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Gadolinium-based linear polymer with temperature-independent proton relaxivities: a unique interplay between the water exchange and rotational contributions†‡

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ABSTRACT: Macromolecular complexes of Gd(III) chelates are widely investigated as MRI contrast agents. In addition to the potential increase in relaxivity, they have a further advantage over the Gd(III) chelates of an extended lifetime in the blood pool, which is necessary for magnetic resonance angiography applications. When designing macromolecular complexes of Gd(III) chelates, it is important to know how the parameters that determine relaxivity are affected in comparison with those of the chelate. This paper reports variable-temperature EPR, variable-temperature and -pressure, multiple field ^{17}O NMR and variable-temperature NMRD studies on a linear Gd(DTPA–bisamide)–poly(ethylene glycol) copolymer. The rate [$k_{ex}^{298} = (4.8 \pm 0.1) \times 10^5$ s^{-1}] and mechanism (dissociatively activated) of the water exchange are identical with those on the corresponding chelate. The rotational correlation time ($\tau_R = 232$ ps) is not much longer than that of the monomer unit restricted to rotate around a single axis, indicating large flexibility of the ethylene glycol chain. The proton relaxivities of the linear polymer complex are virtually independent of temperature, a result of an offset between the opposite dependences of the outer- and inner-sphere contributions with temperature.

KEYWORDS: NMR; ^{17}O NMR; EPR; NMRD; MRI contrast agents; macromolecular Gd(III) complexes

INTRODUCTION

Polymeric complexes of Gd(III) chelates are being investigated very actively as potential contrast agents for magnetic resonance imaging (MRI). One important reason for the popularity of these complexes is that a potentially much higher proton relaxivity can be obtained by slowing the rotational motion of the Gd(III) chelate by covalently binding it to a macromolecule. In addition to the potential increase in relaxivity, macromolecular contrast agents generally offer another advantage in comparison with chelates: an increased lifetime in the blood with less extravasation. Several approaches have been tested in recent years,[3–7] and Gd(III) complexes incorporated in a linear polymer chain represent one of them. However, the relaxivity gain obtained by increasing the size of the molecule was often far less than expected owing to a high internal flexibility of the macromolecules or a non-rigid attachment of the chelate to the macromolecule. In only a few cases could considerably higher relaxivities be attained.[3,7] For macrocyclic Gd(III) complexes, one important issue is how the rate of water exchange between the inner-coordination sphere of the Gd^{3+} ion and the bulk solvent and electronic relaxation rate, will be influenced when a Gd(III) chelate is attached to or incorporated in a polymer molecule.

In the last few years we have accumulated substantial knowledge on how small structural changes in monomer poly(aminocarboxylate) ligands affect the rate and mechanism of water exchange of the Gd(III) complex.[8–11] Although NMRD results have been reported for many polymeric Gd(III) complexes, exact water exchange rates have been measured only for a macrocyclic complex attached to different generations of a PAMAM dendrimer (NMRD = nuclear magnetic relaxation dispersion, which is the measurement of proton relaxation rate as a function of the magnetic field from 2.35×10^{-4} to 1.18 T). For the others, NMRD data were fitted with a ‘reasonable estimation’ of the value of the water exchange rate. Often, this ‘reasonable estimation’ is valid because the water exchange rate is large enough not to have any influence on the relaxivity whatsoever; this is especially true for macromolecular

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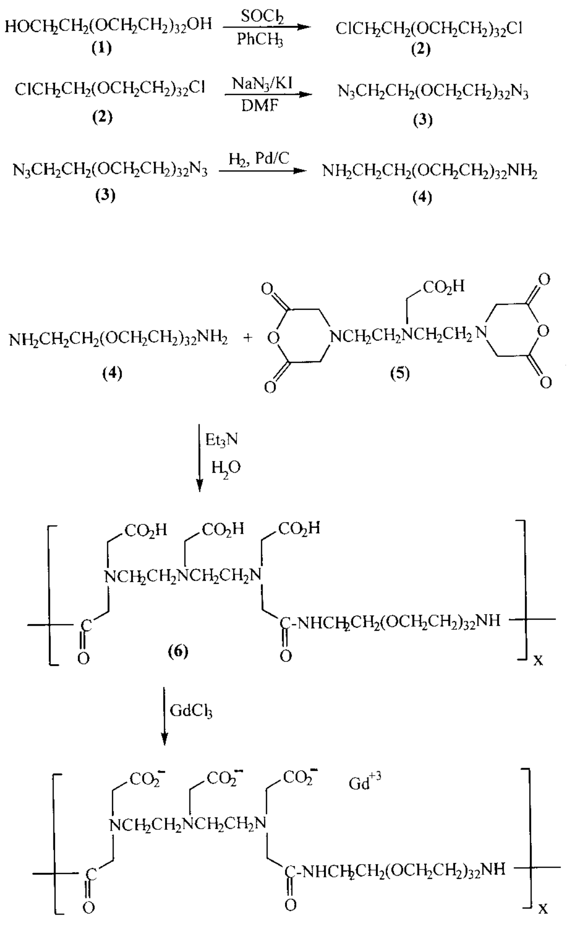

Scheme 1. Synthesis of α,ω-poly(ethylene glycol) diamine–DTPA copolymer and its Gd(III) complex.

systems with high flexibilities and low relaxivities. However, knowing the exact water exchange rate becomes critical for systems with long rotational correlation times, where relaxivity may be partially or, for very slowly rotating complexes, totally limited by water exchange.

The objective of the present work was to address how the water exchange rate is affected when a Gd(III) chelate is incorporated in a linear polymer chain. The polymeric Gd(DTPA–BA)–PEG complex [DTPA = 1,1,4,7,7-pentakis(carboxymethyl)-1,4,7-triazaheptane] that we chose for this study is a poly(ethylene glycol)-based polymer with DTPA-bisamide groups in the chain for complexing the Gd(III) [(DTPA–BA)–PEG = α,ω-poly(ethylene glycol) diamine–DTPA copolymer; for the structure, see **6** in Scheme 1]. Despite its large size, its relaxivity (per Gd^{3+} ion) is only a factor of about 1.5 higher than that of the similar monomeric Gd(III)–DTPA–bisamide chelate, owing to fast internal rotational motions. A variable-temperature NMRD, EPR and multiple-field ^{17}O NMR study was carried out for the Gd(DTPA–BA)–PEG complex in aqueous solution. The NMRD, EPR and ^{17}O NMR data were treated in a simultaneous, multi-parameter fit analysis which we introduced and tested so far only for monomer and dimer Gd(III) complexes. The mechanism of water exchange was also determined by variable pressure ^{17}O NMR measurements.

