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Organotin compounds alter the physical organization of phosphatidylcholine membranes

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Abstract

Organotin compounds have a broad range of biological activities and are ubiquitous contaminants in the environment. Their toxicity mainly lies in their action on the membrane. In this contribution we study the interaction of tributyltin and triphenyltin with model membranes composed of phosphatidylcholines of different acyl chain lengths using differential scanning calorimetry, ^{31}P-nuclear magnetic resonance, X-ray diffraction and infrared spectroscopy. Organotin compounds broaden the main gel to liquid-crystalline phase transition, shift the transition temperature to lower values and induce the appearance of a new peak below the main transition peak. These effects are more pronounced in the case of tributyltin and are quantitatively larger as the phosphatidylcholine acyl chain length decreases. Both tributyltin and triphenyltin increase the enthalpy change of the transition in all the phosphatidylcholine systems studied except in dilauroylphosphatidylcholine. Organotin compounds do not affect the macroscopic bilayer organization of the phospholipid but do affect the degree of hydration of its carbonyl moiety. The above evidence supports the idea that organotin compounds are located in the upper part of the phospholipid palisade near the lipid/water interface.

Keywords: Model membrane; Differential scanning calorimetry; X-Ray diffraction; Infrared spectroscopy

1. Introduction

Organotin compounds are organometallic compounds which are ubiquitous contaminants in the environment. They are used extensively as stabilizers in the production of plastics, agricultural pesticides, preservatives of paper and textiles and antifoulant paints. The high biological activity of these compounds towards aquatic organisms has a deleterious impact on aquatic ecosystems [1]. Humans are exposed to organotin compounds by inhalation, absorption and the consumption of contaminated food and water.

The extensively used organotin compounds, tributyltin (TBT) and triphenyltin (TPT) (Fig. 1), have broad biological activities. Although their mechanism of toxicity is not well understood, it appears to be strongly dependent on organotin lipophilicity [2,3]. TBT and TPT are membrane-active molecules and their toxicity mainly involves the membrane as

Abbreviations: DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimiristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; ^{31}P-NMR, ^{31}P-nuclear magnetic resonance; SAXD, small angle X-ray diffraction; T_m, temperature at the midpoint of the transition

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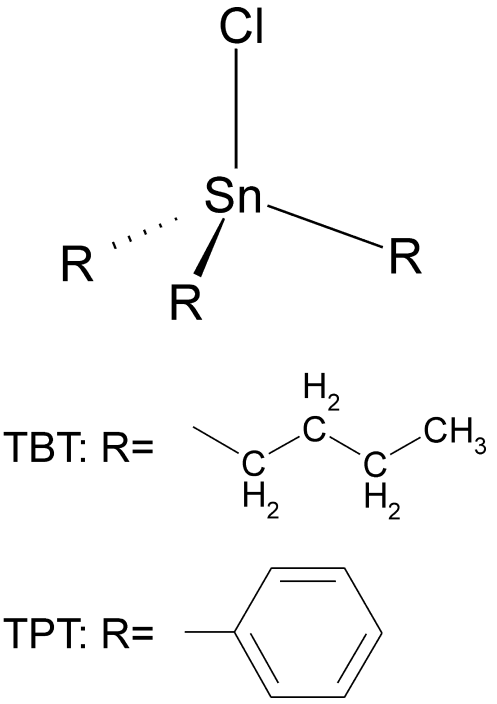

Fig. 1. Chemical structures of tri-*n*-butyltin chloride (TBT) and tri-*n*-phenyltin chloride (TPT).

their site of action. Their mode of action includes haemolysis [3], the release of Ca^{2+} from sarcoplasmic reticulum [4], the inhibition of phosphatidylserine-induced histamine release [5], the alteration of mitochondrial membrane permeability [6], perturbation of the membrane sector of mitochondrial ATP synthase [7], inhibition of membrane-bound enzymes [8] and the induction of apoptosis in lymphocytes [9]. Although the interaction between organotin compounds and membranes may play an important role in their toxic mechanism, very little is known for certain, especially as regards TBT- and TPT-phospholipid molecular interactions. Fluorescence polarization [10] has shown that tributyltin chloride interacts more effectively with model membranes than tributyltin acetate, suggesting that the effect of tributyltin on liposomal membranes is dependent on the anion moiety and the phospholipid characteristics. Recent studies on the release of liposome-bound praseodymium and the change in stability of planar membranes [11] have suggested that the lipophilicity and polarity of organotin compounds and the surface potential and environment of the lipid molecules are important factors in the interaction between these compounds and model membranes.

In an attempt to further understand the influence of organotin compounds on the lipid component of membranes we made a detailed study of the effect of TBT and TPT on the thermotropic and structural properties of phosphatidylcholine, the most important phospholipid in eucaryotic membranes, using differential scanning calorimetry (DSC), ^{31}P-nuclear magnetic resonance (^{31}P-NMR), small angle X-ray diffraction (SAXD) and infrared spectroscopy.

2. Materials and methods

2.1. Materials

1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimiristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) were obtained from Avanti Polar Lipids (Birmingham, AL). Tri-*n*-butyltin chloride and tri-*n*-phenyltin chloride were obtained from Sigma-Aldrich (Spain). All other reagents were of the highest purity available.

