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NOTE

The effect of phenyltin chlorides on the phase polymorphism of dipalmitoylphosphatidylcholine

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The effects of diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT) on polymorphic phase behaviour of aqueous dispersions of a dipalmitoylphosphatidylcholine (DPPC) bilayer were investigated by means of ^{31}P NMR. It is suggested that DPhT induces interdigitated gel phase formation and TPhT induces hexagonal phase formation. The toxic activities of DPhT and TPhT seem to be connected with their ability to induce changes in the membrane structure.

Keywords: diphenyltin dichloride; triphenyltin chloride; inverted hexagonal phase; interdigitated gel structure; ^{31}P NMR

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INTRODUCTION

Although organometallic compounds exhibit toxic activity, they are widely used in many fields of industry, for example in the production of plastics, wood protection agents, textiles, leather and in agriculture as pesticides (fungicides, bactericides and herbicides).[1–4] The toxicity depends on both the chemical structure and the number of organic groups attached to the metal atom. This may be linked to differences in their interactions with membranes, particularly to their different localization in the membrane. Probably, for this reason the interaction of phenyltin compounds with model membranes has been studied for some time.[5–10]

In previous papers[6,8] we suggested that triphenyltin chloride (TPhT) is localized in the hydrocarbon core of the bilayer and at higher concentrations induces inverted hexagonal phase (H_{II}) formation. However, diphenyltin dichloride (DPhT) is probably localized on the surface of the bilayer and at higher concentrations promotes the induction of an interdigitated gel ($L_{\alpha I}$) structure in phospholipid membranes. The ability of phenyltin compounds to induce either non-bilayer or hexagonal structures is very interesting from a theoretical point of view, and may essentially contribute to the explanation of the toxicity of the compounds studied. So, it seemed to us worthwhile to undertake additional studies using other methods in order to confirm the ability of TPhT to induce a non-bilayer arrangement of phospholipids in membranes. Towards this aim we used ^{31}P NMR spectroscopy. ^{31}P NMR spectra of unsonicated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) dispersions are known[11,12] to be very sensitive to the bilayer structure and can be used to distinguish between a bilayer and non-bilayer arrangement of phospholipids in membranes.

MATERIALS AND METHODS

Chemicals

DPhT and TPhT were purchased from Alfa (Karlsruhe). The compounds were used without further purification. DPPC was purchased from Avanti Polar Lipids, Birmingham, Alabama. 99.98% D_2O was purchased from Dr Glaser AG Basel.

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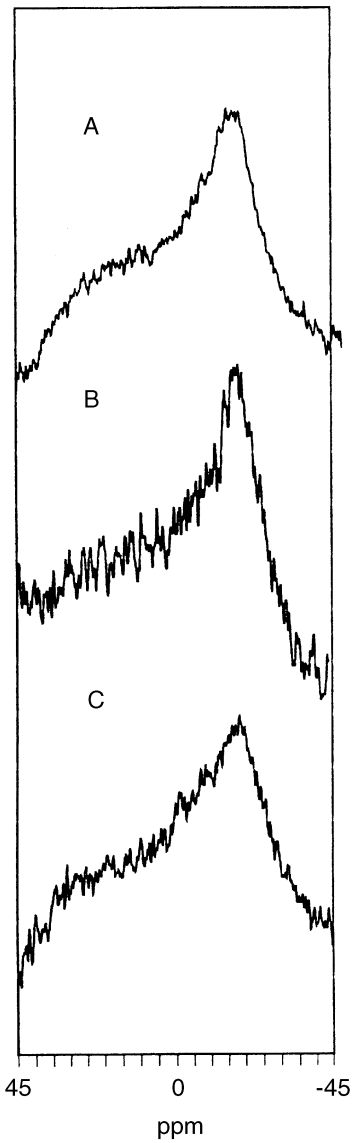

Figure 1 ^{31}P NMR spectra of aqueous unsonicated DPPC dispersions in the absence (A) and presence of DPhT (B) or TPhT (C). The molar ratios of DPhT/DPPC and TPhT/DPPC were 0.20. The data were obtained at 30 °C.

