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High Relaxivity and Stability of a Hydroxyquinolinate-Based Tripodal Monoaquagadolinium Complex for Use as a Bimodal MRI/Optical Imaging Agent

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Supporting Information

ABSTRACT: An octadentate ligand based on triazacyclononane and 8-hydroxyquinolinate/phenolate binding units leads to very soluble, highly stable lanthanide complexes. The monoaquagadolinium complex shows a high relaxivity as a result of the unusually long rotational correlation time, fast water exchange rate, and slow electronic relaxation. The ligand also acts as sensitizer of the near-IR luminescence emission of the Yb and Nd ions. It appears as an excellent candidate for use as a bimodal imaging agent.

Gadolinium complexes are widely used to improve contrast in magnetic resonance imaging (MRI), a routine diagnostic examination in modern medicine with high anatomical resolution but poor sensitivity.[1] The efficiency of commercial MRI contrast agents, all based on monoaquagadolinium complexes of poly(aminocarboxylates), is too low for the application of MRI to molecular imaging. The efficiency of a contrast agent is gauged by its relaxivity, which is the enhancement per millimole of added complex of the measured longitudinal relaxation rate of the water protons. Considerable efforts have been devoted in the past years to synthesize very efficient contrast agents in order to improve the MRI sensitivity.[1,2] The relaxivity can be considerably increased through the simultaneous optimization of mainly four molecular properties of the gadolinium Gd^{III} complex, namely, the number q of water molecules coordinating Gd^{III}, the exchange rate k_{ex} of a coordinated water molecule with bulk water, the rotational correlation time τ_r of the complex, and the longitudinal relaxation time T_{1e} of the Gd^{III} electronic spin. Few studies have been directed toward identifying ligand architectures forming very stable lanthanide complexes that both efficiently sensitize the visible or near-IR (NIR) luminescence emission of Nd^{III}, Eu^{III}, Tb^{III}, or Yb^{III} and afford a high relaxivity to Gd^{III}.[3] Such bimodal optical/MRI reporters are of high interest because they combine the high resolution of MRI with the high sensitivity of optical imaging.[4] However, the presence of water molecules coordinating the lanthanide ion, which is required to obtain an efficient contrast agent in the case of gadolinium, is deleterious for the luminescence of the other lanthanide ions, rendering the design of bimodal probes challenging. Lanthanide complexes of quinolinate-based ligands are attracting an increasing number of studies because of their interesting NIR luminescence emission properties.[5] The 8-hydroxyquinolinate-based lanthanide podates are good candidates for the design of NIR-emitting luminescent tags for biomedical application because of their good stability, low cytotoxicity, sizable luminescence quantum yields in water, and ability to interact with proteins.[6]

More recently, we demonstrated that the NIR luminescence emission remains sizable in the lanthanide(III) complexes of the tripodal hydroxyquinolinate ligand thqN-SO_3^{3-} (Scheme 1) that contain two water molecules coordinated to the lanthanide center, rendering multidentate hydroxyquinolinate ligands particularly attractive for the developement of bimodal optical/MRI reporters.[3a] However, we found that the relaxivity (at physiological pH 7.4 and 25 °C) of the gadolinium complex remain lower (5.2 $s^{-1}\cdot mM^{-1}$ at 200 MHz and 5.7 $s^{-1}\cdot mM^{-1}$ at 20 MHz) than expected for a bisaquagadolinium complex probably due to a slow water exchange rate in this complex. Here, we describe the interesting relaxivity, stability and luminescence properties of lanthanide complexes of the octadentate ligand dhqtcn-SO_3^{3-}-containing two hydroxiquinolinate groups and one phenolate group connected by a triazacyclononane core. The triazacyclononane appeared as an attractive ligand scaffold because of its capacity to enhance the solubility and stability of Gd chelates and to yield a fast water exchange rate and a long electronic relaxation time, which are physical properties both favorable to an increase of the relaxivity.[7]