2.2. Differential scanning calorimetry

The lipid mixtures for differential scanning calorimetry (DSC) measurements were prepared by combining chloroform/methanol (1:1) solutions containing 4 μmol phospholipid and the appropriate amount of organotin compounds as indicated. The organic solvents were evaporated under a stream of dry N_2, free of O_2, and the last traces of solvents were removed by a further 3 h of evaporation under high vacuum. Multilamellar liposomes were prepared in 0.1 mM EDTA, 100 mM NaCl, 10 mM HEPES (pH 7.4) buffer by mixing with a bench mixer, always keeping the samples at a temperature above the gel to liquid-crystalline phase transition temperature of the lipid. The suspensions were centrifuged at 13 000 rpm in a bench microfuge and the pellets were collected into small aluminium pans. The pans were sealed and scanned in a Perkin-Elmer DSC-4 or DSC-7 calorimeter, using a reference pan containing buffer. The instrument was calibrated using indium as standard. The heating rate was 4°C/min in all the experiments. To determine the total amount of

phospholipid contained in a pan, the pan was carefully opened and the lipid was dissolved with chloroform/methanol (1:1). The phosphorous content was determined using Böttcher's method [12]. Decomposition of the multicomponent melting curves was carried out using MicroCal's Origin software. Briefly, this computer program regards the multicomponent melting thermograms as a linear combination of multiple, independent, two-state transitions. The construction of partial phase diagrams was based on the heating thermograms for a given mixture of phospholipid and organotin compounds at various organotin compound concentrations. The onset and completion temperatures for each transition peak were plotted as a function of the molar fraction of organotin compounds. These onset and completion temperature points formed the basis for defining the boundary lines of the partial temperature-composition phase diagram.

2.3. ^{31}P-Nuclear magnetic resonance

The samples for ^{31}P-NMR were prepared by combining of chloroform/methanol (1:1) solutions containing 40 mg of phospholipid and the appropriate amount of organotin compounds. Multilamellar vesicles were formed as described above. The suspensions were centrifuged at 13 000 rpm in a bench microfuge and the pellets were placed in conventional 5 mm NMR tubes. The ^{31}P-NMR spectra were obtained in the Fourier Transform mode in a Varian Unity 300 spectrometer. All chemical shift values are quoted in parts per million (ppm) with reference to pure lysophosphatidylcholine micelles (0 ppm), positive values referring to low-field shifts. All spectra were obtained in the presence of a gated broad band decoupling (10 W input power during acquisition time) and accumulated free induction decays were obtained from up to 2500 scans. A spectral width of 25 000 Hz, a memory of 32K data points, a 2 s interpulse time and a 90° radio frequency pulse were used. Prior to Fourier transformation, an exponential multiplication was applied, resulting in a 100 Hz line broadening.

2.4. Small angle X-ray diffraction

Samples for SAXD were prepared by mixing 15 mg of phospholipids and the appropriate amount of organotin compounds in chloroform/methanol (1:1). Multilamellar vesicles were formed as described above. After centrifugation at 13 000 rpm, the pellets were mounted in aluminum holders with cellophane windows. Nickel-filtered Cu K_α ($\lambda = 1.54$ Å) X-rays were generated by a Philips PW1830 X-ray Generator. X-Rays were focussed using a flat gold-plated mirror and recorded using a Bio-Logic model 210 linear position sensitive detector (Echirolles, France). The sample temperature was controlled to ± 0.5°C using a circulating water bath. X-Ray diffraction profiles were obtained for 10 min exposure times after 5 min of temperature equilibration. The detector was calibrated using crystalline cholesterol ($d = 33.6$ Å).

2.5. Infrared spectroscopy

For the infrared measurements, multilamellar vesicles were prepared in 40 μl of ^2H_2O as described above. Samples were placed between two CaF_2 windows (25×2 mm) separated by 50 μm Teflon spacers and transferred to a Symta cell mount. Infrared spectra were obtained in a Nicolet MX-1 FT-IR spectrometer. Each spectrum was obtained by collecting 27 interferograms. The ^2H_2O spectra taken at the same temperature were subtracted interactively using either GRAMS/32 or Spectra-Calc (Galactic Industries, Salem, MA), as described previously [13].

3. Results

The influence of TBT and TPT on the thermotropic phase transition of DPPC is depicted in Fig. 2. In the absence of organotin compounds, DPPC exhibited two endotherms upon heating, a pretransition at 35°C and the main gel to liquid-crystalline phase transition at 41°C. The presence of low concentrations of organotin compounds, such as the 0.02 molar fraction, abolished the thermotropic pretransition of DPPC. Increasing concentrations of TBT (Fig. 2A) progressively broadened the main transition and caused a shift to lower temperatures. In the samples containing a high proportion of TBT, the presence of two endotherms was apparent. The incorporation of TPT (Fig. 2B) produced a less

marked broadening of the transition than in the case of TBT. The effect of TPT on the T_m of the transition also differed from that of TBT. The presence of low concentrations of TPT, such as 0.02 and 0.05 molar fraction, decreased T_m, but when the concentration increased to 0.1, 0.2 and 0.3 molar fraction the T_m was increased. When the concentration of TPT reached 0.4 and 0.5 molar fraction, T_m fell again. At high concentrations of TPT two endotherms were apparent, as in the case TBT, although at the highest concentration studied (0.5 molar fraction) only one endotherm was recorder with TPT.

Fig. 3 shows the enthalpy change for the gel to liquid-crystalline phase transition of mixtures of DPPC and organotin compounds at different molar fractions. The presence of increasing concentrations of TBT produced a progressive and significant increase in the enthalpy change of the transition. The presence of TPT produced a similar increase, but it seemed to level off at concentrations above 0.2 molar fraction.

