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DSC STUDY ON THE INTERACTION BETWEEN BIS-2-(ETHYLHEXYL) PHTHALATE AND OTHER *o*-PHTHALIC ACID ESTERS AND DIPALMITOYL PHOSPHATIDYLCHOLINE LIPOSOMES

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Abstract

Phthalic acid esters, and in particular bis-2-(ethylhexyl) phthalate, are common environmental contaminants with long-term toxic and carcinogenic effects that readily dissolve in lipid substances. DSC measurements of hydrated multilamellar dipalmitoyl phosphatidylcholine liposomes in the presence of different amounts of bis-2-(ethylhexyl) phthalate, dioctyl phthalate, dibutyl phthalate (DBP) and diethyl phthalate (DEP) were considered. The effects on the main transition temperature, ΔH, and the shape and width of the transition were studied.

A decrease in T_m, without an accompanying decrease in ΔH, and increases in both the asymmetry and the width of the main transition peak were observed.

In some cases, the calorimetric curve showed complex peak structures arising from the coexistence of various aggregates of different sizes. This was particularly evident in the liposomes with DEP and DBP contents ranging from 5.0 to 7.5% m/m.

For all the considered model systems, the effects were noticeable even in the presence of small amounts of phthalates; in the presence of greater amounts, the overall structure of the bilayer was in some cases strongly modified, with the appearance of new different phases.

Since the function of a membrane-associated protein is dependent on the lipid structure, phthalates could modify the function by modifying the membrane structure.

Keywords: DSC, phase transition, phospholipid, phthalates, plasticizers

Introduction

The esters of 1,2-benzenedicarboxylic acid (*o*-phthalic acid) with aliphatic alcohols are widely used as high-boiling temperature solvents and plasticizers in rubber and plastic manufacturing. For this purpose, especially bis-2-(ethylhexyl) phthalate (DEHP) is used, but the diethyl and dibutyl esters are also employed.

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They increase the flexibility and workability of high molecular mass polymers and are commonly used in PVC (polyvinyl chloride) manufacturing, where they generally account for 20–30% by mass of the final product, but the amount may be as low as a few percent or as high as more than 60% [1].

Phthalate esters, and particularly DEHP, can be found in plastic tubing, floor-tiles, furniture, automobile upholstery, food packaging, soft plastic toys for children, and so on. They are also used in biomedical devices, as tubing, blood vessels and plastic or plastic-coated bioprostheses [2]. The success of these compounds arises from their useful chemical-physical properties: they are hydrophobic, high-boiling point oily liquids with large liquid-temperature ranges; they are inert to most chemicals and also exhibit a low acute toxicity.

The solubility of phthalates in water is generally very low and decreases even more as the length of the side-chains increases. As they are not chemically bound to the polymeric matrix, they are able to migrate from the plastic and can be extracted by lipid substances, in which they readily dissolve. Their use in food packaging and the consequent dietary uptake, even if reduced, exposes most of the population to the long-term effects of these chemicals. They are also present in noticeable amounts in plastic and rubber soft toys that may be put in children's mouths, thereby exposing them to a greater uptake.

Recent studies on the effects on living organisms of long-term exposure to phthalates, and particularly to DEHP, the most widely used and one of the most common environmental contaminants, revealed that they can induce hepatomegaly and hepatic carcinoma, and DEHP is actually listed as a carcinogenic substance [3, 4].

Pre-neoplastic changes and profound alterations in membrane-associated components have been observed in rats exposed to DEHP; the liver appeared to be the target organ [5–7]. It induces damage in biomembranes with disturbances in permeability and function, as revealed by studies on erythrocytes [5, 8, 9]. Damage to both mitochondria and cytoplasm membranes in man [10] was observed after exposure to DEPH, which also causes peroxysome proliferation and displays reproductive and developmental toxicity in a variety of mammalian and non-mammalian species [11].

