

Dy-DTPA Derivatives as Relaxation Agents for Very High Field MRI: The Beneficial Effect of Slow Water Exchange on the Transverse Relaxivities

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Proton longitudinal and transverse relaxivities of Dy(DTPA)²⁻ and Dy-DTPA bisamide derivatives (Dy(DTPA-BA): Dy-DTPA bisamide, Dy(DTPA-BEA): Dy-DTPA bisethylamide, Dy(DTPA-BnBA): Dy-DTPA bis-*n*-butylamide, and Dy(DTPA-BBMA): Dy-DTPA bisbismethylamide) were analyzed between 0.47 T and 18.8 T. Curie longitudinal relaxation was clearly observed at magnetic fields larger than 2.4 T, but the longitudinal relaxivities are limited by the fast rotation of the complexes. Rotational correlation times were separately assessed by deuterium relaxometry of the diamagnetic deuterated lanthanum analogs. Transverse relaxivity, which depends on the square of the magnetic field and on the residence time of the coordinated water molecule (τ_M), was more than 7.5 times larger at 18.8 T and 310 K for Dy(DTPA-BA) and Dy(DTPA-BEA) as compared to Dy(DTPA)²⁻. This difference is mainly related to the slower water exchange of the bisamide complexes, as confirmed by the values of τ_M measured by oxygen-17 relaxometry. Such Dy-complexes, characterized by relatively long τ_M values (τ_M^{310} larger than 100 ns but smaller than 1 μ s), thus appear to be useful as negative T_2 (or transverse) contrast agents for high-field imaging. This was demonstrated by the spin-echo images of phantoms obtained at 4.7 T on samples containing Dy(DTPA)²⁻ and Dy(DTPA-BEA). *Magn Reson Med* 47:1121–1130, 2002. © 2002 Wiley-Liss, Inc.

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Among the lanthanide ions, gadolinium is known as the best nuclear relaxation enhancer of longitudinal nuclear relaxation because of its long electronic relaxation time. Its stable complexes are thus widely used as T_1 contrast agents for MRI. Complexes of other paramagnetic lanthanides (III) are usually utilized as shift agents in NMR spectroscopy or as susceptibility agents in MRI. For example, Dy-complexes have been proposed to measure cerebral blood volume (1) and to delineate healthy and damaged tissues (2–7) by MRI. After injection, Dy-complexes are localized in the extracellular compartment of healthy

tissues, and as a result of their relatively large magnetic susceptibility, they induce local field gradients that result in a T_2^* decrease. However, this type of T_2^* effect requires quite large concentrations of the compound. In damaged areas where cell membranes are disrupted, Dy-complexes are homogeneously distributed and have very little influence on the T_2^* at the magnetic fields used in MRI.

For lanthanide ions characterized by very short electronic relaxation times, such as Dy(III), Pr(III), Sm(III), Ho(III), Er(III), and Yb(III), a sizable contribution to longitudinal nuclear proton relaxation resulting from the presence of a large static magnetic moment at high magnetic fields has been proposed by Bertini et al. (8). This contribution, first described by Guéron (9) and called Curie relaxation, originates from the dipolar interaction between the water protons and a large static magnetic moment arising from the electrons. In 1991, Aime et al. (10) suggested taking advantage of the dependence of the proton transverse relaxation rate vs. the square of the magnetic field to reduce the water proton signal in high-field NMR spectra. These authors also showed that the efficacy of the water suppression depends on the structure of the complex, and they attributed this to different exchange rates of the coordinated water with the bulk. More recently, because of the above-mentioned high-field effect on the water protons, Dy(III) complexes were suggested as potential negative contrast agents for MRI (11,12).

In the present work, we report experimental evidence of the Curie longitudinal relaxation of different Dy-complexes of similar size, and illustrate the influence of the magnetic field and the residence time of the coordinated water molecule on the proton transverse relaxation rate. Five open-chain Dy(III) complexes were studied: 1) the parent compound Dy(DTPA)²⁻ (DTPA: 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid); and four bisamide derivatives: 2) Dy(DTPA-BA) (DTPA-BA: 3,9-bis(2-amino-2-oxoethyl)-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid); 3) Dy(DTPA-BEA) (DTPA-BEA: 3,9-bis(2-ethylamino-2-oxoethyl)-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid); 4) Dy(DTPA-BnBA) (DTPA-BnBA: 3,9-bis(2-*n*-butylamino-2-oxoethyl)-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid); and 5) Dy(DTPA-BBMA) (DTPA-BBMA: 3,9-bis(2,2-bismethylamino-2-oxoethyl)-6-(carboxymethyl)-3,6,9-triazaundecanedioic acid) (Fig. 1). The longitudinal proton relaxation rates of the Dy(III) complexes were measured between 0.47 T and 18.8 T in order to analyze the Curie contribution to T_1 . Deuterium relaxometry was performed on the diamagnetic lanthanum complexes of the specifically-labeled ligands to assess the rotational correlation times (τ_R) of the lanthanide complexes. The proton transverse relaxation rates measured over the same range

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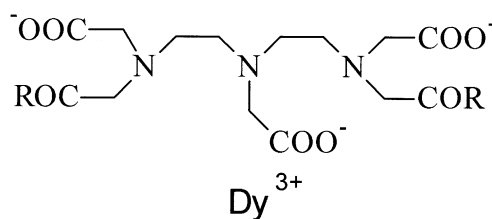


FIG. 1. Structure of 1) Dy(DTPA)²⁻ (R = O⁻), 2) Dy(DTPA-BA) (R = -NH₂), 3) Dy(DTPA-BEA) (R = -NH-CH₂-CH₃), 4) Dy(DTPA-BnBA) (R = -NH-(CH₂)₃-CH₃), and 5) Dy(DTPA-BBMA) (R = -N(CH₃)₂).

of magnetic fields, as well as the induced proton chemical shifts, were used to estimate the water residence time of the coordinated water molecule (τ_M). τ_M values were independently obtained by analysis of the temperature dependence of the O-17 NMR T_1 and T_2 of water. These residence times were compared to those of their Gd(III) analogs. Finally, the efficacy of Dy-complexes with relatively long τ_M values as negative contrast agents for high-field MRI is illustrated.

MATERIAL AND METHODS

Chemicals

All chemicals were purchased from Aldrich or Fluka (Bornem, Belgium) and were used without further purification. Synthesized compounds were characterized by NMR and mass spectrometry. Proton NMR spectra were recorded at 300 MHz on a Bruker AMX 300 spectrometer (Bruker, Karlsruhe, Germany). Mass spectra (LSIMS) were obtained using a VG Autospec mass spectrometer (VG Analytical, Manchester, UK). Samples were dissolved in water and deposited on a glycerol matrix.

High-performance liquid chromatography (HPLC) was used to control the purity of the ligands and complexes. It was performed on a Waters 600 multisolvent delivery system equipped with a Rheodyne injection valve (20 μ L loop) and controlled by Millennium software (Waters, Milford, MA). A Novapak C18 column (4.56 mm \times 150 mm) was used. Elution was performed with a linear gradient from pure 0.05 M triethylammonium acetate (pH = 6) to 100% methanol at a flow rate of 1 mL/min for 20 min. A UV/diode array detector was used to monitor the elution of the ligand or the complex (254 or 270 nm).