Experimental

Synthesis of the polymeric ligand and its Gd(III) complex

Polyoxyethylene bis(chloride) (2). To a solution of poly(ethylene glycol) (**1**, average molecular weight 145 000) (1000 g, 0.69 mol) in 1.5 l of toluene (60–80 °C) was added $SOCl_2$ (200 ml, 2.76 mol) and DMF (10 ml) dropwise over 10 min. The reaction mixture was heated on a steam-bath for 1 h. Analysis by TLC [CH_2Cl_2–MeOH; 6 : 1) indicated that a very small amount of starting material still remained. An additional 20 ml of $SOCl_2$ were added to the reaction and it was heated on the steam-bath for another 20 min to drive the reaction to completion. After cooling the reaction to 0 °C in an ice-bath, 1 M NaOH (2.5 l) was carefully introduced to neutralize the solution and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (3 × 1 l) and the combined CH_2Cl_2 layers were washed with water (2 × 1 l) and dried with $MgSO_4$. The solution was filtered and concentrated under reduced pressure to give a light yellow oil. The product was precipitated by adding TBME (*tert*-butyl methyl ether) (2 l) to the residue with cooling and stirring. The filtered product was dried in an oven under vacuum overnight to yield 941 g (94%) of the title compound as a white solid. Analysis: calculated for $C_{64}H_{128}O_{31}Cl_2$: C, 52.49; H, 8.81; Cl, 4.84. Found: C, 51.94; H, 8.43; Cl, 5.00%.

Polyoxyethylene bis(azide) (3). To a suspension of polyoxyethylene bis(chloride) (**2**, 500 g, 0.336 mol) and KI (139 g, 0.841 mol) in 1500 ml of DMF was added NaN_3 (109 g, 1.68 mol). The suspension was heated on a steam-bath at 70 °C for 12 h to give a yellowish solution. After cooling the reaction to room temperature, 2.5 l of water were added and the solution was extracted with CH_2Cl_2 (3 × 1 l). The combined CH_2Cl_2 layers were washed with water (3 × 1 l), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a light yellow oil. The product was precipitated by adding TBME (1 l) to the residue with cooling and stirring. The filtered product was dried in an oven under vacuum overnight at room temperature to yield 452 g (90%) of the PEG diazide as a white solid. Analysis: Calculated for $C_{64}H_{128}O_{31}N_6$: C, 52.09; H, 8.73; N, 5.69. Found: C, 52.50; H, 8.58; N, 5.11%.

Polyoxyethylene bis(amine) (4). To a solution of polyoxyethylene bis(azide) (**3**, 176 g, 0.117 mol) in 1 l of 1 M HCl was added Pd/C (17.6 g). The suspension was hydrogenated at 45 psi for 15 h. An aliquot withdrawn from the reaction indicated no more starting material by TLC [CH_2Cl_2–MeOH (4 : 1)] and the catalyst was removed by carefully filtering through a short plug of Celite. The filtrate was neutralized with 10% NaOH to pH 5–6 and extracted with CH_2Cl_2 (3 × 600 ml) to remove the impurities. The aqueous solution was neutralized with 35% NaOH to pH > 10 and extracted with CH_2Cl_2 (2 × 500 ml). The CH_2Cl_2 layer was washed with water (500 ml) and brine (500 ml) and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give a light yellow oil. The product was precipitated by adding TBME (1 l) to the residue with cooling and stirring. The filtered product was dried in an oven under vacuum at room temperature overnight to give 138 g (81% yield) of the title compound as a white solid. Analysis: Calculated for $C_{64}H_{132}O_{31}N_2$: C, 53.92; H, 9.33; N, 1.96. Found: C, 53.24; H, 9.35; N, 1.66%.

α,ω-Poly(ethylene glycol) diamine–DTPA copolymer (6) and its Gd(III) complex [Gd(DTPA–BA)–PEG]. Note: WFI (water for injection) was used for this entire process. To a solution of polyoxyethylene bis(amine) (**4**, 550 g, 0.380 mol) in 5500 ml of water was added triethylamine (159 ml, 1.14 mol) and DTPA dianhydride (**5**, 149 g, 0.418 mol). The suspension was stirred at room temperature and gave a clear solution after 10 min. The reaction mixture was stirred for an additional 50 min and a solution of $GdCl_3 . 6H_2O$ (156 g, 0.418 mol) in 2000 ml of water was added. The reaction mixture was checked with 4-(2-pyridylazo)resorcinol (PAR) to ensure that only a slightly excess of $GdCl_3 . 6H_2O$ was added. The complexed solution (pH 2) was neutralized to pH ≈ 5 with 10% NaOH and then diafiltered using a Pellicon diafiltration unit with a 10K cutoff filter for 10 turnovers. A 10% NaCl (USP grade) solution (filtered through a 0.22 μm filter) was used for the first four turnovers and water was used for the remaining six turnovers. At the end of the diafiltration, the solution was concentrated to half of its original volume (pH 7), filtered through a 0.22 μm filter and then lyophilized, leaving 532 g (80%) of a sponge-like product containing 7.09% (m/m) Gd with an average molecular weight (MW) of 20 200 (polydispersity = 1.63) and a relaxivity of 6.13 l mmol^{-1}.

Molecular weights and molecular weight distributions were obtained by size-exclusion HPLC, comparing the polymers with a set of PEG standards obtained from American Polymer Standards. A Perkin-Elmer Series 200 analytical HPLC system fitted with a series of three Supelco 30 cm × 7.8 mm i.d. size-exclusion columns (Progel-TSK G2000 SWXL, Progel-TSK G3000 SWXL and Progel-TSK G4000 SWXL) was employed. Detection effected with a refractive index detector. The method involved 20 μl injections of 1 mg ml^{-1} solutions of the polymer samples, a mobile phase of acetonitrile–phosphate buffer (pH 6.8) (30:70), a flow rate of 0.6 ml min^{-1} and a 60 min isocratic elution program. Turbochrome software was used to control the program and Turbogel software for MW analysis.

Elemental analysis (Galbraith Laboratories): calculated for $[C_{80}H_{152}GdN_5O_{40}]_x$: C, 48.50; H, 7.73; N, 3.53. Found (corrected for H_2O): C, 48.14; H, 7.91; N, 3.29%.

