Influence of Dodecyltrimethylammonium Halides on Interaction of Phenyltin Compounds with Model Membranes

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The effects were studied of dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB) and dodecyltrimethylammonium iodide (DTAI) on thermotropic phase behaviour of phosphatidylcholine bilayers, as well as on ^{1}H NMR and ^{31}P NMR spectra, in the presence of diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT). The obtained results indicate that in the presence of the surfactant studied the interaction of phenyltin compounds with model membranes was changed and the changes depended on the kind of the counterion. The surfactants studied (especially DTAC) decrease the ability of phenyltin compounds to induce structural changes in the bilayer. It is suggested that DTAB, and especially DTAC, prevent DPhT induced interdigitated phase formation as well as formation of an inverted hexagonal phase (H_{II}) in the case of TPhT/DPPC liposomes.

Introduction

Despite of toxic activity organometallic compounds are widely used in industry for example in the production of plastics, wood protection, antifoulings agents, textiles and leather, and in agriculture as pesticides (Fent, 1996; Crowe, 1987a; Crowe, 1987b). In part the toxicity may be due to their interaction with membranes and consequent alteration of the structure of membranes. For that reason the interaction of organometallic compounds with model and also biological membranes are intensively studied (Ambrosini *et al*., 1996; Gray *et al*., 1987; Eng *et al*., 1991; Musmeci *et al.,* 1992; Hamasaki *et al.,* 1995; Langner *et al*., 1998; Radecka *et al.,* 1997; Różycka-Roszak *et al.,* 1997).

In previous papers (Różycka-Roszak, *et al*., 2000; Różycka-Roszak and Pruchnik, 2001) we studied the effect of diphenyltin dichloride (DPhT), triphenyltin chloride (TPhT) and tetraphenyltin (TTPhT) on thermotropic phase behaviour and structure of model membranes. We found that DPhT and TPhT significantly affect phase transition and structure while TTPhT showed a small effect. In this paper we studied the influence of phenyltin compounds on thermotropic phase behaviour in the presence of surfactants. Accordingly we studied DPhT and TPhT (showing a significant effect on structure) and the commonly used surfactants like dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB) and dodecyltrimethylammonium iodide (DTAI). The effects of DTAC, DTAB and DTAI on thermotropic phase behaviour of phosphatidylcholine bilayers were studied previously (Różycka-Roszak and Pruchnik, 2000). The surfactants were added either to the water phase or directly to the lipid phase (a mixed film was formed). The effects of particular surfactants on the main phase transition (T_m) were more pronounced when they were added to the water phase instead of the lipid phase. That is why in this paper the surfactants were added to the water phase. Surfactants are water pollutant and enter the membrane through the water phase. Our studies were prompted by the frequent use of metalloorganic compounds in the presence of surfactants.

As done previously we applied differential scanning calorimetry (DSC) and used nuclear magnetic resonance (^{1}H NMR and ^{31}P MR), which is one of the most powerful techniques to study biological and model membranes (Fenske, 1993; Wu, 1996).

Materials and Methods

Chemicals

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and egg yolk lecithin were purchased

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from Avanti Polar Lipids, Birmingham, Alabama, USA.

Dodecyltrimethylammonium chloride (DTAC) and dodecyltrimethylammonium bromide (DTAB) were purchased from Fluka, Switzerland.

Dodecyltrimethylammonium iodide (DTAI) was prepared by mixing a concentrated aqueous NaI solution with an aqueous dodecyltrimethylammonium chloride solution at room temperature. A precipitate was obtained which was redissolved in warm water and precipitated again after cooling. The solution was filtered and recrystallized from EtOH. The purity was checked by ^{1}H NMR. Also, a satisfactory elemental analysis was obtained.

Diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT) were purchased from Alfa (Karlsruhe, Germany). The compounds were used without further purification.

99.98% D_2O was purchased from Dr. Glaser AG Basel, Switzerland.

