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Gd^{3+} chelates of interest in magnetic resonance imaging (MRI): studies using ^{17}O NMR and EPR at several magnetic fields

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Abstract

The combination of ^{17}O NMR relaxation rate and chemical shift measurements and EPR linewidth measurements at several magnetic fields provides a powerful probe of water exchange, rotational dynamics and electronic relaxation in aqueous solutions of Gd^{3+} complexes. This information is important for the understanding of the proton relaxivity of the complexes. Variable pressure measurements show a change of water exchange mechanism from associatively activated on $[Gd(H_2O)_8]^{3+}$ and $[Gd(PDTA)(H_2O)_2]^-$ to probably limiting dissociative on the MRI contrast agents $[Gd(DTPA)(H_2O)]^{2-}$, $[Gd(DOTA)(H_2O)]^-$ and $[Gd(DTPA\text{-}BMA)(H_2)O)]$. This leads to relatively slow water exchange rates on the latter three complexes. For $[Gd(DTPA\text{-}BMA)(H_2O)]$, the exchange rate is sufficiently slow to have an effect on the inner sphere proton relaxivity, and hence on the interpretation of NMRD profiles.

1. Introduction

Chelate complexes of Gd^{3+} with polyaminocarboxylate ligands are used as contrast agents in biomedical magnetic resonance imaging (MRI) because of their high proton relaxivity, a consequence of the high electron spin and relatively slow electronic relaxation of Gd^{3+} [1]. Proton relaxivity is determined by the structure and dynamics of the complexes and the surrounding water, and by the electronic relaxation of Gd^{3+} [2]. ^{17}O NMR can provide information on the water exchange rate, k_{ex}, between the inner coordination sphere and bulk water, the rotational correlation time, τ_c (often written τ_R), of the complex and the longitudinal electronic relaxation rate, $1/T_{1e}$. EPR linewidth measurements give the transverse electronic relaxation rate, $1/T_{2e}$. All these factors influence proton relaxivity and hence the contrast produced by the complexes.

^{17}O NMR and EPR measurements were first combined to measure water exchange rates on Gd^{3+} complexes in aqueous solution by Southwood-Jones *et al.* [3]. They found that water exchange is rapid on $[Gd(H_2O)_8]^{3+}$, $k_{ex}^{298} = (1.2 \pm 0.1) \times 10^9$ s^{-1}, and is about three times slower on $[Gd(PDTA)(H_2O)_2]^-$ (see Fig. 1 for the structure of $PDTA^{4-}$). This exchange rate decrease has been questioned, since it contrasts with the rate increase found for d-transition metal carboxylates [1]. The determination of k_{ex} depends on the separation of contributions from $1/T_{1e}$ and k_{ex} and so is influenced by the interpretation of the EPR results. We have made new EPR measurements, at a wider range of magnetic fields, for aqueous solutions of $[Gd(H_2O)_8]^{3+}$ and $[Gd(PDTA)(H_2O)_2]^-$ [4]. We found that a modified theoretical approach was necessary to explain the magnetic field dependence of the EPR linewidths. We also repeated the ^{17}O NMR measurements at high magnetic fields (4.7 T and 9.4 T) in order to better separate k_{ex} [5].

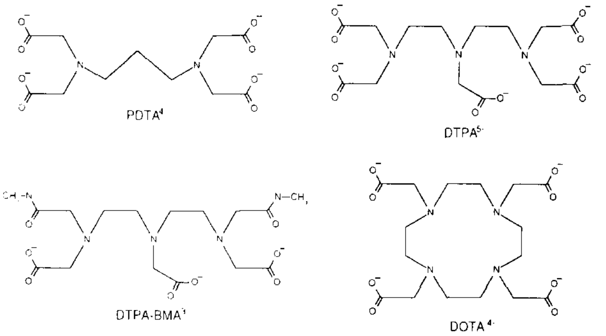

Fig. 1. The structure of the ligands $PDTA^{4-}$ (propylenediaminetetraacetate), $DTPA^{5-}$ (diethylenetriamine-pentaacetate), $DTPA\text{-}BMA^{3-}$ (diethylenetriamine-pentaacetate-bismethylamide) and $DOTA^{4-}$ (tetraazacyclododecane-tetraacetate).

