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How Does Internal Motion Influence the Relaxation of the Water Protons in Ln(III)DOTA-like Complexes?

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Abstract: Taking advantage of the Curie contribution to the relaxation of the protons in the Tb(III) complex, and the quadrupolar relaxation of the ^{17}O and ^2H nuclei on the Eu(III) complex, the effect of the internal motion of the water molecule bound to $[Ln(DOTAM)(H_2O)]^{3+}$ complexes was quantified. The determination of the quadrupolar coupling constant of the bound water oxygen $\chi_O(1 + \eta_O^2/3)^{1/2} = 5.2 \pm 0.5$ MHz allows a new analysis of the ^{17}O and ^1H NMR data of the $[Gd(DOTA)(H_2O)]^-$ complex with different rotational correlation times for the Gd(III)$-O_{water}$ and Gd(III)$-H_{water}$ vectors. The ratio of the rotational correlation times for the Ln(III)$-H_{water}$ vector and the overall rotational correlation time is calculated $\tau_{RH}/\tau_{RO} = 0.65 \pm 0.2$. This could have negative consequences on the water proton relaxivity, which we discuss in particular for macromolecular systems. It appears that the final effect is actually attenuated and should be around 10% for such large systems undergoing local motion of the chelating groups.

Introduction

Gd(III) polyaminocarboxylate complexes are commonly used as MRI contrast agents. Among the macrocyclic systems, the DOTA ligand (DOTA = 1,4,7,10-tetraaza-1,4,7,10-tetrakis-(carboxymethyl)cyclododecane) was early recognized as an excellent candidate for this kind of application due to the outstanding thermodynamic stability of its complexes with Ln(III) ions. Intensive research is performed to better understand their physicochemical properties, which should allow smart development of new agents.[1,2] Particularly, it is well known that lengthening the rotational correlation time should increase the relaxivity. This can be attained with dendrimeric[3,4] or polymeric[5-10] chelates, as well as noncovalent interactions with proteins.[11-13] Unfortunately, due to internal motions of the chelate, the relaxivity did not reach the expected high values.[14] For every chemist's intuition, it is clear that the inner-sphere water also undergoes internal motion. Indeed, this motion has already been considered in the past, for example, by Dwek.[15] However, this phenomenon has never been directly observed so far. Recently, the ^1H and ^{17}O NMR signals of the water bound to $[Eu(DOTAM)(H_2O)]^{3+}$ complex (DOTAM = 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane) were detected, opening a new field of investigations.[16] On the basis of the Curie relaxation in the environment of Tb(III) as well as quadrupolar relaxation of the ^{17}O and ^2H nuclei of the bound water in the Eu(III) complex, we present experimental evidence for this internal motion. The rotational correlation times of the Ln(III)$-H_{water}$ and Ln(III)$-O_{water}$ vectors are compared, and a method to calculate the quadrupolar coupling constant $\chi_O(1 + \eta_O^2/3)^{1/2}$ of the bound water oxygen is applied.

Experimental Section

Sample Preparation. The ligand and the complex were prepared as previously described.[16,17] The solutions were prepared by dissolution of the solid complex $[Ln(DOTAM)(H_2O)](SO_3CF_3)_3$ in CD_3CN and H_2O/D_2O with a drop of TMS as an internal reference. For ^{17}O NMR measurements we used 40% ^{17}O-enriched proton normalized water (Yeda Co. Ltd,). The following samples were prepared: (a) 6.33 mg

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(1) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.
(2) Merbach, A. E.; Toth, E. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Wiley: New York: 2001.
(3) Tóth, E.; Pubanz, D.; Vauthey, S.; Helm, L.; Merbach, A. E. *Chem. Eur. J.* **1996**, *2*, 209−217.
(4) Wiener, E. C.; Brechbiel, M. W.; Brothers, H.; Magin, R. L.; Gansow, O. A.; Tomalia, D. A.; Lauterbur, P. C. *Magn. Reson. Med.* **1994**, *31*, 1−8.
(5) Kellar, K.; Henrichs, P. M.; Hollister, R.; Koenig, S. H.; Eck, J.; Wei, D. *Magn. Reson. Med.* **1997**, *38*, 712−716.
(6) Spanoghe, M.; Lanens, D.; Dommisse, R.; Van der Linden, A.; Alderweireldt, F. *Magn. Reson. Imaging* **1992**, *10*, 913−917.
(7) Vexler, V. S.; Clement, O.; Schmitt-Willich, H.; Brasch, R. C. *J. Magn. Reson. Imaging* **1994**, *4*, 381−388.
(8) Desser, T. S.; Rubin, D. L.; Muller, H. H.; Qing, F.; Khodor, S.; Zanazzi, G.; Young, S. U.; Ladd, D. L.; Wellons, J. A.; Kellar, K. E.; Toner, J. L.; Snow, R. A. *J. Magn. Reson. Imaging* **1994**, *4*, 467−472.
(9) Ladd, D. L.; Hollister, R.; Peng, X.; Wei, D.; Wu, G.; Delecki, D.; Snow, R. A.; Toner, J. L.; Kellar, K.; Eck, J.; Desai, V. C.; Raymond, G.; Kinter, L. B.; Desser, T. S.; Rubin, D. L. *Bioconjugate Chem.* **1999**, *10*, 361−370.
(10) Tóth, E.; Helm, L.; Kellar, K.; Merbach, A. E. *Chem. Eur. J.* **1999**, *5*, 1202−1211.
(11) Tóth, E.; Connac, F.; Helm, L.; Adzamli, K.; Merbach, A. E. *J. Biol. Inorg. Chem.* **1998**, *3*, 606−613.
(12) Aime, S.; Botta, M.; Fasano, M.; Crich, S. G.; Terreno, E. *J. Bioinorg. Chem.* **1996**, *1*, 312−319.
(13) Lauffer, R. B.; Brady, T. J.; Brown, R. D. I.; Baglin, C.; Koenig, S. H. *Magn. Reson. Med.* **1986**, *3*, 541−548.
(14) Dunand, F. A.; Aime, S.; Merbach, A. E. *J. Am. Chem. Soc.* **2000**, *122*, 1506−1512.
(15) Dwek, R. A. *Nuclear Magnetic Resonance (NMR) in Biochemistry*; Clarendon Press: Oxford, England, 1973.
(16) Aime, S.; Barge, A.; Botta, M.; De Sousa, A. S.; Parker, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2673−2675.
(17) Amin, S.; Morrow, J. R.; Lake, C. H.; Churchill, M. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 773−775.

