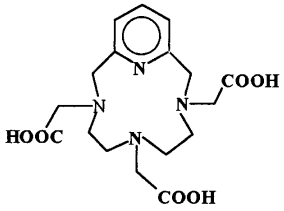
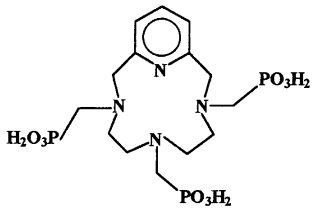


DO3A



PCTA-[12]



PCTP-[12]

Scheme 1

phosphonic acid)-1,4,7,10-tetrazacyclododecane], r_{1p}^{H} assumes a value similar to those found for the monoaquo complexes, because of the large contribution of a relaxation mechanism involving water molecules strongly bound in the second coordination sphere of the Gd(III) ion.[13,14] In contrast, the analogous phosphinic complexes are characterized by relaxivity values about 50% lower, typical of a relaxation mechanism involving only water molecules in the outer coordination sphere of the paramagnetic centre.[15]

In spite of the fact that the relaxivity (the inner sphere component, see below) is directly proportional to the number q of metal-bound water molecules, stable complexes of Gd(III) with heptadentate ligands and possibly two water molecules have only seldom been considered. The prototype of such complexes is GdDO3A (Scheme 1), a macrocyclic complex featuring a tetraazacyclodecane ring and three acetic arms arranged around the metal ion to form a capped square antiprismatic geometry. In the solid state, enneacoordination of Gd(III) is achieved by coordination of a water molecule and an oxygen atom of an adjacent carboxylate group.[16] In the analogous derivative with three methylacetic groups, GdDO3MA, two water molecules occupying axial and equatorial positions are actually observed in one of the two crystallographically independent Gd(III) complexes present in the x-ray solid-state structure.[17] These two water molecules are characterized by very similar Gd—O distances (*ca.* 2.5 Å).

We have investigated in detail the relaxometric properties of GdDO3A by a combined ^{1}H and ^{17}O NMR approach and the results are reported here and compared with those obtained for two related complexes which contain a pyridine moiety in the macrocyclic ring and three acetic (PCTA[12]) or methylenephosphonic (PCTP[12]) arms, respectively (Scheme 1).

EXPERIMENTAL

NMR measurements

The longitudinal water proton relaxation rate was measured by using a Stelar (Mede, Italy) Spinmaster spectrometer operating at 20 MHz, by means of the standard inversion–recovery technique (16 experiments, two scans). A typical 90° pulse width was 3.5 μs and the reproducibility of the T_1 data was ±0.5%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper–constantan thermocouple (uncertainty ±0.1 °C).

The proton $1/T_1$ NMRD profiles were measured over a continuum of magnetic field strength from 0.000 24 to 1.2 T (corresponding to 0.01–50 MHz proton Larmor frequency) on the Koenig–Brown field-cycling relaxometer installed at the NMR relaxometry laboratory of the University of Turin. The relaxometer works under complete computer control with an absolute uncertainty in $1/T_1$ of ±1%. Details of the instrument and of the data acquisition procedure are given elsewhere.[18]

Variable-temperature ^{17}O NMR measurements were recorded on a JEOL EX-90 (2.1 T) spectrometer, equipped with a 5 mm probe, by using a D_2O external lock. Experimental settings were spectral width 10 000 Hz, pulse width 7 μs, acquisition time 10 ms, 1000 scans and no sample spinning. Solutions containing 2.6% of ^{17}O isotope (Yeda, Israel) were used. The observed transverse relaxation rates (R_{2obs}^{O}) were calculated from the signal width at half-height. The ^{1}H and ^{13}C NMR spectra of the PCTP[12] ligand were measured in a JEOL EX-400 spectrometer (9.4 T), using D_2O (99.8%, Merck, Darmstadt, Germany) as solvent. *tert*-Butyl alcohol (1%) and phosphoric acid were used as internal references ($\delta^{1H} = 1.29$, $\delta^{13C} = 31.3$ and $\delta^{31P} = 0$ ppm) for $^{1}H/^{13}C$ and ^{31}P NMR spectra, respectively.

Syntheses of ligands and their Gd(III) complexes

The GdDO3A complex was kindly provided by Bracco (Milan, Italy). PCTA[12] and its Gd(III) complex were synthesized following the published procedure.[19]