The bisamide ligands were synthesized by reacting DTPA bisanhydride with ammonia, ethylamine, *n*-butylamine, or bismethylamine (13–15). Gd(III), La(III), and Dy(III) complexes were prepared by mixing aqueous solu-

tions of equimolar amounts of hydrated LnCl₃ and ligands. The pH was adjusted to 6.5–7. The absence of free Ln(III) was checked with arsenazo III indicator. The neutral complexes were passed through a Sep-Pak column (Accell Plus QMA cartridges; Waters, Brussels, Belgium) to eliminate any charged species, and were then freeze-dried. The concentration of Dy(III) in the stock solutions of the complexes was determined by atomic emission spectrometry (Jobin-Yvon 38+; Jobin-Yvon ISA, Longjumeau, France).

Relaxometry

¹H T_1 and T_2 measurements were performed at 0.47, 4.7, 7.05, 11.75, 14.1, and 18.8 T, respectively, on Minispec PC-20, MSL-200, AMX-300, AMX-500, AMX-600, and Avance 800 from Bruker. The deuterium and oxygen-17 measurements were carried out on 2-mL samples contained in 10-mm-o.d. tubes on a Bruker AMX-300 spectrometer equipped with a broadband probe. No field-frequency lock was used except for the measurements of the ¹⁷O chemical shifts. Field homogeneity was optimized on the proton free induction decay observed with the decoupling coil. Temperature was regulated by air or nitrogen flow (Bruker BVT-2000 unit). Deuterium longitudinal relaxation rates were measured using the inversion recovery Fourier transform (IRFT) sequence on solutions containing the labeled diamagnetic lanthanum complex dissolved in deuterium-depleted water (Aldrich). The experimental data were fitted with a three-parameter minimization routine. In diamagnetic molecules, the relaxation rates of the quadrupolar deuterium nucleus originate predominantly from quadrupolar interactions, which are modulated by the rotational motion. As previously described (16), the analysis of the data thus allows one to estimate the rotational correlation time τ_R . ¹⁷O T_1 were measured using the IRFT sequence, and experimental data were fitted with a three-parameter routine. Diamagnetic transverse relaxation times of ¹⁷O water (pH \approx 6.5) and transverse relaxation times of Dy-complexes solutions were measured at natural abundance using a Carr-Purcell-Meiboom-Gill sequence and a two-parameter fit of the data. ¹⁷O transverse relaxation times of water (natural abundance) in solutions containing Gd-complexes were calculated from the linewidth. All ¹⁷O NMR spectra were proton-decoupled. The concentrations used (Table 1) were lower than 60 mM in order to avoid any significant increase of the viscosity and, hence, of the rotational correlation time (17).

Imaging

Imaging was performed on an Avance 200 instrument (Bruker) at room temperature (multislice, multi-echo se-

Table 1
Concentrations (in mM) of the Various Ln Complexes Used in the Different NMR Measurements

Ligand	¹ H relaxometry of Dy-complex	¹ H chemical shift of Dy-complex	² H relaxometry of La-complex	¹⁷ O relaxometry of Dy-complex	¹⁷ O relaxometry of Gd-complex
DTPA	1.60	20.45	50.00	58.44	50.00
DTPA-BA	2.52	8.33	19.00	49.96	22.59
DTPA-BEA	5.15	10.25	7.50	38.40	24.75
DTPA-BnBA	4.26	10.00	23.18	36.34	27.72
DTPA-BBMA	2.62	12.53	44.00	50.96	21.32

quence; TR = 362.5 ms; TE = 15 ms; NA = 1; matrix = 256 × 256; FOV = 6 cm; slice thickness = 2 mm; total acquisition time = 1 min 32 s).

RESULTS AND DISCUSSION

Proton Longitudinal Relaxation Rate

The proton paramagnetic longitudinal relaxation rate ($1/T_1^p$) in Dy-complexes solution can be described as the sum of inner ($1/T_1^{is}$) and outer ($1/T_1^{os}$) sphere contributions. The inner-sphere contribution (Eq. [1]) depends on the ratio of the Dy-complex and water concentrations ($f = [\text{Dy-complex}]/[\text{water}]$), on the number of water molecules in the first coordination sphere of the complex (q), on the residence time of the coordinated water molecules exchanging with the bulk (τ_M), and on the relaxation rate of the bound nuclei ($1/T_{1M}$) which is the sum of dipolar ($1/T_{1M}^{DD}$) and Curie dipolar ($1/T_{1M}^C$) contributions (Eqs. [2] and [3]) (18).

$$\frac{1}{T_1^{is}} = fq \frac{1}{T_{1M} + \tau_M} \quad [1]$$

$$\frac{1}{T_{1M}^{DD}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \gamma_I^2 \mu_B^2 g_j^2 J(J+1) \frac{1}{r^6} \left[\frac{3\tau_{C1}}{1 + \omega_I^2 \tau_{C1}^2} + \frac{7\tau_{C2}}{1 + \omega_S^2 \tau_{C2}^2} \right] \quad [2]$$

$$\frac{1}{T_{1M}^C} = \frac{2}{5} \left(\frac{\mu_0}{4\pi} \right)^2 \gamma_I^2 B_0^2 \mu_B^4 g_j^4 J^2 (J+1)^2 \frac{1}{r^6} \frac{1}{(3k_B T)^2} \left[\frac{3\tau_{CC}}{1 + \omega_I^2 \tau_{CC}^2} \right] \quad [3]$$

In Eqs. [2] and [3], γ_I is the gyromagnetic ratio of the observed nucleus, μ_B is the Bohr magneton, g_j is the Landé factor of Dy ($g_j = 1.333$), J is the total spin quantum number (15/2), r is the distance between Dy(III) and the observed nucleus, B_0 is the applied magnetic field, k_B is the Boltzmann constant, T is the temperature, ω_I and ω_S are the angular precession frequencies of the proton and the electron, and τ_{Ci} ($i = 1, 2$), the correlation times modulating the dipolar interaction, are related to τ_R , the rotational correlation time of the hydrated complex, to $\tau_{S1,2}$, the electronic relaxation times, and to τ_M ($\tau_{Ci}^{-1} = \tau_R^{-1} + \tau_M^{-1} + \tau_{Si}^{-1}$).

The electronic relaxation times (τ_{S1} and τ_{S2}) of Dy(III) have been reported to be equal and field-independent in the range of interest of our study (8). They will be noted as τ_S . At room temperature, τ_S of Dy(III) is of the order of 10^{-13} s, τ_M is expected to range between 10^{-8} and 10^{-6} s, and τ_R is $\approx 10^{-10}$ s for small complexes. Consequently, τ_{C1} and τ_{C2} are equal and determined exclusively by the values of τ_S . τ_{CC} , the correlation time for the Curie contribution depends on τ_R and τ_M ($\tau_{CC}^{-1} = \tau_R^{-1} + \tau_M^{-1}$) and for Dy-complexes of small size is thus equal to τ_R . The dipolar inner-sphere contribution is thus modulated by τ_S , whereas the Curie inner-sphere dipolar relaxation is modulated by τ_R . The Curie contribution depends on the square of the magnetic field and becomes significant at high fields only.

