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Effect of Organotin Compounds on Membrane Lipids: Fluorescence Spectroscopy Studies

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The temperature dependence of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) fluorescence anisotropy has been studied to investigate the influence of mono-n-butyltin chloride (MBTC), di-n-butyltin chloride (DBTC) and tri-n-phenyl chloride (TPTC) on the physicochemical state of dipalmitoyl phosphatidylcholine. Below the lipid chain melting transition (T_m), fluorescence anisotropy values of DPH and TMA-DPH are increased by the presence of the organotins, without important modifications of the phase transition temperature. A possible difference in localization of the organotin compounds is suggested by the differential effect of the probes. It is suggested that there is localization in the hydrophobic core of the bilayer for TPTC, and at the head-group level for DBTC, and a homogeneous distribution in the bilayer for MBTC. Similar studies have been performed in liposomal suspensions of cardiolipin, phosphatidylserine and egg phosphatidylcholine.

Keywords: organotin compounds; liposomes; fluorescence; 1,6-diphenyl-1,3,5-hexatriene; 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene

INTRODUCTION

Organotin derivatives are used in many different industrial applications. These compounds are utilized mostly as stabilizers for PVC plastics (used for packaging, bottles and potable-water piping), industrial and agricultural biocides and industrial catalysts.[1,2] Their biological effects have attracted much attention in recent years, since organotins and their degradation products may represent a potential cause of environmental pollution. It is known that the tri-, di- and mono-alkyltins display remarkable difference in their biological activities and, in general, their toxicity to mammals decreases from tri- to mono-organotins.[2] Moreover, the toxicity of these molecules is linked also to the nature of the organic ligands: within the same series of compounds the relative potency decreases in the order ethyl > methyl > propyl > phenyl > hexyl > octyl.[1] Organotin-induced phenomena such as mitochondrial respiratory chain inhibition and red blood cell lysis suggest that some tin derivatives may initiate an action at the membrane level, involving proteins and/or phospholipids (PLs).[1,2] Low concentrations of dialkyltins may interact with plasma membrane-associated or cytoskeletal-associated sulphide groups.[1] On the other hand, higher concentrations of the dialkyltins reduce the viability of isolated thymocytes exposed *in vitro*, attesting to the cytotoxic potential of these substances.[1] In contrast to the dialkyltins, trialkyltin derivatives reportedly have little affinity for sulphydryl groups. It is generally believed that low concentrations of trialkyltin derivatives inhibit oxidative phosphorylation, acting as cardiolipin anion CL^-/OH^- exchangers across the mitochondrial membranes, resulting in a disturbance of the existing proton gradient.[2] However, trialkyltins are also believed to bind to mitochondria and to produce an oligomycin-like inhibition of coupled respiration. The evidence that organotin compounds may interact with membrane proteins does not exclude the possibility of an interaction with phospholipids. Since these compounds are soluble in organic solvents, this property could lead to the hypothesis that the primary step in organotin action could be the solubilization in the phospholipid bilayer. Organotins could modify the physical state of lipids and, as a consequence, the lipid–protein interaction.

The interaction between lipids and proteins is central to the understanding of membrane function. Lipid–protein interactions involve alterations in secondary, tertiary or quaternary protein structure induced by changes in the composition or physical state of the lipid. Since it is not clear what is the primary target of these

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compounds, which, nevertheless, could have different effects on the membrane, we have chosen three different organotin compounds (monobutyltin chloride, dibutyltin chloride and triphenyltin chloride) to study their interactions with different kinds of phospholipids.

In order to characterize the interactions of organotin compounds with different lipidic classes, we have studied their effect on the steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) and its charged derivative 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) embedded in multilamellar liposomes of neutral or negatively charged phospholipids. The parallel use of these fluorescent probes in the same system allows the comparison of membrane physicochemical characteristics at different depths in the bilayer.

EXPERIMENTAL

Materials

Di-n-butyltin chloride (DBTC), mono-n-butyltin chloride (MBTC) and triphenyltin chloride (TPTC) were obtained from Aldrich. Dipalmitoyl phosphatidylcholine (DPPC), egg phosphatidylcholine (EPC), bovine heart cardiolipin (CL) and phosphatidylserine (PS) were purchased from Avanti Polar (St Louis, MO, USA). DPH and TMA-DPH were obtained from Molecular Probes (Eugene, OR, USA).