of [Tb(DOTAM)(H$_2$O)](SO$_3$CF$_3$)$_3$, 500 μL of CD$_3$CN, and 5 μL of H$_2$O (c_{Tb} = 12.45 mM); (b) 7.68 mg of [Yb(DOTAM)(H$_2$O)](SO$_3$-CF$_3$)$_3$, 500 μL of CD$_3$CN, and 5 μL of H$_2$O (c_{Yb} = 14.61 mM); (c) 6.26 mg of [Eu(DOTAM)(D$_2$O)](SO$_3$CF$_3$)$_3$, 500 μL of CD$_3$CN, and 5 μL of D$_2$O (c_{Eu} = 12.40 mM); (d) 6.26 mg of [Eu(DOTAM)(H$_2$O)](SO$_3$-CF$_3$)$_3$, 500 μL of CD$_3$CN, and 5 μL of H$_2$^{17}O (c_{Eu} = 12.40 mM).

Variable Field and Temperature NMR. The ^{1}H, ^{2}H, and ^{17}O NMR spectra weremeasured on Bruker DRX-600, DPX-400, and DRX-200 spectrometers. The temperature was stabilized by Bruker VT-2000 and BVT-3000 temperature control units and checked by a substitution method.[18]

Data Treatment. The NMR signals are fitted by a Lorentzian function with the program NMRICMA 2.8 for MATLAB (L. Helm, A. Borel, Institut de Chimie Moléculaire et Biologique, Ecole Polytechnique Fédérale de Lausanne, Switzerland). The half-width of the ^{1}H NMR lines are corrected by subtracting the half-width of the TMS line (field inhomogeneity, line broadening). The inhomogeneity is neglected in the case of ^{17}O NMR. The longitudinal relaxation times are measured using the inversion recovery method. The fitting of the data (line width, T_1) is performed by the programs VISUALISEUR 2.3.0 and OPTIMISEUR 2.3.0 for MATLAB (F. Yerly, 1999−2001, Institut de Chimie Moléculaire et Biologique, Ecole Polytechnique Fédérale de Lausanne, Switzerland).

Results

Curie Relaxation. The Curie contribution to the longitudinal relaxation rate of a proton in the environment of a given lanthanide trivalent cation depends on the magnetic field (B_0), the Ln(III)−H distance (r), and the rotational correlation time (τ_R). Its importance at high magnetic fields for lanthanides aqua ions other than Gd(III) was shown by Bertini et al.[19] It is described by eq 1, where γ_I is the magnetogyric ratio of the proton, μ_B the Bohr magneton, k_B the Boltzmann constant, T the temperature, μ_0 the vacuum permeability, and μ_{eff} the effective magnetic moment of the lanthanide cation.

$$\frac{1}{T_1^{Curie}} = \frac{6}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 \mu_B^4 \mu_{eff}^4}{(3k_B T)^2 r^6} B_0^2 \left(\frac{\tau_R}{1 + (\omega\tau_R)^2}\right) \quad (1)$$

Assuming that the Ln(III)−H distances for the cyclen protons in solution are the same as in the crystal structure, one can calculate the overall rotational correlation time (τ_{RO}) at a given temperature. The validity of this assumption is shown by the work of Aime et al., who calculated the Ln(III)−H distances of the [Tb(DOTA)(H$_2$O)]$^-$ complex in aqueous solution from a known τ_{RO}.[20] The longitudinal relaxation rate of the cyclen protons of the [Tb(DOTAM)(H$_2$O)]$^{3+}$ complex (12.45 mM, CD$_3$CN/H$_2$O 100:1) were measured at 4.7, 9.4, and 14.1 T and 260 K (Figure 1). The crystallographic Ln(III)−H$_{cyclen}$ distances measured on Tb(III), Gd(III), or Eu(III) DOTA-like complexes are equal ($\pm$1%); therefore, we are allowed to use the distances obtained for the corresponding Eu(III) complex [Eu(DOTAM)-(H$_2$O)]$^{3+}$.[21−23] The field dependence of the longitudinal relax-
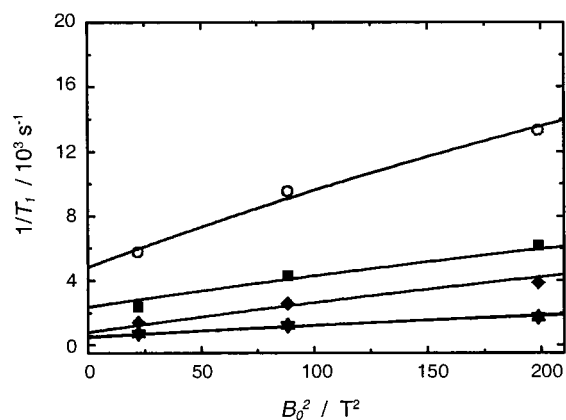
(18) Ammann, C.; Meier, P.; Merbach, A. E. *J. Magn. Reson.* **1982**, *46*, 319−321.
(19) Bertini, I.; Capozzi, F.; Luchinat, C.; Nicastro, G.; Xia, Z. *J. Phys. Chem.* **1993**, *97*, 6351−6354.
(20) Aime, S.; Botta, M.; Ermondi, G.; Barbero, L. *J. Chem. Soc., Dalton Trans.* **1992**, 225−228.
(21) Amin, S.; Voss, D. A., Jr.; Horrocks, D., Jr.; Lake, C. H.; Churchill, M. R.; Morrow, J. R. *Inorg. Chem.* **1995**, *34*, 3294−3300.
(22) Aime, S.; Barge, A.; Bruce, J. I.; Botta, M.; Howard, J. A. K.; Moloney, J. M.; Parker, D.; De Sousa, A. S.; Woods, M. *J. Am. Chem. Soc.* **1999**, *121*, 5762−5771.
(23) Alderighi, L.; Bianchi, A.; Calabi, L.; Dapporto, P.; Giorgi, C.; Losi, P.; Paleari, L.; Paoli, P.; Rossi, P.; Valtancoli, B.; Virtuani, M. *Eur. J. Inorg. Chem.* **1998**, 1581−1584.