The ligand H_3dhqtcn-SO_3 was synthesized in four steps from commercial 1,4,7-triazacyclononane, Tris-HCl diisopropylethylamine, and 2-benzyloxybenzaldehyde with a overall yield of 12%. The complexes [Ln(dhqtcn-SO_3)] (Ln = Nd, Eu, Gd, Y, Yb) were prepared in situ by reacting the protonated ligand with the appropriate lanthanide chloride salt followed by adjustment of the pH. The resulting complexes show high solubility in water (>30 mM). The absence of free gadolinium was checked by the xylenol orange test.[8]

The number of water molecules bound to the lanthanide ion in a water solution was determined to be $q = 0.9 \pm 0.1$ for Eu^{3+} from the measurement of the luminescence lifetimes of the Eu($^5D_0$) excited states of the [Eu(dhqtcn-SO_3)] complex. Analogous structural features can be expected for the complex of the Gd^{III} ion, which has a similar ionic radius.

The monoaqua complex [Gd(dhqtcn-SO_3)(H_2O)] (**1**) displays a very high relaxivity for a small monoaquagadolinium

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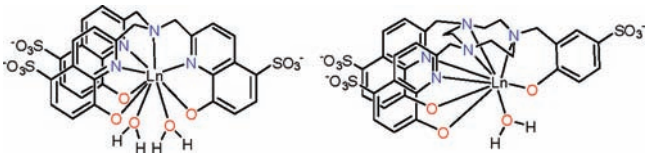
Scheme 1. Structures of the Lanthanide Complexes $[Ln(thqN\text{-}SO_3)(H_2O)_2]$ and $[Ln(dhqtcn\text{-}SO_3)(H_2O)]$



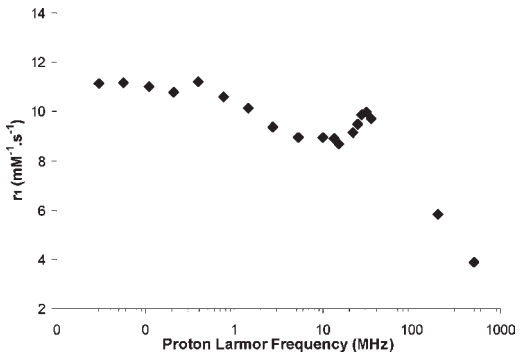


Figure 1. ^{1}H NMRD profile at 298 K at pH = 7.4 in a 4 mM H_2O solution of $[Gd(dhqtcnSO_3)(H_2O)]$ (♦).

chelate [9.1 $s^{-1}\cdot mM^{-1}$ at 20 MHz, (0.47 T)], which is more than twice that of the q = 1 $[Gd(dota)(H_2O)]^{-1}$ complex (4.2 $s^{-1}\cdot mM^{-1}$; H_4dota = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and which remains high at 200 MHz (5.8 $s^{-1}\cdot mM^{-1}$ at 200 MHz). In order to understand the origin of this high relaxivity, we decided to acquire further NMR relaxation data. Studies at medium- and high-field B_0, typically 1.5 T and above, can be particularly informative because the influence of the relaxation of the Gd^{3+} electronic spin $S = {}^7/_2$ is weaker, even negligible, and much easier to model.[9] The profile in Figure 1 of the longitudinal relaxivity r_1, obtained from the T_1 values of the water protons in a 4 mM aqueous solution of **1**, was first measured at pH = 7.4 and at 298 K over the frequency range 0.03−35 MHz with the help of a Stelar fast field-cycling relaxometer. The relaxation times T_1 and $T_{1\rho}$ of the water protons of **1** were also measured at 200 and 500 MHz. From a qualitative point of view, the large experimental values of r_1 at 30 MHz and above, together with its local maximum $r_{1,max}$ around this frequency, indicate a rather long value of the rotational correlation time τ_r of the complex.[1,2] This presumption was confirmed by the values measured at 298 K and 500 MHz in D_2O of the relaxation times of selected protons of the complex $[Y(dhqtcn\text{-}SO_3)(D_2O)]$ (**2**) containing the diamagnetic Y^{3+} ion of nearly the same size as Gd^{3+}.[1] These protons are chosen as indicators of the rotational correlation time τ_r according to eq S16 in the Supporting Information and lead to a value of τ_r = 260 ps in H_2O. It is about 3 times larger than the one reported for $[Gd(dota)(H_2O)]^{-1}$ and is mostly at the origin of the high relaxivity of **1** at 30 MHz. The observed relaxivity is high for a modest molecular weight chelate. Only one example of high relaxivity was previously reported for a small monoaquagadolium(III) complex [r_1 = 8.2 $s^{-1}\cdot mM^{-1}$ at 20 MHz (0.47 T)].[10]

