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A Synergistic Effect of Select Organotin Compounds and Ionic Surfactants on Liposome Membranes

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Organometallic compounds and surfactants constitute a potential threat to the environment. For that reason we have embarked on a study of their joint action on membranes. Model lecithin liposome membranes were modified with the cationic surfactant trimethyldodecylammonium bromide or the anionic surfactant sodium dodecylsulfonate, and the effect of tripropyltin chloride on the process of calcium (Ca^{2+}) and praseodymium (Pr^{3+}) desorption from the liposome membrane was studied.

Kinetic constants for the process of Ca^{2+} ion desorption from lecithin liposome membranes were determined using the radiotracer method. The percentage of Pr^{3+} ion desorption from liposome membranes was measured by the ^1H NMR method.

Trimethyltin, triethyltin and tripropyltin alone caused increased Ca^{2+} and Pr^{3+} desorption from liposome membranes with increasing concentration of the compounds and alkyl chain length. For both the processes studied, a cationic surfactant brought about a lower effectiveness of tripropyltin and an anionic surfactant resulted in a higher effectiveness.

The effect observed can be explained by changes in the surface charge of the membrane, induced by the surfactant modifiers and by the concomitant change in the partition coefficient of the organotin. The results obtained indicate a protective or harmful joint action of the surfactants used with tripropyltin on membranes. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Organotin compounds are used in many industrial applications[1–4] and also as agricultural biocides.[5–8] Their biological effects have attracted much attention in recent years, since organotins and their degradation products may constitute a contamination threat to the natural environment.[9–11]

The toxic properties of organotin compounds depend on the biological object and on the chemical structure of the compounds.[12–14] Toxicity of trialkyltin compounds has been confirmed in the case of mammals and algae,[15–17] for instance. Since these compounds are soluble in organic solvents, this property could lead to the hypothesis that the primary step in organotin action could be solubilization in the phospholipid bilayer. The molecular nature of the trialkyltin–membrane interaction is not yet clear, so we thought it useful to undertake investigations in this area. As the difficulty in studying this effect in natural biological membranes lies in their complexity and lability, we have used in our studies—as have only a few other authors studying the interaction of organotin compounds with membranes[18,19]—a model membrane, i.e.

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phospholipid small unilamellar vesicles (SUVs).

The calcium ion plays an important and well-known role in living organisms, and has been investigated very extensively in biological and model systems.[20–22] It is very well adsorbed onto the surfaces of the phospholipid bilayer[23,24] and its permeability through the lipid bilayer is very low, which allows the flux of permeating calcium ions to be neglected in desorption measurements.[23] Also the paramagnetic praseodymium ion, when added to the suspension after liposomes have been formed, anchors in the external layer of the liposome membrane and slowly penetrates into its interior[25–27] and can also be desorbed from the membrane.

We chose calcium and praseodymium ion desorption as phenomena which can give information not only about ion desorption or release processes but also about the properties of the membrane surface. The process of calcium ion desorption studied by using the radioactive tracer method and a ^{1}H NMR technique using paramagnetic praseodymium ions were also found to be useful for studying the interaction between biologically active compounds and model membranes.[28,29]

A binary mixture of an organotin compound with another active agent is often more effective than either of its components separately, as was reported for pesticidal compositions.[5]

The natural environment is affected not only by organometallic compounds but also by many other agents, such as surfactants; the coexistence of such compounds in the environment may influence their effect on biological objects.[5] In our earlier studies we paid attention to the molecular mechanism of the interaction effect of cationic and anionic surfactants on the structure and function of model phospholipid membranes.[30–34] Thus, it seemed useful to undertake investigations on the cooperative action of compounds of the two groups mentioned, in order to find out to what extent the surfactant-induced modification of the membrane affects calcium and praseodymium ion desorption processes from phospholipid liposome membranes induced by organometallic compounds.

MATERIALS AND METHODS

Materials

Egg lecithin (PC) was prepared according to the method described by Singleton *et al.*[35] Tri-n-alkyltin (TAT chlorides), i.e. trimethyltin chloride (TMT), triethyltin chloride (TET) and tripropyltin chloride (TPT), were purchased from Alfa. Trimethyldodecylammonium bromide (TMDA) and dodecanesulfonic acid sodium salt (sodium dodecylsulfonate; AS_{12}) were purchased from Fluka. Praseodymium chloride ($PrCl_3 \cdot 6H_2O$) and heavy water (D_2O) were obtained from Aldrich and Świerk (Poland), respectively. All chemicals were of analytical grade.