Liposomes preparation

The chosen lipids DBTC, MBTC and TPTC were dissolved in chloroform. DPH and TMA-DPH were added to the phospholipid solution up to a final fluorescent probe/lipid P_i molar ratio of 1:800. The desired amounts of DBTC, MBTC and TPTC in chloroform were added to these solutions then dried under a nitrogen flux and kept under vacuum for 2 h. The lipid films were rehydrated in 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes)–100 mM KCl buffer, pH 7.4, at a temperature well above the chain melting transition (T_m) of the chosen phospholipid. Multilamellar liposomes (MLVs) were obtained by vortexing several times to ensure complete dispersion. The final lipid concentration was determined by phosphorus assay[4] and was 0.3 mM in all cases.

Fluorescence measurements

Fluorescence measurements were performed on a Perkin–Elmer MPF-66 spectrofluorimeter equipped with a 7300 data station. The temperature of the samples was controlled by an external bath circulator (Haake F3). We used, as fluorescent probes, the hydrophobic molecule DPH[5] and its charged derivative TMA-DPH, which has amphipathic properties.[6] For steady-state fluorescence anisotropy (r) measurements, the excitation and emission wavelengths were respectively 360 nm and 430 nm. The degree of DPH and TMA-DPH anisotropy was obtained by Equation [1], where g is an instrumental correction factor; I_{II} and $I_{\perp}$ are, respectively, the emission intensities polarized vertically and horizontally to the direction of polarized light. Steady-state anisotropy has been widely used to monitor membrane fluidity.[7] Three different experiments were performed for each sample. In each experiment, the measurements were repeated five to six times. Results of fluorescence are represented as means ± S.D.

$$r = \frac{I_{II} - I_{\perp} g}{I_{II} + 2 I_{\perp} g} \qquad [1]$$

RESULTS

Fluorescence polarization in phosphatidylcholine liposomes

Steady-state fluorescence anisotropy was performed by using two different probes located in different regions in the lipid bilayer. DPH locates in the hydrocarbon core of the bilayer, while its charged derivative, TMA-DPH, is anchored close to the bilayer surface because of its charged amino group.[9,10] Anisotropy data of DPH in DPPC MLVs are shown in Fig. 1 as a function of both temperature and DBTC, MBTC and TPTC concentrations. The phase transition temperature is only slightly modified by relatively high concentrations of MBTC, with a small increase in the range of the transition. The main effect shown in Fig. 1, below the main transition temperature, is an increase in the anisotropy values measured at each MBTC concentration tested. No effect is evident in the liquid-crystalline phase. DBTC has small effects on the phase transition temperature measured by DPH; no other effect is detectable

