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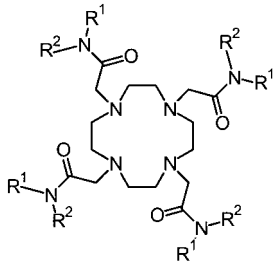
The Amide Protons of an Ytterbium(III) dota Tetraamide Complex Act as Efficient Antennae for Transfer of Magnetization to Bulk Water**

Shanrong Zhang, Lydie Michaudet, Shawn Burgess, and A. Dean Sherry*

Current diagnostic contrast agents (CAs) for magnetic resonance imaging (MRI) are largely based on paramagnetic gadolinium complexes that shorten the relaxation time of bulk-water protons in tissue by rapid exchange of at least one gadolinium-bound inner sphere water molecule with bulk solvent.[1] Recently, Balaban et al.[2] demonstrated that image contrast can be altered by applying a frequency-selective RF pulse at the resonance frequency of an NH or OH group of an intrinsic amino acid, sugar, nucleotide, or other metabolite prior to collection of the imaging data. An advantage of a chemical exchange saturation transfer (CEST) agent over a paramagnetic relaxation agent is that image contrast can be switched on and off at will. A disadvantage is that the amount of CEST agent required to produce significant water contrast is unrealistically high,[3] although a later report demonstrated that the CEST effect can be amplified considerably by using polymers that contain a large number of amide NH groups.[4] As the chemical shifts of diamagnetic NH or OH protons are typically within 5 ppm of that of bulk water, it may ultimately prove difficult to avoid off-resonance direct saturation of the bulk-water signal or indirect saturation via water tightly bound to tissue macromolecules. The latter effect provides the basis of magnetization-transfer (MT) imaging.[5]

We recently reported that the weakly paramagnetic complex $[Eu(\mathbf{2})]^{3+}$, which has a bound-water signal near $\delta =$ 50 ppm with an exchange lifetime of $\tau_M^{298} \approx 350$ μs, acts as an MT contrast agent.[6] Aime et al.[7] also demonstrated that the MT effect can be used to measure pH by using two different exchange sites (OH in $[Eu(\mathbf{3})]^-$ and NH in $[Yb(\mathbf{3})]^-$) to eliminate the concentration dependence. Although paramagnetic systems offer the advantage over diamagnetic systems of having exchangeable protons that are shifted well away from the bulk-water signal,[2, 3] they suffer from a similar lack of sensitivity. One way to increase the sensitivity of a paramagnetic MT agent and thereby make it more practical would be to increase the number of exchangeable protons[4] at a hyperfine-shifted site. Here the Yb^{3+} complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetamide (**1**), which has eight exchangeable, hyperfine-shifted amide protons, is reported as a prototype high-sensitivity MT agent. The eight amide protons of this complex should in principle allow a fourfold reduction in concentration compared to an agent with a single exchangeable bound water molecule.[8]



$R^1 = H_a,\ R^2 = H_b$ **1**
$R^1 = H,\ R^2 = CH_2COOEt$ **2**
$R^1 = H,\ R^2 = CH_2COO^-$ **3**

A crystal of $[Yb(\mathbf{1})(H_2O)](CF_3SO_3^-)_3 \cdot 4H_2O$ was grown from water at room temperature and studied by X-ray diffraction at 153 K (Figure 1).[9] The geometry around the Yb^{3+} ion is a typical square antiprism with average N-C-C-N and N-C-C-O torsion angles of 58.3 and $-22.5°$, respectively. The Yb^{3+} ion is nine-coordinate with average macrocyclic Yb–N and Yb–O bond lengths of 2.608 and 2.301 Å, respectively, and a Yb^{3+}–O_{water} distance of 2.335 Å. The Yb^{3+} – macrocyclic ligand distances are similar to those

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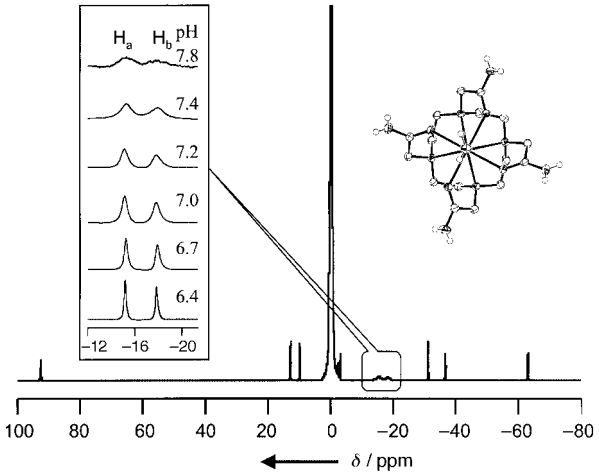