The outer-sphere contribution is also the sum of dipolar ($1/T_{1OS}^{DD}$) and Curie ($1/T_{1OS}^C$) contributions (Eqs. [4] and [5]) (19,20)

$$\frac{1}{T_{1OS}^{DD}} = \frac{C_{DD}}{aD} \times \left\{ 6 \left[J(J+1) - S_c \coth \frac{\chi}{2J} - S_c^2 \right] \cdot j_o(\omega_I, \tau_D, \tau_S) + 7 \cdot \coth \frac{\chi}{2J} \cdot S_c \cdot j_e(\omega_S, \tau_D, \tau_S) \right\} \quad [4]$$

$$\frac{1}{T_{1OS}^C} = \frac{32\pi}{45000} \left(\frac{\mu_0}{4\pi} \right)^2 \gamma_I^2 \mu_B^2 g_j^2 Na[C] \frac{1}{aD} S_c^2 \{ j^A(\omega_I, \tau_D) \} \quad [5]$$

where C_{DD} is equal to $(16\pi/135000)(\mu_0/4\pi)^2 \gamma_I^2 \mu_B^2 g_j^2 Na[C]$, with Na being the Avogadro number and $[C]$ the molar concentration of the paramagnetic center; a is the distance of closest approach; D is the relative diffusion constant; τ_D , the translational correlation time, is equal to a^2/D ; S_c is the time-averaged or “Curie” spin (19), and χ is equal to $J \cdot B_0 \cdot \mu_B \cdot g_j / kT$. $j_o(\omega, \tau_D, \tau_S)$, the spectral density function for the dipolar interaction, depends on τ_D and τ_S (20), whereas $j^A(\omega, \tau_D)$ depends only on τ_D (19). At low fields, the dipolar outer-sphere term, which for Dy(III) is modulated by τ_S , is expected to be predominant, whereas at high fields the Curie term modulated by τ_D should become significant. The total longitudinal relaxation rates are thus expected to be small at low magnetic fields because they are modulated by the very short electronic relaxation time of Dy(III). In addition, since τ_S is likely to be similar for all the complexes studied in this work, comparable low-field longitudinal relaxation rates are expected. At high magnetic fields, the Curie inner- and outer-sphere contributions, modulated by τ_R and τ_D , should become important, and a similar increase of r_1 with the magnetic field should thus be observed for Dy(III) complexes of similar size.

The proton longitudinal relaxivity (r_1 , defined as the relaxation rate enhancement brought by 1 millimole of the paramagnetic center per liter of water) of the five Dy-complexes is, as expected, comparable (Fig. 2). At low fields ($\nu_0 < 80$ MHz), no significant effect of the temperature was noticed, in good agreement with the τ_S control of this part of the curve. At higher fields, a slight r_1 decrease was observed with increasing temperature as a result of the faster rotation and translation of the complexes.

The data were fitted using Eqs. [1]–[5] with the following assumptions: $q = 1$, $r = 3.1 \times 10^{-10}$ m, $a = 3.6 \times 10^{-10}$ m, and the relative diffusion constant set to the reported values for pure water (21) ($D^{298} = 2.62 \times 10^{-9}$ m²s⁻¹, $D^{310} = 3.5 \times 10^{-9}$ m²s⁻¹). Two parameters, τ_S and τ_R , were extracted from the fitting (Table 2). The values obtained for τ_S are somewhat smaller than those reported for Dy(III) aqua ion ($\tau_S^{298} = 0.39$ ps and $\tau_S^{308} = 0.38$ ps) (8) and for Dy(DOTA)⁻ ($\tau_S^{310} = 0.33$ ps) (11,12). τ_R values obtained by the analysis of the longitudinal relaxation of deuterium on the diamagnetic La(III)-complexes specifically deuterated are in good agreement with the values obtained by fitting of the proton r_1 data (Table 2).

It should be noted that for Dy-DTPA derivatives at 310 K, the outer-sphere dipolar contribution is larger than the dipolar inner-sphere contribution, a fact already reported for Dy(DOTA)⁻ (12), and that the Curie inner-

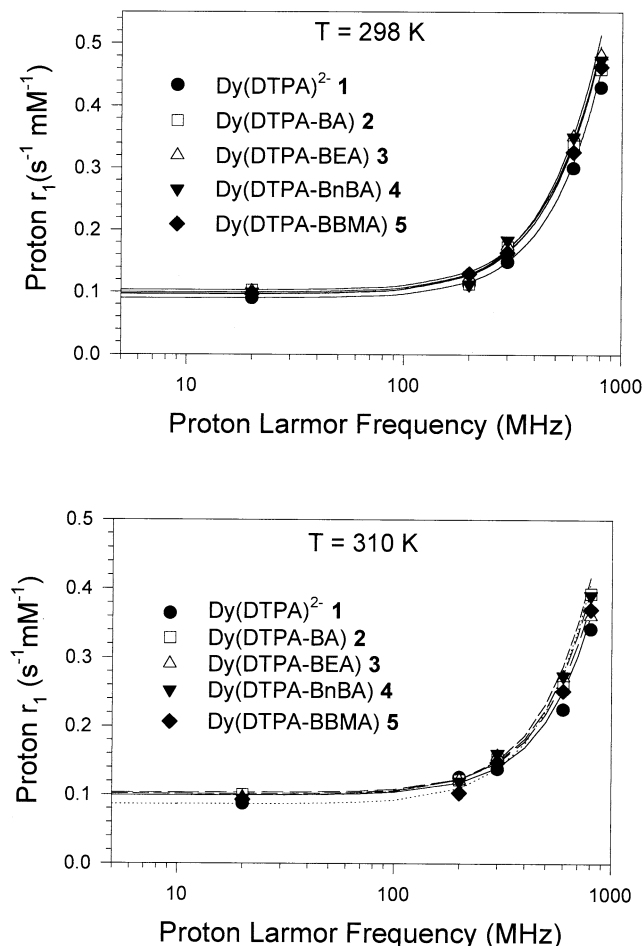


FIG. 2. Proton longitudinal relaxivity vs. proton Larmor frequency at 298 K (top graph) and 310 K (bottom graph).

sphere contribution is only slightly larger than the outer-sphere one (Fig. 3). In Eq. [2], it is assumed that the mean-squared fluctuating component of the spin is constant at all fields, although it is reduced from its zero-field value of $J(J+1)/3$ as the Curie spin grows. Exact values can be obtained by substituting $J(J+1)/3$ with $[J(J+1) - S_c \coth(\chi/2J) - S_c^2]$. However, this correction leads to differences of <4% at 18.8 T, and can therefore be neglected in a first approximation.

