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Organotin compounds promote the formation of non-lamellar phases in phosphatidylethanolamine membranes

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Abstract

Organotin compounds are important contaminants in the environment. They are membrane active molecules with broad biological toxicity. We have studied the interaction of tri-*n*-butyltin chloride and tri-*n*-phenyltin chloride with model membranes composed of different phosphatidylethanolamines using differential scanning calorimetry, X-ray diffraction, ^{31}P-nuclear magnetic resonance and infrared spectroscopy. Organotin compounds laterally segregate in phosphatidylethanolamine membranes without affecting the shape and position of the lamellar gel to lamellar liquid–crystalline phase transition thermogram of the phospholipid. This is in contrast with their reported effect on phosphatidylcholine membranes [Chicano et al. (2001) Biochim. Biophys. Acta 1510, 330–341] and emphasises the importance of the nature of the lipid headgroup in determining how the behaviour of lipid molecules is affected by these toxicants. Interestingly, we have found that organotin compounds disrupt the pattern of hydrogen-bonding in the interfacial region of dielaidoylphosphatidylethanolamine membranes and have the ability to promote the formation of hexagonal H_{II} structures in this system. These results open the possibility that some of the specific toxic effects of organotin compounds might be exerted through the alteration of membrane function produced by their interaction with the lipidic component of the membrane.

Keywords: Organotin compound; Model membrane; Differential scanning calorimetry; ^{31}P-nuclear magnetic resonance; X-ray diffraction; Infrared spectroscopy

1. Introduction

Organotin compounds have several industrial and agricultural applications as plastic stabilisers and catalyst and as molluscicides and fungicides [1], and they are important contaminants in the environment [2]. The toxicity of organotin compounds is very broad, they cause neurotoxicity in animals and humans [3] and are known to have detrimental effects on the immune response [4].

The organotin compounds, tri-*n*-butyltin chloride (TBT) and tri-*n*-phenyltin chloride (TPT) (Fig. 1) are

Abbreviations: DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DMPE, 1,2-dimiristoyl-*sn*-glycero-3-phosphoethanolamine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry; PE, phosphatidylethanolamine; ^{31}P-NMR, ^{31}P-nuclear magnetic resonance; SAXD, small angle X-ray diffraction; T_m, temperature at the midpoint of the transition; TBT, tri-*n*-butyltin chloride; TPT, tri-*n*-phenyltin chloride

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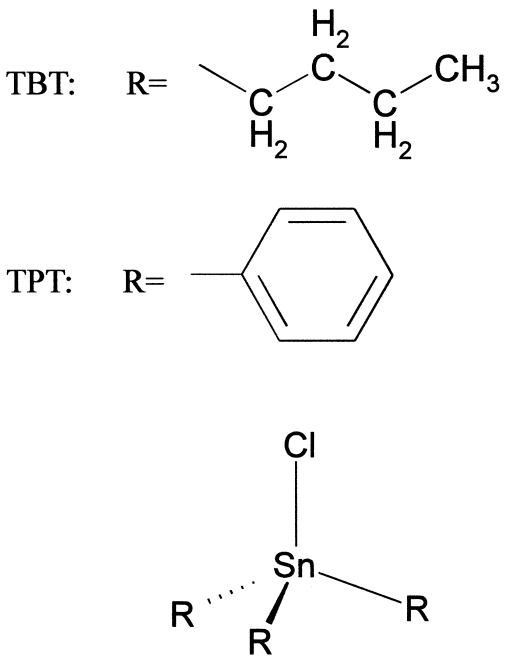

Fig. 1. Chemical structures of TBT and TPT.

membrane active molecules, and their mechanism of action appears to be strongly dependent on organotin lipophilicity [5,6]. They function as ionophores [7] and produce haemolysis [6], release of calcium from sarcoplasmic reticulum [8], alteration of phosphatidylserine-induced histamine release [9], alteration of mitochondrial membrane permeability [10], perturbation of membrane enzymes [11,12] and induction of apoptosis in lymphocytes [13]. Organotin compounds have been shown to affect cell signalling, they activate protein kinase C [14] and increase free arachidonic acid through the activation of phospholipase A_2 [15].

Due to the hydrophobicity of organotin compounds, their interaction with membranes may play an important role in their toxic mechanism, however, very little is known especially as regards organotin–phospholipid interactions. Fluorescence polarisation measurements [16] suggested that the effect of TBT on liposomal membranes is dependent on the anion moiety. Studies on the release of liposome-bound praseodymium [17] indicated that lipophilicity and polarity of organotin compounds and the surface potential and environment of the lipid molecules are important factors in their interaction with membranes. From the study of the interaction of several organotin compounds (differing in their polar and hydrophobic moieties) with erythrocytes [18] it was concluded that the different effects can result from a different location of organotin compounds in the lipid bilayer. Differential scanning calorimetry (DSC) and infrared spectroscopy studies showed that TBT affected the thermotropic properties of dipalmitoylphosphatidylcholine suggesting a location of the toxicant in the hydrophobic region of the membrane [19]. We have recently shown that the effects on the thermotropic properties of phosphatidylcholine are more pronounced in the case of TBT than in the case of TPT being quantitatively larger as the phosphatidylcholine acyl chain length decreases, and also that organotin compounds do not affect the macroscopic bilayer organisation of phosphatidylcholine but do affect the degree of hydration of its carbonyl moiety [20].