Radioactive tracer experiments

Small unilamellar liposomes (SUVs) were prepared from egg-yolk lecithin by using sodium cholate in Liposomat (Dianorm).[36] The solution used to form vesicles contained a 9.5 mM veronal acetate buffer of pH 7.5 and 0.3 mM $CaCl_2$ labelled with radioactive ^{45}Ca. During vesicle formation calcium cations were adsorbed at the outer and inner liposome membranes.[23] The radioactive tracer was removed from the external medium during liposome preparation.

The measuring set-up was composed of 16 vessels, each containing an outer chamber with a coaxially mounted inner cylindrical chamber with cellophane side walls. The chambers were kept at 25 °C. The inner chamber was filled with the liposome suspension (relative Ca^{2+}/lecithin concentration ratio equal to 1:10), and the outer one with the solution alone. Defined amounts of the organotin compounds studied were added to both compartments to give identical concentrations on both sides of the cellophane wall. The final concentrations ranged between 0.5 and 6.0 mM. In experiments with liposome membrane modification by AS_{12} or TMDA surfactants, defined amounts of stock solutions of those compounds were added at first to both chambers. After one hour of incubation of the liposomes with surfactants, the appropriate amounts of TPT were added to both compartments in concentrations equimolar with the surfactants studied. Aliquots were taken at chosen time intervals and their radioactivity was measured with a Packard liquid scintillation counter. The experiments were repeated four to six times for each compound studied.

The theoretical calculation of the transport and desorption measurements previously described[37] was used, with minor modifications. Briefly, in order to determine the rate constant of the ion desorption process, three-compartmental analy-

sis was used. Calcium ions released from the liposome membrane (first compartment) are in the inner chamber (second compartment) from which they pass through a cellophane membrane to the outer chamber (third compartment). The flux of the ions observed results from the desorption process and permeation from the interior of the liposomes. However, the latter flux is negligibly small because of the very low concentration of Ca^{2+} in the bulk inner medium and its very low permeability through the lipid bilayer.[23]

Solving a system of balanced kinetic equations for the amount of radiotracer present in each compartment, one obtains the following solution for relative radioactivity, U (Eqn [1]):

$$U = (A_\infty - A)/A_\infty = [\beta/(\beta-\alpha)]\, e^{-\alpha t} - [\alpha/(\beta-\alpha)]\, e^{-\beta t} \quad [1]$$

where A_∞ = equilibrium radioactivity (in cpm), determined as $A_\infty = [V_o/(V_o+V_i)]A + [V_i/(V_o+V_i)]A_i$; A_i and A = radioactivity of samples taken from the inner and outer chamber, respectively; V_i and V_o = volume of the inner and outer chamber; t = time; α = rate constant of the calcium ion desorption process from liposome membrane; β = rate constant of the calcium ion transport through cellophane membrane (β was determined in a separate experiment).

Plots of logarithm of the relative radioactivity, ln U, against time were constructed from experimental points. Theoretically calculated curves from Eqn [1] were fitted to them using a computer-programmed Newton iteration method that allows us to determine the optimal value of the rate constant α.

^{1}H NMR experiments

The stock solution of lecithin in chloroform was stored at -20 °C. The lecithin was dried in a nitrogen atmosphere and dispersed in D_2O. The final concentration of lecithin was 25 mg ml^{-1}. The suspensions were sonicated for 30 min with a 20 kHz sonicator with a titanium probe. During sonication the dispersions were thermostated at 0–2 °C. The lecithin dispersion were divided into 0.5 ml samples and, in the case of unmodified liposomes, first Pr^{3+} ions and then the TATs or TMDA were added. In the case of modified liposomes, anionic AS_{12} or cationic TMDA compound was incubated with liposomes for 1 h and after incubation Pr^{3+} ions and TPT compounds were added and NMR data were collected. The concentration ratios of the AS_{12} or TMDA modifiers relative to lecithin concentration were 0.029; 0.057; 0.084 and 0.110. This means that the highest concentration of modifier in the samples was equal to 3.6 mM. The relative concentration ratios of Pr^{3+} ions to lecithin were, for unmodified samples and samples modified by AS_{12}, 1:6.76, and for samples modified by TMDA, 1:3.38.

