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Lanthanide-Loaded Paramagnetic Liposomes as Switchable Magnetically Oriented Nanovesicles

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Osmotically shrunken liposomes loaded with paramagnetic lanthanide(III) complexes orient in a static magnetic field according to the sign of their magnetic susceptibility anisotropy ($\Delta\chi$). The magnitude and sign of $\Delta\chi$ are modulated by the magnetic properties of the Ln^{III} ion, by the structural characteristics of the metal chelate, and by the stereochemical arrangement of the lipophilic substituents.

The past decade has witnessed an increasing attention toward the design and applications of magnetically oriented nanoparticles, such as bicelles and phospholipidic nanotubes.[1,2] The peculiar properties of such systems basically rely on their asymmetric shape. In this context, liposomes (unilamellar or multilamellar vesicles consisting of phospholipidic bilayers) could not have been used because they typically have spherical shape and, as such, they cannot align within an external magnetic field.

Although it was early anticipated that liposomes may be transformed in magnetically oriented particles by acting on the membrane composition, on the lamellarity, and on the method of preparation of the vesicles,[3] no detailed study aimed at generating magnetically oriented liposomes has yet been reported on large and small unilamellar vesicles.

One route to generating nonspherical liposomes relies on control of the osmotic forces during the different steps of their preparation.[4] In fact, when subjected to an hyperosmotic stress, liposomes may readily assume nonspherical shapes (e.g., discoidal, oblate, cigarlike, etc.). The alignment of nonspherical particles in a magnetic field is driven by the interaction between the anisotropy of the magnetic susceptibility tensor ($\Delta\chi$) of the vesicle components and the applied static field.[5] Furthermore, the presence of a paramagnetic lanthanide(III) chelate payload is expected to provide an excellent means of modulating $\Delta\chi$ values, as has already been successfully demonstrated in the case of bicelles.[6,7]

In this Communication, we report, for the first time, that the incorporation of lanthanide-based paramagnetic amphiphiles in liposome bilayers can switch the orientation of the vesicle in the field not only according to the magnetic properties of the Ln^{III} ion (as observed in the case of bicelles) but also as a function of the structure of the membrane-incorporated amphiphilic metal complex. Moreover, it will be shown that the alignment direction of these nanoparticles can be successfully assessed by measuring the ^1H NMR chemical shift separation ($\Delta_{intralipo}$) between the intraliposomal and bulk water resonances.

Basically, $\Delta_{intralipo}$ is the sum of two terms:

$$\Delta_{intralipo} = \delta_{intralipo} - \delta_{bulk\ water} = \Delta^{BMS} + \Delta^{pseudo}$$

where Δ^{BMS} (BMS = bulk magnetic susceptibility) is the contribution arising from the partial alignment of the magnetic moments of the compartmentalized paramagnetic species by the static field and Δ^{pseudo} represents the pseudocontact contribution arising from the exchange of metal-

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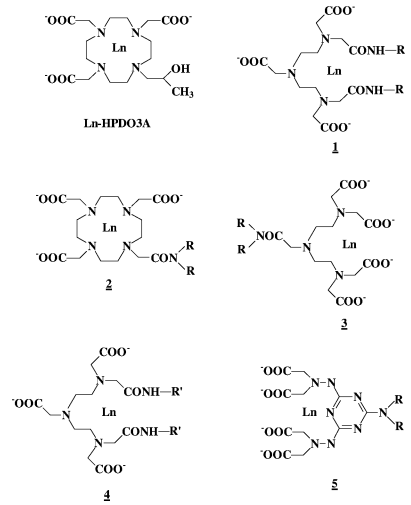
Chart 1. Hydrophilic and Amphiphilic Ln^{III} Complexes (Ln = Tm, Dy, and Gd) Investigated in This Work



coordinated water with the ensemble of water molecules entrapped in the aqueous core of the liposome.

Though a quantitative description of Δ^{BMS} is only possible for a highly symmetric shape and for given orientations to $\mathbf{B}_0$,[3] this contribution is directly proportional to both the effective magnetic moment of the Ln^{III} ion, μ_{eff} (positive for all paramagnetic Ln^{III} ions),[8] and the payload of paramagnetic species. The sign of the Δ^{BMS} term defines the orientation of the vesicle in the static magnetic field. It is worth reminding that the compartmentalization of a paramagnetic species in a cylinder parallel to $\mathbf{B}_0$ induces a positive Δ^{BMS}, whereas a negative shift difference is observed when the cylinder is perpendicular to the field.[9]