Sample preparation

For ^{17}O NMR measurements, Gd(DTPA–BA)–PEG solutions of 0.059 mol kg^{-1} (pH 5.1) and 0.016 mol kg^{-1} (pH 4.7) Gd(III) concentrations (for variable temperature) and 0.025 mol kg^{-1} (pH 5.0; for variable pressure) were prepared. To improve the sensitivity, ^{17}O-enriched water (10% $H_2^{17}O$, Yeda R&D) was added to the Gd complex solutions to yield a 2% ^{17}O enrichment. The EPR study was performed at a 0.025 mol kg^{-1} Gd(III) concentration. The absence of free Gd^{3+} ion in all solutions was verified by using xylenol orange indicator.[14] For the NMRD measurements a solution of 0.897 mmol l^{-1} Gd(III) concentration was used.

^{17}O NMR measurements

Transverse and longitudinal relaxation rates and chemical shifts were measured as a function of temperature at four different magnetic fields using Bruker spectrometers: AMX2-600, 14.1 T, 81.4 MHz; AM-400, 9.4 T, 54.2 MHz; a 1.41 T, 8.14 MHz and a 0.572 T, 3.3 MHz electromagnet connected to an AC-200 console. The technique used for the variable-temperature and -pressure ^{17}O NMR measurements has been described in detail previously.[15]

EPR measurements

The EPR spectra were recorded at the X-band (0.34 T) using a Bruker ESP 300E spectrometer. The spectrometer was operated in the continuous-wave mode. The samples were contained in 1 mm quartz tubes. The cavity temperature was stabilized using electronic temperature control of gas flowing through the cavity. The temperature was verified by substituting a thermometer for the sample tube. The peak-to-peak linewidth was measured from the recorded spectra using the instrument software.

NMRD

$1/T_1$ NMRD profiles were measured on a field-cycling relaxometer in the Department of Neurosurgery, New York Medical College (Valhalla, NY, USA). The specialized instrumentation used for the measurement, and the accuracy and reproducibility of the measurement have been described previously.[16,17]

Data analysis

Simultaneous least-squares fitting was performed with the program Scientist for Windows by Micromath, version 2.0. The reported errors in Table 1 correspond to one standard deviation obtained by the statistical analysis.

Theoretical

A detailed description of the treatment of EPR, ^{17}O NMR and NMRD data has been published recently.[13] However, to supplement the forthcoming discussion, it is convenient to review the theory of proton, ^{17}O and electronic relaxation. The theories are reviewed qualitatively below but, for quantitative purposes, all of the equations used in the fitting procedure can be found in the Appendix.

Variable-temperature EPR measurements

The overall transverse electronic relaxation rates, $1/T_{2e}$, were obtained from the measured peak-to-peak EPR linewidths of the derivative spectrum.[18] The electron spin relaxation rates for metal ions in solution with $S \geqslant \frac{1}{2}$ are mainly governed by a transient zero-field-splitting mechanism (ZFS), induced by distortions of the complex in solution. The ZFS contribution is characterized by two parameters: the mean-square ZFS energy (Δ^2) and the correlation time for the modulation of the ZFS (τ_v). Although it represents only a small contribution to the total relaxation, a magnetic field-independent spin rotation (SR) relaxation mechanism was also included in previous studies on Gd(III) complexes. It is quantified by the deviations from the free electron g_L value ($\delta g_L{}^2$).

Variable temperature ^{17}O NMR.

The reduced relaxation rates, $1/T_{1r}$ and $1/T_{2r}$, and chemical shifts, $\Delta\omega_r$, were calculated from the measured ^{17}O NMR relaxation rates and chemical shifts of the paramagnetic solutions and of the reference, acidified water. Outer-sphere contributions to the ^{17}O reduced relaxation rates are negligible, which is an advantageous feature of ^{17}O NMR.

The longitudinal ^{17}O relaxation rates in Gd^{3+} solutions are dominated by dipole–dipole and quadrupolar interactions and give direct access to the rotational correlation time of the Gd—O vector (τ_R). For transverse ^{17}O relaxation, the scalar interaction is the most

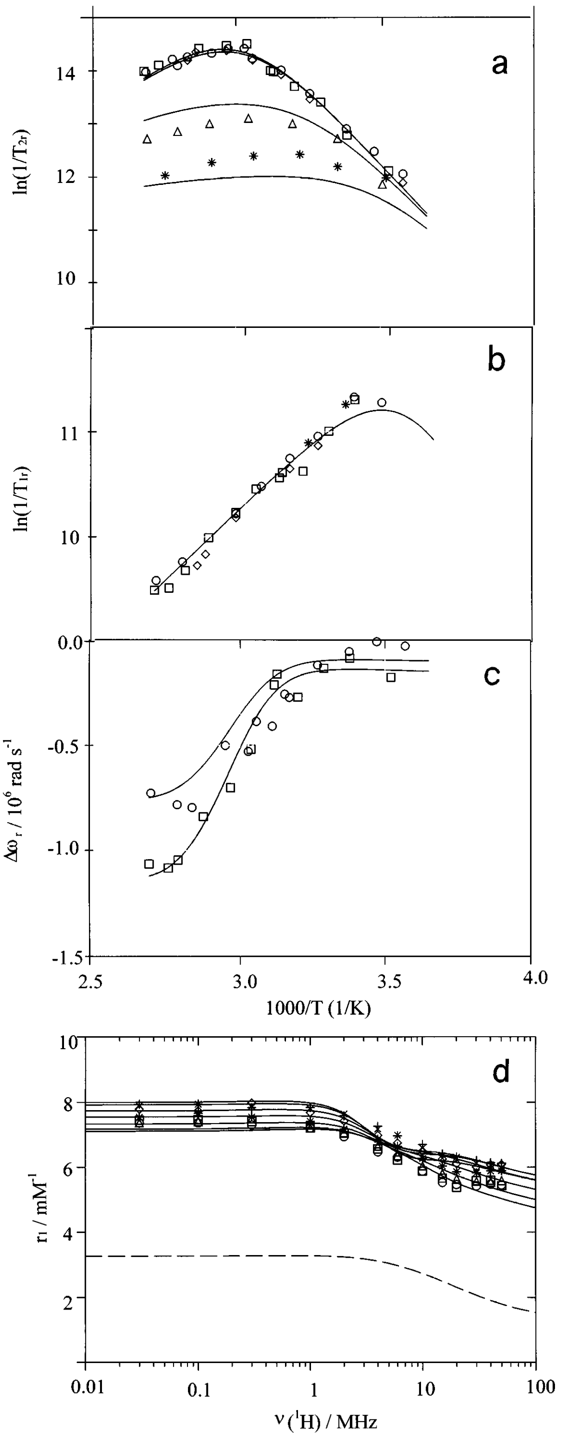

Figure 1. Temperature dependence of reduced transverse (a), longitudinal (b) ^{17}O relaxation rates and chemical shifts (c) at $B = 14.1$ T (□), 9.4 T, $c_{Gd} = 0.059$ M (○), 9.4 T, $c_{Gd} = 0.016$ M (◇), 1.41 T (△) and 0.572 T (∗); NMRD profiles (d) at 35 (□), 30 (○), 25 (△), 20 (∗), 15 (◇), 10 °C (+) and 5 °C (×). The lines represent the simultaneous least-squares fits to all data points as described in the text. The dashed line below the NMRD profiles shows the outer sphere contribution to proton relaxivity at 35 °C as calculated from the fitted parameters.

