

The Gadolinium(III)–Water Hydrogen Distance in MRI Contrast Agents

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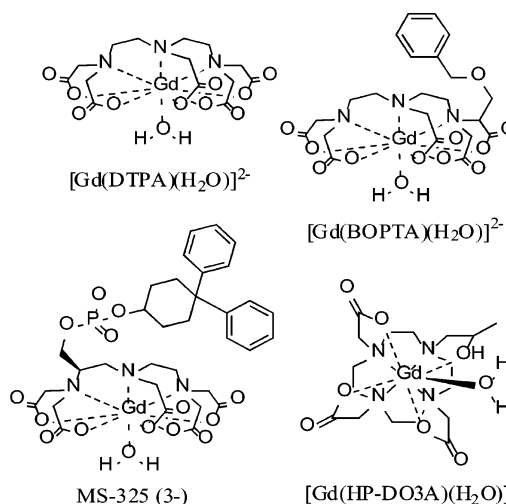
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The ion–nuclear distance of Gd(III) to a coordinated water proton, $r_{\text{Gd-H}}$, is central to the understanding of the efficacy of gadolinium-based MRI contrast agents. The dipolar relaxation mechanism operative for contrast agents has a $1/r^6$ dependence. Estimates in the literature for this distance span 0.8 Å (2.5–3.3 Å). This study describes a direct determination of $r_{\text{Gd-H}}$ using the anisotropic hyperfine constant T_1 determined from pulsed ENDOR spectra. Five Gd(III) complexes were examined: $[\text{Gd}(\text{H}_2\text{O})_8]^{3+}$, $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, $[\text{Gd}(\text{BOPTA})(\text{H}_2\text{O})]^{2-}$, MS-325, and $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$. The distance, $r_{\text{Gd-H}}$, was the same within error for all five complexes: 3.1 ± 0.1 Å. These distance estimates should aid in the design of new contrast agents, and in the interpretation of other molecular factors influencing relaxivity.

Gadolinium-based MRI contrast agents function by catalytically relaxing water protons. Contrast agents typically contain one inner-sphere water molecule (see Chart 1) that is in fast exchange with bulk solvent. The relaxivity, r_1 , is the extent to which the ion can change the relaxation rate of solvent protons. Increased relaxivity allows the contrast agent to be administered at a lower dose or enables the imaging of low-concentration targets and is the focus of extensive academic and industrial research.¹ Relaxivity depends on the electronic properties of the ion, the number of water molecules in the inner and second coordination spheres, the ion–hydrogen distance and the mean lifetime of these waters, and the rotational diffusion of the complex (Supporting Information). There is increased understanding of the various parameters² that affect relaxivity, and this knowledge has been applied to the design of improved contrast agents such as MS-325^{2c} (EPIX Medical and Schering AG), yet the ion–water proton distance remains poorly understood. For inner-sphere water, the relaxation mechanism is predominantly

Chart 1



dipolar and has a $1/r^6$ dependence where r is the Gd–H distance.¹ This distance, $r_{\text{Gd-H}}$, is critical to the correct interpretation of relaxivity data, and to the design of improved contrast agents. Muller and co-workers^{3a,b} state that the Gd–H distance is reduced in derivatives of $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ such as $[\text{Gd}(\text{BOPTA})(\text{H}_2\text{O})]^{2-}$, MS-325, and $[\text{Gd}(\text{EOB-DTPA})(\text{H}_2\text{O})]^{2-}$ relative to $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ itself by as much as 0.3 Å, despite the fact that the Gd–O_{water} distance is very similar for these four compounds (2.45–2.50 Å). Cohen et al.^{3c} report $r_{\text{Gd-H}}$ values ranging from 2.95 to 3.10 Å for a series of similar tripodal complexes. These studies imply that the Gd–H distance may be optimized to produce compounds of higher relaxivity.

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(1) (a) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293. (b) *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; Wiley: New York, 2001. (c) *Topics in Current Chemistry*; Krause, W., Ed.; Springer-Verlag: Berlin, 2002; Vol. 221.

