Dendrimeric Gd(III) complex of a monophosphinated DOTA analogue: optimizing relaxivity by reducing internal motion†

Jakub Rudovský,[a] Petr Hermann,*[a] Mauro Botta,[b] Silvio Aime*[c] and Ivan Lukeš[a]

Received (in Cambridge, UK) 14th December 2004, Accepted 8th March 2005
First published as an Advance Article on the web 18th March 2005
DOI: 10.1039/b418712a

A marked increase of relaxivity has been observed upon rigidifying the internal frame of Gd-containing PAMAM dendrimers: the effect has been attained by either protonation of the dendrimer or by forming supramolecular adducts with cationic polyaminoacids.

Paramagnetic Gd^{III} complexes are currently used in clinical settings as contrast enhancing agents (CA) for nuclear magnetic resonance imaging (MRI).[1,2] Their efficiency, expressed as millimolar relaxivity (r_1), can be improved through the optimization of the three correlation times involved in the paramagnetic relaxation process.[3] Two of them, namely the exchange lifetime of the coordinated water molecule(s), τ_M, and the electronic relaxation time, τ_S, are essentially defined by the characteristics of the coordination cage of the gadolinium ion, whereas the molecular reorientational correlation time, τ_R, can be modulated by binding the complex to systems of different dimensions.[1,3,4] Therefore, the search has been addressed to Gd^{III} chelates endowed with the proper τ_M and τ_S values and bearing suitable groups for further conjugation to a high-molecular substrate.

We have recently synthesized a monophosphinated DOTA-like ligand, $DO3AP^{ABn}$,[5] which could be modified to isothiocyanate for use as a reactive anchoring group. The corresponding Gd^{III} chelate displays a τ_M and a τ_S value in the optimal range for the attainment of a high relaxivity once the mobility of the complex is suitably slowed down.[5] It was previously shown that a route to moderate increase of the reorientational correlation time of Gd^{III} complexes is provided by their linking to the surface of polyamidoamine (PAMAM) dendrimers.[6,7] However, it was found that the relaxivity of Gd^{III} complexes of DTPA- or DOTA-like ligands bound to PAMAM dendrimers shows a sort of "saturation effect" upon increasing the size of the adduct. This finding was accounted for in terms of undesired mobility of the anchoring spacer and overall flexibility of the dendrimeric backbone.[8] In order to get more insight into the phenomenon of the "quenching" of the relaxivity of Gd-containing dendrimers, we have synthesized a new series of four low-generation PAMAM conjugates with Gd-$DO3AP^{ABn}$.

