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Tuning water-exchange rates on (carboxymethyl)iminobis-(ethylenenitrilo)tetraacetate (dtpa)-type gadolinium(III) complexes†

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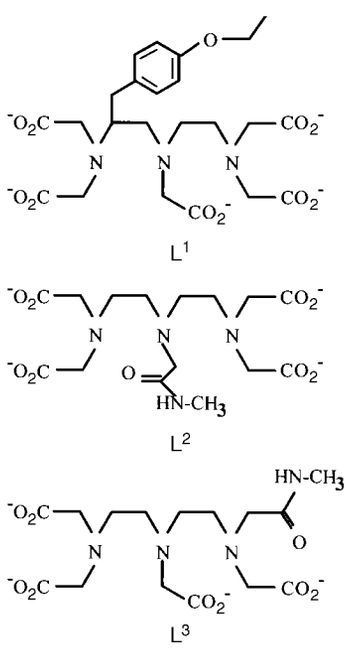
A variable-temperature and -pressure, multiple-field ^{17}O NMR study has been performed on the gadolinium(III) complexes of an ethoxybenzyl (L^1) and symmetric (L^2) and asymmetric (L^3) mono(methylamide) derivatives of (carboxymethyl)iminobis(ethylenenitrilo)tetraacetate (dtpa) in order to study water exchange and rotational dynamics. Electronic relaxation parameters were obtained from EPR measurements. The water-exchange rates on the $[GdL^2(H_2O)]^-$ and $[GdL^3(H_2O)]^-$ complexes [$k_{ex}^{298} = (1.9 \pm 0.1) \times 10^6$ and $(1.3 \pm 0.1) \times 10^6$ s^{-1}] are smaller than that observed for $[Gd(dtpa)(H_2O)]^{2-}$; that of the ethoxybenzyl derivative $[GdL^1(H_2O)]^-$ is $k_{ex}^{298} = (3.6 \pm 0.1) \times 10^6$ s^{-1}. High positive activation volumes have been obtained for all three complexes studied ($\Delta V^{\ddagger} = 10.6$–$12.7$ cm^3 mol^{-1}), indicating dissociatively activated water exchange. As a general rule, when amide groups substitute for carboxylates in gadolinium(III) polyaminopolycarboxylate complexes, the water-exchange rate is decreased by about a factor of 4 per substituted carboxylate, but the mechanism of the process is not affected. However, no influence on the water exchange is observed as a result of the introduction of large groups on the carbon backbone of the ligand, outside the first co-ordination sphere.

Highly stable lanthanide complexes formed with polyaminopolycarboxylate ligands are of practical importance in biology and in medicine.[2] One of the fields where the practical applications are particularly important is magnetic resonance imaging (MRI). In MRI paramagnetic metal complexes are used to increase the image contrast.[3,4] The first clinically utilised contrast enhancement agent was $[Gd(dtpa)]^{2-}$, which distributes in the extracellular space and significantly increases proton relaxation rates [H_5dtpa = (carboxymethyl)iminobis(ethylenenitrilo)-tetraacetic acid].

In recent years a lot of effort has been directed towards the development of non-ionic contrast agents, since $[Gd(dtpa)]^{2-}$, administered intravenously, results in undesired side effects due to relatively high osmotic pressure. A clinically used non-ionic contrast agent is [GdL′] {H_3L' = *N*′-carboxymethyl-*N*,*N*″-bis-[(*N*-methylcarbamoyl)methyl]iminobis(ethyleneimino)diacetic acid}.[5] The stability constant of [GdL′] is lower than that of $[Gd(dtpa)]^{2-}$, but due to the high selectivity of the ligand for Gd^{3+} against the essential trace elements (*e.g.* Zn^{2+}), [GdL′] is a safe contrast agent.[6]

Another important aspect is the synthesis and study of organ-specific contrast agents which could be used in MRI more safely at lower concentrations. The weak lipophilic character of $[Gd(dtpa)]^{2-}$ has been increased with the attachment of an ethoxybenzyl group to $dtpa^{5-}$ to give L^1. The complex formed $[GdL^1]^{2-}$ shows both renal and hepatobiliary excretion which makes it a potential liver-specific contrast agent.[7]

Gadolinium(III) complexes as potential contrast agents must have a high thermodynamic and kinetic stability and high proton relaxivity. The term relaxivity is used to characterise the ability of the complex to enhance the proton relaxation rate in aqueous solution per unit concentration (mmol dm^{-3}). In discussing the relaxation effect of a gadolinium(III) complex the relaxivity can be divided into two parts: (1) outer-sphere relaxivity, resulting from long-range interactions between Gd^{3+} and bulk water, and (2) inner-sphere relaxivity due to short-range interactions with inner-sphere water molecule(s), mediated to the bulk by chemical exchange of water. The inner-sphere contribution to total relaxivity depends on the number of water molecules in the inner sphere, on the proton-exchange rate, on the rotational correlation time and on the electronic relaxation rates. The proton-exchange rate can be taken as identical to the exchange rate of the entire water molecules, at least around physiological pH, as was confirmed recently for several gadolinium(III) complexes.[8–10] In order to ensure a high thermodynamic and kinetic stability all the ligands in the commercially available contrast agents are octadentate, and in the inner sphere of Gd^{3+} there is only one water molecule.



Scheme 1

† High-pressure NMR kinetics. Part 80. Part 79 is ref. 1.
Supplementary data available (No. SUP 57227, 19 pp.): variable temperature (or pressure) relaxation rates, chemical shifts, EPR parameters and UV/VIS spectra. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1997, Issue 1.

The water exchange of the bis(amide) derivative [GdL′-(H_2O)], studied by ^{17}O NMR spectroscopy, has been found to be 10 times slower than that of $[Gd(dtpa)(H_2O)]^{2-}$.[8,11] In order to get more information on the role of the amide group in water-exchange kinetics we have chosen two mono(methylamide) derivatives of $dtpa^{5-}$: in the symmetric L^2 the amide group is attached to the central nitrogen atom, while in the asymmetric L^3 it is on a terminal nitrogen.