Figure 1. Longitudinal relaxation rates field dependence of selected protons of [Tb(DOTAM)(H$_2$O)]$^{3+}$ at 260 K (12.45 mM, CD$_3$CN/H$_2$O 100:1). ○, water proton (crystalline Ln(III)−H = 2.95 Å); both axial ■ and ◆ (3.66 and 3.69 Å); and both equatorial ▲ and ▼ (4.30 and 4.34 Å) cyclen protons.
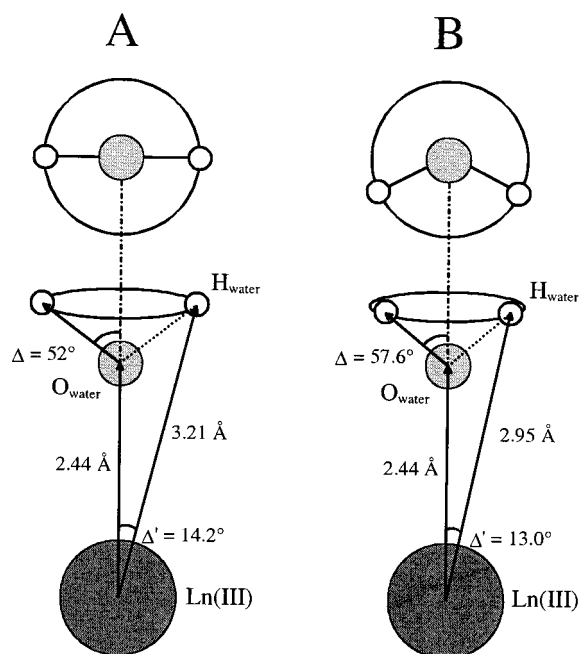

Figure 2. Schematic representation of the Ln(III)−water entity showing the distances and angles used in the analysis: **A**, with the water dipole axis aligned with the Ln(III)−O vector; **B**, position from the crystallographic data.

ation rates produced by the Curie interaction allows us to calculate the rotational correlation time from eq 1: τ_{RO} = 101 $\pm$ 12 ps. In the same way, one can calculate the rotational correlation time of the Ln(III)−H$_{water}$ vector for two models (see Figure 2): (**A**), assuming that the water molecule aligns its symmetry axis with the Ln(III)−O$_{water}$ vector, and an H−O−H angle of 104°, the Ln(III)−H$_{water}$ distance is r_{LnH} = 3.21 Å and τ_{RH} = 117 $\pm$ 20 ps; (**B**) the Ln(III)−H$_{water}$ distance is directly measured from the X-ray structure[17] r_{LnH} = 2.95 Å and τ_{RH} = 60 $\pm$ 10 ps. The same measurements were performed on the Yb(III) complex cyclen protons under the same conditions. Using the X-ray distances of a similar Yb(DOTA-tetraamide) complex,[24] we obtain τ_{RO} = 115 $\pm$ 11 ps, in good agreement with the Tb(III) complex value. The τ_{RH}/τ_{RO} ratio

(24) Batsanov, A. S.; Beeby, A.; Bruce, J. I.; Howard, J. A. K.; Kenwright, A. M.; Parker, D. *Chem. Commun.* **1999**, 1011−1012.

for the Tb(III) complex is then 1.2 ± 0.3 and 0.6 ± 02 for models **A** and **B**, respectively.

Quadrupolar Relaxation. In the extreme narrowing conditions, the quadrupolar contribution to the transverse relaxation of the ^{17}O nucleus is described by eq 2, where I is the nuclear spin of the quadrupole, χ_O is the quadrupolar coupling constant, η_O is an asymmetry parameter, and τ_{RO} is the rotational correlation time of the main axis of the electric field gradient. For a water molecule bound to a trivalent lanthanide cation, this axis is most probably along the $Ln(III)-O_{water}$ vector and the associated rotational correlation time is the one of the whole complex.

$$\frac{1}{T_{iQ}} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi_O{}^2 (1 + \eta_O{}^2/3) \tau_{RO} \qquad i = 1, 2 \quad (2)$$

In the case of Eu(III) one can approximate that the transverse relaxation rate of the bound water oxygen is only due to the exchange with the bulk water and the quadrupolar interaction. Indeed, $T_1 = T_2$ within the experimental error, and the Curie contribution is negligible in this case (see Supporting Information). The quadrupolar transverse relaxation rate is readily calculated in the slow exchange limit from eq 3 as the exchange contribution is well known.[14]

$$\frac{1}{T_i} = \frac{1}{T_{iQ}} + k_{ex} \qquad i = 1, 2 \quad (3)$$

The transverse relaxation rates were measured at several temperature between 225 and 262 K for the Eu(III) complex and the quadrupolar contribution was evaluated at 260 K, assuming an Arrhenius temperature dependence. The knowledge of the rotational correlation time at 260 K (108 ± 20 ps, taking the average between the Tb(III) and Yb(III) values) from the Curie relaxation allows us to calculate the quadrupolar coupling constant with eq 2, yielding $\chi_O (1 + \eta_O{}^2/3)^{1/2} = 5.2 \pm 0.5$ MHz.