The DSC endotherms shown in Fig. 2 for the main

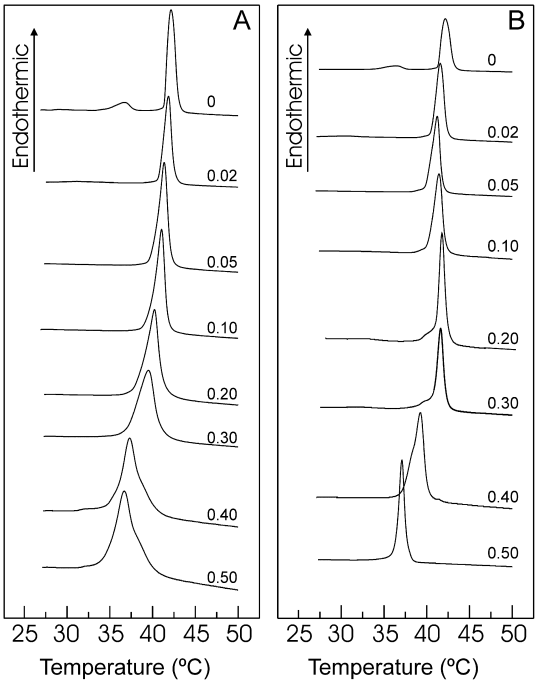

Fig. 2. DSC thermograms for DPPC and mixtures of DPPC/TBT (A) and DPPC/TPT (B). The concentration of TBT and TPT in the membrane (molar fraction) is expressed on the curves.

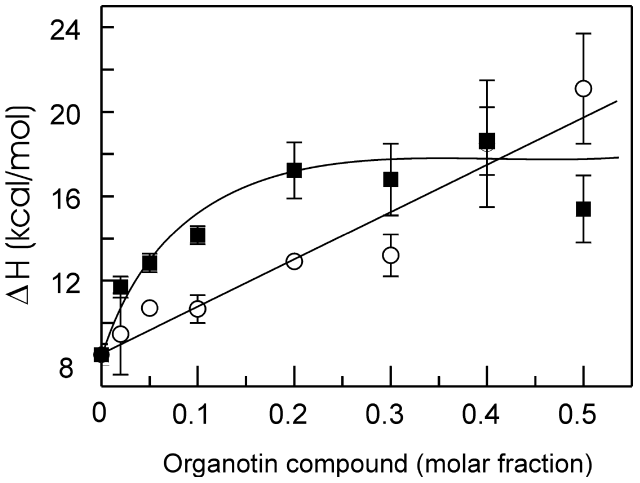

Fig. 3. The enthalpy change for the gel to liquid-crystalline phase transition of mixtures of DPPC/TBT (○) and DPPC/TPT (■) at different organotin compound concentrations. Data represent the average of three different experiments (standard error bars are shown when larger than the symbols).

phase transition of samples containing organotin compound concentrations in excess of 0.2 molar fraction could be fitted to two components, which were resolved by the fitting procedure described above. As an example, the scans of systems containing 0.3 molar fraction of TBT and 0.4 molar fraction of TPT, along with the theoretical curves for the decomposition that best fits the experimental values, are shown in Fig. 4B and D, respectively. The effect of increasing concentrations of organotin compounds on the transition enthalpy change of the two components resolved by the curve fitting procedure is shown in Fig. 4A,C. In both cases, the amplitude of the lower melting component increased at the expense of the higher melting component when the concentration of organotin compounds was increased. In the case of TBT (Fig. 4A) the higher melting component was still present at a 0.5 molar fraction of the organotin compound, while in the case of TPT at 0.5 molar fraction the higher melting component has completely disappeared and the amplitude of the lower melting component includes the whole main transition.

^{31}P-NMR spectroscopy was used to check whether organotin compounds affect the phase behaviour of DPPC. Samples containing TBT and TPT always gave rise to an asymmetrical ^{31}P-NMR line shape, with the high-field peak and low-field shoulder char-

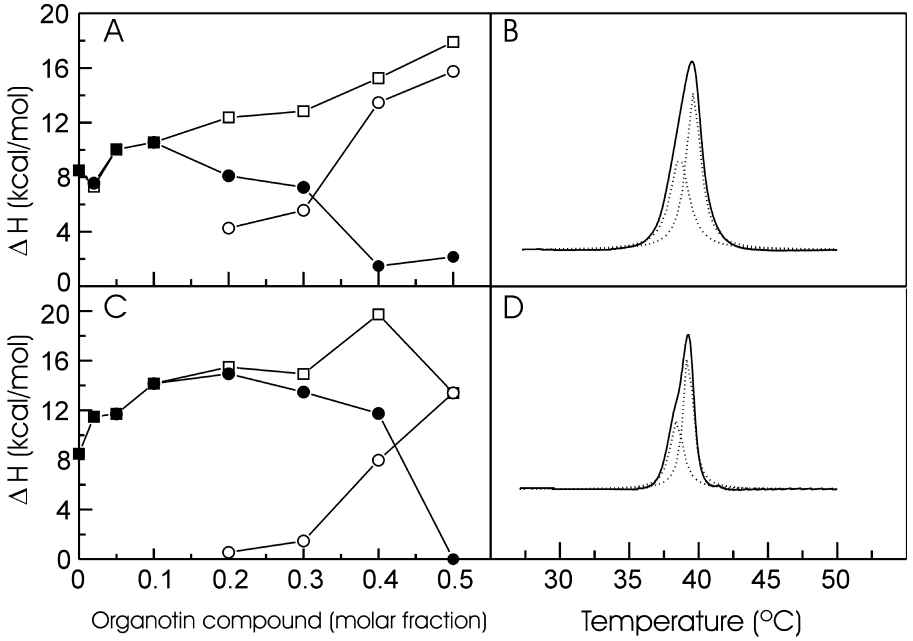

Fig. 4. Effect of increasing concentrations of organotin compounds on the transition enthalpy change of the two components of the different DPPC/TBT (A) and DPPC/TPT (C) mixtures resolved by the curve fitting procedure; enthalpy change of the overall transition (□), upper melting component (●) and lower melting component (○). Illustration of the curve fitting procedure used to resolve the components of the DSC thermograms exhibited by DPPC membranes containing different organotin compounds: 0.3 molar fraction TBT (B) and 0.4 molar fraction TPT (D). Solid line, experimental curves; dotted lines, theoretical curves.

acteristic of bilayer structures, while the line shape in the gel state was broader than in the liquid-crystalline state [14] (data not shown). This indicates that the presence of organotin compounds does not change the phospholipid phase organization, which remains in the lamellar phase over the whole range of temperatures studied. It also shows that the different endotherms present in the various thermograms correspond solely to lamellar gel to lamellar liquid-crystalline phase transitions.

