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ORIGINAL PAPER

Relaxivities of paramagnetic liposomes: on the importance of the chain type and the length of the amphiphilic complex

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Abstract Nuclear magnetic relaxation dispersion (NMRD) profiles of unilamellar DPPC liposomes incorporating Gd-DTPA-bisamides with alkyl chains of 12 to 18 C atoms in their external and internal layers were recorded in order to study the influence that the chain length and structure of Gd-bisamides incorporated in the liposomal membrane have on their proton relaxivity. The NMRD profiles recorded at 310 K show that the relaxivity reaches a minimum value when the carbon chain lengths of the phospholipid and of the Gd complex match and is at a maximum in the presence of a carbon–carbon double bond. For these DPPC paramagnetic liposomes, the longer the aliphatic chains of the complex, the larger will be its immobilization in the membrane. In addition, the presence of an unsaturated carbon–carbon bond in the alkyl chain of the Gd complex induces an increase of its mobility and of its water exchange rate with, as a result, a much greater efficiency as an MRI contrast agent.

Keywords Liposomes · Paramagnetic complexes · MRI · NMRD profiles · Contrast agents

Introduction

Liposomes are supramolecular structures of great interest in medicine, biology and pharmacy (Konduri et al. 2003; Kedei et al. 2004; Freyschmidt-Paul et al. 2003; Cattel et al. 2003; Shoji and Nakashima 2004; Johnston 2004; Krugner-Higby et al. 2003; Lasic 1993). Paramagnetic liposomes have been used as MRI contrast agents (Torchilin 1996, 1997; Lokling et al. 2004a, 2004b; Leclercq et al. 2003; Bertini et al. 2004; Lattuada and Lux 2003). Two types of such magnetic liposomes exist according to the location of the paramagnetic complex, either in the membrane (Leclercq et al. 2003; Bertini et al. 2004; Lattuada and Lux 2003), or encapsulated in the liposome cavity (Lokling et al. 2004a, 2004b). Additionally, self-associating polychelate amphiphilic polymers can be used to increase the concentration of the paramagnetic metal ions in supramolecular structures and thus enhance the image's contrast (Trubetskoy et al. 1996).

This work focuses on amphiphilic gadolinium paramagnetic complexes and on their insertion in the liposome membrane. Previous studies (Kimpe et al. 2003) performed on mixed micelles of phospholipids with 16 C atoms incorporating various aliphatic Gd-DTPA-bisamides (chain lengths of 12 to 18 C atoms) showed a maximum relaxivity for micelles with Gd bisamide complexes with a chain length of 14 and 16 C atoms. The present study aimed at investigating the possible influence of the alkyl chain length and of the presence of an unsaturated chain of the chelate on the relaxivity of various paramagnetic liposomes. Unilamellar liposomes constituting 1,2-sn-glycero-3-phosphatidylcholin (DPPC) and Gd-DTPA-bisamide derivatives (Gd-DTPA-$BC_{12}A$, Gd-DTPA-$C_{14}A$, Gd-DTPA-$C_{16}A$, Gd-DTPA-$BC_{18}A$ and Gd-DTPA-BC_{18}Aunsat (Fig. 1)) were synthesized and characterized by photon correlation spectroscopy (PCS) and proton relaxometry.

Materials and methods

Chemicals

All reagents were commercially available (Aldrich or Fluka, Bornem, Belgium and Acros, Geel, Belgium) and

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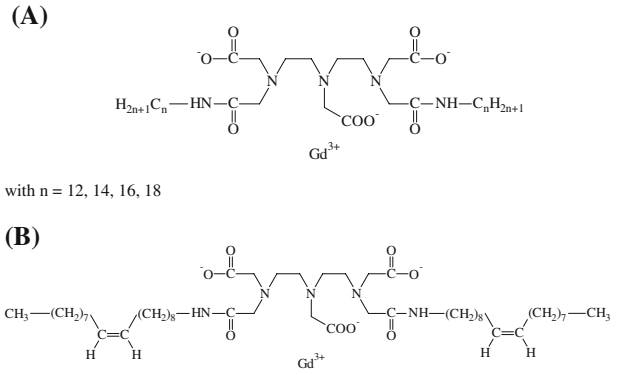

Fig. 1 Chemical structure of the Gd-DTPA-bisamide derivatives. **a** Gd-DTPA-$BC_{12}A$ ($n = 12$), Gd-DTPA-$BC_{14}A$ ($n = 14$), Gd-DTPA-$BC_{16}A$ ($n = 16$), Gd-DTPA-$BC_{18}A$ ($n = 18$). **b** Gd-DTPA-BC_{18}Aunsat

were used without further purification. DPPC was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland).

