J|A|C|S COMMUNICATIONS

Published on Web 05/19/2010

Large Relaxivity Enhancement of Paramagnetic Lipid Nanoparticles by Restricting the Local Motions of the Gd^{III} Chelates

Filip Kielar,[†] Lorenzo Tei,[†] Enzo Terreno,[‡] and Mauro Botta*,[†]

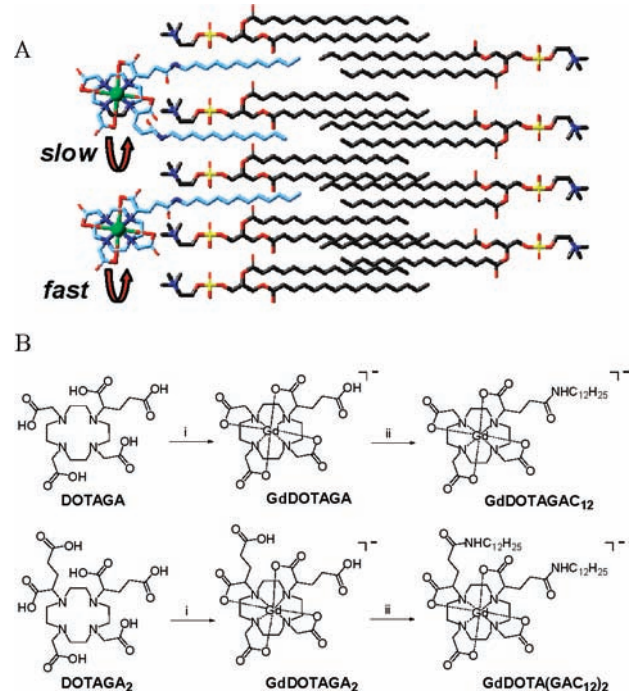
Dipartimento di Scienze dell' Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Alessandria, Italy, and Department of Chemistry IFM and Molecular Imaging Centre, Università di Torino, Torino, Italy

Received February 22, 2010; E-mail: mauro.botta@mfn.unipmn.it

The use of paramagnetic Gd^{III} chelates represents a common approach for altering the intensity of magnetic resonance imaging (MRI) proton spins to enhance the image contrast.[1] However, currently used Gd-based contrast agents possess only a fraction of the efficacy (relaxivity, r_1) predicted by theoretical models.[2] An attractive strategy for developing high-relaxivity systems is the use of complexes that self-aggregate into lipid nanoparticles such as micelles (5−50 nm) and liposomes (50−500 nm).[3] These systems possess high molecular relaxivity resulting from both the additive effect of all of the Gd^{III} centers and the slow global rotational motion that enhances the r_1 of each complex. The interest in these systems also arises from the fact that the biodistribution of micelles and liposomes is highly dependent on their physicochemical properties and can thus be chemically manipulated.[3,4] However, in spite of the high relaxivities per particle found for these systems, the r_1 values for the individual gadolinium centers are typically modest [10−20 mM^{-1} s^{-1} at 298 K, 20 MHz (0.47 T)] and well below theoretical expectations. The two major limiting factors are the use of neutral Gd complexes (DTPA bisamides and DOTA monoamides) exhibiting slow water exchange ($k_{ex} = 1/\tau_M$)[3,5] and/or fast local rotation of the Gd^{III} complex around its linker to the nanoparticle. The effect of slow water exchange has recently been studied in detail, and several stable Gd complexes exhibiting fast coordinated water exchange are currently available.[2b] The problem of the poor motional coupling between the paramagnetic unit and the nanoparticle is also highly relevant but in just a few cases it has been explicitly considered.[6]