The lipophilic complex $[GdL^1]^{2-}$ exhibits a higher proton relaxivity than its hydrophilic analogue $[Gd(dtpa)]^{2-}$, which may be the consequence of slower tumbling due to the bulky ethoxybenzyl group. Furthermore, it is not known what effects large substituents on the carbon chain will have on water-exchange rates. A previous study on gadolinium(III) complexes of different sugar-based bis(amide) derivatives of dtpa indicated that the water-exchange rate is not significantly influenced by the ligand structure outside the inner co-ordination sphere. However, these ligands had different substituents on the amide nitrogens, and not on a carbon.[12]

It has become increasingly important to know how different modifications in the ligand structure influence the water-exchange rate of the gadolinium(III) complex. New-generation MRI contrast agents will probably be complexes attached to, or incorporated in, macromolecules. These compounds have rotational correlation times which are long enough for the water exchange to influence, or even to limit, the overall proton relaxivity at imaging fields, as has been shown for dendrimer-based contrast agents.[13] Therefore, for these macromolecular agents further improvement in efficiency cannot be obtained without tuning, *i.e.* increasing the water-exchange rate, which requires a complete understanding of the influencing parameters.

Oxygen-17 NMR spectroscopy is an efficient technique for studying water-exchange parameters. The oxygen of the co-ordinated water molecule, directly bound to the paramagnetic Gd^{3+} ion, is a more sensitive antenna than are protons. Another advantage of this method is that the outer-sphere contribution to the relaxation is negligible.[11] However, it has been observed in many cases that in interpreting ^{17}O NMR transverse relaxation rates one faces problems, mainly due to the fact that the electronic relaxation parameters obtained only from ^{17}O NMR are rather ill-defined. Therefore, it is desirable to determine these parameters by EPR spectroscopy as an independent technique, which gives direct access to transverse electronic rates.[14] The ideal case would be to use all the three techniques that are at one's disposal to characterise relaxation parameters of MRI contrast agents, *i.e.* ^{17}O NMR, EPR and NMRD (nuclear magnetic resonance dispersion, which measures proton relaxation as a function of magnetic field).[10] In the present study we have used EPR and variable-temperature and -pressure ^{17}O NMR spectroscopy at three fields to study the $[GdL^1]^{2-}$, $[GdL^2]^-$ and $[GdL^3]^-$ complexes. The EPR and ^{17}O NMR data were analysed together in a simultaneous multiple-parameter least-squares fitting procedure which gives more reliable results than separate fits. We have also performed a variable-temperature corresponding UV/VIS study on the europium(III) complexes in order to check the presence of co-ordination equilibria in solution.

Experimental

Sample preparation

The polyamino carboxylates H_5L^1, H_4L^2 and H_4L^3 and the $[GdL^1]^{2-}$ complex were synthesized and kindly provided by J. Platzek and H. Schmitt, Schering AG (Berlin), and were used without further purification. Stock solutions of $Ln(ClO_4)_3$ (Ln = Gd or Eu) were prepared by dissolving Ln_2O_3 (NUCOR Corp., 99.99%) under reflux in a slight excess of $HClO_4$ (Merck, p.a., 70%) in double-distilled water, followed by filtration. The concentration was determined by titration with Na_2H_2edta (H_4edta = ethylenedinitrilotetraacetic acid) solution using xylenol orange as indicator and urotropin for pH regulation. All solutions were prepared by weight. For the preparation of the complexes $[GdL^2]^-$ and $[GdL^3]^-$, weighed quantities of solid ligands were dissolved in double-distilled water, then a weighed amount of $Ln(ClO_4)_3$ stock solution was added dropwise to form the chelate complexes with a ligand excess of 2–3%. The pH of the solutions was adjusted using weighed amounts of 1 mol dm^{-3} NaOH (p.a. Merck) and measured with a combined glass electrode, calibrated with Metrohm buffer solutions. The absence of free Ln^{3+} ion in the solution was verified by using xylenol orange indicator.[15] To improve sensitivity ^{17}O-enriched water (10% $H_2{}^{17}O$, Yeda R&D Co.) was added to the gadolinium complex solutions to produce solutions with about 2% enrichment. The compositions of all solutions are given in Table 1.

Table 1 Composition of the solutions used in variable-temperature (1–5), variable-pressure (6–8) ^{17}O NMR measurements, in EPR measurements (3–5) and in spectrophotometric measurements (9–11)

Solution	Sample	$[Ln^{3+}]$/mol kg^{-1}	$10^3 P_m$	pH
1	Acidified water			3.4
2	$[GdL^1(H_2O)]^{2-}$	0.0207	0.373	5.5
3	$[GdL^1(H_2O)]^{2-}$	0.0521	0.938	5.6
4	$[GdL^2(H_2O)]^-$	0.0492	0.885	5.6
5	$[GdL^3(H_2O)]^-$	0.0472	0.850	5.5
6	$[GdL^1(H_2O)]^{2-}$	0.0502	0.903	5.4
7	$[GdL^2(H_2O)]^-$	0.0547	0.985	5.4
8	$[GdL^3(H_2O)]^-$	0.0518	0.931	5.4
9	$[EuL^1(H_2O)]^{2-}$	0.0201	0.361	5.5
10	$[EuL^2(H_2O)]^-$	0.0199	0.358	5.6
11	$[EuL^3(H_2O)]^-$	0.0202	0.362	5.4

UV/VIS spectrophotometry

The UV/VIS measurements were done on a Perkin-Elmer Lambda 19 spectrophotometer in thermostatted cells with a 10 cm optical pathlength. The transitions were measured at 276, 303 and 363 K.

^{17}O NMR measurements

The technique used for the variable-temperature and -pressure ^{17}O NMR measurements has been previously described.[8,11,13]

EPR measurements

The EPR spectra were recorded at X-band (0.34 T) using a Bruker ESP 300E spectrometer operated in continuous-wave mode. The samples were contained in 1 mm glass tubes. The cavity temperature was stabilised using electronic temperature control of the gas flowing through the cavity. It was verified by substituting a thermometer for the sample tube. Measurements were made from 273 up to 365 K. The peak-to-peak linewidth was mesured from the recorded spectra using the instrument software.