Proton Transverse Relaxation Rate

Like the longitudinal relaxation rate, the proton paramagnetic transverse relaxation rate ($1/T_2^p$) contains inner-

Table 2

Values of τ_s and τ_R Obtained by Fitting of the Experimental Proton r_1 Data of the Five Dy-Complexes at 298 K and 310 K

	τ_s^{298} (ps)	τ_s^{310} (ps)	τ_R^{298} (ps) ^a	τ_R^{310} (ps) ^a
Dy(DTPA) ²⁻ 1	0.14	0.15	80 (85)	55 (60)
Dy(DTPA-BA) 2	0.17	0.16	75 (79)	65 (57)
Dy(DTPA-BEA) 3	0.16	0.15	95 (110)	70 (78)
Dy(DTPA-BnBA) 4	0.16	0.15	95 (104)	80 (74)
Dy(DTPA-BBMA) 5	0.15	0.13	95 (98)	80 (70)

^aValues in parentheses were obtained by ²H NMR on the La-complexes.

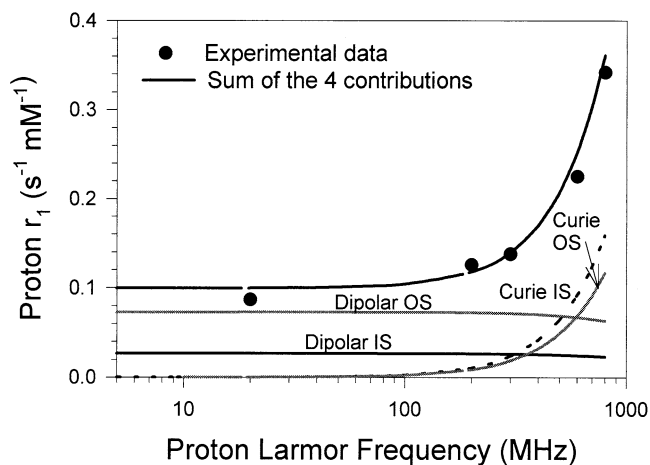


FIG. 3. Proton NMRD data of Dy(DTPA)²⁻ at 310 K and the dipolar inner-sphere (dipolar IS), Curie inner-sphere (Curie IS), dipolar outer-sphere (dipolar OS), and Curie outer-sphere (Curie OS) contributions to the observed relaxivity. The parameters used for the calculation of these contributions are those described in the text.

sphere ($1/T_2^{is}$) and outer-sphere ($1/T_2^{os}$) contributions. The inner-sphere contribution is given by (18):

$$\frac{1}{T_2^{is}} = fq \frac{1}{\tau_M} \frac{1}{\left(\frac{1}{\tau_M} + \frac{1}{T_{2M}}\right)^2} + \Delta\omega_M^2 \quad [6]$$

where f , q and τ_M are as defined above; and $1/T_{2M}$ the transverse relaxation rate of the coordinated water molecule, results from dipolar ($1/T_{2M}^{DD}$), dipolar Curie ($1/T_{2M}^C$), and Curie contact ($1/T_{2M}^{CC}$) contributions (Eqs. [7] and [8]). For proton, the contact term (not shown) is much smaller than the dipolar and Curie ones, and can thus be neglected. $\Delta\omega_M$, the chemical shift of the coordinated water molecule, is proportional to the magnetic field and is the sum of contact ($\Delta\omega_M^{con}$) and pseudocontact terms.

$$\frac{1}{T_{2M}^{DD}} = \frac{1}{15} \left(\frac{\mu_0}{4\pi}\right)^2 \gamma_i^2 \mu_B^2 g_j^2 J(J+1) \times \frac{1}{r^6} \left[4\tau_{C1} + \frac{3\tau_{C1}}{1 + \omega_1^2 \tau_{C1}^2} + \frac{7\tau_{C2}}{1 + \omega_3^2 \tau_{C2}^2} \right] \quad [7]$$

$$\frac{1}{T_{2M}^C} + \frac{1}{T_{2M}^{CC}} = \frac{1}{5} \left(\frac{\mu_0}{4\pi}\right)^2 \gamma_i^2 B_0^2 \mu_B^4 g_j^4 J^2 (J+1)^2 \times \frac{1}{r^6} \frac{1}{(3k_B T)^2} \left[4\tau_{CC} + \frac{3\tau_{CC}}{1 + \omega_1^2 \tau_{CC}^2} \right] + \frac{4}{3} (\Delta\omega_M^{con})^2 \tau_M \quad [8]$$

Two extreme situations can exist. First, if the water exchange is fast as compared to the relaxation rate of the bound water molecule and to the chemical shift difference between bound and bulk water ($\tau_M^{-1} \gg T_{2M}^{-1}$ and $\tau_M^{-1} \gg \Delta\omega_M$), the expression of the inner-sphere relaxation rate simplifies to Eq. [9].

$$\frac{1}{T_2^{is}} \cong fq \left(\frac{1}{T_{2M}} + \tau_M \Delta\omega_M^2 \right) \quad [9]$$

At a low magnetic field, $\tau_M \Delta\omega_M^2$ is much smaller than $1/T_{2M}$ and, consequently, $1/T_2^{is}$ is equal to $f \cdot q \cdot T_{2M}^{-1}$. At higher fields, when $\tau_M \Delta\omega_M^2$ becomes larger than T_{2M}^{-1} , $1/T_2^{is}$ is equal to $f \cdot q \cdot \tau_M \cdot \Delta\omega_M^2$.

The second extreme situation is met when the water exchange is slower, $\tau_M^{-1} \leq \Delta\omega_M$, at intermediate and high fields, but $\tau_M^{-1} \gg T_{2M}^{-1}$. At a very low field, $\Delta\omega_M$ is small and $1/T_2^{is} = f \cdot q \cdot T_{2M}^{-1}$. At intermediate and high fields, Eq. [6] can then be written as

$$\frac{1}{T_2^{is}} = fq \frac{\tau_M \Delta\omega_M^2}{1 + \tau_M^2 \Delta\omega_M^2} \quad [10]$$

As long as $\tau_M^2 \Delta\omega_M^2$ remains much smaller than 1, $1/T_2^{is}$ depends on the square of the magnetic field ($1/T_2^{is} = f \cdot q \cdot \tau_M \cdot \Delta\omega_M^2$); but at high fields when $\tau_M^2 \Delta\omega_M^2$ becomes much larger than 1, the inner-sphere contribution is limited by τ_M ($1/T_2^{is} = f \cdot q / \tau_M$) and becomes field-independent.

The dipolar and Curie outer-sphere contributions are given by (19):

$$\frac{1}{T_{2OS}^{DD}} = \frac{C_{DD}}{aD} \cdot \left\{ \left[J(J+1) - S_c \coth \frac{\chi}{2J} - S_c^2 \right] \cdot (3j_o(\omega_l, \tau_D, \tau_S)) + 4j_o(o, \tau_D, \tau_S) \right\} + \frac{C_{DD}}{aD} \cdot 6.5 \cdot \coth \frac{\chi}{2J} \cdot S_c \cdot j_o(\omega_s, \tau_D, \tau_S) \quad [11]$$

$$\frac{1}{T_{2OS}^C} = \frac{16\pi}{45000} \left(\frac{\mu_0}{4\pi} \right)^2 \gamma_I^2 \mu_B^2 g_j^2 N a [C] \times \frac{1}{aD} S_c^2 \{ 3j^A(\omega_{\pi D}) + 4j^A(0, \tau_D) \} \quad [12]$$

It can thus be expected that the low-field total transverse relaxivity will be small and similar to the longitudinal relaxivity, since it is modulated by the electronic relaxation time τ_S , and the contribution of T_{2M} will be significant only if the exchange is very fast (Eq. [9]). When the magnetic field increases, the relaxivity becomes larger and depends on B_0^2 and on τ_M until $\Delta\omega_M^{-1}$ gets close to τ_M .