The aim of this study was the synthesis of a DOTA-like Gd^{III} complex functionalized with two hydrophobic chains on adjacent donor groups, endowing it with the ability to self-assemble into micelles and be incorporated into liposomes. Zhang et al.[6a] clearly demonstrated that the strategy of multisite interaction favors a relaxivity enhancement resulting from increased immobilization of the complex upon binding (Scheme 1A). In fact, the presence of two aliphatic chains on adjacent acetic arms was expected to reduce considerably the local rotational motion of the Gd^{III} chelate (described by the correlation time τ_{Rl}) in comparison with the global rotation of the nanoparticles (τ_{Rg}). Four complexes were synthesized for this purpose starting from 1,4-DO2A(O*t*Bu)$_2$ and DO3A(O*t*Bu)$_3$.[7] These were then alkylated using an alkyl bromide prepared in two steps from L-glutamic acid, affording DOTA structures with one or two pendant glutamic acid arms, respectively, that were then deprotected with TFA. The complexation was carried out in aqueous media at pH 6, yielding the basic complexes GdDOTAGA and GdDOTAGA$_2$. Amide coupling reactions with dodecyl amine and HPLC purification yielded the desired amphiphilic complexes GdDOTAGAC$_{12}$ and GdDOTA(GAC$_{12}$)$_2$ (Scheme 1B).

Scheme 1. (A) Schematic Representation of Embedding of Complexes GdDOTAGAC$_{12}$ (Bottom) and GdDOTA(GAC$_{12}$)$_2$ (Top) in a Lipid Bilayer; (B) Synthesis of the GdDOTAGA$_2$ and GdDOTA(GAC$_{12}$)$_2$ Complexes[a]



[a] Reaction conditions in (B): (i) $GdCl_3$, H_2O, pH 6; (ii) $C_{12}H_{25}NH_2$, DMF, TNTU, DIPEA.

Table 1. Selected Refinement Parameters of the $1/T_1$ NMRD Profiles (298 K) for the Gd Complexes Discussed in This Work[a]

	DOTA[b]	GA	GA$_2$	GAC$_{12}$	(GAC$_{12}$)$_2$
r_1 (mM^{-1} s^{-1})[c]	4.7	5.3	5.9	15.4	34.8
τ_{Rl} (ps)	−	−	−	210 ± 20	820 ± 110
τ_{Rg} (ps)	77 ± 4	81 ± 4	98 ± 3	2900 ± 420	4700 ± 400
S^2	−	−	−	0.14 ± 0.01	0.70 ± 0.03
τ_M (ns)	251 ± 7	128 ± 9	108 ± 8	220 ± 8	297 ± 12

[a] See the Supporting Information for full details. [b] Data from ref 11. [c] At 20 MHz (0.47 T).

The solution NMR relaxometric study of GdDOTAGA and GdDOTAGA$_2$ revealed that their properties are quite similar to those of the parent GdDOTA and related derivatives (Table 1).[2a,b] In particular, they retain one coordinated water molecule ($q = 1$). The ability of the corresponding amphiphilic conjugates to self-assemble into supramolecular structures was clearly evidenced in their NMR

[†] Università del Piemonte Orientale "A. Avogadro".
[‡] Università di Torino.

10.1021/ja101518v

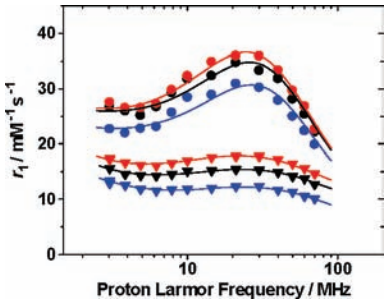


Figure 1. ^{1}H NMRD profiles expressed per Gd for micelles of GdDOTA-GAC_{12} (▼) and GdDOTA$(GAC_{12})_2$ (●) at (red) 283, (black) 298, and (blue) 310 K.

