Polypeptide Imaging Agents

Supramolecular Adducts between Poly-L-arginine and [Tm^{III}dotp]: A Route to Sensitivity-Enhanced Magnetic Resonance Imaging–Chemical Exchange Saturation Transfer Agents**

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The last few years have witnessed a growth in interest in a novel class of contrast media for magnetic resonance imaging (MRI) applications, the so-called chemical exchange saturation transfer (CEST) agents,[1–4] primarily because of their ability to act as responsive agents for parameters of diagnostic relevance.[5–8]

Such agents possess mobile protons that, upon irradiation of their resonance frequency, transfer saturated magnetization to the bulk water resonance.[9] In an MR image this results in a loss of signal (negative agent). Several parameters control the extent of the saturation transfer (ST) effect. Some of them are strictly dependent upon the instrumental modality through which the selectivity and power of the irradiation rf field is applied. Others are related to the characteristics of

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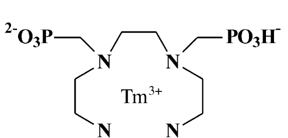
the CEST agent, namely the chemical shift separation $\Delta\omega$ (in rad Hz) between the resonance of the mobile protons and water, the exchange rate of the mobile protons k_{ex}, the number of exchangeable protons n, and the longitudinal relaxation time of the bulk water protons in the presence of the irradiating rf field T_1^{sat}. As long as the condition $\Delta\omega > k_{ex}$ holds, ST and k_{ex} are directly related. Therefore, paramagnetic systems displaying large $\Delta\omega$ values are candidates of choice because they allow the exploitation of large k_{ex} values.[2,4,6–8] Currently, the main limitation of the CEST agents is their relatively low sensitivity. The most sensitive systems so far reported yield ST effects of 5 % (which ensures the detection of the contrast in the MR image) at a concentration of 0.5 mM.[6,8]

van Zjil and co-workers tackled the sensitivity problem by investigating the ST properties of several cationic diamagnetic macromolecular systems (polyaminoacids and dendrimers). They found an ST effect for the most sensitive system of almost 50 % for a 100 μM solution of a poly-L-lysine of 488 kDa.[3] Clearly, the high sensitivity displayed by this system is a result of the huge number of irradiated mobile protons (about 2300 backbone amide protons per molecule versus 2 or 8 water or amide protons for the most sensitive of the paramagnetic agents). Although these findings represent an important step in the search for CEST agents with improved sensitivity, it is evident that a much greater improvement could be achieved if such an exchangeable proton pool were endowed with much larger $\Delta\omega$ values. On this basis, we deemed it of interest to explore a new route to enhance the sensitivity of CEST agents. Our approach is based on the formation of supramolecular adducts between a species containing a high number of exchanging protons and a paramagnetic shift reagent that is able to markedly increase its $\Delta\omega$ value in such a way that $|\Delta\omega| > k_{ex}$. A further advantage lies in the fact that the resonance frequency of the mobile protons is shifted away from its diamagnetic position upon interacting with the paramagnetic agent, thus the observed ST effect is not affected by the contribution from protons belonging to endogenous molecules.

As a model system to prove the efficacy of the proposed approach, we have considered the supramolecular adduct formed by the cationic polypeptide poly-L-arginine (53.5 kDa) and the negatively charged lanthanide complex $[Tm(Hdotp)]^{4-}$ (at pH 7.4), whose ability to act as an NMR shift reagent for cationic species is well documented.[10–12]



$[Tm(Hdotp)]^{4-}$

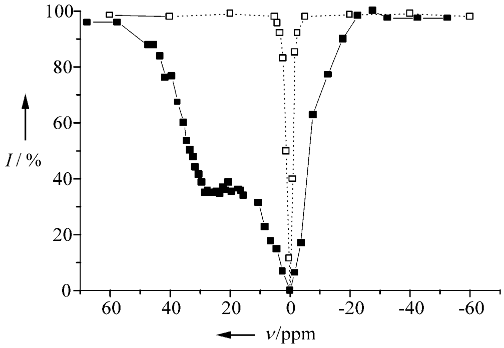


Figure 1. CEST spectra (7.05 T, pH 7.4 and 312 K) showing the intensity of the NMR signal of the bulk water protons (normalized to 100) as a function of the irradiation frequency (in ppm). Open squares: poly-L-arginine 0.11 mM. Filled squares: poly-L-arginine 0.11 mM in the presence of 2.0 mM $[Tm(Hdotp)]^{4-}$. Irradiation conditions: pulse: rectangular, duration: 2 s, power: 25.0 μT.

In the absence of the shift reagent, the exchange rate of the mobile guanidine protons of the polypeptide at pH 7.4 and 312 K is so fast that no ST can be measured (Figure 1, open squares). When $[Tm(Hdotp)]^{4-}$ is added to the poly-L-arginine solution, a remarkable transfer of saturated magnetization takes place when the rf irradiating field is set at 20–30 ppm downfield of the water resonance (Figure 1, filled squares). This is an unambiguous indication of the formation of a tightly associated ion pair. It is likely that a $[Tm(Hdotp)]^{4-}$ chelate sweeps along the polypeptide surface, thus causing an overall shift of all the guanidine protons whose saturation causes a strong decrease in the intensity of the water signal. An 18:1 ratio between the paramagnetic chelate and the polypeptide has been found to provide optimal ST effects. Higher ratios yielded extensive precipitation of the adduct as a consequence of the decrease in the effective residual electric charge.