Further information on the structural characteristics of DPPC/organotin compound systems was obtained by SAXD. Phospholipids, when organized into multilamellar structures, should give rise to reflections with relative distances of 1:1/2:1/3... [15]. Fig. 5 shows the diffraction pattern profiles corresponding to pure DPPC and DPPC containing organotin compounds at different temperatures. Pure DPPC produced two reflections with relative distances of 1:1/2, which is consistent with its expected multilamellar organization. This technique not only defines the macroscopic structure itself, but also provides the interlamellar repeat distance in the lamellar phase. The largest first-order reflection component corresponds to the interlamellar repeat distance, which is comprised of the bilayer thickness and the thickness of the water layer between bilayers. DPPC in the gel state (25°C, Fig. 5A) gave rise to a first-order reflection with a *d*-value of approx. 65 Å. This value increased to reach a maximum at approx. 70 Å after the pretransition and near the main transition (38°C, Fig. 5B) and then decreased above the chain melting temperature to approx. 61 Å (50°C, Fig. 5C), in agreement with previous reports [16,17]. Samples containing organotin compounds gave rise to two reflections which relate as 1:1/2 in the whole range of temperatures under study, confirming that the presence of these toxicants does not alter the lamellar structural organization of DPPC. No significant change in the interlamellar repeat distance was noted. However, it can be seen from Fig. 5B that at 38°C the system containing 0.4 molar fraction of TBT presented a distance of approx. 63 Å, which was about 7 Å smaller than the corresponding distance of the pure lipid, probably reflecting the observed decrease in the transition temperature. It is interesting to note that the presence of organotin compounds broadened the reflections and lowered

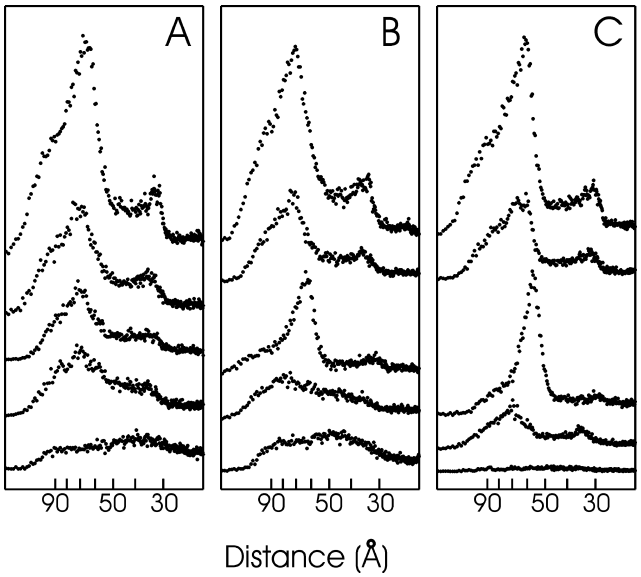

Fig. 5. X-Ray diffraction profiles at 25°C (A), 38°C (B) and 50°C (C) obtained from (top to bottom) pure DPPC, DPPC containing 0.1 molar fraction of TBT, DPPC containing 0.4 molar fraction of TBT, DPPC containing 0.1 molar fraction of TPT and DPPC containing 0.4 molar fraction of TPT.

their intensity. This was most apparent in the case of the system containing 0.4 molar fraction of TPT, where no sharp reflections but only broad scattered bands were observed.

Using the DSC data and information of the phospholipid structural organization obtained from ^{31}P-NMR and SAXD, partial phase diagrams were constructed for the DPPC component in mixtures of the phospholipid and organotin compounds. The onset and completion temperatures of the heating thermograms shown in Fig. 2 provided the points necessary for obtaining the solid and fluid lines of the phase diagrams, respectively. In the case of the DPPC/TBT system (Fig. 6A) both the solid and the fluid lines displayed near ideal behaviour, the temperature decreasing as the TBT concentration increased. The system evolved from a lamellar gel phase (G phase) to a lamellar liquid-crystalline phase (F phase) through a coexistence region (G+F) which became wider as more TBT was added to the system. In the presence of TPT (Fig. 6B), the temperature of the solid line decreased with increasing concentrations of TPT in the membrane. However, the fluid line behaved differently, with the completion temperatures first decreasing from 0 to 0.05 TPT molar fraction. When the concentration of TPT was raised from 0.1 to 0.3 molar fraction, the completion temperature did not decrease further, but instead, slightly increased. Finally, at TPT concentrations higher than 0.4 molar fraction the completion temperature decreased again. This indicates that at very low and at very high concentrations of TPT, when the completion temperature decreases, TPT is miscible with DPPC. However, at intermediate TPT concentrations (ranging from 0.1 to 0.3 molar fraction) the fluid line is bell shaped, indicating that in this range of TPT concentrations fluid immiscibility oc-