Sample preparation

Multilamellar vesicles (MLVs) for differential scanning calorimetry (DSC) were prepared in the following way. DPPC and appropriate amounts of di- or triphenyltin chlorides 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 water solution of DTAC, DTAB or DTAI of appropriate concentration were added and the flask and its contents were heated to 60 °C in a water bath. 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 25 mg/ml. Lipid suspension was loaded into the sample cell of a DSC microcalorimeter of Mettler Toledo Thermal Analysis System D.S.C. 821^e. Scan rates of 2 °C/min were employed. The measurement was repeated, at least for three independent sample preparations. Small unilamellar vesicles (SUV) for ^{1}H-NMR were prepared in the following way. Egg yolk lecithin and appropriate amounts of DPhT and TPhT were dissolved in chloroform and then the mixture was evaporated to form a film on the flask wall. Traces of chloroform were removed by a stream of dry nitrogen. Then a D_2O solution of DTAC and DTAB of appropriate concentration was added. The lipid film was dispersed by agitating the flask on a vortex mixer to give a milky suspension of liposomes. This suspension was sonicated at 0 °C for ½ h with a 20 kHz sonicator.

^{1}H-NMR spectra were recorded on an Avance Bruker DRX 300 Spectrometer at 300.13 MHz.

Samples were enclosed in 5 mm diameter NMR tubes. Signals were acquired using a 6173.84 Hz spectral window, 10.6 μs pulse width and 2.65 s acquisition time. Digital resolution was 150.065 Hz/cm or 0.5 ppm/cm.

The residual water signal was used as a chemical shift reference. All experiments were performed at 300 K.

^{31}P NMR spectra were recorded on an Avance Bruker DRX 300 Spectrophotometer at 121.51 MHz. The same sample were used for ^{31}P NMR as for ^{1}H NMR. Signals were acquired using 38535.645 Hz spectral windows, 12.0 μs pulse width and 0.85 s acquisition time. Digital resolution was 182.24 Hz/cm or 1.5 ppm/cm. Chemical shift values are given relative to 85% H_3PO_4.

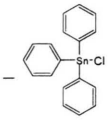
Results

Differential scanning calorimetry (DSC)

The effects of 2, 5 and 10 mM of DTAC, DTAB and DTAI on the phase transitions of DPPC liposomes in the absence of phenyltin compounds and containing different amounts of DPhT or TPhT were compared in Table I. After addition of DTAX to DPPC liposomes (without phenyltin compounds) the main phase transition was asymmetrical or even separated into two peaks (Różycka-Roszak and Pruchnik, 2000). After addition of DTAX to liposomes containing DPhT or TPhT the main phase transition of DPPC did not separate into two peaks, and remained almost symmetrical and significantly narrow than in the absence of phenyltin compounds.

In Fig. 1 were compared the effects on T_m of particular surfactants at chosen concentrations of phenyltin/DPPC liposomes. The upper curves referred to phenyltin/DPPC liposomes (without surfactant) studied before (Różycka-Roszak *et al.*, 2000). As was reported before and also shown by Fig. 1 the phenyltin compounds affected the main phase transition of DPPC in different ways. DPhT scarcely affected T_m while TPhT decreased T_m sig-

Table I. Comparison of the effects of surfactants on the phase transitions in the absence and presence of phenyltin compounds.

DPhT –  diphenyltin dichloride; TPhT –  triphenyltin chloride; DTAX – dodecyltrimethylammonium halides; T_m-main phase transition temperature; $T½$ – the halfwidth of main phase transition temperature; n_{TPhT} – number of moles TPhT; n_{DPhT} – number of moles DPhT; n_{DPPC} – number of moles DPPC.