The chelates $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist, Schering), $[Gd(DTPA\text{-}BMA)(H_2O)]$ (Omniscan, Ny-

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comed Imaging) and $[Gd(DOTA)(H_2O)]^-$ (Dotarem, Guerbet) are already used as MRI contrast agents (see Fig. 1 for the structures of the ligands). The presence of one inner sphere water molecule allows a strong relaxivity effect *via* rapid water exchange. We have made multiple field ^{17}O NMR measurements on aqueous solutions of these three chelates [6,7], and multiple field EPR measurements for $[Gd(DTPA\text{-}BMA)(H_2O)]$ [4]. We discuss the influence of the parameters obtained from these measurements on the proton relaxivity of the complexes.

The activation volume is an extremely useful parameter in the determination of the mechanism of solvent exchange reactions [8]. We, therefore, complemented our ^{17}O NMR studies with variable pressure measurements, and so determined the activation volumes for water exchange. We discuss these results in terms of the water exchange mechanism on the different complexes.

2. EPR Linewidth measurements

The EPR spectrum of Gd^{3+} in solution is, in general, a superposition of Lorentzian lines with different linewidths and intensities, centred at a Landé g-factor, $g_L = 2.0$ [9]. We recorded EPR spectra for solutions of $[Gd(H_2O)_8]^{3+}$, $[Gd(PDTA)(H_2O)_2]^-$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$ at four magnetic fields. The spectra were always Lorentzian, so that the peak-to-peak linewidth, ΔH_{pp}, is proportional to an overall transverse relaxation rate, $1/T_{2e}$ [10]. The temperature and magnetic field dependence of ΔH_{pp} is shown in Fig. 2.

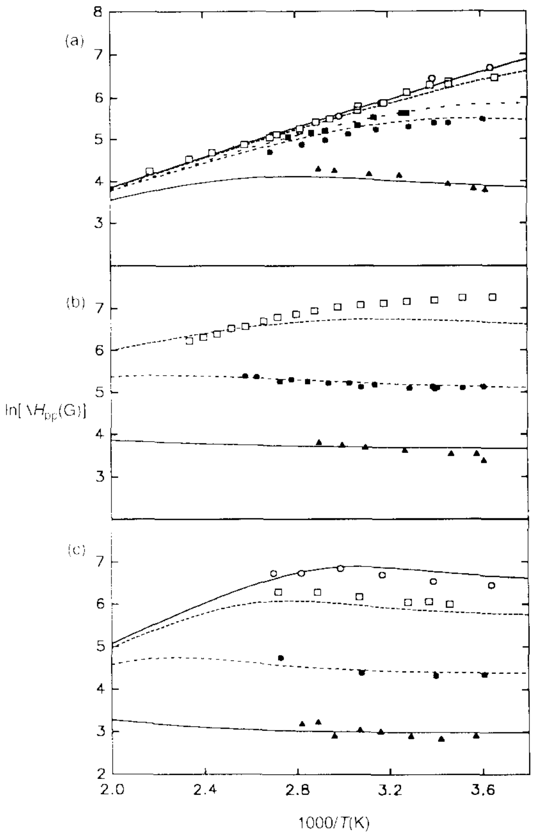

Fig. 2. (Taken from ref. 4.) Peak-to-peak EPR linewidths for (a) $[Gd(H_2O)_8]^{3+}$, (b) $[Gd(PDTA)(H_2O)_2]^-$ and (c) $[Gd(DTPA\text{-}BMA)(H_2O)]$ in aqueous solution, measured at (○) S-band (0.14 T), (□) X-band (0.34 T), (■) K-band (0.90 T)[14], (●) Q-band (1.2 T) and (▲) 2 mm band (5.0 T). The curves result from fits (three parameters per complex) of a theoretical model to the temperature dependence of the linewidth at the different fields (see text).

It has been proposed that the EPR linewidths of metal ions with spin $S > 1/2$ in solution are determined by a zero field splitting (ZFS) induced by distortions of the ligand field of the complexes [11,12]. Analytical expressions have been obtained for the mean electronic relaxation rates [13]. We found, however, that the mean transverse relaxation rate does not adequately describe the data in Fig. 2.

We used instead the formalism of Hudson and Lewis [9] to calculate the longitudinal and transverse relaxation matrices for Gd^{3+}. We diagonalised the matrices numerically to obtain four different longitudinal and transverse relaxation rates with different intensities. The longitudinal relaxation is dominated by one relaxation rate, and is described well by the mean longitudinal relaxation rate [13].

