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A R_2/R_1 Ratiometric Procedure for a Concentration-Independent, pH-Responsive, Gd(III)-Based MRI Agent

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Gd(III)-based paramagnetic complexes are currently used as contrast agents for MRI investigations.[1] Their contrast-enhancing ability is usually assessed by measuring their longitudinal relaxivity (r_1), which represents the relaxation enhancement of water protons in solutions containing the paramagnetic agent at 1 mM concentration. The higher the relaxivity, the larger the signal enhancement detected in the corresponding T_1-weighted images. The most distinctive property of this class of agents relies on the fact that, by properly designing the contrast agent, its relaxivity may be made dependent upon a specific physicochemical characteristic of the microenvironment in which it distributes. Several systems have been reported whose relaxivity is made dependent on pH, temperature, P_{O2}, enzymatic activity, ion and metabolite concentrations, respectively.[2] However, such peculiar responsive properties have not been properly exploited in practice, because the in vivo MRI response cannot be unambiguously ascribed to a change in the relaxation rates if the actual concentration of the paramagnetic complex is unknown. So far, this problem has been tackled by an indirect determination of the local concentration of the agent by using a reference compound whose relaxivity is not dependent on the parameter of interest.[3] The practical limits of the latter approach demands the design of improved responsive probes.

In this communication, we propose a novel method based on a ratiometric approach that consists of measuring the ratio between the transverse and the longitudinal paramagnetic contribution to the water protons relaxation rate, i.e., R_{2p}/R_{1p}. For an aqueous solution of a Gd(III) complex containing one labile water molecule coordinated to the metal center, the inner-sphere contribution to the paramagnetic water proton relaxation rates at magnetic field strength higher than 0.2 T is commonly described by the following equations:

$$R_{1p} \simeq \frac{P_M}{T_{1M}+\tau_M} \Rightarrow \frac{1}{T_{1M}} = \frac{6}{15}\frac{K^{DIP}}{r_H^6}\left(\frac{\tau_C}{1+\omega_H^2\tau_C^2}\right) \quad (1)$$

$$R_{2p} \simeq \frac{P_M}{T_{2M}+\tau_M} \Rightarrow \frac{1}{T_{2M}} = \frac{1}{15}\frac{K^{DIP}}{r_H^6}\left(4\tau_C + \frac{3\tau_C}{1+\omega_H^2\tau_C^2}\right) \quad (2)$$

where P_M is the molar fraction of water protons bound to Gd(III) ion (equal to [GdL]/55.6), τ_M is their residence lifetime, r_H their distance from the metal center, ω_H their Larmor frequency (rad·s^{-1}), and τ_C their molecular correlation time ($\tau_C^{-1} = \tau_M^{-1} + \tau_R^{-1} + \tau_{1S}^{-1}$ with τ_R = rotational correlation time and τ_{1S} = longitudinal electronic relaxation time). K^{DIP} is a constant value ($3.887\cdot10^{-42}$ m^6·s^{-2}) related to the dipolar interaction between the electron and the nuclear spins. From eqs 1−2, it is clear that the R_{2p}/R_{1p} ratio is determined by τ_M, τ_C, and ω_H values, but it is independent of the concentration of the paramagnetic agent.

A theoretical simulation, based on eqs 1−2, indicates that at the magnetic field strengths available in MRI (>0.2 T), the R_{2p}/R_{1p} ratio starts to be sensitive to the rotational mobility of the Gd(III) complex only for τ_R values longer than 0.5 ns (Figure 1).

It is noteworthy that, at a fixed magnetic field strength, τ_R and τ_M have an opposite effect on the R_{2p}/R_{1p} ratio. In fact, τ_R affects the R_{2p}/R_{1p} ratio through its effect on τ_C and consequently on T_{iM} (i = 1,2), whereas τ_M influences the R_{2p}/R_{1p} term through the limiting effect on R_{ip} via the ($T_{iM} + \tau_M$) terms. On the basis of the available theory and in order to be effective as ratiometric responsive probe, a Gd(III) complex must have a τ_R value ≥1 ns, and furthermore, the rotational mobility and/or the residence lifetime of the metal-bound water molecule/s must be dependent on the parameter of interest. To validate this approach, we investigated the pH responsiveness of a macromolecular adduct based on poly-L-ornithine whose terminal amino groups have been partly functionalized with a macrocyclic Gd(III) complex by using a squarate moiety as a linker ([(GdDOTAam)$_{33}$−Orn$_{205}$], Chart 1). The pH responsiveness, in terms of R_{1p}, of an analogous paramagnetic macromolecule was already assessed[4] as a consequence of a change in the molecular reorientational time caused by a reversible, pH-dependent transition between α-helical and random coil conformation.