Most of the studies on the interaction between organotin compounds and phospholipid membranes have been carried out using phosphatidylcholines. We believe that the knowledge of the interaction of organotin compounds with other important membrane phospholipids is necessary for a proper understanding of the influence of organotin compounds on membranes. In order to further understand the influence of organotin compounds on the lipid component of membranes we made a study of the effect of TBT and TPT on the thermotropic and structural properties of phosphatidylethanolamine (PE), one of the most important phospholipids in eukaryotic membranes, using DSC, small angle X-ray diffraction (SAXD), ^{31}P-nuclear magnetic resonance (^{31}P-NMR) and infrared spectroscopy.

2. Materials and methods

2.1. Materials

1,2-Dimiristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) and 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE) were obtained from Avanti Polar Lipids Inc. (Birmingham, AL, USA). TBT and TPT were obtained from Sigma-Aldrich (Spain). All other reagents were of the highest purity available.

2.2. DSC

The lipid mixtures for 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, 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-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 in chloroform:methanol (1:1). The phosphorous content was determined using the Böttcher's method [21]. 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. SAXD

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 placed in aluminium 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 focused 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).

2.4. ^{31}P-NMR

The samples for ^{31}P-NMR were prepared by combining 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 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 32 K 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.

2.5. Infrared spectroscopy

For the infrared measurements, multilamellar vesicles were prepared in 40 μl of D_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 D_2O spectra taken at the same temperature were subtracted interactively using either GRAMS/32 or Spectra-Calc (Galactic Industries, Salem, MA, USA), as described previously [22].

3. Results

The effect of TBT on the thermotropic transitions of saturated PE bearing acyl chains with 14 (DMPE) and 16 (DPPE) carbon atoms is depicted in Fig. 2. In the absence of organotin compounds DMPE and DPPE exhibited only one endotherm upon heating, located at 50°C and 65°C respectively. The presence of TBT did not affect the shape of the gel to liquid–crystalline phase transition of DMPE and DPPE. The influence of TPT on the phase transition of PE was very similar to that described above for TBT (data not shown). The effect of organotin compounds on the enthalpy change and the temperature at the midpoint of the transition (T_m) of the thermotropic transition of DMPE and DPPE are presented in Fig. 3. Organotin compounds heightened the enthalpy change of the transition (Fig. 3A), and the effect was quantitatively larger for the shorter DMPE homologue. The influence of TPT was less pronounced than that of TBT, so that it only slightly affected the enthalpy change of DPPE. Interestingly, both compounds had almost no effect on the T_m of the transition (Fig. 3B).

SAXD was used to check whether organotin compounds affected the phase behaviour of DMPE. 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. SAXD measurements of pure DMPE systems revealed one diffraction band with a repeat distance of ca. 57.5 Å in the gel phase (Fig. 4A), this value decreased above the chain melting temperature to ca. 49 Å (Fig. 4B), which is in agreement with previous data [23] and it is consistent with its expected multilamellar organisation. Samples containing organotin compounds always gave rise to the same first-order reflection in the whole range of temperatures under study, indicating that the presence of these toxicants did not alter the lamellar structural organisation of DMPE. No significant change in the interlamellar repeat distance was noted.

To investigate the effect of organotin compounds on the interfacial region of DMPE molecule, infrared spectroscopy was used. Fig. 5 shows the carbonyl

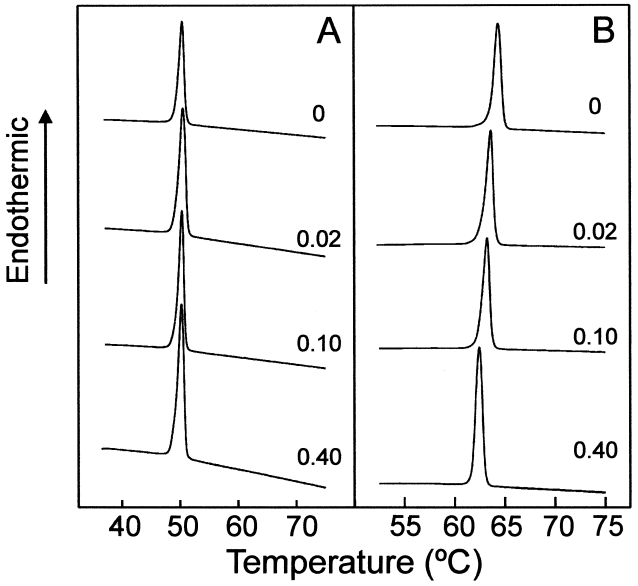

Fig. 2. DSC thermograms for DMPE and mixtures of DMPE/TBT (A) and DPPE and mixtures of DPPE/TBT (B). The concentration of TBT in the membrane (molar fraction) is expressed on the curves.

