Improved liposomes-based Ca(II) responsive MRI contrast agents

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PURPOSE

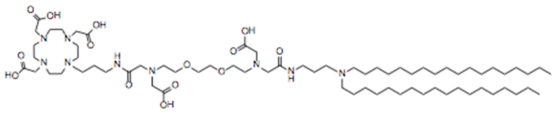
The motivation of this work was to assess the MRI performance of liposomes loaded with an amphiphilic Ca(II) sensitive Gd(III) complex with the aim of improving both the overall sensitivity in the MRI detection of the probe and the Ca(II) responsiveness. The structure of the probe consisted of two coordination cages: one (macrocyclic, DO3A-like) selective for Gd(III), and the other (linear, EGTA-like) selective for Ca(II) linked to two C18 aliphatic chains. Similar ligands has been already demonstrated to act as Ca(II) sensor under the form of monomer, dimer, and dendrimer.[1]

METHODS

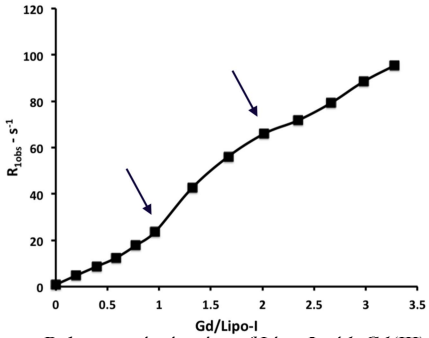
Liposomes were prepared using the conventional method based on the hydration (at 55°C) of a thin lipid film with an isotonic buffer at pH 7.4 followed by sequential extrusion. Liposomes were formulated with DPPC (85 % in moles), DSPE-PEG2000 (5 %), and the amphiphilic ligand **I** (10 %). Size and polydispersion index (PDI) of the nanovesicles were measured by Dynamic Light Scattering (DLS). NMRD dispersion profiles (reporting the longitudinal relaxivity as a function of the magnetic field strength in the range 0.01-70 MHz) were acquired on a Fast Field Cycling relaxometer (Stelar, Mede (PV), Italy).

RESULTS:

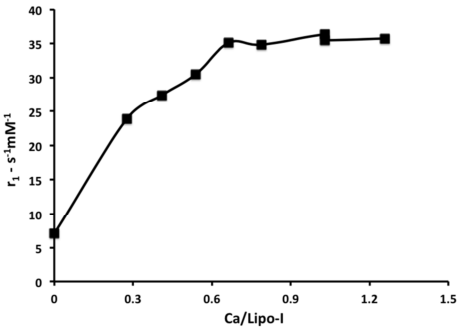
The hydrodynamic diameter of the liposomes was 140 ± 8 nm with a PDI value around 0.1. Upon titrating liposomes with Gd(III), the relaxivity showed a non linear behavior, where three regions can be detected corresponding to the binding of the metal to: A) the DO3A-like cage, B) the EGTA-like cage, and C) the phosphatidyl heads exposed on the liposomes surface. This finding has been confirmed by performing the titration on liposomes not containing ligand **I**. To remove the paramagnetic ion bound to sites B and C, a controlled excess of EDTA was added. This ligand binds to Gd(III) stronger than sites B and C, but weaker than the DO3A-like cage. The relaxivity of the Gd-I-liposomes, measured after exhaustive dialysis to remove Gd-EDTA and EDTA ligand and normalized to the millimolar concentration of Gd(III), was around 7.0 $s^{-1}mM^{-1}$, thus indicating that no water molecules are bound to the paramagnetic center. However, the relaxivity hump observed in the NMRD profiles suggests the presence of relaxation contribution arising from second-sphere water protons. Interestingly, upon addition of Ca(II) the relaxivity increased markedly until a Ca/Gd molar ratio of 1.



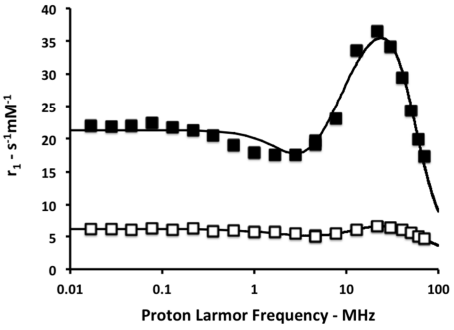
Ligand **I**



Relaxometric titration of Lipo-**I** with Gd(III) (21 MHz, 25°C).



Relaxometric titration of Lipo-Gd-**I** with Ca(II) (21 MHz, 25°C).



NMRD profiles (25°C) for Lipo-Gd-**I** (open labels) and Lipo-Gd-Ca-**I** (filled labels).

DISCUSSION:

Ligand I was successfully incorporated in liposomes without affecting size and polydispersion of the nanoparticles. The new ligand **I** was designed to form a low-relaxivity Gd(III) complex where the metal is fully coordinated by the seven donor atoms of the DO3A-like cage and two donors from the empty EGTA-like cage. The NMRD profile of such a species shows a relaxivity hump consistent with relaxation contributions from outer- and second-sphere water protons. Upon addition of equimolar amount of Ca(II), a five-fold increase in relaxivity was observed. This unprecedented large enhancement reflected the change in the hydration state of Gd(III) (from 0 to 1) that occurs when one donor atom of the EGTA-like cage detaches from Gd(III) and moves to coordinate Ca(II) along with other five donor atoms of the EGTA-like cage. Moreover, the binding of Ca(II) rigidifies the overall structure, thus causing a further increase in relaxivity.

CONCLUSION:

The herein presented results demonstrate that the incorporation of Ca(II) responsive Gd(III) complexes into liposomes can represent a valuable option to improve the overall performance of this class of smart MRI probes.

REFERENCES

1. Gündüz S, Nobuhiro N, Vibhute S et al., Dendrimeric Calcium-responsive MRI Contrast Agents with Slow *in vivo* Diffusion. Chem. Commun. 2014 DOI: 10.1039/c4cc07540d.