(2) Representative examples. (a) Increasing inner-sphere water: Xu, J.; Franklin, S. J.; Whisenhunt, D. W., Jr.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, *117*, 7245. (b) Effect of water exchange: Powell, D. H.; Ni Dhubghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333. (c) Effect of rotation: Caravan, P.; Cloutier, N. J.; Greenfield, M. T.; McDermid, S. A.; Dunham, S. U.; Bulte, J. W. M.; Amedio, J. C., Jr.; Looby, R. J.; Supkowski, R. M.; Horrocks, W. DeW., Jr.; McMurry, T. J.; Lauffer, R. B. *J. Am. Chem. Soc.* **2002**, *124*, 3152. (d) Electronic relaxation: Rast, S.; Borel, A.; Helm, L.; Belorizky, E.; Fries, P. H.; Merbach, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 2637. (3) (a) Vander Elst, L.; Maton, F.; Laurent, S.; Seghi, F.; Chapelle, F.; Muller, R. N. *Magn. Reson. Med.* **1997**, *38*, 604. (b) Muller, R. N.; Raduchel, B.; Laurent, S.; Platzeck, J.; Pierart, C.; Mareski, P.; Vander Elst, L. *Eur. J. Inorg. Chem.* **1999**, 1949. (c) Cohen, S. M.; Xu, J.; Radkov, E.; Raymond, K. N.; Botta, M.; Barge, A.; Aime, S. *Inorg. Chem.* **2000**, *39*, 5747.

Reported $r_{\text{Gd-H}}$ values range from 2.5 to 3.3 Å,⁴ with values of 2.90–3.13 Å more common. Often these distances are determined³ indirectly by fitting Gd(III) enhanced proton relaxation rate data. There is some ambiguity in this approach because $r_{\text{Gd-H}}$ is correlated with the correlation time, τ_c , and relaxation arising from waters in the second and outer spheres. Other workers^{1,2} have used X-ray structures by taking the Gd–O distance and either assuming H positions or locating the H atoms within the structure. Distances determined from H locations using X-ray diffraction may be biased short because the electron density is distributed along the O–H bond and does not reside at the H nucleus.⁵ Clarkson et al.⁶ used electron spin–echo envelope modulation spectroscopy (ESEEM) to estimate rather short Gd–D_{water} distances for the DTPA and EDTA complexes of Gd in D₂O ($r_{\text{Gd-D}} \sim 2.7$ Å). Similarly, Yim and Makinen^{4a} reported short $r_{\text{Gd-H}}$ (2.5–2.9 Å) in a continuous wave (CW) electron–nuclear double resonance (ENDOR) study of Gd(III) complexes in glassy water–methanol solutions. However, both studies assumed that the analysis for a Gd(III) complex ($S = 7/2$) can be made in the same manner as for a $S = 1/2$ ion. This assumption was recently shown to be incorrect,⁷ casting doubt on the validity of these short distances.

ENDOR studies of Gd(III) doped into single-crystal hosts (lanthanum ethylsulfate^{8a} and lanthanum nicotinate^{8b}) revealed that the hyperfine interaction (hfi) tensor of the coordinated water protons has a nearly axial symmetry, with the isotropic hfi constant a_{iso} being close to zero ($|a_{\text{iso}}| < 0.25$ MHz). This indicates that the spin density delocalization on the water proton is negligible, and the proton anisotropic hfi is primarily determined by the dipole interaction with the electron spin on the Gd³⁺ ion. The anisotropic hfi constant T_{\perp} can be used as a direct measure of the Gd–H distance $r_{\text{Gd-H}}$:⁹

$$r_{\text{Gd-H}} \approx \sqrt{\frac{3g_e\beta_e g_n\beta_n\rho}{hT_{\perp}}} \quad (1)$$

Encouraged by the single crystal work, systematic pulsed ENDOR studies of Gd-based MRI contrast agents (Chart 1) were initiated. The DTPA complex and two DTPA derivatives were chosen to determine if DTPA substitution and/or