dispersion (NMRD) profiles, which showed the characteristic peak at ~20 MHz typical of slowly tumbling systems (Figure 1). Dynamic light scattering (DLS) data showed a ~2-fold difference in the mean diameter of these aggregates, giving 7 and 16 nm for $GdDOTAGAC_{12}$ and GdDOTA$(GAC_{12})_2$, respectively. The NMRD data for these complexes in the aggregated form at three temperatures were fitted using the Lipari−Szabo approach for the description of the rotational dynamics.[6] The large difference in the relaxivities of the two amphiphilic complexes (101% at 20 MHz and 298 K) was mainly the result of their different order parameter S^2 (indicating the coupling between local and global motions) and τ_{RI} values, thus showing that the rotational flexibility was significantly reduced for GdDOTA$(GAC_{12})_2$. To the best of our knowledge, the r_1 value of the latter complex [34.8 mM^{-1} s^{-1} at 298 K and 20 MHz (0.5 T)] is the highest reported to date for Gd-based paramagnetic micelles with $q = 1$.[8] It should be noted that even though the observed relaxivity increment was attenuated at higher fields, extrapolation of the observed data suggested that a 35% increment was still maintained at 128 MHz (3 T).

Next, the two amphiphilic complexes were incorporated into liposomes. The phospholipids and the formulation used (85 mol % DPPC, 5 mol % DSPE−PEG2000, 10 mol % Gd−chelate) were chosen in order to prepare stealth nanovesicles similar to those currently used as probes for MR molecular imaging protocols.[1b] The liposomes were prepared using a thin-layer deposition/extrusion technique, and their analysis using DLS showed that their diameters were in the range 45−60 nm. The r_1 values of the complexes embedded in liposomes were 17.0 and 40.0 mM^{-1} s^{-1} [298 K, 20 MHz (0.47 T)] for $GdDOTAGAC_{12}$ and GdDOTA$(GAC_{12})_2$, respectively. The remarkable relaxivity gain observed (135% at 0.47 T; 99% at 1.41 T) for GdDOTA$(GAC_{12})_2$ relative to the monosubstituted derivative results from the strong reduction in the flexibility of the system associated with the hindered local rotation about the two aliphatic chains (Figure 2). These data can be compared with related values for GdDMPE−DTPA, a neutral DTPA monoamide derivative carrying a fatty acid chain that exhibits slow water exchange ($r_1 = 12$ at both 0.47 and 1.41 T).[9] Next, we note that increasing the rate of water exchange resulted in an enhancement of r_1 by 42%, whereas simultaneous optimization of water exchange and local rotation increased the relaxivity by ~233% (Figure 2, left). A phantom-imaging experiment was carried out to demonstrate how this relaxation enhancement translates into image contrast. Solutions of the two complexes incorporated into liposomes were imaged together with solutions of the commercially used contrast agent Prohance (Figure 2).[2a] The experiment clearly showed that superior contrast was generated with GdDOTA-$(GAC_{12})_2$ at concentrations equivalent to those of the other complexes.

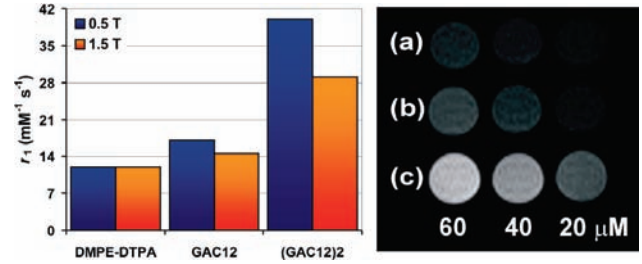


Figure 2. (left) Relaxivity per Gd for liposomes based on GdDMPE−DTPA, $GdDOTAGAC_{12}$, and GdDOTA$(GAC_{12})_2$ at 20 MHz (0.47 T) and 60 MHz (1.41 T). (right) T_1-weighted multislice spin-echo phantom images (TR/TE/NEX = 250/8/16) for solutions of (a) Prohance[2a] as a control and liposomes of (b) $GdDOTAGAC_{12}$ and (c) GdDOTA$(GAC_{12})_2$ at 20, 40, and 60 μM concentrations [298 K, 40 MHz (1 T)].