The long rotational correlation time can be ascribed to the large size of the complex associated with a rigid structure. The structure rigidity was confirmed by the proton NMR spectrum of complex **2**, which shows 29 signals for the 29 protons of the complex (Figure S6 in the Supporting Information). The presence of six signals for the diastereotopic methylene protons of the ligand arms and of 12 signals for the diastereotopic protons of triazacyclononane is in agreement with the presence of a helical rigid structure, with all eight ligand donor atoms remaining bound to the metal center on the NMR time scale.

The fit of the experimental value of the relaxation time T_2 of the water ^{17}O versus temperature measured for **1** at 9.4 T (Figure S11 in the Supporting Information) shows a short value of the coordinated water molecule residence time τ_m^{298} = 20−100 ns at 298 K.[1,11] This value is significantly shorter than the one reported for $[Gd(dota)(H_2O)]^{-1}$ (244 ns).[11]

These fitted values for τ_r and τ_m were derived from NMR experiments independent of the ^{1}H NMRD profile of water. Setting the average distance between the Gd^{3+} center and the protons of coordinated water to r_H = 3.2 Å, the Solomon−Bloembergen−Morgan (SBM) high-field theory of relaxivity[9] at 298 K predicts r_1 = 8.7 $s^{-1}\cdot mM^{-1}$, $r_{1\rho}$ = 11.0 $s^{-1}\cdot mM^{-1}$ and 5.7 and 9.6 $s^{-1}\cdot mM^{-1}$ at 200 and 500 MHz, respectively, which compare favorably with their experimental counterparts (r_1 = 7.1 $s^{-1}\cdot mM^{-1}$, $r_{1\rho}$ = 11.7 $s^{-1}\cdot mM^{-1}$ and 4.8 and 10.8 $s^{-1}\cdot mM^{-1}$ at 200 and 500 MHz, respectively). This gives further validation to the values of τ_r and τ_m, which in conjunction with q and r_H are sufficient to characterize the relaxivity at the standard imaging fields at 1.5 T and above. Note that the somewhat high but, nevertheless, reasonable value[2c] of r_H = 3.2 Å indicates that the water molecule may be at a somewhat larger distance from Gd^{3+} than in most complexes. This observation is consistent with the fact that the hyperfine constant $A/\hbar$ in **1** is smaller (-1.6×10^6 rad·s^{-1}) than the value $A/\hbar = -3.8 \times 10^6$ rad·s^{-1} found or accepted for many complexes.[11] Note that a small value of $A/\hbar = -2.1 \times 10^6$ rad·s^{-1} was already reported for a polydentate pyridinecarboxylate ligand.[12] Moreover, the high values of the relaxivity r_1 in the frequency range 30−200 MHz, beyond its maximum $r_{1,max}$ near 30 MHz, indicate that the electronic spin relaxation time T_{1e} is long enough not to be a limiting factor. This favorable situation takes place even though, at low frequency, a fast electronic relaxation significantly quenches the relaxivity, as revealed by its values, which are just slightly above $r_{1,max}$. Remember that the low-frequency values of r_1 would be about (10/3) $r_{1,max}$ if the electronic relaxation was infinitely slow.[9c] Note that the fastness of the electronic spin relaxation at low frequency results from the slow modulation of the static zero-field-splitting Hamiltonian by the Brownian rotation of the complex with long τ_r. With increasing frequency above 1 MHz, this modulation mechanism becomes less and less operative so that electronic spin relaxation slows down considerably to such an extent that it no longer affects the relaxivity.

