Optimization of the Relaxivity of MRI Contrast Agents: Effect of Poly(ethylene glycol) Chains on the Water-Exchange Rates of Gd^{III} Complexes

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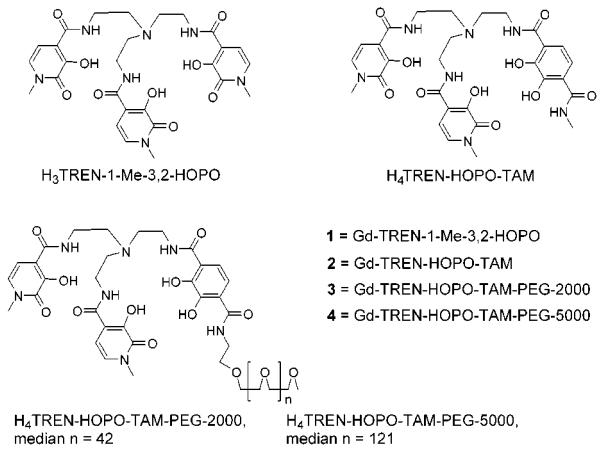
The use of Gd^{III} complexes as contrast agents in magnetic resonance imaging (MRI) has proven invaluable in the diagnosis of several internal abnormalities.[1] The range of medical applications for which contrast agents are useful is likely to increase in the future with the development of target-specific contrast agents of increased relaxivity, such as MS-325 which binds with HSA in the blood pool, resulting in a substantial increase in rotational correlation time, τ_R, and relaxivity.[2,3] Theory predicts that to attain optimal relaxivities, it will be necessary to optimize both τ_M (τ_M is the inverse of the water-exchange rate, k_{ex}) and τ_R (the rotational correlation lifetime).[4] Currently, the relaxivity of commercial complexes is restricted by slow water exchange.[1]

We have previously reported a series of Gd^{III} complexes of hexadentate ligands which include Gd−TREN-1-Me-3,2-HOPO (**1**) and Gd−TREN-HOPO-TAM, (**2**) (Chart 1).[5−9] **1** has been shown to be thermodynamically stable in the presence of the competing physiological metal ions Ca^{II} and Zn^{II} and is therefore not anticipated to release toxic quantities of Gd^{III} in vivo. Furthermore, **1** and **2** exhibit relaxivities of 10.5 mM^{-1} s^{-1} (20 MHz, 37 °C) and 8.8 mM^{-1} s^{-1} (20 MHz, 25 °C) respectively, values considerably higher than the values typically observed for commercial complexes.[1] However, the most remarkable feature of these complexes is the exceptionally short values of τ_M (typically 8 to 15 ns). These values are considerably shorter than those observed for commercial complexes. Furthermore, these values are near optimal for attaining maximum relaxivities for molecules of very high τ_R. Several methods have been reported of increasing τ_R including physical attachment of Gd^{III} complexes to polymers or dendrimers,[10] or exploiting noncovalent interactions between the complex and proteins in vivo.[11−13] As a preliminary investigation into slowly tumbling derivatives of **2**, we have synthesized Gd−TREN-HOPO-TAM-PEG-2000 (**3**) and Gd−TREN-HOPO-TAM-PEG-5000 (**4**) in which poly(ethylene glycol) (PEG) moieties of average molecular weights 2000 and 5000

Chart 1



respectively are attached to the ligand (Chart 1). The PEG group was chosen for two reasons. First, the high water solubility associated with PEG chains is anticipated to increase the rather low solubility of the parent complexes. Additionally, although it has been previously found that rapid internal motions within a PEG chain result in only a modest increase in τ_R,[14] it has also been demonstrated that PEG chains can bind to HSA across a wide pH range.[15] We were therefore interested in exploiting this noncovalent interaction to effect an increase in τ_R and relaxivity.