Phthalate esters are referred to as substances whose mode of action is 'polar narcosis' or 'type II narcosis', reflecting the fact that these chemicals typically possess polar functional moieties [1, 12]. In the development of these toxicity effects, the interactions with the biomembrane seem to play a key role.

The interactions with biomembranes are important for at least two reasons: first, the lipid fraction displays high affinity toward phthalate esters, and can extract them from the environment, concentrating them to a significant extent; secondly, the alterations induced in the biomembrane structure can modify the biological behaviour of the molecules interacting directly with the membranes, such as transmembrane proteins and enzymes, leading to toxic effects. The extraction of DEHP and its concentration to significant levels in erythrocytes has been observed in blood samples packed in plastic bags [2].

In this work, the modifications induced by the interactions of some widely used phthalate esters with model lipid membranes were studied by differential scanning

calorimetry (DSC), mimicking physiological conditions. To study the effects of different side-chains, diethyl, di-*n*-butyl, bis-(2-ethylhexyl) and di-*n*-octyl phthalate were considered.

DSC is a thermodynamic technique of great value in studies of lipid thermotropic phase behaviour in model phospholipid liposomes and biological membranes. It has been widely used to study membrane interactions with molecules that can penetrate into the hydrophobic core of the lipid bilayer, such as cholesterol [13–15] or narcotic substances [16, 17]. It has also been used to study the modifications of the hydrophobic core induced by interactions with non-penetrating molecules that interact only with the external polar surface of the biomembrane, such as polyamines or pesticides [18, 19].

In this work, dipalmitoyl phosphatidylcholine (DPPC) liposomes were used as a model of cellular membranes, both because lecithins are the major components in mammalian membranes and also because they show a sharp, strong, thermotropic transition near the physiological temperature, arising from the transition from the gel to the liquid-crystal phase. For these reasons, DPPC liposomes are the most widely used model systems of biomembranes.

Materials and methods

DPPC was purchased from Sigma Chemical (synthetic, *DL* type; purity grade 99%) and used without further purification. DEHP and dibutyl phthalate (DBP) of 'selectrophore grade', dioctyl phthalate (DOP) and diethyl phthalate (DEP) of analytical grade, were purchased from Fluka. Their purities, tested by GC, were found to be ≥99% and they were therefore used without further purification. Twice distilled water and high-purity, low-residue chloroform (Fluka for residue analysis) were also used. Other chemicals were ACS reagent grade Merck products.

Samples were prepared by adding the appropriate amount of phthalate ester in $CHCl_3$ to DPPC previously dissolved in the same solvent. $CHCl_3$ was then removed under a nitrogen stream and gentle heating (1 h at 60°C), the last traces being removed in vacuum. Multilamellar liposomes (vesicles) were prepared by adding physiological saline solution at pH 7.0 (0.9% NaCl, m/m) with a low amount ($\cong 10^{-3}$ M) of phosphate buffer at pH 7.0 to get a final lipid concentration of about 20% m/m. The suspension was sonicated for 3 min at very low power (<0.5 W) to avoid overheating, resulting in homogeneous, gelatinous samples.

Before measurement, all samples were held for 30 min above the melting temperature (50°C) and then for 1 h at room temperature. DSC measurements were made with a Mettler-Toledo DSC 821 calorimeter in the temperature range 25–55°C. The heating and cooling rates were fixed at 2.0°C min^{-1}. To calibrate temperature and enthalpy scales in the considered range, caprylic acid was used. Heating and cooling cycles were repeated at least 3 times to ensure the reproducibility and constancy of the thermal parameters. In all cases, the measured deviations were within the expected experimental error (±0.1°C for T_m and ±5% for ΔH values).

Samples were prepared at least 2 times to ensure reproducibility within different lots of samples. The amount of DPPC and the ratio DPPC/phthalate ester were measured on the same samples as used in the DSC measurements, after drying in vacuum overnight at 80°C. By weighing the residue, we obtained the sum of DPPC, phthalic acid ester and sodium chloride, whose amount can be calculated after the loss of water. GC confirmed the amount of phthalate ester on dissolution of the residue in $CHCl_3$. A Perkin Elmer 8600 gas chromatograph equipped with a PTE 5 capillary column and an FID detector was used. In accordance with the negligible vapour pressures of the phthalate esters in the given temperature ranges, GC measurements confirmed that the losses during the drying of the samples were negligible.