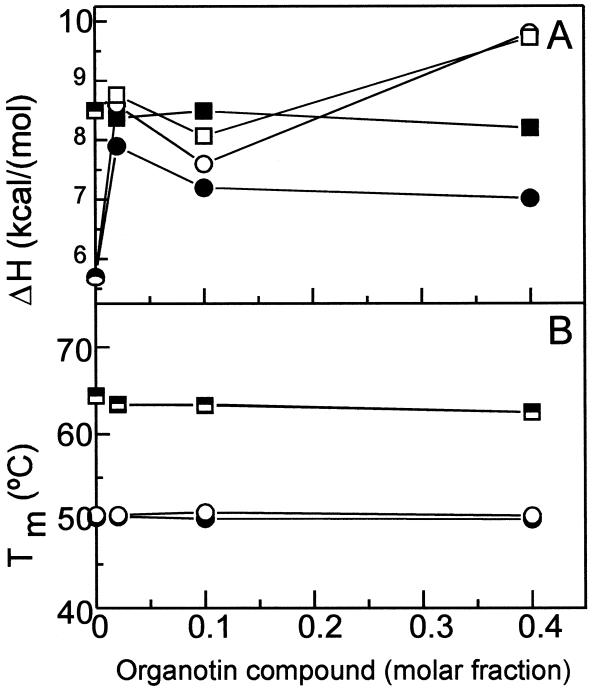

Fig. 3. Main phase transition enthalpy change (A) and midpoint temperature (B) for DMPE (circles) and DPPE (squares) containing different concentrations of TBT (open symbols) and TPT (closed symbols).

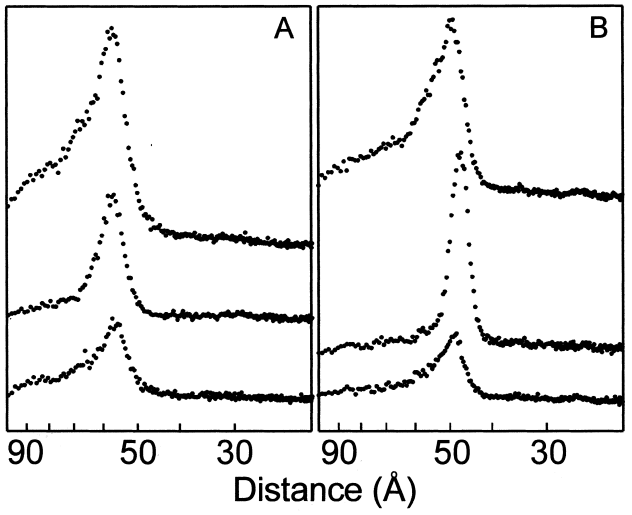

Fig. 4. X-Ray diffraction profiles at 35°C (A) and 60°C (B), obtained from (top to bottom) pure DMPE, DMPE containing 0.3 molar fraction of TBT and DMPE containing 0.3 molar fraction of TPT.

stretching band for pure DMPE and mixtures of DMPE/organotin compounds. Pure DMPE showed absorption maxima at 1739 cm^{-1} and 1737 cm^{-1} in the gel and liquid–crystalline state, respectively. As shown in Fig. 5, the presence of organotin compounds did not change the wavenumber of the absorption maxima of this band.

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. Pure DMPE spectra represent a summation of the component bands centred at 1743 and 1728 cm^{-1}, attributed to dehydrated and hydrated carbonyl groups, respectively [24]. The spectra from Fig. 5 were subjected to curve fitting to two bands centred at 1743 and 1728 cm^{-1}. These bands were simulated by a Gaussian–Lorentzian function, for which best fit estimates of band shape were obtained with an approximately 70% Gaussian contribution. The relative areas of these simulated bands were calculated for DMPE and mixtures with organotin compounds, and it was found that the presence of TBT and TPT did not substantially modify the contribution of the components bands as compared with the pure phospholipid (data not shown).

We next studied the interaction between organotin compounds and DEPE, an unsaturated PE which undergoes a thermotropic transition from the lamellar phase to the hexagonal H_{II} phase. The effect of organotin compounds on the thermotropic phase transitions of DEPE is shown in Fig. 6. Aqueous dispersions of DEPE can undergo a gel to liquid–crystalline phase transition in the lamellar phase and, in addition, a lamellar to hexagonal H_{II} structural phase transition. This is shown in the scan corresponding to pure DEPE (Fig. 6, upper part). The lamellar gel to lamellar liquid–crystalline phase transition takes place at 37.2°C and the lamellar to hexagonal H_{II} structural phase transition occurs at 63.8°C in agreement with previous data [25]. The latter has a much smaller transition enthalpy due