Data analysis

The simultaneous least-squares fitting was performed by the program SCIENTIST® for WINDOWS™ by MICROMATH®, version 2.0.[16] The reported errors in Table 2 correspond to one standard deviation obtained by the statistical analysis.

Results

UV/VIS spectroscopy

In the UV/VIS spectra a single absorption band was observed for the $[EuL^1]^{2-}$, $[EuL^2]^-$ and $[EuL^3]^-$ complexes in the range $579 < \lambda < 581$ nm (579.8 nm at 303 K). This $^7F_0 \longrightarrow {}^5D_0$ transition band of the Eu^{3+} ion is very sensitive to changes in the

co-ordination environment. Thus it offers a good tool to check the presence of differently solvated species in solution, as was shown for some polyaminopolycarboxylate complexes.[18,19] Since only a single band was observed in the temperature range studied it reliably excludes any solvation equilibrium.

EPR measurements

The measured peak-to-peak linewidths, ΔH_{pp}, of the derivative spectrum can be related to the overall transverse electronic relaxation rate, $1/T_{2e}$, *via* equation (1), where g_L is the isotropic Landé *g* factor ($g_L = 2.0$ for Gd^{3+}).[20]

$$\frac{1}{T_{2e}} = \frac{g_L \mu_B \pi \sqrt{3}}{h} \Delta H_{pp} \quad (1)$$

Variable-temperature ^{17}O 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[21] as in equations (2)–(4),

$$\frac{1}{T_{1r}} = \frac{1}{P_m}\left(\frac{1}{T_1} - \frac{1}{T_{1A}}\right) = \frac{1}{T_{1m} + \tau_m} + \frac{1}{T_{1os}} \quad (2)$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left(\frac{1}{T_2} - \frac{1}{T_{2A}}\right) = \frac{1}{\tau_m}\frac{T_{2m}^{-2} + \tau_m^{-1}T_{2m}^{-1} + \Delta\omega_m^2}{(\tau_m^{-1} + T_{2m}^{-1})^2 + \Delta\omega_m^2} + \frac{1}{T_{2os}} \quad (3)$$

$$\Delta\omega_r = \frac{1}{P_m}(\omega - \omega_A) = \frac{\Delta\omega_m}{(1 + \tau_m T_{2m}^{-1})^2 + \tau_m^2\Delta\omega_m^2} + \Delta\omega_{os} \quad (4)$$

where $1/T_{1m}$, $1/T_{2m}$ are the relaxation rates of the bound water, $\Delta\omega_m$ is the chemical shift difference between bound and bulk water (in the absence of a paramagnetic interaction with the bulk water), P_m is the mole fraction of bound water and τ_m is the residence time of water molecules in the inner co-ordination sphere. The total outer-sphere contributions to the reduced relaxation rates and chemical shift are represented by $1/T_{1os}$, $1/T_{2os}$ and $\Delta\omega_{os}$, and it has been shown that in equations (2) and (3) they can be neglected.[11] The maxima observed in the temperature dependence of $\ln(1/T_{2r})$ and the inflection in the plots of $\Delta\omega_r$ in Figs. 1–3 are characteristic of a changeover from the 'fast exchange' limit at high temperatures to the 'slow exchange' limit at low temperatures. At high temperatures the inner-sphere contribution to $\Delta\omega_r$ is given by the chemical shift of the bound water molecules, $\Delta\omega_m$, which is determined by the hyperfine interaction between the electron spin of Gd^{3+} and the ^{17}O nucleus *via* equation (5), where *S* is the electron spin ($\frac{7}{2}$ for Gd^{3+}), $A/\hbar$ is the hyperfine or scalar coupling constant and *B* is the magnetic field.[22] The outer-sphere contribution to $\Delta\omega_r$ has a similar temperature dependence to $\Delta\omega_m$ and is given by equation (6), where C_{os} is an empirical constant.

$$\Delta\omega_m = \frac{g_L \mu_B S(S+1)B}{3k_B T}\frac{A}{\hbar} \quad (5)$$

$$\Delta\omega_{os} = C_{os}\Delta\omega_m \quad (6)$$

The oxygen-17 longitudinal relaxation rates in Gd^{3+} solutions are dominated by the dipole–dipole and quadrupolar mechanisms,[11] and are given by equations (7)–(10),[23,24] where $\gamma_s = g_L\mu_B/\hbar$ is the electron gyromagnetic ratio (1.76×10^{11} rad s^{-1} T^{-1} for $g_L = 2.0$), γ_I the nuclear gyromagnetic ratio (-3.626×10^7 rad s^{-1} T^{-1} for ^{17}O), *r* the effective distance between the electron charge and the ^{17}O nucleus (the metal–oxygen distance in the point-dipole approximation), *I* the nuclear spin ($\frac{5}{2}$ for ^{17}O), χ the quadrupolar coupling constant and η an asymmetry parameter {we use here the value for acidified water, $\chi[1 + (\eta^2/3)]^{\frac{1}{2}} = 7.58$ MHz};[25] τ_R is the rotational correlation time of the Gd–O vector, which, in the case of monomer complexes, can be considered as the rotational correlation time of the whole molecule. For the oxygen–metal distance we use an estimation of $r = 0.25$ nm based on neutron-diffraction measurements of lanthanide aqua ions in solution.[26] The right choice of this distance is extremely crucial for the absolute value of the rotational correlation time, since *r* enters into the 6th power in equation (8). In a recent publication, where ^{17}O, NMRD and EPR data have been treated together in a simultaneous multiple-parameter fitting procedure, the value of the effective Gd–O distance was also fitted, with the quadrupolar coupling constant kept fixed at the same time.[10] The values found for several gadolinium(III) complexes are about 10% shorter than 0.25 nm. In our case the lack of NMRD data prevents us fitting the Gd–O distance. Therefore, here we use the same *r* value as in several previous ^{17}O NMR studies on gadolinium(III) complexes in order to obtain a valid comparison of the rotational correlation times.[8,11,13,17] With this *r* value the dipole–dipole mechanism contributes to 68% of $1/T_{1m}$.