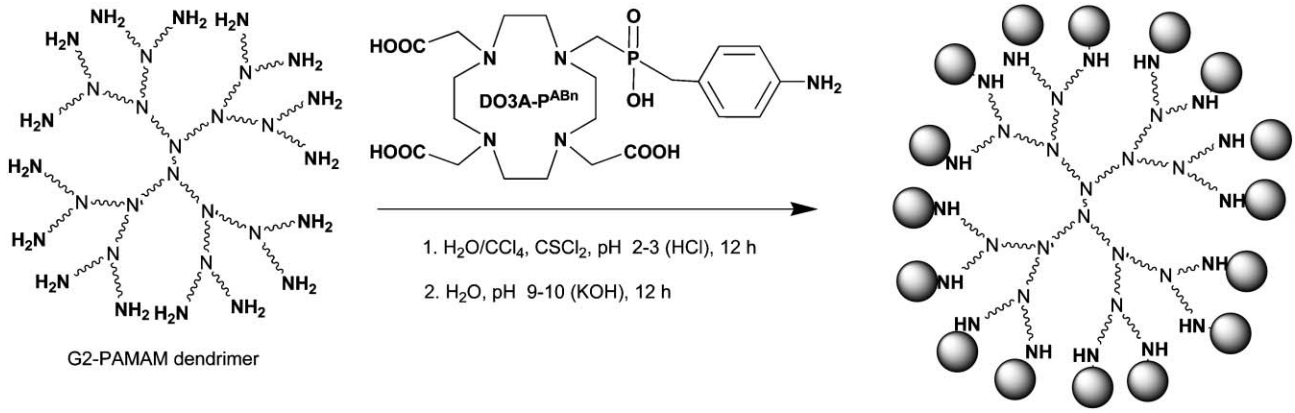
In this communication we report on the relaxometric characterization of the generation-two (G2) full-loaded conjugate, containing 16 gadolinium chelates on the surface of the dendrimer (Scheme 1). The highly reactive isothiocyanate derivative of the title ligand was obtained by mixing of the $DO3AP^{ABn}$ with thiophosgene under biphasic (H_2O/CCl_4) acidic conditions. The G2-16 conjugate was subsequently obtained by reacting, in aqueous media, the isothiocyanate with the sixteen primary amino groups of the PAMAM dendrimer. The reaction was carried out at pH = 9 and at 30 °C for several hours, in the presence of an excess of the ligand. Purification of the final conjugate was provided by ultrafiltration and the final product was characterized by ^1H NMR measurement and elemental analysis.‡ The corresponding Gd^{III} complex was synthesized by mixing the conjugate with a twofold excess of $GdCl_3$ at pH 7. The resulting cloudy solution was stirred at 50 °C overnight. Finally, a large excess of EDTA was added and the resulting clear mixture was stirred for an additional 12 h under identical conditions. All the low-molecular weight impurities were finally removed by ultrafiltration. The amount of metal loaded was assessed by relaxometric titration of the free conjugate with $GdCl_3$ where a full complexation of 16 Gd^{III} ions was confirmed.‡ The relaxivity of G2-16Gd assumes the value of 20.4 $mM^{-1}\ s^{-1}$.§ The observed r_1 is not limited by the exchange lifetime of the coordinated water molecule as the ^{17}O NMR R_2 data gave a τ_M^{298} value of *ca.* 30 ns (see ESI, Fig. S1†). Interestingly, the proton relaxivity of this system was found to be pH-dependent and, at pH < 6, it increases to *ca.* 24.8 $mM^{-1}\ s^{-1}$. As the relaxivity of Gd-$DO3AP^{ABn}$ is constant in the pH range 2.5 to 12,[5] the observed behavior has to be related to an effect associated with the protonation of donor groups on the dendrimer backbone. We speculate that the protonation of the ethylenediamine core of the dendrimer as well as of branching tertiary amines may rigidify the backbone of the dendrimer itself[9] and/or induce the formation of hydrogen bonds with neighboring oxygen atoms of the Gd-chelates. The occurrence of these intra-branch interactions yields a decrease in the internal mobility of each paramagnetic complex and, in turn, an increase of the observed relaxivity.

On this basis, it was decided to investigate the effect on the relaxivity of formation of ionic pairs between the negatively charged G2-16Gd dendrimer (net charge: −16) and polycationic species. The experiment consists of the titration of the paramagnetic system with an increasing amount of the substrate and the measurement of the enhancement of the water proton relaxation rate that occurs upon binding interaction. This increase of the relaxation rate is expressed in terms of relaxation enhancement, ε^*, which is given by the following Equation (1):

$$\varepsilon^* = \left(R_{1obs}^{Gd-Sub} - R_{1obs}^{Sub}\right) / \left(R_{1obs}^{Gd} - R_{1obs}^{water}\right) \quad (1)$$

† Electronic supplementary information (ESI) available: Fig. S1 – VT-^{17}O R_2 NMR data for G2-16Gd conjugate; Fig. S2 – relaxometric titration of G2-16Gd solution with meglumine; Eq. S1 – full set of equations for K_A determination. See http://www.rsc.org/suppdata/cc/b4/b418712a/

*petrh@natur.cuni.cz (Petr Hermann)
silvio.aime@unito.it (Silvio Aime)



Scheme 1 Reaction sequence leading to the full-loaded G2-PAMAM dendrimeric conjugate (simplified representation).

