Imaging Agents

DOI: 10.1002/anie.201007105

Yb^{III}-HPDO3A: A Dual pH- and Temperature-Responsive CEST Agent**

*Daniela Delli Castelli, Enzo Terreno, and Silvio Aime**

The use of contrast agents (CAs) has been introduced about 25 years ago.[1] Since then, a number of paramagnetic gadolinium(III) complexes have been proposed to tackle relevant bio-medical issues by endowing the systems with targeting and responsive capabilities.[2] Responsive agents suffer from the drawback that the exploitation of the responsiveness of their relaxivity towards given physico-chemical parameters requires the knowledge of the actual concentration of the agent in the region of interest.[3] This drawback may be overcome by exploiting the frequency-encoding contrast that can be generated by chemical exchange saturation transfer (CEST) agents.[4,5] In fact, it has been demonstrated that such probes may allow the detection of a concentration independent magnetic resonance imaging (MRI) response by means of ratiometric approaches. The latter require the design of agents provided with two pools of exchanging protons that show a different dependence of their CEST properties (e.g. exchange rate or chemical shift) toward the pH value of interest. Alterations of the pH values are involved in several pathological states, and, consequently, there is a high demand for developing in vivo protocols to monitor pH values. Within the field of CEST agents, this task has been so far addressed by using either diamagnetic or paramagnetic agents.[6–9] The use of the latter class of agents (ParaCEST) is particularly useful because the larger chemical shift separation present in paramagnetic complexes allows the exploration of faster proton-exchange rates, that, in turn, may result into a marked sensitivity enhancement. ParaCEST agents have also been investigated as temperature reporters that exploit the effects of the exchange rates[10] or chemical shift [11] of the mobile protons, and the overall stability of the agents.[12] As it might be of interest (e.g. for imaging-guided hyperthermia treatments) to assess simultaneously pH and temperature, it has been deemed of relevance to merge the two responses into the same compound. To select a system with a good potential for a clinical translation, the choice has been directed toward Ln^{III}-HPDO3A complexes (HPDO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) in virtue of the successful clinical use of Gd^{III}-HPDO3A (ProHance). Thus, Ln^{III}-HPDO3A complexes should have thermodynamic and kinetic stabilities, in vivo distribution, and excretion properties very similar to those shown by ProHance. Recently, it has been demonstrated that hydroxylic protons can be successfully used for generating CEST contrast when they are close enough to the paramagnetic center.[13,14] Hence, as the exchange rate of such protons is expected to be pH dependent, these chelates may act as pH sensitive ParaCEST agents.

Concerning the choice of the lanthanide, it has been reported that, when the mobile pool is different from metal coordinated water protons, Yb^{III} is the most efficient ion because of to the good compromise between shifting ability and T_2 shortening.[10,15] Hence, the Yb^{III}-HPDO3A complex was selected for achieving the purpose of this work. The ^{1}H NMR spectrum of this complex (Figure 1) indicates the presence in solution of two species that strongly differ in the line width of their resonances.

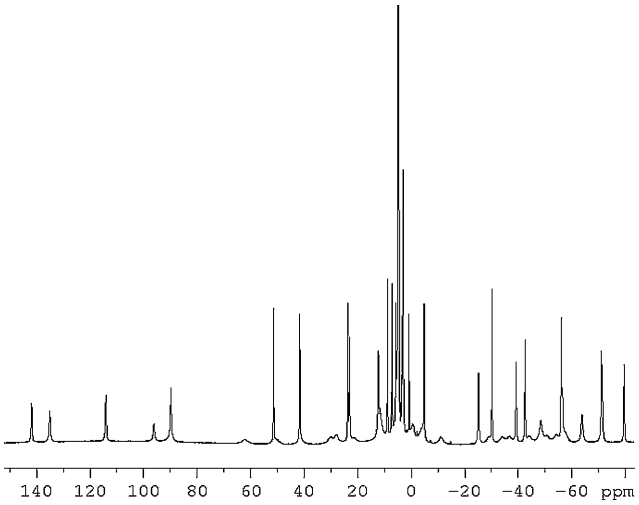


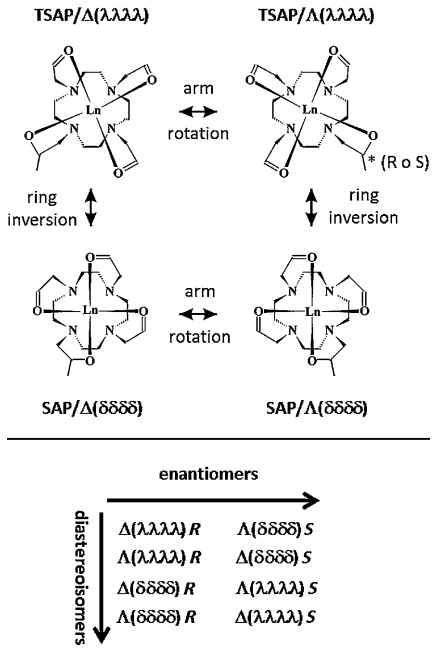
Figure 1. 600 MHz ^{1}H NMR spectrum of Yb^{III}-HPDO3A at 5 °C in D_2O.