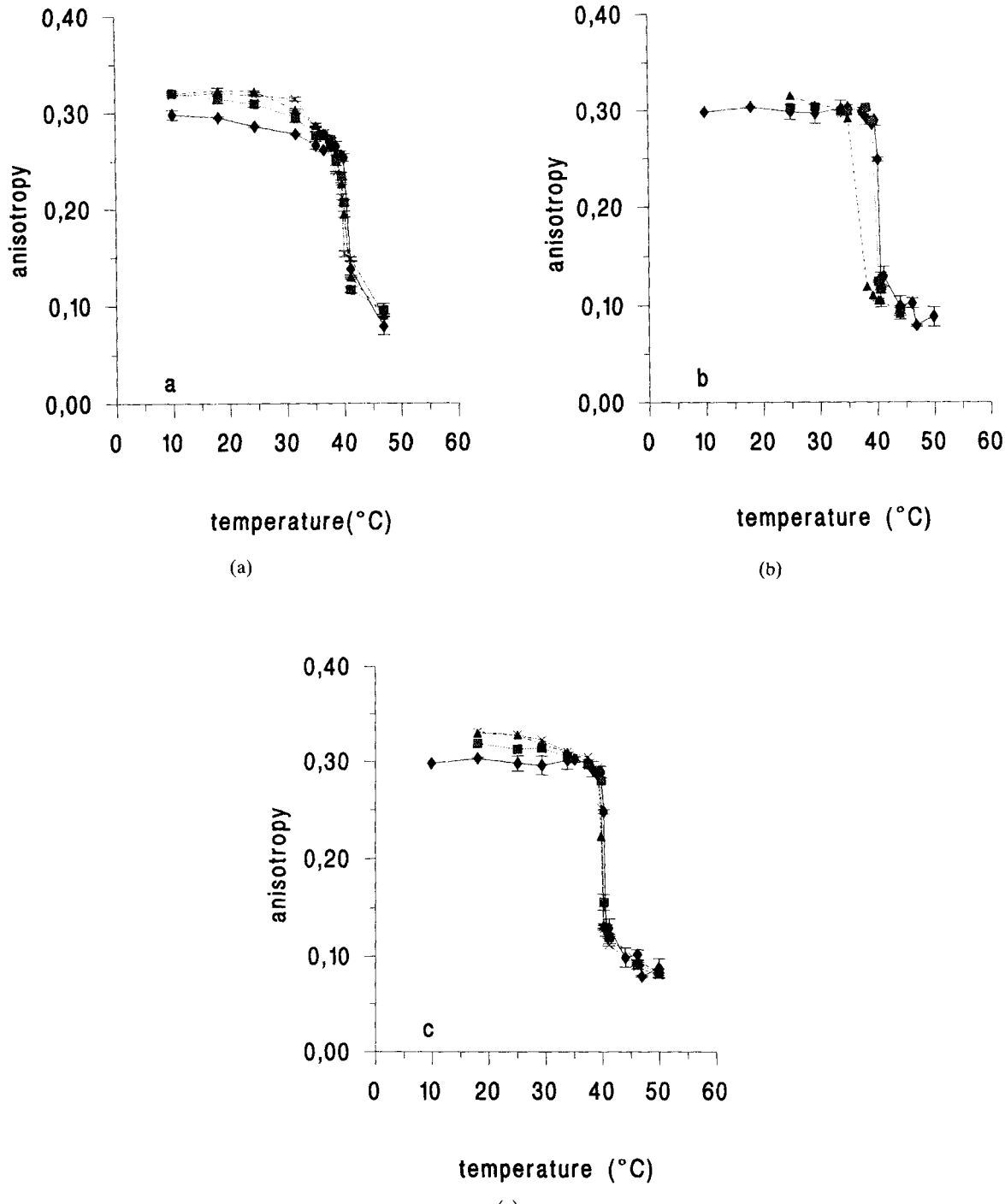

Figure 1 Effect of increasing concentrations of (a) MBTC, (b) DBTC and (c) TPTC on the phase transition of DPPC liposomes, as determined by DPH steady-state fluorescence anisotropy. Liposomes, containing increasing concentrations of organotins, were suspended in 10 mM Hepes/100 mM KCl, pH 7.4. Final probe/lipid molar ratio was 1 : 800 and lipid concentration was 0.3 mM. (a) ■, 22 μM MBTC; △ 27.5 μM MBTC; (×) 40 μM MBTC; ◆, DPH in pure DPPC. (b) ■, 22 μM DBTC; △, 40 μM DBTC; ◆, DPH in pure DPPC. (c) ■, 5 μM TPTC; △, 22 μM TPTC; ×, 40 μM TPTC; ◆, DPH in pure DPPC.