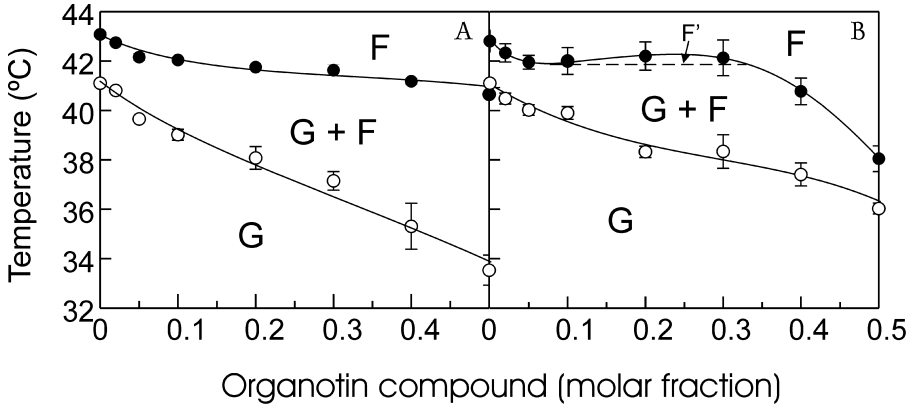

Fig. 6. Partial phase diagrams for DPPC in DPPC/TBT mixtures (A) and DPPC/TPT mixtures (B). Open (○) and closed (●) circles were obtained from the onset and completion temperatures of the main gel to liquid-crystalline phase transition. The phase designations are as follows: G, gel phase; F, liquid-crystalline phase; F′, immiscible DPPC/TPT domains in the liquid-crystalline phase. Data represent the average of three experiments (standard error bars are shown when larger than the symbols).

curs. The DPPC/TPT system evolved from a lamellar gel phase (G phase) to a lamellar liquid-crystalline phase (F phase) through a coexistence region (G+F) which is narrower than that observed for the DPPC/TBT system, while in a 0.1–0.3 molar fraction concentration range of TPT the system showed fluid phase immiscibility, in which different lamellar liquid-crystalline phases coexist (F′).

To investigate the effect of organotin compounds on different parts of the DPPC molecule, infrared spectroscopy was used. Fig. 7 shows the C=O stretching band for DPPC and organotin compounds. Pure DPPC showed absorption maxima at 1733 cm^{-1} and 1730 cm^{-1} in the gel and liquid-crystalline state, respectively. The presence of organotin compounds increased the wave number of the absorption maxima to 1737 cm^{-1} and 1734 cm^{-1} in the case of TBT and to 1742 cm^{-1} and 1740 cm^{-1} in the case of TPT at temperatures below and above the gel to liquid-crystalline phase transition, respectively.

It is known that the carbonyl groups of diacylphospholipids may be found in lipid vesicles in hydrated and dehydrated states, their proportion depending on the physical state of the phospholipid bilayer [18]. Pure DPPC spectra represent a summation of the component bands centred at 1742 and 1727 cm^{-1} (attributed to dehydrated and hydrated C=O groups, respectively) [19]. The spectra from Fig. 7 were subjected to curve fitting to two bands

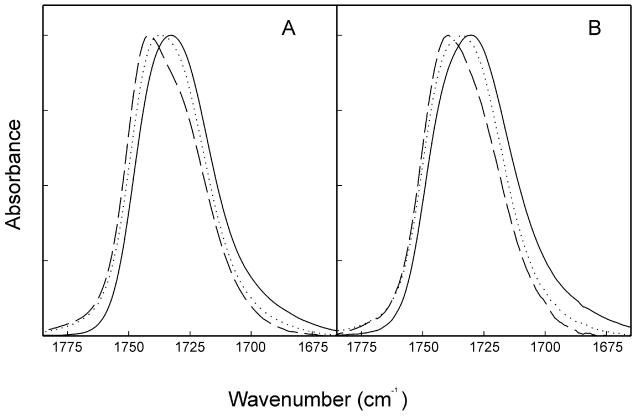

Fig. 7. Infrared spectra of the C=O stretching band of DPPC (solid line) and DPPC containing 0.3 molar fraction of TBT (dotted line) or 0.3 molar fraction of TPT (dashed line) at 25°C (A) and 50°C (B).

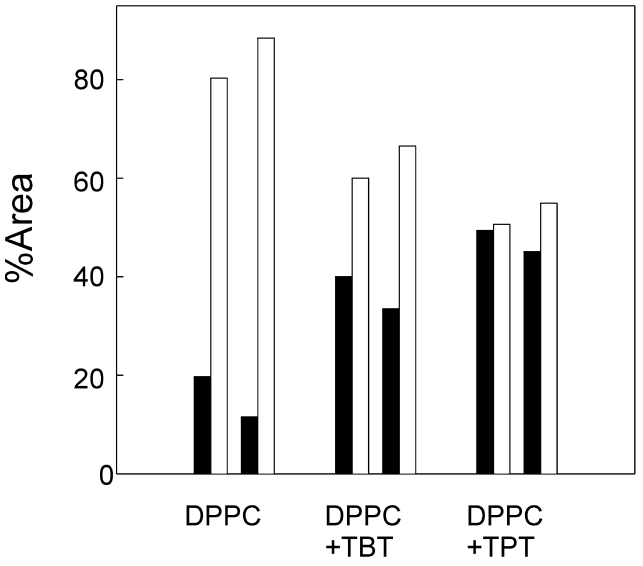

Fig. 8. Relative area of the dehydrated (black) and hydrated (white) components of the C=O stretching band for DPPC and DPPC containing 0.3 molar fraction of TBT and TPT, at 25°C (bars at the left) and 50°C (bars at the right).

centred at 1742 and 1727 cm^{-1}. These bands were simulated by a Gaussian-Lorentzian function, for which best fit estimates of band shape were obtained with an approx. 70% Gaussian contribution. The relative areas of these simulated bands were calculated for DPPC and mixtures with organotin compounds (Fig. 8). It can be seen that the presence of TBT and TPT substantially increased the contribution of the dehydrated component compared with the pure phospholipid, both below and above the phase transition.

When the CH_2 stretching bands of DPPC were studied in the presence of organotin compounds, no change in the frequency of these absorption bands were found below the phase transition temperature. However, an increase of around 1–2 cm^{-1} was observed when the phospholipid was in the liquid-crystalline state (data not shown).