In principle, this macrocyclic complex may be present in solution as eight isomeric forms that differ in the layout of the acetate arms, in the conformation of the macrocyclic ring, and in the configuration of the chiral center (*R* or *S*; Scheme 1).

Among the possible eight isomers, only four diastereoisomers can be distinguished in the NMR spectrum, and among them, only two were detected. Because NMR experiments performed at different temperatures suggested that the isomers do not interconvert, it is likely that the two isomers correspond to the *R* and *S* forms of the same conformer,

[*] Dr. D. Delli Castelli, Prof. E. Terreno, Prof. S. Aime
Dipartimento di Chimica I.F.M., Unversità di Torino
Via Nizza n°52, Torino (Italy)
Fax: (+39) 011-670-5487
E-mail: silvio.aime@unito.it

[**] Economic and scientific support from MIUR (FIRB and PRIN projects), EC-FP6-projects MEDITRANS, and EU-COST D38 Action, EC-FP7-projects ENCITE, PIIMDMT, and Nano-IGT (Regione Piemonte). HPDO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, CEST = chemical exchange saturation transfer.



Scheme 1. Schematic representation of the isomeric forms of Yb^{III}-HPDO3A in solution.

respectively. The signals that belong to the hydroxyl groups of these isomers were assigned upon comparison of the NMR spectra recorded in water and D_2O, respectively. At pH 5 and 20 °C, the hydroxyl proton that belongs to the most shifted isomer resonates at $\delta = 99$ ppm from water, and can be barely detected in the NMR spectrum, while the OH group that belongs to the less shifted isomer absorbs at $\delta = 71$ ppm from bulk water (Figure 2).

Figure 3 reports the Z-spectra (i.e. the intensity of the water signal as a function of the saturation frequency offset) acquired at 20 °C and 37 °C for a 20 mM solution of Yb^{III}-HPDO3A (7 T, pH 7.3). Both spectra clearly show the presence of the two exchanging pools that belong to the OH protons of the two isomers.

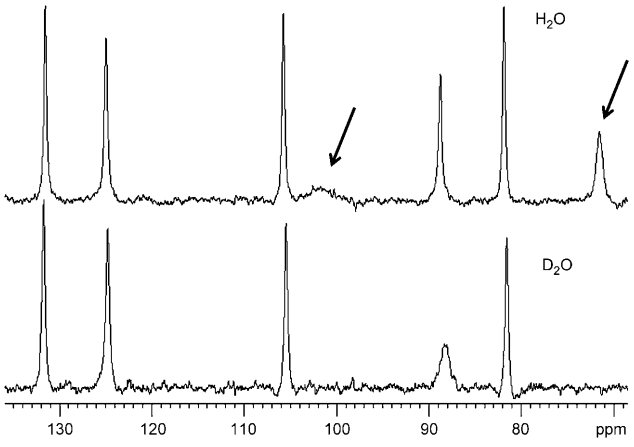


Figure 2. 600 MHz ^{1}H NMR spectra of Yb^{III}-HPDO3A in D_2O and H_2O (20 °C, pH 5.0).

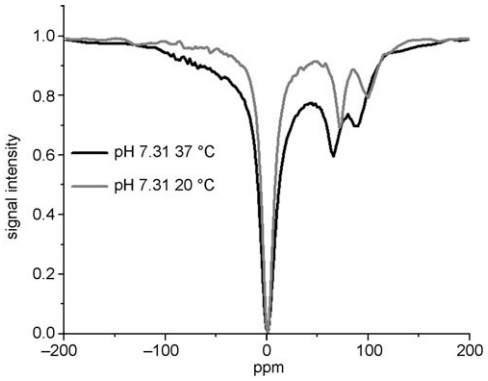


Figure 3. Z-spectra of Yb^{III}-HPDO3A at 20 °C and 37 °C (7 T, 20 mM, pH 7.3, irradiation power 24 μT).

This finding allows to exploit the two signals to set up a ratiometric calibration that will report about the pH values irrespective of the probe concentration (Figure 4). This is the first example in which the ratiometric method involves mobile pools belonging to two isomers of the same molecule.

