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ORIGINAL CONTRIBUTION

Calorimetric and spin-label ESR studies of PEG:2000-DPPE containing DPPC/lyso-PPC mixtures

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Abstract The thermotropic behavior of dipalmitoylphosphatidylcholine (DPPC) multibilayers containing up to 10 mol% of lyso-palmitoylphosphatidylcholine (lyso-PPC) with and without low content of poly(ethylene glycol:2000)-grafted dipalmitoylphosphatidylethanolamine (PEG:2000-DPPE) has been studied by high sensitivity differential scanning calorimetry (DSC) and electron spin resonance (ESR) using the spin probe di-*tert*-butyl-nitroxide (DTBN). The three lipids, dispersed in buffer at appropriate concentrations, form thermosensitive liposomes used as site-specific drug-delivery systems. Without polymer–lipids, the DPPC main transition temperature is downshifted of 1.2–1.3 °C at the highest lyso-PPC content. The molar enthalpy and the cooperative unit of the DPPC main transition first decrease rapidly, then more slowly and finally slightly increase with lyso-PPC content. Moreover, in the mixed dispersions, the membrane fluidity increases at any temperature. The addition up to 5 mol% of PEG:2000-DPPE to DPPC/10 mol% lyso-PPC mixtures does not affect neither the thermotropic phase behavior nor the transition cooperativity and the fluidity of the dispersions.

Keywords DPPC · Lyso-PPC · Polymer–lipid · DSC · Spin-probe ESR · Transition cooperativity · Membrane fluidity

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Introduction

Supramolecular structures from self assembly of amphiphilic lipid molecules (liposomes, vesicles, micelles, nanoparticles, etc.) are widely used for basic biomembrane research and biotechnological applications [1, 2]. In the biomedical area, liposomes, for their ability to encapsulate both hydrophilic and hydrophobic substances, have found wide use as drug carriers [1, 3]. Different liposome formulations have been developed that combine prolonged lifetime in the blood circulation, resistance to protein adsorption, and adhesion to cellular surfaces, escape of diverse elements of the immune system, efficient release at the target site [1, 3–5]. After the establishment of a maximum in permeability of lipid membranes for polar ionic solutes at the chain-melting temperature, where the gel and the fluid phases coexist [6–8], Needham et al. [5, 9] have developed a liposomal formulation for drug delivery in which release can be triggered by mild hyperthermia that can be applied topically. These thermosensitive liposomes are composed of the common diacyl lipid dipalmitoylphosphatidylcholine (DPPC), low concentration of the highly bilayer compatible lysolipid lyso-palmitoylphosphatidylcholine (lyso-PPC), and appropriate amount of a polymer–lipid. The last is formed by dipalmitoylphosphatidylethanolamine (DPPE) which has covalently attached to its polarhead the poly(ethylene glycol) (PEG) of average molecular weight of 2,000 Da (PEG:2000-DPPE) that confers steric stabilization to the phospholipid dispersions.

It is, therefore, of great interest to study the physical properties of lipid membranes at the main phase transition, which are of crucial importance for the ability of liposomes to transport and to release drugs at the intracellular target site.

In the present study, we have investigated the thermotropic phase behavior of mixtures of DPPC and lyso-PPC up to 10 mol%. To the multibilayer dispersions composed of DPPC and 10 mol% lyso-PPC, we have added increasing concentration of the polymer–lipid PEG:2000-DPPE up to 5 mol%. Experiments were carried out by means of high sensitivity differential scanning calorimetry (DSC) at low scan rate and electron spin resonance (ESR) spectroscopy using the spin probe di-*tert*-butyl-nitroxide (DTBN), that partitions between the aqueous and the fluid lipid hydrocarbon environments. The measurements show that the DPPC main transition temperature, T_m, downshifts progressively with the amount of lyso-lipid added to the mixtures. At the highest lyso-PPC content, T_m is depressed of about 1.2–1.3 °C. A biphasic behavior is, instead, observed for the molar enthalpy, ΔH, and the cooperativity of the main phase transition of DPPC which decrease at low content and then increase at high content. Moreover, the presence of lyso-PPC in the host lipid matrix of DPPC leads to a loosening of the lipid packing density and to an increase of the fluidity of the membranes at any concentration and temperature. The addition of the polymer–lipid PEG:2000-DPPE to the binary system DPPC/lyso-PPC (9:1 molar ratio) does not significantly affects the temperature of the main transition, the transition cooperativity, and the membrane fluidity.