The effect of organotin compounds on the thermotropic transitions of saturated phosphatidylcholines bearing acyl chains with 12 (DLPC), 14 (DMPC), 16 (DPPC) and 18 (DSPC) carbon atoms is depicted in Figs. 9 and 10. In the absence of organotin compounds, DMPC, DPPC and DSPC exhibited two endotherms upon heating: a lower temperature-lower enthalpy pretransition and a higher temperature-higher enthalpy main transition. Since the pretransi-

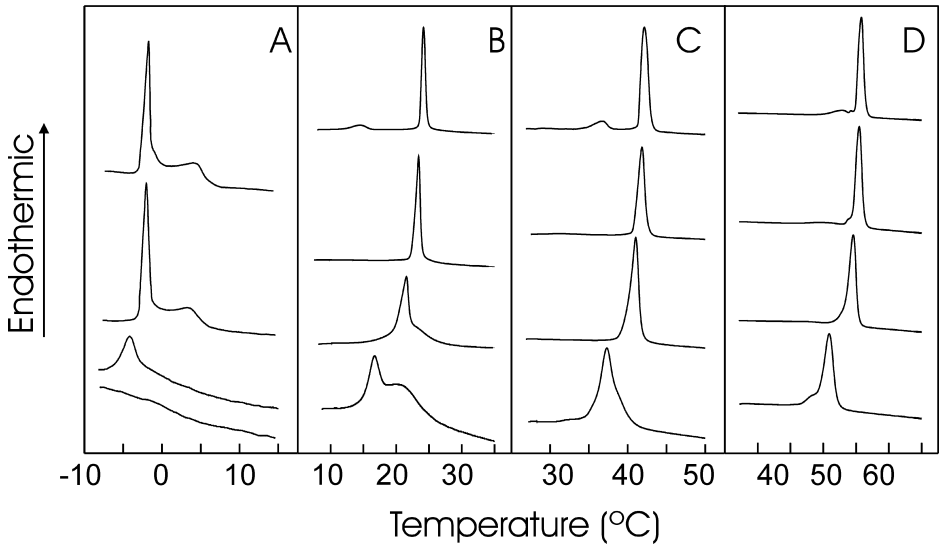

Fig. 9. DSC thermograms for DLPC (A), DMPC (B), DPPC (C) and DSPC (D) containing different concentrations of TBT. From top to bottom: pure phospholipid, 0.02 molar fraction TBT, 0.1 molar fraction TBT and 0.4 molar fraction TBT.

tion temperature increased more steeply with increasing hydrocarbon chain length than did the main transition temperature, in agreement with previous reports [20], the temperature interval between this two events decreased as the length of the phosphatidylcholine chains increased. Pure DSPC (Fig. 9D and 10D), as recently reported [21], displayed an additional extremely low enthalpy transition within one degree of the main phase transition, which is termed submain transition. DLPC (Fig. 9A and 10A) did not show pretransition but exhibited a sharp peak at −2.5°C and an additional broad transition at around 5°C in agreement with previous reports [22,23]. The influence of TBT on the different phosphatidylcholines is shown in Fig. 9. The presence of 0.02 molar fraction of TBT abolished the pretransition in DMPC (Fig. 9B) and DPPC (Fig. 9C) systems. However, at the same concentration of TBT, the pretransition and the submain transition were still clearly seen in the thermogram corresponding

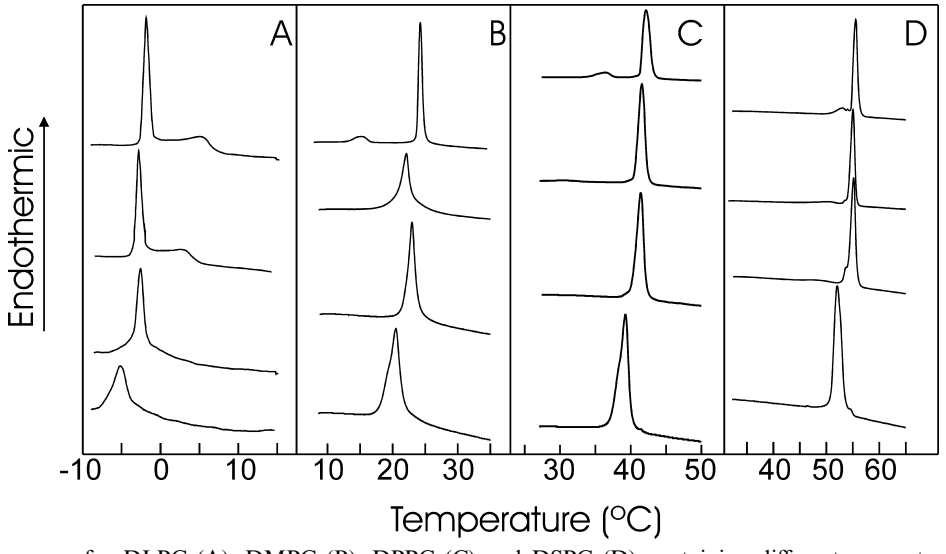

Fig. 10. DSC thermograms for DLPC (A), DMPC (B), DPPC (C) and DSPC (D) containing different concentrations of TPT. From top to bottom: pure phospholipid, 0.02 molar fraction TPT, 0.1 molar fraction TPT and 0.4 molar fraction TPT.