$$\frac{1}{T_{1m}} = \frac{1}{T_{1dd}} + \frac{1}{T_{1q}} \quad (7)$$

$$\frac{1}{T_{1dd}} = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{S(S+1)\hbar^2\gamma_I^2\gamma_s^2}{15r^6}\left(6\tau_{d1} + \frac{14\tau_{d2}}{1 + \omega_s^2\tau_{d2}^2}\right) \quad (8)$$

$$\frac{1}{\tau_{dj}} = \frac{1}{\tau_m} + \frac{1}{T_{je}} + \frac{1}{\tau_R}; \quad j = 1 \text{ or } 2 \quad (9)$$

$$\frac{1}{T_{1q}} = \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}\chi^2\left(1 + \frac{\eta^2}{3}\right)\tau_R \quad (10)$$

A simple exponential temperature dependence is assumed for the rotational correlation time, τ_R [equation (11)], where

$$\tau_R = \tau_R^{298}\exp\{E_R/R[(1/T) - (1/298.15)]\} \quad (11)$$

τ_R^{298} is the rotational correlation time at 298.15 K and E_R the activation energy of rotation.

In the case of the transverse relaxation the scalar contribution, $1/T_{2sc}$, is the most important one [equation (12)]. Here ω_s is the Larmor frequency of the metal electron spin and $1/\tau_{sj}$ the sum of the exchange rate constant and the electron-spin relaxation rate. As the τ_{s1} term dominates in equation (12) the contribution of the transverse electronic relaxation to oxygen-17 transverse relaxation can be neglected.

$$\frac{1}{T_{2m}} \approx \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\tau_{s1} + \frac{\tau_{s2}}{1 + \omega_s^2\tau_{s2}^2}\right); \quad \frac{1}{\tau_{sj}} = \frac{1}{\tau_m} + \frac{1}{T_{je}}, j = 1 \text{ or } 2 \quad (12)$$

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 (z.f.s.), induced by distortions of the complex. In previous studies a magnetic field-independent electronic relaxation mechanism had also to be included in order to describe oxygen-17 relaxation rates [equation (13)].[8] This spin-rotation (s.r.)

$$\frac{1}{T_{je}} = \left(\frac{1}{T_{je}}\right)^{zfs} + \left(\frac{1}{T_{je}}\right)^{sr}; \quad j = 1 \text{ or } 2 \quad (13)$$

$$\left(\frac{1}{T_{1e}}\right)^{zfs} = \frac{1}{25}\Delta^2\tau_v[4S(S+1)-3](J_1 + 4J_2) \quad (14)$$

$$\left(\frac{1}{T_{2e}}\right)^{zfs} = \frac{1}{50}\Delta^2\tau_v[4S(S+1)-3](J_0 + 5J_1 + 2J_2) \quad (15)$$

$$J_j = [1 + (j\omega_s\tau_v)^2]^{-1} \quad (16)$$

$$\left(\frac{1}{T_{je}}\right)^{sr} = \frac{\delta g_L^2}{9\tau_R}; \quad j = 1 \text{ or } 2 \quad (17)$$

relaxation mechanism arises from the asymmetry of the complex and represents only a small contribution. The z.f.s. terms can be expressed by the McLachlan equations (14) and (15) in the limit of $\omega_s\tau_v \ll 1$.[26] The s.r. contribution is given to a good approximation by equation (17),[8] where δg_L is the deviation from the free-electron value of g_L along the principal axis of the g_L tensor. In equations (14)–(16) Δ^2 is the trace of the square of the zero-field-splitting tensor and τ_v is the correlation time for the modulation of the z.f.s. This modulation may arise from transient distortions or from rotation of the complex. We assume that the temperature dependence of τ_v has an Arrhenius behaviour [equation (18)].

$$\tau_v = \tau_v^{298} \exp\{E_v/R[(1/T) - (1/298.15)]\} \quad (18)$$

The binding time (or exchange rate, k_{ex}) of water molecules in the inner sphere is assumed to obey the Eyring equation (19),

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h}\exp\left(\frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT}\right) = \frac{k_{ex}^{298} T}{298.15}\exp\left[\frac{\Delta H^\ddagger}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \quad (19)$$

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

The reduced oxygen-17 transverse and longitudinal relaxation rates and reduced chemical shifts as well as the transverse electronic relaxation rates for the three gadolinium(III) complexes are presented in Figs. 1–3 as a function of temperature. We performed a simultaneous least-squares fit of the EPR and ^{17}O NMR data in Figs. 1–3 using equations (1)–(19) with the following fitted parameters: k_{ex}^{298} (or $\Delta S^\ddagger$), $\Delta H^\ddagger$, $A/\hbar$, C_{os}, τ_R^{298}, E_R, τ_v^{298}, E_v, Δ^2 and δg^2. Since no concentration dependence was found for the reduced transverse and longitudinal relaxation rates and chemical shifts for the $[GdL^1]^{2-}$ complex, the $1/T_{1r}$, $1/T_{2r}$ and $\Delta\omega_r$ values measured in solutions of different concentrations were fitted together. The resulting curves are shown in Figs. 1–3 and the fitted parameters in Table 2. There is a rather large variation in the values of the electronic relaxation parameters for the five polyaminopolycarboxylate complexes compared in Table 2. The main reason is that the values for $[Gd(dtpa)(H_2O)]^{2-}$ and $[GdL'(H_2O)]$ were calculated either from only EPR data[8,14] or from a simultaneous fit of EPR, ^{17}O NMR and also NMRD data,[10] thus we will not interpret this variation in terms of any significant structural differ-