The experimental data obtained on the five Dy-complexes are shown in Fig. 4. At low magnetic fields, the transverse relaxivities are comparable for the five complexes, and similar to the longitudinal ones ($r_2 \sim 0.1$ – $0.15 \text{ s}^{-1} \text{ mM}^{-1}$), and barely affected by temperature. At higher fields ($\nu_0 > 100 \text{ MHz}$), a field-dependent increase of r_2 is observed. The largest r_2 are observed for complexes 2 and 3, and the smallest one for complex 1. The marked decrease of r_2 when temperature is raised and the absence of leveling-off of r_2 at high fields indicate that no τ_M limitation of r_2 occurs. At high magnetic fields, r_2 depends on both τ_M and $\Delta\omega_M$. Since $\Delta\omega_M$ should not be very different among the complexes because of the similarity of their structures, the highest r_2 should correspond to the longest τ_M , and the marked decrease of r_2 at high fields when temperature is raised should be mainly attributable to the subsequent shortening of τ_M .

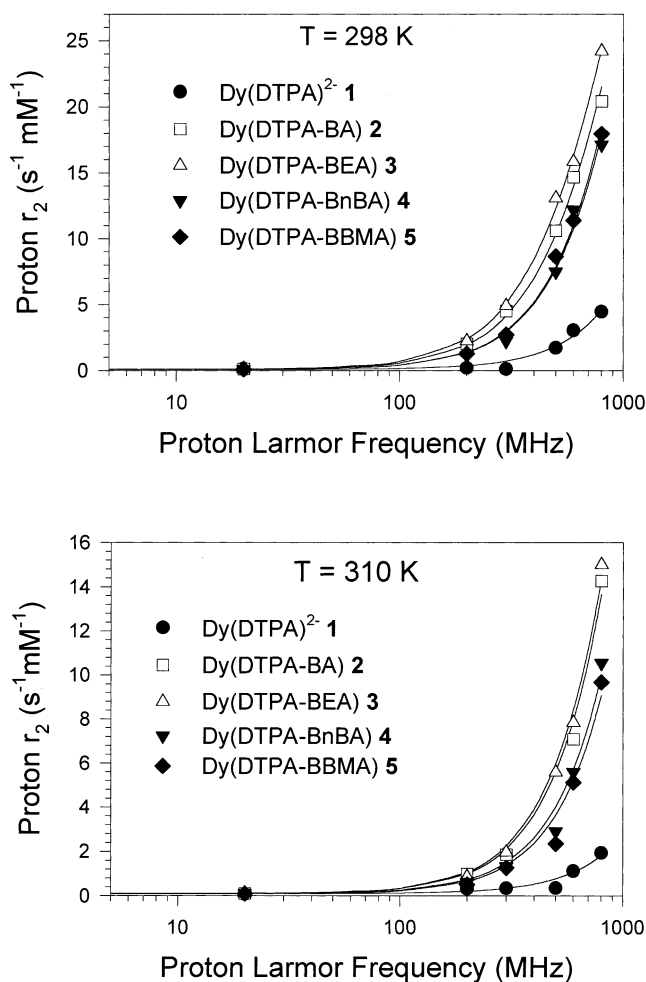


FIG. 4. Proton transverse relaxivity vs. proton Larmor frequency at 298 K (top graph) and 310 K (bottom graph).

Fitting of the data was performed using Eqs. [6]–[8], [11], and [12]. The contact Curie term calculated with the value of $\Delta\omega_M^{con}$ obtained as described below is quite small and does not contribute significantly to the final r_2 value. The parameters q , r , a , and D were set to the values used for the fitting of r_1 , while τ_R and τ_S were fixed to the values obtained from the analysis of r_1 data. Two parameters, τ_M and $\Delta\omega_M$, were fitted. To have an independent estimation of $\Delta\omega_M$, chemical shifts were measured at different temperatures ($T = 290$ – 335 K) and 7.05 T on solutions of the Dy-complexes containing minute amounts of t-butanol. In these experiments, the susceptibility effects were taken into account by measuring the shift between the resonances of the methyl group and the water protons. The paramagnetic chemical shift ($\Delta\omega^P$) is then given by

$$\Delta\omega^P = fq \frac{\Delta\omega_M}{\left(1 + \frac{\tau_M}{T_{2M}} \right)^2 + \tau_M^2 \Delta\omega_M^2} \quad [13]$$

For Dy-complexes, $\tau_M \ll T_{2M}$, and thus $\Delta\omega^P$ depends on the chemical shift of the bound nuclei ($\Delta\omega_M$) and on the residence time τ_M :

Table 3

Values of τ_M and $\Delta\omega_M$ Obtained by the Fitting of the Experimental Proton r_2 Data of the Five Dy-Complexes at 298 K and 310 K

	τ_M^{298} (ns)	τ_M^{310} (ns)	$\Delta\omega_M^{298}$ ($10^5 \text{rad s}^{-1} \text{T}^{-1}$)	$\Delta\omega_M^{310}$ ($10^5 \text{rad s}^{-1} \text{T}^{-1}$)
Dy(DTPA) ²⁻ 1	29	12	1.47	1.42
Dy(DTPA-BA) 2	220	100	1.44	1.50
Dy(DTPA-BEA) 3	225	110	1.52	1.50
Dy(DTPA-BnBA) 4	115	51	1.56	1.66
Dy(DTPA-BBMA) 5	115	48	1.60	1.63

$$\Delta\omega^p = fq \frac{\Delta\omega_M}{1 + \tau_M^2 \Delta\omega_M^2} \quad [14]$$

