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Gd-Loaded Liposomes as T_1, Susceptibility, and CEST Agents, All in One

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Gadolinium complexes are currently used in research and clinical settings as contrast agents for MRI investigations thanks to their outstanding ability of catalyzing the relaxation rate of water protons. In fact, Gd^{3+} ion with its seven unpaired electrons distributed in the seven f orbitals was recognized early on as the candidate of choice for the development of paramagnetic T_1-relaxation agents.[1] On the other hand other paramagnetic lanthanide(III) ions, displaying a nonisotropic distribution of f-electrons, have been considered as candidates for the design of susceptibility T_2-agents.[2] Recently, the shift reagent abilities of paramagnetic Ln(III) complexes (except Gd^{3+}) have been exploited in a novel class of chemical exchange saturation transfer (CEST) agents.[3] The latter class consists of systems containing an exchangeable pool of protons whose resonance is selectively saturated by the application of a suitable rf field. The transfer of the saturated magnetization to the bulk water signal causes a decrease in its signal intensity that results in a darkening effect in the corresponding MR image. A further development in this approach has led to the use of lanthanide complexes as shift reagents for water molecules confined in the intraliposomal cavity. This results in a dramatic sensitivity enhancement as the number of exchangeable protons is now determined by the huge number of water molecules entrapped in the liposomal vesicles.[4] In a spherical liposome the chemical shift of the entrapped water is the result of the interaction with the lanthanide shift reagent, which in turn is a result of the asymmetrical distribution in the seven f-orbitals of the Ln(III) ion. Thus, the observed shift is determined solely by the hyperfine interaction between the paramagnetic center and the protons of the coordinated water. It is negligible for Gd(III) complexes.

In principle, the ^{1}H-chemical shift of water molecules entrapped in a liposome cavity in the presence of paramagnetic metal complexes can receive a substantial contribution if the shape of the internal compartment is no longer spherical. This contribution is associated with changes in the bulk magnetic susceptibility of the compartment and not with the dipolar effect resulting from the interaction with a metal ion characterized by a nonisotropic electron distribution. Therefore, a Gd(III) complex in a nonspherical liposomal cavity may act as shift reagent.

It has been reported that the exposure of unilamellar liposomes to hyperosmotic stress may induce changes in the vesicle shape from the commonly accepted spherical or quasi-spherical shapes to prolate or oblate shapes.[5]

In this communication, it is shown how a Gd(III) agent entrapped in a liposome acquires shift reagent properties, thus yielding a system that works as T_1-, T_2-, and CEST contrast agent at the same time.

PEGylated liposomes (135 nm in diameter, polidispersity index = 0.12) were obtained by hydrating a thin layer mixture of saturated phospholipids (DPPC/DSPE-PEG2000, 95/5 molar ratio, DPPC = dipalmitoylphosphocholine, DSPE = distearoylphosphoethanolamine) with an aqueous solution of the neutral complex Gd-HPDO3A (0.040 M, HPDO3A = 1,4,7,10-tetraazacyclododecane-1-hydroxymethylpropyl-4,7,10-triacetic acid). Then, the suspension was extruded five times on polycarbonate filters of 200 nm. The resulting unilamellar vesicles were extensively purified by means of dialysis against a buffer solution (NaCl, HEPES buffer 38:1 molar ratio) of the same osmolarity (0.040 Osm) as the hydration solution. As expected, in the NMR spectrum of the liposome suspension only a single resonance, corresponding to both the bulk water signal and the intraliposomal one, is detected (Figure 1a). Upon increasing the osmolarity of the suspension by adding NaCl, the liposomes shrink, thus releasing part of the intraliposomal water in order to reach the same osmolarity as the outside medium. This phenomenon is accompanied with the loss of the spherical shape of the vesicles. The occurrence of this change is clearly detected by the observation of a progressive downfield shift of the resonance of the entrapped water molecules (Figure 1 b,c).