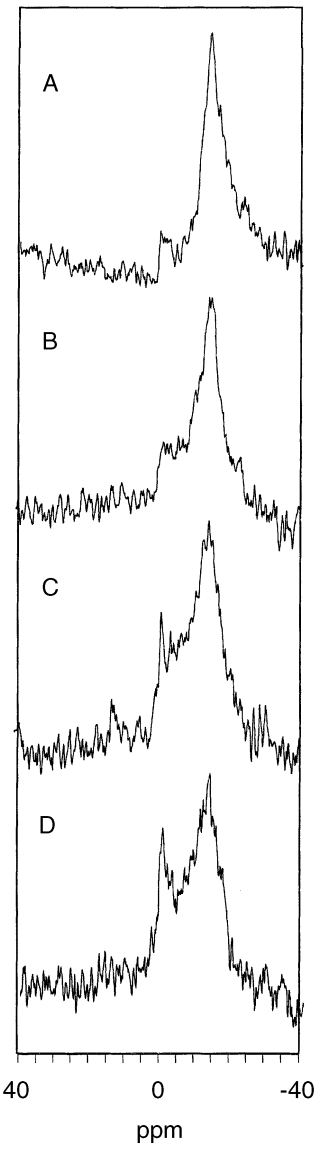

Figure 2 ^{31}P NMR spectra of aqueous unsonicated DPPC dispersions in the absence (A) and presence of DPhT or TPhT. The molar ratio of DPhT/DPPC was 0.20:1 (B) and those of TPhT/DPPC were 0.07 (C) and 0.20:1 (D). The data were obtained at 60 °C.

Sample preparation

Multilamellar vesicles (MLV) for ^{31}P NMR were prepared from DPPC and appropriate amounts of DPhT or TPhT. DPPC and DPhT or DPPC and TPhT were dissolved in chloroform. The mixture was evaporated to form a thin film on the flask wall. Traces of chloroform were removed with a stream of dry nitrogen. Then D_2O was added. The lipid film was dispersed by agitating the flask on a vortex mixer to give a milky suspension of liposomes. The final lipid concentration was 55 mg cm^{-3}. Samples were enclosed in 5 mm diameter NMR tubes.

^{31}P NMR spectra were recorded on an Avance Bruker DRX 300 spectrometer at 121.5 MHz. Signals were acquired using a 13368.98 spectral window, 14.0 μs pulse width and 1.23 s acquisition

time. Digital resolution was 578.55 Hz cm^{-1} or 4.76 ppm cm^{-1}. Chemical shift values are given relative to 85% H_3PO_4. Experiments were performed at 30 and 60 °C.

RESULTS AND DISCUSSION

The ^{31}P NMR spectra of aqueous dispersions of DPPC and DPPC/DPhT and DPPC/TPhT mixtures were taken in the gel state (at 30 °C) and in the liquid crystalline state (at 60 °C) and are presented in Figs 1 and 2 respectively. In the gel state, the line shape is known to be more broadened compared with the liquid-crystalline state, where the line shape is considerably narrower.[13]

Phospholipids in the bilayer phase exhibit a line shape with a high-field peak and low-field shoulder, whereas phospholipids in hexagonal phases show its opposite behaviour (low-field peak and high-field shoulder). The line shapes of aqueous dispersions of DPPC (Figs 1 and 2) resemble those for the bilayer phase. After addition of TPhT the line shape changes. This suggests that the addition of TPhT induces changes in the bilayer structure. At 30 °C at a molar ratio TPhT/DPPC of 0.20 a low intensity shoulder appeared at the low side of the main signal at about 1 ppm. (Fig. 1C). At 60 °C this peak is more pronounced (Fig. 2C); a peak of low intensity can also be distinguished in the spectrum at lower molar ratio 0.07 (Fig. 2D). Therefore, the intensity of the peak increases with increasing concentration of TPhT. The position of the new peak on the low-field shoulder may indicate formation of an inverted hexagonal phase (H_{II}). The spectrum seems to here superimposed a hexagonal-like ^{31}P NMR line shape on the bilayer line shape. This, in turn, indicates the coexistence of lamellar and hexagonal phase arrangements. Thus, TPhT seems to be a promoter of formation of a non-lamellar phase in the lamellar phase state.

After the addition of DPhT the characteristic shape of a lamellar phase is maintained at both temperatures. This is in agreement with the suggestion that DPhT induces formation of an interdigitated gel phase, since it is also a lamellar phase.

The formation of interdigitated bilayers in biological membranes has not yet been observed, but the possibilities have been suggested by many studies carried out using model membranes.[14–16] It is suggested that interdigitated structure formation, as well as a hexagonal gel phase,[14–16] plays an important role in regulating many functions of biological membranes. So, the toxicity of DPhT and TPhT is probably due to their ability to induce structural changes in the bilayer.

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