(1)

In Eq. 1, the observed longitudinal relaxation rates refer to solutions containing the complex and the substrate (Gd–Sub), the substrate (Sub), the complex (Gd), and the pure solvent (water), respectively. The limiting value of ε^*, corresponding to the fully bound complex, is denoted with ε_b. By titrating an aqueous solution of the paramagnetic dendrimer with cationic polyaminoacids of different polymerization degree (PD), *i.e.* poly(Arg) (PD $\sim$ 56 and 320) and poly(Lys) (PD $\sim$ 61), the observed relaxivity increases, to reach a plateau of *ca.* 34 mM^{-1} s^{-1} (ε_b = 1.6, irrespective of the size of the poly(Arg)) and 28 mM^{-1} s^{-1} (ε_b = 1.4, Fig. 1), respectively. In order to estimate the strength of the interaction, an effective affinity constant (K_A) was calculated by means of the proton relaxation enhancement (PRE) method (see ESI, Eqs S1†), under the assumption that the interacting sites are equivalent.[10] In fact, since the exact speciation in solution is unknown and the number of relevant experimental points is limited due to formation of a precipitate in charge-balanced mixtures (Fig. 1), only the products $n{\cdot}K_A$ can be obtained ($n{\cdot}K_A^{pLys61}$ = 570 $\pm$ 17 mM^{-1}, $n{\cdot}K_A^{pArg56}$ = 250 $\pm$ 10 mM^{-1}, $n{\cdot}K_A^{pArg320} > 10^4$ mM^{-1}), where n is the number of independent interacting sites. These findings support the view that the relaxation enhancement is not primarily associated with the formation of multimeric structures involving crosslinking of more polyaminoacid chains by a single unit of the dendrimeric conjugate. Rather, the independence of the relaxivity enhancement observed from the length and nature of the polyaminoacid chain indicates that the polycationic species act as a sort of "glue" that reduces the internal mobility of the Gd-chelates, in a way analogous to that above surmised for the protonation of the dendrimeric backbone. In order to further support this hypothesis, we measured the $1/T_1$ NMRD profiles of G2-16Gd in the absence and in the presence of polyarginine over a broad range of frequencies (Fig. 2). Clearly, the high-field relaxivity peak associated with the molecular correlation time τ_R moves to a lower field upon formation of the ion-pair interaction to indicate the increase of the τ_R value.[3] Another possible contribution to the observed relaxivity gain could arise from the possible formation of a strongly hydrogen-bonded network of second-sphere water molecules induced by the electrostatic interaction.[11] This effect has been previously observed and is responsible for a large increase of the relaxivity of anionic Gd^{III} complexes in the presence of cationic substrates.[11] To this purpose, the G2-16Gd conjugate was titrated with the low-molecular weight organic base meglumine (*N*-methyl-D,L-glucamine). In spite of the known ability of meglumine to induce a marked increase of the relaxivity through ion-pairing interactions with negatively charged complexes,[12] no changes in relaxivity were detected in our case (see ESI, Fig. S2†).

In conclusion, it may be worth noting that this novel route to relaxation enhancement arising from ion pair formation with large-sized substrates may be exploited, besides in the class of dendrimers, also in other systems.

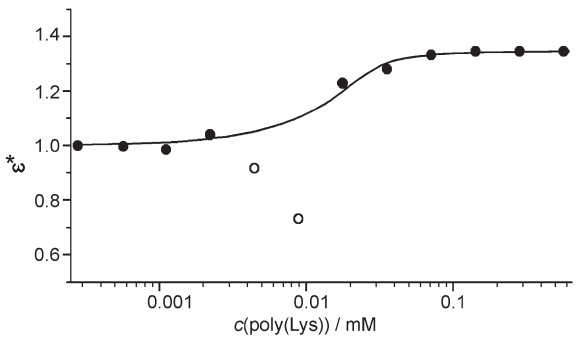


Fig. 1 Relaxometric titration (at 20 MHz) of the G2-16Gd conjugate (22 μM) with poly(Lys) (mean PD $\sim$ 61). The open circles represent the data points where a partial precipitation occurred. Data were obtained at pH 7.5 and 25 °C.

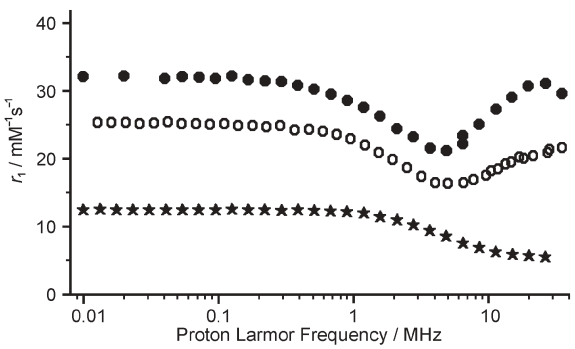


Fig. 2 ^1H NMRD profiles (pH 7.5 and 25 °C) of the G2-16Gd dendrimer (open circles), its adduct with poly(Arg)-320 (filled circles) and of free Gd-DO3AP^{ABn} (filled stars).