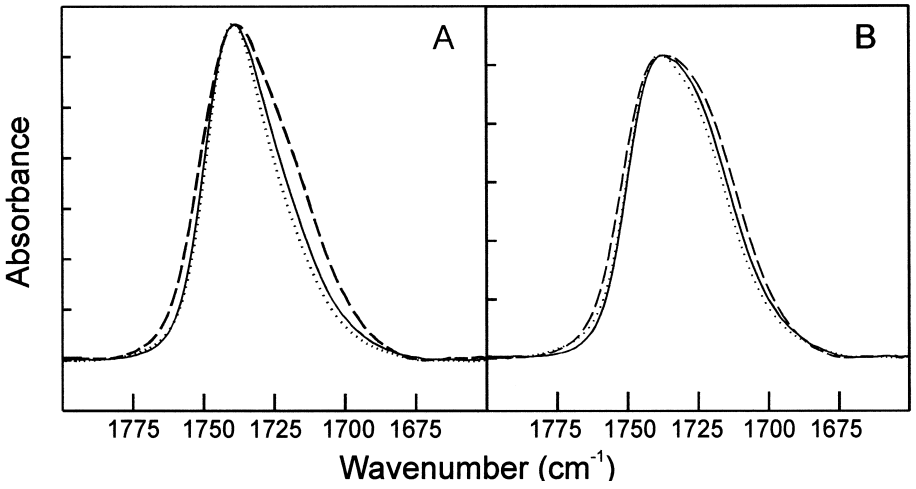

Fig. 5. Infrared spectra of the carbonyl stretching band of DMPE (solid line) and DMPE containing 0.3 molar fraction of TBT (dashed line) or 0.3 molar fraction of TPT (dotted line) at 30°C (A) and 60°C (B).

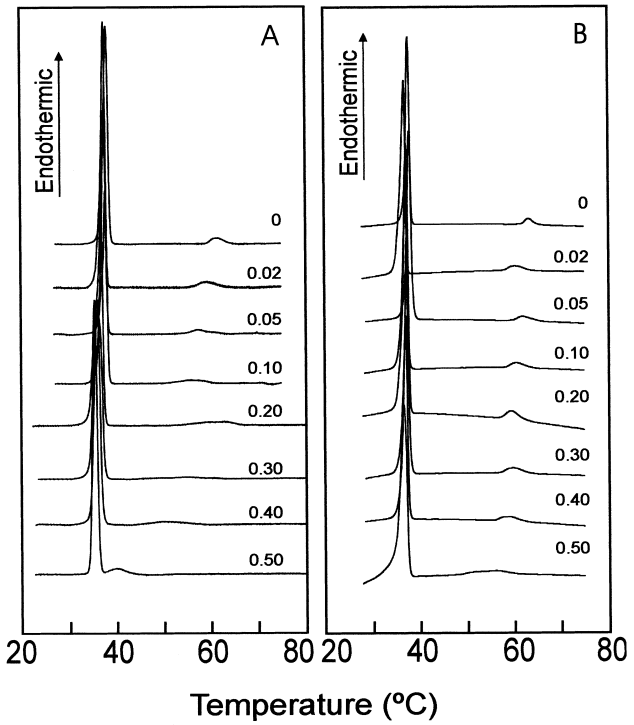

Fig. 6. DSC thermograms for DEPE and mixtures of DEPE/TBT (A) and DEPE/TPT (B). The concentration of TBT and TPT in the membrane (molar fraction) is expressed on the curves.

to the fluid character of both the lamellar and the hexagonal H_{II} phase [26]. The presence of organotin compounds had no apparent effect on the lamellar gel to lamellar liquid–crystalline phase transition of DEPE. However the effect of organotin compounds on the lamellar to hexagonal H_{II} phase transition was evident, resulting in a broadening and shifting of the transition peak to lower temperatures, this effect was more prominent in the case of TBT (Fig. 6A) than in the case of TPT (Fig. 6B)

Information on structural characteristics of the DEPE/organotin compounds systems was obtained with the use of SAXD. Fig. 7 shows the diffraction patterns corresponding to pure DEPE and DEPE containing organotin compounds at different temperatures. It can be seen that, similar to what was found above for DMPE, DEPE systems in the lamellar states showed only the first-order reflection. It has been previously observed [27,28] that in DEPE systems in the lamellar phases no higher order reflections are found. For pure DEPE (Fig. 7A) below the gel to liquid–crystalline phase transition (30°C) the interlamellar repeat distance in the gel state was ca. 66 Å. The transition to a liquid–crystalline phase was accompanied by a decrease of ca. 10 Å in first-order repeat distance, due to the decrease in the effective acyl chain length. Increasing the temperature in the liquid–crystalline state produced a reduction of the interlamellar distance from 55.1 Å at 45°C to 53.5 Å at 55°C, due to the resulting increase in chain motion

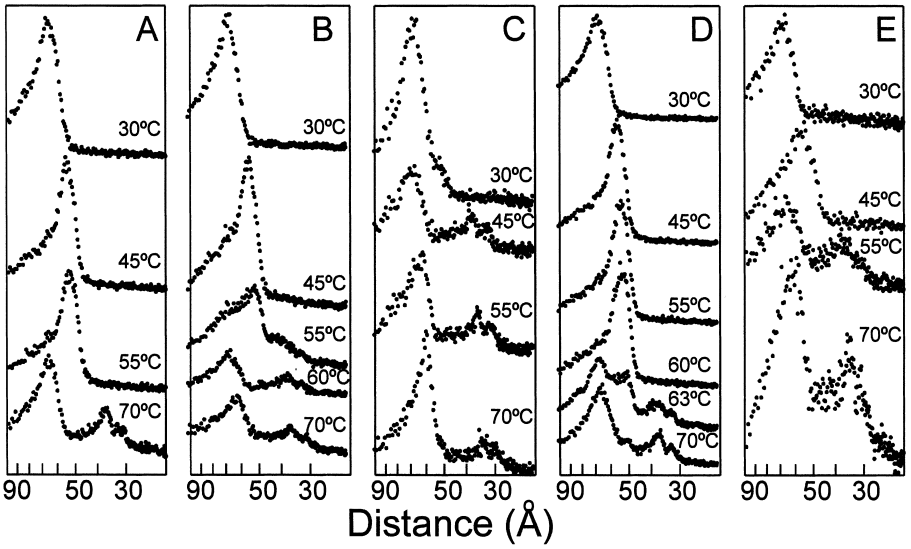

Fig. 7. X-Ray diffraction profiles obtained for pure DEPE (A), DEPE containing 0.1 molar fraction of TBT (B), DEPE containing 0.4 molar fraction of TBT (C), DEPE containing 0.1 molar fraction of TPT (D) and DEPE containing 0.4 molar fraction of TPT (E) at different temperatures.