If the water exchange is fast as compared to the chemical shift difference ($\tau_M \ll 1/\Delta\omega_M$), then $\Delta\omega^p$ is proportional to $\Delta\omega_M$. The observed reduced chemical shifts ($\Delta\omega^R = \Delta\omega^p/fq$) of the five Dy-complexes range from 1.3×10^5 to $1.6 \times 10^5 \text{ rad s}^{-1} \text{T}^{-1}$ at 310 K, and from 1.3×10^5 to $1.8 \times 10^5 \text{ rad s}^{-1} \text{T}^{-1}$ at 298 K. These values are very close to those obtained by fitting the proton r_2 data, and confirm that at 7.05 T and 310 K the water exchange is not slow as compared to the chemical shift difference ($\tau_M^2 \Delta\omega_M^2 < 0.1$) and that τ_M is thus smaller than 280 ns. This value of $\Delta\omega_M$ results from contact ($\Delta\omega_M^{con}$) and pseudocontact ($\Delta\omega_M^{dip}$) contributions. To estimate the contact contribution, the chemical shift induced on water protons by Gd(DTPA)²⁻ was measured. The obtained value ($1.05 \times 10^4 \text{ rad s}^{-1} \text{T}^{-1}$ at 310 K), which is a pure contact contribution, allows the calculation of a hyperfine coupling constant of $4.6 \times 10^5 \text{ rad s}^{-1}$ (Eq. [15]). Assuming that the hyperfine coupling constant is similar for Gd³⁺ and Dy³⁺ ions, a value of $\Delta\omega_M^{con}$ equal to $9.4 \times 10^3 \text{ rad s}^{-1} \text{T}^{-1}$ is calculated for Dy³⁺ (Eq. [16]). This value is, as expected, much smaller than the measured value of $\Delta\omega_M$ (see above), confirming that for Dy³⁺ the pseudocontact contribution to $\Delta\omega_M$ is predominant.

$$\Delta\omega_M^{con} = B_0 \mu_B g_j S(S+1) \frac{1}{3k_B T} \frac{A}{\hbar} \quad [15]$$

$$\Delta\omega_M^{con} = B_0 \mu_B g_j (g_j - 1) J(J+1) \frac{1}{3k_B T} \frac{A}{\hbar} \quad [16]$$

The results of the fitting (Table 3) show that at both 310 K and 298 K, τ_M of complexes 2 and 3 are comparable and longer than those of complexes 4 and 5, while complex 1 (Dy(DTPA)²⁻) is characterized by the shortest τ_M . The linear dependence of r_2 vs. B_0^2 is observed for B_0 values ranging from 1 T to 10 T for compounds 4 and 5, and from 1 T to 4 T for compounds 2 and 3. Above these B_0 values, this linear dependence is no longer observed due to the increasing value of $\tau_M^2 \Delta\omega_M^2$ (see Eq. [10]). It should also be pointed out that the outer-sphere contribution is not negligible at low fields but at high fields its contribution is very low, particularly for Dy-complexes with slow water exchange.

In summary, the analysis of the longitudinal and transverse proton relaxation rates of the Dy-complexes show that: 1) the electronic relaxation time is similar for all complexes ($\tau_s \sim 0.15 \text{ ps}$); 2) τ_R being of the same order of

magnitude for all complexes, their r_1 values are similar at all magnetic fields; and 3) the r_2 of complexes with longer τ_M are markedly larger at high magnetic fields.

Oxygen-17 Longitudinal and Transverse Relaxation Rates

The analysis of the dependence on temperature of the transverse relaxation rate of oxygen-17 of water solutions is a convenient route for determining the exchange parameters of water molecules coordinated to paramagnetic ions. It has been extensively used to characterize gadolinium complexes (16,22–24) and has been applied to a few dysprosium complexes (Dy(DTPA-BMA), Dy(PDTA)⁻) (25–27). To obtain an alternative estimation of τ_M of our five Dy-complexes, oxygen-17 T_1 and T_2 measurements were performed at temperatures ranging from 278 K to 345 K.

Theory predicts that the paramagnetic longitudinal relaxation rate of oxygen-17 results mainly from the inner-sphere contribution described by Eq. [1]. In addition to the dipolar and dipolar Curie relaxation contributions (Eqs. [2] and [3]), two other terms have to be taken into account: the scalar or contact ($1/T_{1M}^{SC}$) and the quadrupolar ($1/T_{1M}^Q$) contributions:

$$\frac{1}{T_{1M}^{SC}} = \frac{2}{3} J(J+1) \left(\frac{A}{\hbar} \right)^2 \left[\frac{\tau_{SC}}{1 + \omega_s^2 \tau_{SC}^2} \right] \quad [17]$$

$$\frac{1}{T_{1M}^Q} = \frac{1}{T_{2M}^Q} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \left(\frac{e^2 q Q}{h} \right)^2 \left(1 + \frac{\eta^2}{3} \right) \tau_R \quad [18]$$

where I is the nuclear spin of oxygen-17 (5/2); $e^2 q Q/h$ is the nuclear quadrupolar coupling constant; η is the asymmetry parameter of the field gradient; and τ_{SC}^{-1} is equal to $(\tau_s^{-1} + \tau_M^{-1})$ and at 7.05 T is thus equal to τ_s^{-1} for Dy(III) complexes. The dipolar and the scalar contributions are therefore modulated by τ_s , whereas the Curie and quadrupolar mechanisms are modulated by τ_R . For T_2 relaxation, Eq. [6] can be used, but contact (Eq. [19]) and quadrupolar (Eq. [18]) terms have to be added to the dipolar (Eq. [7]) and Curie (Eq. [8]) contributions. $\Delta\omega_M$ was assumed to be predominantly determined by $\Delta\omega_M^{con}$.

$$\frac{1}{T_{2M}^{SC}} = \frac{1}{3} J(J+1) \left(\frac{A}{\hbar} \right)^2 \left[\tau_s + \frac{\tau_s}{1 + \omega_s^2 \tau_s^2} \right] \quad [19]$$

The temperature dependence of the different parameters is assumed to be described by Eqs. [16], [20], and [21]

$$\tau_R = \tau_R^{298} \exp \left(\frac{E_R}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right) \quad [20]$$

$$\frac{1}{\tau_M} = \frac{k_B T}{h} \exp\left(\frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT}\right) \quad [21]$$

where E_R is the activation energy of the rotation; ΔS^\ddagger and ΔH^\ddagger are respectively the entropy and enthalpy of the water exchange process; and τ_S is supposed to have a temperature dependence analogous to that of τ_R with an activation energy, E_S .

At our measuring field of 7.05 T, it is expected that the predominant contribution to T_{1M} will be the quadrupolar one. On the other hand, τ_M , likely to be ranging between 10^{-6} to 10^{-8} s, has no effect on T_1 . The temperature dependence of T_1 is thus determined by τ_R , and small differences are thus expected among the five Dy-complexes. For transverse relaxation, since τ_M is smaller than T_{2M} , two limiting situations can be encountered:

- 1) When $\tau_M^2 \Delta\omega_M^2 \ll 1$ (fast exchange or low fields, for example), $1/T_2^p$ is the sum of two terms: T_{2M}^{-1} , which results mainly from the quadrupolar contribution, and the product $\tau_M \Delta\omega_M^2$ (Eq. [9]). In this case, a temperature decrease will induce an increase of the transverse relaxation rate since T_{2M}^{-1} , τ_M , and $\Delta\omega_M$ increase.
- 2) When the exchange rate is very slow (at low temperatures, for example), $1/T_2^p$ is equal to $f \cdot q \cdot \tau_M^{-1}$ and a temperature decrease will result in a decrease of the reduced transverse relaxation rate $1/T_2^R$ ($1/T_2^R = (f \cdot q \cdot T_2^p)^{-1}$) due to the enhancement of τ_M .