Materials and methods

Materials

The synthetic lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso-PPC) and the spin probe di-*tert*-butyl-nitroxide (DTBN) in ethanol solution were from Sigma/Aldrich (St. Louis, MO, USA), whereas the polymer–lipid of high purity (>99%) 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-poly(ethylene glycol) with PEG of average molecular mass 2,000 Da (PEG:2000-DPPE) was purchased from Avanti Polar Lipids (Birmingham, AL. USA). The reagent grade salts for the 10 mM phosphate buffer solution (PBS) at pH=7.2 were from Merck (Darmstadt, Germany). All materials were used as purchased with no further purification. Bidistilled water was used throughout.

DSC measurements

Multilayers of the lipid mixtures were prepared by dissolving the required amounts of the lipids in chloroform. The solvent was evaporated in a nitrogen gas stream and the resultant thin lipid film was kept under vacuum overnight. The dried samples were then fully hydrated with PBS, by heating and vortexing for ca. 30 min at 50 °C, i.e., above the chain melting transition temperature of DPPC. The final lipid concentration was 1 mg/ml.

The calorimetric scans, which give the excess heat capacity at constant pressure (Cp_{exc}) vs temperature, were recorded using a high sensitivity differential scanning calorimeter, model VP-DSC (MicroCal, Northampton, MA, USA). The samples were degassed before the scans and the thermograms were recorded on heating and on cooling between 20 and 50 °C with scan rate of 4 °C/h. This very slow scan rate is appropriate for lipid membranes which present sharp main phase transition.

The calorimetric data were analyzed using MicroCal ORIGIN dedicated software (MicroCal Software). To test the reproducibility of the measures, samples were scanned three times. The main phase transition temperature, T_m, is the temperature at which the peak of the thermograms is maximum, whereas $\Delta T_{1/2}$ is the width at half-height of the Cp_{exc} profiles. The molar enthalpy, ΔH, of the main phase transition was obtained from the area under the DSC peak and the mass of phospholipids in each sample (see inset of Fig. 1).

Spin-label ESR measurements

Spin-labeled lipid mixtures for ESR measurements were prepared as described for the DSC measurements except that the dried samples were fully hydrated with a 5×10^{-4} M solution of DTBN in PBS at pH 7.2. The concentration of lipid in the dispersions was of 73 mg/ml.

The lipid dispersions were sealed in 1-mm (i.d.) 100-μl glass capillaries and incubated at 4 °C for 24 h before ESR measurements.

The ESR spectra were recorded with a 9-GHz Bruker (Karlsruhe, Germany) spectrometer, model ESP 300 and digitized with the spectrometer's built-in computer using OS-9 compatible ESP 1600 Data System spectral acquisition software.

Sample capillaries were inserted in a 4-mm (i.d.) quartz ESR tube containing light silicone oil for increased thermal stability and were centered in a standard TE_{102} rectangular ESR cavity (ER 4201, Bruker). Sample temperature was controlled with a Bruker Eurotherm ER 4111 VT variable temperature control unit. ESR spectra were recorded at thermal equilibrium starting from low temperatures at a microwave power of 10 mW, i.e., well below saturation, using a 100-kHz field modulation frequency for phase sensitive detection and 0.25 G_{p-p} as magnetic field modulation amplitude.

In an aqueous environment, the small spin probe DTBN undergoes rapid isotropic motion on the conventional ESR timescale with rotational correlation time $\tau_c<10^{-9}$ s. When dissolved in an aqueous lipid dispersion, it partitions between the aqueous and the fluid lipid phases [10–12]. The corresponding ESR spectrum is the superposition of