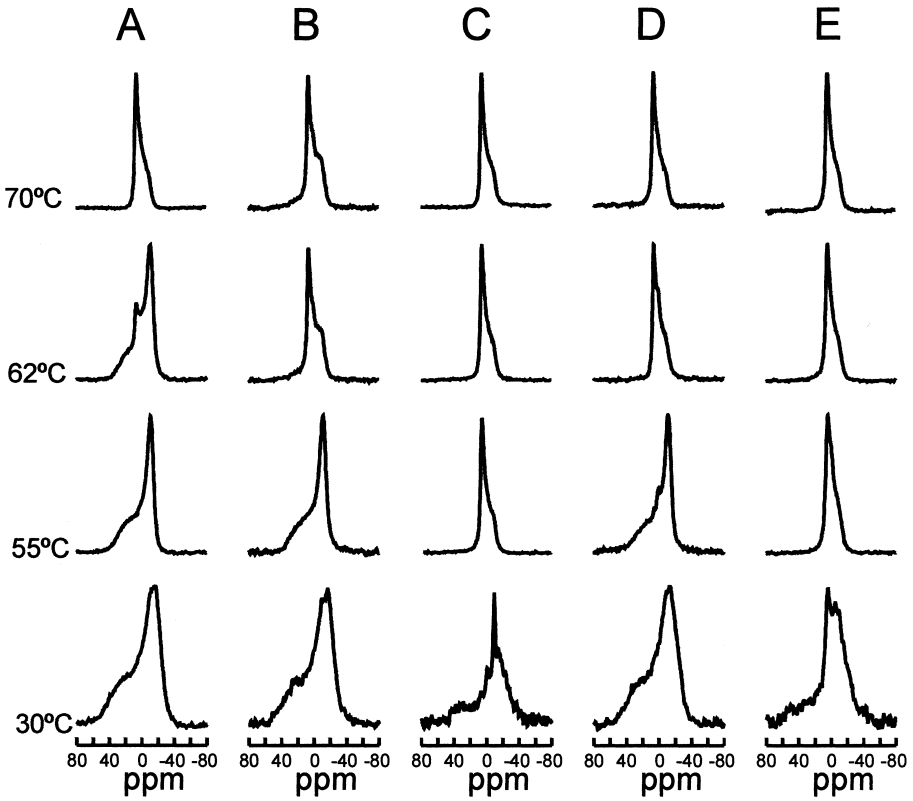

Fig. 8. ^{31}P-NMR spectra at different temperatures corresponding to pure DEPE (A), DEPE containing 0.1 molar fraction of TBT (B), DEPE containing 0.4 molar fraction of TBT (C), DEPE containing 0.1 molar fraction of TPT (D) and DEPE containing 0.4 molar fraction of TPT (E).

which leads to a reduction of the effective chain length. A similar behaviour has been previously reported [29]. Lipids organised in hexagonal H_{II} structures give rise to reflections at distances which relate as $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}...$ [30]. In our case, pure DEPE in the hexagonal H_{II} phase, i.e. at 70°C (Fig. 7A) gave rise to three reflections with distances which relate as $1:1/\sqrt{3}:1/\sqrt{4}$. The presence of 0.10 molar fraction of TBT produced the appearance of reflections which distances related as $1:1/\sqrt{3}$, characteristic of lipid organised in hexagonal H_{II} structures (Fig. 7B), at 55°C, temperature at which pure DEPE was organised in the lamellar liquid–crystalline state. When the concentration of TBT was increased to 0.4 molar fraction (Fig. 7C) the hexagonal H_{II} diffraction pattern appeared even at lower temperatures (45°C). Qualitatively similar results, though less prominent, were found in the presence of TPT, the hexagonal H_{II} diffraction pattern appeared at 63°C and at 55°C in the presence of 0.10 and 0.40 molar fraction of TPT, respectively (Fig. 7D,E).

The effect of organotin compounds on the thermotropic phase transitions of DEPE was further investigated by ^{31}P-NMR (Fig. 8). DEPE when organised in bilayer structures (Fig. 8A) gives rise to a characteristic asymmetrical ^{31}P-NMR lineshape, with a high-field peak and a low-field shoulder [31]. For DEPE organised in hexagonal H_{II} phase (Fig. 8A, 70°C) additional motional averaging is experienced due to diffusion of the phospholipids around the cylinders of which this phase is composed. These results in a two-fold reduction in effective chemical shift anisotropy and a reversed asymmetry (i.e. a high-field shoulder and a low-field peak) [32]. In the presence of TBT and TPT the characteristic spectrum corresponding to the hexagonal H_{II} phase appeared at lower temperatures (Fig. 8B–E). It is shown that at temperatures at which the pure phospholipid is organised in extended bilayer structures, after the addition of organotin compounds all the phospholipid is organised in hexagonal H_{II} phase.

