Poly-β-cyclodextrin based platform for pH mapping *via* a ratiometric $^{19}F/^{1}H$ MRI method†

Eliana Gianolio, Roberta Napolitano, Franco Fedeli, Francesca Arena and Silvio Aime*

Received (in Cambridge, UK) 20th July 2009, Accepted 26th August 2009
First published as an Advance Article on the web 21st September 2009
DOI: 10.1039/b914540k

The *in vitro* validation of a new pH mapping MRI method based on a supramolecular poly-β-cyclodextrin–^{19}F–Gd adduct is reported.

Mapping pH is an important task in medical imaging as changes in pH usually accompany the development of various pathologies such as tumours, strokes and infections.

Several attempts to map pH by MRI have been reported.[1–10] One method relies on the use of paramagnetic Gd(III) complexes whose structures have been designed in order to make their relaxivities (r_{1p}) responsive to the pHs of the microenvironments in which they are distributed. In order to be usable, the method requires an independent knowledge of the concentration of the paramagnetic complex; otherwise the observed changes in water protons T_1 may be ascribed either to changes in the relaxivity (r_{1p}) or to changes in the concentration.[11] Sherry, Gillies *et al.*[12,13] tackled this issue in *in vivo* pH mapping experiments by assuming that the local concentration of the pH-responsive agent was the same as that of a related non-responsive paramagnetic complex. Clearly this approach may be misleading as minor structural changes in the complex may result in dramatic effects on its biodistribution.

In principle, one may recover the concentration data by introducing on the surface of the pH-responsive agent a concentration reporting moiety consisting, for instance, of an ensemble of highly sensitive NMR heteronuclei (*e.g.* ^{19}F, ^{31}P, ^{13}C). The major drawback of this approach is related to the broadening of the heteronuclear resonance caused by its proximity to the fast relaxing Gd(III) ion. It is evident that the occurrence of broadened resonances invariably leads to inaccuracies in the determination of the concentration parameter.

Herein an alternative route to developing a ratiometric Gd/^{19}F method for pH mapping is reported. Instead of being covalently bound, the Gd(III) and ^{19}F containing moieties are "hosted" by the same carrier, which is a poly-β-cyclodextrin (poly-β-CD) substrate consisting of 8–10 β-CD units. This ensures that the ^{19}F nuclei are sufficiently far from the Gd(III) centre so as to avoid drawbacks in the signal acquisition. The binding of the Gd-complex‡ and the ^{19}F-containing reporter§ to the poly-β-CD substrate has been ensured by the functionalization of both molecules with an adamantane moiety that is known to strongly bind[14] the β-CD cavity (Fig. 1).

The pH responsiveness is provided by a Gd(III)-chelate whose coordination cage consists of a tetraazamacrocycle bearing three acetate and one substituted sulfonamide arms. The protonation–deprotonation step of the latter functionality takes place at pH = 6.7. Upon deprotonation, the sulfonamide enters into the coordination cage of the Gd(III) ion by replacing the two inner sphere water molecules (Scheme 1). This change in hydration of the paramagnetic centre results in a dramatic effect on the observed relaxivity of the complex that becomes strongly pH-dependent at values close to neutrality (Fig. 2a). Upon formation of the supramolecular adduct with the poly-β-CD substrate, the lengthening of the molecular reorientation time yields an increase in relaxivity that is particularly pronounced at pH < 6 and at a magnetic field of *ca.* 1 T (Fig. 2a). This gain in relaxivity enhancement observed at acidic pHs and at fields around 1 T is clearly depicted in Fig. 2b, where the NMRD profiles of the supramolecular adduct at pH 5.8 and 8.4 are reported. In acidic conditions, a strong relaxivity peak centred around 1 T is observed for the Gd-complex endowed with two inner sphere water molecules, whereas in basic conditions a lower enhancement is obtained in the case of the outer sphere system.