to DSPC (Fig. 9D), both transitions disappearing when the concentration of TBT was increased. TBT affected the DMPC, DPPC and DSPC main transition in a qualitatively similar way, broadening and shifting the transition to lower temperatures with the appearance of a second component in the thermogram. However, the effect was quantitatively larger as the acyl chain was shortened, and was therefore more prominent in the case of DMPC (Fig. 9B). Low concentrations of TBT brought the two transitions of DLPC closer (Fig. 9A), while high concentrations induced the broadening and shifting of the transition to lower temperatures until the transition was so broad that it was difficult to distinguish from the thermogram. The influence of TPT on the phase transition of phosphatidylcholines (Fig. 10) was qualitatively similar to that described above for TBT although less pronounced, so that the main transition was not so broad and the presence of the second endotherm was less apparent. The pretransition and the submain transition of DSPC (Fig. 10D) could still be observed at 0.1 molar fraction of TPT. Finally even at 0.4 molar fraction of TPT a broad transition was clearly present in the case of DLPC (Fig. 10A). The effect of organotin compounds on the enthalpy change and the net shift in T_m of the thermotropic transition for the different phosphatidylcholines are presented in Fig. 11. Organotin compounds heightened the enthalpy change of the transition in all the phosphatidylcholine systems studied except in the DLPC system, where it decreased with TBT (Fig. 11A) or did not change with TPT (Fig. 11B). Both compounds shifted T_m to lower temperatures although the net shift was larger in the case of TBT (Fig. 11C) than in that of TPT (Fig. 11D). In the latter case, increasing concentrations of TPT first produced a decrease of T_m, followed by an increase and finally a pronounced decrease of the temperature of the transition (Fig. 11D).

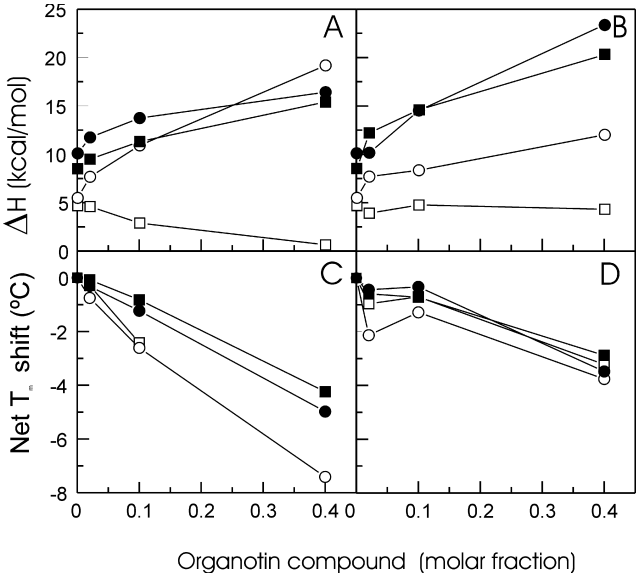

Fig. 11. Overall main phase transition enthalpy change for phosphatidylcholine/TBT (A) and phosphatidylcholine/TPT (B) systems, and net transition temperature shift for phosphatidylcholine/TBT (C) and phosphatidylcholine/TPT (D) systems. □, DLPC; ○, DMPC; ■, DPPC; ●, DSPC.

4. Discussion

In this study, the nature of the interaction between organotin compounds and membranes has been investigated using lipid vesicles formed by phosphatidylcholines, an important class of phospholipid. DSC was used to characterize the influence of TBT and TPT on the thermotropic properties of phosphatidylcholines of different acyl chain lengths, and the effect of TBT and TPT on the macroscopic organization and structural properties of DPPC was further studied by means of ^{31}P-NMR, SAXD and infrared spectroscopy.

The profile of a DSC thermogram representing the phospholipid phase transition is determined by the transition temperature and the enthalpy change. The overall effects of the presence of increasing amounts of TBT or TPT on the thermotropic phase transitions of DMPC, DPPC and DSPC can be resumed as follows. First, they make the pretransition disappear at very low organotin compound concentration, second, they produce the broadening of the main transition peak and a shifting of the transition temperature to lower values, and third, they induce the appearance of a new peak below the main transition peak. These effects are more pronounced in the case of TBT than in the case of TPT. These observations are compatible with the hydrophobic butyl and phenyl moieties aligning themselves with the prevailing directions of the phospholipid acyl chain, where they can disrupt its packing, reduce the cooperativity of the transition and shift the phase transition temperature to lower values. The interaction of

the phospholipid acyl chains with the hydrophobic moieties of TBT and TPT would enhance the hydrophobic interactions in the chains. As a result of this, the enthalpy change of the transition from the gel to the liquid-crystalline phase is drastically increased in the presence of organotin compounds. A more detailed study of the effect of organotin compounds on the enthalpy change of DPPC phase transition shows that while the presence of TBT produces a proportional increase in the enthalpy change, the presence of TPT above 0.2 molar fraction does not produce any further increase. The appearance of a second, lower melting component in the thermograms when the concentration of organotin compounds is increased can be explained by the formation of TBT- or TPT-enriched domains similar to those described for other toxicants like abietic acid [24]. These effects are more pronounced for shorter phospholipid acyl chains, probably because the relative fraction of the length of the acyl chain that interacts with organotin compounds increases as the acyl chain becomes shorter. Decomposition of the thermograms corresponding to organotin compounds and DPPC suggests that the proportion of TBT- and TPT-enriched domains increases with the organotin compound content. This tendency to form enriched domains might explain why the TBT aggregates associated with cell membranes as intercalations in the lipid bilayer are able to induce changes in the shape of red cell membranes, leading to haemolysis [25]. It is interesting to note that, in the case of DSPC, the submain transition, despite its calorimetric width of only 0.15°C and enthalpy change only a fraction of that of the main transition, is clearly observed at low TBT concentrations (Fig. 9D) and at moderate TPT concentrations (Fig. 10D). In the latter case this submain transition even increases in area, while the presence of a small peak in the upper part of the main transition at 0.4 molar fraction of TPT suggests that this transition may take place after the main transition. It has been suggested [26] that the submain transition is a manifestation of a decoupling of two transitions corresponding to two basic mechanical variables, the acyl chain conformational variable, which describes the degree of internal acyl chain molecular order, and the translational variable, which describes the lateral position of the molecule in the plane of the bilayer. It has also been suggested that this submain transition involves lattice melting, whereas the acyl chain melting takes place at a higher temperature (at the main transition) [26]. It seems that the presence of organotin compounds not only stabilizes the acyl chains in their conformational state (as described above), but also impedes the translational movements of the phospholipid molecules in the plane of the bilayer. The effect of organotin compounds on DLPC differs from that discussed above for the other phosphatidylcholines. This is perhaps to be expected considering that DLPC has been considered unique in the way that its properties appear to deviate from those of its longer chain homologues [22,27]. There is strong evidence that the ordered phase involved in the thermotropic transition of DLPC is a crystalline phase rather than a gel phase [22,27] and it has been suggested that the transition from the ordered phase to the liquid-crystalline phase proceeds via a partially disordered intermediate phase [28,29]. In this sense, the presence of organotin compounds would perturb the packing of the DLPC molecules in the crystalline state and hence explain the observed decrease in the transition temperature. Since the steric and van der Waals interchain interactions in this phase are quite similar to those of the anhydrous lipid crystals [30], organotin compounds will also diminish such interactions, which is consistent with the small decrease in the enthalpy change (TBT) or the absence of such an effect (TPT) observed here.