The PCTP[12] ligand was synthesized according to the following procedure. To a solution of 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene[19] (100 mg, 0.49 mmol) in 37% hydrochloric acid (0.25 ml, 3.00 mmol), water (0.25 ml) and phosphorous acid (246 mg, 300 mmol), paraformaldehyde (300 mg, 10.00 mmol) was added portionwise over 60 min at 110–115 °C. The mixture was maintained at the same temperature for an additional 60 min. The solution was allowed to reach room temperature and then cooled to 0 °C. Ethanol (4 ml) was added dropwise; the crude product precipitated and was collected by filtration. Purification was effected by repeated dissolution in water (0.5 ml) and precipitation with ethanol (0.5 ml). The title compound (140 mg, 58.5% yield) was obtained sufficiently pure for the subsequent studies. ^{1}H NMR (400 MHz, D_2O, pH 7): δ (ppm) 7.98 (1H, t, $^{3}J = 8.0$ Hz), 7.48 (2H, d, $^{3}J = 8$ Hz), 4.96 (4H, s), 3.60 (4H, m), 3.43 (4H, d, $^{2}J_{H,P} = 12$ Hz), 3.02 (4H, m), 2.97 (2H, d, $^{2}J_{H,P} = 11.0$ Hz). ^{13}C NMR (100 MHz, D_2O, pH 7): δ (ppm) 152.3, 141.3, 124.2, 60.8, 56.4, 55.4, 53.5, 52.7. ^{31}P NMR (121.4 MHz, D_2O; pH 7): δ (ppm) 16.9 [1P], 7.68 [2P]. FAB-MS (NBA): calculated for $C_{14}H_{27}N_4O_9P_3$, 488; found, 489 $[M + H]^+$, 511 $[M + Na]^+$, 527 $[M + K]^+$, 392, 298, 204. Elemental analysis calculated for $C_{14}H_{27}N_4O_9P_3 \cdot 4HCl \cdot H_2O$, C 25.85, H 5.12, N 8.62; found, C 25.78, H 5.10, N 8.59%.

The GdPCTP[12] complex was synthesized by dissolving the ligand (0.2 mmol) in H_2O (5 ml) and adjusting the pH of the solution to 7.5 with 1 M NaOH. To this solution, 3 ml of an aqueous solution of $GdCl_3$ (0.2 mmol) was added dropwise, maintaining the pH at 7.5 with 1 M NaOH. At room temperature the complex formation was instantaneous. The pH of the solution was then increased to 8–9 by adding 1 M NaOH in order to precipitate the excess of uncomplexed Gd(III) ions. The solution was then evaporated under reduced pressure and the residue dried overnight at 70 °C.

THEORY

^{1}H water relaxation rate

The longitudinal water proton relaxivity r_{1p}^H is defined as the paramagnetic contribution to the observed water proton relaxation rate (R_{1obs}^H) of a 1 mM solution of the paramagnetic metal complex:

$$r_{1p}^H = R_{1obs}^H - R_{1d}^H \quad (1)$$

where R_{1d}^H is the water proton relaxation rate in the presence of an equimolar amount of a diamagnetic analogue of the paramagnetic compound. The observed relaxivity results from contributions arising from water molecules in the inner and the outer coordination spheres:

$$r_{1p}^H = R_{1p}^{His} + R_{1p}^{Hos} \quad (2)$$

R_{1p}^{His} represents the contribution arising from the exchange of the water directly coordinated to the paramagnetic metal ion and is given by

$$R_{1p}^{His} = \frac{1.8 \times 10^{-5} q}{T_{1M}^H + \tau_M{}^H} \quad (3)$$

where q is the hydration number, T_{1M}^H is the longitudinal relaxation time of the inner-sphere water protons and $\tau_M{}^H$ is their residence lifetime. The Solomon–Bloembergen theory[20] provides the magnetic field dependence of T_{1M}^H which, for a Gd(III) chelate, is given by

$$\frac{1}{T_{1M}^H} = \frac{2}{15} \frac{\gamma_H{}^2 g_e{}^2 \mu_B{}^2 S(S+1)}{r_H{}^6} \times \left[\frac{3\tau_{c1}}{1+\omega_H{}^2\tau_{c1}^2} + \frac{7\tau_{c2}}{1+\omega_S{}^2\tau_{c2}^2} \right] \quad (4)$$

where S is the electron spin quantum number [7/2 for Gd(III)], γ_H is the proton nuclear magnetogyric ratio, μ_B is the Bohr magneton, g_e is the Landé factor for the free electron, r_H is the distance between the metal ion and the inner-sphere water protons, ω_H and ω_S are the proton and electron Larmor frequencies ($\omega_S = 658\omega_H$), respectively, and τ_{ci} ($i = 1, 2$) are the correlation times related to the modulation of the dipolar electron–proton coupling. Such an interaction may be modulated by the reorientation of the paramagnetic species, τ_R, by the residence lifetime, τ_M, and by the electronic relaxation times, T_{iE}:

$$\tau_{ci}^{-1} = \tau_R^{-1} + \tau_M^{-1} + T_{iE}^{-1} \quad (5)$$

By analogy with the nuclear relaxation time, the electronic relaxation processes also depend on the magnetic field strength. For Gd(III) complexes T_{iE} are related to the modulation of the zero field spitting (ZFS) of the electronic spin states due to the dynamic distortions of the ligand field interaction and, according to the Bloembergen–Morgan theory,[21] their magnetic field dependence is given by the following equations:

$$T_{1E}^{-1} = \frac{1}{25} \Delta^2 \tau_v [4S(S+1) - 3] \times \left(\frac{1}{1+\omega_S{}^2\tau_v{}^2} + \frac{4}{1+4\omega_S{}^2\tau_v{}^2} \right) \quad (6)$$

$$T_{2E}^{-1} = \frac{1}{50} \Delta^2 \tau_v [4S(S+1) - 3] \times \left(3 + \frac{5}{1+\omega_S{}^2\tau_v{}^2} + \frac{2}{1+4\omega_S{}^2\tau_v{}^2} \right) \quad (7)$$

where Δ^2 is the trace of the square of the transient ZFS tensor and τ_v is the correlation time related to its modulation.