			DTAX Concentration [mM] 2		5		10	
			Cl^-	Br^-	Cl^-	Br^-	Cl^-	Br^-
DPPC		T_m	41.30	40.71	39.70	39.68	37.74	38.00	35.37
		$T½$	0.43	1.36	1.36	1.09	1.93	1.75	2.01
n_{DPhT}/n_{DPPC}	0.03	T_m	41.40	40.01	39.02	39.35	38.97	35.38	34.68
		$T½$	1.24	1.20	1.10	1.76	1.88	1.74	3.29
	0.07	T_m	41.70	40.02	38.51	38.55	38.84	35.12	36.05
		$T½$	1.70	1.65	1.56	1.92	1.27	1.99	2.41
	0.20	T_m	42.20	40.00	36.61	38.09	37.34	34.89	36.71
		$T½$	3.50	1.85	1.81	2.27	2.63	2.86	2.25
n_{TPhT}/n_{DPPC}	0.03	T_m	41.2	39.90	39.50	38.21	38.41	35.32	36.72
		$T½$	0.75	1.55	1.30	1.46	1.56	1.72	1.88
	0.07	T_m	40.63	39.50	39.89	37.35	38.60	34.12	35.84
		$T½$	1.65	2.40	2.23	1.87	1.71	2.62	2.36
	0.20	T_m	38.80	38.40	39.82	36.78	37.65	32.8	33.68
		$T½$	1.90	2.45	2.32	1.78	1.96	2.53	2.42

nificantly. The addition of a surfactant decreased T_m of phenyltin/DPPC liposomes significantly and T_m was decreased more in the case of DPhT/DPPC liposomes than TPhT/DPPC liposomes.

Besides, in the presence of phenyltin compounds T_m was decreased less by DTAB (especially at low concentration) than by DTAC; the opposite happened when phenyltin compounds were absent (Różycka-Roszak and Pruchnik, 2000).

^1H NMR

The effect of DTAB and DTAC on ^1H NMR spectra of sonicated egg yolk PC (lecithin) dispersions containing DPhT and TPhT in molar ratios 0.03 were shown in Fig. 2. Surfactant concentration was 10 mM. DTAI was not used, due to its small solubility.

^1H NMR resonance of the trimethylammonium group of lecithin, $[N(CH_3)_3]_L$, remained almost unchanged in sonicated egg-yolk PC (lecithin) dispersions containing phenyltin compounds and surfactants studied. Also, the position of ^1H NMR resonance of the trimethylammonium group of the surfactant head group, $N(CH_3)_3$ remained practically unchanged. Anyway, the signal were of significantly low intensity in comparison to pure water but of greater intensity than it was in lipid dispersion without phenyltin compounds (Różycka-Roszak and Pruchnik, 2000). This suggests that DTAC and DTAB were embedded into liposomes but in the presence of phenyltin compounds less than in their absence. Besides, the $N(CH_3)_3$ signal intensity of DTAC was lower than that of DTAB. This finding suggest that DTAC was more embedded into lecithin dispersions containing phenyltin than DTAB although in the absence of phenyltin compounds DBT was more embedded than DTAC (Różycka-Roszak and Pruchnik, 2000).

The effect of DTAB and DTAC on ^1H NMR spectra of sonicated egg yolk PC (lecithin) dispersions containing TPhT in molar ratios 0.20 was

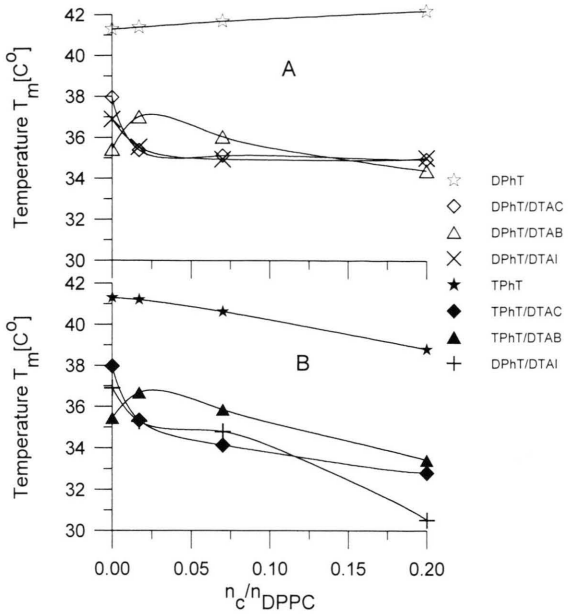


Fig. 1. Main phase transition temperatures (T_m) as a function of DPhT (A) and DPhT (B) concentrations in the presence and the absence of 10 mM dodecyltrimethylammonium halides. n_c – number of moles phenyltin compounds; n_{DPPC} – number of moles DPPC

presented in the Table II. The addition of DTAX caused a significant decease in halfwidth of the of the spectrum.