^{1}H NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer at a temperature of 30 °C. The 300 MHz ^{1}H NMR parameters were: spectral window 6173 Hz; digital resolution 145.2145 Hz/cm or 0.48348 ppm cm^{-1}; pulse width 10.7 μs; acquisition and delay times were 2.65 s and 1 s, respectively.

RESULTS

In Fig. 1, an example of the relation between logarithm of relative radioactivity of samples and time is presented. It can be seen (Fig. 1) that radioactivity of samples in the outer chamber increases with time and is dependent on TPT concentration.

The final results, presented in Figs 2, 3 and 4,

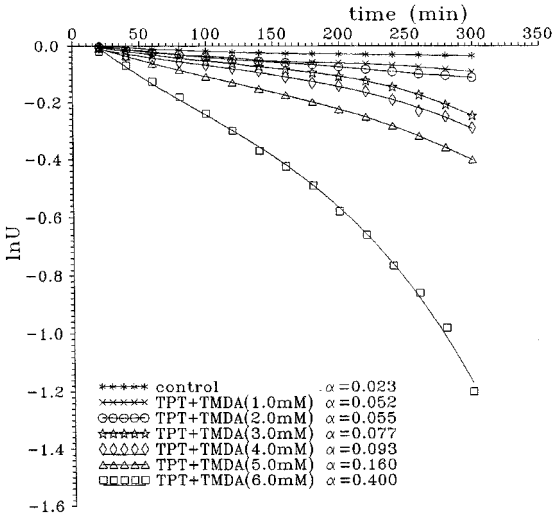

Figure 1 A representative relationship between logarithm of relative radioactivity, ln U, and time for several concentrations of the TPT+TMDA modifiers. $U=(A_\infty - A)/A_\infty$, where A is the radioactivity of the sample taken from the outer chamber and A_∞ is the radioactivity of the sample at infinite time. The theoretical curves (solid lines) have been fitted to experimental points. Values of the kinetic constants α, given in the legend, have been determined from a three-compartmental analysis.

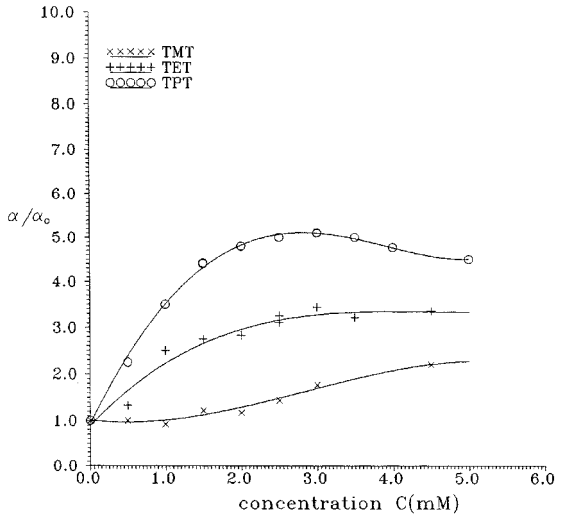

Figure 2 Relative rate constant, α/α_o, for the calcium ion desorption process from liposome membranes against concentration of the TMT, TET and TPT compounds. α and α_o are kinetic constants for modified and unmodified membrane, respectively.

show the relation between the relative rate constant α/α_o of the calcium ion desorption process and the concentration of the compounds studied. It can be seen in Fig. 2 that, in the concentration range studied, an increased concentration of the TMT compound caused a small increase in the rate constant, a little greater increase for the TET compound and the greatest

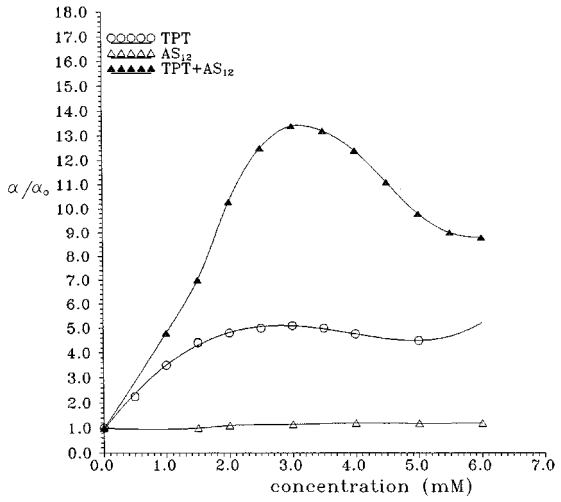

Figure 3 Relative rate constant, α/α_o, for the calcium ion desorption process from liposome membranes against concentration of the AS_{12}, TPT and AS_{12}+TPT compounds. α and α_o are kinetic constants for modified and unmodified membrane, respectively.