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

where Δ^2 is mean square ZFS energy, S is the electron spin and ω_s is the electron resonance frequency. The correlation time, τ_v for modulation of the ZFS may originate from rotation of the complex or from fluctuations of the distortion axis. For transverse relaxation, the four relaxation rates have comparable intensities for $\omega_s\tau_v \lesssim 1$. We calculated the inverse mean relaxation time, which, for $\omega_s\tau_v \lesssim 10$, gives a reasonable representation of the calculated overall spectrum and can be fitted empirically by

$$\frac{1}{\langle T_{2e}\rangle} = \Delta^2\tau_v\left[\frac{(5.26\pm0.53)}{1.0+(0.372\pm0.065)\omega_s^2\tau_v^2} + \frac{(7.18\pm0.85)}{1.0+(1.24\pm0.18)\omega_s\tau_v}\right] \tag{2}$$

We fitted eqn. (2) to ΔH_{pp} with Δ^2, τ_v^{298} and E_v as fitted parameters, where we assumed τ_v has an Arrhenius temperature dependence with activation energy E_v and value τ_v^{298} at 298.15 K. The fitted curves in Fig. 2

reproduce the observed field and temperature dependence for all three complexes. The deviation from the experimental points for $[Gd(PDTA)(H_2O)_2]^-$ at low field reflects the limitations of the approximations used. The fitted parameters allow the calculation of both transverse and longitudinal relaxation rates at different temperatures and magnetic fields.

3. ^{17}O NMR relaxation rate and chemical shift measurements

The temperature and magnetic field dependence of the reduced ^{17}O relaxation rates and chemical shift, $1/T_{1r}$, $1/T_{2r}$, and $\Delta\omega_r$, for $[Gd(H_2O)_8]^{3+}$ in aqueous solution is shown in Fig. 3. The relaxation is in the "fast exchange" regime, where the reduced relaxation rates and chemical shift are equal to those in bound water molecules. $1/T_{1r}$ is dominated by the dipole-dipole and quadrupolar relaxation mechanisms and, to a good approximation, is proportional to the rotational correlation time, τ_c. We assume that τ_c has an Arrhenius temperature dependence with activation energy E_c and value τ_c^{298} at 298.15 K. We fitted the longitudinal relaxation data (upper curve in Fig. 3) to obtain τ_c^{298} and E_c. $1/T_{2r}$ is dominated by the scalar interaction, which, to an excellent approximation, is proportional to the scalar coupling constant, $A/\hbar$ and a correlation time, $\tau_{s1} = 1/(1/T_{1e} + k_{ex})$. The magnetic field dependence of $1/T_{2r}$ originates from the $1/T_{1e}$ term (eqn. (1)). $\Delta\omega_r$ is directly proportional to the magnetic field and $A/\hbar$. We assume that the water exchange rate obeys the Eyring equation

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{\hbar} \exp\left(\frac{\Delta S^{\#}}{R} - \frac{\Delta H^{\#}}{RT}\right)$$
$$= \frac{k_{ex}^{298} T}{298.15} \exp\left\{\frac{\Delta H^{\#}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right\} \qquad (3)$$

where $\Delta S^{\#}$ and $\Delta H^{\#}$ are the entropy and enthalpy of activation for the exchange process and k_{ex}^{298} is the exchange rate at 298.15 K.