Instrumentation

NMR spectroscopy

Proton and carbon 13 NMR spectra were obtained on a Bruker AMX 300 spectrometer (Bruker, Karlsruhe, Germany).

Mass spectrometry

Mass spectra ESI were obtained on a Q-TOF 2 (Micromass, Manchester, UK). Samples were solubilized in methanol.

Proton relaxometry

NMRD profiles were obtained at 310 K on a field cycling relaxometer (Stelar, Mede, Italy). The range of magnetic fields covered varies from 0.47 mT to 0.24 T, which corresponds to frequencies varying from 0.02 to 10 MHz. Additional longitudinal and transverse relaxation rates were measured on Bruker Minispecs pc120 and mq60 at 20 MHz (0.47 T) and 60 MHz (1.41 T), respectively.

Extrusion of liposomes

The extrusion of liposomes was performed on a T001 Lipex extrudor (Lipex Biomembranes, Vancouver, BC, Canada). Biomembranes had a capacity of 10 ml and the driving gas was nitrogen.

Size of liposomes

The average diameter of the liposomes was measured by photon correlation spectroscopy on a Brookhaven apparatus equipped with a goniometer, a photomultiplier BI-PMT/9863 and a BI-9000 AT correlator (Brookhaven Corporation Instruments, New York, USA). The light source is a laser He-Ne working at a wavelength of 632.8 nm.

Synthesis of ligands and gadolinium complexes

Ligands

The "saturated" DTPA-bisamides (DTPA-$BC_{12}A$, DTPA-$BC_{14}A$, DTPA-$BC_{16}A$, and DTPA-$BC_{18}A$) were obtained by reaction of DTPA bisanhydride with the corresponding amine (dodecylamine, tetradecylamine, hexadecylamine and octadecylamine, respectively) as described by Kimpe et al. (2003). DTPA-BC_{18}Aunsat was prepared from oleylamine by the method of Cacheris et al. (1994). All DTPA-bisamides were complexed with gadolinium chloride (Fig. 1). The arsenazo or xylenol orange test confirmed the absence of free gadolinium ion. Their structures were confirmed by proton and carbon-13 NMR spectroscopy and mass spectrometry.

DTPA-$BC_{12}A$: MS: $[M + H]^+ = 728$; ^1H NMR ($CDCl_3$, δ (ppm)): 8.0–7.6 (5 H, m, 3× OH, 2× NH); 3.7–2.9 (22 H, m, 11× CH_2); 1.5 (4 H, m, 2× CH_2); 1.4–1.1 (36 H, m, 18× CH_2); 0.9 (6 H, m, 3× CH_2); ^{13}C NMR ($CDCl_3$, δ (ppm)): 178.2, 177.1, 170.1, 59.9, 58.7, 57.8, 56.4, 52.6, 38.9, 31.9, 29.7, 29.6, 29.5, 29.3, 28.9, 26.3, 25.7, 25.6, 22.7, 14.1.

DTPA-$BC_{14}A$: MS : $[M + Na]^+ = 807$, ^1H NMR ($CDCl_3$, δ (ppm)): 8.4 – 8.0 (5 H, m, 3× OH, 2× NH); 3.6–2.8 (22 H, m, 11× CH_2); 1.5 (4 H, m, 2× CH_2); 1.3 - 1.2 (44 H, m, 22× CH_2); 0.9 (6 H, t, 2× CH_3); ^{13}C NMR ($CDCl_3$, δ (ppm)): 176.3, 170.1, 168.8, 57.6, 51.4, 42.2, 39.8, 38.4, 36.6, 34.2, 32.1, 31.5, 30.0, 29.9, 29.6, 27.5, 27.1, 26.7, 22.9, 14.3.

DTPA-$BC_{16}A$: MS : $[M + 3\ Na]^+ = 907$; ^1H NMR ($CDCl_3$, δ (ppm)): 8.2 - 7.9 (5 H, m, 3× OH, 2× NH); 3.3–2.8 (22 H, m, 11× CH_2); 1.6 (4 H, m, 2× CH_2); 1.3–1.2 (52 H, m, 26× CH_2); 0.9 (6 H, t, 2× CH_3); ^{13}C NMR ($CDCl_3$, δ (ppm)): 176.2, 171.1, 170.4, 59.8, 57.8, 56.4, 52.8, 52.6, 38.9, 32.1, 31.9, 30.7, 29.9, 29.7, 29.6, 29.55, 29.54, 29.51, 29.4, 29.3, 28.8, 25.7, 22.7, 13.9.

