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Optimized Relaxivity and Stability of [Gd(H(2,2)-1,2-HOPO)(H₂O)][−] for Use as MRI Contrast Agent¹

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Abstract

Relaxometry and solution thermodynamic measurements show that Gd-H(2,2)-1,2-HOPO is a good candidate as a contrast agent for Magnetic Resonance Imaging (MRI-CA). Acidic, octadentate H(2,2)-1,2-HOPO forms a very stable Gd(III) complex [pGd 21.2 (2)]. The coordination sphere at the Gd(III) center is completed by one water molecule that is not replaced by common physiological anions. In addition, this ligand is highly selective for Gd(III) binding in the presence of Zn(II) or Ca(II).

The symmetric charge distribution of the 1,2-HOPO chelates is associated with favorably long electronic relaxation time $T_{1,2e}$ comparable to those of GdDOTA. This, in addition to the fast water exchange rate typical of HOPO chelates improves relaxivity to $r_{1p} = 8.2 \text{ mM}^{-1} \text{ s}^{-1}$ (0.47 T). This remarkably high value is unprecedented for small molecule, $q = 1$ MRI-CA.

Introduction

Recently, the Eu(III) luminescence sensitizing properties of 1,2-HOPO chelates were discovered.² Ternary complexes of tetradentate 1,2-HOPO ligands support very efficient Eu(III) sensitization with quantum yields as high as $\Phi_{Eu} 21.5 \%$. However, octadentate ligands such as H(2,2)-1,2-HOPO (**1**) (Chart 1), which were investigated for improved aqueous stability³ gave a quantum yield for $[Eu(\mathbf{1})]^-$ of $\Phi_{Eu} 3.6 \%$, almost one order of magnitude lower. This decrease was ascribed to the presence of one water molecule in the first coordination sphere and a change of symmetry at the Eu(III) center. While both properties are unfavorable for highly luminescent Eu(III) chelates, they increase relaxivity of Gd(III) complexes, which can be used as MRI-CA. Particularly, the electronic relaxation time is one of the limiting factors for most of the commercially available MRI-CA. The favorable electronic relaxation times of 1,2-HOPO chelates versus 3,2-HOPO have been recognized previously for hexadentate TREN-1,2-HOPO (**2**).⁴ However, the relatively low selectivity of **2** for Gd(III) over Zn(II) may represent a problem utilizing this compound as MRI-CA.⁵ For this reason relaxometric and solution thermodynamic properties of $[Gd(\mathbf{1})]^-$ have been investigated.

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Supporting Information Available. Additional stability data, figures and tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Results

The $1/T_1$ NMRD profile of a 0.42 mM aqueous solution of $[\text{Gd}(\mathbf{1})]^-$ was recorded at pH 7.4 at 25 and 37 °C (Figure 1). Relaxivities of $[\text{Gd}(\mathbf{1})]^-$ are very high compared to commercial MRI-CA.⁶ The low field relaxivity r_{1p} 15 $\text{mM}^{-1}\text{s}^{-1}$ (25°C) is twice the corresponding value for $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ (r_{1p} 7.8 $\text{mM}^{-1}\text{s}^{-1}$) and ca. 25 % higher than for $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ with (r_{1p} 12 $\text{mM}^{-1}\text{s}^{-1}$). Furthermore, relaxivities at relevant field strengths r_{1p} 8.2 $\text{mM}^{-1}\text{s}^{-1}$ (20 MHz) and r_{1p} 7.9 $\text{mM}^{-1}\text{s}^{-1}$ (60 MHz) are approximately double to those of both $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ and $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$. These relaxivity values, very high considering $[\text{Gd}(\mathbf{1})]^-$ as a small, monohydrated ($q = 1$) complex, can be rationalized by fitting the NMRD profiles to equations for inner- and outer spheres paramagnetic relaxation (Table 1).⁷ The predominant contribution arises from a longer rotational correlation time, τ_R , about twice that of $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$. This implies a rather rigid structure since the increase in size of $[\text{Gd}(\mathbf{1})]^-$ is efficiently transferred into a corresponding increase of τ_R . Compared to other MRI-CA and Gd(III) chelates studied,⁸ Δ^2 is small and τ_V long, which indicates a very favorable longitudinal electronic correlation time T_{1e} of ca. 70 ns at 60 MHz. This is also the case for $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ ($T_{1e} = 10$ ns at 60 MHz), which, however, is limited by a long water residence time τ_M 472 ps.⁸