Results

Bis-(2-ethylhexyl) phthalate (DEHP)

DSC heating curves of hydrated multilamellar vesicles of pure DPPC and of some significant DPPC/DEHP mixtures are presented in Fig. 1. In the absence of DEHP, DPPC bilayers exhibit two endothermic transitions upon heating: a lower-temperature, lower-enthalpy pretransition and a higher-temperature, higher-enthalpy main transition, whose maximum temperature is generally referred to as T_m.

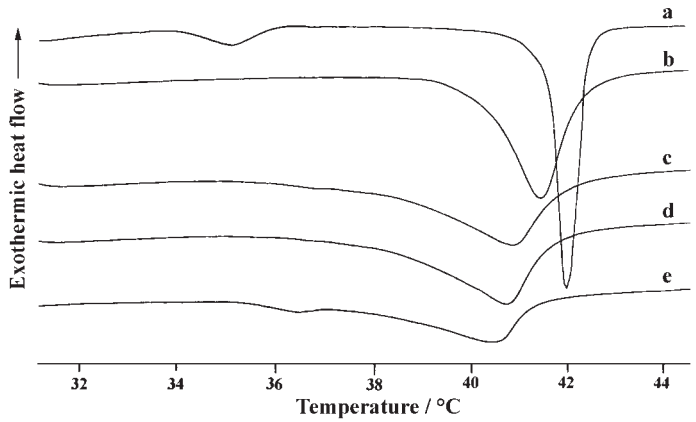

Fig. 1 DSC heating curves of hydrated multilamellar vesicles of DPPC/DEHP mixtures with different DEHP contents (a – 0%; b – 1.0%; c – 3.0%; d – 5.0% and e – 10.0%)

The pretransition arises from the conversion of the lamellar gel (L'_β) phase to the rippled gel (P'_β) phase, and the main transition from the conversion of the (P'_β) gel phase to the lamellar liquid-crystalline (L_α) phase [14, 20]. DSC scans exhibit noticeable effects due to the presence of DEHP on both the pretransition and the main transition of DPPC.

The pretransition is very sensitive to the presence of DEHP and it is no longer detectable in the presence of as little as 0.5% m/m incorporated in the bilayer. The effect on T_m is also noticeable. The values of T_m in all the considered systems are reported in Table 1, and the trends of the main transition temperature as a function of the added amount of different phthalate esters up to 5% m/m are plotted in Fig. 2.

Table 1 Temperature of the maximum (T_m) of the main calorimetric peaks observed for the DPPC/phthalic acid esters considered. The peaks denoted by * are new peaks, not detectable in the low-phthalate systems

% m/m	DEHP	DOP	DBP	DEP
0	41.9	41.9	41.9	41.9
0.5	41.6	41.7	41.5	41.6
1	41.3	41.5	41.2	41.3
2	40.9	41.1	40.6	40.6
3	40.8	40.9	40.0	39.9
4	40.7	40.9	39.3	39.2
5	40.6	40.8	38.5 ca–36.0*	38.5 ca–35.0*
7.5	40.5	40.8	36.0*	35.0*–34.8*
10.0	40.4	40.7	35.9*	35.0*–34.6*
15.0	40.2	40.6	35.9*	35.1*–34.6*
20.0	40.0	40.7	35.8*	35.0*
40.0	40.0	40.6	35.8*	35.0*