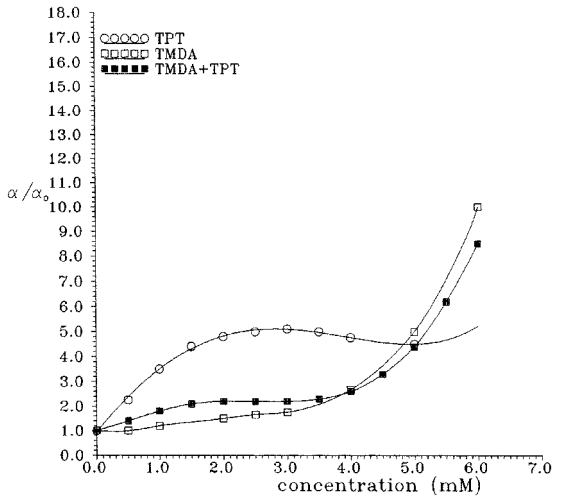

Figure 4 Relative rate constant, α/α_o, for the calcium ion desorption process from liposome membranes against concentration of the TMDA, TPT and TMDA+TPT compounds. α and α_o are kinetic constants for modified and unmodified membrane, respectively.

increase for the TPT compound. Compound TPT causes an increase in its concentration of up to about 3 mM and then α/α_o decreases a little for higher concentrations.

For the most potent compound, TPT, the results of surfactant modification are presented in Figs 3 and 4. In each Figure three curves can be observed: for TPT acting alone (for comparison); for each surfactant acting alone; and for TPT acting together with each surfactant.

No effect can be seen for AS_{12} acting separately (Fig. 3), but for liposomes modified earlier by anionic surfactant the increased equimolar concentration of TPT caused an increase in the rate constant of the calcium ion desorption process up to about 3 mM, and then a decrease. The modifying effect of the cationic surfactant TMDA is presented in Fig. 4. After modifying liposomes with TMDA, a decrease in the rate constant of calcium ion desorption was observed, as compared with a control, up to 5 mM TMDA concentration, and then a sharp increase.

In Fig. 5, curve A shows the complete 300 MHz ^{1}H NMR spectrum for unmodified egg lecithin vesicles in D_2O. The major $(CH_2)_n$, CH_3 and choline headgroup $N^+(CH_3)_3$ resonances of phosphatidylcholines are well recognized.[38,39] Those peak assignments are presented in Table 1. Figure 5, curve B, shows selected spectra of egg PC vesicles in D_2O after addition of Pr^{3+} with an extravesicular egg lecithin, to a Pr^{3+} ion concen-

tration ratio equal to 6.76:1. The initial single peak of the $N^+(CH_3)_3$ resonance is split by the addition of 'shift reagents'. The original 'single' peak is transformed into resolved downfield and upfield components at a distance defined as $\Delta\nu$[Hz].[40] The ratio of the downfield signal from the extravesicular (O) to the upfield resonance from intravesicular (I) choline headgroups was equal to 1.7 for unmodified liposomes, the distance between the two peaks being proportional to the concentration of the praseodymium added to the extravesicular medium.[33,41,42] Figure 5, curve C, shows the spectrum for egg lecithin liposomes modified with the anionic AS_{12} compound, at a concentration relative to lecithin equal to 0.11 after addition of Pr^{3+} ions. Figure 5, curve D, shows the spectrum of the same samples as those in Fig. 5, curve C, after addition of TPT in equimolar concentration relative to AS_{12}. In Fig. 6, A, B, C and D show analogous spectra but for the cationic modifier TMDA and a Pr^{3+}/PC concentration ratio of 1:3.38. The results of studies performed with unmodified liposomes in the presence of increasing amounts of TAT, and with liposome modified with cationic TMDA or anionic AS_{12} compounds in the presence of increasing amounts of TPT, showed a differentiated decrease in the choline headgroup signal splitting ($\Delta\nu$) induced by the compounds added. The percentage of Pr^{3+} desorption from the membrane (being proportional to the decrease in $\Delta\nu$) was calculated as the ratio of the $\Delta\nu$ (Hz) distance in the choline group signal splitting coming from the outer and inner liposome membrane layers after addition of TAT, to the splitting in the absence of TAT. The results of the calculation are presented in Figs 7, 8 and 9. Figure 7 shows the percentage of Pr^{3+} desorption induced by the three TAT compounds versus their relative concentration C_{TAT} (with respect to C_{LEC}) in the suspension of unmodified liposomes. It is easily seen in Fig. 7 that the percentage of ion desorption increases with increasing TAT concentration according to the sequence: TPT > TET > TMT. Figures 8 and 9 show the