DTPA-$BC_{18}A$: MS : $[M + Na]^+ = 919$; ^1H NMR ($CDCl_3$, δ (ppm)): 8.3 - 7.9 (5 H, m, 3× OH, 2× NH); 3.6 – 3.0 (22 H, m, 11× CH_2); 1.6 (4 H, m, 2× CH_2); 1.3 - 1.2 (60 H, m, 30× CH_2); 0.9 (6 H, t, 2× CH_3); ^{13}C NMR ($CDCl_3$, δ (ppm)): 177.1, 171.3, 170.5, 59.8, 57.8, 56.4, 52.8, 52.6, 38.9, 32.9, 31.9, 30.7, 29.9, 29.7, 29.6, 29.55, 29.54, 29.5, 29.4, 29.3, 28.8, 25.7, 21.3, 13.9.

DTPA-BC_{18}Aunsat: MS : $[M + Na]^+ = 915$; ^1H NMR ($CDCl_3$, δ (ppm)): 6.3–5.9 (5 H, m, 3× OH, 2× NH);

5.3 – 5.2 (4 H, m, 4× =CH); 3.6 – 3.0 (22 H, m, 11× CH_2); 1.6 (4 H, m, 2× CH_2); 1.3–1.2 (56 H, m, 28× CH_2); 0.9 (6 H, t, 2× CH_3); ^{13}C NMR ($CDCl_3$, δ (ppm)): 178.2, 174.3, 171.4, 130.1, 59.9, 58.4, 56.4, 52.8, 52.6, 39.3, 38.9, 32.0, 30.5, 29.7, 29.6, 29.5, 29.4, 29.3, 28.8, 27.3, 27.1, 26.9, 25.7, 25.6, 22.8, 14.1.

Complexes

Equimolar amounts of ligand (2.5 mmol) and $GdCl_3.6H_2O$ dissolved in 100 ml of methanol and 2 ml of distilled water were mixed. The pH was adjusted at 5 with pyridine and the solution stirred overnight. The solution was filtered and the solvent was evaporated under vacuum. Xylenol orange or arsenazo tests confirmed the absence of free gadolinium (Barge et al. 2006). The product was treated with 50 ml of distilled water then lyophilized and its structure was confirmed by mass spectrometry.

Gd-DTPA-BC_{12}A : $[M + Na]^+ = 905$; Gd-DTPA-BC_{14}A : $[M + Na]^+ = 961$; Gd-DTPA-BC_{16}A : $[M + Na]^+ = 1017$; Gd-DTPA-BC_{18}A : $[M + Na]^+ = 1073$; Gd-DTPA-BC_{18}Aunsat : $[M + Na]^+ = 1069$.

Synthesis of the liposomes

DPPC (1 equivalent, 0.12 mmol) and Gd complex (0.1 equivalent) were dissolved in a mixture of chloroform (25 ml) and methanol (25 ml). The solvent was evaporated under vacuum to obtain a homogeneous film. This film was dispersed in 4 ml of distilled water. The solution was heated to 55°C and stirred for 45 min. The stirring was stopped and the heating continued for 1 h. Then, liposomes were extruded, at 55°C, on two polycarbonate filters with pores of 1 μm, 800 nm, 400 nm, 200 nm or 100 nm in diameter until the size distribution was monodisperse. The liposomes were used without further purification. This synthesis procedure assumes that all materials (phopholipids and amphiphilic Gd-complexes) are incorporated into the liposomes.

Determination of the Gd concentration in the final liposomal solutions

Determination of the gadolinium concentration by relaxometry

A volume of 150 μl of Triton X-114 was added to 250 μl of the liposomes solution to destroy them. Then, 2.5 ml of HNO_3 and distilled water were added to obtain a volume of 5 ml. The relaxation rate at 20 MHz and 310 K was measured and was used to determine the Gd^{3+} concentration (r_1 Gd^{3+} aquoion = 11.76 s^{-1} mM^{-1}).