The latter structure, caused by the electrostatic repulsion between the positively charged amino groups of the side chains, is stable at pH < 9, whereas at higher pH values the deprotonation of the amino groups promotes the formation of the α-helical conformation.[5]

Figure 2 reports the magnetic field dependence (in the 10−600 MHz range) of the paramagnetic contribution to the longitudinal and transverse relaxation rates at pH 7, 10, and 12, of a solution of [(GdDOTAam)$_{33}$−Orn$_{205}$]. The R_{1p} enhancement observed upon increasing pH (in the 10−100 MHz range) is a clear evidence of the reduction of the rotational mobility of the adduct. From the analysis of the magnetic field dependence (from 0.01 to 600 MHz) carried out by using the available theory of the paramagnetic relaxation, implemented with the Lipari−Szabo model, global reorientational times (at 25 °C) of 1.0, 1.7, and 3.6 ns were obtained at pH 7, 10, and 12, respectively. A similar trend was also observed for the local reorientation motion of the complex (0.23, 0.27, and 0.52 ns, respectively, order parameter, $S^2 = 0.6$). Also the R_{2p} values are pH dependent, but differently from R_{1p}, they are almost field-independent, and consequently, the pH dependence of R_{2p} is maintained even at high fields.

The data reported in Figure 2 unambiguously show that: (i) the R_{2p}/R_{1p} ratio is directly related to the magnetic field strength and (ii) the pH responsiveness of the "relaxometric ratio" improves upon increasing the field. To test the validity of the ratiometric method,

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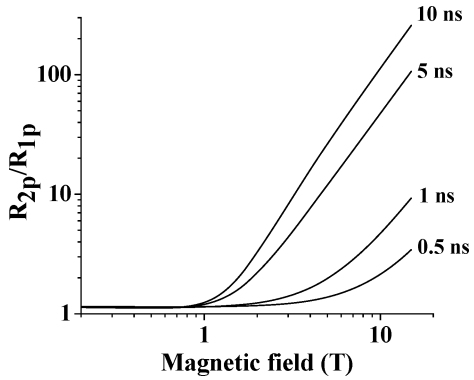


Figure 1. Calculated R_{2p}/R_{1p} values as a function of magnetic field strength for a macromolecular Gd(III) complex ($q = 1$, $r = 3$ Å, $\tau_M = 200$ ns, $\Delta^2 = 2 \cdot 10^{19}$ s^{-2}, $\tau_V = 10$ ps) endowed with τ_R values in the 0.5−10 ns range.

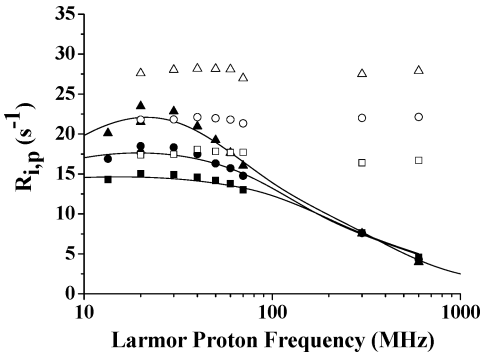
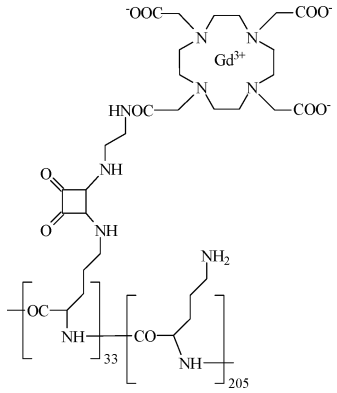


Figure 2. Magnetic field dependence of R_{1p} (filled symbols) and R_{2p} (open symbols) measured at 25 °C and 600 MHz for a solution containing the Gd(III)-based poly-L-ornithine adduct ([Gd] = 1 mM). pH 7 (squares), pH 10 (circles), and pH 12 (triangles).