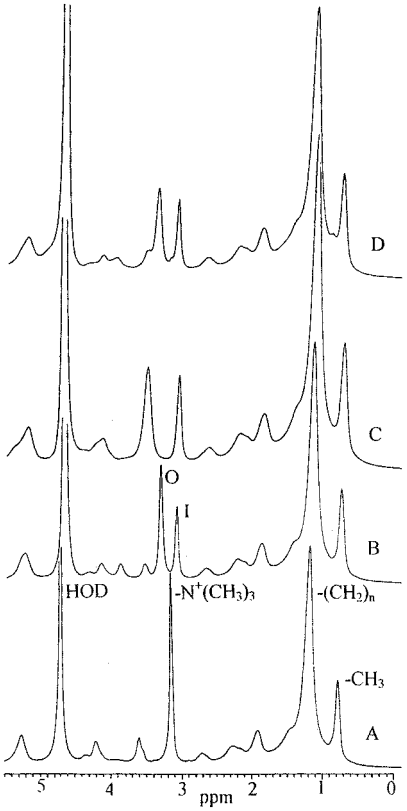

Figure 5 300 MHz ^{1}H NMR spectrum of the egg yolk lecithin liposomes (25 mg lipid/ml) at 30 °C. The HOD peak at 4.700 ppm was used as the reference signal. Curve A, obtained prior to addition of Pr^{3+} to the vesicle suspension; the main signals come from the choline headgroups, $N^+(CH_3)_3$, acyl chain methylenes, $(CH_2)_n$, and terminal methyl groups, CH_3. Curve B, after addition of Pr^{3+} at extravesicular PC/Pr^{3+} concentration ratio = 6.76:1, showing a resolved upfield shift signal from the intravesicular choline headgroup $[N^+(CH_3)_3]_{in}$, and a downfield shift signal from the extravesicular choline headgroup, $[N^+(CH_3)_3]_{out}$. Curve C, liposomes after extravesicular Pr^{3+} addition (at PC/Pr^{3+} concentration ratio = 6.76:1) and modification for 1 h with 1.8 mM AS_{12} compounds. Curve D, spectrum of the samples as in C, after addition of TPT in equimolar concentration relative to AS_{12}.

Table 1. Peak assignments for sonicated egg lecithin liposomes in D_2O before and after addition to external-medium Pr^{3+} cations

Peak assignments[b]	Chemical shift, δ (ppm)[c]
-CH_3	0.827
-$(CH_2)_n$-	1.217
-$N^+(CH_3)_3$	3.187
HOD	4.700
$[-N^+(CH_3)_3]_{out}$	3.340[d]
$[-N^+(CH_3)_3]_{in}$	3.144[d]

[a] Relative concentration of lecithin to Pr^{3-} ions was 6.76:1.
[b] Assignments were made according to Refs 39–42 and standard chemical shift correlation tables.
[c] The peak of HOD at 4.700 ppm was used as the reference signal.
[d] Data after the addition of Pr^{3+}-shift reagent.

percentage of Pr^{3+} ion desorption from liposome membranes induced by the addition of TPT to the suspension of liposomes modified with the anionic AS_{12} compound (Fig. 8) and the cationic TMDA compound (Fig. 9) versus their concentration relative to lecithin. It is apparent in Fig. 8 that the percentage of Pr^{3+} desorption from liposomes modified with the anionic detergent AS_{12}, induced by the added TPT, increases faster with its concentration than it did for unmodified liposomes. A reverse effect can be noted in Fig. 9. Liposome membranes modified with the cationic detergent TMDA showed a markedly lower desorption of Pr^{3+} than unmodified liposomes.