When the osmolarity of the suspension is in the range of biological fluids, the shift of the internal water is ~7 ppm from bulk water. The increase in the shift is accompanied by an increase in line width that is probably accounted for by an enhanced intraliposomal Gd complex concentration and the consequent increase in the R_2 of the encapsulated water protons. Thus, exploiting the BMS shift contribution, we were able to double the shift of the intraliposomal water resonance with respect to the case of the Ln(III)-based shift reagents entrapped in spherical liposomes.[4] Furthermore, the osmotically shrunken Gd-loaded liposomes maintained the characteristic property of T_1 relaxing agents as is shown by the $1/T_1$ NMRD profile recorded over an extended range of Larmor frequencies (0.01−300 MHz) (Figure 2).

The observed relaxivity at 20 MHz and 39 °C (3.2 s^{-1} mM^{-1}) is only slightly lower than that measured for an aqueous solution of Gd-HPDO3A under the same experimental conditions (3.6 mM^{-1} s^{-1}), but significantly higher than the values reported for spherical liposomes with similar membrane composition filled with the same complex at higher concentrations (150 mM).[6] Interestingly, we observed that the r_1 value of the liposomes increased upon osmotic shrinking from 1.6 s^{-1} mM^{-1} (closer to the value reported for spherical vesiscles) to 3.2 s^{-1} mM^{-1}. The relaxivity of Gd-loaded liposomes is dependent on several factors, including the intrinsic relaxivity of the entrapped agent, its local concentration inside the liposome cavity, and the residence lifetime of the intraliposomal water protons, which depends on the liposome size and water permeability of the phospholipidic membrane.[6] The analysis of the ^{1}H NMRD profile of the shrunken liposomes, performed using a simple two-compartment model, suggested that the higher r_1 values of the nonspherical vesiscles are mainly determined by the shortening of the residence lifetime of the intraliposomal water protons (sub-ms range), probably attributable to a change in the membrane permeability (and/or size and shape) of the lipidic vesicles induced by the osmotic stress.

In addition, the system shows a marked enhancement in the transverse relaxation time in the high-field region of the NMRD

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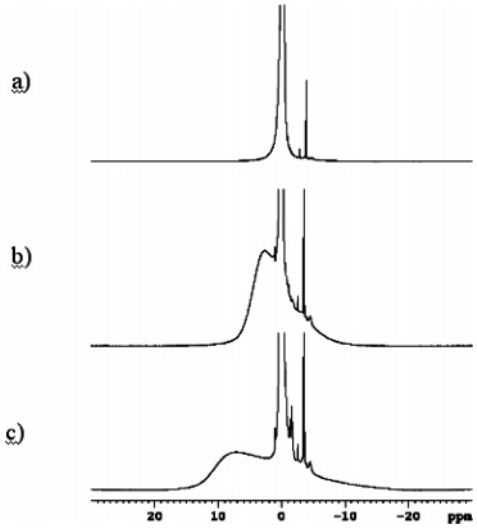

Figure 1. Chemical shift dependence of the intraliposomal water upon changing the osmolarity of the outside medium. (a) 40 mOsm (same osmolarity as in the liposomal cavity); (b) 150 mOsm; (c) 300 mOsm (isotonic). The spectra were collected at 25 °C, pH = 7.4, and 600 MHz.

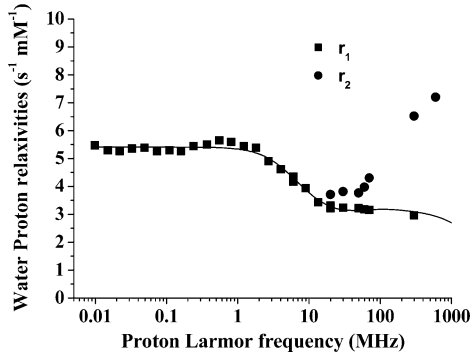

Figure 2. Magnetic field dependence at 39 °C of millimolar longitudinal (r_1) and transverse (r_2) relaxation rates of the osmotically shrunken Gd-filled liposomes. The r_1 data was fitted as discussed in the text.