important contribution. The maxima observed in the temperature dependence of the $1/T_{2r}$ values [Fig. 1(a)] correspond to the change-over from the slow kinetic region at low temperatures ($1/T_{2r}$ is determined by the exchange rate, k_{ex}) to the fast exchange region at high temperatures ($1/T_{2r}$ is determined by transverse relaxation rate of the coordinated water oxygen, $1/T_{2m}$). From the temperature dependence of the binding time ($\tau_m = 1/k_{ex}$), the activation entropy and enthalpy of the exchange process and the exchange rate at 298.15 K (k_{ex}^{298}) can be calculated. The chemical shift of the coordinated water oxygen is determined by the hyperfine interaction between the Gd^{3+} electron spin and the ^{17}O nucleus, thus giving access to the value of the hyperfine or scalar coupling constant ($A/\hbar$). The outer-sphere contribution to the ^{17}O chemical shifts is quantified by an empirical constant, C_{os}.

NMRD

For Gd(III) complexes, the outer-sphere term is significant and the measured $1/T_1$ relaxation rate is a sum of inner- and outer-sphere contributions. The inner-sphere contribution, the relaxivity of the proton of the exchangeable water molecule bound in the inner coordination sphere of the Gd^{3+} ion, is influenced by correlation times involving rotation, water exchange and electronic relaxation, in addition to the number of water molecules bound in the inner coordination sphere of the Gd^{3+} ion and the distance of a bound water proton from the center of the ion. The outer-sphere contribution, which arises from the relative diffusional motions of unbound water molecules and the Gd^{3+} ion, is influenced by correlation times involving diffusional and electronic relaxation, as well as the distance of closest approach between a water proton and the center of the Gd^{3+} ion. The diffusional motion is characterized by the diffusion constant, D_{GdH}, and its activation energy, E_{GdH}. Both inner- and outer-sphere contributions have the electronic relaxation correlation times as common parameters. Although only $1/T_1$ NMRD profiles are measured, the $1/T_2$ NMRD profiles could be predicted, with accuracy, from an analysis of the $1/T_1$ NMRD profiles because both $1/T_1$ and $1/T_2$ are influenced by the same correlation times for proton relaxation.

Unified treatment of EPR, ^{17}O NMR and NMRD data

There are a large number of parameters that affect the experimental data obtained by each of the three techniques. EPR data represent the simplest case, since they are determined only by the electronic transverse relaxation rates. In ^{17}O NMR, electronic relaxation influences the transverse relaxation rates in the fast exchange region. If there is a well defined slow exchange region, the water exchange rate can be directly determined. In this case, the contributions from water exchange and electronic relaxation to the transverse relaxation rate in the fast exchange region are easily separated, and valu-

Table 1. Parameters obtained from the simultaneous fitting of EPR, ^{17}O NMR and NMRD data for Gd(III) complexes

Ligand/parameter	DTPA[a]	DTPA–BMA[a]	(DTPA–BA)–PEG
k_{ex}^{298} (10^6 s^{-1})	3.3 ± 0.2	0.45 ± 0.01	0.48 ± 0.01
$\Delta H^{\neq}$ (kJ mol^{-1})	51.6 ± 1.4	47.6 ± 1.1	47.0 ± 1.0
$\Delta S^{\neq}$ (J mol^{-1} K^{-1})	$+53 \pm 4.7$	$+22.9 \pm 3.6$	$+21.8 \pm 3$
$\Delta V^{\neq}$ (cm^3 mol^{-1})	$+12.5 \pm 0.2$	$+7.3 \pm 0.2$	$+9.2 \pm 0.2$
$A/\hbar$ (10^6 rad s^{-1})	-3.8 ± 0.2	-3.8 ± 0.2	-4.1 ± 0.3
C_{os}	0.18 ± 0.04	0.11 ± 0.04	0.10 ± 0.03
τ_R^{298} (ps)	58 ± 11	66 ± 11	232 ± 10
E_R (kJ mol^{-1})	17.3 ± 0.8	21.9 ± 0.5	22.2 ± 1.0
τ_v^{298} (ps)	25 ± 1	25 ± 1	29.6 ± 0.6
E_v (kJ mol^{-1})[b]	1.6 ± 1.8	3.9 ± 1.4	$\underline{3.9}$
Δ^2 (10^{20} s^{-2})	0.46 ± 0.02	0.41 ± 0.02	0.29 ± 0.01
$\delta g_L{}^2$ (10^{-2} F)	1.2 ± 0.3	0.8 ± 0.2	2 ± 0.1
D_{GdH}^{298} (10^{-10} m^2 s^{-1})	20 ± 3	23 ± 2	26 ± 1
E_{GdH} (kJ mol^{-1})	19.4 ± 1.8	12.9 ± 2.1	18.6 ± 0.1
$\chi(1 + \eta^{2/3})^{1/2}$ (MHz)[b]	$14 \pm 2/\underline{7.58}$	$18 \pm 2/\underline{7.58}$	$15.2 \pm 0.1/\underline{7.58}$
r_{GdO} (Å)[b]	$\underline{2.5}/2.2 \pm 0.09$	$\underline{2.5}/2.12 \pm 0.04$	$\underline{2.5}/2.16 \pm 0.03$

[a] Ref. 13.

[b] The underlined parameters were fixed in the least-squares fit (see text).

able information can also be obtained on electronic relaxation from ^{17}O NMR. Longitudinal ^{17}O relaxation rates are dominated by the dipole–dipole and quadrupolar relaxation mechanisms, for which only rotation determines the correlation time at the fields applied. On the other hand, rotation has no influence on transverse ^{17}O relaxation. As a consequence, most of the parameters can be well separated in analyzing ^{17}O NMR data.

It is not as straightforward to separate the contributions of rotation, water exchange and electronic relaxation to relaxivity, and an exact value for the water exchange rate cannot be obtained from NMRD data. Except for small Gd^{3+} chelates at physiological temperatures, it is necessary to determine independently the water exchange rate in order to fit NMRD profiles properly.[7] Because the outer-sphere contribution is significant, the separation of the inner- and outer-sphere contributions to the total relaxivity, when NMRD is treated separately, is often done by a stepwise iterative process involving the electronic relaxation times which are common to both the inner- and outer-sphere contributions,[20] or by subtracting the NMRD profile of a Gd^{3+} chelate containing no exchangeable water molecule in the inner-coordination sphere of the Gd^{3+} ion (only outer-sphere contribution) from the total relaxivity.