The outer-sphere term, R_{1p}^{Hos}, describes the contribution arising from the water molecules diffusing near the paramagnetic chelate and, according to the model of Hwang and Freed,[22] may be related to the minimum distance between the metal and the diffusing water molecules, a, the relative solute–solvent diffusion coefficient, D, and the electronic relaxation times, T_{iE}:

$$R_{1p}^{Hos} = C^{os}\left(\frac{1}{aD}\right)[7J(\omega_S) + 3J(\omega_H)] \quad (8)$$

where C^{os} is a constant (5.8×10^{-13} s^{-2} M^{-1}) and the dependence on the electronic relaxation times is expressed in the non-Lorentzian spectral density functions $J(\omega_i)$.

^{17}O water relaxation rate

The residence lifetime of a water molecule directly coordinated to a paramagnetic metal ion ($\tau_M{}^O$) may be evaluated by measuring the temperature dependence of the paramagnetic contribution (R_{2p}^O to the observed ^{17}O water solvent transverse relaxation rate:

$$R_{2p}^O = R_{2obs}^O - R_{2d}^O \quad (9)$$

where, in analogy with the previous case, the diamagnetic term R_{2d}^O is measured on a solution containing a diamagnetic analogue of the chelate of interest. R_{2p}^O is related to $\tau_M{}^O$ through the values of $\Delta\omega_M{}^O$ (which is the ^{17}O chemical shift difference between coordinated and bulk water molecule) and R_{2M}^O (which is the transverse relaxation rate of the coordinated water oxygen):[23]

$$R_{2p}^O = \frac{qC}{55.6} \tau_M^{O-1} \frac{R_{2M}^{O2} + \tau_M^{O-1} R_{2M}^O + \Delta\omega_M^{O2}}{(R_{2M}^O + \tau_M^{O-1})^2 + \Delta\omega_M^{O2}} \quad (10)$$

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

$$\Delta\omega_M{}^O = \frac{g_e \mu_B S(S+1) B_0}{3k_B T} \frac{A}{\hbar} \quad (11)$$

where B_0 is the magnetic field strength (2.11 T in this work) and $A/\hbar$ is the Gd–^{17}O scalar coupling constant [the value of which for polyaminocarboxylate Gd(III) complexes may be reasonably fixed at -3.8×10^6 rad s^{-1}].[12]

For a relatively small-sized Gd(III) chelate and at 2.11 T, R_{2M}^{O} is dominated by the electron–nucleus scalar interaction:

$$R_{2M}^{O} = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_s^2\tau_{E2}^2}\right) \quad (12)$$

$$\tau_{Ei}^{-1} = T_{iE}^{-1} + (\tau_M^{O})^{-1} \quad (13)$$

Finally, the temperature dependence of R_{2p}^{O} is expressed in terms of the Eyring relationship for τ_M^{O} and τ_v:

$$(\tau_j)_T^{-1} = \frac{(\tau_j^{-1})^{298.15}T}{298.15}\exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \quad (14)$$

where j refers to the two different dynamic processes involved (j = v, M) and ΔH_j is the corresponding activation enthalpy.

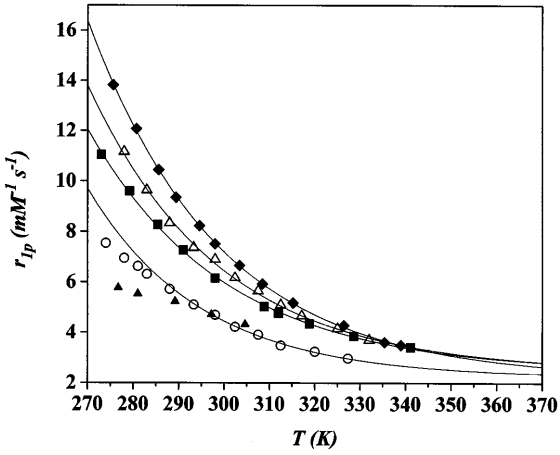


Figure 1. Temperature dependence of the longitudinal water proton relaxivity at 20 MHz and pH 7 of GdPCTP[12] (◆), GdPCTA[12] (△), GdDO3A (■), GdDOTA (○) and GdDOTA-MA (▲).