The Δ^{pseudo} contribution is unaffected by the orientation of the vesicle, and in the presence of a fast exchange of the metal-coordinated water molecule, it is directly proportional to the chemical shift of the water protons at the metal site (Δ_M) weighted by the molar fraction of the intraliposomal water protons visiting the encapsulated paramagnetic center. Δ_M is related to the anisotropy of the magnetic susceptibility of the paramagnetic molecule that is proportional to Bleaney's constant (C_j, whose sign and magnitude is characteristic for each Ln^{III} ion)[8] and modulated by the crystal-field coefficients of the complex. In addition to the magnetic properties, Δ_M is also strongly influenced by the position of the coordinated water protons with respect to the main axis of the χ tensor of the paramagnetic species.[10]

Nonspherical, osmotically shrunken liposomes were prepared by hydrating a thin film with a given lipidic composition (DPPC/DSPE-PEG2000 95/5 mol %) with an aqueous solution containing a neutral paramagnetic agent (Ln-HPDO3A; Chart 1) at a concentration of 40 mM.

The hydrated lipids, organized in multilamellar vesicles, were extruded 10-fold on polycarbonate filters with a pore

Table 1. $\Delta_{intralipo}$ Values Measured at 14.1 T and 298 K for Liposomes Entrapping Ln-HPDO3A Complexes

encapsulated Ln-HPDO3A	Bleaney's factor	μ_{eff}	$\Delta_{intralipo}$ (ppm)	
			spherical	nonspherical
Gd	0	7.94	0	7.2
Dy	<0	10.6	−4.0	14.1
Tm	>0	7.6	3.5	10

diameter of 200 nm. Then, the resulting unilamellar liposomes were dialyzed against an isotonic (i.e., hyperosmotic with respect to the intraliposomal compartment) buffer.[4,11] This procedure led to almost monodisperse (PDI < 0.1) nonspherical liposomes with hydrodynamic diameters in the range of 130–150 nm for all preparations, as determined by dynamic light scattering measurements. Spherical liposomes were prepared by following the same procedure except for the higher concentration of the shift reagent in the hydration solution (300 mM) in order to avoid the osmotic stress of the liposomes during the dialysis.

Three paramagnetic HPDO3A complexes differing in the sign of the C_j constant of the Ln^{III} ion (Tm → $C_j > 0$, Dy → $C_j < 0$, and Gd → $C_j = 0$) were used. On the basis of the C_j value, it is expected that the Δ^{pseudo} contribution induced by the intraliposomal entrapment of these complexes should be positive, negative, and null for Tm, Dy, and Gd, respectively. The $\Delta_{intralipo}$ values reported in Table 1 outline that the theoretical expectation was only met for the spherical liposomes, whereas for the nonspherical vesicles, the measured shifts followed a different trend.

The invariance of the $\Delta_{intralipo}$ sign for the latter systems, in addition to the larger shift values, is a clear indication about the occurrence of a large Δ^{BMS} effect for the osmotically shrunken vesicles. Because these liposomes should have the same (nonspherical) shape, this result can be accounted for only in terms of the orientation of the vesicles in the field, and it suggests that the three types of liposomes adopt the same orientation. Furthermore, the magnitude of the shift results from the combination between the μ_{eff} values of the entrapped Ln^{III} ions and the sign of the Δ^{pseudo} term (dependent on C_j values). The association between the negative susceptibility anisotropy of the phospholipids of the liposome bilayer and the positive $\Delta_{intralipo}$ values suggests that the shrunken liposomes are oriented with their longer axis parallel to $\mathbf{B}_0$ (Scheme 1; the $\Delta\chi < 0$ case on the left).

Next, we have explored the possibility of switching the orientation of the nonspherical liposomes through modulation of the magnetic susceptibility anisotropy of the liposome bilayer. To do this, the amphiphilic Ln^{III} complexes (Ln = Tm, Dy, and Gd) of ligand **1** (Chart 1, already used for switching the magnetic orientation of bicelles)[6] were incorporated in the membrane of osmotically shrunken liposomes, entrapping the corresponding Ln-HPDO3A shift reagent.

The data reported in Table 2 indicate that the $\Delta_{intralipo}$ value is negative when the complex Tm-**1** is incorporated and positive when the liposome membrane is loaded with Dy-**1** or Gd-**1**.