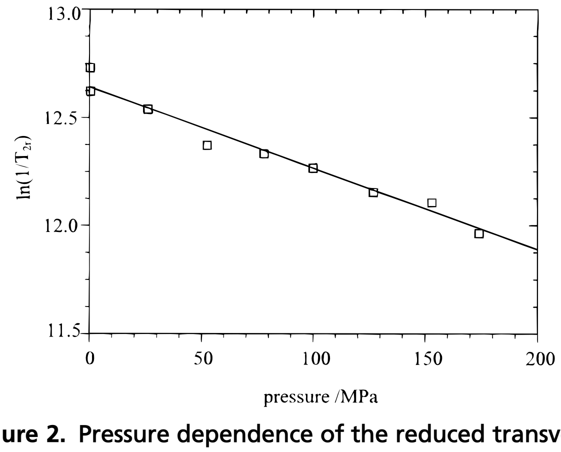

Figure 2. Pressure dependence of the reduced transverse ^{17}O relaxation rates for Gd(DTPA–BA)–PEG at $B = 9.4$ T and $T = 294$ K. The line shows the least-squares fit that results in an activation volume of $\Delta V^{\neq} = +(9.2 \pm 0.2)$ cm^3 mol^{-1}.

One can take advantage of the fact that there are several parameters that are common to two of the three techniques, and perform a global fit of all the experimental data with all the equations that describe their influence.[13] This certainly places additional constraints on the parameters which may decrease the 'quality' of the fits compared to separate fits. From a practical point of view, it is the NMRD data that are most relevant for MRI because the signal in MRI derives from the NMR signal of water protons. Consequently, one of the objectives of combining the three different techniques is to provide more insight into the correlation times that influence the relaxivity, which are difficult, or impossible, to obtain by the analysis of NMRD profiles alone.

Results

The reduced ^{17}O transverse and longitudinal relaxation rates and reduced chemical shifts, and the proton relaxivities for the polymeric Gd(III) complex are presented in Fig. 1. We performed a simultaneous least-squares fit of the NMRD, EPR and ^{17}O NMR data with the following fitted parameters: k_{ex}^{298} (or $\Delta S^{\neq}$), $\Delta H^{\neq}$, $A/\hbar$, C_{os}, τ_R^{298}, E_R, τ_v^{298}, Δ^2, δg^2, D_{GdH}^{298} and E_{GdH}. Furthermore, the data were fitted with either the quadrupolar coupling constant fixed [$\chi(1 + \eta^2/3)^{1/2} = 7.58$ MHz] and the Gd—O distance, r_{GdO}, adjustable, or with $\chi(1 + \eta^2/3)^{1/2}$ adjustable and r_{GdO} fixed (2.5 Å). The values of the transverse electronic relaxation rates, as measured by EPR at several temperatures between 0 and 90 °C, are in the range 7.1–8.6 10^9 s^{-1}, and did not show any temperature dependence. In the fits we fixed the activation energy for the modulation of the ZFS (E_v) at 3.9 kJ mol^{-1}, which is the value previously obtained for the Gd(DTPA–BMA) complex, since it was not sufficiently well defined by the experimental data (DTPA–BMA = 1,7-bis [(*N*-methylcarbamoyl)methyl] -1,4,7-tris-(carboxymethyl)-1,4,7-triazaheptane).[13] As expected, no concentration dependence was found for the reduced transverse and longitudinal ^{17}O relaxation rates and chemical shifts for the complex, thus the $1/T_{1r}$, $1/T_{2r}$ and Δw_r values measured at 9.4 T in solutions of different concentrations were fitted together. As in previous NMRD analysis, the distance of closest approach of a water proton to the Gd(III) centre, a_{GdH}, was fixed at 3.5 Å, and the inner sphere Gd—H distance, r_{GdH}, was fixed at 3.1 Å. The resulting curves are shown in Fig. 1 and the fitted parameters are given in Table 1.

In all previous ^{17}O NMR studies on Gd(III) complexes, the lowest magnetic field used was 1.41 T.[9,11,13] Here we have introduced an extremely low field for ^{17}O NMR (0.572 T; 3.3 MHz) which is already in the range of the NMRD measurements. By measuring ^{17}O transverse relaxation rates also at the fields of the proton relaxation data we were, first, hoping to understand better electronic relaxation, which is a field-dependent and common parameter for both techniques. Unfortunately, the quality of the ^{17}O $1/T_{2r}$ fit at low fields is not good (Fig. 1). This clearly shows that the theory of electronic relaxation as it is available for Gd(III) cannot accurately describe the field dependence when data in such a large field range are treated. The unsatisfactory agreement of experiment and theory of electronic relaxation for Gd(III) systems has already been noted in previous studies.[13] We have also performed a simultaneous fit of EPR, ^{17}O NMR and NMRD data without the ^{17}O transverse relaxation rates at the lowest field (0.572 T), and found that the kinetic (k_{ex}^{298}, $\Delta H^{\neq}$, $\Delta S^{\neq}$) and rotational parameters (τ_R^{298} and E_R) were unaffected.

Variable-pressure ^{17}O NMR

At the magnetic field and temperature chosen ($T = 294$ K), the system is in the slow exchange limit where $1/T_{2r}$ is virtually equal to $1/\tau_m$, hence the decrease in $1/T_{2r}$ with increasing pressure is due to the slowing of the water exchange. The pressure dependence of the reduced transverse ^{17}O relaxation rates, $1/T_{2r}$, is presented in Fig. 2. The results of the fit can be seen in Fig. 2 with the calculated value for the activation volume ($\Delta V^{\neq}$) in Table 1. The fitted value of the water exchange rate at zero pressure and at $T = 294$ K is $(k_{ex})_0{}^T = (3.1 \pm 0.2) \times 10^5$ s^{-1}.

Discussion

Structural information

The Gd(III) is complexed in the polymer by a DTPA–bisamide chelating unit; therefore, by analogy, we expect the coordination of the three nitrogens, three carboxylates and two amide groups to the metal, leaving space for one water molecule in the first coordination sphere. This is confirmed by the value of the ^{17}O scalar coupling constant, which is in the normal range found for Gd(III) complexes with one inner sphere water. The fitted value of the Gd—O distance (2.16 Å) is also very similar to that obtained for [Gd(DTPA–BMA)(H_2O)], which can be regarded as the monomer chelate unit of the polymeric Gd(DTPA–BA)–PEG complex.