Figure 1. 500 MHz ^{1}H NMR spectrum of a 30 mM aqueous solution of $[Yb(\mathbf{1})]^{3+}$ at pH 7.4 and 25 °C ($\delta_{bulk\,water} = 0$ ppm). The inset shows the signals of H_a and H_b as a function of pH. A top view of the crystal structure of $[Yb(\mathbf{1})]^{3+}$ is also shown. For clarity, four additional water molecules and three triflate anions are omitted.

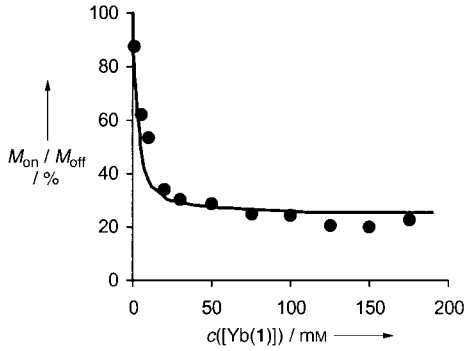

Figure 2. A plot of M_{on}/M_{off} versus concentration of $[Yb(\mathbf{1})]^{3+}$ at 25 °C and pH 7.4. The solid line through the data represents the fit to Equation (1) (see also text). Data were obtained with a Varian Inova-300 NMR spectrometer by simultaneous saturation of both amide protons with a modified water-elimination technique (wet1d pulse sequence, see Varian User Manual). Loops were added to the shaped pulse train (90° e-burp1, typically repeated 0–500 times) at a frequency offset corresponding to the chemical shift of the amide protons to yield a 1500 Hz square bandwidth (pwwet = 3.0 ms and wetpwr = 28 db).

reported for another Yb^{3+} tetraamide complex ($\langle Yb{-}N\rangle$ 2.62 Å and $\langle Yb{-}O\rangle$ 2.28 Å) while the $Yb{-}O_{water}$ distance is significantly shorter ($Yb{-}O_{water}$ 2.44 Å).[10] The high-resolution ^{1}H NMR spectrum of $[Yb(\mathbf{1})]^{3+}$ in solution at pH 7.4 and 25 °C (Figure 1) is consistent with its solid-state structure (see Supporting Information). The spectrum is dominated by one axially symmetric coordination isomer[11] with two magnetically nonequivalent amide protons on each NH_2 group, which appear as two broad peaks at $\delta = -14.5$ and -17.7 ppm. The inset illustrates the different sensitivity of the amide proton linewidths to pH.

Table 1 summarizes the lifetimes τ_M^{298}, as determined by fitting the linewidths at each temperature to standard exchange theory. Interestingly, the exchange lifetimes of the amide protons differed only slightly ($\tau_M^{298}(H_a) \approx 1.3\,\tau_M^{298}(H_b)$). Table 1 shows that both sites meet the slow-exchange condition, $\Delta\omega\,\tau_M^{298} > 1$, at all pH values and should therefore act as efficient antennae for transferring magnetization to bulk water.

Table 1. Amide proton lifetimes τ_M^{298} versus pH for $[Yb(\mathbf{1})]^{3+}$. Experimental values of $\Delta\omega\,\tau_M$ at 7.05 T are also listed.

pH	τ_M^{298} [ms]		$\Delta\omega\,\tau_M^{298}$	
	H_a	H_b	H_a	H_b
6.46	5.00	4.00	139.3	133.7
6.76	2.30	1.78	64.1	59.5
7.06	1.21	0.82	33.6	27.2
7.21	0.92	0.72	25.5	24.2
7.43	0.64	0.52	17.9	17.4
7.84	0.57	0.44	15.9	14.7