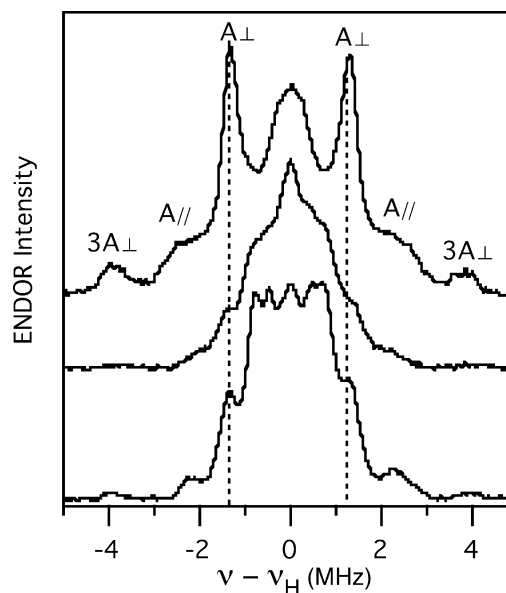


Figure 1. Mims ENDOR spectra of $[\text{Gd}(\text{H}_2\text{O})_8]^{3+}$ (top), MS-325 (middle), and $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$ (bottom) in frozen solutions (4.2 K); mw frequency = 14.411 GHz; mw pulses, 3×10 ns; $\tau = 130$ ns; $T = 30$ s; rf pulse duration, 21 μs ; rf power, 800 W; $\mathbf{B}_0 = 5180$ G (maximum of $1/2 \leftrightarrow -1/2$ transition in EPR spectrum).

total charge affected $r_{\text{Gd-H}}$. $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$, a neutral macrocyclic complex, was chosen as an example of a different ligand geometry and charge. The aquo ion was also examined. The experiments were performed for frozen solutions of 10 mM Gd complex in 1:1 (v/v) H₂O/CD₃OH (methanol added for glassification).

Figure 1 (upper trace) shows the Mims ENDOR¹⁰ spectrum of the aquo ion. The central peak of the spectrum arises from distant matrix protons. The peaks labeled “A_⊥” originate from water ligand protons and correspond to the perpendicular orientation of the $\mathbf{r}_{\text{Gd-H}}$ vector with respect to the direction of magnetic field \mathbf{B}_0 . These lines belong to proton transitions within the $m_S = \pm 1/2$ electron spin manifolds. The lines of similar transitions within the $m_S = \pm 3/2$ manifolds are marked “3A_⊥” (see Supporting Information for assignment details, and the possible effects of methanol coordination). The lines of the $\pm 5/2$ and $\pm 7/2$ manifolds have lower intensity and are outside the shown frequency range.

Model simulations for various orientations of the hfi axis with respect to the crystal field frame revealed that for $D/g\beta B_0 \sim 0.1$ the positions of A_⊥ lines are not sensitive to the crystal field whereas the A_∥ lines, corresponding to $\mathbf{r}_{\text{Gd-H}}/\mathbf{B}_0$ (observed in the spectrum of the Gd aquo ion), are much more sensitive to the crystal field strength, and a realistic model for the crystal field parameters should be used when interpreting these lines. Numerical simulations of the ENDOR spectrum (Supporting Information) allow one to estimate $a_{\text{iso}} \approx -0.1$ MHz and $T_{\perp} \approx -2.58$ MHz. To provide a correct width of the A_⊥ features, either a_{iso} or T_{\perp} had to be Gaussian distributed, with a distribution width of 0.4 MHz. This width reflects a scattering of the hfi parameters that was also observed in studies of Gd(III) in host single crystals.⁸ Ascribing this width solely to T_{\perp} results in $r_{\text{Gd-H}}$

(4) (a) 2.5 Å: Yim, M. B.; Makinen, M. W. *J. Magn. Reson.* **1986**, *70*, 89. (b) 3.3 Å: Koenig, S. H.; Brown, R. D. *Prog. NMR Spectrosc.* **1990**, *22*, 487.

(5) Churchill, M. R. *Inorg. Chem.* **1973**, *12*, 1213.

(6) Clarkson, R. B.; Hwang, J. H.; Belford, R. L. *Magn. Reson. Med.* **1993**, *29*, 521.

(7) Atashkin, A. V.; Raitsimring, A. M. *J. Chem. Phys.* **2002**, *117*, 6121.