RESULTS AND DISCUSSION

^{1}H NMR

Relaxivity. At 20 MHz, 40 °C and a pH close to neutrality, the longitudinal proton relaxivities r_{1p}^{H} of GdDO3A, GdPCTA[12] and GdPCTP[12] are 4.8, 5.1 and 5.3 mM^{-1} s^{-1}, respectively. Under the same experimental conditions, r_{1p}^{H} for the enneacoordinated, monoaquo parent compound GdDOTA is 3.5 mM^{-1} s^{-1}. Such a large difference in the measured relaxivities has been tentatively explained on the basis of a different hydration number of the complexes: 1 for GdDOTA and 2 for the other chelates considered in this work. In fact, from a comparative study of the relaxivities of Gd(III) macrocyclic aminocarboxylate complexes, Zhang *et al.*[24] estimated a contribution of 2.0 ± 0.3 mM^{-1} s^{-1} for the outer-sphere relaxivity and 1.7 ± 0.1 mM^{-1} s^{-1} for each inner-sphere water molecule. Hence the complexes studied here apparently display identical hydration numbers and similar Gd—OH_2 distances. On the basis of this explanation, the small differences in relaxivity among the three complexes with heptadentate ligands may be simply accounted for in terms of slightly different reorientational correlation times, τ_R being the dominant contribution to τ_{ci} at this temperature and magnetic field strength.

Temperature dependence. The temperature dependence of the complexes at 20 MHz for the three complexes was measured in the range 273–340 K and is reported in Fig. 1. Analogous data for the Gd(III) complexes of DOTA and DOTA-MA [10-(2-{[2-hydroxy-1-(hydroxymethyl)ethyl]amino} - 1 - [(phenylmethoxy) - methyl] - 2 - oxoethyl) - 1,4,7,10 - tetraazacyclododecane - 1,4,7-triacetic acid], a monoamide derivative of a DOTA-like ligand, are also shown for comparison. In the range 273–342 K, r_{1p}^{H} increases with decreasing T but, whereas for the complexes of DO3A, PCTA[12] and PCTP[12] the r_{1p}^{H} curve follows a simple exponential law, in the case of GdDOTA and its monoamide derivative a significant deviation from the exponential behaviour is observed below 293–298 K. At this magnetic field strength, r_{1p}^{H} is largely controlled by τ_R (for the inner-sphere component) and D (for the outer-sphere component), which are expected to be very similar for complexes of similar size and, therefore, cannot be responsible for their different temperature dependences. Rather, this difference is indicative of the differences [in Eqn (3)] between T_{1M}^{H} and τ_M^{H} which exhibit opposite temperature dependences, i.e. on lowering the temperature T_{1M}^{H} decreases and τ_M^{H} increases. Very often, for the Gd(III) polyaminocarboxylate complexes, at an observation frequency of 20 MHz, the fast exchange condition ($T_{1M}^{H} \gg \tau_M^{H}$) is fulfilled over a wide range of temperatures. However, in some cases, the exchange rate of the coordinated water may be slow enough to result in a limiting effect on the relaxivity ($T_{1M}^{H} \approx \tau_M^{H}$).[12,25] This is well documented in Fig. 1 by comparing the behaviour of GdDOTA ($\tau_M^{H} = 230$ ns at 298 K) with that of its monoamide derivative, the water exchange rate of which is about four times lower. Clearly, a much more pronounced flattening of the r_{1p}^{H} curve at low temperatures is observed for the latter complex. On this basis, the r_{1p}^{H} data in the temperature range 273–342 K provide a qualitative indication that for the Gd(III) complexes with heptadentate ligands the water exchange rate is faster than that for GdDOTA.

pH dependence. The relaxivities of the Gd(III) complexes, at 20 MHz and 298 K, at various pH values are plotted in Fig. 2. Whereas the values of r_{1p}^{H} are constant in the pH range 4–10, they increase markedly for lower pH values where the stepwise protonation of the ligand results in a partial dissociation of the complex, followed by a release of the metal ion, which, under these experimental conditions, has a relaxivity of 13.0 mM^{-1} s^{-1}. GdPCTP[12] shows a more pronounced increase of r_{1p}^{H} at low pH and presents a maximum of 14.6 mM^{-1} s^{-1} at pH ≈ 2. This could be indicative of the formation of an intermediate species with a higher q value arising

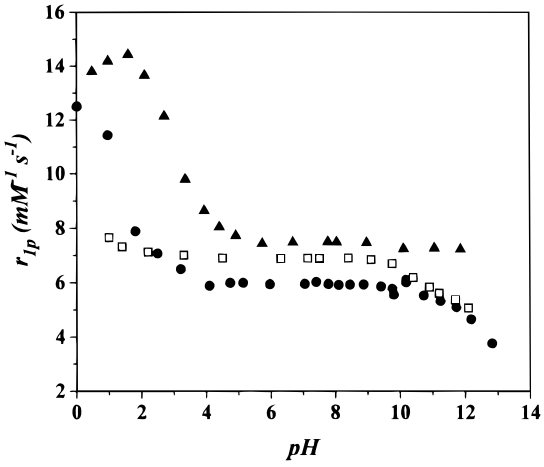


Figure 2. pH dependence of the longitudinal water proton relaxivity at 20 MHz and 25 °C of GdPCTP[12] (▲), GdPCTA[12] (□) and GdDO3A (●).