Results and discussion

Unilamellar liposomes prepared from DPPC and the appropriate amphiphilic gadolinium complex ([DPPC]/[Gd complex] = 10) were characterized through (1) their hydrodynamic size measured by photon correlation spectroscopy, (2) their final gadolinium complex concentration, and (3) their proton relaxivity. This last study included the recording of their proton longitudinal NMRD profile between 0.02 and 60 MHz as well as the measurement of the transverse relaxivities at 20 and 60 MHz at a temperature lower than the phase transition temperature of the phospholipid ($T = 310$ K).

Three syntheses of each type of liposome were carried out. For most of the syntheses, a monodisperse size distribution could be obtained after ten extrusions (Fig. 2), but more extrusions were needed for two samples of liposomes incorporating Gd-DTPA-BC_{16}A, and Gd-DTPA-BC_{18}A (Table 1). The diameter of the vesicle, the relative final concentration of Gd-complex (74 ± 3 % for Gd-DTPA-BC_{12}A, 56 ± 4 % for Gd-DTPA-BC_{14}A, 55 ± 7 % for Gd-DTPA-BC_{16}A, 65 ± 12 % for Gd-DTPA-BC_{18}A, and 84 ± 5% for Gd-DTPA-BC_{18}Aunsat), and the relaxometric data are reproducible for all types of liposomes (Table 1, Figs. 2, 3). The average hydrodynamic size is larger than 130 nm, except for liposomes containing Gd-DTPA-BC_{12}A that are much smaller (around 60 nm). The reason why the size of these latter liposomes is smaller remains unclear. Both longitudinal and transverse relaxivities at 20 and 60 MHz are minimum for liposomes with Gd-DTPA-BC_{16}A and maximum for Gd-DTPA-BC_{18}Aunsat. This trend is also observed for the longitudinal relaxivities at lower magnetic fields as shown by the proton NMRD profiles (Fig. 3). The hump observed in the frequency range extending from 10 to 100 MHz attests, as expected, to a low rotational mobility of the complex, but its amplitude depends on the size of the Gd-complex embedded in the liposomal membrane. On the one hand, the relaxivity of Gd-DTPA-BC_{16}A liposomes at 20 MHz is approximately 45% lower than that of Gd-DTPA-BC_{14}A liposomes. It however remains larger than the relaxivity of the parent complex Gd-DTPA (r_1 = 3.9 s^{-1} mM^{-1} at 20 MHz and 310 K). On the other hand, the relaxivity of Gd-DTPA-BC_{18}Aunsat liposomes is about 2.5 times larger than that of the Gd-DTPA-BC_{18}A liposomes and more than 4 times larger than the relaxivity of Gd-DTPA.

The theoretical adjustment of these experimental proton NMRD data was performed using the classical innersphere and outersphere models (Solomon 1955; Bloembergen 1957; Freed 1978; Table 2). Some parameters were fixed: d, the distance of closest approach (0.36 nm), the distance electron–nucleus r (0.31 nm), and D the relative diffusion