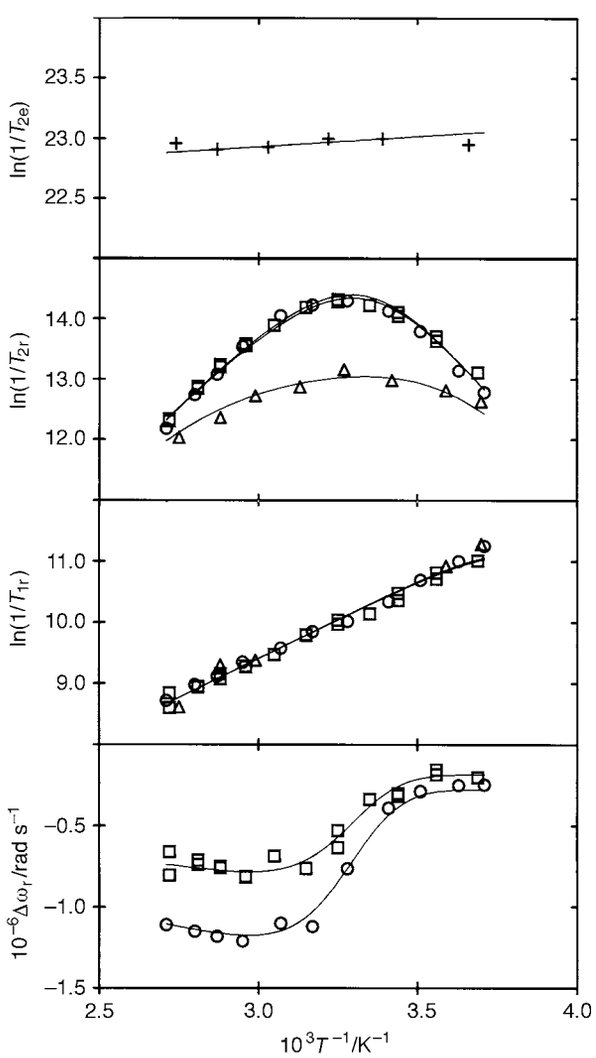


Fig. 1 Transverse electronic relaxation rates at 0.34 T (+), reduced oxygen-17 transverse and longitudinal relaxation rates (s^{-1}) and reduced chemical shifts of aqueous solutions of $[GdL^1(H_2O)]^{2-}$ as a function of inverse temperature. B = 1.41 (△), 9.4 (□) and 14.1 T (○)