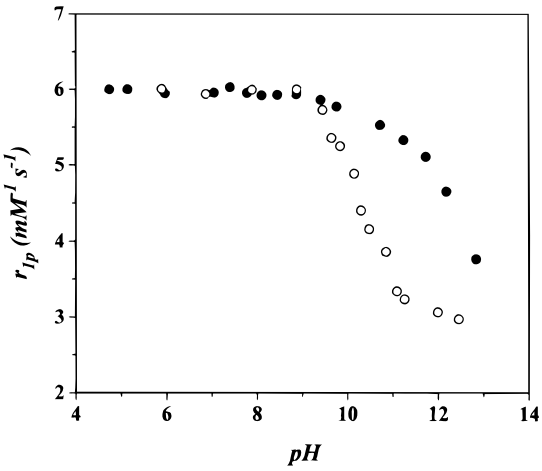


Figure 3. pH dependence of the water proton relaxivity at 20 MHz and 25 °C for a 1 mM solution of GdDO3A with (○) or without (●) carbonate.

from the dissociation of one or two phosphonate groups.

In contrast, in the pH range 10–13 the relaxivity of DO3A and PCTA[12] chelates decreases by *ca.* 40–50%. In the same pH range, no changes are observed in the relaxivity of GdPCTP[12]. Such a decrease in r_{1p}^{H} in the higher pH region is not unusual for Gd(III) chelates with $q > 1$ and it has been reported previously by Kim *et al.*[26] for GdPCTA[12]. It may be attributed to (i) hydrolysis of the complex leading to partial precipitation of the hydroxide, (ii) formation of OH-bridged polymeric species lacking any inner sphere contribution to the relaxivity, (iii) reduction of the q value by substitution of the coordinated water molecules with the OH^- ligand or (iv) formation of ternary complexes with dissolved carbonate anions. In our case, the first two possibilities can be ruled out since the behaviour of r_{1p}^{H} with pH is perfectly reversible. As far as the fourth hypothesis is concerned, it may be useful to recall that the x-ray crystal structure of GdDO3A shows three units of the complex filled by a carbonate ion in a bidentate mode.[16] Moreover, Burai *et al.*[27] have reported recently a study on equilibria involving Gd(III) chelates and CO_3^{2-}, PO_4^{3-} and citrate ions, where it was shown that the formation of ternary complexes with carbonate ions is significant at pH > 8 in the case of GdEDTA ($q = 3$), but negligible for GdDOTA and GdDTPA (both complexes have $q = 1$). Hence similar equilibria are likely to occur in the solutions of GdDO3A and GdPCTA[12].

In Fig. 3, the results relative to the pH dependence of the relaxivity for GdDO3A as measured in a closed cell under an N_2 atmosphere by using CO_2-free KOH and in an open cell by using standard NaOH are compared. The effect of the formation of a ternary complex with carbonate on the longitudinal relaxation rate of the water protons is clear and accounts for most of the relaxivity decrease observed in the higher pH range (11–12.5). Nonetheless, even under controlled conditions the relaxivity decrease cannot be completely eliminated and this may be explained, as mentioned above, by the concomitant partial displacement of the coordinated water molecules by OH^-. In fact, a potentiometric titration analysis (298 K, $I = 0.1$ KCl) gave for the bound water molecules a pK_a value of 12.15 (± 0.03), in excellent agreement with the relaxometric data. Interestingly, in analogy with GdDOTA (and a number of related systems with $q = 1$), the phosphonic PCTP[12] chelate does not show any decrease in r_{1p}^{H} at basic pH and hence one is tempted to explain this behaviour by the presence of a single coordinated water molecule also for GdPCTP[12].

^{17}O NMR

A more reliable evaluation of the hydration number and a quantitative assessment of the water exchange rate is obtained from the analysis, through Eqns (9)–(14), of the temperature dependence of the solvent water ^{17}O transverse relaxation rate. We measured the data for 50 mM solutions of the three complexes at 2.1 T and pH 7 over the temperature range 275–350 K. The results are plotted in Fig. 4 with the corresponding curves representing the results of the best fitting of the data according to Eqns (9)–(14) (Table 1). In spite of the fact that the three complexes exhibit a very similar temperature dependence of the proton relaxivity, their R_{2p}^{O} temperature profiles are different in both shape and amplitude. The profile of GdDO3A is similar to the analogous profiles reported for the complexes of DOTA (Fig. 5, and see below) and DTPA,[28] but with a higher amplitude and a maximum of the curve slightly shifted towards lower temperature (300 K), to indicate a slightly higher value of the water exchange rate. By adopting a standard value of -3.8×10^6 rad s^{-1} for the hyperfine coupling constant $A/\hbar$ and assuming a q value of 2, a good fit of the data with the parameters in Table 1 is obtained. The calculated values of τ_M^{O} (at 298 K) for GdDO3A and GdDOTA (160 and 245 ns, respectively)

