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Novel pH-Reporter MRI Contrast Agents**

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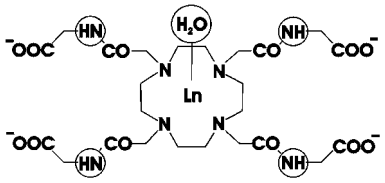
The contrast obtained in magnetic resonance imaging (MRI) relies essentially on differences in the intensity of the ^{1}H water signal. Therefore, the contrast can be augmented by the use of chemicals (contrast agents, CAs) able to enhance the relaxation properties of water protons.[1] Recently, a novel class of paramagnetic CAs has been proposed for MRI applications.[2,3] Such chemicals contain paramagnetically shifted mobile protons whose exchange with the bulk water is slow on the NMR timescale ($|k_{ex}| < |\Delta\omega|$). Thus, irradiation of the mobile protons of the agent determines a decrease of the ^{1}H water signal through the so-called chemical exchange saturation transfer (CEST) effect.[4,5]

One of the major advantages of the CEST agents relies on the possibility of designing responsive agents in which the effectiveness of the CA is not dependent on its concentration. This goal can be pursued when the CEST properties of two independent exchanging pools are monitored in the same experiment (ratiometric method).[6] This possibility has been demonstrated using a mixture of two compounds.[3] An improvement in such ratiometric methods would be obtained if the two proton-exchanging pools were parts of the same molecule (single-molecule CEST procedure). Paramagnetic [Ln(dotamGly)]$^-$ complexes (dotam = 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetrazacyclododecane) would be appropriate for this purpose since they are characterized by the presence of two kinds of mobile protons, namely, the four equivalent amide protons and the two protons of the water molecule coordinated to the Ln^{III} ion. It has been previously shown that the use of a cocktail formed by [Yb(dotamGly)]$^-$ and [Eu(dotamGly)]$^-$ allows the set-up of a ratiometric method for pH measurements by making use of the two exchanging pools provided by the amide protons of the Yb derivative and by the metal-coordinated water protons of the Eu complex, respectively. Herein we demonstrate that the paramagnetic dotamGly complexes of the lighter Ln^{III} ions (Pr, Nd, and Eu) behave as pH-responsive "single-molecule CEST" agents. The efficiency of saturation transfer (ST), once the irradiation time is sufficiently long to reach a steady-state ST value,[3] is directly related to the exchange rate of the irradiated protons (k_{ex}), their molar concentration (defined by the product $n[C]$, where n is the number of irradiated protons and $[C]$ is the molar concentration of the agent), and inversely related to the longitudinal relaxation rate of the bulk water protons during the irradiation $R_{1,irr}$ [Eq. (1)].



$$\mathrm{ST}\,\% = \left(1-\frac{I_S}{I_0}\right)100 = \frac{k_{ex}\,n[C]}{111.2\,R_{1,irr} + k_{ex}\,n[C]}\,100 \qquad (1)$$

The latter parameter, in the presence of a paramagnetic Ln^{III} complex, is mainly determined by the relaxation rate of the metal-coordinated water protons, and this is directly dependent on the intrinsic paramagnetism of the metal ion (μ_{eff}).[7]

I_S and I_0 refer to the intensity of the bulk water signal when the irradiation pulse is set on-resonance (frequency ν^{on}, signal intensity I_S) and off-resonance with respect to the frequency of the bulk water protons ($\nu^{off} = -\nu^{on}$, I_0). The off-resonance measurement is necessary to take into account the direct saturation effect on the bulk water signal which is determined by the irradiation pulse.

The data reported in Figure 1 clearly show that all the three complexes ($[C] = 30$ mM) yield significant ST effects (at 312 K and pH 7.4) upon selective irradiation of their pools of mobile protons. In the case of [Eu(dotamGly)]$^-$, the ST effect from the coordinated water is so efficient that a 10% effect is still detectable when the concentration of the complex is as low as 1 mM. This result makes this system the most efficient CEST agent so far reported if the ST effect is normalized to the number of irradiated spins.[2,4,8] The ST effect from the amide protons $(ST)_{NH}$ follows a reverse order with respect to the

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[**] Financial support from Bracco Imaging S.p.A., MIUR (PRIN), and CNR (PF Oncology, L. 95/95) are gratefully acknowledged. This work has been carried out under the framework of the EU-COST D 18 action.

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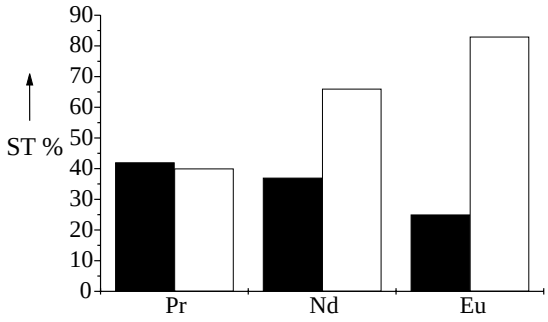

Figure 1. ST effects on the solvent water resonance upon irradiating the amide protons (black bars) and the coordinated water protons (white bars) in 30 mM solutions of [Ln(dotamGly)]$^-$ chelates (312 K, pH 7.4, irradiation time: 4 s).

irradiation of Ln-$\underline{H_2}$O signal, that is, it is higher for the Pr^{III} complex and smaller for the Eu^{III} complex.