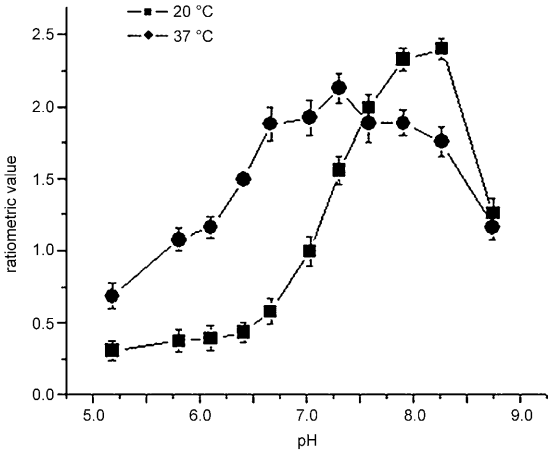


Figure 4. Average and standard deviation of the ratiometric values obtained from three samples of Yb^{III}-HPDO3A (5 mM, 10 mM, and 20 mM) at different pH and temperature values.

The results obtained suggest that an accurate pH determination can be only done in the range 6.5–8.0 at 20 °C and 5.0–7.0 at 37 °C.

The determination of the temperature can be done independently by exploiting the high temperature sensitivity displayed by the lanthanide-induced shift,[11] which is also unaffected by the pH value. Figure 5 reports the variation of the chemical shift of the hydroxylic protons of Yb^{III}-HPDO3A obtained from the analysis of Z-spectra acquired at different temperatures.

The proof-of-concept on the ability of this CEST agent to simultaneously report about pH and temperature is shown in Figure 6. A phantom containing 14 capillaries filled with solutions of Yb^{III}-HPDO3A either at different pH values or at different concentrations was subjected to a MRI investigation. By acquiring the Z-spectrum, it was possible either to

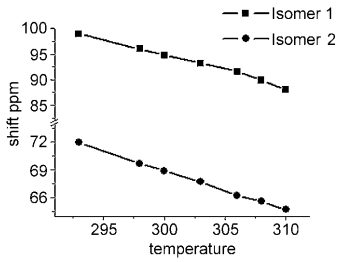


Figure 5. Temperature dependence of the chemical shift of the hydroxylic protons for the two isomers.

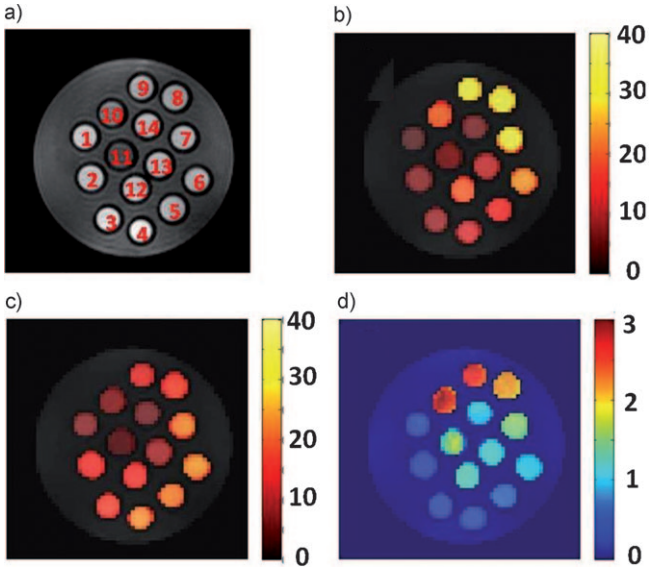


Figure 6. MR images of a phantom containing 14 capillaries containing Yb^{III}-HPDO3A either at different pH or concentration values. a) T_{2w} image, tubes from one to eleven contain a 20 mM solution of Yb-HPDO3A at increasing pH values (5.2, 5.8, 6.1, 6.4, 6.7, 7.0, 7.3, 7.6, 7.9, 8.3, 8.8) while tube twelve contains Yb^{III}-HPDO3A 10 mM at pH 7.0, tube thirteen contains Yb^{III}-HPDO3A 5 mM at pH 7.0, tube fourteen contains Yb^{III}-HPDO3A 3 mM at pH 7.0. b) CEST map acquired on the same phantom after irradiating the frequency corresponding to $\delta = 71$ ppm; c) CEST map acquired on the same phantom after irradiating the frequency corresponding to $\delta = 99$ ppm; d) ratiometric map image. The experiments have been performed at 20 °C.

determine the temperature of the sample from the chemical shift of the OH groups or measure the pH according to the ratiometric calibration curves shown in Figure 4.

In summary, Yb^{III}-HPDO3A, which is present in solution as two major isomeric forms, acts as prototype of a new family of multi-responsive CEST agents. Moreover, Yb^{III}-HPDO3A, as a ParaCEST agent, is effective at quite low concentrations (2 mM), a property that, together with the expected high tolerability, makes this agent particularly suitable for clinical translation.