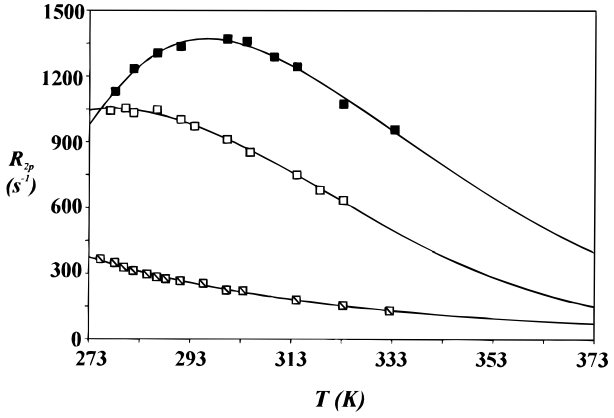


Figure 4. Temperature dependence of the transverse water ^{17}O relaxation rate at 2.1 T and pH 7 for 50 mM solutions of GdDO3A (■), GdPCTA[12] (□) and GdPCTP[12] (▨). The solid curves through the data points were calculated with the parameters in Table 1.

are of the same order of magnitude and this suggests that, in spite of their hydration number differing by one unit, the water exchange takes place through a similar limiting dissociative mechanism. On the other hand, the small difference between their τ_M^O values has a strong influence on the proton relaxivity at low temperature, as clearly shown in Fig. 1. It is worth commenting that, in principle, the complexes with heptadentate ligands could be involved in equilibria between nine-coordinate ($q = 2$) and eight-coordinate ($q = 1$) species, in analogy with the case of the Eu(III) complexes with hexadentate

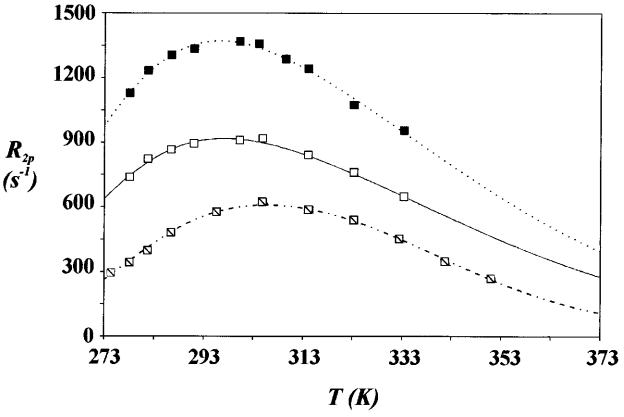


Figure 5. Temperature dependence of the transverse water ^{17}O relaxation rate 2.1 T for 50 mM solutions of GdDO3A [pH 7 (■) and pH 12 (□)] and of GdDOTA at pH 7(▨).

ligands discussed recently.[29] However, in our case neither the ^1H nor ^{17}O relaxation rates show any evidence of the occurrence of a change of the hydration number over the entire range of temperatures investigated.

The profile of GdPCTA[12] is shifted even more towards lower temperature with the maximum near 275 K and in fact the analysis of the data, by assuming $q = 2$, gives a τ_M^O value of 70 ns (at 298 K). This result is surprising if we consider that GdDO3A and GdPCTA[12] show similar T and pH dependences of the longitudinal proton relaxivity and similar thermodynamic stability constants. Hence the structural changes resulting from the introduction of the pyridine ring into the macrocycle seem to have a significant influence only on the water exchange rate for the chelates investigated in this work.

The profile of ^{17}O NMR R_{2p}^O *vs.* T for GdPCTP[12] displays a lower magnitude and a shape which clearly indicates the occurrence of a fast exchange regime down to 275 K. Under such conditions, it is difficult to obtain an accurate determination of the water exchange rate since the observed relaxation rates have only a small dependence on τ_M^O. However, a reasonably good fit of the data can be obtained with $q = 1$, i.e. by assuming that, unlike the other two compounds, Gd(III) forms an octacoordinate complex with PCTP[12]. The water exchange rate is very fast (τ_M^O is a few nanoseconds at 298 K), in analogy with the results reported for other octacoordinate complexes such as the aquo ion, $[Gd(H_2O)_8]^{3+}$, and GdPDTA [H_4PDTA = 1,1,5,5-tetrakis(carboxymethyl)-1,5-diazapentane], where the exchange has been shown to occur by an associative mechanism involving an enneacoordinate transition state.[30]

Finally, it is worth commenting that the high r_{1p}^H measured for the phosphonate derivative and its low hydration number suggests that in this case an additional contribution to the proton relaxivity has to be taken into account. In order to obtain greater insight into the determinants of the paramagnetic relaxation enhancement, we undertook the analysis of the magnetic field dependence of r_{1p}^H.

^{1}H NMR: magnetic field dependence

The proton nuclear magnetic relaxation dispersion (NMRD) profiles of the three complexes at 298 K are reported in Figs 6 and 7. The curves through the data represent the profiles calculated with the parameters

Table 1. ^{17}O NMR best-fitting parameters obtained from the analysis of the temperature dependence of R_{2p}^O for 50 mM aqueous solutions of the Gd(III) complexes

Complex	q	Δ^2 ($s^{-2} \times 10^{19}$)	τ_v (ps)	τ_M (ns)	ΔH_v (kJ mol^{-1})	ΔH_M (kJ mol^{-1})
Gd–DO3A	2	3.5	14	160	1.7	44
Gd–PCTA[12]	2	5.9	15	70	3.6	45
Gd–PCTP[12]	1	7.8	19	6.0	5.0	14