The binding affinity of the adamantane functionalized Gd-complex towards the poly-β-CD substrate has been assessed by the proton relaxation enhancement (PRE) method[15] by titrating a 0.1 mM solution of the paramagnetic complex with

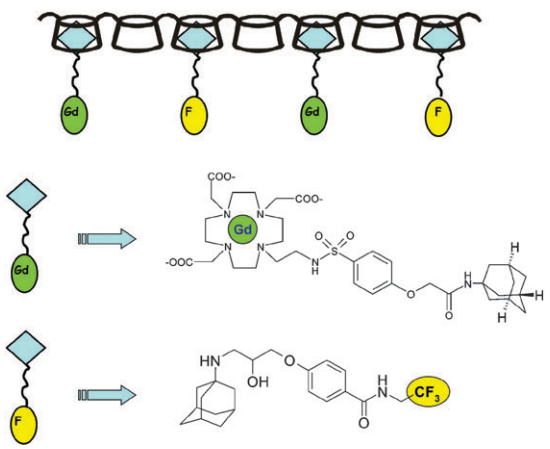
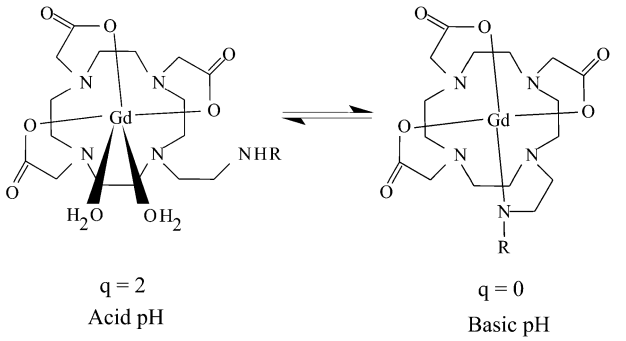


Fig. 1 Schematic representation of the supramolecular adduct between poly-β-CD, the Gd(III)-complex and the ^{19}F-reporting molecule designed for pH mapping.

Dept. of Chemistry IFM and Molecular Imaging Center, Via Nizza 52, Torino, Italy. E-mail: silvio.aime@unito.it; Fax: +39 0116706487; Tel: +39 0116706451

† Electronic supplementary information (ESI) available: Full details on the synthesis of the Gd(III)- and F-containing probes, the determination of their binding affinities toward poly-β-CD and the calibration lines for the determination of Gd concentration are provided. See DOI: 10.1039/b914540k



Scheme 1 Mechanism of action of the pH-responsive Gd(III)-complex: the protonation–deprotonation of the sulfonamide group produces changes in the complex hydration.

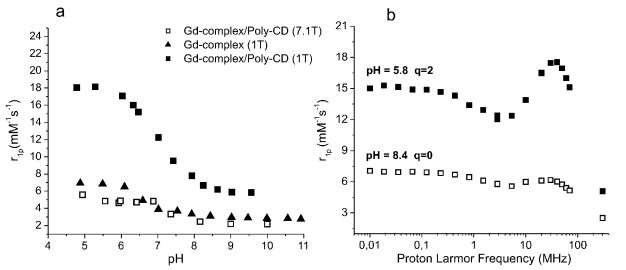


Fig. 2 (a) Profiles of water proton relaxivity as a function of pH measured for the supramolecular adduct poly-CD–^{19}F-reporter–Gd-complex (20 mM/5 mM/1 mM) at 298 K and 1 T (■) and 7.1 T (□) and for the free Gd-complex at 298 K and 1 T (▲); (b) NMRD profiles of the supramolecular adduct poly-CD–^{19}F-reporter–Gd-complex (20 mM/5 mM/1 mM) at 298 K and pH = 5.8 (■) and 8.4 (□).

poly-β-CD (ESI†). Fitting of the titration curve yielded a K_A value of 1300 M^{-1} independent of the pH of the solution. Analogously, the interaction of the ^{19}F-containing reporter§ with poly-β-CD has been assessed by the measurement of the ^{1}H-NMR chemical shift changes upon formation of the inclusion complex (ESI†). The resulting K_A value (14 000 M^{-1}) is one order of magnitude higher than that of the corresponding Gd-complex. The observed behaviour may be related to the overall enhanced hydrophobicity of the latter compound as well as to the possible role of the OH group in forming additional H-bonds with the outer OH groups of the poly-β-CD substrate.