We thank the GAČR (No. 203/03/0168), GAUK, MURST, the EC COST Action D18 and EMIL for support. MB and PH acknowledge the NATO Science Program for a travel grant (contract n° PST.CLG 980045).

Jakub Rudovský,[a] Petr Hermann,*[a] Mauro Botta,[b] Silvio Aime*[c] and Ivan Lukeš[a]

[a]*Department of Inorganic Chemistry, Universita Karlova, Hlavova 2030, 128 40, Prague 2, Czech Republic. E-mail: petrh@natur.cuni.cz; Fax: +420-221951253; Tel: +420-21951263*

[b]*Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale ''Amedeo Avogadro'', Spalto Marengo 33, I-15100, Alessandria, Italy*

[c]*Dipartimento di Chimica I. F. M., Università di Torino, Via P. Giuria 7, I-10125, Torino, Italy. E-mail: silvio.aime@unito.it; Fax: +39-0116707855; Tel: +39-0116707520*

Notes and references

‡ **G2-16** – ^{1}H NMR: 1.95–3.95 (m, 638H, aliphatics); 7.17 (m, 64H, aromatics). The chelate/dendrimer ratio was determined to be 16.5. An average number of 16.9 chelates per dendrimer was calculated from the nitrogen/sulfur ratio, consistent with an empirical molecular mass of the batch of 13500 Da. **G2-16Gd** – Overtitration was detected by the break of the linear dependence of r_1 on c(Gd) at molar ratio Gd/G2-16 = 15.6. All the attempts made to characterize the prepared compounds by MS (ES-Ion Trap and MALDI-TOF) failed, probably because of the multiple-anionic character of the compounds responsible for the unavoidable capture of H^+, Na^+ and K^+ cations during the measurement. A monodispersity higher than 95% is declared by the manufacturer for the G2-PAMAM dendrimer.

§ Unless otherwise stated, the values of r_1 were measured at 20 MHz and 25 °C.

1 A. E. Merbach and É. Tóth, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Wiley, Chichester (UK), 2001.
2 P. A. Rinck, *Magnetic Resonance in Medicine*, ABW Wissenschaftsverlag GmbH, Berlin, 2003.
3 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293.
4 S. Aime, A. Barge, M. Botta and E. Terreno, in *Metal Ions in Biological Systems*, Eds. A. Sigel and H. Sigel, Marcel Dekker Inc., New York, 2003, Vol. 40, Chapter 16.
5 J. Rudovský, J. Kotek, P. Hermann, I. Lukeš, V. Mainero and S. Aime, *Org. Biomol. Chem*, 2005, **3**, 112.
6 R. Esfand and D. A. Tomalia, *Drug Discov. Today*, 2001, **6**, 427.
7 E. C. Wiener, M. W. Brechbiel, H. Brothers, R. L. Magin, A. Gansow, D. A. Tomalia and P. C. Lauterbur, *Magn. Reson. Med.*, 1994, **31**, 1; H. Kobayashi, S. Kawamoto, T. Saga, N. Sato, T. Ishimori, J. Konishi, K. Ono, K. Togashi and M. W. Brechbiel, *Bioconjugate Chem.*, 2001, **12**, 587.
8 É. Tóth, D. Pubanz, S. Vauthey, L. Helm and A. E. Merbach, *Chem. Eur. J.*, 1996, **2**, 1607.
9 W. Chen, D. A. Tomalia and J. L. Thomas, *Macromolecules*, 2000, **33**, 9169.
10 S. Aime, M. Botta, M. Fasano and E. Terreno, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Eds. A. E. Merbach and É. Tóth, John Wiley & Sons, Chichester (UK), 2001, Chapter 5.
11 M. Botta, *Eur. J. Inorg. Chem.*, 2000, 399.
12 S. Aime, M. Botta, E. Terreno, P. L. Anelli and F. Uggeri, *Magn. Reson. Med.*, 1993, **30**, 583.