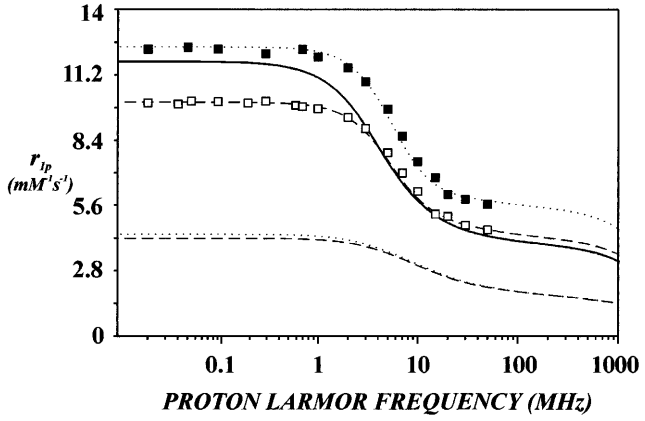


Figure 6. $1/T_1$ NMRD profile of a 1 mM aqueous solution of GdDO3A at pH 7 (■) and pH 12 (□). The solid curve represents the calculated profile for GdDOTA (1 mM, pH 7). The corresponding curves in the lower part represents the outer-sphere contributions to the overall relaxivity.

obtained by a best fitting procedure to Eqns (1)–(8) of the paramagnetic relaxation. In the calculations of the mean residence lifetimes τ_M^H were assumed to coincide with the values of τ_M^O obtained from the ^{17}O data, even though this parameter has a negligible influence on the relaxivity at this temperature. The hydration numbers q were fixed at 2 for GdDO3A and GdPCTA[12] and 1 for GdPCTP[12], and standard values of 3.8 Å and 2.24×10^{-5} cm^2 s^{-1} were used for the outer-sphere relaxation parameters a and D, respectively.[25]

The NMRD profiles of GdDO3A at pH 7 and 12 are shown in Fig. 6 and compared with the calculated profile of GdDOTA. The profiles at 298 K and neutral pH of GdDO3A and of its oxatriazamacrocyclic analogue have been reported recently.[31] As expected on the basis of the higher hydration number, the relaxivity of GdDO3A at pH 7 is higher than that of GdDOTA over the entire range of proton Larmor frequencies. The difference in relaxivity is attenuated in the low magnetic field region, as a consequence of the lower value of the

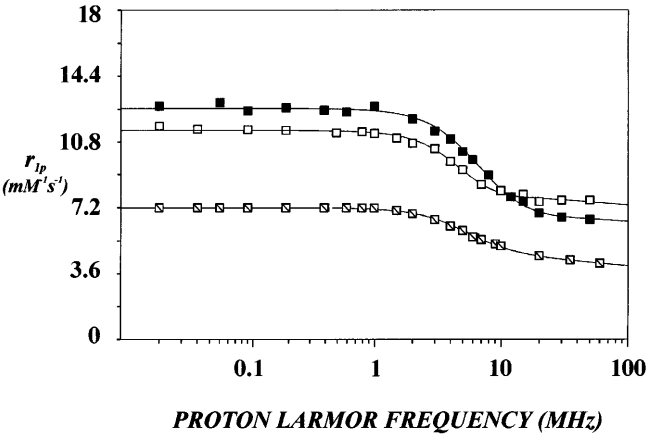


Figure 7. $1/T_1$ NMRD profile of a 1 mM aqueous solution of GdPCTA[12] (■), GdPCTP[12] (□) and GdDOTP (▨). The solid curves through the data points were calculated with the parameters in Table 2.

electronic relaxation time for GdDO3A. At pH 12, the profiles of the two complexes are nearly coincident in the high-field region, where the inner-sphere component of the relaxivity is essentially controlled by the factor $q\tau_R/r_H^6$. This represents a further and clear indication that the relaxivity decrease observed in the high pH region for the Gd(III) complexes with the heptadentate carboxylate ligands arises from a decrease in the hydration number of the complexes due to partial deprotonation of the bound water molecules and coordination of carbonate ions, thus excluding the formation of polymeric species. In fact, although very approximate, the profile of GdDO3A at pH 12 can be well reproduced by simply lowering the q value from 2 to 1.3. Further evidence of the reduction of the hydration number occurring for GdDO3A at basic pH is represented by the analysis of the temperature dependence of the water ^{17}O R_{2p}^O measured for this chelate at pH 12 (Fig. 5). The profile displays R_{2p}^O values lower than those obtained at neutral pH over the entire range of temperature investigated and it was well fitted only by reducing the q value from 2 to 1.

The relaxivities of GdPCTA[12] and GdDO3A are very similar not only at 20 MHz but also at other magnetic field strengths. The two NMRD profiles are in fact almost superimposable. This result is unexpected. Several pieces of evidence have been collected over the recent years of a relationship between the symmetry and the rigidity of a Gd(III) chelate and the value of its electronic relaxation time.[6] The introduction of a pyridine moiety into the macrocyclic ring causes an increase in the stereochemical rigidity of the coordination cage of the chelate and this would have been expected to affect its T_{iE} value. Nonetheless, this hypothesis does not find support in the NMRD data.