Using the DSC data shown in Fig. 6 and the in-

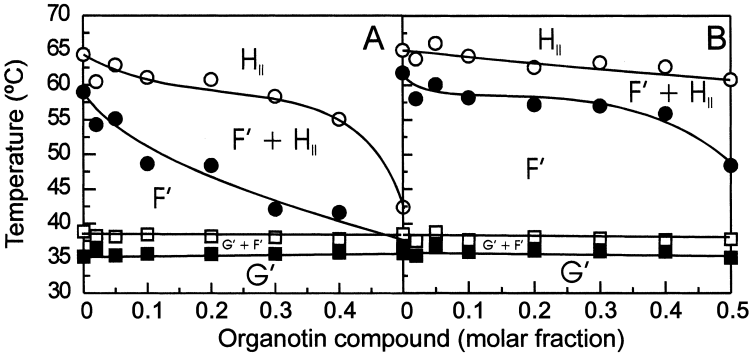

Fig. 9. Partial phase diagrams for DEPE in DEPE/TBT mixtures (A) and DEPE/TPT mixtures (B). Open (□) and closed (■) squares were obtained from the onset and completion temperatures of the main gel to liquid–crystalline phase transition; and open (○) and closed (●) circles were obtained from the onset and completion temperatures of the lamellar to hexagonal H_{II} phase transition. The phase designations are as follows: G′, immiscible DEPE/organotin compounds domains in the gel phase; F′, immiscible DEPE/organotin compounds domains in the liquid–crystalline phase; and H_{II}, hexagonal H_{II} phase.

formation of phospholipid structural organisation obtained from SAXD and ^{31}P-NMR presented in Figs. 7 and 8, a partial phase diagram for DEPE in mixtures with organotin compounds (Fig. 9) was constructed. The onset and completion temperatures of the lamellar gel to lamellar liquid–crystalline phase transition were used to define the solid and fluid boundary lines, whereas the onset and completion temperatures of the lamellar to hexagonal H_{II} phase transition were used to define the lamellar and hexagonal boundary lines. The solid and fluid lines kept horizontal, i.e. at a constant temperature, in the whole range of organotin compounds concentration under study. The temperature of the lamellar and hexagonal lines decreased as the concentration of organotin compounds increased. The system evolved from a lamellar gel phase (G′) with different DEPE–organotin compounds domains, to a lamellar liquid–crystalline phase (F′) with different DEPE–organotin compounds domains, through a very narrow coexistence region (G′+F′) and then to the hexagonal H_{II} phase (H_{II}) through a coexistence region (F′+H_{II}) which was wider in the case of TBT (Fig. 9A) than in the case of TPT (Fig. 9B).

In order to check the influence of organotin compounds on the interfacial region of this phospholipid, we studied the infrared carbonyl stretching band of DEPE and that of DEPE systems containing organo-

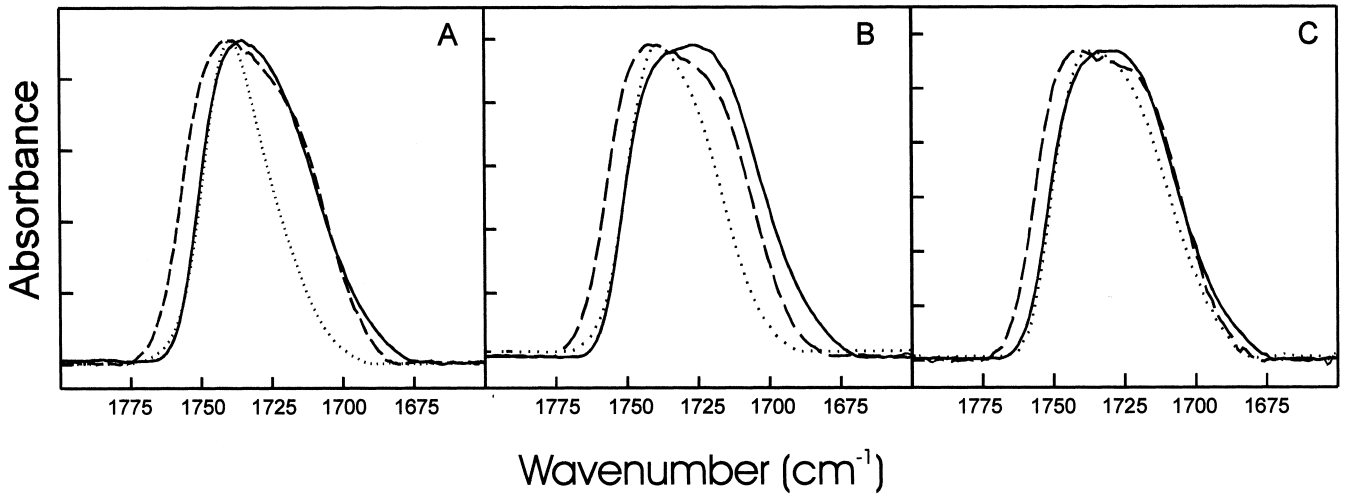

Fig. 10. Infrared spectra of the carbonyl stretching band of DEPE (solid line) and DEPE containing 0.3 molar fraction of TBT (dashed line) or 0.3 molar fraction of TPT (dotted line) at 25°C (A), 53°C (B) and 70°C (C).