The relation between the parameters Δ^2 , τ_V , τ_R , τ_M , and relaxivity r_{1p} at high field has been detailed recently.¹⁰ Further improvement of the relaxivity of $[\text{Gd}(\mathbf{1})]^-$ can be achieved by increasing the rotational correlation time τ_R . T_{1e} can be estimated from Δ^2 and τ_V to be significantly longer than for other HOPO chelates reported.¹¹ Thus T_{1e} and τ_M represent optimal values for a significant improvement of r_{1p} to over 100 $\text{mM}^{-1}\text{s}^{-1}$ by slowing rotational tumbling (Figure 2).

Attaching such a small molecule contrast agent to a large and rigid molecule can significantly improve τ_R . For this reason one may take advantage of the association of a MRI-CA to human serum albumin (HSA).¹² However, no interaction of $[\text{Gd}(\mathbf{1})]^-$ with HSA was observed in serum samples. Attachment of a HSA binding moiety to $[\text{Gd}(\mathbf{1})]^-$ is in progress and will be reported.

The most important parameters for evaluating the toxicity of $[\text{Gd}(\mathbf{1})]^-$ are complex stability and selectivity of **1** for Gd(III) binding versus Zn(II) and Ca(II).¹³ The protonation constants³ illustrate the acidity of **1** compared to 3,2-HOPO chelates.¹⁴ The four acidic protonation steps are associated with deprotonation of the 1,2-HOPO moieties while the tertiary nitrogen atoms represent the two basic protonation sites.

Gd(III) binding of **1** versus the benchmark ligand DTPA (pGd 19.11) was monitored by spectrophotometry. Batch titration techniques were employed because of slow protonation kinetics of the complex preventing variable pH titrations. Conditional stability constants were determined at pH 6.0, 7.4, and 9.0 (Table 2). From this data two protonation constants of the Gd(III) complex and $\log\beta$ values could be calculated. The two basic protonation sites of $[\text{Gd}(\mathbf{1})]^-$ are located at the scaffold amines because relaxivities of GdL^- , GdLH , and GdLH_2^+ are nearly identical (Figures 3 and S5). The high solubility of $[\text{Gd}(\mathbf{1})]^-$ (> 0.5 mM) is not affected by protonation at pK_a 9.7 (5). However, the protonation of the second scaffold amine with pK_a 6.9 (3) reduces the solubility to 0.1 mM. Under physiological conditions the monoprotonated, neutral complex GdLH is dominant (75 %) with one proton being shared by the two amine functions in the scaffold.

Zn(II) and Ca(II) binding of **1** is weak (Table 2) as determined by pM values obtained from competition batch titration versus DTPA. The addition of a fourth 1,2-HOPO moiety to the ligand does not improve the stability of the Zn(II) complex resulting in a high selectivity of **1** for lanthanides versus five or six-coordinate transition metals. The difference between pGd

and pZn is 6.7 (3). This is well beyond the ability of the benchmark compound DTPA (4.2) and comparable to more basic 3,2-HOPO chelates.¹⁵

Calcium binding of **1** is stronger than that of DTPA by almost one order. However, the resulting pCa 8.3 (2) indicates that a 13 log unit excess of Ca(II) is required to remove Gd(III) from [Gd(**1**)][−] although the formation of a mixed complex with Ca(II) cannot be excluded.