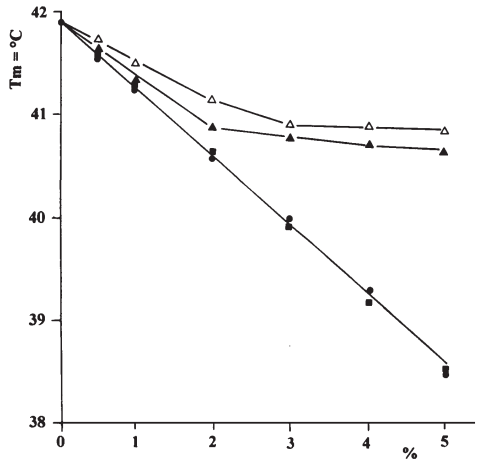

Fig. 2 Plots of T_m values as a function of the added amount of phthalic acid esters in the range 0–5% m/m (Δ – DOP; ▲ – DEHP; ■ – DEP; ● – DBP)

To study the asymmetry of the transition peak in relation to pure DPPC, an asymmetry index (A_s) defined as

$$A_s = \left(\frac{T_{ons} - T_m}{T_{end} - T_m} \right)_{sample} / \left(\frac{T_{ons} - T_m}{T_{end} - T_m} \right)_{pure\ DPPC}$$

was taken into account. Referring to the DSC plot, T_{ons} (onset temperature) is the temperature of the intercept between the baseline and the tangent to the rising part of the graph at its inflection point, and T_{end} (endset temperature) is the same, but refers to the descending part of the calorimetric peak. According to this definition, A_s values greater than 1 denote skewing to a lower temperature, and A_s values lower than 1 denote skewing to a higher temperature.

The half-width of the transition ($\Delta T_{1/2}$) and A_s data for the systems with phthalate ester amounts ≤5% m/m are reported in Table 2.

Table 2 Asymmetry index (A_s) and half-width of the transition ($\Delta T_{1/2}$) for DPPC/phthalic acid ester systems at low phthalate amount

% m/m	DEHP		DOP		DBP		DEP	
	A_s	$\Delta T_{1/2}$	A_s	$\Delta T_{1/2}$	A_s	$\Delta T_{1/2}$	A_s	$\Delta T_{1/2}$
0	1.0	0.4	1.0	0.4	1.0	0.4	1.0	0.4
0.5	2.1	0.7	2.0	0.6	2.0	0.8	1.9	0.6
1	2.5	1.0	2.3	0.9	2.4	1.0	2.6	0.8
2	2.8	1.3	2.2	1.3	3.0	1.3	2.6	1.4
3	3.3	1.5	2.7	1.6	3.1	1.7	2.9	1.7
4	3.5	1.6	2.7	1.6	3.1	1.7	3.0	1.9
5	3.6	1.7	2.9	1.7	–	–	–	–

Figure 2 and Table 1 show that for low DEHP amounts (≤3% m/m) T_m decreases in an approximately linear fashion with increasing added DEHP, and the endothermic transition broadens, becomes asymmetric, and skews to lower temperatures.

If the DEHP amount is ≥10% m/m, the shape of the heating peak is more complex, with the appearance of a secondary, poorly resolved maximum at about 36°C (Fig. 1), whose intensity increases as the DEHP amount is further increased. In the systems with a DEHP content greater than 3% m/m, the values of both T_m and A_s change slightly or remain constant. In contrast, $\Delta T_{1/2}$ seems to increase even more. A further apparent increase in $\Delta T_{1/2}$ is observed in samples with higher concentrations, but this result may be meaningless because of the complex structure of the curve, arising from the overlapping of more than one calorimetric peak.

The presence of two transitions in the DSC plots suggests that another mixed phase is present and phase segregation takes place. Nevertheless, even in the presence of great amounts of added DEHP (40% m/m) the main transition is still present, even if reduced in intensity.