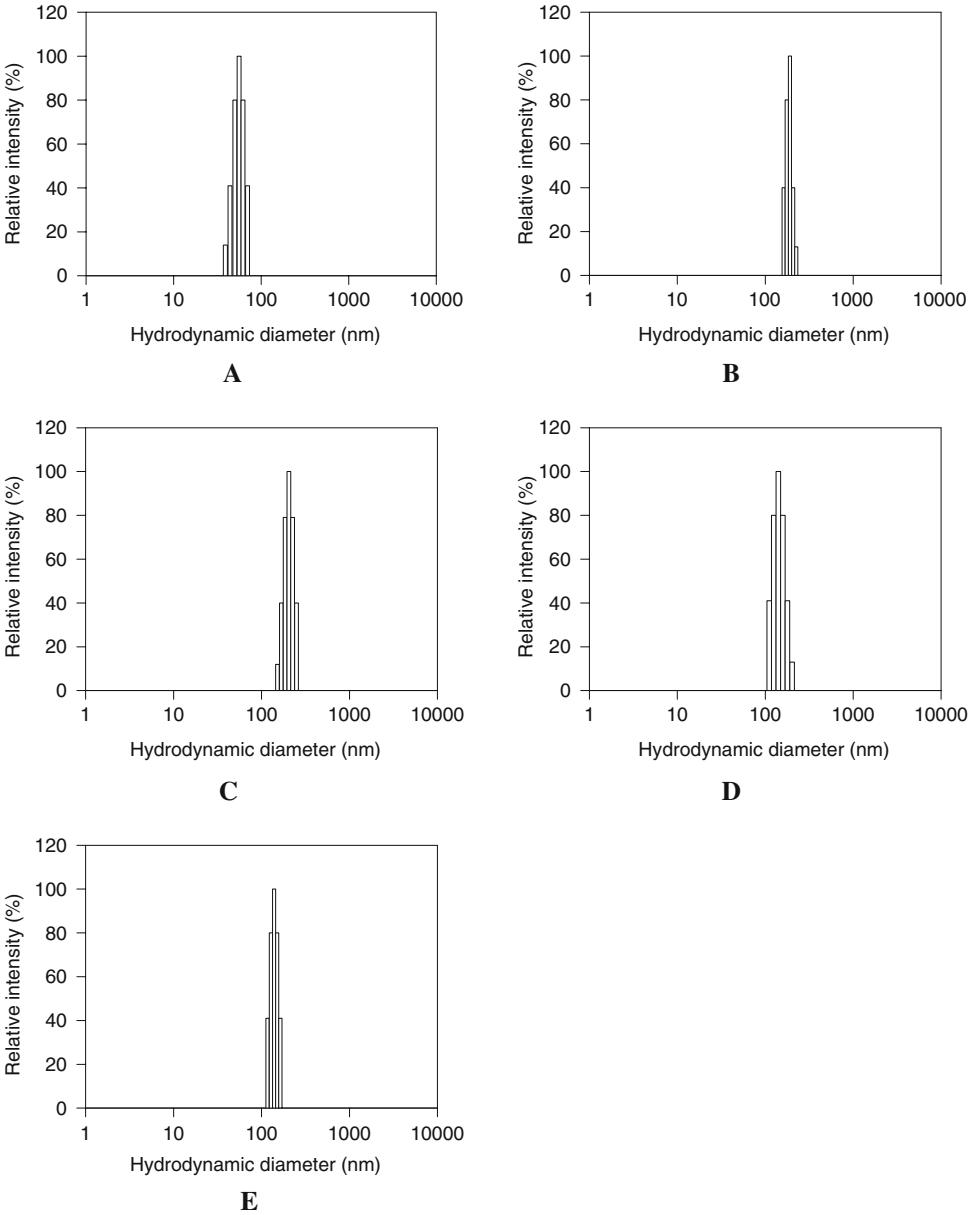

Fig. 2 Typical PCS data of the different types of liposomes with Gd-DTPA-$BC_{12}A$ (**a**), Gd-DTPA-$BC_{14}A$ (**b**), Gd-DTPA-$BC_{16}A$ (**c**), Gd-DTPA-$BC_{18}A$ (**d**) and Gd-DTPA-BC_{18}Aunsat (**e**) incorporated in their bilayer

coefficient (3.10^{-9} m^2 s^{-1}). The number of water molecules in the first coordination sphere was assumed to be equal to 1 by analogy with other Gd-DTPA derivatives. The other parameters, i.e., the rotational correlation time τ_R, the residence time of the coordinated water molecule τ_M, the electronic relaxation time at low field τ_{S0}, and the correlation time modulating the electronic relaxation τ_V were adjusted simultaneously.

The τ_R values obtained from the theoretical fittings are clearly related to the local mobility of the complexes since, for a spherical particle with a diameter of 60 nm, the global rotational correlation time calculated by the Stokes–Einstein theory should be of the order of 20 μs. It seems thus that the saturated complexes with the shorter alkyl chains have higher internal mobility than those with longer chains. It should be pointed out that the simple theoretical model used here is not the most appropriate, since: firstly, it is not adequate for slowly rotating systems; secondly, it does not discriminate local rotational motion from the rotation of the supramolecular structure; and thirdly, it does not distinguish the water exchange rate between internal and external water pools from the water exchange rate of the coordinated water molecule of the gadolinium complex. It should be kept in mind that the contribution of the Gd-complex embedded in the internal layer of the membrane can become negligible if

Table 1 Hydrodynamic diameters, final Gd-complex concentration, longitudinal and transverse relaxivities of the three individual syntheses of the liposomes containing the various Gd-DTPA-bisamide derivatives