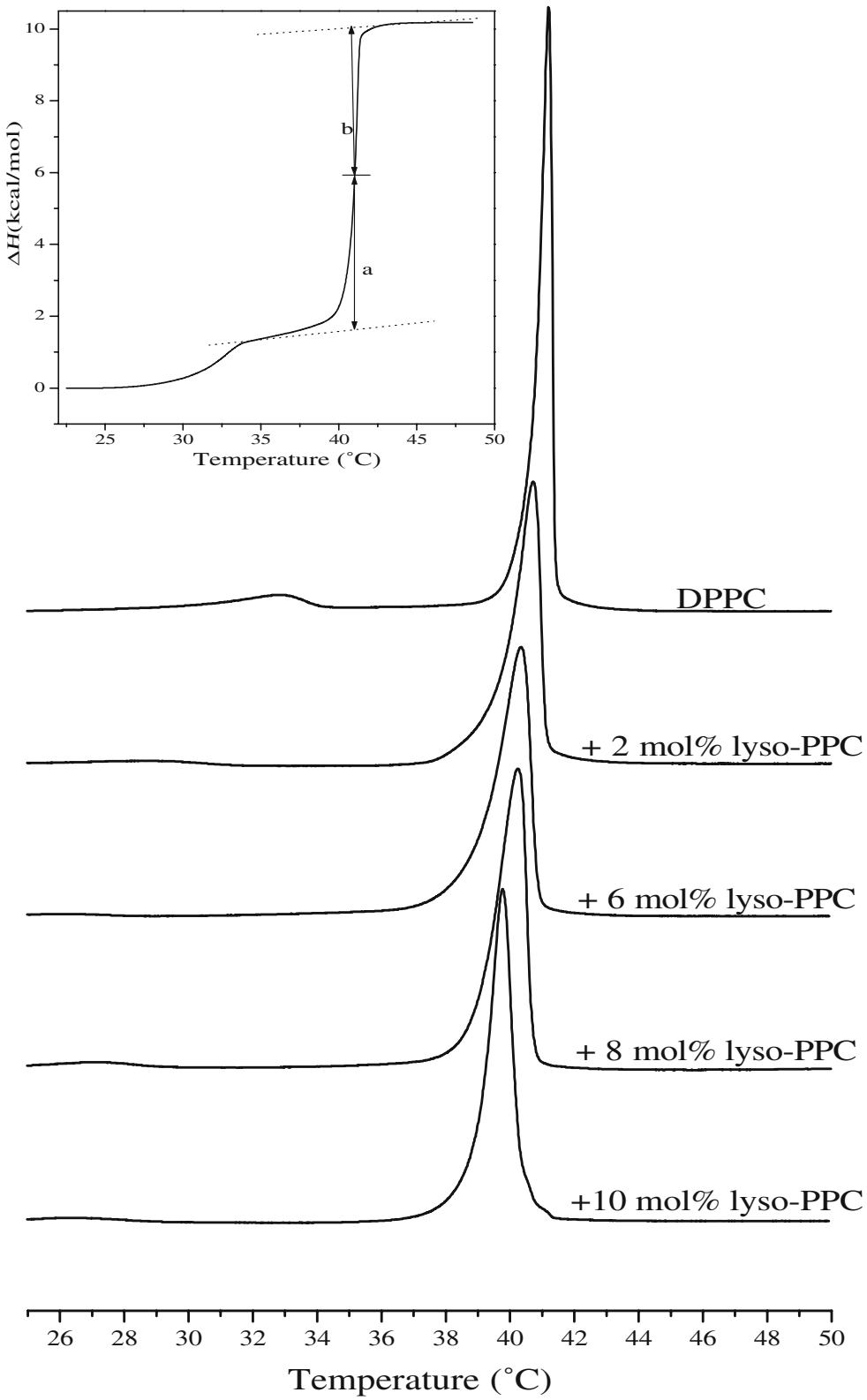

Fig. 1 DSC heating thermograms of DPPC/lyso-PPC mixtures at a scan rate of 4 °C/h. *Inset*: temperature dependence of ΔH for DPPC dispersions

two isotropic ESR patterns: one arising from DTBN in the bulk dispersion medium and the other from the fraction of spin probe in the fluid hydrophobic environment of the lipid multilayers. As the fluid hydrocarbon region of membranes have higher viscosity and lower polarity than the aqueous environment, there are small differences in the isotropic Landè *g*-value and hyperfine splitting separation of the two ESR patterns. At 9 GHz, the two components are partially resolved in the high-field line of the ESR spectra (inset of Fig. 3). The measure of the amplitude of the spin

probe signal in the aqueous phase, H_W, and of that in the fluid lipid one, H_L, allows an estimate of the fraction of DTBN in the fluid hydrophobic region of the bilayer, by means of the partition coefficient, P_c, [12, 13]:

$$P_c = H_L/(H_L + H_W) \quad (1)$$

Taking into account the second integral of the resonance lines, an improved determination of the partition coefficient, P_c^*, can be obtained by:

$$P_c^* = \frac{k}{1 + (k-1) \times P_c} P_c \quad (2)$$

where the value of the parameter k is equal to 3.67 [14].

The partition coefficient is related to membrane fluidity, and plots of this parameter as a function of the temperature give insight into the thermotropic phase behavior of the lipid dispersions [10–13].