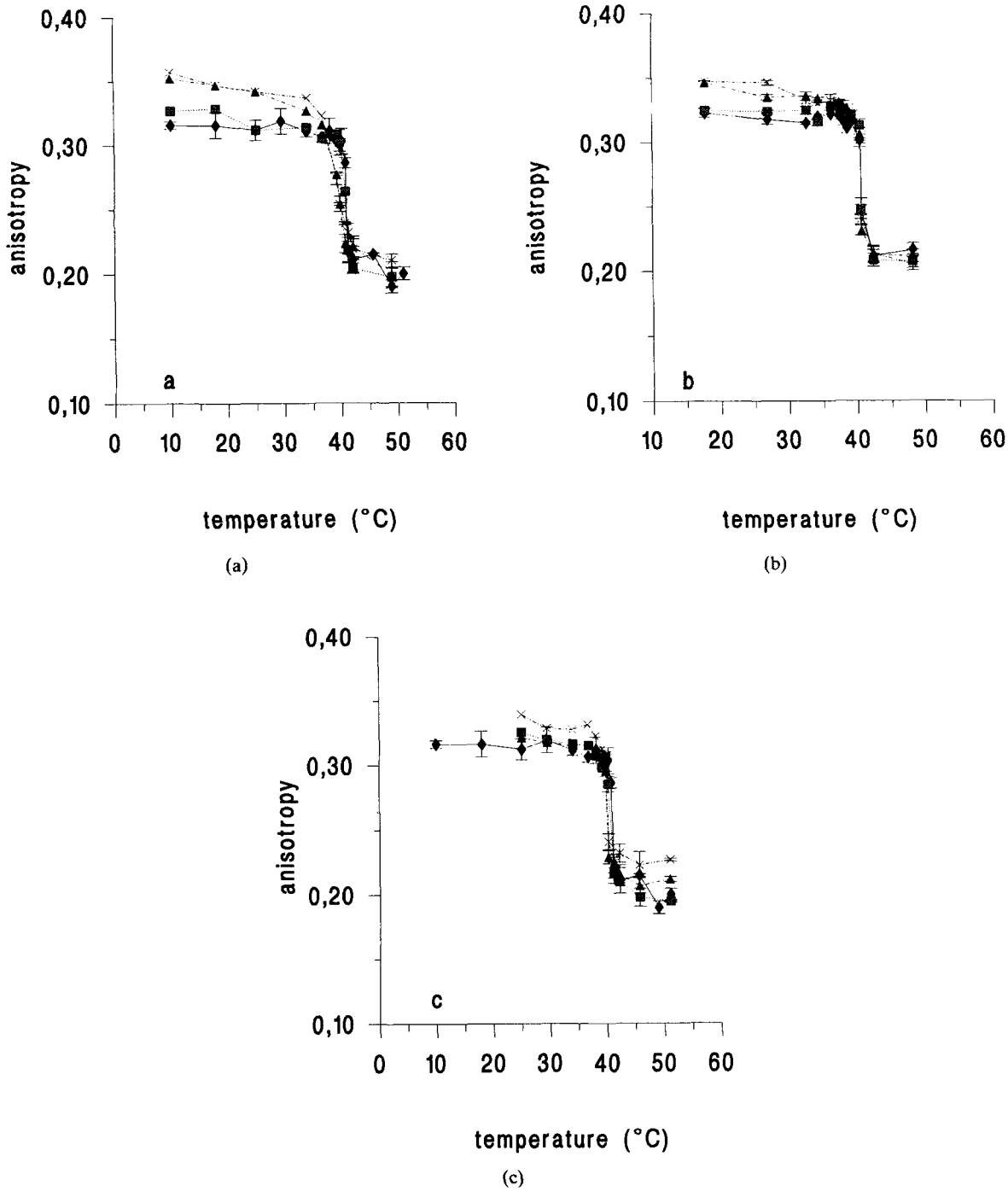

Figure 2 Effect of increasing concentrations of (a) MBTC, (b) DBTC and (c) TPTC on the phase transition of DPPC liposomes, as determined by TMA-DPH steady-state fluorescence anisotropy. Liposomes, containing increasing concentrations of organotins, were suspended in 10 mM Hepes/100 mM KCl, pH 7.4. Final probe/lipid molar ratio was 1:800 and lipid concentration was 0.3 mM. (a) ■, 5 μM MBTC; △, 22 μM MBTC; ×, 40 μM MBTC; ◆, TMA-DPH in pure DPPC. (b) ■, 5 μM DBTC; △, 22 μM DBTC; ×, 27.5 μM DBTC; ◆, TMA-DPH in pure DPPC. (c) ■, 5 μM TPTC; △, 22 μM TPTC; ×, 40 μM TPTC; ◆, TMA-DPH in pure DPPC.