In our analysis, we consider that each Gd^{3+} ion is coordinated by a bisamide ligand. On the base of the average molecular weight (20.2 kDa) and owing to the excess of **5** over **4** in the polymerization step (Scheme 1), one might expect that *ca.* 20% of the Gd(III) chelate is in monoamide–DTPA form at the end of the chains. Unfortunately, we have no data that would unambiguously identify the end groups of the polymer. However, since the polymerization reaction is carried out in water, the hydrolysis of **5** is competing with reaction with **4**. Therefore, excess **5** may not be present at the end of the reaction to form monoamides. Consequently, the assumption that all Gd^{3+} ions are chelated by a bisamide is justified.

Water exchange kinetics

Among the numerous Gd(III) chelates studied so far by ^{17}O NMR in order to determine water exchange rates, there has been only one macromolecular complex, which was a dendrimer-based potential contrast agent.[12] For this dendrimeric Gd(III) complex, the rate and the mechanism of the water exchange was virtually unchanged in comparison with the monomeric chelate. The parameters describing, for the first time, water exchange on a linear polymer complex, [Gd(DTPA–BA)(H_2O)]–PEG, are shown and compared with those of [Gd(DTPA–BMA)(H_2O)] and [Gd(DTPA)(H_2O)]$^-$ in Table 1. The exchange rate is the same as on the monomeric bisamide chelate, indicating that only the donor groups in the first coordination sphere have an influence on this parameter. Any changes outside the inner sphere of the metal, such as incorporation of the ligand into a linear polymer chain, do not affect water exchange. This is an important but not unexpected result, since previous studies on different bisamide derivatives of DTPA led to the same conclusion: the water exchange rate is independent of the nature of the group on the amide nitrogen. That this is also true for linear polymers is very useful for the efficiency in the design of future contrast agents; τ_M for the polymer-bound chelate is simply that of the monomeric chelate, so it is only necessary to measure τ_M for the monomeric chelate. As expected from the identical exchange rates, the mechanism of the water exchange also does not change compared with the monomeric chelate: for both the polymer complex and Gd(DTPA–BMA) the activation volumes indicate a dissociative activation mode.

The decrease in the exchange rate on amide derivatives as compared with the carboxylate complex can be rationalized by the decreased crowding of the first coordination sphere on substitution of carboxylates by amide donor groups. The amide oxygen, as a weaker donor for lanthanide(III) ions, is situated further from the metal center, which results in a less crowded inner sphere. All these complexes undergo a dissociatively activated water exchange, where steric crowding is of great importance: an increased crowding around the metal facilitates the leaving of an outgoing water molecule. As a consequence, carboxylate derivative complexes undergo a more rapid water exchange. On the basis of the numerous amide derivative Gd(IIII) complexes studied so far, we can generally state that the water exchange rate decreases proportionally to the number of carboxylates substituted. For the substitution of one carboxylate by an amide, the decrease is 3–4-fold; for the substitution of two carboxylates, it is about 10-fold.

Rotation

The rotational correlation time is determined by the longitudinal ^{17}O relaxation rates and by the ^{1}H NMRD data. Both methods have the same drawback as far as the absolute value of τ_R is concerned: one has to have a good estimation of the Gd—H distance (r_{GdH}; in NMRD) or the Gd—O distance (r_{GdO}; in ^{17}O NMR), both of which enter as the sixth power in the calculations, thus having a strong influence on the absolute value of τ_R. The exact Gd—H and Gd—O distances for a Gd(III) complex in solution are not known, and only assumptions can be made. The τ_R obtained from ^{17}O data also depends on the quadrupolar coupling constant $[\chi(1 + \eta^2/3)^{1/2}]$, which is also not easily accessible by independent methods. However, when the same Gd—O distance and coupling constant are used for a series of similar complexes, which is a reasonable approach, one can have a good comparison of the rotational correlation times as determined from oxygen longitudinal relaxation rates. ^{17}O NMR has another advantage over NMRD: as mentioned before, outer sphere contributions do not need to be accounted for and τ_R is obtained directly from the experimental data. However, owing to the practical importance of NMRD data for MRI, contrast agents are generally characterized by the rotational correlation times as obtained from NMRD. In the simultaneous treatment of the NMRD and ^{17}O NMR data, a single τ_R is fitted which we attribute to the rotation of the Gd—water vector. Since the motion of the Gd chelate in the polymer chain is anisotropic, this value represents an average as sensed locally by the protons and the oxygen of the coordinated water. There is very often a discrepancy between the τ_R values obtained in separate fits of ^{17}O and ^{1}H NMR data due to the use of the incompatible r_{GdH} and r_{GdO} values. In the simultaneous treatment, we allow for this by fitting either the Gd—water oxygen distance or the quadrupolar coupling constant. The value of the Gd–O distance fitted in this way (2.16 Å) is shorter than that fixed previously in ^{17}O NMR studies (2.5 Å; obtained from neutron diffraction measurements of lanthanide aqua ions in solution). The Gd—water oxygen distances determined by x-ray diffraction for solid Gd(III)–poly(aminocarboxylate) complexes with one inner sphere water are all also shorter than 2.5 Å, so the value that we obtain in the simultaneous fit is more reasonable. The fitted value of the quadrupolar coupling constant (while r_{GdO} is fixed at 2.5 Å) is considerably higher than that of the pure water, which is also reasonable according to a recent study on paramagnetic complexes.[21] In reality, probably both r_{GdO} and the coupling constant deviate from their values of 2.5 Å and 7.58 MHz, respectively.

The rotational correlation time obtained in the simultaneous fit for the [Gd(DTPA–BA)(H_2O)]–PEG polymer is 232 ps, not much longer than the value for a monomeric Gd chelate, e.g. [Gd(DTPA–BMA)(H_2O)] ($\tau_R = 66$ ps), restricted to rotate about a single axis ($\tau_R = 198$ ps). This means that the poly(ethylene glycol) chain has almost no additional rigidity that could increase τ_R.