At a molar ratio of 0.20 was not possible to study lecithin dispersion with DPhT because a precipitate (not detectable by ^{1}H NMR and ^{31}P NMR) was formed (Różycka-Roszak *et al.*, 2000).

^{31}P NMR

The effect of DTAX on ^{31}P NMR spectra of sonicated egg yolk PC (lecithin) dispersions containing phenyltin compounds were presented in the Table III. The addition of DTAX changed significantly both the chemical shift as well as the halfwidth of the spectrum. Sonicated egg-yolk PC (lecithin) dispersions containing DPhT, as well as TPhT, markedly decreased the halfwidth of the spectrum in the presence of DTAX, while the chemical shift was downfield moved with DPhT present and upfield with of TPhT.

Discussion

The different effect of DTAC and DTAB on the interaction of DPhT with model membranes is reflected in the DSC results. The main phase transition temperature of DPPC/DPhT liposomes shifts progressively to lower temperatures with increasing concentration of both DTAB and DTAC (Table I). The addition of DTAB to lipid dispersion containing DPhT (at low DPhT/DPPC ratio) increases T_m while addition of DTAC decreases T_m in comparison to DPPC without phenyltin compounds (Fig. 1). From ^{1}H NMR studies it follows that in the presence of DPhT the DTAC and DTAB are less embedded into liposomes than in the absence of DPhT. So, the lower effect of DTAB on T_m mentioned above may be attributed to a smaller amount of DTAB embedded to liposomes. DTAC decreases T_m in the presence of

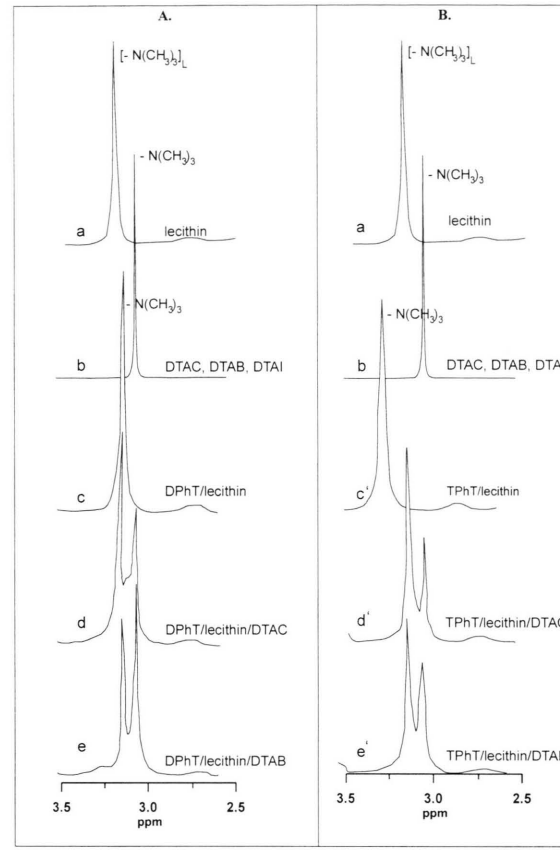


Fig. 2. The effect of DTAX on ^{1}H NMR spectra of egg yolk lecithin liposome suspension in the absence (a) and presence of DPhT (c,d,e)-A and of TPhT (c′,d′,e′)-B. $[N(CH_3)_3]_L$-trimethylammonium group of lecithin, $N(CH_3)_3$ trimethylammonium group of surfactant. Lecithin concentration – 10 mg/ml, surfactant concentration – 10 mM, molar ratio of DPhT/lecithin –0.03.

Table II. Comparison of ^{1}H NMR chemical shifts (ppm) for DPhT/lecithin and TPhT/lecithin dispersion in D_2O with and without 10 mM DTAX.