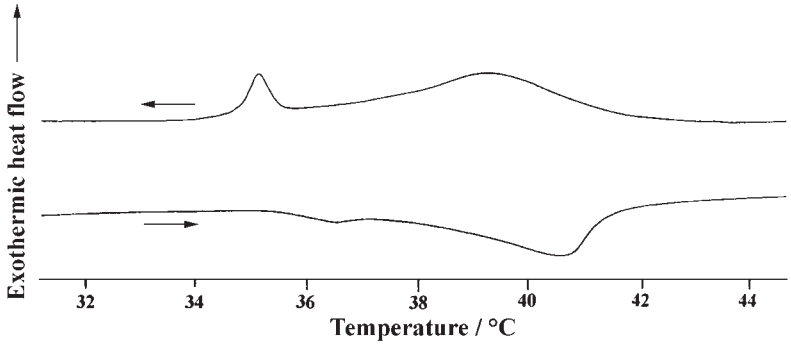

Fig. 3 Heating (lower) and cooling (upper) DSC curves of hydrated multilamellar vesicles of DPPC/DEHP in the presence of 10.0% m/m DEHP

For pure DPPC liposomes, a $\Delta T_{1/2}$ value of 0.4°C and a ΔH value of 8.6 kcal mol^{-1} were found, in good agreement with the literature data [21]. On addition of DEHP, ΔH seems to decrease, and in the presence of 7.5% m/m of added chemical, the observed ΔH value is about 12% lower than that for pure DPPC liposomes. This trend seems to continue with further DEHP addition, but the simultaneous presence of more than one maximum, poorly defined, in the samples with a higher DEHP content, makes it difficult to evaluate the true trend of ΔH for the main transition. Indeed, the observed decrease could also be due to a part of the lipid being present in a different phase, which also gives an endothermic transition, but probably with a lower ΔH.

Cooling calorimetric plots confirm the results obtained from the heating measurements: a regular, small decrease of about 1°C is observed in the T_m value. This shift can be attributed to the heating and cooling rates and to the finite response time of the calorimeter. However, this effect does not influence the interpretation of the data presented here. If the DEHP amounts is ≥10% m/m and several components are present in the DSC curve, the lower-temperature transition is better defined in the cooling measurements (Fig. 3), indicating that the transition reaches equilibrium faster with the cooling rather than with the heating. This effect is probably due to the high lateral mobility in the liquid-crystal phase, which leads to a shorter equilibration time in the cooling process rather than in the heating process, especially as far as lower-temperature transitions are concerned.

Di-n-octyl phthalate (DOP)

Figure 4 depicts the DSC heating plots for some DPPC–DOP mixtures; the T_m trend is shown in Fig. 2 and Table 1, whereas the $\Delta T_{1/2}$ and A_s values are reported in Table 2. The general trend is very similar to that observed in the DPPC–DEHP system; a T_m decrease is observed at a low DOP content, and the T_m value remains constant when the amount of DOP is greater than 3% m/m. In the DPPC–DOP system, the main transition is still present in the presence of large amounts of added DOP (40% m/m), even if its intensity appears reduced.

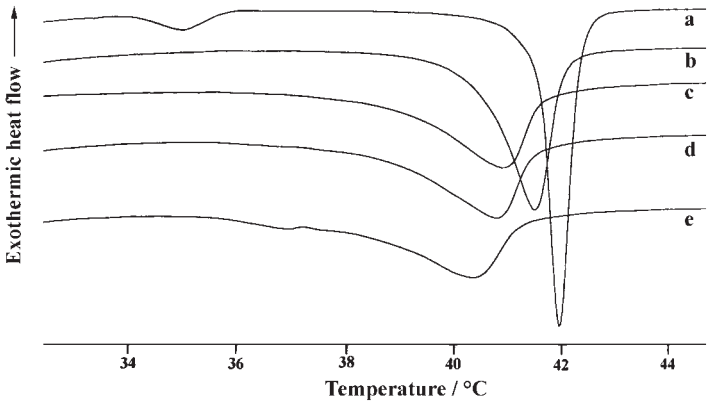

Fig. 4 DSC heating curves of hydrated multilamellar vesicles of DPPC/DOP mixtures with different DOP contents (a – 0%; b – 1.0%; c – 3.0%; d – 5.0% and e – 15.0%)

For DOP ≥10% m/m, the shape of the TG is more complex, with the appearance of a weak, poorly defined maximum at about 37°C, which is more evident in the cooling plots than in the heating plot in this case, too.