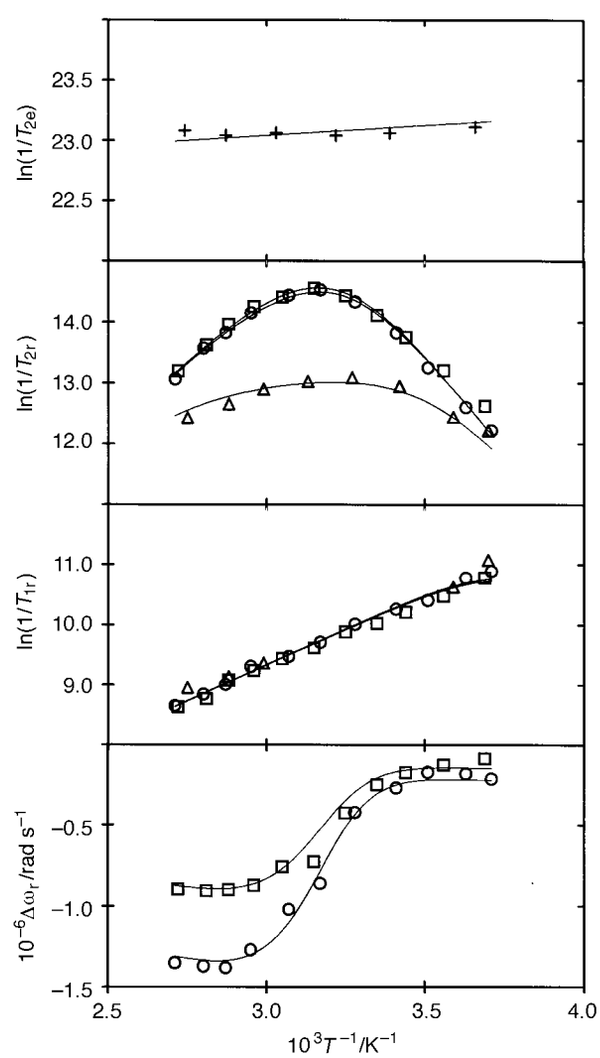


Fig. 2 Plots as in Fig. 1, but for $[GdL^2(H_2O)]^-$

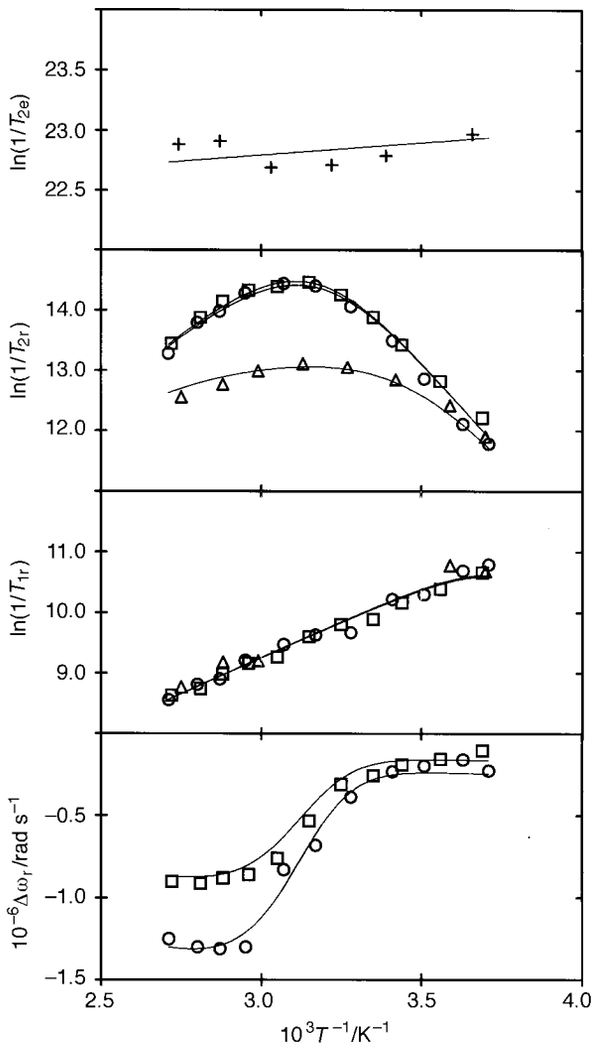


Fig. 3 Plots as in Fig. 1, but for $[GdL^3(H_2O)]^-$

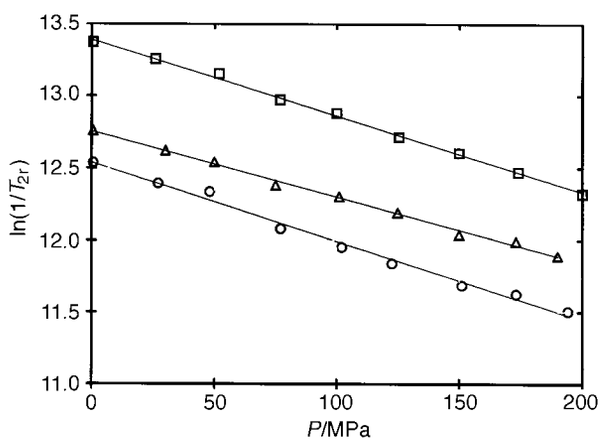


Fig. 4 Pressure dependence of the reduced oxygen-17 transverse relaxation rates (s^{-1}) for aqueous solutions of $[GdL^1(H_2O)]^{2-}$ (□), $[GdL^2(H_2O)]^-$ (△) and $[GdL^3(H_2O)]^-$ (○)

ences. On the other hand, it indicates that electronic relaxation rates by themselves are complicated, even for simple complexes, and the theory available cannot satisfactorily describe them.

Variable-pressure ^{17}O NMR

The pressure dependence of the reduced transverse oxygen-17 relaxation rates, $1/T_{2r}$, for the $[GdL^1]^{2-}$, $[GdL^2]^-$ and $[GdL^3]^-$ complexes at 277.0 K is presented in Fig. 4. At this magnetic field and temperature all the three systems are in the slow-exchange limit which means that $1/T_{2r}$ is practically equal to $1/\tau_m$, thus the decrease in $1/T_{2r}$ with increasing pressure is due to the slowing of the water exchange. The pressure dependence of $\ln(k_{ex})$, as in previous studies,[8,10–13,17,28] was linear and is given by equation (20), where $\Delta V^‡$ is the activation volume and

$$\frac{1}{\tau_m} = k_{ex} = (k_{ex})_0^T \exp\left(-\frac{\Delta V^‡}{RT} P\right) \qquad (20)$$

$(k_{ex})_0^T$ is the water-exchange rate at zero pressure and temperature T. The scalar coupling constant has previously also been shown to be independent of pressure, so we assume that it is constant and equals the value in Table 2. We performed a least-squares fit of the data in Fig. 4 using equations (3), (12), (14) and (20) with $(k_{ex})_0^T$ and $\Delta V^‡$ as fitted parameters. The results of the fit can be seen in Fig. 4 with the calculated values for the parameters in Table 2.

Discussion

Structure of the complexes

The temperature invariance of the UV/VIS absorption spectra lets us conclude that there is no solvation equilibrium in the solution of the LnL complexes (L = L^1, L^2 or L^3), as could be expected for lanthanide(III) complexes of dtpa derivative ligands. On the basis of similarity to $[Ln(dtpa)(H_2O)]^{2-}$ [29–31] and $[LnL'(H_2O)]$ [31,32] complexes it is reasonable to suppose that, besides the three nitrogens and five carboxylates $\{[LnL^1(H_2O)]^{2-}\}$, or three nitrogens, four carboxylates and one amide group $\{LnL^{2,3}(H_2O)]^-\}$, there is one water molecule co-ordinated to the lanthanide ion. This assumption is confirmed by the values of the hyperfine coupling constant, $A/\hbar$, obtained from the ^{17}O chemical shifts in the GdL solutions, which are in the usual range previously observed for several different gadolinium(III) polyaminopolycarboxylate complexes with one water molecule in the inner co-ordination sphere. The scalar coupling constant is a measure of the gadolinium spin density at the oxygen nucleus, thus it gives some information on the $Gd–OH_2$ distance. Although the coupling constants for the two monoamide complexes $\{[GdL^2]^-$ and $[GdL^3]^-\}$ are slightly higher, we would not attach much significance to this concerning the structure of the complex.

Water-exchange rate and mechanism

During the past three years the rate and mechanism of water exchange have been determined for a considerable number of lanthanide(III) aqua and polyaminopolycarboxylate complexes.[8,11–13,17,28] The accumulated data clearly show that both the rate and the mechanism are intimately related to the inner-sphere solution structure of the complexes. For lanthanide(III) aqua ions the water-exchange rates decrease by more than one order of magnitude between $[Gd(H_2O)_8]^{3+}$ and $[Yb(H_2O)_8]^{3+}$.[34,35] From neutron-diffraction measurements it is known that as the ionic radius decreases the co-ordination number of the lanthanide aqua ions changes from nine at the beginning of the series to eight at the end, Sm^{3+} having an average co-ordination number of 8.5.[36] The activation volumes indicate associatively activated water-exchange processes for all the octaaqua ions {from $[Gd(H_2O)_8]^{3+}$ to $[Yb(H_2O)_8]^{3+}$}. The fast water exchange on $[Gd(H_2O)_8]^{3+}$ can therefore be interpreted in terms of activation energy: being relatively close to an equilibrium state between eight- and nine-co-ordinated species, for the $[Gd(H_2O)_8]^{3+}$ ion little energy is required to reach the transition state (co-ordination number nine) in an associatively activated process. The nine-co-ordinate gadolinium(III) polyaminopolycarboxylates compared in Table 2 all have large positive activation volumes, indicative of dissociatively activated water exchange. This may be expected considering that in a nine-co-ordinate lanthanide complex there is no longer space for a second water molecule to enter before the subsequent