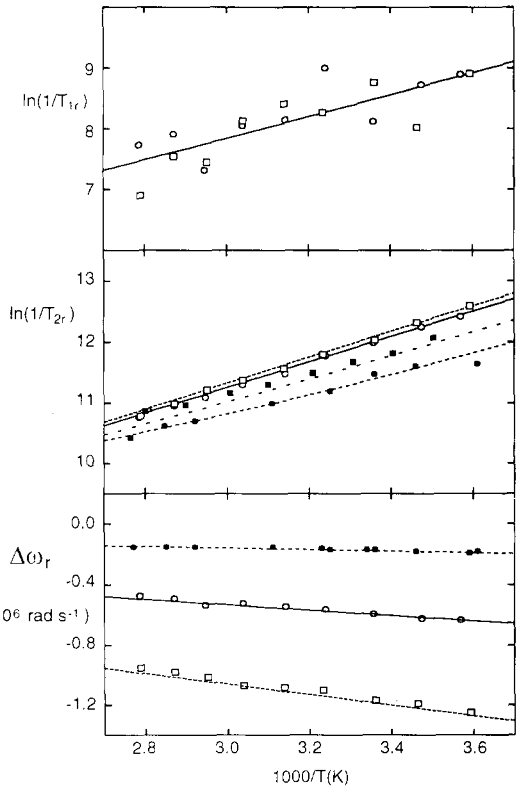

Fig. 3. Reduced ^{17}O relaxation rates for $[Gd(H_2O)_8]^{3+}$ in aqueous solution, measured at (●) 1.4 T, (■) 2.1 T [3], (○) 4.7 T and (□) 9.4 T. The curves result from a two-parameter fit ($1/T_{1r}$) and a three-parameter fit ($1/T_{2r}$ and ω_r simultaneously) of the temperature dependence of the data at the different fields (see text).

We fitted $1/T_{2r}$ and $\Delta\omega_r$, with Δ^2, τ_v^{298} and E_v fixed to the values obtained from our EPR measurements and $\Delta H^{\#}$, $\Delta S^{\#}$ (or k_{ex}^{298}) and $A/\hbar$ as fitted parameters (curves in Fig. 3). The quality of the fit, in particular the field dependence, shows excellent agreement with the parameters obtained from EPR. The contribution from $1/T_{1e}$ diminishes to less than 3% at 9.4 T, so that k_{ex} is well determined by these new high field measurements.

The pressure dependence (up to 200 MPa) of $\ln(1/T_{2r})$ at 9.4 T is linear within experimental error. The pressure dependence of k_{ex} is given by

$$\frac{1}{\tau_m} = k_{ex} = (k_{ex})_0^T \exp\left\{-\frac{\Delta V_0^{\#}}{RT}P + \frac{\Delta\beta^{\#}}{2RT}P^2\right\} \qquad (4)$$

where $(k_{ex})_0^T$ is the exchange rate and $\Delta V_0^{\#}$ is the activation volume at zero pressure and temperature T and $\Delta\beta^{\#}$ is the compressibility coefficient of activation (assumed to be zero). We fitted the results using eqn. (4) to obtain $(k_{ex})_0^T$ and $\Delta V_0^{\#}$.

We analysed the ^{17}O NMR data for the chelates in a similar way. For $[Gd(DTPA)(H_2O)]^{2-}$, $[Gd(DTPA\text{-}BMA)(H_2O)]$ and $[Gd(DOTA)(H_2O)]^-$, we observed a turnover between fast and slow exchange governed regions of $1/T_{2r}$, so that k_{ex} could be determined more easily. For $[Gd(PDTA)(H_2O)_2]^-$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$, we had to treat τ_v^{298} as a fitted parameter in order to fit $1/T_{2r}$. The τ_v^{298} values we obtained, however, were only just outside the errors of the EPR values, and we use the NMR values in the following discussion, since they are more precise. Multiple field EPR measurements have not been made for $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^-$. The values of Δ^2, τ_v^{298} and E_v for these two complexes were obtained solely from NMR measurements at two fields and so are prone to systematic error and will not be discussed here.

4. Results and discussion

Our results are summarised in Table 1. We consider first the water exchange kinetic parameters. The values of k_{ex}^{298} for $[Gd(H_2O)_8]^{3+}$ and $[Gd(PDTA)(H_2O)_2]^-$ differ by factors of 0.7 and 0.3 from the previous results [3]. These new values, obtained at high magnetic field, are more accurate, and reinforce the observation of an exchange rate decrease from $[Gd(H_2O)_8]^{3+}$ to $[Gd(PDTA)(H_2O)_2]^-$. The k_{ex}^{298} for $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^-$ are even lower (about 200 times lower than for $[Gd(H_2O)_8]^{3+}$), and that for $[Gd(DTPA\text{-}BMA)(H_2O)]$ is even lower again (about 2000 times lower than for $[Gd(H_2O)_8]^{3+}$). This rate decrease is accompanied by an increase of $\Delta H^{\#}$ and a change of sign of $\Delta S^{\#}$ and $\Delta V_0^{\#}$. The negative $\Delta V_0^{\#}$ values for $[Gd(H_2O)_8]^{3+}$ and $[Gd(PDTA)(H_2O)_2]^-$ indicate associatively activated water exchange. The large positive $\Delta V_0^{\#}$ values for the other three chelates indicate dissociatively activated water exchange. This can be understood if the $DTPA^{5-}$, $DTPA\text{-}BMA^{3-}$ and $DOTA^{4-}$ chelates can accommodate only one inner sphere water molecule. The incoming water molecule cannot participate in water exchange, which will have a dissociative activation mode, and probably a limiting, dissociative D mechanism. Without the participation of the incoming water molecule, more energy is required to break the bond between the outgoing water molecule and the highly charged Gd^{3+}, leading to the higher $\Delta H^{\#}$ and lower k_{ex}^{298} values.