A decrease in ΔH occurs on increase of the amount of added DOP. The observed decrease is less evident in this case than for the DPPC–DEHP system, and it is near the limits of experimental error. Indeed, a decrease of about 10% in the ΔH value when the DOP concentrations range from 0 to 7.5% m/m was evaluated. In this case too, a further decrease in the overall ΔH can be observed on further addition of DOP, but the complex structure of the plot, resulting from the overlapping of more than one maximum, made correct evaluation difficult, as in the DPPC–DEHP system. The trends in both $\Delta T_{1/2}$ and A_s are similar to those observed in the presence of DEHP (Table 2).

Dibutylphthalate (DBP) and diethylphthalate (DEP)

Figures 5 and 6 present some DSC plots for the DPPC–DBP and DPPC–DEP systems, respectively. The two systems behave similarly, but differ in some respects from the behaviour observed previously for the longer side-chain esters. Indeed, when the presence of the phthalate ester is greater than 5% m/m, the main transition is no longer detectable, and other well-defined peaks are present at lower temperature.

In the presence of small amounts of phthalate (up to 4% m/m), a roughly linear decrease in T_m with a simultaneous broadening of the peak is observed. The decrease occurs to some extent for both esters, as can be deduced from Fig. 2 and Table 1. In this concentration range, both $\Delta T_{1/2}$ and A_s tend to increase.

When the phthalate esters are present in greater amounts, only the lower-temperature transition peak is still present, whose maximum temperature and half-width $\Delta T_{1/2}$ depend only slightly on the ester concentration. In the DPPC–DEP system, this peak has a doublet structure, with maximum temperatures at 34.6 and 35.0°C (Fig. 6).

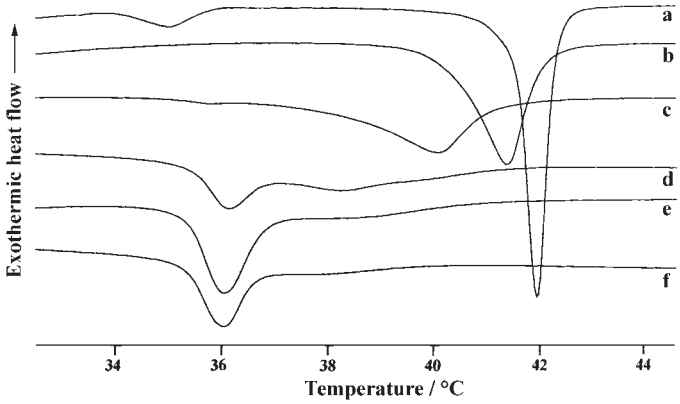

Fig. 5 DSC heating curves of hydrated multilamellar vesicles of DPPC/DBP mixtures with different DBP contents (a – 0%; b – 1.0%; c – 3.0%; d – 5.0%; e – 7.5% and f – 10.0%)

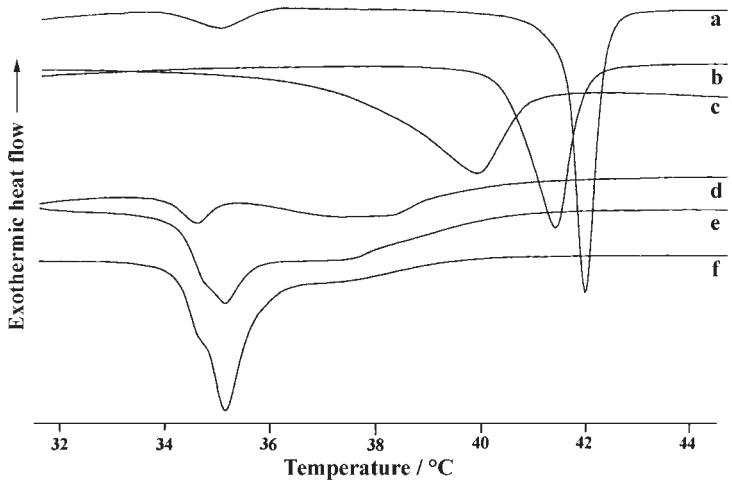

Fig. 6 DSC heating curves of hydrated multilamellar vesicles of DPPC/DEP mixtures with different DEP contents (a – 0%; b – 1.0%; c – 3.0%; d – 5.0% e – 7.5% and f – 10.0%)