Transition cooperativity

The main phase transition in lipid bilayers is described in terms of growing fluid lipid domains with temperature increase [13, 15]. During the transition, the lipids are in three different states: lipids in the ordered phase, lipids in fluid phase, and lipids in the region between ordered and fluid phases, i.e., the interfacial region, which take up the transition [16]. An important parameter that characterizes the ordered-to-fluid lipid main phase transition of phospholipid bilayers is the transition cooperativity. The cooperative unit, CU=$\sigma^{-1/2}$, is a parameter that describes the degree of intermolecular cooperation between phospholipid molecules undergoing the main phase transition, i.e., the number of lipid molecules undergoing melting simultaneously. The cooperativity parameter, σ, is an index of the cooperativity of the transition: the higher the σ, the smaller is the cooperativity [13, 16].

The cooperative unit can be determined from DSC and ESR data as reported elsewhere [13, 16]. Briefly, from the temperature dependence of the molar enthalpy (see inset of Fig. 1) and/or from the variation of the partition coefficient with temperature (see Fig. 3), the degree of transition, θ, of the main transition can be evaluated as:

$$\theta = \frac{a}{a+b} \quad (3)$$

Near the transition temperature, i.e., for $T \approx T_m$, θ depends linearly on $1/T$ [13, 16] and it is valid:

$$\frac{d\theta}{dT}(T \approx T_m) = \frac{1}{4\sqrt{\sigma}} \frac{\Delta H}{RT^2} \quad (4)$$

$$\theta(T \approx T_m) = \text{Cte} - \frac{\alpha}{T} \quad (5)$$

where ΔH is the molar enthalpy of the main phase transition and

$$\alpha = \frac{\Delta H}{4R\sqrt{\sigma}} \quad (6)$$

is the slope of the linear plot of θ vs $1/T$.

Thus, from the plot of θ vs $\frac{1}{T}$ near the transition temperature, first α and then the mean size of the cooperative unit, CU=$\sigma^{-1/2}$, can be determined.

Results

DSC measurements on DPPC/lyso-PPC mixtures

DSC heating scans of multilamellar dispersions of DPPC in the absence and in the presence of different amounts of lyso-PPC are shown in Fig. 1. Repeated heating and cooling scans on the same samples, although with a slight hysteresis, gave identical Cp_{exc} profiles, indicating that all the DSC scans were reversible (data not shown). For any system considered, the thermograms do not show multiple components of distinct peaks. This suggests that the single chained lyso-lipids at the concentrations considered mix well with the double-chained DPPC lipids both in the gel and in the fluid phase. The characteristics of the DPPC main phase transition, i.e., temperature, amplitude, and width, as well as its thermodynamic parameters (see Table 1) are in good agreement with the lipid mesophase and the experimental method used [17, 18]. The incorporation of increasing concentration of lyso-PPC up to 10 mol% into liposomes of DPPC has an effect on the gel to liquid–crystalline phase transition of DPPC. A progressive decrease of the main transition temperature, T_m, is observed which is at most of about 1.3 °C when 10 mol% of the lyso-lipid are mixed with diacyl lipids of DPPC. Instead, it is interesting to note that the linewidth at half height of the transition, $\Delta T_{1/2}$, markedly increases (of ~0.5 °C) at the lowest lyso-PPC percentage into the mixture, then slowly increases and finally tends to decrease. A biphasic behavior is also observed for the molar enthalpy, ΔH, vs [lyso-PPC]. In fact, it first decreases rapidly of 1 kcal/mol on going from DPPC to DPPC + 2 mol% lyso-PCC, then reaches a minim value of 7.6 kcal/mol at 6 mol% and finally increases reaching 8.2 kcal/mol at 10 mol% of lyso-PPC. These results imply that the cooperativity of the chain melting transition of DPPC is also affected by the presence of the lyso-lipids. To evaluate the cooperative unit, CU, of the main transition of the lipid dispersions from the DSC data, the degree of transition, θ, was estimated from the plots of the temperature dependence of the molar enthalpy (see "Materials and methods" section). The dependence of θ on $1/T$ for different mixtures of DPPC and lyso-PPC are

Table 1 Main transition temperature, T_m, linewidth at half height, $\Delta T_{1/2}$, molar enthalpy, ΔH, and cooperative unit, CU, for DPPC/lyso-PPC binary mixtures in PBS deduced from DSC and ESR measurements

Sample	DSC				ESR	
	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (kcal/mol)	CU	T_m (°C)	CU
DPPC	41.2	0.40	8.8	147	40.8	111
+2 mol % lyso-PPC	40.7	0.90	7.8	80	40.5	72
+4 mol % lyso-PPC	40.5	0.95	7.7	75	40.2	52
+6 mol % lyso-PPC	40.3	1.15	7.6	69	40.0	33
+8 mol % lyso-PPC	40.1	1.05	8.0	75	39.5	34
+10 mol % lyso-PPC	39.9	0.95	8.2	78	39.6	37

reported in Fig. 2. From these plots, the α-parameters have been determined and finally the CU values evaluated. The results of these calculations are given in Table 1. As expected, the DSC data show that the incorporation of the lyso-lipid into DPPC liposomes affects in a concentration dependent manner the cooperativity of the main phase transition. Indeed, the cooperative unit is approximately halved on adding only 2 mol% of lyso-PPC to the multibilayers of DPPC; it decreases further to 69 at 6 mol% of lyso-PPC and then it slightly increases when the lyso-lipid concentration is raised up to 10 mol%.