Since all Dy-complexes studied in this work are quite similar in size, their oxygen-17 T_1 , which depends on τ_R , should be comparable whereas their oxygen-17 T_2 , which is influenced by τ_M , should be larger for complexes with slower water exchange when temperature is decreased (Eqs. [9] and [10]) until very slow exchange conditions are reached. As expected, the T_1 data are quite similar for all complexes and $1/T_2^R$ increases on lowering the temperature (Fig. 5) indicating that the exchange rate is fast (or intermediate) (Eqs. [9] and [10]). For $\text{Dy}(\text{DTPA})^{2-}$, the differences between transverse and longitudinal relaxation rates are much smaller than for the four other complexes, indicating a faster water exchange of this complex. As already observed by proton relaxometry, complexes 2 and 3, on the one hand, and complexes 4 and 5, on the other hand, behave similarly: the former relax faster at low temperatures than the latter.

The simultaneous fit of transverse and longitudinal oxygen-17 data gave the results shown on Fig. 5 and Table 4. In these fittings, r was set to 2.4×10^{-10} m, A/h was allowed to fluctuate around the values reported for Gd-complexes, and the quantity equal to $(e^2 q Q/h)(1 + \eta^2/3)^{1/2}$ was assumed to be temperature-independent. The fitted values of τ_M , τ_R , and τ_S are in good agreement with those obtained by proton and deuterium relaxometry. The fitted value of $\Delta\omega_M$ can be compared to the chemical shift measured in experiments performed to determine the number of coordinated water molecules of Dy-complexes (16,28,29). When one water molecule is in the first coordination sphere of Dy(III), a chemical shift of 43 ppm is typically observed at 330 K for a 1-molar solution of Dy-complex (16,28,29), which corresponds to a $\Delta\omega_M$ of $0.6 \times$

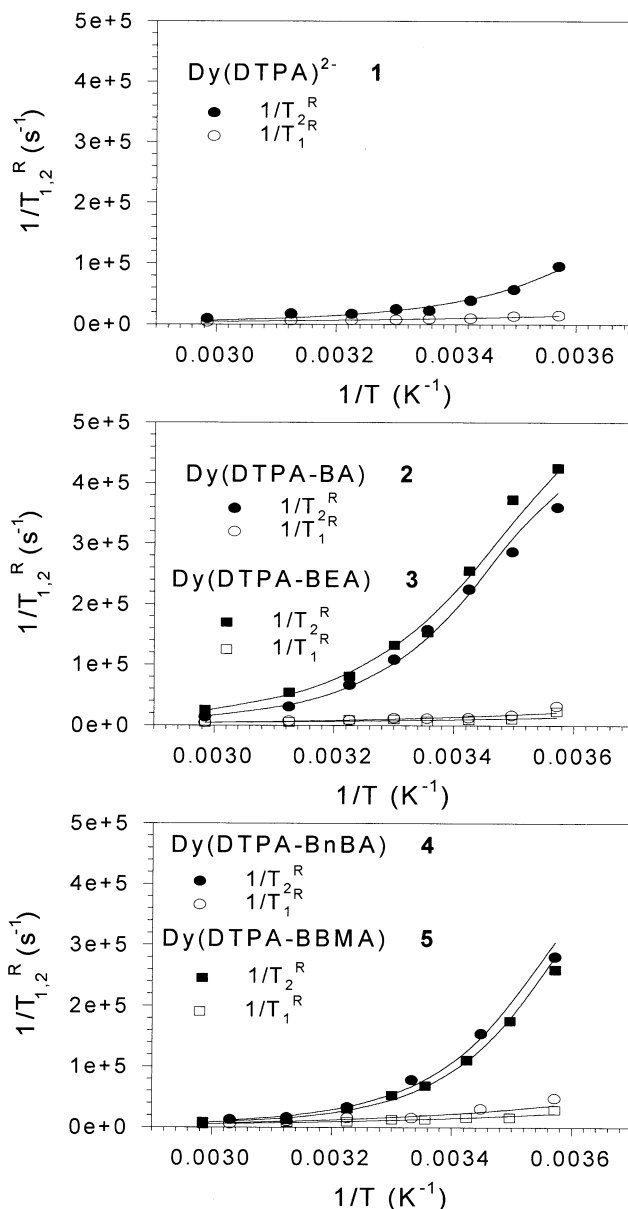


FIG. 5. Oxygen-17 transverse and longitudinal reduced relaxation rates ($1/T_{1,2}^R = 1/f \cdot q \cdot T_{1,2}^p$) vs. the reciprocal of the temperature for 1) $\text{Dy}(\text{DTPA})^{2-}$ (top graph), 2) $\text{Dy}(\text{DTPA-BA})$, and 3) $\text{Dy}(\text{DTPA-BEA})$ (middle graph); and 4) $\text{Dy}(\text{DTPA-BnBA})$ and 5) $\text{Dy}(\text{DTPA-BBMA})$ (bottom graph).

10^6 rad s^{-1} at 7.05 T. This value is in good agreement with the values obtained from the fittings of the T_1 and T_2 O-17 data ($\Delta\omega_M = 0.5 \times 10^6$ to 0.6×10^6 rad s^{-1}) and with that reported for $\text{Dy}(\text{DTPA-BMA})$ (25,26). The values of $(e^2 q Q/h)(1 + \eta^2/3)^{1/2}$ obtained in this study range between 9.4 and 13.4 MHz and agree well with the fitted values obtained by Powell et al. (23) for different Gd-complexes. It should also be noted that $(T_{2M})^{-1}$ is mainly determined by the quadrupolar and Curie contact mechanisms. At 280 K, when the water exchange is relatively slow, the Curie contact mechanism contributes to at least 75% of $(T_{2M})^{-1}$. At 330 K, its contribution decreases but remains significant (at least 20% of $(T_{2M})^{-1}$).

Table 4

Values of τ_S , τ_R , τ_M , $[(e^2qQ/h)(1+\eta^2/3)^{1/2}]$ and A/\hbar Obtained by Fitting of the Experimental Oxygen-17 T_2 and T_1 Data of the Five Dy-Complexes at 298 K and 310 K

	T = 298 K					T = 310 K		
	τ_S^{298} (ps)	τ_R^{298} (ps)	τ_M^{298} (ns)	$(e^2qQ/h)(1+\eta^2/3)^{1/2}$ (MHz)	A/\hbar (10^6 rad s^{-1})	τ_S^{310} (ps)	τ_R^{310} (ps)	τ_M^{310} (ns)
Dy(DTPA) ²⁻	0.12	85	28	10.4	3.8	0.12	65	13.5
Dy(DTPA-BA)	0.18	75	221	12.9	3.5	0.18	53	96
Dy(DTPA-BEA)	0.13	107	207	9.1	4.1	0.13	85	104
Dy(DTPA-BnBA)	0.12	105	116	13.9	3.2	0.11	71	42
Dy(DTPA-BBMA)	0.18	95	103	12.0	3.2	0.18	69	38

τ_M values of the Gd analogs of complexes 1–5, obtained by oxygen-17 T_2 analysis as described previously (16,24), are shown in Table 5. As expected, the bisamide derivatives have longer τ_M than the parent compound (23,29–32), but all bisamides do not have the same water exchange rate (F. Botteman, personal communication). The bisamides substituted by small groups (Gd(DTPA-BA) and Gd(DTPA-BEA)) have longer τ_M than Gd(DTPA-BnBA) or the tertiary bisamide Gd(DTPA-BBMA). While a similar trend is observed for Dy and Gd DTPA derivatives, the τ_M of the former complexes are about one order of magnitude shorter than those reported for the latter ones. This difference has been observed earlier for DTPA-BMA complexes, for which values of 283 ns and 2.2 μ s were reported at 298 K for Dy and Gd complexes, respectively (23,25). Similarly, Aime et al. (10) reported a τ_M^{298} value for Dy-(DOTA)⁻ much smaller than that observed for Gd-(DOTA)⁻. More recently, Caravan et al. (33) showed that the dysprosium analog of MS-325, a Gd(DTPA)²⁻ derivative, is characterized by a shorter τ_M than the gadolinium complex (24,34).