(8) (a) DeBeer, R.; Biesboef, F.; Van Ormondt, D. *Physica* **1976**, *83B*, 314. (b) Fields, R. A.; Hutchinson, C. A., Jr. *J. Chem. Phys.* **1985**, *82*, 1711.

(9) Here g_e = electron g -factor, g_n = proton g -factor, β_e = Bohr magneton, β_n = nuclear magneton, ρ = spin density on Gd, h = Planck's constant. Equation 1 assumes $\rho = 1$; to the best of our knowledge, the direct measurement of spin density transfer from Gd³⁺ to oxygen was not reported; however, ¹⁹F ENDOR measurements for a similar system, Eu²⁺(f⁷):CaF₂ (Ranon, U.; Hyde, *J. Phys. Rev.* **1966**, *141*, 259), show that the spin density on a F ligand does not exceed 0.1%. Therefore, even for a 9-coordinated f-ion the total spin density delocalization would not exceed 1% and can be neglected.

(10) Mims, W. B. *Proc. R. Soc. London* **1965**, *283A*, 452.

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distributed within the limits from 3 to 3.2 Å (i.e., $r_{\text{Gd-H}} \approx 3.1 \pm 0.1$ Å).

ENDOR spectra of MS-325 and [Gd(HP-DO3A)(H₂O)] are also shown in Figure 1. Spectra of [Gd(BOPTA)(H₂O)]²⁻ and [Gd(DTPA)(H₂O)]²⁻ were identical with that of MS-325 (Supporting Information). In MS-325 the A_{\perp} lines of the water ligand protons have much smaller intensity because only one coordination site is accessible for the solvent. Still, these lines are readily observable and the splitting between them is the same as that between the A_{\perp} lines in the upper trace. The A_{\perp} lines for [Gd(HP-DO3A)(H₂O)] are more intense, likely, because of the contribution of the OH proton from the macrocyclic ligand that resides at the same distance from Gd as protons of the water ligand (see Chart 1). The A_{\parallel} lines of the water ligand protons in the spectra of the contrast agents are not readily observable because of greater crystal field strength and because the $3A_{\perp}$ lines from second-sphere protons contribute in this region (A_{\perp} lines for these protons ~ 0.9 and ~ 1.6 MHz). However, a_{iso} for the water ligand protons in the contrast agents should be similar to that in the aquo ion and also relatively small because of poor overlap of the Gd f-orbitals with the OH bond orbitals. Therefore, the distance estimate for the studied contrast agents is the same as that for the aquo ion, $r_{\text{Gd-H}} \approx 3.1 \pm 0.1$ Å. The uncertainty in the positions of maxima of weakly resolved A_{\perp} lines in MS-325 (~ 0.1 MHz) does not affect this estimate because it is significantly smaller than the T_{\perp} distribution width used to obtain the range of $r_{\text{Gd-H}}$ variation.

The $r_{\text{Gd-H}}$ value found for the aquo ion agrees very well with solution neutron diffraction studies of the aquo ions of Sm(III) and Dy(III) in D₂O.¹¹ MS-325, [Gd(BOPTA)(H₂O)]²⁻, and [Gd(DTPA)(H₂O)]²⁻ have almost identical ENDOR spectra and $r_{\text{Gd-H}}$. Contrary to other reports,^{3a,b} the $r_{\text{Gd-H}}$ values determined here suggest that there is little influence of the coligand substitution pattern, coligand type, or total charge on the Gd(III)–water hydrogen distance. Differences in relaxivities among these compounds are primarily due to different rotational correlation times because of differing size/charge; there may also be differences in second-sphere relaxivity. Further studies are planned at higher operational frequencies to detect A_{\parallel} features and involving other nuclei (¹⁷O, ¹⁵N) in addition to protons to better understand the electronic structure of the complexes and possibly improve the distance estimate.

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Supporting Information Available: Experimental conditions, additional spectra, simulations, and effect of distance on relaxivity. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(11) Cossy, C.; Helm, L.; Powell, D. H.; Merbach, A. E. *New J. Chem.* **1995**, *19*, 27.