The presence of organotin compounds does not affect the macroscopic organization of DPPC, which, as revealed by ^{31}P-NMR and SAXD, remains lamellar even at high organotin compound concentrations. Neither TBT nor TPT affects the interlamellar repeat distance, although at high concentrations they lead to the disappearance of the sharp Bragg reflections, indicating that domains enriched in organotin compound reduce the long-range order in the multilamellar system.

When organotin compounds are incorporated into phospholipid systems, they will change the transition temperature of the phospholipid, if both types of molecule are miscible. The phase diagram shows that the temperature of both the solid and liquid lines decreases as more TBT is added to the system. This indicates that DPPC and TBT are miscible in the gel and in the liquid-crystalline phase, and that

the intercalation of TBT molecules in the phospholipid palisade perturbs its thermotropic properties. The phase diagram corresponding to the DPPC/TPT system presents some differences with respect to the above diagram, the region of phase coexistence at high TPT concentration being smaller than in the case of TBT, and, more interestingly, fluid phase immiscibility is observed in the range of TPT concentrations between 0.1 and 0.3 molar fraction. The increased T_m observed at intermediate concentrations of TPT in all the phosphatidylcholine systems studied reflects the bell shape of the fluid line. Bell-shaped fluid immiscibilities have previously been described for mixtures of DPPC and anaesthetics [31], and DPPC and fatty acids [32]. Such fluid phase immiscibility may arise from a tendency of TPT to aggregate in the membrane, a tendency which may be explained by the greater propensity (compared with the butyl groups of TBT) of the aromatic rings of TPT to interact between themselves rather than with the phospholipid bilayer due to the different hydrophobicities and stereochemistries of the molecules. The aggregative behaviour of TPT would explain why the thermotropic properties of phosphatidylcholine are less perturbed by this molecule than by TBT. The formation of TPT aggregates diminishes the interaction between TPT and the phospholipids, and hence the shift of the transition temperature, the formation of enriched domains and the effect on the enthalpy change of the transition would be less pronounced than in the case of TBT. This aggregative behaviour of TPT would also help explain the observation that TPT is less toxic [33] and induces less drastic lesions [34] than TBT.

The effect of organotin compounds on the C=O stretching band of DPPC suggests that these compounds interact with the interfacial region of the phospholipid and make the carbonyl groups less accessible to water. It is known that the mobility of lipid molecules decreases with decreasing hydration because of an enhanced intermolecular steric hindrance [35]. TBT has been shown to inhibit the crystallization of $(Na^+ + K^+)$-ATPase in microsomal membrane preparations, a process requiring molecular mobility within the membrane [36] and, more recently, it has been proposed that organotin compounds freeze the structure of the F_0 sector of the ATP synthase complex in a way that prevents the subunit molecular motions required for rapid proton flux [7]. Both effects can be partly explained by increasing hydrophobic interactions and the enhanced dehydration effects produced by these toxic compounds on the phospholipid molecule.

One explanation for the observed increase in the enthalpy change of the phospholipid transition is that these compounds might induce interdigitation of the phospholipid acyl chains in the same way as has been described for molecules like ethanol [37]. However, our SAXD results show no evidence of a reduction in the interlamellar repeat distance in the presence of organotin compounds to support this hypothesis. It has been shown that a significant decrease in diphenylhexatriene fluorescence intensity occurs during the transition of DPPC from the gel to the interdigitated phase [38]. Again, we observed no such quenching transition in the presence of organotin compounds (data not shown), clearly indicating that interdigitation is not induced by TBT or TPT.

All the above evidence supports the hypothesis that organotin compounds are located in the upper part of the phospholipid palisade. The butyl and phenyl groups intercalate between the initial methylene segments, increasing the hydrophobic interactions and affecting the hydration of the interfacial region. To summarize, this study has shown that organotin compounds are incorporated in the most abundant phospholipid of eucaryotic membranes, i.e. phosphatidylcholine, where they perturb its thermotropic and structural properties. TBT and TPT alter the packing of the phospholipids, increase the enthalpy change of the transition and induce the formation of membrane-enriched domains, phenomena which are more evident as the phospholipid acyl chain becomes shorter. Organotin compounds affect the interfacial region of DPPC but do not affect the macroscopic bilayer organization of the phospholipid. The observed interaction between organotin compounds and phospholipids promotes physical perturbations, which could affect membrane function and may mediate some of their toxic effects. Further studies on the interaction between organotin compounds and other relevant membrane phospholipids will be required to understand the interaction of these toxicants with the membrane and the influence

that such interactions might have on the toxicity mechanism of these compounds.

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