The second approach to estimate the internal motion of the bound water molecule takes advantage of the ^2H and ^{17}O quadrupolar relaxation. The rotational correlation time of the $Ln(III)-O_{water}$ vector (τ_{RO}) can be easily calculated from the ^{17}O quadrupolar coupling constant reported above and the measured transverse relaxation rate of the bound water oxygen (eqs 2 and 3). The temperature dependence of τ_{RO} can be described by an Arrhenius law to yield $\tau_{RO}^{298} = 48.6 \pm 3$ ps and $E_{RO} = 14.4 \pm 0.5$ kJ mol^{-1} (Supporting Information, Figure S2).

To obtain the rotational correlation time of the $Eu(III)-D_{water}$ vector, the longitudinal relaxation rates of the free and bound water deuterium were measured at various temperature between 225 and 251 K where the exchange does not significantly influence the inversion recovery profiles. The quadrupolar contribution to the longitudinal relaxation rates can be easily calculated within the slow exchange approximation (eq 3). To control the validity of the approximation, the free and bound water deuterium longitudinal relaxation rates were also analyzed simultaneously with the exact equations for the relaxation rates reported by Leigh.[25] The quadrupolar contributions calculated with and without the slow exchange approximation are identical, as can be seen in the Supporting Information (Figure S3).

In the *Encyclopedia of NMR,* Woessner reported the correlation functions for axially symmetrical ellipsoids with internal motion.[26] In our case, we consider that the ellipsoid (the complex) has an isotropical rotational motion; i.e., its rotational motion can be described by a unique rotational correlation time (τ_{RO}). In this model, it is assumed that the internal motion is only due to the rotational diffusion of the water molecule around the $Eu(III)-O_{water}$ axis (see Figure 2). The spectral density function corresponding to this model is given by eq 4.

$$J(\omega) = A \frac{\tau_A}{1 + (\omega\tau_A)^2} + B \frac{\tau_B}{1 + (\omega\tau_B)^2} + C \frac{\tau_C}{1 + (\omega\tau_C)^2} \quad (4)$$

where,

$$A = \frac{1}{4}(1 - 3\cos^2 \Delta)^2, \quad B = \frac{3}{4}\sin^2 2\Delta, \quad C = \frac{3}{4}\sin^4 \Delta \quad (5)$$

$$\frac{1}{\tau_A} = \frac{1}{\tau_{RO}}, \quad \frac{1}{\tau_B} = \frac{1}{\tau_{RO}} + \frac{1}{\tau_{Ri}}, \quad \frac{1}{\tau_C} = \frac{1}{\tau_{RO}} + \frac{4}{\tau_{Ri}} \quad (6)$$

The extreme narrowing conditions valid for τ_{RO} in the quadrupolar relaxation also holds for τ_A, τ_B, and τ_C so that eq 4 can be replaced by eq 7.

$$J(\omega) = A\tau_A + B\tau_B + C\tau_C \quad (7)$$

Δ is the angle between the symmetry axis of the water molecule and the relaxation vector of the deuterium which is fixed to $52°$ or $57.6°$ (see Figure 2). The rotational correlation time of the $Ln(III)-O_{water}$ vector (τ_{RO}) is chosen to describe the isotropic overall rotation. τ_{Ri} is the rotational correlation time characteristic for the internal rotational diffusion.

The electric field gradient responsible for the relaxation of the deuterium quadrupole is along the $O-D$ bound. Hence, the quadrupolar contribution to the longitudinal relaxation is described by eq 8.

$$\frac{1}{T_{1Q}} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi_D{}^2 (1 + \eta_D{}^2/3) J(\omega) \quad (8)$$

On the basis of the work by Leyte et al. on Mg(II) aquo complexes, the values $\chi_D = 193$ kHz and $\eta_D = 0.12$ were taken for the deuterium quadrupolar coupling constant and asymmetry parameter.[27] This seems acceptable since χ_D varies of only 20% between the free water and the water bound to Mg(II) and the influence on τ_{RD} of an error of 10% on χ_D is less than 1%.

The T_{1Q} values of the bound water deuterium calculated within the slow exchange limit were fitted to eqs 7 and 8 to yield, for **A**, $\tau_{Ri}^{298} = 6.6 \pm 4$ ps and $E_{Ri} = 46.0 \pm 6$ kJ mol^{-1}, and for **B**, $\tau_{Ri}^{298} = 7.5 \pm 5$ ps and $E_{Ri} = 46.1 \pm 6$ kJ mol^{-1} (Figure 3). The rotational correlation times of the $Ln(III)-D_{water}$ (τ_{RD}) correspond to the spectral density function for an angle $\Delta' = 14.2°$ or $13°$ in eq 7 for **A** and **B**, respectively (see Figure 2).

The following parameters were calculated: for **A**, $\tau_{RD}^{298} = 41.5 \pm 5$ ps and $E_{RD} = 15.4 \pm 0.5$ kJ mol^{-1}, and for **B**, $\tau_{RD}^{298} = 42.7 \pm 5$ ps and $E_{RD} = 15.3 \pm 0.5$ kJ mol^{-1}. The ratios

(25) Leigh, J. S., Jr. *J. Magn. Reson.* **1971**, *4*, 308−311.