The noticeable improvement in the sensitivity brought about by such a supramolecular system is evident by measuring the ST effect of solutions containing a different absolute concentration of the interacting species (at the fixed $[Tm(Hdotp)]^{4-}$/[poly-L-Arg] ratio of 18:1; Figure 2). Inspection on the results reported in Figure 2 shows that a 5 % ST effect can be observed at very low concentrations of polymer (1.7 μM) and metal complex (30 μM), thus demonstrating the great sensitivity enhancement that can be attained by this approach.

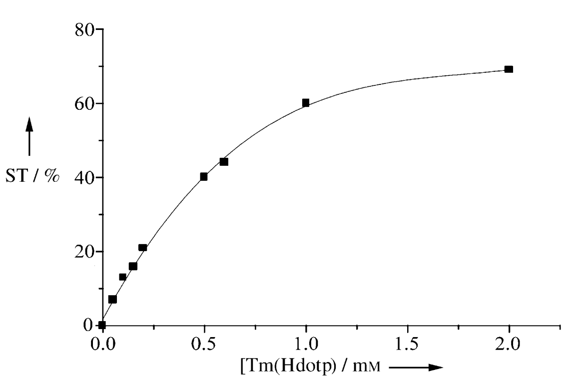


Figure 2. ST effect as a function of the absolute concentration of $[Tm(Hdotp)]^{4-}$ of solutions containing a fixed $[Tm(Hdotp)]^{4-}$/[poly-L-Arg] ratio of 18:1. Experimental conditions: 7.05 T, pH 7.4, 312 K, irradiation pulse: train of 2000 e-burp pulses (1 ms each), irradiation frequency: ± 6000 Hz from the water protons, irradiation power: 86.7 μT.

Finally, the CEST properties of the $[Tm(Hdotp)]^{4-}$/poly-L-Arg system have been assessed by means of an in vitro MRI experiment. Figure 3 shows an MR difference (on-off) CEST image (see the Experimental Section) of a phantom made of four specimens containing $[Tm(Hdotp)]^{4-}$ and poly-L-arginine (at a fixed concentration ratio of 18:1), where the concentration of the metal complex ranges from 50 to 500 μM. The acquisition of such a CEST image takes about 24 minutes (irradiation time of 2 s), whereas the same experiment with a typical diamagnetic CEST agent is much more time consuming. In fact, the long T_1^{sat} values displayed by the diamagnetic systems require much longer irradiation times (usually 10 s) to fully exploit the maximum ST effect.

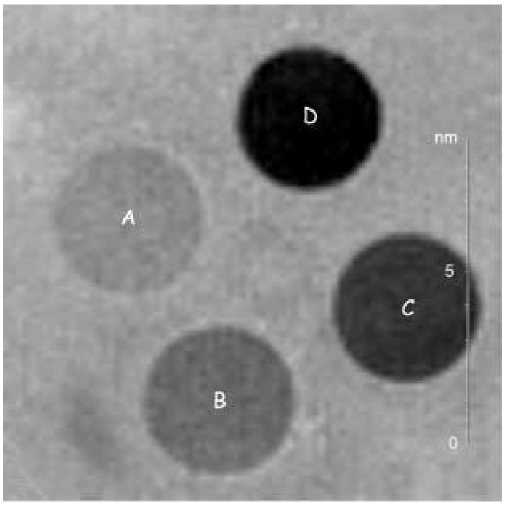


Figure 3. Difference spin-echo MR images (on-off) of a phantom consisting of four solutions of $[Tm(Hdotp)]^{4-}$/poly-L-arginine at the following concentrations: A: 50 μM/2.8 μM, B: 100 μM/5.5 μM, C: 200 μM/11.1 μM,D: 500 μM/27.8 μM. Experimental conditions: 7.05 T, pH 7.4, 312 K, echo sequence: TR/TE/NE = 6 s/5 ms/1, irradiation pulse: train of 2000 sinc pulses (1 ms each), irradiation frequency: ±6000 Hz from the water protons, irradiation power: 47.8 μT.

In summary, the results reported herein show that a new class of CEST agents can be envisaged. They are based on the formation of reversible adducts between a diamagnetic system, the pool of a high number of exchanging protons, and a paramagnetic shift reagent that enables them to combine a large chemical shift value with the shortening of the water T_1 value. Such a control of the determinants of the CEST effect provides extremely sensitive systems, in terms of the concentrations of both the metal complex and the polymer. The observed sensitivity enhancement markedly decreases the detection limit of an MRI-CEST experiment, and it now approaches the concentration limit achieved in conventional Gd^{III}-based contrast agents.

Experimental Section

$[Tm(Hdotp)]^{4-}$ has been prepared according to the literature.[11] Poly-L-arginine (DP 270, 53.3 kDa) was purchased from Sigma. ST measurements were carried on a Bruker Avance300 spectrometer. ST values (expressed as percentages) were calculated by using the following equation:

$$ST\,\% = \left(1 - \frac{I_s}{I_0}\right) 100$$

where 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 ($\nu^{off} = -\nu^{on}$, I_0) with respect to the frequency of the bulk water protons. The off-resonance measurement is necessary to take into account the direct saturation effect on the bulk water signal by the irradiation pulse.

The MR images were obtained on the same spectrometer equipped with a microimaging probe (inner diameter 30 mm). The image reported in Figure 3 is the difference between the image acquired by irradiating the resonance frequency of the guanidine protons (on-image, 6000 Hz from water protons) and that acquired off-resonance (off-image, −6000 Hz from the water protons). Both images were obtained using a conventional spin-echo sequence (TR/TE/NE = 6 s/5 ms/1) preceded by a train of sinc pulses (2000, 1 ms each, irradiation power 47.8 μT).

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