In Fig. 7, the NMRD profiles of the Gd(III) complexes of PCTA[12] and PCTP[12] are compared. The relaxivity at 20 MHz of the phosphonate derivative is 7.5 mM^{-1} s^{-1}, significantly higher than the value of 6.9 mM^{-1} s^{-1} found for GdPCTA[12]. This represents an evident contradiction with what was expected from the relative number of inner-sphere water molecules, 1 for the phosphonate and 2 for the carboxylate derivative. On the other hand, the relaxivity profile of the phosphonate derivative is lower than that of GdPCTA[12] for any frequency below 10 MHz. This difference in the shape and magnitude of the NMRD profiles cannot be accounted for by a difference in the reorientational correlation time for the two metal chelates or by an unusually short Gd—H distance of the coordinated water for GdPCTP[12]. In order to explain this enhanced relaxivity we have to consider the additional contribution to the paramagnetic relaxation of hydrogen-bonded water molecules in the second coordination sphere of the anionic complex. The importance of such a contribution depends on the overall residual charge and the nature of the donor groups on the surface of the complex and it has been shown to account for about 50% of the relaxivity of GdDOTP.[14] In fact, GdDOTP with $q = 0$ has a relaxivity at 25 °C of

Table 2. Best-fitting parameters obtained from the analysis of the NMRD profiles for the Gd(III) complexes at 25 °C and pH 7.0

Complex	q	Δ^2 ($s^{-2} \times 10^{19}$)	τ_v (ps)	τ_R (ns)	r (Å)
Gd–DO3A	2	4.6	14	66	3.15
Gd–PCTA[12]	2	2.8	28	70	3.10
Gd–PCTP[12]	1	7.8	19	106	3.06

4.7 mM^{-1} s^{-1}, similar to that of the monoaquo complexes DTPA and DOTA. Therefore, we have to consider the paramagnetic relaxation enhancement of GdPCTP[12] as given by the sum of three contributions arising from inner-sphere, outer-sphere and second coordination sphere water molecules, respectively. A quantitative evaluation of the latter component was pursued by subtracting the 75% of the NMRD profile of GdDOTP (in order to consider the different number of phosphonate groups between the two chelates) from the corresponding profile of GdPCTP[12] and by fitting the difference profile to the equations for the inner-sphere relaxation. Following this procedure, reasonable values for the relaxation parameters (Table 2) were obtained only with $q = 1$, thus confirming the results of the ^{17}O study.

CONCLUSIONS

This detailed analysis of the ^{1}H and ^{17}O NMR relaxometric properties of the Gd(III) complexes of DO3A, PCTA[12] and PCTP[12] has outlined the advantages of the use of heptadentate ligands with respect to the commonly used octadentate DTPA, DOTA and related ligands. In fact, whereas the thermodynamic stability of the chelates with heptadentate ligands is still very satisfactory (i.e. log $K_f \geqslant 22$) for *in vivo* applications, their relaxivities, at 40 °C and 20 MHz, display enhancements of *ca.* 40% with respect to GdDTPA and GdDOTA. We have shown that, in the case of GdDO3A and GdPCTA[12], the increased relaxivity is due to the presence of two water molecules in the inner coordination sphere, whereas in the case of GdPCTP[12] there is a substantial contribution from water molecules in the second coordination sphere. Hence the phosphonate groups in the latter complex cause either increased steric hindrance around the metal ion, which results in a decreased hydration number ($q = 1$), or the occurrence of a number of water molecules tightly bound on the surface of the complex, the contribution of which to the observed relaxivity appears comparable to that arising from a water molecule directly coordinated to the paramagnetic centre. On the other hand, analogous behaviour is observed in the case of the octadentate DOTP ligand, whose Gd(III) complex displays an r_{1p}^{H} value similar to that of GdDOTA in spite of the fact that it does not possess any water molecule in the inner coordination sphere.

Even more remarkable, in view of the preparation of suitable functionalized complexes to be linked to slowly moving systems, are the results concerning the exchange rate of the coordinated water molecule.

All three complexes have a decreased lifetime of the coordinated water molecule with respect to complexes with octadentate ligands such as GdDOTA. When $q = 2$ the effect is less pronounced, suggesting that in these enneacoordinated chelates the exchange rate is determined by a dissociative mechanism whose rate-determining step is the dissociation of one coordinated water molecules by a pathway analogous to that occurring in related complexes with octadentate ligands and $q = 1$. Conversely, the exchange lifetime becomes very short in GdPCTP[12], suggesting that, in this system with a coordination number of 8, the exchange mechanism that occurs is associative, i.e. the rate-determining step is the formation of an enneacoordinated intermediate with $q = 2$. This result is fuly consistent with previous observations on $[Gd(H_2O)_8]^{3+}$ and $[GdPDTA(H_2O)_2]^{-}$, whose fast exchange rates of the coordinated water molecules was accounted for the occurrence of an associative exchange mechanism.

In summary, GdPCTP[12] displays a number of interesting properties (high K_f, high r_{1p}^{H}, fast water exchange rate) which make this chelate a very promising candidate for further applications as a CA for MRI.

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