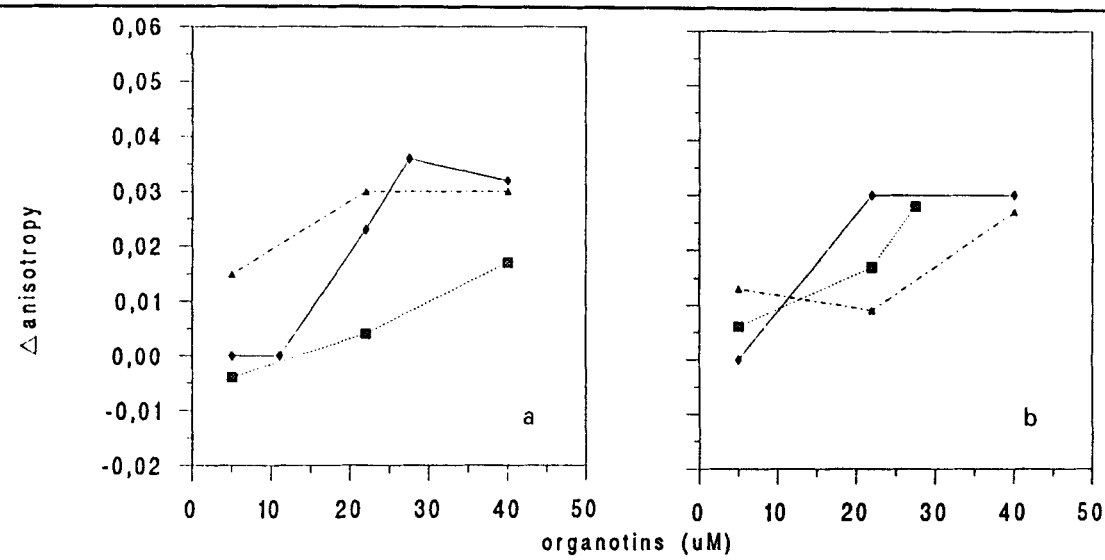

Figure 3 Effect of increasing concentrations of MBTC (◆), DBTC (■) and TPTC (△) on (a) DPH and (b) TMA-DPH anisotropy (Δanisotropy) in DPPC liposomes at 25 °C. Δanisotropy was calculated as the difference between the anisotropy of the chosen sample and the anisotropy of the control. Liposomes, containing increasing concentrations of organotins and the chosen probe, were suspended in 10 mM Hepes 100 mM KCl, pH 7.4. Final probe/lipid molar ratio was 1:800 and lipid concentration was 0.3 mM.

by this probe. Also TPTC has only small effects on the phase transition temperature measured by DPH, with respect to the control. However, at temperatures below the main transition, TPTC increases DPH anisotropy values. This effect is concentration-dependent. Above the main transition no changes of the bilayer physical state are detectable.

In order to study the effect of these molecules at the head-group level of DPPC, we used the fluorescent molecule TMA-DPH, a cationic derivative of DPH, anchored by its positive charge to the polar part of the membrane. MBTC action on DPPC, as detected by TMA-DPH is shown in Fig. 2(a). The higher concentrations tested (22 μM and 40 μM) cause an approx. 1.5 °C of the T_m and an increase of anisotropy values, below the main transition, which is similar for both concentrations tested. A small increase of anisotropy values is measurable also in the liquid-crystalline phase (Fig. 2a). The only evident effect of DBTC at the head-group level (probed by TMA-DPH) is a concentration-dependent raising of anisotropy values in the gel phase (Fig. 2b). TPTC causes a small decrease in T_m values (<1 °C at the higher concentrations tested) and a raising of anisotropy values both in the gel and in the liquid-crystalline phases, in a concentration-dependent way. Figure 3 shows the change in DPH (Fig. 3a) and TMA-DPH (Fig. 3b) anisotropy values expressed as Δanisotropy Δanisotropy was calculated as the difference between the anisotropy of the chosen sample and the anisotropy of the control.

The comparison between the effect on DPH (Fig. 3a) and TMA-DPH (Fig. 3b) anisotropy data shows that TPTC has its highest effect in the hydrophobic core of the bilayer, where the maximum increase in anisotropy is reached starting from 20 μM. At the head-group level, TMA-DPH anisotropy values are similarly increased only at TPTC concentrations higher than 20 μM. The opposite effect is evident for DBTC, which is more effective at the phospholipidic head-group

Table 1 Steady-state fluorescence anisotropy of DPH in cardiolipin, EPC and PS liposomes in the presence of increasing concentrations of organotin derivatives[a]