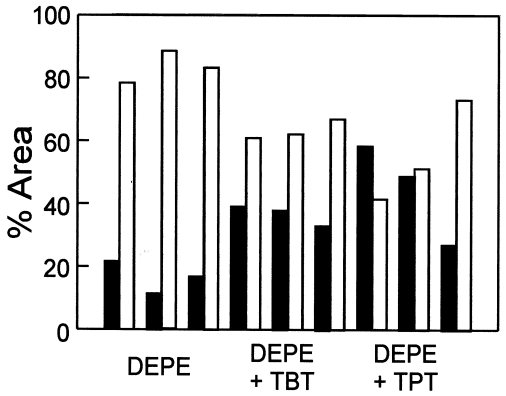

Fig. 11. Relative area of the dehydrated (black) and hydrated (white) components of the carbonyl stretching band for DEPE and DEPE containing 0.3 molar fraction of TBT and TPT, at 25°C (bars at the left), 53°C (bars at the centre) and 70°C (bars at the right).

tin compounds, and the results are presented in Fig. 10. This band was sensitive to both DEPE phase changes. The carbonyl stretching band for pure DEPE showed an absorption maximum at 1735 cm^{-1} in the lamellar gel phase (Fig. 10A), which decreased to a value of 1728 cm^{-1} after the lamellar gel to lamellar liquid–crystalline phase transition (Fig. 10B). As it was shown previously [33], the shift in frequency at the lamellar to hexagonal H_{II} phase transition is opposite to that observed in the chain melting transition, the carbonyl stretching band showing an absorption maximum in the hexagonal H_{II} phase at 1730 cm^{-1} (Fig. 10C). It can be seen that the presence of both TBT and TPT produced a shift of the band maximum to higher wavenumbers in the three different phase states. The relative areas of the simulated bands component were calculated for DEPE and mixtures with organotin compounds (Fig. 11). It can be seen that, in the three different phases under study, the lamellar gel, the lamellar liquid–crystalline and the hexagonal H_{II} phase, the presence of TBT and TPT substantially increased the contribution of the dehydrated component compared with the pure phospholipid.

4. Discussion

The thermotropic properties and polymorphic phase behaviour of mixtures of different PE's and organotin compounds have been examined to establish the extent of intermolecular interaction between both types of molecules. The interaction between molecules can be evidenced by the change of the thermotropic properties of the pure component of a mixture. The profile of a DSC thermogram of a phospholipid phase transition is determined by the transition temperature and the enthalpy change. DSC experiments showed that the bilayer gel to bilayer liquid–crystalline phase transition of saturated PE was not very much influenced by organotin compounds. If the phase transition of the phospholipid remains unaltered by the inclusion of a molecule intrinsic to the membrane, it should be concluded that the foreign molecule is not interacting with the phospholipid. Neither the shape nor the position of the thermograms were drastically affected by organotin compounds, however, the enthalpy of the transition was slightly increased. We therefore must conclude that the interaction between organotin compounds and the phospholipid acyl chains is not very efficient. This is in contrast with the marked perturbation exerted by organotin compounds on the phase transition of phosphatidylcholines, where they broadened the main transition peak and shifted the transition temperature to lower values [20]. It is known that the small polar headgroup of PE together with the formation of hydrogen-bonding allows a very close packing of these phospholipids [34,35]. The finding that hydrophilic interactions in PE bilayers tend to be considerably stronger than those of phosphatidylcholine bilayers may explain many of the differences in the physical properties of these two classes of phospholipids, in particular, the presence of strong polar interactions may account for the higher melting temperatures observed for PE bilayers [36]. We have recently suggested that organotin compounds tends to aggregate and form enriched domains in phosphatidylcholine membranes [20]. It seems that the highly packed PE molecules are not miscible with the organotin compounds and this, together with the tendency of these toxicants to aggregate, will produce the lateral segregation of the organotin compounds in the PE membrane, explaining why these compounds only slightly affect the thermotropic transition of the phospholipid. From SAXD experiments it is concluded that organotin compounds did not affect the macroscopic lamellar organisation of DMPE and that the phospholipid could accommodate orga-

notin compounds aggregates in both the gel and liquid–crystalline state without altering the interlamellar repeat distance. These results demonstrate that the nature of the lipid polar headgroup is an important determinant of how the behaviour of bilayer lipid molecules is affected by these membrane-penetrating compounds even when lipid–organotin compounds interactions are expected to be primarily hydrophobic.