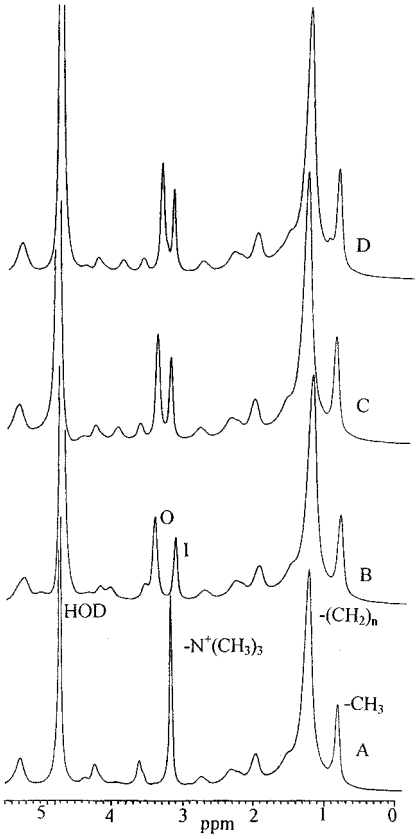

Figure 6 300 MHz ^1H NMR spectra of lecithin liposomes at 30 °C. The HOD peak at 4.700 ppm was used as the reference signal. Curve A, the pure lecithin liposome (25 mg PC/ml). Curve B, after addition of Pr^{3+} ions at PC/Pr^{3+} concentration ratio = 3.38:1. Curve C, lecithin liposomes after extravesicular Pr^{3+} addition (at PC/Pr^{3+} concentration ratio = 3.38:1) and modification for 1 h with 1.83 mM TMDA compounds. Curve D, after addition to the samples as in C of TPT in equimolar concentration relative to TMDA.

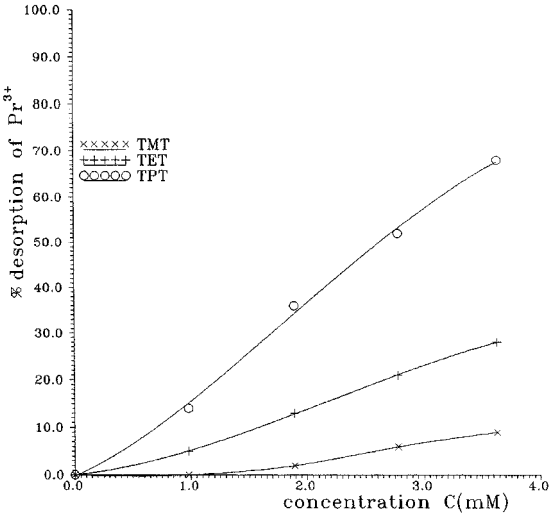

Figure 7 Percentage of Pr^{3+} ion desorption versus concentration of TAT compound added to the lecithin liposome dispersion (25 mg lecithin/ml). Percentage desorption was calculated as the change in the signal splitting caused by the addition of TAT compound to a liposome dispersion in the presence of constant extravesicular concentration (relative to lecithin) of Pr^{3+} ions relative to the signal splitting caused by Pr^{3+} ion in the absence of TAT.

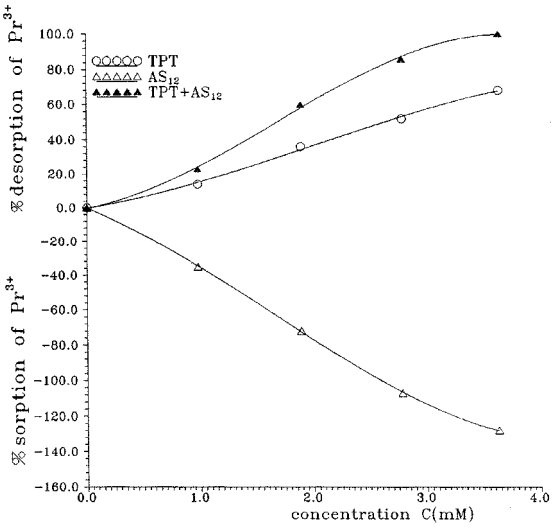

Figure 8 Percentage of Pr^{3+} ion desorption (or sorption) versus the concentration of compound added to the lecithin liposome (25 mg lecithin/ml) dispersion. Percentage desorption (or sorption) was calculated as the change in signal splitting caused by addition of the compounds studied to the liposome dispersion (unmodified or modified by AS_{12}) in the presence of a constant extravesicular concentration of Pr^{3+} ion relative to the change in signal splitting cause by Pr^{3+} ion without any addition. Pr^{3+}/lecithin concentration ratio = 1:6.76.