Table 2 Kinetic and NMR parameters as obtained from the simultaneous fit of ^{17}O NMR and EPR data

Parameter	$[Gd(H_2O)_8]^{3+}$ [a]	$[Gd(dtpa)(H_2O)]^{2-}$ [a]	$[GdL'(H_2O)]$ [a]	$[GdL^1(H_2O)]^{2-}$	$[GdL^2(H_2O)]^{-}$	$[GdL^3(H_2O)]^{-}$
$10^{-6}\ k_{ex}^{298}$/s^{-1}	830 ± 95 (804 ± 60)	4.1 ± 0.3 (3.3 ± 0.2)	0.43 ± 0.02 (0.45 ± 0.01)	3.6 ± 0.1	1.9 ± 0.1	1.3 ± 0.1
$\Delta H^{\ddagger}$/kJ mol^{-1}	14.9 ± 1.3 (15.3 ± 1.3)	52.0 ± 1.4 (51.6 ± 1.4)	46.6 ± 1.3 (47.6 ± 1.1)	49.1 ± 0.8	50.6 ± 1.0	48.6 ± 1.1
$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	−24.1 ± 4.1 (−23.1 ± 4.1)	+56.2 ± 5.0 (+53.0 ± 4.7)	+18.9 ± 4.0 (+22.9 ± 3.6)	+45.2 ± 3	+40.2 ± 3	+35.7 ± 4
$\Delta V^{\ddagger}$/cm^3 mol^{-1}	3.3 ± 0.2	+12.5 ± 0.2	+7.3 ± 0.2	+12.3 ± 0.2	+10.6 ± 0.3	+12.7 ± 0.4
$10^{-6}A/\hbar$/rad s^{-1}	−5.33 ± 0.2 (−5.3 ± 0.1)	−3.8 ± 0.2 (−3.8 ± 0.2)	−3.6 ± 0.3 (−3.8 ± 0.2)	−3.4 ± 0.1 (−3.6 ± 0.1) [b]	−4.3 ± 0.2	−4.2 ± 0.2
$10^{12}\ \tau_R^{298}$/s	29 ± 2 (41 ± 2)	103 ± 10 (58 ± 11)	167 ± 5 (66 ± 11)	178 ± 3	155 ± 4	143 ± 4
E_R/kJ mol^{-1}	15.1 ± 1.5 (15.0 ± 1.3)	18 ± 2 (17.3 ± 0.8)	21.6 ± 0.1 (21.9 ± 0.5)	21.2 ± 0.4	20.0 ± 0.5	19.8 ± 0.6
C_{os}	0.0 (0.0)	0.13 ± 0.06 (0.18 ± 0.04)	0.13 ± 0.06 (0.11 ± 0.04)	0.22 ± 0.04 (0.14) [b]	0.14 ± 0.03	0.16 ± 0.03
$10^{12}\ \tau_v^{298}$/s	7.2 ± 0.7 (7.3 ± 0.5)	0.25 ± 0.01 (25 ± 1)	34 ± 8 (25 ± 1)	4.0 ± 0.2	3.1 ± 0.2	3.0 ± 0.2
E_v/kJ mol^{-1}	15.4 ± 1.1 (18.4 ± 1.4)	1.6 ± 1.8 (1.6 ± 1.8)	9 ± 2 (3.9 ± 1.4)	1.7 ± 0.9	1.6 ± 1.0	1.9 ± 1.2
$10^{-20}\Delta^2$/s^{-2}	0.93 ± 0.04 (1.19 ± 0.09)	0.15 (0.46 ± 0.02)	0.38 ± 0.02 (0.41 ± 0.02)	2.3 ± 0.1	3.1 ± 0.2	2.6 ± 0.2
$\delta g_L^2/10^{-2}$	0.0	(1.2 ± 0.3)	1.7 ± 0.4 (0.8 ± 0.2)	2.3 ± 0.3	2.2 ± 0.3	2.0 ± 0.3

[a] The values were calculated only from ^{17}O NMR data, except for the electronic relaxation parameters which were obtained from EPR measurements. For $[Gd(H_2O)_8]^{3+}$ see ref. 17, for $[Gd(dtpa)(H_2O)]^{2-}$ ref. 11 and for $[GdL'(H_2O)]$ ref. 8. The values in parentheses were obtained by a simultaneous fit of ^{17}O NMR, EPR and NMRD data, and are taken from ref. 10. [b] The fitted value of C_{os} (0.22) is not unreasonable, but somewhat higher than those for other similar complexes. Consequently, it results in a smaller coupling constant. By fixing C_{os} at 0.14 we can obtain -3.6×10^6 rad s^{-1} for $A/\hbar$.

departure of the bound water molecule. On the other hand, the eight-co-ordinate transition state must be very unstable energetically, since for these types of complexes only the co-ordination number of nine is observed all along the lanthanide series.[31–33,37,38] The relative instability of the transition state, thus the high activation energy needed, results in a decreased rate constant. So the difference in the inner-sphere structure and therefore the difference in the mechanism is the reason why water exchange on lanthanide(III) polyaminopolycarboxylate complexes is generally much slower when compared to the gadolinium(III) aqua ion.

Let us now consider the differences in water-exchange rate between different, nine-co-ordinate, linear polyaminopolycarboxylates of Gd^{III} with one inner-sphere water molecule (Table 2). Although the mechanism is always dissociative, there is a ten-fold decrease in k_{ex} on going from the pentacarboxylate $[Gd(dtpa)(H_2O)]^{2-}$ to the bis(amide) derivative $[GdL'(H_2O)]$, with the values for the two monoamide complexes being in between these. The diminution of the water-exchange rate with the substitution of a carboxylate by an amide as co-ordinating group has recently been found for several macrocyclic gadolinium polyaminopolycarboxylate complexes.[10,13] Amide derivatives of dota (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate) have been synthesized in the last few years with the aim of diminishing the total complex charge, or, at the same time, forming dimeric complexes or linking the complex to macromolecules. For the gadolinium(III) complex of each amide dota derivative so far studied the water-exchange rate is in the range of $(1.1–1.6) \times 10^6$ s^{-1},[10,13] about one fourth of k_{ex} on $[Gd(dota)(H_2O)]^-$.[11] On the basis of all the k_{ex} values available for amide derivatives of either dtpa or dota we can generally state that the replacement of one carboxylate group by an amide decreases the water-exchange rate of the gadolinium(III) complex by a factor of about 4.