(26) Woessner, D. E. In *Encyclopedia of NMR*; Grant, D. M., Harris, R. K., Eds.; John Wiley and Sons: Chichester, England, 1996; pp 1068−1084.
(27) Struis, R. P. W. J.; de Bleijser, J.; Leyte, J. C. *J. Phys. Chem.* **1987**, *91*, 6309−6315.
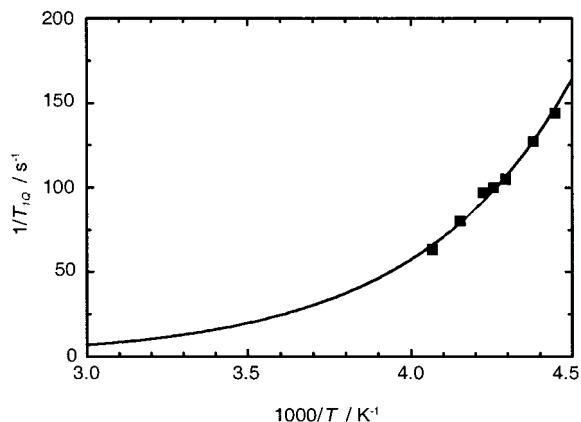
Figure 3. Temperature dependence of the quadrupolar longitudinal relaxation rate of the bound water deuterium of $[Eu(DOTAM)(D_2O)]^{3+}$ at 9.4 T as calculated with the slow exchange approximation (12.4 mM, CH$_3$-CN/D$_2$O 100:1). The lines represent the best fit to the rates as described in the text.

$\tau_{RD}/\tau_{RO} = 0.85 \pm 0.15$ (**A**) and $\tau_{RD}/\tau_{RO} = 0.88 \pm 0.15$ (**B**) at 298 K are constant over the temperature range since the activation energies are fairly equal.

Multifit Gd(III). The simultaneous analysis of EPR, ^{17}O NMR, and ^{1}H NMRD data on the polyaminocarboxylate Gd(III) complex is also a method to elucidate the internal motion of the bound water molecule. This type of analysis commonly afforded $\chi_O(1 + \eta_O^2/3)^{1/2} > 10$ MHz with the Gd(III)$-$O$_{water}$ and Gd(III)$-$H$_{water}$ distances fixed to 2.5 and 3.1 Å, respectively, and a unique rotational correlation time to describe the tumbling of the Gd(III)$-$O$_{water}$ and Gd(III)$-$H$_{water}$ vectors.[28] In fact, it seems now rather obvious that the high value of the quadrupolar coupling constant could compensate (see eq 2) the need for a longer rotational correlation time to describe the ^{17}O longitudinal relaxation data. Hence, we performed a new analysis of the $[Gd(DOTA)(H_2O)]^-$ data[28] where $\chi_O(1 + \eta_O^2/3)^{1/2}$ is fixed to 5.2 MHz and the ratio τ_{RH}/τ_{RO} is taken as a parameter. Following the recent progress on the electronic relaxation of Gd(III), we did not consider the EPR and low-field NMRD (<10 MHz) data in the analysis.[29] To obtain comparable parameters, the Ln(III)$-$O$_{water}$ and Ln(III)$-$H$_{water}$ distances were fixed to 2.44 and 2.95 (or 3.21) Å, respectively, as reported in Figure 2 (the X-ray distances for the $[Gd(DOTA)(H_2O)]^-$ complex are 2.458 and 2.94 Å[30]). The experimental data are well described using ratios $\tau_{RH}/\tau_{RO} = 0.65 \pm 0.1$ (**A**) and $\tau_{RH}/\tau_{RO} = 0.35 \pm 0.1$ (**B**). The experimental data as well as the calculated curves are represented in Figure 4 for the model **A** (the analysis with model **B** is similar and is not reproduced here). The parameters used in the simultaneous fit are reported in the Supporting Information (Table S5).

A popular tool in the NMR study of local motions is the model-free approach of Lipari and Szabo,[31,32] and one might consider using it to describe the proton relaxivity in the simultaneous fit. However, it seems unnecessary in the case of small complexes such as $[Gd(DOTA)(H_2O)]^-$. Since τ_{RH} and
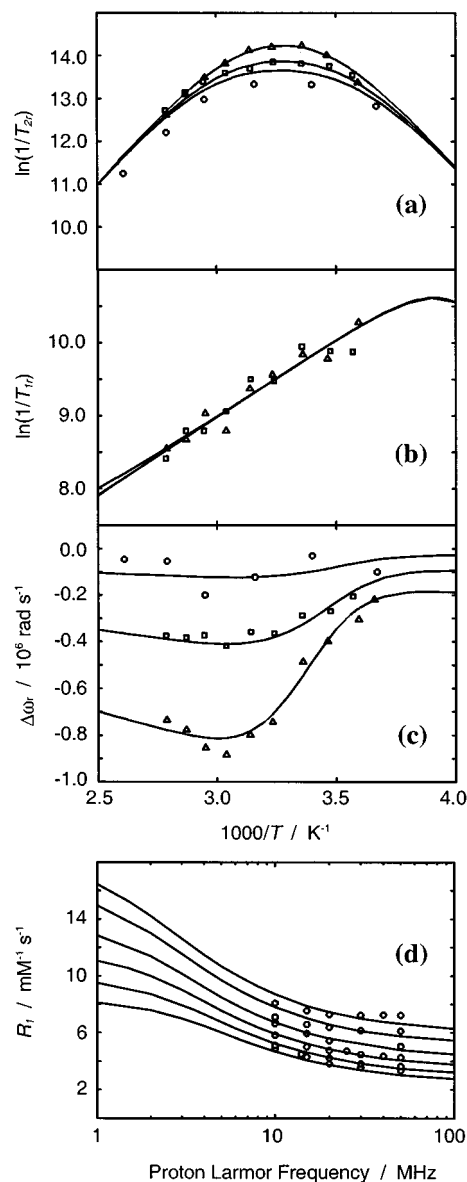
(28) Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Lebedev, Y.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333−9346.
(29) Rast, S.; Borel, A.; Helm, L.; Belorizky, E.; Fries, P. H.; Merbach, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 2637−2644.
(30) Dubost, J. P.; Leger, J. M.; Langlois, M. H.; Meyer, D.; Schaefer, M. *C. R. Acad. Sci. Paris* **1991**, *312*, 349−354.
(31) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4559−4570.
(32) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546−4559.