The effect of organotin compounds on the lamellar gel to lamellar liquid–crystalline phase transition of DEPE was similar to that commented above for DMPE, i.e. they did not affect the shape and position of the thermograms. The most interesting finding is that organotin compounds were able to promote the formation of hexagonal H_{II} structures in DEPE systems. Incorporation of increasing amounts of organotin compounds resulted in a progressive decrease of the transition temperature of the lamellar to hexagonal H_{II} phase transition, indicating that DEPE molecules interacting with organotin compounds gave rise to a broad phase transition which is shifted to lower temperatures. This interpretation was confirmed by our SAXD data which evidenced that organotin compounds were very effective promoters of the hexagonal H_{II} phase, such that the characteristic reflections of the hexagonal H_{II} phase appeared at temperatures at which pure DEPE, in the absence of organotin compounds, was organised in the lamellar state. This was further evidenced by the fact that the hexagonal H_{II} phase ^{31}P-NMR spectrum also appeared at temperatures lower than that of the pure DEPE.

The observation that both the solid and fluid lines in the DEPE–organotin compounds partial phase diagram remained horizontal along the whole range of toxicants under study, evidences the formation of organotin domains laterally segregated in both the lamellar gel and the lamellar liquid–crystalline state of DEPE. However, these organotin compounds domains greatly affected the formation of the hexagonal H_{II} phase, with both the lamellar and the hexagonal H_{II} boundary lines going down as more organotin compound is present in the system, suggesting that organotin compounds are more miscible with DEPE in the hexagonal H_{II} phase. The ability of organotin compounds to promote hexagonal H_{II} structures was higher for TBT than for TPT. This is compatible with our finding [20] that the effects on the structural properties of phosphatidylcholines were more pronounced in the case of TBT, and could arise from the higher 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. This higher aggregative behaviour of TPT would help to explain the observation that TPT is less toxic [37] and induces less drastic lesions [38] than TBT. Similar formation of hydrophobic aggregates which exert a limited perturbation of the bilayer properties but greatly influence the macroscopic polymorphic organisation of the lipid has been previously described for coenzyme Q [39].

The effect of organotin compounds on the carbonyl stretching band of DEPE suggests that these compounds affect the interfacial region of the phospholipid and make the carbonyl groups less accessible to water. This is in contrast with the lack of perturbation on the interfacial region observed for DMPE, which probably arises from the fact that the interfacial region of pure DMPE is already highly dehydrated, as evidenced by its propensity to form condensed crystal-like phases upon long-term incubation at low temperatures [40]. Organotin compounds influence the behaviour of the interfacial region of DEPE, and this is probably because, as suggested previously for the interaction between model peptides with PE bilayers [41], the presence of organotin compounds aggregates may disrupt the pattern of hydrogen-bonding and/or ionic interactions in the lipid bilayer, even in the absence of specific interactions between the toxicants aggregates and the polar headgroups of the PE.

The ability of lipids to adopt different non-lamellar structures has been thoroughly studied. The cone-shaped molecule of PE makes them compatible with inverted structures such as the hexagonal H_{II} phase [32]. The presence of organotin compounds aggregates in the bilayer will perturb the lipid matrix increasing the hydrophobic volume and this will result in a more effective cone-shaped PE molecule which will facilitate the formation of hexagonal H_{II} structures. A similar mechanism has been previously

suggested for the promotion of non-lamellar structures found for coenzyme Q [39] and vitamin K_1 [42]. It has been suggested that the strength of hydration of PE is of paramount importance in determining the ability of this phospholipid to form non-lamellar phases [43], and direct evidence has been reported for the partial dehydration of PE bilayers on approaching the hexagonal H_{II} phase [44]. Hence, it is obvious how the dehydrating effect exerted by organotin compounds on the interfacial region of PE discussed above, will also favour the formation of non-lamellar structures in this phospholipid system. The promotion of non-lamellar structures by organotin compounds might contribute to the observed alterations in membrane structure responsible for the toxicity of these compounds [6,8,10].

There is experimental evidence indicating a correlation between the physical state (i.e. the phase state of cellular membranes), determined by their lipid composition, and the physiological state of the cell [45,46]. Considerable interest has been focused on the role of 'hexagonal H_{II} phase propensity' and its effects on membrane properties. There is increasing evidence that as membrane bilayers become more prone to enter the hexagonal H_{II} phase they can activate membrane proteins [47]. It has been shown that organotin compounds are able to activate protein kinase C [14] and phospholipase A_2 [15], and the activities of both enzymes have been associated with the presence of non-lamellar forming lipids and found to depend on the physical state of the membrane [48–51]. A possibility emerges that organotin compounds, besides to directly interact with membrane proteins, could affect membrane function by altering the physical properties of the lamellar phase. In this connection, it is relevant to note that similar ideas, i.e. that particular compounds may exert their activity by interacting with the lipid bilayer, have been put forwarded previously, regarding the action of antimicrobial peptides, as well as the effects of drugs in general, as shown for cyclosporin A [52].

In summary, this study has shown that organotin compounds interact differently with PE than with phosphatidylcholines [20]. TBT and TPT laterally segregate in the PE membrane, slightly affecting the lamellar gel to lamellar liquid–crystalline phase transition of the phospholipid. However, these toxicants affect the interfacial region of DEPE and make the carbonyl groups less accessible to water, being able to promote hexagonal H_{II} structures in this system. We believe that these results are useful to get insight into the interaction of organotin compounds with membranes and to understand the effects of these toxicants, opening the possibility that some of the specific effects of TBT and TPT might be exerted through the alteration of membrane function produced by their interaction with the lipidic component of the membrane.

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