profile. For the aqueous solution of the free complex r_2 is definitively comparable to r_1 at any Larmor frequency, whereas the behavior observed for the entrapped agent clearly reports the occurrence of large magnetic susceptibility effects, as already previously reported for similar systems.[6]

In Figure 3, the MR imaging properties of the nonspherical Gd-loaded liposomes are reported. The phantom consists of two coaxial tubes of which the inner contains a suspension of the shrunken liposomes in the isotonic medium (total [Gd] = 2.5 mM) and the outer only neat water. The images have been acquired at 39 °C on a MR scanner operating at 7 T.

As expected, the proton density image does not show any detectable difference between the inner and outer compartments

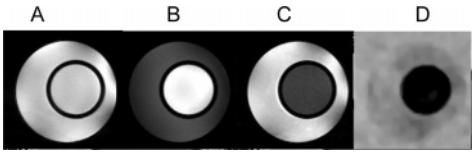

Figure 3. MR images (7 T, 39 °C) of the phantom described in the text. (A) Spin−echo proton density image, (B) spin−echo T_1-weighted image, (C) spin−echo RARE T_2-weighted image, and (D) spin−echo RARE CEST on−off difference image upon irradiating at 7 ppm from bulk water (saturation pulse: single rectangular pulse, B_1 6 μT, length 2 s).

(Figure 3A). Instead the T_1-weighted image shows the inner tube hyperintense with respect to the outer one, reflecting the large T_1 difference between neat water (∼2.5 s) and the Gd-loaded liposome suspension (0.13 s at 300 MHz) (Figure 3B). In the T_2-weighted image (Figure 3C), the inner compartment appears very dark as its T_2 value is nearly 15 times shorter than T_2 of neat water. Finally, the CEST-MR difference image (Figure 3D) was obtained by subtracting from the image acquired by irradiating the intraliposomal water resonance at 7 ppm from bulk water, the image acquired by changing the saturation frequency offset to −7 ppm. By this procedure the direct saturation effects of the bulk water signal are ruled out. A ST value of 5% was calculated for a liposome concentration of about 30 nM, thus showing a sensitivity that is among the highest reported for a CEST agent. This result is clearly due to the saturation of a large pool of exchangeable protons in the liposomal cavity. Interestingly, the short T_1 of the water protons appears not to represent a limit in the presence of a large CEST effect.

In conclusion, the results reported herein have shown that a Gd complex may be endowed with shift reagent properties when confined in a nonspherical liposomal compartment, thus yielding a system that acts, at the same time, as efficient T_1-, T_2-, and CEST agents.

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References

(1) Tóth, E.; Helm, L.; Merbach, A. E. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Tóth, E, Eds.; John Wiley & Sons: Chichester, UK, 2001; Chapter 2, p 45.
(2) (a) Vander Elst, L.; Roch, A.; Gillies, P.; Laurent, S.; Bottemann, F.; Bulte, J. W. M.; Muller, R. N. *Magn. Res. Med.* **2002**, *47*, 1121. (b) Caravan, P.; Greenfield, M. T.; Bulte, J. W. M. *Magn. Res. Med.* **2001**, *46*, 917.
(3) Woods, M.; Woessner, D. E.; Sherry, A. D. *Chem. Soc. Rev.* **2006**, *35*, 500.
(4) Aime, S.; Delli Castelli, D.; Terreno, E. *Angew. Chem., Int. Ed.* **2005**, *44*, 5513.
(5) Pencer, J.; White, G. F.; Hallett, F. R. *Biophys. J.* **2001**, *81*, 2716.
(6) Fossheim, S. L.; Fahlvik, A. K.; Klaveness, J.; Muller, R. N. *Magn. Res. Imaging* **1999**, *17*, 83.

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