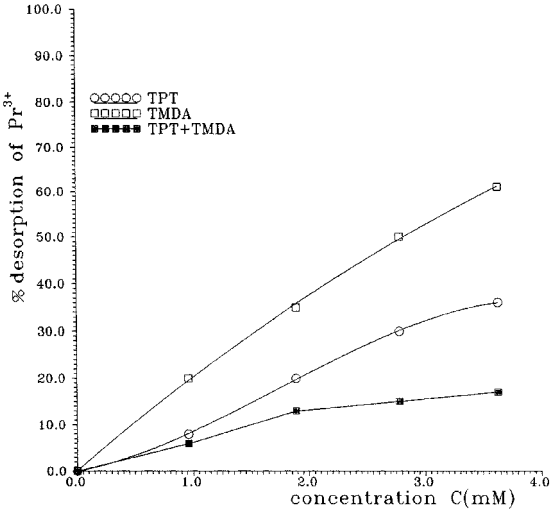

Figure 9 Percentage of Pr^{3+} ion desorption versus concentration of the compound added to the lecithin liposome (25 mg lecithin/ml) dispersion. Percentage desorption was calculated as the change in signal splitting caused by addition of the compound studied to the liposome dispersion (unmodified or modified by TMDA) in the presence of a constant extravesicular concentration of Pr^{3+} ion relative to the change in signal splitting caused only by Pr^{3+} without any addition. Pr^{3+}/lecithin concentration ratio = 1:3.38.

DISCUSSION

It can be seen from the experimental procedure and results described above, and from other papers,[23] that calcium ions are adsorbed at both surfaces of liposomes. The binding sites are localized at the phosphate groups which are the carriers of the negative part of the delocalized charge of the lecithin polar head groups. Praseodymium ions interact also with negatively charged phosphate groups but in our experiments only at the outer layer of the liposome membrane. Single ions like calcium or praseodymium complex more than one phosphate binding center.[43] The presence of adsorbed ions means that, contrary to the membranes of 'pure' lecithin which are electrically neutral in the range of pH from 3 to 10,[44] the membranes used in our experiments have mostly an initially strong positive charge.

The presence of trialkyltin compounds in the liposome dispersion influenced both the calcium ion desorption process and that of praseodymium ions in a similar way. Increased concentration of organotin compounds caused an increased release of both the ions from the membrane up to a concentration of about 3 mM (Figs 2 and 7).

For higher concentrations of TAT compounds it can be seen that the rate constant of calcium ion desorption from the membrane (Fig. 2) assumes a definite character. Two effects acting in opposition may be responsible for such a course of the curves. Both Ca^{2+} ions and TAT compounds can induce a fusion or aggregation of liposomes,[18,45] with a resultant decrease in the surface available for Ca^{2+} desorption. Although desorption is favored by increasing concentration, both of these effects almost cancel out.

Trimethyltin chloride (M_3SnCl) dissolves in water to form a diaquo cation $\{M_3Sn(H_2O)_2\}^+$ and the longer-chain trialkyltin chlorides, which are less soluble in water, probably give similar cationic diaquo species on hydration.[46] Heywood *et al.*[18] suggested that such TAT diaquo ions are weakly associated with the phosphate group of the phospholipid membrane. One may suppose that the TAT ions compete with Ca^{2+} and Pr^{3+} ions for binding centers in the phosphate group, causing their release. The longer the alkyl chains of the compounds studied, the stronger an effect was observed. A similar sequence was observed for toxicity of many biological compounds.[17] This means that the increasing alkyl chain lengths of the organotins, TPT > TET > TMT, may cause an increase in their hydrophobicity, resulting in greater partitioning of the organotin compounds in the lipid phase.[47]

As the tripropyltin chloride causes the strongest effect, for further experiments we have chosen that compound.

Our results, in agreement with those obtained previously in our laboratory with other different surfactants,[30–34,48] and also those reported by other authors,[49,50] suggest that adding such compounds to liposome dispersions results in incorporation of the compounds into the lipid membrane. It can be expected that the hydrophobic part of the compound is localized in the hydrocarbon region of the bilayer, and the hydrophilic part is close to the polar part of the bilayer. The greater the amount of incorporated positively charged TMDA ions, the higher the positive charge of the membrane surface, and certainly in the case of negatively charged AS_{12} ions the negative membrane surface charge will increase.

An increased negative surface charge, because of the incorporation of AS_{12} compound in the liposome membrane, should result in a decreased desorption of the positively charged Ca^{2+} ions

and of a stronger sorption of the Pr^{3+} ions. This effect can be observed for Pr^{3+} ions in Fig. 8, but in the case of Ca^{2+} ions this can almost not be observed because of a very small release of ion from unmodified membrane.