ESR measurements on DPPC/ lyso-PPC mixtures

In the inset of Fig. 3, the high field regions of the ESR spectra, at 37 °C of the spin probe DTBN in lyso-PPC containing DPPC membranes, at three different molar ratio are shown. The presence of increasing concentration of the lyso-lipid causes a progressive increase of the intensity of the lipid component, H_L, in the spectra. This corresponds to an increase of the amount of the spin probe that permeates into the membranes composed by mixtures of DPPC and lyso-PPC. The augmented partition of the spin probe into the hydrophobic region is also seen in the temperature dependence of the partition coefficient, P_c^*, for different binary mixtures of DPPC/lyso-PPC shown in the main body of Fig. 3. The P_c^* values are equal to zero for DPPC in the gel state and they start to increase progressively on approaching the pretransition at ca. 32.5 °C, and then a large variation of P_c^* is seen for temperatures around the chain-melting transition of the DPPC multilayers at 40.8 °C. In the presence of the lyso-lipid mixed with DPPC, the spin probe DTBN has an appreciable partition in the fluid hydrophobic region of the dispersions even at the lowest temperatures considered, and the P_c^* values increase notably at the main transition of the lipid dispersions. Moreover, as already evidenced with DSC results, the main transition temperatures, T_ms, are progressively downshifted with increasing content of lyso-PPC and the shifts are at most of 1.2 °C. Following the same procedure used for DSC

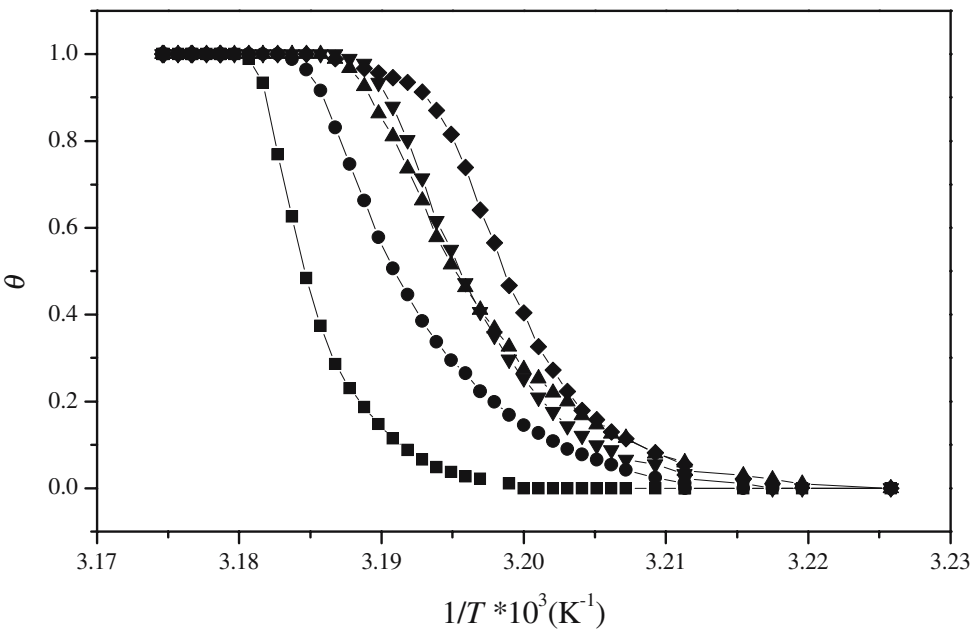

Fig. 2 Degree of transition, θ, as a function of $1/T$ of DPPC multilayers containing different percentage of lyso-PPC: (*filled square*) DPPC alone, (*filled circle*) DPPC + 2 mol% lyso-PPC, (*filled triangle*) DPPC + 6 mol% lyso-PPC, and (*filled inverted triangle*) DPPC + 10 mol% lyso-PPC. Data are obtained from DSC results