Sample	Mean diameter (nm)	Final [Gd-complex] (mM)	r_1 at 20 MHz (s^{-1} mM^{-1})	r_2 at 20 MHz (s^{-1} mM^{-1})	r_1 at 60 MHz (s^{-1} mM^{-1})	r_2 at 60 MHz (s^{-1} mM^{-1})
Gd-DTPA-BC_{12}A						
1	58[a]	2.26	13.31	15.24	12.58	17.42
2	68[a]	2.24	12.85	14.03	11.45	15.61
3	56[b]	2.46	11.90	12.90	10.89	14.77
Mean ± SD	61 ± 6	2.32 ± 0.12	12.69 ± 0.72	14.06 ± 1.17	11.64 ± 0.86	15.93 ± 1.35
Gd-DTPA-BC_{14}A						
1	161[a]	1.72	9.69	10.74	8.59	11.60
2	176[a]	1.61	8.92	10.32	8.01	10.82
3	176[a]	1.83	9.86	10.90	8.51	11.55
Mean ± SD	171 ± 9	1.72 ± 0.11	9.49 ± 0.50	10.65 ± 0.30	8.37 ± 0.31	11.32 ± 0.44
Gd-DTPA-BC_{16}A						
1	144[c]	1.64	5.83	6.56	4.95	7.09
2	201[a]	1.52	5.01	5.57	4.27	6.18
3	190[a]	1.94	4.43	4.98	3.98	5.73
Mean ± SD	178 ± 30	1.70 ± 0.22	5.09 ± 0.70	5.70 ± 0.80	4.40 ± 0.50	6.33 ± 0.69
Gd-DTPA-BC_{18}A						
1	91[d]	1.74	7.24	8.23	6.22	8.70
2	149[a]	2.48	7.24	8.13	6.54	9.48
3	167[a]	1.84	5.92	6.70	5.27	7.71
Mean ± SD	136 ± 40	2.02 ± 0.40	6.80 ± 0.76	7.69 ± 0.86	6.01 ± 0.66	8.63 ± 0.89
Gd-DTPA-BC_{18}Aunsat						
1	136[a]	2.66	15.76	18.22	16.44	24.28
2	136[a]	2.48	16.52	18.99	17.07	25.76
3	127[e]	2.72	17.84	20.64	18.27	28.06
Mean ± SD	133 ± 5	2.62 ± 0.13	16.71 ± 1.05	19.28 ± 1.24	17.26 ± 0.93	26.03 ± 190

[a] Liposomes extruded ten times on filters of 400 nm

[b] Liposomes extruded three times on filters of 800 nm then seven times on filters of 200 nm

[c] Liposomes extruded ten times on filters of 400 nm then ten times on filters of 200 nm

[d] Liposomes extruded 10 times on filters of 400 nm then 20 times on filters of 100 nm

[e] Liposomes extruded three times on filters of 1 μm then seven times on filters of 200 nm

the water exchange through the membrane becomes very slow with a resulting very low "global" relaxivity. For DPPC/DPPG (95/5 w/w) unilamellar liposomes at 310 K, Fossheim et al. (1999) reported a water exchange time through the bilayer increasing from 2.5 ms to 13.7 ms depending of the size of the liposomes. They showed that the water exchange rate through the bilayer ($1/\tau$) increases with the inner surface area-to-volume ratio S/V (Eq. 1) and reported a permeability (P) of 3.3×10^{-4} cm s^{-1} at 310 K

$$1/\tau = P \times S/V. \quad (1)$$

If it is assumed that the Gd-complex has little influence on the water permeability of our DPPC liposomes; those incorporating Gd-DTPA-BC_{14}A and Gd-DTPA-BC_{16}A should have similar water exchange rate through the bilayer (calculated values: 8–8.5 ms), whereas it should be slightly lower for the DPPC/Gd-DTPA-BC_{18}A liposomes (calculated value: 6.2 ms). Such large values of the water exchange between inner water and bulk water should thus induce a quenching of the relaxivity contribution of Gd-complexes located in the inner layer. Such quenching was recently reported in a study comparing the relaxivity of DPPC liposomes incorporating amphiphilic Gd-complexes (Gd-DTPA-BC_{14}A) either in both sides of the liposomal bilayer or only in the external layer of the membrane (Laurent et al. 2008).