The proof of concept of this approach has been achieved *in vitro* by acquiring the ^{1}H and ^{19}F-MR images of a phantom consisting of four tubes filled with solutions of poly-β-CD–^{19}F-reporter–Gd-complex at different values of concentration and pH. In order to guarantee that both the Gd-complex and the fluorine reporter are fully bound to the poly-β-CD substrate, the observed K_A values suggested that the supramolecular adduct components have to be present in the following molar ratio: poly-β-CD : ^{19}F-reporter : Gd-complex = 20 : 5 : 1. The phantom (Fig. 3) consists of five capillaries containing the following solutions: (1) $NaPF_6$ 25 mM as a reference specimen for the quantitative assessment of ^{19}F present in the other four capillaries; (2) poly-CD 10 mM, F-reporter 2.5 mM, Gd-complex 0.5 mM at pH = 6.8; (3) poly-CD 13 mM, F-reporter 3.25 mM, Gd-complex 0.65 mM at pH = 7.2; (4) poly-CD 30 mM, F-reporter 7.5 mM, Gd-complex 1.5 mM at pH = 7.6; (5) poly-CD 20 mM, F-reporter 5 mM, Gd-complex 1 mM at pH = 8.2.

Upon acquiring a T_1-weighted ^{1}H-MR image (7.1 T),¶ the obtained signal intensities (Fig. 3a) do not reflect in any way the order of the pH values of the solutions contained in the capillaries numbered 2–5. Actually tube 4 displays a markedly higher intensity as a consequence of the highest concentration of the paramagnetic probe. In Fig. 3b the ^{19}F image of the five capillaries recorded at 7.1 T has been reported; the ^{19}F signal intensity in each capillary is obtained on the basis of the ratio of the signal generated by that capillary to the signal generated by the reference 25 mM $NaPF_6$ solution in capillary 1. Clearly, the obtained ^{19}F signal intensities correctly reflect the amounts of fluorine in the specimens numbered 2–5 and therefore they can be used to normalize the Gd(III) concentrations in the T_1-weighted ^{1}H-MR images (calibration curves in ESI†). This operation leads to the transformation of the relaxation data (R_1^{obs}) into relaxivity (r_{1p}) values, thus establishing the

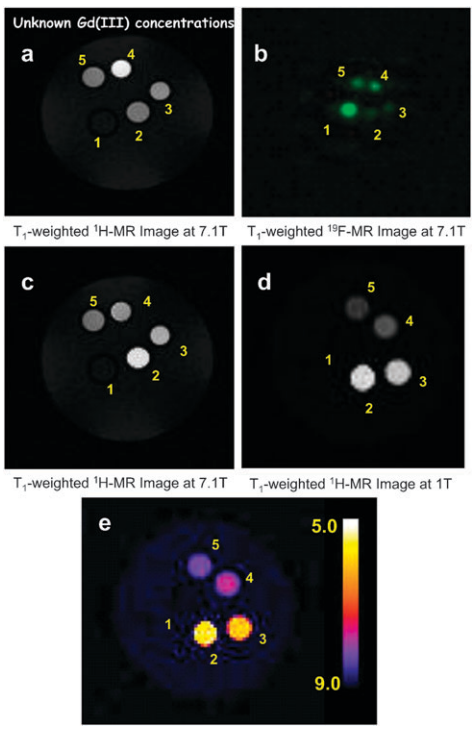


Fig. 3 MR Images of a phantom containing the following solutions: (1) $NaPF_6$ 25 mM; (2) poly-CD 10 mM, F-reporter 2.5 mM, Gd-complex 0.5 mM at pH = 6.8; (3) poly-CD 13 mM, F-reporter 3.25 mM, Gd-complex 0.65 mM at pH = 7.2; (4) poly-CD 30 mM, F-reporter 7.5 mM, Gd-complex 1.5 mM at pH = 7.6; (5) poly-CD 20 mM, F-reporter 5 mM, Gd-complex 1 mM at pH = 8.2. (a) T_1-weighted spin-echo ^{1}H-MR image acquired at 7.1 T (TR/TE/NEX (80/4.4/32), FOV 1.6 cm, 1 slice 1 mm); (b) T_1-weighted spin-echo ^{19}F-MR Image acquired at 7.1 T (TR/TE/NEX (2000/1.4/384), FOV 2 cm, 1 slice 3 mm); (c) T_1-weighted spin-echo ^{1}H-MR image acquired at 7.1 T and normalized to the concentrations found from image (b); (d) T_1-weighted spin-echo ^{1}H-MR image acquired at 1 T (TR/TE/NEX (80/7.2/32), FOV 2 cm, 1 slice, 2 mm) and normalized to the concentrations found from image (b); (e) pH-map image derived from image (d).

relationship between the latter data and the pH values; this is shown in Fig. 3c.