In summary, our experimental results confirm that long τ_M values are beneficial for providing efficient T_2 dysprosium contrast agents for proton high-field imaging. However, it should be noted that τ_M should be kept smaller than 1 μ s to prevent a loss of relaxivity (Fig. 6), since in this case $1/T_2^s$ will be field-independent and inversely proportional to τ_M .

Proton Imaging

Spin-echo images obtained at 4.7 T and room temperature on two samples containing the same concentration of Dy-(DTPA)²⁻ (Dy-complex with a short τ_M) and Dy(DTPA-BEA) (Dy-complex with a long τ_M) are shown in Fig. 7.

On the first echo image (TE = 15 ms), attenuation of the signal of the Dy(DTPA-BEA) solution is already observed; on the fifth echo image, the signal coming from this solu-

tion is no longer detected. It should be emphasized that Dy-complexes with long τ_M can be much more efficient as T_2 relaxation agents than their Gd-analogs at high magnetic fields: for example, r_2 of Dy(DTPA-BA) is more than three times larger at 800 MHz and 310 K than that of Gd(DTPA-BA) because of the larger chemical shifts induced by Dy³⁺ as compared to Gd³⁺.

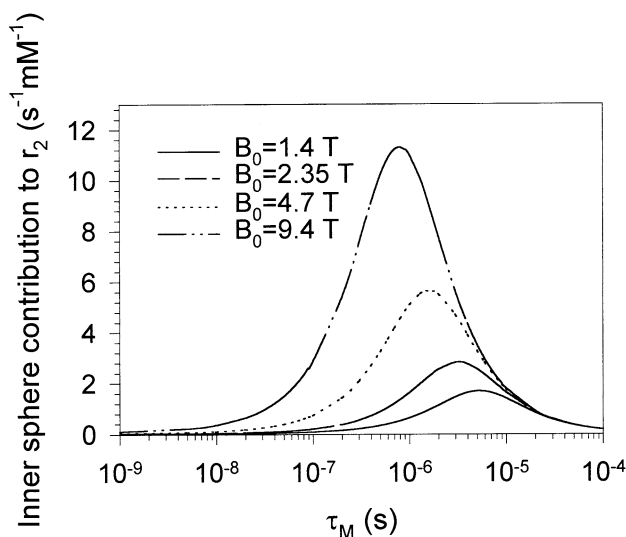
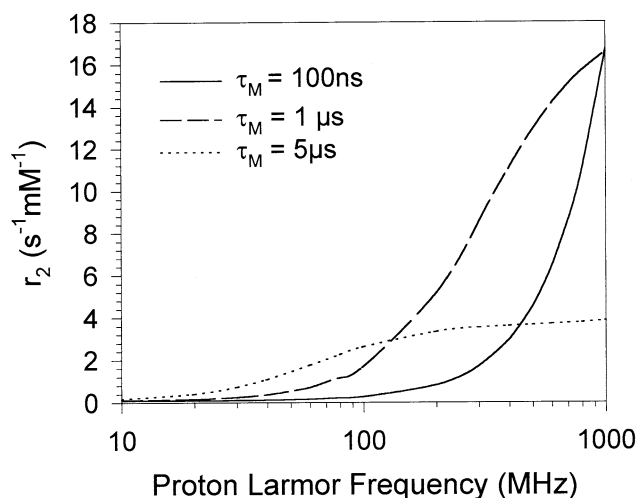


FIG. 6. Effect of τ_M on the proton transverse relaxivity of a Dy-complex at 310 K ($\tau_S = 0.15$ ps, $\tau_R = 65$ ps, $\Delta\omega_M = 1.33 \cdot 10^5$ rad s^{-1} T⁻¹, $q = 1$).

Table 5

τ_M Values Obtained by Fitting of the T_2 Data of the Gd-Complexes at 298 K and 310 K

	τ_M^{298} (ns)	τ_M^{310} (ns)
Gd(DTPA) ²⁻	331	143
Gd(DTPA-BA)	2386	1171
Gd(DTPA-BEA)	2046	927
Gd(DTPA-BnBA)	1552	713
Gd(DTPA-BBMA)	1383	624

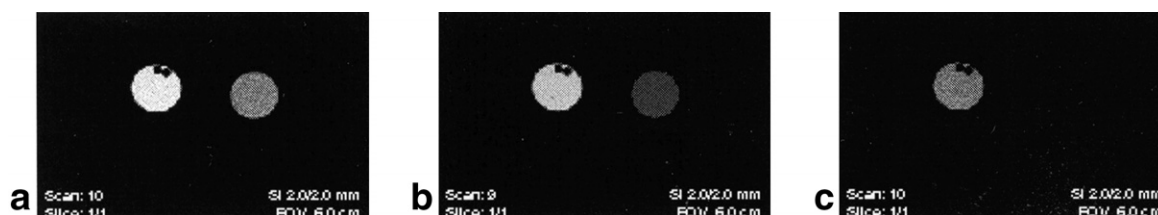


FIG. 7. Spin-echo images of tubes containing 10 mM of Dy(DTPA)²⁻ (left tube) and Dy(DTPA-BEA) (right tube) at different echo times (TE = 15 ms, TR = 362.5 ms): (a) first echo, (b) third echo, and (c) fifth echo. The black circles in the left tube are empty capillary tubes.

CONCLUSIONS

The Curie contribution to proton longitudinal relaxation has been clearly observed at high magnetic fields for Dy-(DTPA)²⁻ and Dy-DTPA bisamide derivatives, but its extent is limited by the small size and, thus, the fast rotation of the complexes analyzed in this work. Complexes with longer τ_R could be slightly more efficient as T_1 relaxation agents as long as: 1) their τ_R remains smaller than the residence time of their coordinated water molecule, and 2) the value of $\omega_1\tau_R$ is smaller than 1. If τ_R is larger than either τ_M or ω_1^{-1} , further increase of the molecular size will not induce any additional enhancement of the longitudinal relaxivity. As expected, the transverse relaxivity of these complexes depends on the square of the magnetic field and on the residence time of the coordinated water molecule (τ_M). Consequently, Dy-complexes with long τ_M are potential negative contrast agents at high magnetic fields because of their very efficient transverse relaxivity.

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