The rotational correlation times, τ_c^{298}, show the expected progression, being longer for the large chelates than for $[Gd(H_2O)_8]^{3+}$. The ZFS modulation time, τ_v^{298}, is always shorter than τ_c^{298}. This suggests the model of a transient ZFS, modulated by fluctuations of the distortion axis rather than by rotation of the complexes.

We now consider the implications of the measured parameters for the proton relaxivity of the complexes, which is given by [2]

$$R_1 = \frac{q}{55.6}\,(T_{1m} + \tau_m^{H})^{-1} \tag{5}$$

where q is the number of inner sphere water molecules and τ_m^H is the residence time of protons in the inner sphere (τ_m for water molecules is an upper limit for τ_m^H). The proton relaxation rate in inner sphere water, $1/T_{1m}$, is dominated by the dipole-dipole mechanism

$$\frac{1}{T_{1m}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar\gamma_I^2\gamma_s^2}{r^6} S(S+1)\left[\frac{3\tau_{d1}}{1+\omega_I^2\tau_{d1}^2} + \frac{7\tau_{d2}}{1+\omega_s^2\tau_{d2}^2}\right] \tag{6}$$

[2] where γ_I and γ_s are the proton and electron gyromagnetic ratios, ω_I is the proton Larmor frequency, S is the electron spin, r is the Gd^{3+}-proton distance (we estimate $r \approx 0.31$ nm from neutron diffraction studies of Ln^{3+} aqua ions [15]) and $1/\tau_{di} = 1/\tau_c + 1/T_{ie} + 1/\tau_m^H$. We assume that τ_m^H can be approximated by the water residence time, $\tau_m = 1/k_{ex}$.

The values of k_{ex} and $1/\tau_c$, and the calculated frequency dependence of $1/T_{1m}$, $1/T_{1e}$ and $1/T_{2e}$ at 37 °C for $[Gd(H_2O)_8]^{3+}$, $[Gd(PDTA)(H_2O)_2]^-$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$ are illustrated in Fig. 4. In all three cases, $k_{ex} \ll 1/\tau_c$, so that τ_m^H does not influence τ_{di} and hence $1/T_{1m}$ *via* eqn. (6). The dominant contributions to τ_{di} are $1/\tau_c$, $1/T_{1e}$ and $1/T_{2e}$, electronic relaxation being important for $[Gd(PDTA)(H_2O)_2]^-$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$ at low fields. For $[Gd(H_2O)_8]^{3+}$ and $[Gd(PDTA)(H_2O)_2]^-$, $k_{ex} \gg 1/T_{1m}$, so that τ_m^H should not influence the relaxivity in eqn. (5). For $[Gd(DTPA\text{-}BMA)(H_2O)]$, on the other hand, k_{ex} is only four times greater than $1/T_{1m}$, so that τ_m^H will make a moderate contribution in eqn. (5) and will reduce the relaxivity by about 20%.

TABLE 1. Parameters obtained from least squares fits of (a) the ^{17}O NMR data and (b) the EPR data