A plot of M_{on}/M_{off} (simultaneous saturation at both NH sites) versus concentration at pH 7.4 and 25 °C is shown in Figure 2. Bulk-water magnetization in the presence (M_{on}) or absence (M_{off}) of selective RF irradiation at an exchange site is given by Equation (1),[12] where k_{obs} is the pseudo-first-order

$$\frac{M_{on}}{M_{off}} \approx \left(\frac{1}{1 + k_{obs}\,T_{1sat}}\right) \qquad (1)$$

exchange rate (n[CA]/(110 τ_M^{NH}), where n is the number of exchanging sites),[13] and T_{1sat} is the spin-lattice relaxation time of the bulk-water protons during saturation of the exchanging NH protons; T_{1sat} also depends on the concentration of paramagnetic agent and the exchange lifetime of the amide proton(s) being saturated. If one makes the simplifying assumption that $T_{1sat}^{-1} \approx T_{1obs}^{-1} = r_1[CA] + T_{1dia}^{-1}$ (where r_1 is the water relaxivity (mM^{-1}s^{-1}) of $[Yb(\mathbf{1})]^{3+}$ and T_{1dia} is the spin-lattice relaxation time of water in the absence of $[Yb(\mathbf{1})]^{3+}$), then it is easy to show that M_{on}/M_{off} becomes independent of [CA] at high concentrations. This indicates that M_{on}/M_{off} will be dominated by three parameters: the number of exchangeable sites n, the exchange lifetime τ_M, and the paramagnetic relaxivity r_1 when $r_1[CA] \gg T_{1dia}^{-1}$. This is experimentally shown in Figure 2: M_{on}/M_{off} is sensitive to the concentration of $[Yb(\mathbf{1})]^{3+}$ below about 30 mM but nearly independent of concentration above about 50 mM. Fitting this theory to the data of Figure 2 gave $r_1 = 0.04$ mM^{-1}s^{-1}, $T_{1dia} = 3.0$ s, and $\tau_M^{NH} = 0.58$ ms, with an agreement factor of 0.90. The low r_1 value is consistent with Yb^{3+} having a much lower magnetic moment than Gd^{3+}. The fitted value of τ_M^{NH} is identical to the average value of τ_M^{NH} for the two H_a and H_b sites determined by variable-temperature NMR spectroscopy (Table 1) and similar to those estimated for $[Eu(\mathbf{3})]^{3+}$ ($\tau_M^{NH} \approx 0.1$–10 ms in the pH range 3–9 at 25 °C (unpublished data)) and for other $[Ln(\mathbf{3})]^-$ complexes ($\tau_M^{NH} \approx 0.29$–0.40 ms at 37 °C and pH 8.1, $Ln^{3+} = Ho^{3+}$, Er^{3+}, Tm^{3+}, and Yb^{3+}).[7]

The pH dependence of M_{on}/M_{off} with selective presaturation of either H_a, H_b, or both is shown in Figure 3 for 30 mM $[Yb(\mathbf{1})]^{3+}$. At the minimum of these curves, about 52 and 42% of the bulk-water signal was eliminated upon saturation of H_a or H_b alone, respectively, while about 70% was eliminated upon simultaneous saturation of both NH groups. This compares to about 13 and 38% decrease in the bulk-water signal with 1 and 5 mM $[Yb(\mathbf{1})]^{3+}$, respectively (Figure 2). Large MT effects have been reported for macromolecules: about 43% saturation of bulk water was achieved with 0.1 mM poly-L-lysine (corresponds to 467 mM of NH groups) and 51%

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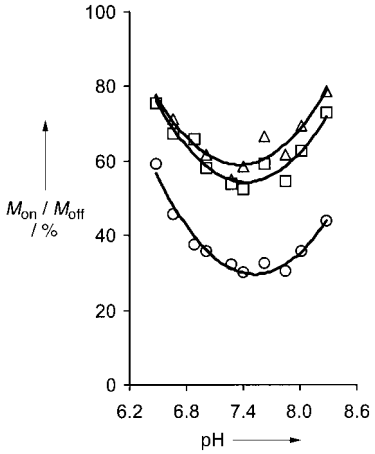

Figure 3. A plot of M_{on}/M_{off} versus pH for $[Yb(\mathbf{1})]^{3+}$ at 30 mM and 25 °C. Data were obtained on the same instrument (see Figure 2 caption), by using a RF bandwidth of 600 Hz for saturating H_a or H_b (pwwet = 7.5 ms and wetpwr = 20 db) and 1500 Hz for saturating both simultaneously. The symbols □, △, and ○ represent data points for saturating H_a, H_b, or both, respectively.