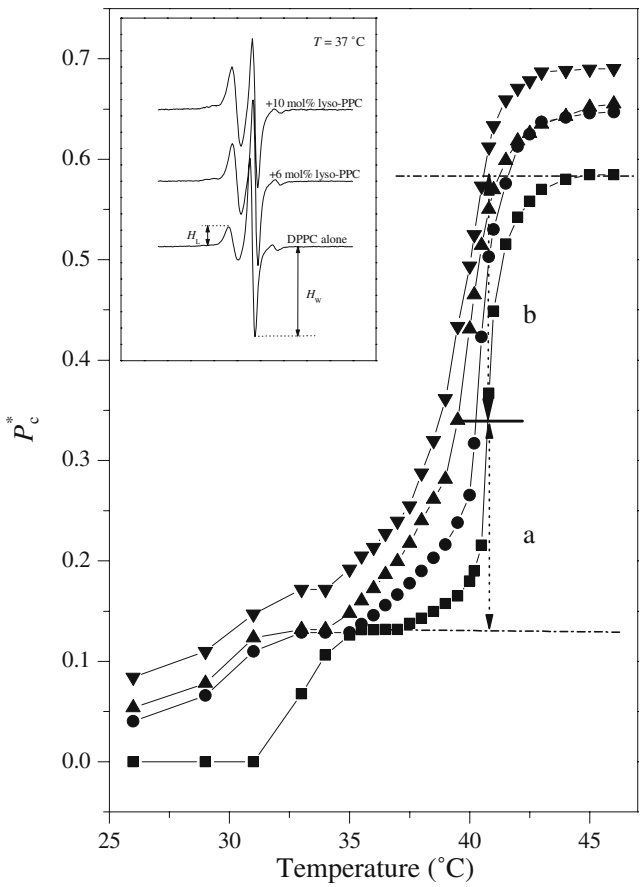

Fig. 3 Temperature dependence of the partition coefficient, of the spin probe DTBN in dispersions of (*filled square*) DPPC alone, (*filled circle*) DPPC + 2 mol% lyso-PPC, (*filled triangle*) DPPC + 6 mol% lyso-PPC, and (*filled inverted triangle*) DPPC + 10 mol% lyso-PPC. *Inset*: high-field lines of the ESR spectra of DTBN in different dispersion of DPPC/lyso-PPC at T=37 °C

data, the cooperative unit of the main transition of the lipid dispersions from the spin-label ESR data has been evaluated (see Table 1). As expected, the values of the cooperative unit are dependent on the technique used. Nevertheless, it is worthy to note that the same trend of the values of the cooperative unit vs lyso-PPC content is found both from DSC and from ESR.

DSC and ESR measurements on DPPC/10 mol% lyso-PPC/ PEG:2000-DPPE dispersions

The effect of the polymer–lipid PEG:2000-DPPE on the calorimetric and spectroscopic behavior of the binary mixture composed of DPPC and 10 mol% of lyso-PPC has also been investigated. Our results show that the polymer–lipid does not significantly influence neither the characteristics of the main phase transition of the DPPC/10 mol% lyso-PPC matrix nor the fluidity of the lipid dispersions. This is deduced from the DSC profiles given in Fig. 4 and from the plots of P_c^* vs T (not shown) for ternary mixtures of DPPC/10 mol% lyso-PPC/ PEG:2000-DPPE, and from the parameters reported in Table 2.

Discussion

High sensitivity DSC at low scan rate and ESR spectroscopy with the spin probe DTBN have been used to investigate the effects of lyso-PPC up to 10 mol% on the main phase transition of DPPC multilayers and the fluidity of the binary mixture of DPPC/lyso-PPC multilayers. The influence of increasing concentration of the polymer–lipid PEG:2000-DPPE on the mixed DPPC/10 mol% lyso-PPC lipid dispersions have also been investigated.

The experimental results indicate that the single chained lyso-lipid, having the same zwitterionic polarhead and the same hydrocarbon chain length of the double chained DPPC molecules, mix well in the dispersions and form stable lamellar phases at the molar ratios considered. However, the lyso-lipid affects in a concentration-dependent manner the molecular properties of the DPPC dispersions. The increased partition of DTBN in the fluid hydrocarbon region suggests that the wedge-shaped lyso-PPC molecules lead to a progressive loosening of the lipid packing density, increase the fluidity, and stabilize the fluid state of the DPPC membranes. Indeed, T_m is reduced of about 1.2–1.3 °C in the liposomes composed of DPPC and 10 mol% of lyso-PPC. In dispersions of the same lipid composition, similar T_m downshifts have been reported by DSC heating scans at 2 °C/min [5] and at 60 °C/h [19, 20]. Although small, the shifts are relevant because they bring the DPPC main transition temperature closer to the physiological body temperature. For this property, mixtures of DPPC and 10 mol% lyso-PPC have been proposed as lipid matrix upon to base the thermosensitive liposomes [5]. The increased fluidity of the lyso-PPC containing DPPC dispersions observed at the main transition temperature correlates well with the enhancement of the phase transition permeability of the entrapped carboxyfluorescein from DPPC liposomes by incorporation of lyso-PPC [5].