Effect of rotation and water exchange kinetics on relaxation behavior

For the linear polymeric Gd(III) complex used in this study, the relaxivities at all field strengths are virtually independent of temperature in the range 5–35 °C. This is particularly interesting at high fields (corresponding to proton Larmor frequencies of 10 MHz and higher), which is most relevant for MR imaging, where τ_R is the dominating correlation time for Gd(III) complexes. Both τ_R and τ_M contribute only to the inner-sphere contribution. A decrease in temperature causes an increase in τ_R, and thus an increase in the relaxivity, provided that the T_{1m}^{H} of the water proton bound in the inner coordination sphere of the Gd^{3+} ion is larger than τ_M. This is true, e.g., for $Gd(DTPA)^{2-}$, where the relaxivities at 5 °C are higher than at 35 °C by a factor of about two; the increase represents the increase in τ_R (which is proportional to the ratio of the viscosity to absolute temperature) corresponding to this temperature difference. However, for Gd(DTPA–BMA) the relaxivities are only slightly higher at 5 °C than at 35 °C. Although τ_R is increased to the same extent as for $Gd(DTPA)^{2-}$, the value of τ_M (which also increases with decreasing temperature) is much longer, and it is so large that it limits the relaxivities.[20] The slight temperature dependence of the relaxivities for Gd(DTPA–

BMA) and the temperature independence of the relaxivities for the linear polymer of this study are two features whose differences arise from differences in their inner-sphere contributions. The outer-sphere contribution is independent of rotational motion and should consequently have relatively equal contributions for both the linear polymer and Gd(DTPA–BMA). Because the outer-sphere relaxivity depends only on diffusional motion (at high fields), and is independent of τ_M, it increases with decreasing temperature. In contrast, the inner-sphere relaxivity can decrease with decreasing temperature if τ_M is long enough, as it is for Gd(DTPA–BMA). It is possible, then, that the opposite changes of the inner- and outer-sphere relaxivities with temperature can be offset exactly, leading to temperature independent total relaxivities; this is the case for the linear polymer used in this study. The reason for the temperature independent relaxivities for this linear polymer, whereas they are temperature-dependent for Gd(DTPA–BMA), is the larger τ_R of the polymer. Since the values of τ_M are equal for both of them, and the outer-sphere contributions are very similar, the larger τ_R results in a larger inner-sphere contribution at 35 °C for the polymer, which leads to its larger relaxivities. At this temperature τ_M is shortest and has the least influence on the relaxivities. When the temperature is lowered, both τ_R and τ_M increase, and at a certain temperature the inner-sphere contribution becomes significantly limited by τ_M. Consequently, the relaxivities for Gd(DTPA–BMA) have an opportunity to increase (although slightly) on decreasing the temperature below 35 °C before they become limited by τ_M. However, for the linear polymer (slower rotation), r_1 does not become significantly greater on lowering the temperature because its relaxivities at 35 °C, a temperature where exchange limitations are not significant, are only slightly higher than those of Gd(DTPA–BMA) at 5 °C, a temperature where exchange limitations are severe. The value of τ_R at 35 °C is too large in comparison with that of Gd(DTPA–BMA), and the increase in τ_M with decreasing temperature below 35 °C [although the same as that for Gd(DTPA–BMA)] is too large to provide an opportunity for the relaxivities of the linear polymer to increase with decreasing temperature. It is interesting that when a more slowly rotating polymer is synthesized that still has the Gd(DTPA–BMA)-type chelate (thus the same τ_M), the relaxivities can decrease with decreasing temperature, since the inner-sphere contribution will be higher at 35 °C. Indeed, this behavior has been reported for rigid linear Gd(III)–DTPA–bisamide–alkyl copolymers.[7]

Conclusion

The rate and mechanism of water exchange between the inner coordination sphere of the Gd^{3+} ion and the bulk for Gd(III) poly(aminocarboxylate) chelates are unaffected when incorporated into linear polymer chains. This is an important finding relevant to the efficiency of the design and synthesis of MRI contrast agents; the macromolecular complexes of the chelate do not have to be synthesized before the necessary water exchange information can be obtained. The rotational correlation time of the Gd(DTPA–BA)–PEG polymer is only about that of its monomer chelate unit restricted to rotate around a single axis, owing to the internal flexibility of the poly(ethylene glycol) moieties. Consequently, the relaxivities of this polymer are only slightly greater than that of Gd(DTPA–BMA).

An interesting feature of the Gd(DTPA–BA)–PEG polymer is that its relaxivities are virtually independent of temperature at all field strengths. This is a result of a combination of slow water exchange and a rotational correlation time that is slightly higher than that of Gd(DTPA–BMA), which causes the inner-sphere contribution to decrease with decreasing temperature to an extent that exactly offsets the increase of the outer-sphere contribution with decreasing temperature. The temperature independence of its proton relaxivities makes this compound a good candidate for use as a standard to calibrate MRI signal intensities and T_1 values. Provided that the T_1 of the standard is not so long that the temperature dependence of the solvent makes a significant contribution, the T_1 of the standard will not change with positioning from the patient, where large ranges in temperature (body temperature to room temperature) are possible.

Supporting Information

The following supplementary material is available from the corresponding author: reduced transverse and longitudinal ^{17}O relaxation rates and chemical shifts as a function of temperature (Tables S1–S5); proton relaxivities as a function of the magnetic field (Table S6), variable temperature transverse electronic relaxation rates (Table S7) and reduced transverse ^{17}O relaxation rates as a function of pressure (Table S8) (eight pages).

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APPENDIX

We give here all the equations that are used in the treatment of EPR, ^{17}O NMR and NMRD data.

Electronic relaxation

The ZFS terms can be expressed by Eqns (A1) and (A2),[A1,A2] where Δ^2 is the trace of the square of the zero-field-splitting tensor, τ_v is the correlation time for the modulation of the ZFS with the activation energy E_v and ω_s is the Larmor frequency of the Gd^{3+} electron spin:

$$\left(\frac{1}{T_{1e}}\right)^{\mathrm{ZFS}} = \frac{1}{25}\Delta^2\tau_v[4S(S+1)-3] \times\left(\frac{1}{1+\omega_S{}^2\tau_v{}^2}+\frac{4}{1+4\omega_S{}^2\tau_v{}^2}\right) \quad \text{(A1)}$$

$$\left(\frac{1}{T_{2e}}\right)^{\mathrm{ZFS}} = \Delta^2\tau_v\left(\frac{5.26}{1+0.372\omega_S{}^2\tau_v{}^2}+\frac{7.18}{1+1.24\omega_S\tau_v}\right) \quad \text{(A2)}$$

$$\tau_v = \tau_v^{298}\exp\left[\frac{E_v}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right] \quad \text{(A3)}$$

The contribution arising from spin rotation is given by Eqn (A4),[19] where $\delta g_L{}^2$ is the deviation from the free electron g_L value and τ_R is the rotational correlation time:

$$\left(\frac{1}{T_{ie}}\right)^{\mathrm{SR}} = \frac{\delta g_L{}^2}{9\tau_R} \qquad i = 1, 2 \quad \text{(A4)}$$

$$\tau_R = \tau_R^{298}\exp\left[\frac{E_R}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right] \quad \text{(A5)}$$