A further increase in the sensitivity of the pH assessment can be obtained from acquiring the T_1-weighted ^{1}H-MR image at 1 T where the relaxivity gap between $q = 2$ and $q = 0$ conditions is maximized (Fig. 3d).

In summary, the proof of concept reported here shows that a novel, highly accurate pH mapping can be pursued by MRI using the heteronuclear ^{19}F signal to normalize the relaxation enhancement values induced by a pH-responsive Gd-complex. Moreover, the use of poly-β-CD as a macromolecular carrier can be further exploited to host analogous adamantane functionalized moieties to endow the supramolecular adduct with targeting as well as multimodal capabilities.

For an *in vivo* translation of the proposed method, it appears necessary to improve the binding affinity of the adamantane functionalized Gd-complex to the poly-β-CD substrate. The system used here shows a good binding to HSA (K_A = 13 000 M^{-1}). Thus, at the physiological protein concentration of 0.6 mM and a 1 mM Gd-complex concentration (and poly-β-CD and ^{19}F-reporter in the above established ratio), *ca.* 20% of the paramagnetic complex will be bound to HSA.

Finally, as it is known that in Gd-DO3A derivatives,‡ the two inner sphere water molecules can be replaced by the coordination of endogenous oxoanions,[1,16,17] an improvement in the design of the pH-responsive coordination cage should be pursued. It has already been shown that the introduction of carboxyalkyl substituents on the acetate arms inhibits anion binding while maintaining the pH responsiveness.[1,18]

Economic and scientific support from MIUR (FIRB RBI P06293N_001 and PRIN 2007W7M4NF projects), EC-FP6-projects DiMI (LSHB-CT- 2005-512146), EMIL (LSHC-CT-2004-503569), MEDITRANS (Targeted Delivery of Nanomedicine: NMP4-CT-2006-026668), ENCITE (European Network for "Cell Imaging and Tracking Expertise" 201842) and EU-COST D38 Action is gratefully acknowledged.

Notes and references

‡ The synthesis of the ligand involved alkylation of the DO3A–tris-*tert*-butylester with *N*-(benzyloxycarbonyl)-2-bromoethylamine (CH_3CN, K_2CO_3, 4 days at 50 °C). Following chromatographic purification on silica, cleavage of the benzyloxycarbonyl group has been achieved by hydrogenation with Pd/C at atmospheric pressure to yield the free primary amino group. This group allowed the subsequent coupling with 4-(methyloxycarbonylmethylenoxy)-benzenesulfonyl chloride (CH_3CN, triethylamine, 3 days at RT), obtained by reaction of methyl phenoxyacetate with chlorosulfonic acid in dichloromethane. After purification by chromatography on silica and hydrolysis of the methyl ester (MeOH–H_2O, pH 12, 1 hour), the carboxylic acid was coupled with 1-adamantamine (DMF, HATU, 20 hours at RT). Following chromatographic purification on silica, treatment with CF_3COOH removed the *tert*-butyl protecting groups and reaction with $GdCl_3$ in water at pH 7 led to the isolation of the neutral complex.

§ The ^{19}F-containing reporter has been synthesized from the alkylation of 1-adamantamine by 4[(2,3-epoxy)propyloxy]phenylacetic acid phenyl methyl ester (CH_3CN, DIPEA, 5 days at 50 °C), obtained from the reaction of benzyl 4-hydroxybenzoate with epibromohydrin (DMF, K_2CO_3, 6 hours at 70 °C). Cleavage of the benzyl group has been achieved by hydrogenation with Pd/C at atmospheric pressure to yield the carboxylic acid, that has been coupled to 2,2′,2″-trifluoro-ethylamine (DMF, DIPEA, HBTU, 16 hours at RT). The final product has been carefully purified to > 98% by liquid chromatography on Amberchrom® CG161 resin.