PEG monoamines of average molecular weights 2000 and 5000 were attached to the ligand TREN-HOPO-TAM using an adaptation of a previously described synthetic procedure,[6] to give the complexes **3** and **4**. Characterization by UV/vis spectroscopy, electrospray time-of-flight (ES-TOF) mass spectrometry, and elemental analyses for C, H, N, and Gd confirmed formation of the desired complexes.

Both **3** and **4** were found to be of high solubility in H_2O, allowing τ_M to be determined by a variable temperature ^{17}O NMR study of the transverse relaxation rate of $H_2{}^{17}O$ (R_2) at 2.1 T. τ_M was also remeasured for **2** at the same field strength using the same technique. Analysis of the profiles also allows the number of coordinated water molecules, q to be evaluated. Interestingly, it was found that $q = 2$ for **2**, and $q = 1$ for **3** and **4**. An independent evaluation of q for **4** was obtained by fitting the nuclear magnetic resonance dispersion (NMRD) profile with a theoretical curve generated from a given set of relaxivity parameters. The best fit was obtained with $q = 1$, in agreement with the value obtained from the ^{17}O NMR study. The values of $q = 2$ and $\tau_M = 8 \pm 1$ ns obtained for **2** are consistent with previous studies.[5,6] The reduction in q that occurs in the presence

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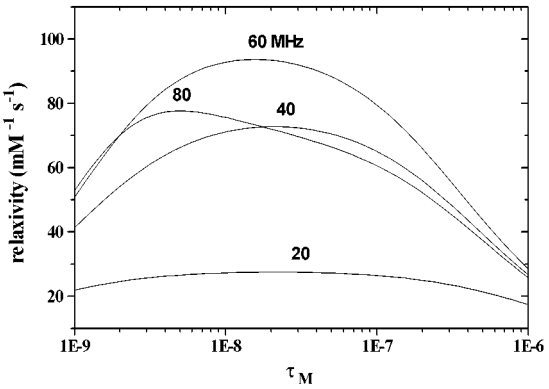

Figure 1. Calculated relaxivity at 20, 40, 60, and 80 MHz as a function of τ_M for a macromolecular complex ($\tau_R = 10$ ns). Typical parameters of **2** were utilized: $q = 2$, $r = 3.0$ Å, $\Delta^2 = 1.0 \times 10^{20}$ s^{-2}, $\tau_V = 20$ ps, $a = 3.8$ Å, $D = 2.24 \times 10^{-5}$ cm^2 s^{-1}.

of a PEG chain can be explained by partial displacement of the two water molecules by the PEG oxygen donors. Significantly, there appears to be an increase in τ_M as the PEG chain is lengthened, with values of 19 ± 2 ns and 31 ± 2 ns for **3** and **4**, respectively.

Variations in τ_M have previously been observed for alkyl-DTPA-bisamide copolymers of the formula [Gd(DTPA-BA)−$(CH_2)_n]_x$, where $\tau_M = 2300$, 1500 and 2000 ns for $n = 6$, 10 and 12, respectively.[16] The proportional change in τ_M for these complexes is relatively small compared to that observed between **2**, **3** and **4**. Furthermore, the values of τ_M observed for **2**, **3** and **4** span a range that is considered optimal for achieving maximum values of relaxivity, whereas the values of τ_M observed for [Gd-(DTPA-BA)−$(CH_2)_n]_x$ lie far outside this range. The optimal value for τ_M depends on several variables, in particular the field strength of the MRI scanner. Previous reports have suggested optimal τ_M values of a few tens of nanoseconds.[1,17] To investigate the likely optimal values of τ_M for the complexes reported herein, we have calculated optimal τ_M across a range of field strengths that are typical in MRI (Figure 1). It can be seen that the optimal value of τ_M decreases with increasing magnetic field strength, so that at the commonly used MRI field strengths of 20 and 60 MHz, the optimal values of τ_M are 30 and 15 ns. As more powerful MRI machines are being introduced into hospitals it can be seen that the optimal value of τ_M decreases further. It can also be seen that the values of τ_M for **2**, **3** and **4** traverse the range of useful field strengths, implying that the variation of the PEG length could potentially be used to fine-tune τ_M, so that it is optimized for a particular field strength.