An amide group is co-ordinated less strongly towards the lanthanide ion than a carboxylate, which is reflected by smaller stability constants of the amide complexes compared to carboxylates in solution,[6,39] and by the long gadolinium-amide oxygen distances in the solid state, compared to carboxylate oxygen distances {*e.g.* the average gadolinium-carboxylate oxygen distance in $Na_2[Gd(dtpa)(H_2O)]$ is 0.240 nm,[40] and the amide oxygen distance in the dtpa bis(benzylamide) complex of Gd^{III} is 0.244 nm}.[41] The consequence of the longer amide oxygen compared to carboxylate oxygen distance is a less crowded inner sphere in amide as compared to carboxylate complexes. In dissociatively activated water-exchange processes the steric crowding is of primary importance: a tightly co-ordinating ligand encourages the water molecule to leave, thus favouring the dissociative-activation step. The significance of crowding at the water binding site was nicely demonstrated by a ^{17}O NMR study on the whole lanthanide series of L′ complexes.[42] On progressing from the middle to the end of the series the eight-co-ordinate transition state becomes more and more accessible since the radius of the lanthanide ion decreases, and the result is a large increase in the water-exchange rate from $[EuL'(H_2O)]$ to $[HoL'(H_2O)]$.

Beside steric crowding the diminished charge of the ligand may also play an important role. The positive charge of the lanthanide ion is not shielded as much by the co-ordination of an amide group as compared to that by a carboxylate. The leaving water molecule will experience a stronger electrostatic attractive force from the metal centre which makes the dissociative step energetically disfavoured.

Moreover, the two monoamide complexes $[GdL^2]^-$ and $[GdL^3]^-$ offer a good opportunity to look at the influence of small changes of the inner-sphere structure on water-exchange rates. The difference in k_{ex} between the two complexes (Table 2) may be rationalised in terms of thermodynamic stability. The stability constant for the symmetric complex, with the amide group on the central nitrogen, is slightly higher than that of the asymmetric complex (log $\beta = 19.8$ and 19.5 for the symmetric and asymmetric complexes, respectively).[43] This difference was explained by a more rigid solution structure for the former complex: the two, strongly co-ordinating iminodiacetate groups pull the central amide closer to the Gd^{3+} than is possible in the case of the asymmetric molecule. The increased crowding around the metal centre results in faster water exchange.

The water-exchange rate on the gadolinium(III) complex of the ethoxybenzyl pentacarboxylate L^1 is practically the same as on $[Gd(dtpa)(H_2O)]^{2-}$.[11] {Muller and co-workers[44] have recently found a slightly higher value for $[GdL^1(H_2O)]^{2-}$ in an independent ^{17}O NMR study, carried out at a single field ($k_{ex}^{298} = 5.0 \times 10^6$ s^{-1}).} The invariability of the exchange rate on the addition of a bulky group indicates that structural changes outside the inner co-ordination sphere do not have any effect on the water exchange. Furthermore, the substituent on the amide nitrogen has practically no influence either on the water exchange, for both dtpa- and dota-type complexes: sugar derivatives,[12] bis(amides) incorporated in linear polymers,[45] several different monoamides in dimers[10] or dendrimers.[13]

Rotation

The rotational dynamics of gadolinium(III) complexes as potential MRI contrast agents is a crucial point in determining proton relaxivity. For all the commercially available contrast agents the proton relaxivity is limited by slow rotation at imaging fields. The order of τ_R values (Table 2), obtained from ^{17}O NMR spectroscopy, reflects the molecular size; the two monoamides tumble somewhat more slowly than $[Gd(dtpa)(H_2O)]^{2-}$, but faster than the slightly larger bis(amide). For the ethoxybenzyl derivative $[GdL^1]^-$ rotation becomes slower due to the presence of the bulky substituent. The longer rotational correlation time can reasonably account for the increased proton relaxivity ($R_1^H = 7.9$ dm^3 $mmol^{-1}$ s^{-1}) compared to that of $[Gd(dtpa)(H_2O)]^{2-}$ ($R_1^H = 6.0$ dm^3 $mmol^{-1}$ s^{-1}; 10 MHz, 25 °C). A similar increase in proton relaxivity as compared to that of the $[Gd(dota)]^-$ ($R_1^H = 3.56$ dm^3 $mmol^{-1}$ s^{-1}) was observed for the gadolinium(III) complexes of dota-like ligands with substituents of similar size to that of the ethoxybenzyl group: a nitrophenyl derivative ($R_1^H = 5.4$ dm^3 $mmol^{-1}$ s^{-1}),[46] and two polyhydroxy(benzyloxy)propionamide derivatives ($R_1^H = 4.49$ and 5.19 dm^3 $mmol^{-1}$ s^{-1}; 20 MHz, 39 °C).[47] For all three complexes the proton relaxivity gain was related to the larger molecular size, therefore to the reduced tumbling rate.

Conclusion

Our understanding of the relation between the polyaminopolycarboxylate ligand structure and the water-exchange rates of their gadolinium(III) complexes has been increased. Modifications of the co-ordinating groups, *i.e.* changes in the inner co-ordination sphere of the metal ion, dramatically affect the rate, but not the mechanism, of water exchange. The replacement of carboxylates with amide functions results in a decreased rate, with the effect proportional to the number of carboxylates substituted. On the basis of the activation volumes, the exchange process is always dissociatively activated. Modifications to the carbon backbone of the ligand (outside the inner co-ordination sphere) do not influence water exchange. These findings make possible the fine-tuning of water-exchange rates, and therefore are of great significance in designing new ligands for MRI contrast agents.

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