¶ Magnetic resonance images have been acquired on a Bruker Avance300 spectrometer operating at 7.1 T equipped with a microimaging probe and on an Aspect scanner (Netanya, Israel) operating at 1 T using standard T_1-weighted multislice multiecho sequences.

1 M. P. Lowe, D. Parker, O. Reany, S. Aime, M. Botta, G. Castellano, E. Gianolio and R. Pagliarin, *J. Am. Chem. Soc.*, 2001, **123**, 7601.
2 S. R. Zhang, K. C. Wu and A. D. Sherry, *Angew. Chem., Int. Ed.*, 1999, **38**, 3192.
3 S. Aime, M. Botta, S. Geninatti, G. B. Giovenzana, G. Palmisano and M. Sisti, *Chem. Commun.*, 1999, 1577.
4 S. Aime, A. Barge, M. Botta, J. A. K. Howard, R. Kataky, M. P. Lowe, J. M. Moloney, D. Parker and A. De Sousa, *Chem. Commun.*, 1999, 1047.
5 R. Hovland, C. Glogard, A. J. Aasen and J. Klaveness, *J. Chem. Soc., Perkin Trans. 2*, 2001, 929.
6 M. Woods, S. Zhang, E. Von Howard and A. D. Sherry, *Chem.–Eur. J.*, 2003, **9**, 4634.
7 S. Aime, A. Barge, D. Delli Castelli, F. Fedeli, A. Mortillaro, F. U. Nielsen and E. Terreno, *Magn. Reson. Med.*, 2002, **47**, 639.
8 J. A. Pikkemaat, R. T. Wegh, R. Lamerichs, R. A. van de Molengraaf, S. Langereis, D. Burdinski, A. Y. F. Raymond, H. M. Janssen, B. M. F. de Waal, N. P. Willard, E. W. Meijer and H. Grull, *Contrast Media Mol. Imaging*, 2007, **2**, 229.
9 M. Oishi, S. Sumitani and Y. Nagasaki, *Bioconjugate Chem.*, 2007, **18**, 1379.
10 A. M. Kenwright, I. Kuprov, E. De Luca, D. Parker, S. U. Pandya, P. K. Senanayake and D. G. Smith, *Chem. Commun.*, 2008, 2514.
11 S. Aime, F. Fedeli, A. Sanino and E. Terreno, *J. Am. Chem. Soc.*, 2006, **128**, 11326.
12 N. Raghunand, C. Howison, A. D. Sherry, S. R. Zhang and R. J. Gillies, *Magn. Reson. Med.*, 2003, **49**, 249.
13 M. L. Garcia-Martin, G. V. Martinez, N. Raghunand, A. D. Sherry, S. R. Zhang and R. J. Gillies, *Magn. Reson. Med.*, 2006, **55**, 309.
14 J. Carrazana, A. Jover, F. Meijide, V. H. Soto and J. Vasquez Tato, *J. Phys. Chem. B*, 2005, **109**, 9719.
15 S. Aime, M. Botta, M. Fasano and E. Terreno, in *The chemistry of Contrast Agents*, ed. A. Merbach and E. Toth, John Wiley & Sons, Chichester, UK, 2001, pp. 193–241.
16 E. Terreno, M. Botta, P. Boniforte, C. Bracco, L. Milone, B. Mondino, F. Uggeri and S. Aime, *Chem.–Eur. J.*, 2005, **11**, 5531.
17 S. Aime, E. Gianolio, E. Terreno, G. B. Giovenzana, R. Pagliarin, M. Sisti, G. Palmisano, M. Botta, M. P. Lowe and D. Parker, *J. Biol. Inorg. Chem.*, 2000, **5**, 488.
18 J. I. Bruce, R. S. Dickins, L. J. Govenlock, T. Gunnlaugsson, S. Lopinski, M. P. Lowe, D. Parker, R. D. Peacock, J. J. B. Perry, S. Aime and M. Botta, *J. Am. Chem. Soc.*, 2000, **122**, 9674.