Figure 4. Temperature dependence of reduced ^{17}O transverse (a) and longitudinal (b) relaxation rates at 9.4 (△), 4.7 (□), and 1.41 T (○); (c) reduced ^{17}O chemical shift 9.4 (△), 4.7 (□), and 1.41 T (○); (d) NMRD profiles at (from top to bottom) 277, 283, 291, 298, 305, and 312 K. In all cases, the lines represent the fit obtained from the simultaneous analysis of all data reported and a Gd(III)$-$H$_{water}$ distance of 3.21 Å.

τ_{RO} are short on the NMR time scale ($\sim$100 ps) the model-free approach of Lipari and Szabo (L$-$S) is unable to distinguish between an internal motion of the water molecule or a faster isotropic tumbling of the Ln$-$H vector compared to the Ln$-$O vector. Therefore, we assumed an isotropic tumbling and used a single adjustable parameter (the τ_{RH}/τ_{RO} ratio) instead of two (S^2 and τ_l) as in the L$-$S equations. The case of macromolecular complexes, where the L$-$S approach is required to account for local motions of the chelate, is discussed later in the text (see Discussion).

Discussion

In the Curie experiments, the principal source of error is the Ln(III)$-$H distance as r^6 is taken into account. Fortunately, the distances reported for DOTA derivative complexes with diverse lanthanides differ by less than 1%, which means an error of

Table 1. Ln(III)−O$_{water}$ and Ln(III)−H$_{water}$ Rotational Correlation Times Calculated As Described in the Text

	Curie relaxation[a]		quadrupolar relaxation[b]		multifit Gd(III)[c]	
	A[d]	B[e]	A[d]	B[e]	A[d]	B[e]
τ_{RO}/ps	101 ± 12	101 ± 12	48.6 ± 3	48.6 ± 3	105 ± 12	105 ± 12
τ_{RH}/ps	117 ± 20	60 ± 10	41.5 ± 5	42.7 ± 5	68 ± 3	37 ± 2
τ_{RH}/τ_{RO}	1.2 ± 0.3	0.6 ± 0.2	0.85 ± 0.15	0.88 ± 0.15	0.65 ± 0.1	0.35 ± 0.1

[a] Rotational correlation times at 260 K, in a CD$_3$CN/H$_2$O 100:1 mixture. [b] Rotational correlation times at 298 K, in a CD$_3$CN/H$_2$O 100:1 mixture. At 260 K, τ_{RO} = 108 ± 20 ps (see text). [c] Rotational correlation times at 298 K, in H$_2$O. [d] Calculated with the model **A** from Figure 2. [e] Calculated with the model **B** from Figure 2.

less than 6% on the rotational correlation time of the cyclen protons. Furthermore, due to the "rigidity" of the DOTA cage, the Ln(III)−H distances in solution for the cyclen protons should be the same as the crystallographic values. The choice of limiting cases with Ln(III)−H$_{water}$ distances of 2.95 and 3.21 Å seems reasonable since 3.0−3.1 Å is the accepted value for most of the reported ^{1}H NMRD analysis.[1] Furthermore the Ln(III)−O$_{water}$ distance fixed to the crystallographic value (r_{LnO} = 2.44 Å) is justified in view of XAFS experiments in water solution on the [Gd(DOTA)(H$_2$O)]$^-$ complex, which afforded a Ln(III)−O$_{water}$ distance r_{LnO} = 2.447 Å[33] essentially equal to the crystal structure distance r_{LnO} = 2.458 Å.[30]

The values for $\chi_O(1 + \eta_O^2/3)^{1/2}$ are smaller than the 7.58 MHz obtained for the bulk water in acidic medium[34] and much smaller than the one fitted in the simultaneous analysis of EPR, ^{17}O NMR, and ^{1}H NMRD data of Gd(III) chelates:[28] $\chi_O(1 + \eta_O^2/3)^{1/2}$ = 10 MHz for DOTA,[4−] 14 MHz for DTPA[5−], and even 18 MHz for DTPA−BMA[3−] (DTPA = *N,N,N′,N″,N″*-pentacarboxymethyl-1,4,7-triazapentane; DTPA−BMA = *N,N″*-bis[(*N*-methylcarbamoyl)methyl)]-*N,N′,N″*-triscarboxymethyl-1,4,7-triazapentane). Leyte et al. measured $\chi_O(1 + \eta_O^2/3)^{1/2}$ = 6.5 ± 0.4 MHz for the bound water molecule in aqueous Mg(II).[27] If we consider that the electric field gradient produced by the metallic cation and felt by the oxygen atom is proportional to the charge of the cation (*q*) and to 1/r^3, where *r* is the metal−oxygen distance, we can estimate the [17]O quadrupolar coupling constant for the bound water molecule in aqueous Gd(III): $\chi_O(1 + \eta_O^2/3)^{1/2}$ = 5.7 ± 0.4 MHz, which is very similar to our value ($\chi_O(1 + \eta_O^2/3)^{1/2}$ = 5.2 ± 0.5 MHz).

The structural difference of the compounds with D$_2$O or H$_2$O is negligible in the analysis with the model of Woessner. The approximation of an internal motion only due to the rotational diffusion neglects the tilting motions that certainly take place. In principle, the comparison of the internal correlation time τ_{Ri} and the lifetime of the hydrogen bonds between the water molecule under study and other water molecules would provide useful information about the nature of the internal motion, since rotation around the metal−oxygen axis certainly requires the breaking of such bonds. However, in the case of [Ln(DOTA)-(H$_2$O)]$^-$, the information one may gather in this manner is very limited. Molecular dynamics simulations of the solvation of [Gd(DOTA)(H$_2$O)]$^-$ [35] have shown that the second shell water molecules (residence time 27.4 ps) are not necessarily hydrogen-bound to the inner-sphere water (14.8% probability only) but rather to the carboxylic oxygens. Nevertheless, for our purpose, we only need a parameter including the information of the various internal tumbling sources that can be compared to the

overall tumbling of the molecule. Unfortunately, the model limits the quantitative determination of the internal motion, with Δ = 52° and an infinitely rapid internal motion ($\tau_{Ri} \ll \tau_{RO}$), the ratio of the rotational correlation times has a minimum value of τ_{RH}/τ_{RO} = 0.82.