	$[Gd(H_2O)_8]^{3+}$	$[Gd(PDTA)(H_2O)_2]^-$	$[Gd(DTPA)(H_2O)]^{2-}$	$[Gd(DTPA\text{-}BMA)(H_2O)]$	$[Gd(DOTA)(H_2O)]^-$
(a) k_{ex}^{298} (s^{-1})	$(8.30 \pm 0.95) \times 10^8$	$(1.02 \pm 0.10) \times 10^8$	$(4.1 \pm 0.3) \times 10^6$	$(4.3 \pm 0.2) \times 10^5$	$(4.8 \pm 0.4) \times 10^6$
(a) $\Delta H^{\#}$ (kJ mol^{-1})	14.9 ± 1.3	11.0 ± 1.4	52.0 ± 1.4	46.6 ± 1.3	48.8 ± 1.6
(a) $\Delta S^{\#}$ (J K^{-1} mol^{-1})	-24.1 ± 4.1	-54.6 ± 4.6	$+56.2 \pm 5.0$	$+18.9 \pm 4.0$	$+46.6 \pm 6.0$
(a) $\Delta V_0^{\#}$ (cm^3 mol^{-1})	-3.3 ± 0.2	-1.5 ± 0.5	$+12.5 \pm 0.2$	$+7.3 \pm 0.2$	$+10.5 \pm 0.2$
(a) $A/\hbar$ ($\times 10^6$ rad s^{-1})	-5.3 ± 0.2	-4.9 ± 0.2	-3.8 ± 0.2	-3.6 ± 0.3	-3.4 ± 0.3
(a) τ_c^{298} (s)	$(2.9 \pm 0.2) \times 10^{-11}$	$(7.9 \pm 0.3) \times 10^{-11}$	$(1.0 \pm 0.1) \times 10^{-10}$	$(1.67 \pm 0.05) \times 10^{-10}$	$(9.0 \pm 1.5) \times 10^{-11}$
(a) E_c (kJ mol^{-1})	15 ± 1.5	19 ± 1	18 ± 2	21.6 ± 0.1	17 ± 3
(b) Δ^2 ($\times 10^{20}$ s^{-2})	0.93 ± 0.04	0.80 ± 0.04		0.38 ± 0.02	
τ_V^{298} (s)	(b) $(7.2 \pm 0.7) \times 10^{-12}$	(a) $(1.6 \pm 0.2) \times 10^{-11}$		(a) $(3.4 \pm 0.8) \times 10^{-11}$	
E_V (kJ mol^{-1})	(b) 15 ± 1	(a) 10 ± 2		(a) 9 ± 2	

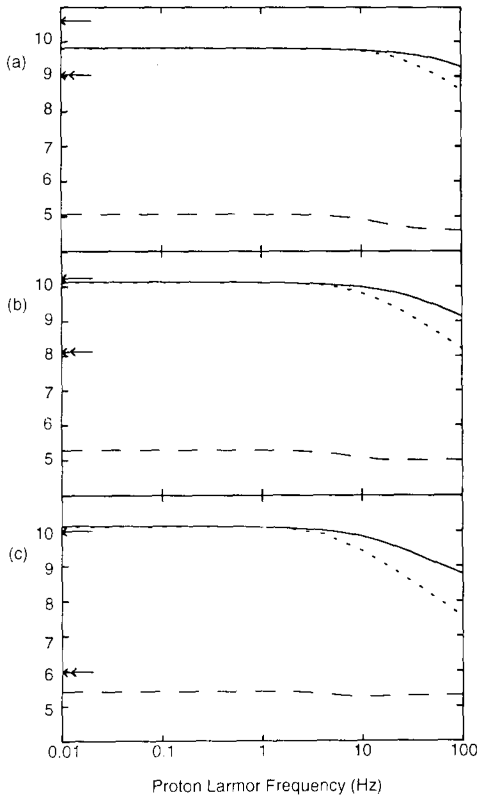

Fig. 4. Values of $\log(k_{ex})$ (double arrow) and $\log_{10}(1/\tau_c)$ (single arrow) and calculated frequency dependence of the electronic relaxation rates, $\log_{10}(1/T_{1e})$ (short dashed line) and $\log_{10}(1/T_{2e})$ (full line), and of the inner sphere proton longitudinal relaxation rates, $\log_{10}(1/T_{1m})$ (long dashed line), for (a) $[Gd(H_2O)_8]^{3+}$, (b) $[Gd(PDTA)(H_2O)_2]^-$ and (c) $[Gd(DTPA\text{-}BMA)(H_2O)]$ in aqueous solution at 37 °C.

From Fig. 4, one can see that $1/T_{1m}$ is similar for all the complexes. One can therefore estimate that, for the τ_m^H term in eqn. (5) to be negligible, τ_m^H must be shorter than 10^{-6} s (or $k_{ex} > 10^{-6}$ s^{-1}). Clearly this is the case for $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^-$, so that we do not expect the slow water exchange rates on these complexes to influence their proton relaxivity.

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