	Egg lecithin	DPhT/lecithin				TPhT/lecithin			
		0.03		0.20		0.03		0.07	
Group									
$N(CH_3)_3$	3.19	3.14		3.12		3.29		2.94	
+ DTAX,	X:	Cl^-	Br^-	Cl^-	Br^-	Cl^-	Br^-	Cl^-	Br^-
$N(CH_3)_3$		3.16 3.07	3.17 3.09	3.16 3.07	3.16 3.09	3.18 3.08	3.18 3.10	3.04	3.02

Table III. Comparison of ^{31}P NMR spectrum of DPhT/lecithin and TPhT/lecithin vesicles in D_2O with and without 10 mM DTAX.

	Egg lecithin	DPhT/lecithin				TPhT/lecithin			
		0.03		0.07		0.03		0.20	
Chemical shift [ppm]	–0.33	–1.16		–1.55		–0.36		–0.67	
Halfwidth [Hz]	60.70	97.18		103.30		66.77		224.60	
DTAX,	X:	Cl^-	Br^-	Cl^-	Br^-	Cl^-	Br^-	Cl^-	Br^-
Chemical shift [ppm]		–0.63	–0.61	–0.74	–0.77	–0.43	–0.47	–0.81	–0.98
Halfwidth [Hz]		59.97	69.08	61.80	96.42	64.83	69.08	151.10	169.32

DPhT more than in the absence although DTAC is also less embedded into liposomes in the presence of DPhT. This may suggest that DTAC and probably DTAB (but in smaller degree), makes DPhT locate in the lipid bilayer region although DPhT was suggested to be localized near the polar region of the bilayer in the absence of DTAX (Różycka-Roszak *et al.*, 2000). In a previous paper (Różycka-Roszak *et al.*, 2000) we suggested that DPhT induces interdigitated gel phase formation. The significant shift and broadening of the ^{31}P NMR spectrum after addition of DPhT (TableIII) was explained by the formation of oriented bilayers or bilayer fragments. The bilayers were suggested to be formed from interdigitated vesicles because they do not interdigitate in a stable way. In the presence of DTAX (especially DTAC) the shift and linewidth of ^{31}P NMR signal significantly decreased. This may suggest that DTAX prevents DPhT an induced interdigitated gel phase formation and in consequence DTAX prevents formation of bilayers, too. DTAC seems to prevent the interdigitation process more than DTAB. The DSC results suggest that DTAC makes DPhT to locate in the lipid bilayer region. In the interdigitated gel phase a compound is expected to be localized near the polar region of the bilayer. So, the location DPhT in the lipid bilayer may explain why DTAX prevent interdigitation.

TPhT was suggested (Różycka-Roszak *et al.*, 2000) to be at least partially embedded into the lipid bilayer. That is why TPhT broadens the main phase transition significantly. After addition of DTAX to TPhT/DPPC dispersion the main phase

transition remains almost unchanged (narrow) even at a TPhT/DPPC molar ratio of 0.20. At that ratio (without DTAX) the marked fluidization effect was observed not only by DSC but also by ^{1}H NMR (Różycka-Roszak *et al.*, 2000). Besides, ^{31}P NMR lineshape was changed: a low-field shoulder appeared and the inverted hexagonal phase (H_{II}) formation was likely to occur. Such phases are known to be involved in the molecular mechanism of membrane aggregation and bilayer destabilization resulting in membrane fluidization. After addition of DTAC and DTAB the fluidization effect was significantly diminished as follows from the DSC, ^{1}H NMR and ^{31}P NMR results. This may be due to DTAX which prevents inverted hexagonal phase formation. After addition of TPhT the ^{31}P NMR signal is upshifted, probably due to TPhT localized in the interface.

The surfactants studied (especially DTAC) decrease essentially the ability of phenyl tin compounds to induce structural changes in the bilayer. In a previous work (Różycka-Roszak *et al.*, 2000) we suggested that the toxicity of phenyltin compounds may be related to structural changes they induce in the bilayers. So we hypothesise that the addition of a surfactant, especially DTAC, decreases the toxicity of the phenyltin compounds studied.

Acknowledgement

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