The simultaneous analysis of the ^{17}O NMR and ^{1}H NMRD data on the Gd(III) complex including the τ_{RH}/τ_{RO} ratio was performed without the need for a new parameter as the [17]O quadrupolar coupling constant for the bound water molecule was fixed to $\chi_O(1 + \eta_O^2/3)^{1/2}$ = 5.2 MHz. The other parameters obtained are reasonable as compared to the one previously published (see Supporting Information, Table S5).[28] Note that we used the approximate expressions of Powell et al.[28] to describe the electron spin relaxation rates. The more complete theory of Rast et al.[29] is a better choice for future studies. This new approach combines the classical zero-field splitting modulation by molecular vibrations and collisions with a static crystal field modulated by rotation. Since the Gd(III)−O$_{water}$ vector may be seen as an integral part of the coordination polyhedron, it should be safe to use the rotation correlation time τ_{RO} in the description of the electron spin relaxation.

In the case of the Curie relaxation (Tb(III)) or the multifit of the Gd(III) complexes, the relaxation depends directly on the rotational correlation time of the Ln(III)−H$_{water}$ vector and is proportional to 1/r^6.[6] The importance of the difference between the two geometrical models (**A** and **B**) can be explained by the difference of the Ln(III)−H$_{water}$ distances 2.95 and 3.21 Å, r^6_A/r^6_B = 0.6, which is close to the τ_{RH}/τ_{RO} value obtained from the fits (Curie, 0.6/1.2 = 0.5; Multifit Gd(III), 0.35/0.65 = 0.54). On the other hand, the electric field gradient that governs the quadrupolar relaxation of the water deuterium is aligned with the O−D bond. In the analysis of the [17]O and ^{2}H quadrupolar relaxation following the model of Woessner, the Ln(III)−O−D angle plays the main role in the description of the internal motion. The angle Δ is very similar in the two geometrical model (**A** and **B**), and consequently, the τ_{RH}/τ_{RO} ratios are similar as well (0.85 and 0.88).

The Curie method is in principle the best one since we have a direct link between the observable and the rotational correlation times. However, it is unfortunately restricted to a very small class of compounds, namely, those where the bound water proton NMR signal is observable. Furthermore, it is strongly dependent on the accuracy of the Ln−H distance, as can be seen from the aberrant τ_{RH}/τ_{RO} ratio (larger than 1) obtained for model **A**. This shows that the distance of 3.21 Å is certainly overestimated, indicative of a possibly tilted inner-sphere water molecule in solution. For all these reasons, the multifit method with two rotational correlation times offers a convenient alternative, even if the accuracy of the results is inherently less. Notwithstanding the Curie model **A** value (taken as 1.0), the

(33) Benazeth, S.; Purans, J.; Chalbot, M.-C.; Nguyen-van-Duong, M. K.; Nicolas, L.; Keller, F.; Gaudemer, A. *Inorg. Chem.* **1998**, *37*, 3667−3674.
(34) Halle, B.; Wennerstrom, H. *J. Chem. Phys.* **1981**, *75*, 1928−1943.
(35) Borel, A.; Helm, L.; Merbach, A. E. *Chem. Eur. J.* **2001**, *7*, 600−610.

average ratio obtained from the two methods above is 0.65 ± 0.2. The third method, based on the analysis of ^{2}H and ^{17}O quadrupolar relaxation rates, is limited by Woessner's model of motion and thus unable to account for τ_{RH}/τ_{RO} ratios lower than ~0.8 (depending on the exact geometry of the model).

We now address the impact of our findings for macromolecular MRI contrast agents. The problem of global and local motion has been a subject of considerable interest for dynamic NMR in the past decades. In the field of MRI contrast agents, the ^{17}O and ^{1}H NMR study of macromolecular complexes (i.e., chelates bound to proteins, linear polymers, or dendrimers) required using specific approaches, such as the one of Lipari and Szabo,[31,32] to take such effects into account and assess their influence on relaxivity.[10,36] The Lipari−Szabo model-free approach expresses the spectral densities $J(\omega)$ as functions of the global correlation time of the macromolecule τ_g (~1000−10 000 ps), a local correlation time for the chelating group τ_l (typically ~500 ps) and a generalized order parameters S^2 (0.1−0.4 in general), as given in eq 9 for the well-known dipolar relaxation equations of Solomon[37] and Bloembergen.[38]

$$J(\omega_I;\tau_{di}) = \left(\frac{S^2\tau_{dig}}{1 + \omega_I^2\tau_{dig}^2} + \frac{(1 - S^2)\tau_{di}}{1 + \omega_I^2\tau_{di}^2} \right) \quad (9)$$

$$\frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau_g} + \frac{1}{T_{ie}} \qquad i = 1, 2 \quad (10)$$

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m} + \frac{1}{\tau} + \frac{1}{T_{ie}} \quad (11)$$

$$\frac{1}{\tau} = \frac{1}{\tau_g} + \frac{1}{\tau_l} \quad (12)$$

Obviously the global correlation time is not affected by the internal motion of the bound water molecule and remains the same from the point of view of ^{17}O and ^{1}H NMR. The τ_{RH}/τ_{RO} ratio discussed in this paper should only have consequences on τ_l and/or S^2. However, since the Lipari−Szabo approach is model-free, these are only effective parameters and their exact calculation for the water protons and oxygen, respectively, is not easily performed in general. We will now consider two simple cases and assess the consequences of the water internal motion on the Lipari−Szabo parameters τ_l and/or S^2.