In brief, the origin of the high relaxivity at the imaging fields is a long rotational correlation time associated with a fast water exchange and an adequately slow electronic relaxation. The relaxivity of **1**, which is one of the highest reported for non-macromolecular monoaqua chelates, suggests that hydroxyquinolinates are an attractive alternative to aminocaboxylates for the development of high-relaxivity contrast agents.

The ^{1}H NMRD relaxivity profiles of a 0.2 mM aqueous solution of **1** were measured at pH = 7.4 and at 298 K over the frequency range 0.03−35 MHz in bovine serum or in the presence of bovine serum albumin (BSA) only (Figure S10 in the Supporting Information). They show similar significant increases in the relaxivity with maximum values of r_1 = 26.8

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and 28.2 $s^{-1}\cdot mM^{-1}$, respectively, at 35 MHz, which are consistent with an increase of τ_r expected from the association of a small chelate to a large macromolecule. The determination of the extent of association and of the relaxivity of the BSA-bound complex is in progres.

As a result of the interaction with albumin, the steep relaxivity maximum of **1** in physiological serum is very high. Contrast agents with a strong affinity for serum albumin are of high interest for MRI angiography applications.[13] Moreover, the sharp increase of the relaxivity slope of **1** upon binding to BSA shows that this complex could also be used as a smart targeting agent in δ-relaxation-enhanced magnetic resonance.[14] To evaluate the possibility of the application of lanthanide complexes of dhqtcn-SO_3^{3-} as imaging probes, a preliminary study of the stability at physiological pH was undertaken. A pGd value of 19.8 was measured for the monoaqua complex **1** by spectrophotometric competition titration. The observed good metal−ligand complementarity plays an important role in the observed stability and leads to a high kinetic inertness. Notably, preliminary competition experiments with 100-fold excess of $dtpa^{-5}$ indicate a high kinetic inertness essential for in vivo applications. The determination at pH = 7.4 of the stability constant of the Zn^{2+} complex of dhqtcn-SO_3 (pZn = 14.8) demonstrates a good selectivity toward Gd^{3+}. We also investigated the emission properties of the Nd^{3+} and Yb^{3+} complexes to evaluate their potential use as NIR-emitting luminescent probes in biomedical applications. Ligand excitation at 375 nm results in sizable NIR luminescence of the complexed Nd^{3+} and Yb^{3+} ions in water, and the emission spectra exhibit the characteristic three bands originating from the $Nd(^4F_{3/2})$ level to the $^4I_{9/2}$, $^4I_{11/2}$, and $^4I_{13/2}$ sublevels and from one band from the $Yb(^2F_{5/2})$ levels, respectively (Figures S8 and S9 in the Supporting Information). The measured absolute quantum yields, which amount to 9×10^{-5}% for Nd^{3+} and 1.8×10^{-2}% for Yb^{3+}, show that the H_3dhqtcn-SO_3 tripod is an efficient sensitizer of the NIR emission of these ions in spite of the presence of one coordinated water molecule. The observation of sizable NIR emission quantum yields for hydrated lanthanide complexes is remarkable, and only two examples of hydrated lanthanide complexes showing similar properties have been reported before.[3a,b] In summary, the complexes $[Ln(dhqtcn\text{-}SO_3)(H_2O)]$ with their very high thermodynamic stability comparable to that of commercial contrast agents, associated with their kinetic inertness, their high relaxivity under physiological conditions, which further increases significantly and sharply in serum, and their sizable quantum yields are very attractive systems as bimodal probes endowed with high relaxivities and fluorescent properties.

■ ASSOCIATED CONTENT

Supporting Information. Full experimental procedures and spectroscopic and relaxometric data. This material is available free of charge via the Internet at http://pubs.acs.org.

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