Sample	Cardiolipin	EPC	PS
Control	0.134 ± 0.007	0.094 ± 0.005	0.109 ± 0.005
5 μM DBTC	0.123 ± 0.006	0.097 ± 0.008	0.105 ± 0.003
22 μM DBTC	0.123 ± 0.005	0.093 ± 0.004	0.109 ± 0.002
40 μM DBTC	0.122 ± 0.005	0.095 ± 0.008	0.105 ± 0.002
5 μM MBTC	0.135 ± 0.005	0.098 ± 0.003	0.116 ± 0.005
22 μM MBTC	0.149 ± 0.007	0.096 ± 0.004	0.122 ± 0.005
40 μM MBTC	0.162 ± 0.009	0.096 ± 0.003	0.110 ± 0.005
5 μM TPTC	0.134 ± 0.007	0.096 ± 0.002	0.107 ± 0.003
22 μM TPTC	0.162 ± 0.008	0.092 ± 0.001	0.105 ± 0.003
40 μM TPTC	0.171 ± 0.009	0.094 ± 0.003	0.106 ± 0.002

[a] Data were obtained at 25 °C. Values are presented as means ± S.D.

level than in the hydrophobic core of DPPC at 25 °C (Fig. 3a, b). Both fluorescent probes show a similar increase in anisotropy values in the presence of MBTC, suggesting a homogeneous distribution of this molecule within DPPC suspensions at the temperature tested.

Fluorescence polarization in egg phosphatidylcholine (EPC), cardiolipin (CL) and phosphatidylserine (PS) liposomes

CL, PS and EPC were chosen to study the effects of organotin derivatives on phospholipids with unsaturated chains and with different headgroups. Table 1 shows the results obtained at 25 °C, where both lipids are in the liquid-crystalline phase. A great increase in DPH fluorescence anisotropy, compared with the control value, is evident in cardiolipin liposomes when MBTC and TPTC are present. This effect is detectable for 40 μM MBTC (+21% increase) and for 22 μM (+21%) and 40 μM (+28%) TPTC. No important effects on DPH anisotropy values are induced by DBTC.

In the EPC and PS liposomes, MBTC, DBTC or TPTC does not greatly modify the DPH fluorescence anisotropy, compared with the control (Table 1).

DISCUSSION

Phospholipids represent the major components of what in most membranes is a complex mixture of many different varieties of lipid molecules. Membrane lipids must significantly affect protein function, since membrane proteins generally remain tightly bound to the lipid bilayer and it is likely that the protein structure is at least partially determined by its dissolution in the bilayer.[11]

Biological membranes contain a large variety of different molecular species of lipids. This diversity and the specificity of the lipidic composition of different membrane types has led to questions regarding the functional roles of the different lipidic species. It was therefore proposed that the presence of different lipids is required to provide appropriate fluidity characteristics in a given membrane. In addition, local domains of appropriate lipid composition could perhaps modulate local fluidity characteristics, possibly influencing protein function.[11] Moreover, the physicochemical characteristics of lipids may modulate other important features of membranes, such as permeability to ions and water.[11] For these reasons a modification of physicochemical characteristics of lipids, as a consequence of the interaction of exogenous molecules, can be important in modulating membrane activities.

In the present work, the temperature dependence of DPH and TMA-DPH fluorescence anisotropy was studied to investigate the influence of MBTC, DBTC and TPTC on the physicochemical state of DPPC liposomes. Our data indicate that all organotin compounds tested affect the fluorescence anisotropy of probes embedded in the lipid membrane. In fact, these molecules modify fluorescence parameters also at temperatures well below the DPPC phase transition. However, relatively low concentrations of TPTC have an effect only on the probe localized in the hydrophobic core of the bilayer (DPH), and only a 40 μM concentration affects the fluorescence of the TMA-DPH at the membrane surface. In contrast, DBTC has an effect only at the membrane surface, and MBTC is effective at each level.