Chart 1. Ratiometric pH-Responsive Probe Investigated



the relaxometric ratio has been measured (600 MHz, 25 °C) at different pH values for solutions of [(GdDOTAam)$_{33}$−Orn$_{205}$] at three different Gd(III) concentrations (Figure 3, left). The small deviation observed at each pH value between the relaxometric ratios for the three solutions fully supports the validity of this approach.

The pH dependence of the R_{2p}/R_{1p} ratio (600 MHz, 25 °C) is displayed in the right side of Figure 3, where each data point and the corresponding error bars have been calculated from the data

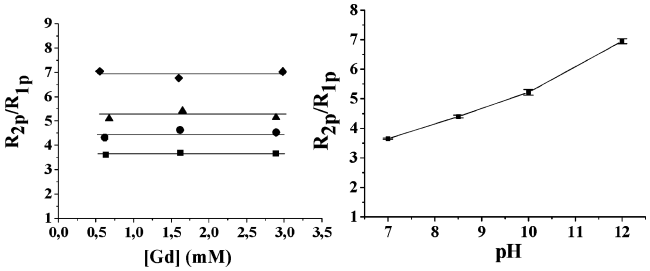


Figure 3. (Left) Dependence of the relaxometric ratio on the concentration of Gd(III) for [(GdDOTAam)$_{33}$−Orn$_{205}$] at four pH values: pH 7 (squares), pH 8.5 (circles), pH 10 (triangles), and pH 12 (diamonds) (600 MHz. 25 °C). (Right) Corresponding pH dependence of the relaxometric ratio calculated from the data point reported on the left.

reported in the left side. The error in the pH reading is estimated to be around ±0.1.

From the analysis of the data reported in Figure 3, the increase of the R_{2p}/R_{1p} values upon increasing pH is mainly determined by the significant involvement of τ_R in determining the transverse relaxation rate. Larger R_{2p}/R_{1p} values could have been obtained in the presence of larger molecular reorientational time and faster exchange rate of the water molecule coordinated to the Gd(III) ion (a τ_M of 0.9 μs has been calculated at 25 °C from ^{17}O NMR measurements).

In conclusion, the results reported in this communication show that the long-standing problem of developing Gd(III)-based MRI agents whose responsiveness is independent from their absolute concentration could now be tackled by the proper design of a slow-tumbling paramagnetic complex whose rotational mobility, water exchange rate, and/or electronic relaxation rates are made specifically dependent on the parameter of interest. Until now, the acquisition of parametric MR images was thought to be the domain of the new class of CEST agents.[6] The finding that Gd-complexes may also be made suitable for this purpose is particularly valuable in view of the outstanding sensitivity shown by such relaxation agents.

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References

(1) Merbach, A. E.; Tóth, E. The *Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; John Wiley & Sons: Chichester, UK, 2001.
(2) (a) Jacques, V.; Desreux, J. F. *Top. Curr. Chem.* **2002**, *221*, 123. (b) Zhang Z. D.; Nair, S. A.; McMurry, T. J. *Curr. Med. Chem.* **2005**, *12*, 751. (c) Aime, S.; Botta, M.; Terreno, E. *Adv Inorg. Chem.* **2005**, *57*, 173 and references therein.
(3) (a) Raghunand, N.; Howison, C.; Sherry, A. D.; Zhang, R. S.; Gillies, R. J. *Magn. Res. Med.* **2003**, *49*, 249. (b) Garcia-Martin M. L.; Martinez G. V.; Raghunand, N.; Sherry, A. D.; Zhang, S.; Gillies, R. J. *Magn. Res. Med.* **2006**, *55*, 309.
(4) Aime, S.; Botta, M.; Crich, S. G.; Giovenzana, G.; Palmisano, G.; Sisti, M. *Chem. Commun.* **1999**, 1577.
(5) Hammes, G. C.; Roberts, P. B. *J. Am. Chem. Soc.* **1969**, *91*, 1812.
(6) Aime, S.; Barge, A.; Delli Castelli, D.; Fedeli, F.; Mortillaro, A.; Nielsen, F. U.; Terreno, E. *Magn. Res. Med.* **2002**, *47*, 639.

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