Experimental Section

The HPDO3A ligand has been kindly gifted from Bracco Imaging S.p.A. (Colleretto Giacosa (TO), Italy). Yb_2O_3 was purchased from Sigma–Aldrich. Yb^{III}-HPDO3A was prepared by mixing in a stoichiometric ratio the lanthanide oxide and the ligand (1:2 molar ratio) in water and letting the mixture react for two weeks under stirring and heating at (80 °C). High resolution ^{1}H NMR spectra were recorded on a Bruker Avance 600 spectrometer. CEST-MRI experiments were performed at 7 T on a Bruker Avance 300 spectrometer equipped with a Micro2.5 microimaging probe. A RARE spin-echo sequence (rare factor 64) with an echo time of 3.3 ms and a repetition time of 5 s was used (Isotropic field of view 10 mm, acquisition matrix 64×64). The sequence was preceded by a saturation scheme consisting of a rectangular pulse (2 s long) with a RF intensity of 24 μT. Z-spectra were successively analyzed by using a home-made software compiled in MATLAB platform.[16] The total paramagnetic concentration of the solution was determined by measuring the bulk magnetic susceptibility of the solution on a Bruker Avance 600 spectrometer (14 T). Ratiometric values at each pH value have been calculated on three samples (5 mM, 10 mM, and 20 mM, respectively) as described in the literature.[7,8]

Received: November 11, 2010
Published online: January 18, 2011

Keywords: CEST agents · imaging agents · lanthanides · magnetic resonance imaging

[1] E. Toth, L. Helm, A. E. Merbach in *The chemistry of contrast agent in medical magnetic resonance imaging, Vol. 1* (Eds.: A. E.Merbach, E. Toth), Wiley-VCH, Weinheim, **2001**, pp. 45–119.
[2] C. F. G. C. Geraldes, S. Laurent, *Contrast Media Mol. Imaging* **2009**, *4*, 1–23.
[3] L. M. De Leon-Rodriguez, A. J. M. Lubag, C. R. Malloy, G. V. Martinez, R. J. Gillies, A. D. Sherry, *Acc. Chem. Res.* **2009**, *42*, 948–957.
[4] K. M. Ward, A. H. Aletras, R. S. Balaban, *J. Magn. Reson.* **2000**, *1*, 79–87.
[5] E. Terreno, D. Delli Castelli, S. Aime, *Contrast Media Mol. Imaging* **2010**, *5*, 78–98.
[6] J. Zhou, J.-F. Payen, D. A. Wilson, R. J. Traystman, P. C. M. van Zjil, *Nat. Med.* **2003**, *9*, 1085–1090.
[7] D. Longo, W. Dastrù, G. Digilio, J. Keupp, S. Langereis, S. Lanzardo, S. Prestigio, O. Steinbach, E. Terreno, F. Uggeri, S. Aime, *Magn. Reson. Med.* **2010**, DOI: 10.1002/mrm.22608.
[8] S. Aime, D. Delli Castelli, E. Terreno, *Angew. Chem.* **2002**, *114*, 4510–4512; *Angew. Chem. Int. Ed.* **2002**, *41*, 4334–4336.
[9] Y. Wu, T. Soesbe, C. G. E. Kiefer, P. Zhao, A. D. Sherry, *J. Am. Chem. Soc.* **2010**, *132*, 14002–14003.
[10] E. Terreno, D. Delli Castelli, G. Cravotto, L. Milone, S. Aime, *Invest. Radiol.* **2004**, *39*, 235–243.
[11] S. Zhang, C. R. Malloy, A. D. Sherry, *J. Am. Chem. Soc.* **2005**, *127*, 17572–17573.
[12] S. Langereis, J. Keupp, J. L. J. van Velthoven, I. H. J. de Roos, D. Burdinski, J. A. Pikkemaat, H. Gruell, *J. Am. Chem. Soc.* **2009**, *131*, 1380–1381.
[13] M. Woods, D. E. Woessner, P. Zhao, A. Pasha, M.-Y. Yang, C.-H. Huang, O. Vasalitiy, J. R. Morrow, A. D. Sherry, *J. Am. Chem. Soc.* **2006**, *128*, 10155–10162.
[14] C.-H. Huang, J. R. Morrow, *J. Am. Chem. Soc.* **2009**, *131*, 4206–4207.
[15] S. Aime, A. Barge, D. Delli Castelli, F. Fedeli, A. Mortillaro, F. U. Nielsen, E. Terreno, *Magn. Reson. Med.* **2002**, *4*, 639–648.
[16] J. Stancanello, E. Terreno, D. Delli Castelli, C. Cabella, F. Uggeri, S. Aime, *CMMI* **2008**, *4*, 136–149.