In contrast to the trend of the main phase transition temperature, the other transition parameters, i.e., the linewidth at half height, $\Delta T_{1/2}$, the molar enthalpy, ΔH, and the cooperative units, CU, show a biphasic behavior with the content of lyso-PPC mixed with DPPC. It is likely that lyso-PPC at low concentration (≤6 mol%) weakens the molecular packing of DPPC bilayers reducing the hydrophobic interactions between the hydrocarbon lipid chains. In turn, this reduces the enthalpy and limits the cooperativity of the transition. At higher concentration (>6 mol%) the lyso-lipid could affect the structural organization of the DPPC bilayers. We have evidence (data not shown) that low amount of lyso-PPC influences the dynamic properties

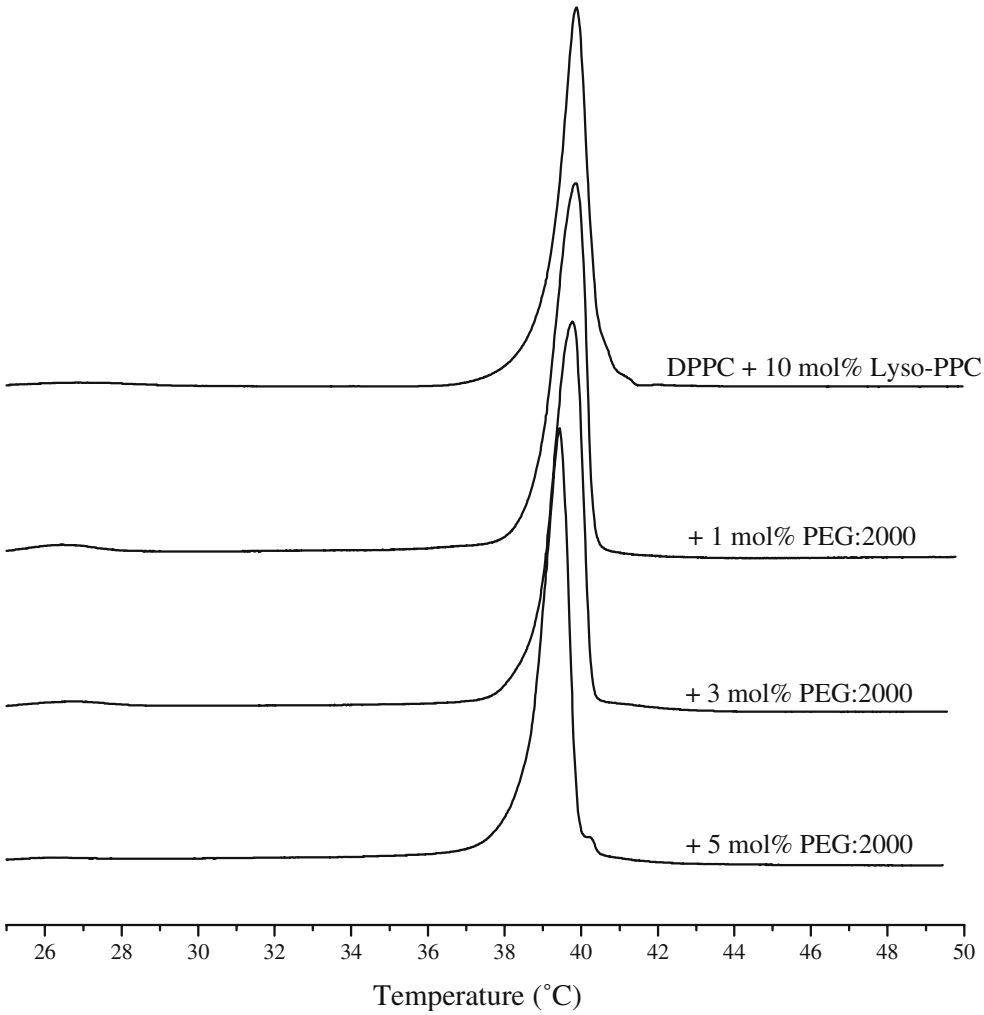

Fig. 4 DSC heating thermograms of DPPC/10 mol% lyso-PPC/PEG:2000-DPPE ternary mixtures at a scan rate of 4 °C/h