For liposomes with the unsaturated alkyl chain Gd-DTPA-BC_{18}Aunsat, the relaxation rates are markedly larger than for its saturated analogue. This difference seems to be related to a significantly faster water

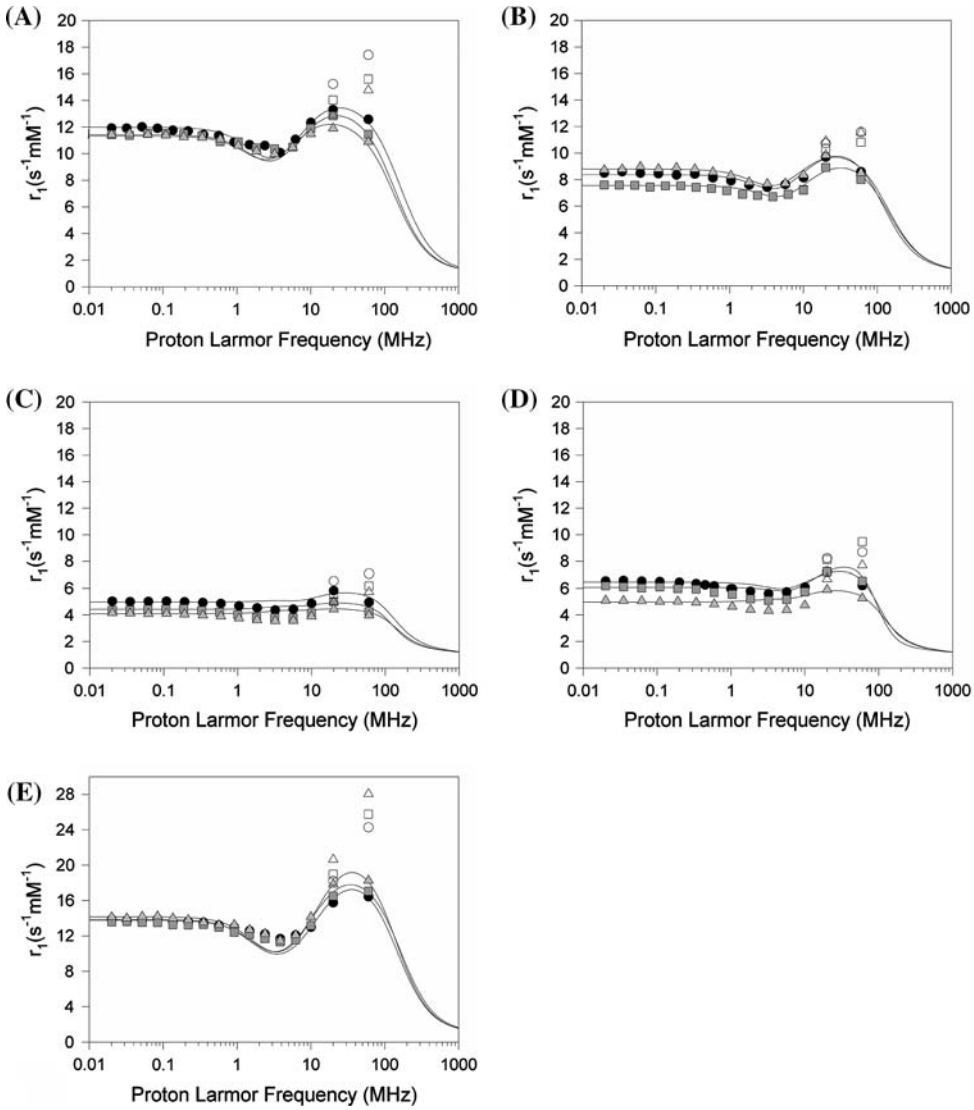

Fig. 3 NMRD profiles of the different types of liposomes with Gd-DTPA-$BC_{12}A$ (**a**), Gd-DTPA-$BC_{14}A$ (**b**), Gd-DTPA-$BC_{16}A$ (**c**), Gd-DTPA-$BC_{18}A$ (**d**) and Gd-DTPA-BC_{18}Aunsat (**e**) incorporated in their bilayer *Closed symbols*: r_1, *open symbols*: r_2; *circles*: sample 1, *squares*: sample 2, *triangles*: sample 3; $T = 310$ K)

exchange rate (decreased global τ_M) related to the presence of the C = C. It is well known that a carbon–carbon double bond increases the flexibility inside the membrane layers and the water permeability. Recently, paramagnetic pegylated liposomes made of saturated DSPC, saturated Gd-DTPA bisamide with 18 carbon atoms, and cholesterol were also shown to have a lower relaxivity at 37°C than their analogue made of unsaturated phospholipids and Gd-DTPA-derivatives (Strijkers et al. 2005). Similarly, the authors found a faster water exchange rate for the unsaturated liposomes. In addition, it seems that the Gd-complex has a much larger mobility (shorter τ_R), which could be explained by a looser structure of the membrane due to the carbon–carbon double bond.