The pH dependence of the ST efficiency (at 312 K) in a separate irradiation of the two pools of protons in the Pr, Nd, and Eu complexes is reported in Figure 2. $(ST)_{H_2O}$ is not pH-dependent in the interval investigated because the prototropic

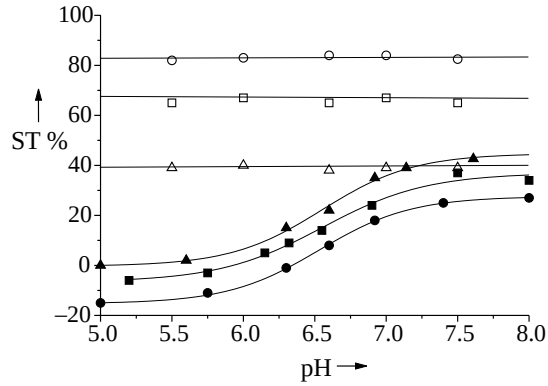

Figure 2. pH-dependence of the ST efficiency measured at 312 K upon irradiation (4 s) of the amide protons (filled symbols) and of the metal-coordinated water protons (open symbols) for 30 mM solutions of [Ln(dotamGly)]$^-$. Pr: triangle, Nd: square, Eu: circle.

exchange rate is still slower than the exchange rate of the whole water molecule. Conversely, $(ST)_{NH}$ is pH dependent in the 6–8 range. At pH < 6, the value of k_{NH} is too small to transfer saturation (Table 1), whereas at pH > 8, the value of k_{NH} becomes too high, thus approaching the coalescence condition where ST can no longer be detected. The ratiometric method is based on the assessment of a ST ratio through Equation (2).

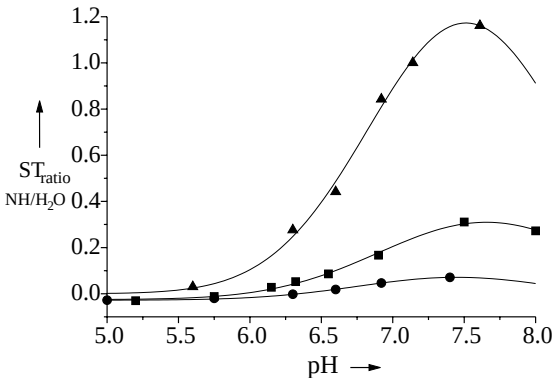

Figure 3. Ratiometric plot for [Ln(dotamGly)]$^-$ complexes (312 K, irradiation time: 4 s, selective e-burp pulse, [Ln(dotamGly)] = 30 mM). Pr^{III}: triangle, Nd^{III}: square, and Eu^{III}: circle.

$$\frac{\left(\frac{I_0-I_S}{I_0}\right)_{NH}}{\left(\frac{I_0-I_S}{I_0}\right)_{H_2O}} = \frac{n^{NH}\,k_{NH}\,R_{1,irr}^{H_2O}}{n^{H_2O}\,k_{H_2O}\,R_{1,irr}^{NH}} \qquad (2)$$

In Figure 3 the ST ratio calculated according to the above equation are reported for [Ln(dotamGly)]$^-$ (Ln = Eu, Pr, Nd) at 312 K. Although the three complexes are characterized by similar shaped ratiometric plots, the ST ratio and consequently the accuracy and the sensitivity of the pH responsiveness is, at 7.05 T, considerably higher for the Pr^{III} complex. The ratiometric method based on a "single-molecule CEST" agent overcomes the main limitations associated to the procedure based on the use of a cocktail of two paramagnetic complexes, for which the same biodistribution pattern has to be assumed. Furthermore, the total concentration of metal-containing agent administered may be lower in the case of a single agent. Conversely, the use of the cocktail could be advantageous to enhance the sensitivity of the response, because the ST ratio can be made dependent on the concentration ratio between the two CEST agents.[3]

In conclusion, the data reported in this work illustrate that paramagnetic CEST agents may be considered as attractive alternatives to the conventional Gd^{III}- or Fe^{III}-based CAs for MRI applications. In fact, ratiometric procedures such as that described here provide a route to overcome the major conceptual problem usually encountered with Gd-based responsive systems, for which it is difficult, if not impossible, to differentiate a change in the concentration of the paramagnetic agent from the variation in relaxivity eventually occurring in response to a change of a given parameter of interest.

Table 1. Exchange rates and chemical shift separation for the amide protons and the metal-coordinated water protons (312 K, pH 6, 7.05 T) in the [Ln(dotamGly)]$^-$ series.

Complex	$^{312}k_{H_2O}$ [s^{-1}]	$^{312}\Delta\omega_{H_2O}$ [rad s^{-1}]	$^{312}k_{NH}$ [s^{-1}]	$^{312}\Delta\omega_{NH}$ [rad s^{-1}]
[Pr(dotamGly)]$^-$	1.06×10^5	-1.32×10^5	128.5	2.5×10^4
[Nd(dotamGly)]$^-$	3.92×10^4	-9.4×10^4	125.0	2.02×10^4
[Eu(dotamGly)]$^-$	4.20×10^4	9.4×10^4	127.0	-7.85×10^3

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Experimental Section

The $[Ln(dotamGly)]^-$ complexes were synthesized accordingly to the procedure reported in the literature.[3]

All the measurements were carried out on a Bruker Avance 300 spectrometer at 312 K. The concentration of the metal complexes in the samples were determined by means of the Evans' method by using *tert*-butyl alchool as a reference.[9] The exchange rates of the two proton pools, k_{H_2O} and k_{NH}, were evaluated at pH 6.1 by analyzing the temperature dependence of the transverse relaxation rate of the bulk water signal and of the amide protons, respectively, in aqueous solutions of $[Ln(dotamGly)]^-$.[10]

The ST measurements were performed by using a saturation pulse (4 s) which constituted a train of selective e-burp pulses, whose duration and power were optimized according to the signal to be irradiated (20 ms and 1.35 μT for the amide protons and 1 ms and 17.1 μT for the metal-coordinated water protons).

Received: May 14, 2002 [Z19701]

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