For the sake of simplicity, let us assume that the ratio between the proton local correlation time τ_{lH} and the oxygen local correlation time τ_{lO} is the same as the τ_{RH}/τ_{RO} ratio for the free DOTA-like complexes, say 0.66. The order parameter, which characterizes the degree of spatial restriction of the local motion with correlation time τ_l, should be very similar if not equal. As τ_g grows longer, its contribution to the local term of the spectral density will vanish. If we now consider reasonable parameters ($\tau_g = 10^{-8}$ s, $\tau_{lO} = 3 \times 10^{-10}$ s, $\tau_m = 10^{-7}$ s, $T_{1e} \cong T_{2e} = 10^{-8}$ s, $S^2 = 0.3$), the spectral densities are readily calculated at a proton frequency of 50 MHz ($\omega \cong 3 \times 10^{-10}$ s^{-1}). If $\tau_{lH} = \tau_{lO}$, we have $J(3 \times 10^8$ s$^{-1}) = 6.7 \times 10^{-10}$ s. Now if $\tau_{lH} = 0.66 \times \tau_{lO}$, we obtain $J(3 \times 10^8$ s$^{-1}) = 6.0 \times 10^{-10}$ s. The spectral density is only decreased by ~10% when the bound water

internal motion is taken into account. We see that since the global contribution $S^2\tau_{dig}/(1 + \omega_I^2\tau_{dig}^2)$ is not affected by this internal motion, the final effect on macromolecular agents will be less than on the monomers (such as [Gd(DOTA)(H$_2$O)]$^-$) for a reasonable choice of parameters (provided $1 > S^2 > 0$).

Now in the limiting case where $S^2 = 1$ (no local motion of the chelate with respect to the supporting macromolecule), the previous analysis is obviously not valid. Clearly Woessner's model as discussed earlier (or a more elaborated description of the internal motion) is applicable in this case. Since the dominant contribution to the dipolar correlation time τ_{di} is the rotational one, the full effect of the shorter proton rotational correlation time will appear in the spectral density. Thus, a more general approach might be to consider two order parameters S_H^2 and S_O^2 and only one local correlation time. However, the only consistency check that could be performed on these parameters is that S_O^2 must be higher than S_H^2. No further information can be obtained in a model-free approach. To summarize, we can say that the local motion of the chelating group in macromolecular complexes leads to a smaller effect of the bound water internal motion on the spectral densities, and thus on relaxivity (−10% with our choice of parameters). The complex only suffers from the full effect of the reduced proton rotational correlation time (−34%) when the chelating group is rigidly bound to the macromolecular system.

It is now evident that the rotational correlation time associated with the Ln(III)−H$_{water}$ vector is only ~1.5 times shorter than the one associated with the Ln(III)−O$_{water}$ vector. In fact, the internal motion is independently revealed by molecular dynamics simulations on polyaminocarboxylate Gd(III) complexes, which provided a ratio $\tau_{RH}/\tau_{RO} = 0.7$.[39] These results show that the bound water internal motion cannot be the main limiting factor in attaining high relaxivity with Gd(III) complexes.

Conclusion

Taking advantage of the Curie contribution to the relaxation of the protons in the Tb(III) complex, and the quadrupolar relaxation of the ^{17}O and ^{2}H nuclei on the Eu(III) complex, a quantitative estimation of the internal motion of the water molecule bound to Ln^{III}(DOTA-like) complexes was performed. The rotational correlation times for the Ln(III)−H$_{water}$ vector is a factor ~1.5 shorter than the overall correlation time and should consequently not be a limiting factor for attaining high relaxivities with Gd(III) complexes. The determination of the quadrupolar coupling constant $\chi_O(1 + \eta_O^2/3)^{1/2}$ of the bound water oxygen allowed a new analysis of the ^{17}O and ^{1}H NMR data of the [Gd(DOTA)(H$_2$O)]$^-$ complex with different rotational correlation times for the Gd(III)−O$_{water}$ and Gd(III)−H$_{water}$ vectors yielding reasonable parameters. This type of analysis could be generalized and should be very helpful for the understanding of potential Gd(III)-based contrast agents. It can be seen that the bound water internal motion also affects macromolecular complexes, but the final effect when combined with the local motion of the chelating group should be generally less than what is observed for the monomeric species.

(36) Dunand, F. A.; Toth, E.; Hollister, R.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2001**, *6*, 247−255.
(37) Solomon, I. *Phys. Rev.* **1955**, *99*, 559.
(38) Bloembergen, N. *J. Chem. Phys.* **1957**, *27*, 572−573.

(39) Yerly, F.; Hardcastle, K. I.; Helm, L.; Aime, S.; Botta, M.; Merbach, A. E. *Chem. Eur. J.,* in press.

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Supporting Information Available: Experimental field-dependent relaxation rates of selected protons for [Tb(DOTAM)-$(H_2O)]^{3+}$ (Table S1) and [Yb(DOTAM)$(H_2O)]^{3+}$ (Table S2, Figure S1); experimental temperature dependence of the ^{17}O transverse and longitudinal relaxation rates of the bound water of [Eu(DOTAM)$(H_2O)]^{3+}$ (Table S3); experimental temperature dependence of the ^2H longitudinal relaxation rates of the bound and free water of [Eu(DOTAM)$(H_2O)]^{3+}$ (Table S4, Figure S3);

temperature dependence of the quadrupolar contribution to the transverse relaxation rate of the bound water ^{17}O of [Eu-(DOTAM)$(H_2O)]^{3+}$ (Figure S2); Parameters from the simultaneous analysis of the ^{17}O and ^1H NMR data of [Gd(DOTA)-$(H_2O)]^-$ (Table S5); calculation of the Curie contribution to the ^{17}O transverse relaxation rate of the [Eu(DOTAM)$(H_2O)]^{3+}$ inner-sphere water molecule. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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