Organotin compounds dissolved in aqueous solution form different cations, depending on the number of organic ligands. Although it is difficult to foresee whether the particular features of these compounds could be influenced by the interaction with phospholipids, some generalizations may be attempted. The trialkyltin form a trigonal bipyramidal diaquo cation with equatorial alkyl groups, while dialkyltins form hydrated tetra-aquo cations in which the atom occupies an octahedral *trans*-R_2-SnX_4 geometry. Monoalkyltins give a dimeric hydroxyl-bridged molecule containing two water molecules.[12] The different numbers and types of ligands influence both the polarity and structural characteristics of each organotin compound, and could be the origin of the different actions at the phospholipid membrane level. A triorganotin derivative, containing sterically hindering hydrophobic groups such as phenyl, may be expected to be localized in the hydrocarbon core of the bilayer. In contrast, it is reasonable to forecast a localization in the phospholipid polar region for the diorganotin derivative, containing four water molecules. It is not easily to foresee the localization of the monobutyltin derivative. Since the MBTC-induced modifications of anisotropy data are evident with both probes used, a homogeneous distribution of this molecule in the bilayer is suggested.

Phospholipids can differ in length and unsatu-

ration of acyl chains and in structural and chemical characteristics of head-groups. The phase behaviour of lipids and other characteristics (e.g. molecular shape and intermolecular hydrogen bonds) may be affected by these differences. Moreover, the physical chemistry of PLs is greatly influenced by the net charge carried by the head-group, which can bring about a redistribution of protons, cations and anions at the water/lipid interface.[13] Thus, PC and phosphatidylethanolamine (PE) are zwitterionic phospholipids, while CL, phosphoctidylglycerol (PG), PS and phosphatidylinositol (PI), at physiological pH, have negative charges. Under our experimental conditions, DBTC, MBTC and TPTC did not modify anisotropy values of DPH embedded in PS and egg PC liposomes. On the contrary MBTC and TPTC caused an increase in DPH anisotropy in CL liposomes. The comparison between results obtained on DPPC, containing saturated acyl chains, and liposomes obtained from PLs, differing in head-groups and/or acyl chains, suggests that the interaction of organotins with biomembranes could be dependent on their PL composition. These data could indicate that the differences in susceptibility of mammalian tissues to the action of organotins could be due, in part, to the differences in phospholipid composition of cellular membranes. Moreover they could explain, at least partially, the differences in intracellular localization of these compounds.[14]

Although these results cannot demonstrate that the toxic effect of organotins may be linked to their action on PLs, an alteration of membrane 'fluidity', caused by the presence of organotins, may be directly linked to the activation of enzymes bonded to the membranes. For these reasons further studies are necessary to clarify the possible biological implications of the interaction of organotins with membrane PLs.

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REFERENCES

1. I. J. Boyer, *Toxicology* **55**, 253 (1989).
2. N. J. Snoeij, A. H. Penninks and W. Seinen, *Environ. Res.* **44**, 335 (1987).
3. A. Ambrosini, E. Bertoli, F. Tanfani and G. Zolese, *Chem. Phys. Lipids* **59**, 189 (1991).
4. M. Kates, in: *Techniques in Lipidology*, Vol. 3, Elsevier, Amsterdam, 1972, pp. 355–360.
5. M. Shinitzky and Y,. Barenholz, *Biochim. Biophys. Acta* **515**, 367 (1978).
6. L. W. Engel and F. G. Prendergast, *Biochemistry* **20**, 7338 (1981).
7. B. R. Lentz, *Chem. Phys. Lipids* **50**, 171 (1989).
8. L. W. Engel and F. G. Prendergast, *Biochemistry* **20**, 7338 (1981).
9. M. Cranney, R. B. Cundall, G. R. Jones, J. T. Richards and E. W. Thomas, *Biochem. Biophys. Acta* **735**, 418 (1983).
10. A. M. Kleinfeld, in: *Membrane Fusion*, Wilschut, J. and Hoekstra, D. (eds), pp. 3–33, Marcel Dekker, N.Y., 1991.
11. D. W. Deamer and J. Bramhall, *Chem. Phys. Lipids* **40**, 167 (1986).
12. A. G. Davies and P. J. Smith, in: *Comprehensive Organometallic Chemistry*, Wilkinson, G., Stone, F. G. A. and Abel, E. W. (eds), Pergamon, Oxford, 1982, pp. 519–627.
13. J. F. Tocanne and J. Teissiè, *Biochem. Biophys. Acta* **1031**, 111 (1990).
14. Y. Arakawa, T. Iizuka and C. Matsumoto, *Biomed. Res. Trace Elements* **2**(3), 321 (1991).