At a very high concentration (40% m/m), the 35.0°C endothermic transition is the only one detectable.

The ΔH in the concentration range 0–4% m/m seem to suggest that no changes or only very small decreases have taken place. Indeed, the observed decrease of less than 6–7% in both systems falls within the limits of experimental errors and thus no definite conclusion can be drawn. The ΔH values in the higher-concentration systems cannot be related to those in the lower-concentration systems because of the presence of a complex peak structure. Cooling measurements confirm the results of the heating measurements.

Discussion

In the presence of very small amounts of phthalate esters, the most evident effect in all cases is the decrease in T_m, together with a significant broadening of the main transition. The maximal excess heat capacity, related to the height of the peak, is strongly reduced, but the enthalpy changes associated with the main transition are reduced only a little or not at all.

The interactions between lipid bilayers and many neutral and charged molecules have been considered in recent years. For small neutral molecules, such as short-chain alcohols, the systems are reasonably explained by a model of solution where the solute is found partly in the liquid-crystalline phase and partly in the gel phase in lipid regions [22].

In accordance with this model, a linear decrease in the freezing temperature was observed with an increase in the molar fraction of the solute. This decrease did not depend on the nature of the solute. Longer-chain alcohols, however, do not follow this model, and increases in T_m have also been observed [22].

The decrease in T_m can be explained from a molecular point of view by considering that all these molecules increase the free volume, i.e. the average volume accessible to a single lipid molecule [16]. Theoretical models of the gel to liquid-crystalline phase transition predict a decrease in T_m upon the introduction of the free volume into the bilayer [23–25]. The decrease in T_m results from the tendency of the bilayer to maximize its entropy by filling the void volume by *trans–gauche* isomerization, and this effect increases as the void volume increases. The smaller decrease in temperature observed after the incorporation of long-chain alcohols in the DPPC bilayers is explained by hypothesizing that the free volume effects, which one would expect to decrease the gel to liquid-crystalline transition temperature, are offset by the increase in the van der Waals interactions between the acylic lipid chains [16].

The differences observed in the systems considered are in agreement with the previously described theory. Indeed, the greatest T_m decrease was observed in the presence of the short side-chain esters. In contrast, the *n*-octyl ester, which can better fill the space by inserting itself between the acylic chains in the DPPC vesicles, exhibits the lowest T_m decrease. The effect of the long, but branched side-chains of DEHP is intermediate: the observed ΔT_m is greater than that for DOP, but lower than those for the DEP and DBP systems. In the DEHP and DOP systems, the decrease in T_m stops as the content of the ester reaches 3–4% m/m, and the main transition is still detectable in the presence of a large amount of phthalate. This is probably due to the development of relatively strong van der Waals interactions between the acyl lipid chains and the long side-chains in the esters.

For all the systems considered, the pretransition disappears in the presence of only 0.5% m/m of the phthalate ester, thereby confirming the great sensitivity of the pretransition to the presence of other substances. This effect has already been observed in different systems [16, 17, 21] and could be due to a reduction in the structural differences between rippled and lamellar gel phases, leading to a reduced enthalpy and entropy of the pretransition [16]. The pretransition involves a two-dimensional reorganization of the

lipid bilayer structure and is generally ascribed to an interplay between the packing constraints of the bulky phosphatidylcholine headgroups and the long hydrophobic acyl chains, which leads to a transition with a changed orientation of the acyl chains with respect to the bilayer normal [20]. The insertion of even a small amount of foreign substances between the acyl chains could force the bilayer to the rippled gel phase, with disappearance of the transition.