In summary, we have rationally designed a bis-substituted GdDOTA derivative bearing two adjacent glutamic acid arms that is amenable to conjugation to a variety of chemical moieties and/or macromolecular scaffolds characterized by enhanced rotational immobilization. Micelles and liposomes incorporating this paramagnetic building unit show an unprecedented relaxivity enhancement relative to the derivative bearing only a single glutamic acid arm due to a favorable water exchange rate and optimized rotational rigidity. In addition, these GdDOTA derivatives are more stable and much more kinetically inert than other Gd chelates typically used in micelles and liposomes.[10]

Acknowledgment. This research was supported by funding from Regione Piemonte (PIIMDMT and Nano IGT Projects) and ESF COST Action D38.

Supporting Information Available: Detailed experimental procedures and characterization data for the four complexes discussed in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Villaraza, A. J. L.; Bumb, A.; Brechbiel, M. W. *Chem. Rev.* **2010**, *110*, 2921. (b) Aime, S.; Delli Castelli, D.; Geninatti Crich, S.; Gianolio, E.; Terreno, E. *Acc. Chem. Res.* **2009**, *42*, 822.

(2) (a) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293. (b) Aime, S.; Botta, M.; Terreno, E. *Adv. Inorg. Chem.* **2005**, *57*, 173.

(3) (a) Accardo, A.; Tesauro, D.; Luigi, A.; Pedone, C.; Morelli, G. *Coord. Chem. Rev.* **2009**, *253*, 2193. (b) Mulder, W. J. M.; Strijkers, G. J.; van Tilborg, G. A. F.; Griffioenz, A. W.; Nicolay, K. *NMR Biomed.* **2006**, *19*, 142.

(4) Vucic, E.; Sanders, H. M. H. F.; Arena, F.; Terreno, E.; Aime, S.; Nicolay, K.; Leupold, E.; Dathe, M.; Sommerdijk, N. A. J. M.; Fayad, Z. A.; Mulder, W. J. M. *J. Am. Chem. Soc.* **2009**, *131*, 406.

(5) Kimpe, K.; Parac-Vogt, T. N.; Laurent, S.; Pierart, C.; Van der Elst, L.; Muller, R. N. *Eur. J. Inorg. Chem.* **2003**, 3021.

(6) (a) Zhang, Z.; Greenfield, M. T.; Spiller, M.; McMurry, T. J.; Lauffer, R. B.; Caravan, P. *Angew. Chem., Int. Ed.* **2005**, *44*, 6766. (b) Rudovsky, J.; Botta, M.; Hermann, P.; Hardcastle, K. I.; Lukes, I.; Aime, S. *Bioconjugate Chem.* **2006**, *17*, 975. (c) Avedano, S.; Tei, L.; Lombardi, A.; Giovenzana, G. B.; Aime, S.; Longo, D.; Botta, M. *Chem. Commun.* **2007**, 4726. (d) Polášek, M.; Hermann, P.; Peters, J. A.; Geraldes, C. F. G. C.; Lukeš, I. *Bioconjugate Chem.* **2009**, *20*, 2142.

(7) Li, C.; Wong, W.-T. *Tetrahedron* **2004**, *60*, 5595.

(8) Schühle, D. T.; Schatz, J.; Laurent, S.; Vander Elst, L.; Muller, R. N.; Stuart, M. C. A.; Peters, J. A. *Chem.—Eur. J.* **2009**, *15*, 3290.

(9) Bertini, I.; Bianchini, F.; Calorici, L.; Colagrande, S.; Fragai, M.; Franchi, A.; Gallo, O.; Gavazzi, C.; Luchinat, C. *Magn. Reson. Med.* **2004**, *52*, 669.

(10) Overoye-Chan, K.; Koerner, S.; Looby, R. J.; Kolodziej, A. F.; Zech, S. G.; Deng, Q.; Chasse, J. M.; McMurry, T. J.; Caravan, P. *J. Am. Chem. Soc.* **2008**, *130*, 6025.

(11) Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333.

JA101518V