The observed increase in τ_M with increasing length of PEG chain can be accounted for by considering the concentration of water molecules in the immediate vicinity of the Gd^{III} complex. Water exchange on the Gd^{III} center in **2**, **3** and **4** is thought to proceed via an associative mechanism involving an eight coordinate ground state and a nine coordinate intermediate, whereas amino-carboxylate complexes generally exchange water via a dissociative mechanism since they have nine-coordinate ground states (Figure 2).[6,14] Since the water exchange rate for an associative mechanism depends on the water concentration, it can be seen that a decrease in the local concentration of water would be expected to lead to a decrease in water exchange rate (increase in τ_M).

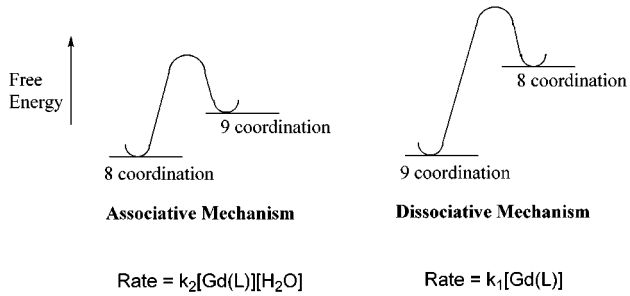

Figure 2. Free-energy diagrams for associative and dissociative mechanisms of water exchange for Gd^{III} complexes.

The relaxivity of **4** at pH = 7.5 is 9.1 mM^{-1} s^{-1} (20 MHz, 25 °C), which compares to 8.8 mM^{-1} s^{-1} (20 MHz, 25 °C) for **2**. The increase in relaxivity observed upon addition of the PEG chain is very modest considering the large increase in molecular weight, reflecting the decrease in q and the effect of rapid internal motions of the PEG chain on τ_R. We were interested to see whether previously reported binding interactions between PEG and HSA would effect an increase in τ_R and hence relaxivity.[15] The longitudinal relaxation rate (R_1) of water protons in a 0.25 M solution of **4** was measured with increasing concentrations of HSA at 20 MHz and 25 °C. From these data, the relaxivity of the **4**−HSA adduct was calculated to be 74 ± 14 mM^{-1} s^{-1} with a formation constant, K_a of 186 ± 50 M^{-1}. This represents relatively weak binding, which would result in a mixture of bound and unbound complex under physiological concentrations of HSA. Also, as a consequence of the weak binding, it was not possible to accurately quantify the relaxivity parameters of the **4**−HSA adduct by NMRD or ^{17}O NMR. Nevertheless, the relaxivity observed for **4**−HSA is considerably higher than the highest value reported previously of 53.2 mM^{-1} s^{-1} (per Gd^{III} center),[18] reflecting an optimized water-exchange rate and a slow rotational correlation time.

In summary, we have demonstrated for the first time how the water-exchange rate on a Gd^{III} center can be fine-tuned within a range that is optimal for the development of MRI contrast agents of increased relaxivity. Furthermore, the optimal value of τ_M, together with the increase in τ_R that occurs upon binding of the PEG with HSA results in an exceptionally high value of relaxivity. These properties make Gd−TREN-HOPO-TAM-PEG complexes excellent candidates for components in the development of diagnostic MRI contrast agents of increased sensitivity.

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Supporting Information Available: NMRD profile for **4**, plot of relaxivity as a function of τ_M showing the changes of the relaxivity at 20 MHz that occur when Δ^2 is divided by 2 and τ_V is increased from 20 to 50 ns, plot of ^{17}O NMR transverse relaxation rates as a function of temperature, ^1H longitudinal relaxation rate of H_2O as a function of HSA concentration for **4**, characterization data for **3** and **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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