Oxygen-17 NMR

From the measured ^{17}O NMR relaxation rates and angular frequencies of the paramagnetic solutions, $1/T_1$, $1/T_2$ and ω, and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and ω_A, one can calculate the reduced relaxation rates and chemical shift, $1/T_{1r}$, $1/T_{2r}$ and $\Delta\omega_r$, which may be written as in Eqns (A6)–(A8),[A3] where $1/T_{1m}$, $1/T_{2m}$ are the relaxation rates of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water.

$$\frac{1}{T_{1r}} = \frac{1}{P_m}\left(\frac{1}{T_1}-\frac{1}{T_{1A}}\right) = \frac{1}{T_{1m}+\tau_m} \quad \text{(A6)}$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left(\frac{1}{T_2}-\frac{1}{T_{2A}}\right) = \frac{1}{\tau_m}\left[\frac{T_{2m}^{-2}+\tau_m{}^{-1}T_{2m}^{-1}+\Delta\omega_m{}^2}{(\tau_m^{-1}+T_{2m}^{-1})^2+\Delta\omega_m{}^2}\right] \quad \text{(A7)}$$

$$\Delta\omega_r = \frac{1}{P_m}(\omega-\omega_A) = \frac{\Delta\omega_m}{(1+\tau_m T_{2m}^{-1})^2+\tau_m{}^2\Delta\omega_m{}^2}+\Delta\omega_{os} \quad \text{(A8)}$$

$\Delta\omega_m$ is determined by the scalar coupling constant, $A/\hbar$, according to Eqn (A9), where B represents the magnetic field.

$$\Delta\omega_m = \frac{g_L\mu_B S(S+1)B}{3k_B T}\frac{A}{\hbar} \quad \text{(A9)}$$

The outer-sphere contribution to the ^{17}O chemical shift is proportional to $\Delta\omega_m$, where C_{os} is an empirical constant:

$$\Delta\omega_{os} = C_{os}\Delta\omega_m \quad \text{(A10)}$$

The ^{17}O longitudinal relaxation rates are given by Eqn (A11),[A4,A5] where γ_S is the electron and γ_I is the nuclear gyromagnetic ratio ($\gamma_S = 1.76\times10^{11}$ rad s^{-1} T^{-1}, $\gamma_I = -3.626\times10^7$ rad s^{-1} T^{-1}), r is the effective distance between the electron charge and the ^{17}O nucleus, I is the nuclear spin ($\frac{5}{2}$ for ^{17}O), χ is the quadrupolar coupling constant and η is an asymmetry parameter:

$$\frac{1}{T_{1m}} = \left[\frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_I{}^2\gamma_S{}^2}{r_{\mathrm{GdO}}^6}S(S+1)\right]\times\left(6\tau_{d1}+14\frac{\tau_{d2}}{1+\omega_S{}^2\tau_{d2}^2}\right)+\frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}\chi^2(1+\eta^2/3)\tau_R \quad \text{(A11)}$$

In the transverse relaxation the scalar contribution, $1/T_{2sc}$, is the most important one [eqn (A12)]. In Eqn (A12), $1/\tau_{sj}$ is the sum of the exchange rate constant and the electron spin relaxation rate.

$$\frac{1}{T_{2m}} \approx \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\tau_{s1} + \frac{\tau_{s2}}{1+\omega_s{}^2\tau_{s2}^2}\right) \quad \text{(A12)}$$

$$\frac{1}{\tau_{sj}} = \frac{1}{\tau_m} + \frac{1}{T_{je}} \qquad j = 1, 2$$

The binding time (or exchange rate, k_{ex}) of water molecules in the inner sphere is assumed to obey the Eyring equation [Eqn (A13)], where $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are the entropy and enthalpy of activation for the exchange process and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\begin{aligned}\frac{1}{\tau_m} &= k_{ex}\\ &= \frac{k_B T}{h}\exp\left(\frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{RT}\right)\\ &= \frac{k_{ex}^{298}T}{298.15}\exp\left[\frac{\Delta H^{\neq}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \quad \text{(A13)}\end{aligned}$$

The pressure dependence of $\ln(k_{ex})$ is linear [Eqn (A14)], where $\Delta V^{\neq}$ is the activation volume and $(k_{ex})_0{}^T$ is the water exchange rate at zero pressure and temperature T.

$$\frac{1}{\tau_m} = k_{ex} = (k_{ex})_0{}^T \exp\left(-\frac{\Delta V^{\neq}}{RT}P\right) \quad \text{(A14)}$$

NMRD

The measured proton relaxivities [normalized to 1 mM Gd(III) concentration] contain both inner sphere and outer sphere contributions:

$$r_1 = r_{1is} + r_{1os} \quad \text{(A15)}$$

The inner sphere term is given by Eqn (A16), where q is the number of inner sphere water molecules.

$$r_{1is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^{H} + \tau_m} \quad \text{(A16)}$$

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}{}^{H}$ can be expressed as in Eqn (A17):[A6,A7]

$$\frac{1}{T_{1m}{}^{H}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_S{}^2\gamma_I{}^2}{r_{GdH}^6}S(S+1) \times \left(\frac{3\tau_{d1}}{1+\omega_I{}^2\tau_{d1}^2} + \frac{7\tau_{d2}}{1+\omega_S{}^2\tau_{d2}^2}\right) \quad \text{(A17)}$$

where r_{GdH} is the effective distance between the Gd(III) electron spin and the water protons, ω_I is the proton resonance frequency and τ_{di} is given by

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m} + \frac{1}{\tau_R} + \frac{1}{T_{ie}} \qquad i = 1, 2 \quad \text{(A18)}$$

The outer-sphere contribution can be described by Eqn (A19),[A8,A9] where N_A is the Avogadro constant and J_{os} is a spectral density function.

$$r_{1os} = \frac{32N_A\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_S{}^2\gamma_I{}^2}{a_{GdH}D_{GdH}}S(S+1) \times [3J_{os}(\omega_I, T_{1e}) + 7J_{os}(\omega_S, T_{2e})] \quad \text{(A19)}$$

$$J_{os}(\omega, T_{je}) = \mathrm{Re}\left[\frac{1 + \frac{1}{4}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{1/2}}{1 + \left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{1/2} + \frac{4}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right) + \frac{1}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right)^{3/2}}\right] \qquad j = 1, 2 \quad \text{(A20)}$$

For the temperature dependence of the diffusion coefficient for the diffusion of a water proton away from a Gd(III) complex, D_{GdH}, we assume an exponential temperature dependence, with an activation energy E_{DGdH}:

$$D_{GdH} = D_{GdH}^{298}\exp\left[\frac{E_{DGdH}}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right] \quad \text{(A21)}$$

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