The $\Delta T_{1/2}$ increase is a general aspect for the main transition peak when phthalates are present and it is monotonous in respect of the amount of the ester. The trend is not linear, the effect being more evident for the lower concentrations than for the higher concentrations. The broadening indicates that the apparent cooperativity of the transition is reduced, as is the average number of molecules per cooperative unit, as deduced according to Mabrey and Sturtevant [26].

It should be stressed, however, that the reality is probably better described by a modification of a cluster size distribution in the presence of the phthalate esters. In multilamellar vesicles of pure DPPC, it has been shown [17], by application of the Kolmogorov-Abrami theory to the transition kinetics, that the domains of the minor phase during the fusion process are highly compact and the optimal mode to minimize the Gibbs free energy is the formation of large, nearly circular domains, in which all molecules melt cooperatively. In the presence of phthalate esters, similarly to what was observed in DPPC multilamellar vesicles containing dibucaine, whose DSC curves are very similar to those observed in our systems, the foreign substance could stabilize the gel to liquid-crystal phase domain boundaries that become more ramified and smaller, thereby explaining the broadening of the equilibrium excess heat capacity curve [17]. This interpretation is consistent with the results of Monte Carlo simulation [27] used to study the effect of an anaesthetic on the lipid phase transition. This model leads to the conclusion that foreign molecules preferentially localize in the boundary region between lipid clusters of different phases, resulting in the formation of more clusters of smaller size [28]. Moreover, the size reduction could also be due to the kinetic heterogeneity induced by the presence of the foreign molecules.

In all cases, however, the broadening of the heat capacity curve indicates that local fluctuations in the composition have taken place and these could have a great modifying effect on the biological properties of the membrane. Indeed, in the previously cited Monte Carlo simulation study [27], it was proven that they could have a dramatic effect on the bilayer fluctuations in the phase transition region, inducing localized zones of very high concentrations, even if the overall concentration of foreign molecules is low. Since the biological properties of membrane-related proteins are very sensitive to their microenvironment, significant changes could take place.

The $\Delta T_{1/2}$ of the lower-temperature transition present in the systems in which great amounts of DEP and DBP are present (Figs 5 and 6), and also the peak temperature T_m, are influenced only a little or not at all by the composition, suggesting that the phase transition takes place in a well-defined and constant-composition phase. It can probably be depicted as a mixed phase constituted by an ordered disposition of phthalate and lipid molecules, as was observed in mixed liposomes of surfactants and phospholipids [29].

The simultaneous presence of many calorimetric peaks, some with complex structures, in the systems with DEP and DBP contents ranging from 5 to 7.5% m/m could also be due to the coexistence of various aggregates of different sizes, such as bilayers, vesicles and cylindrical and globular structures. The progressive dissociation of these aggregates with the increase of temperature leads to complex, low-cooperativity phase transition processes, similar to those recently observed in Laudan-containing lipid vesicles [30].

The asymmetry of the main transition peak, skewed toward low temperatures, suggests that the phthalate esters are preferentially soluble in the liquid-crystal phase and distribute preferentially on the boundaries of the fused regions.

The asymmetry index, as previously defined, increases rapidly as phthalate esters are added and it tends to remain roughly constant, within the experimental errors, when the amounts added are greater than 1–2% m/m. This seems to indicate that a partition equilibrium is reached between the boundaries of the gel and liquid-crystal domains.

In conclusion, our data suggest the occurrence of interactions between phthalate esters and biomembranes. In the model systems considered, the effects are noticeable even in the presence of small amounts of esters; in the presence of larger amounts, the overall structure of the bilayer is strongly modified in some cases, with the appearance of new phases.

As the function of a membrane-associated enzyme is coupled with lipid structural fluctuations, it is possible that phthalates modify the enzyme function by modifying the fluctuations.

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