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Scheme 1. Schematic Representation of the Preferred Orientation of the Osmotically Shrunken Liposomes (Here Assumed To Be Discoidal) in the Static Field $\mathbf{B}_0$ According to Their Magnetic Susceptibility Anisotropy (White Circles, Paramagnetic Entrapped Species; Red Circles, Incorporated Paramagnetic Compound)

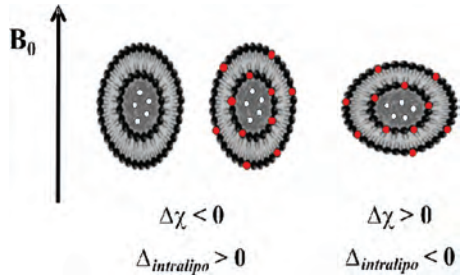

Table 2. $\Delta_{intralipo}$ Values Measured at 14.1 T and 298 K for Nonspherical Liposomes Incorporating Amphiphilic Ln^{III} Complexes and Entrapping Ln-HPDO3A

incorporated amphiphilic Ln^{III} complex	$\Delta_{intralipo}$ (ppm)
Gd-**1**	5.0
Gd-**2**	13.9
Dy-**1**	15.7
Dy-**2**	−45.0
Tm-**1**	−18.3
Tm-**2**	19.0
Tm-**3**	12.0
Tm-**4**	−31.0
Tm-**5**	10.5

In analogy to what was observed with bicelles, the sign change for the $\Delta_{intralipo}$ value in the presence of Tm-**1** can be interpreted in terms of a 90° flip of the field alignment of the liposome as a result of the sign change of the magnetic susceptibility anisotropy of the liposome bilayer because of the positive $\Delta\chi$ value of Tm-**1** (Scheme 1; the $\Delta\chi > 0$ case). Conversely, the incorporation of Dy-**1** inverts the sign of $\Delta_{intralipo}$ because of the expected sign change of the magnetic anisotropy from Tm to Dy, whereas the incorporation of Gd-**1** results in a positive $\Delta_{intralipo}$ value because this ion cannot alter the natural magnetic susceptibility anisotropy of the phospholipids constituting the liposome membrane ($C_j = 0$). In order to gain further insight on the role of the incorporated paramagnetic chelate in defining the field alignment of the nonspherical vesicles, Ln^{III} complexes of different ligands (Chart 1) have been tested. Concerning the macrocyclic ligand **2**, the sign of the $\Delta_{intralipo}$ values for Gd was opposite those for Tm and Dy, but, surprisingly, it changed direction upon passing from ligand **1** to ligand **2**.

This unexpected behavior can be interpreted in terms of a "sign switch" of the magnetic anisotropy of the bilayer induced by the incorporated amphiphile. This phenomenon might be related to the characteristics of the insertion mode of the amphiphilic complexes into the bilayer. It has been reported that Ln-**1** is present in solution as a mixture of several diastereosomers,[12] in which the aliphatic chains are pointing in different directions, whereas the two aliphatic chains are likely almost parallel in the solution structure of Ln-**2**. On this basis, the two complexes might display a different incorporation modality in the phospholipidic bilayer, thus modulating the magnetic anisotropy of the membrane.

Further support to this view was gained by looking at the $\Delta_{intralipo}$ values measured for the other Tm-based amphiphiles (Table 2). According to the linking mode of the hydrocarbon tails to the coordination cage of the ligands and regardless of the typology (linear or macrocyclic) of the coordination cage, Tm-**4** resembles Tm-**1**, whereas Tm-**3** and Tm-**5** are similar to Tm-**2**. Interestingly, the measured $\Delta_{intralipo}$ values parallel such a structural motif, thus suggesting that the magnetic susceptibility anisotropy of the amphiphilic complex in the liposome membrane seems to be driven by its incorporation characteristics.

Because the $\Delta_{intralipo}$ value induced by amphiphilic Gd^{III} complexes in nonspherical liposomes is defined only by the Δ^{BMS} contribution, the quite large difference in the shift magnitude between Gd-**1** and Gd-**2** preparations might be accounted for in terms of a different shape (or small effects on the orientation) induced by the incorporation of the two amphiphiles. For the systems where Δ^{pseudo} contributes to $\Delta_{intralipo}$, it is also relevant to consider the role played by the Δ_M value of the amphiphile pointing inward in the cavity. To this regard, it is worth reminding that DTPA-like cages typically have much smaller Δ_M values than macrocyclic DOTA-like structures[13] (see the $\Delta_{intralipo}$ difference between Tm-**2** and Tm-**3** incorporated liposomes).

In conclusion, we have demonstrated that osmotically shrunken paramagnetic liposomes orient in a static magnetic field depending on the sign of the magnetic susceptibility anisotropy of their bilayer. Importantly, this property can be controlled by modulating the magnetic susceptibility anisotropy of the Ln^{III} ion incorporated in the membrane and the characteristics of the amphiphilic complex. Furthermore, the direction of the magnetic alignment of such nanovesicles can be easily determined by the sign of the ^1H NMR chemical shift of the intraliposomal water protons. One may envisage a number of applications for these liposomes, whose orientation in the magnetic field can be modulated at will. As an example, they have recently been proposed as innovative and multimodal MRI probes[4,11] and, furthermore, they might have a great potential as magnetically aligned systems for carrying out high-resolution NMR structural studies of proteins and other macromolecular systems such as alternative platforms to bicelles.

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