The comparison of the mean relaxivity of the four different types of DPPC liposomes synthesized in the present work (Fig. 4) and characterized by a similar size clearly shows a relationship between the length and the type of the alkyl chains of the Gd-DTPA-bisamide derivatives. The relaxivities decrease when the length of the saturated chain increases from C14 to C16 and then increases for the C18 chain length.

It thus seems that when the length of the alkyl chains of the paramagnetic chelate is similar to the length of the phospholipid chains of the main component of the liposomal membrane (in this work DPPC), the immobilization of the paramagnetic complex within the membrane is increased and the "global" water exchange is slowed down with, as a global result, a lower efficiency as an MRI contrast agent. On the other hand, the presence of an unsaturated carbon–carbon bond in the chain is beneficial for the relaxivity mainly because of a much faster water exchange rate.

Table 2 Parameters obtained by the theoretical adjustment of the proton NMRD profiles at 37°C

Sample	τ_R (ns)	τ_M (μs)	τ_{S0} (ps)	τ_V (ps)
Gd-DTPA-BC_{12}A				
1	2.5	1.27	199	36
2	3.7	1.43	194	37
3	4.0	1.58	217	41
Mean ± SD	3.4 ± 0.8	1.43 ± 0.16	203 ± 12	38 ± 26
Gd-DTPA-BC_{14}A				
1	6.3	2.06	112	30
2	5.0	2.29	92	28
3	4.8	2.08	132	30
Mean ± SD	5.4 ± 0.8	2.17 ± 0.13	112 ± 20	29 ± 1
Gd-DTPA-BC_{16}A				
1	14.2	4.5	48.7	28.2
2	21.0	5.8	42	29
3	24.0	2.8	38	27
Mean ± SD	19.7 ± 5.0	4.4 ± 1.5	42.9 ± 5.4	28.1 ± 1.0
Gd-DTPA-BC_{18}A				
1	24.0	3.1	77.5	26.7
2	22.0	3.0	63	27
3	20.0	4.4	48	32
Mean ± SD	22 ± 2	3.5 ± 0.8	63 ± 15	28.6 ± 3
Gd-DTPA-BC_{18}Aunsat				
1	2.1	0.78	186	26
2	1.7	0.70	173	31
3	2.0	0.64	172	28
Mean ± SD	1.9 ± 0.2	0.71 ± 0.07	177 ± 7.8	28.3 ± 2.5

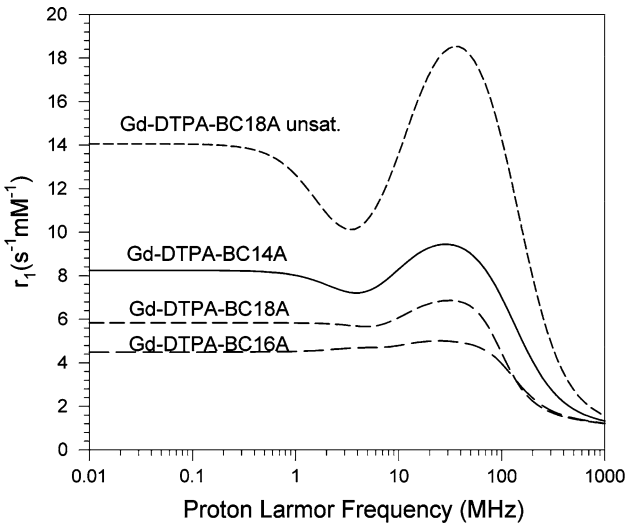

Fig. 4 Comparison of the average NMRD profiles of four different kinds of liposomes

Conclusion

The syntheses of DPPC liposomes with Gd-DTPA bisamide complexes in their external and internal layers show a good reproducibility for their proton relaxivity, their hydrodynamic size and Gd-complex final concentration (>55 % of the initial Gd-complex concentration). At 310 K, the incorporation of Gd-DTPA bisamide complexes with saturated alkylated chains into DPPC liposome membranes results in a minimum relaxivity when the chain lengths of both liposome components are similar. On the contrary, the presence of a carbon–carbon double bond has a significant beneficial effect on the relaxivity. At a temperature lower than the phase transition temperature of the main phospholipidic component (T_m of DPPC = 314 K), the relaxivity of paramagnetic DPPC liposomes is thus mainly related to the matching of the carbon chain length and of the chemical structure of the phospholipid and the Gd-complex, the best efficiency as an MRI contrast agent being obtained when the membrane is less structured.

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