[Gd(**H1**)] and [Eu(**H1**)] show no affinity for carbonate, biphosphate, and fluoride as established by luminescence spectroscopy (Figure 4) and relaxometry at 60 MHz (Supp. Info), respectively, upon addition of 1100 to 1600 equivalents of anion. The lack of changes in luminescence intensity or relaxation rate observed indicates affinities K_A for all three anions well below 10 M^{-1} . Thus, binding constants of [Eu(**H1**)] are far lower than the already uncritical affinities of Gd-DOTA, Gd-DTPA, and Gd-DTPA-BMA for biphosphate ranging from 100 to 160 M^{-1} .¹¹

Summary and Outlook

[Gd(**1**)][−] reveals excellent properties for utilization as MRI-CA. The very high relaxivity is achieved by optimizing all relevant parameters for this small molecule. Solution thermodynamic assessment predicts very low toxicity based on the high stability of the Gd(III) complex and the excellent selectivity of **1** for Gd(III) over Zn(II) and Ca(II). In addition the low affinity for phosphate, carbonate and protein interactions are valuable for good performance as MRI-CA *in vivo* and clean excretion. Current efforts aim on exploring strategies to reduce rotational tumbling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 $1/T_1$ NMRD profile of a solution (pH 7.4) of $[Gd(1)]^-$ at 25 and 37 C.

Figure 2. Simulation of relaxivity r_{1p} for $[\text{Gd}(\mathbf{1})]^-$ calculated as a function of τ_R using parameters of Table 1 (298 K).

Figure 3.
Calculated speciation of a 0.1 mM solution of $[\text{Gd}(\mathbf{1})]^-$.

Emission at J = 2 band

Figure 4. Obtained emission spectra between 600 and 625 nm (J = 2 band) of $[\text{Eu}(\mathbf{1})]^-$ (black; 17 μM) and after addition of 1100 equivalents of phosphate (blue), 1100 eq. carbonate (green) or 1600 eq. fluoride (blue) at pH 7.4 after 24 hr. equilibration.

Chart 1.
Potential MRI-CA based on 1,2-HOPO chelates.

Table 1

Parameters from refining NMRD profiles of $[\text{Gd}(\mathbf{1})]^-$ at 25 and 37 C. The third column lists those for $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]$ as comparison.⁹

Parameters	$[\text{Gd}(\mathbf{1})]^-$ (298 K)	$[\text{Gd}(\mathbf{1})]^-$ (310 K)	$[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]$ (298 K)
r_{lp} ($\text{mM}^{-1}\text{s}^{-1}$)	8.2	6.3	3.9
20 MHz			
Δ^2 (s^{-2} ; $\times 10^{19}$)	0.9 (1)	1.0 (2)	1.60
τ_{V} (ps)	51 (2)	47 (3)	11
τ_{R} (ps)	157 (6)	107 (2)	77
τ_{M} (ns)	10*	8*	473
q	1*	1*	1
r (Å)	3.0*	3.0*	3.13
a (Å)	4*	4*	-
D (cm^2s^{-1} ; $\times 10^5$)	2.24*	3.0 (1)	2.2

* these parameters were fixed during refinement.

Table 2Metal Binding Constants of **1**.*

$\log\beta_{\text{GdL}}$	21.5 (5)	$\text{pGd}^{9.0}$	21.7 (1)
$\log\beta_{\text{GdLH}}$	31.2 (2)	$\text{pGd}^{7.4}$	21.2 (2)
$\log\beta_{\text{GdLH}_2}$	38.1 (3)	$\text{pGd}^{6.0}$	20.0 (2)
pZn	14.5 (1)	pCa	8.3 (2)

* $\text{pM} = -\log[\text{M}^{\text{free}}]$ in a solution with $c_{\text{L}}^{\text{tot}} 10 \mu\text{M}$ and $c_{\text{M}}^{\text{tot}} 1 \mu\text{M}$ at pH 7.4 or as specified by superscript.