of chain-labeled lipids incorporated in the mixed DPPC/lyso-PPC dispersions. In fact, the motion of a label localized near the terminal methyl end of the chain (16-PCSL) is markedly restricted, whereas that of a label localized near the polar/apolar interface (5-PCSL) is more favored. These are experimental evidences, detected by spin-label ESR, for chain interdigitation induced by a threshold concentration of a variety of inducers in saturated symmetrical chain PCs [21–24]. It is worthy to point out that DPPC chain interdigitation in the gel phase induced by lyso-PPC at concentration below 30 mol% has been reported using X-ray diffraction and fluorescent probes [19, 20], whereas McIntosh et al. [25] have reported interdigitation in DPPC/lyso-PPC at 1:1 mol/mol ratio. Relative to the main transition which normally occurs between gel and fluid noninterdigitated lamellar phases, in many samples, the transition from interdigitated gel phase to fluid phase has almost the same thermodynamic characteristics and slightly increased molar enthalpy [21, 26]. Moreover, it has been found that the transition enthalpy of the gel to liquid crystalline phase transition of DPPC/lyso-PPC mixtures from 0 to 80 mol% lyso-PPC has a

Table 2 As in Table 1, but for the ternary systems DPPC/10 mol% lyso-PPC and different amount of PEG:2000-DPPE

Sample	DSC			ESR
	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔH (kcal/mol)	T_m (°C)
DPPC+10 mol % lyso-PPC	39.9	0.95	8.2	39.5
+0.5 mol % PEG:2000-DPPE	39.9	1.00	8.3	39.7
+1 mol % PEG:2000-DPPE	39.8	1.00	8.3	39.7
+3 mol % PEG:2000-DPPE	39.7	1.07	8.6	39.5
+5 mol % PEG:2000-DPPE	39.4	0.90	8.7	39.5

tendency to decrease but it jumps to higher values in the concentration range of 5÷10 mol% and at 50 mol% [5]. The authors ascribed the observed ΔH increase in the complex DPPC/lyso-PPC at 1:1 molar ratio to the formation of the interdigitated phase [25]. Although the formation of an interdigitated gel phase in mixtures of DPPC and 8–10 mol% of lyso-PPC need to be ascertained with further study, it could correspond to the observed augmented enthalpy and cooperativity, accounting for the formation of a more stable gel phase.

Loading the DPPC/10 mol% lyso-PPC binary mixtures with increasing concentrations of the polymer-lipid PEG:2000-DPPE up to 5 mol% does not give rise to appreciable variations of the molecular physico-chemical properties of the mixed bilayers. Previously, we found that the lateral pressure exerted between the polymer chains in the brush regime of the polymer-lipids PEG:2000-DPPE (i.e., concentration ≥1–1.5 mol%) increases the DPPC chain mobility in the gel phase and shifts the chain melting temperature of DPPC liposomes of −1 to −2 °C [27, 28]. Most probably, in the host lipid matrix composed of DPPC and 10 mol% of lyso-PPC, the interaction between the polymer–chains is hindered, and the molecular packing imposed by the lyso-lipid remains unmodified by the polymer–lipid, which exerts only a steric protective effect. It is worthy to note that the addition of PEG:2000-DPPE does not influence the spectral lineshape of 5- and 16-PCSL spin-labels at any temperature, and single component spectra are obtained at any composition. In other words, the structure of the lamellar phase of DPPC/10 mol% lyso-PPC is not perturbed by the polymer-lipid. Furthermore, this suggests that the three lipid components form homogeneous mixtures at the concentration considered.

In conclusion, our DSC and ESR results put in evidence that stable lamellar phases are formed by incorporating up to 10 mol% of lyso-PPC and low concentration of the polymer-lipid PEG:2000-DPPE in the host lipid matrix of DPPC. In particular, the liposome formulation of thermosensitive liposomes consisting of DPPC + 10 mol% lyso-PPC + 4 mol% of PEG:2000-DPPE shows reduced main phase transition temperature relative to DPPC membranes, augmented fluidity, and additional stability. From a biomedical application point of view, the function of the lyso-lipid additive is to bring the chain melting transition closer to physiological temperatures and to optimize the release characteristics. The polymer coating, instead, confers stealth properties at the thermosensitive liposomes and ensures long circulation time in the blood stream.

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