with 1 mM PAPAM dendrimer (256 mM of NH groups),[4] but $[Yb(\mathbf{1})]^{3+}$ appears to exhibit even more efficient MT simply on the basis of these concentration comparisons. The shape of the curves of Figure 3 reflects the sensitivity of the MT effect to proton exchange. This, in combination with the τ_M^{298} values of Table 1, leads to the conclusion that $\Delta\omega\tau_M$ values in the range 15–18 are optimal for this system. To check the general validity of this value, we also measured the pH dependence of τ_M^{298} and M_{on}/M_{off} for $[Eu(\mathbf{3})]^-$, a complex with monosubstituted amido groups containing only four NH protons, with a chemical shift of $\delta = -6$ ppm. Here again, we found that $\Delta\omega\tau_M \approx 15$ was optimal (Figure 4). This was further confirmed by saturating the resonance of the Eu^{3+}-bound water in $[Eu(\mathbf{3})]^-$ at 4.7 T.[14]

In summary, the eight exchangeable amide protons in $[Yb(\mathbf{1})]^{3+}$ appear as two magnetically inequivalent signals at $\delta = -14.5$ and -17.7 ppm. These could be saturated by using selective RF pulses, either separately or simultaneously, to yield an easily measurable MT effect at concentrations similar to that used for other clinical MRI contrast agents. Most importantly, this study revealed in two different paramagnetic complexes, $[Yb(\mathbf{1})]^{3+}$ and $[Eu(\mathbf{3})]^-$, with similar τ_M^{NH} but different $\Delta\omega$ values optimal MT efficiency for $\Delta\omega\tau_M$ values near 15. This provides a useful guideline for developing even more efficient MT agents.

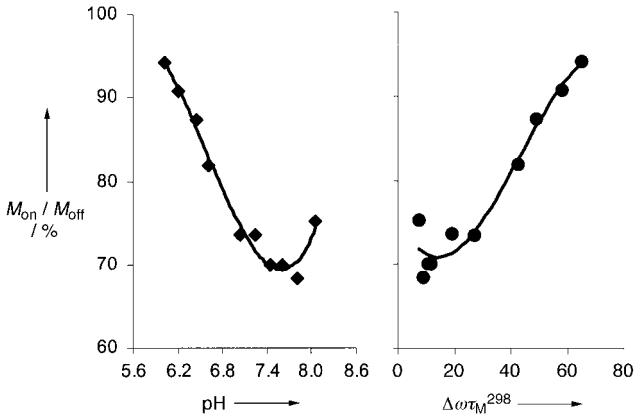

Figure 4. A plot of M_{on}/M_{off} versus pH (left) and versus $\Delta\omega\tau_M^{298}$ (right) for $[Eu(\mathbf{3})]^-$ at 30 mM and 25 °C. Data were obtained on the same instrument (see Figure 2 caption) by using a RF irradiation (with a bandwidth of 300 Hz, pwwet = 15 ms and wetpwr = 16 db) centered at the resonance of four amide protons (single peak at $\delta \approx -6$ ppm). A low saturation power was used to avoid any direct off-resonance saturation.

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[8] $[Eu(\mathbf{2})]^{3+}$ has four exchangeable amide protons but the chemical shift of those protons (−5 ppm upfield of bulk water) offers no advantage over diamagnetic agents.

[9] X-ray data for $[Yb(C_{16}H_{32}H_8O_4)(H_2O)](CF_3SO_3^-)_3 \cdot 4H_2O$: $M = 1110.83$, triclinic, space group $P\bar{1}$, $\rho_{calcd} = 1.879\ Mg\,m^{-3}$, $Z = 2$, $a = 12.8780(2)$, $b = 12.9861(3)$, $c = 13.0747(3)$ Å, $\alpha = 86.483(1)$, $\beta = 64.549(1)$, $\gamma = 84.192(1)°$, $V = 1963.83(7)$ Å^3, $T = 153$ K. Large, colorless prisms grew by slow evaporation of an aqueous solution of the complex. The crystal was cut from a larger crystal and had approximate dimensions $1.4 \times 0.4 \times 0.24$ mm. The data were collected on a Nonius Kappa CCD diffractometer with a graphite monochromator with $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods by using SIR92 and refined by full-matrix least-squares methods on F^2 with anisotropic displacement parameters for the non-hydrogen atoms by using SHELXL-97. The hydrogen atoms on carbon atoms were calculated in ideal positions with displacement parameters set to $1.2\,U_{eq}$ of the attached atom. The hydrogen atoms bound to the amide nitrogen atoms were located in a ΔF map and refined with isotropic displacement parameters. The hydrogen atoms of the water molecules were also located in a ΔF map. Final residual $wR2 = 0.0760$ for all data and $R1 = 0.0326$. CCDC-175397 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit @ccdc.cam.ac.uk).

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