The observed desorption of Ca^{2+} and Pr^{3+} ions from liposome membrane in the presence of TMDA compound (Figs 4 and 9) is the result of competition between these cations and cationic surfactant for the negatively charged binding sites localized on the polar moieties of the lecithin molecules.[18,30–34]

It can be observed in Fig. 3 that AS_{12} acting alone does not increase the rate constant of the calcium ion desorption process, and that the TPT compound acting alone increases the rate constant by no more than five-fold, but when both the compounds act together they cause a 14-fold increase as compared with the control and a three-fold increase as compared with TPT for a 3 mM concentration. This result can be explained in that the incorporated AS_{12} negative ions cause, at constant Ca^{2+} and Pr^{3+} concentrations, a decrease in the positive membrane potential, which is the greater the higher the AS_{12} ionic surfactant concentration; then a membrane neutralization and eventual increase in negative membrane potential occurs. This causes a stronger binding of Ca^{2+} ions, and in the case of Pr^{3+} a greater number of adsorbed ions. Lowering of positive membrane potential or increase of negative membrane potential also facilitates membrane penetration of TPT ions.

The partition coefficient of TPT compound seems to be the dominant factor for concentrations within the range 0–3 mM, which results in an increased number of released Ca^{2+} ions. A maximum occurs at a concentration equal to about 3 mM, and then a decrease in the rate constant of desorption indicates that electrical interactions dominate between Ca^{2+} ions and the negatively charged membrane.

The results presented in Fig. 8 on the desorption of Pr^{3+} ions from liposome membranes modified with AS_{12}, induced by the TPT compound, indicate that the modifier acts as in the case of calcium ions, i.e. an increase of Pr^{3+} ion desorption occurs as compared with the control. The range of AS_{12} and TPT concentrations where a maximum might occur was not investigated. The reason for this is that the praseodymium concentration would have to be a few times higher than that of calcium and thus exceed its critical micelle concentration (CMC) in the solution studied.[51]

Contrary to the anionic surfactant, the cationic surfactant causes a decrease in the effectiveness of TPT action. As follows from Fig. 4, the cationic surfactant TMDA when acting alone causes a very weak increase in the Ca^{2+} ion desorption process for concentrations lower than about 4 mM, and is several times less effective than TPT in its separate action on the membrane. The reason must be the greater hydrophobicity of the TPT than the TMDA compound and hence a greater partition coefficient between the membrane and medium. However, pretreatment of a membrane with a cationic surfactant causes, with a very weak desorption of calcium ions, an increase in positive membrane potential that makes it more difficult for the TPT aquo ions to enter the membrane, with resultant weakening of their effect on the desorption process. With concentrations of the compounds greater than 4 mM one observes a rapid increase in the effectiveness of TMDA in ion desorption, which is observed also with other compounds of this type and explained by the 'threshold' effect.[52]

As seen in Fig. 9, the cationic surfactant TMDA in its action by itself on the liposome membrane causes a substantial increase in the number of membrane-released Pr^{3+} ions with increasing concentrations of the surfactant up to *ca* 4 mM, whereas Ca^{2+} desorption for the same concentrations is slight (Fig. 4). The great effectiveness of the surfactant in Pr^{3+} desorption as compared with Ca^{2+} release seems to be due to the Pr^{3+} concentration, which is several times higher than the Ca^{2+} concentration. However, a prior modification of liposome membranes with a cationic surfactant results, as was the case with Ca^{2+} ions, in an increase in positive membrane potential, which makes it more difficult first for Pr^{3+} ions and then also for TPT aquo ions to penetrate the membrane. The resultant weakening of the desorption process is seen also in Fig. 9 (compared with the release from unmodified membrane).

CONCLUSIONS

Trialkyltin (TAT) compounds exert an effect on the surface phenomena of the model biological membrane, which is the phospholipid liposome membrane. The way the adsorption (or desorption) process proceeds depends on the elec-

trical properties of both the compounds studied and the membrane. The effectiveness of the TAT compounds depends on their concentration and increases with increasing length of their alkyl chains, which may be due to increasing hydrophobicity and the concomitant increase in the partition coefficient between the lipid phase and medium.

Modifying a membrane with surfactants changes the electrical properties of the membrane, with a resultant change in effectiveness of the TAT compounds. Depending on the surfactants charge, the effect can be enhanced or totally inhibited.

This synergistic effect, where the binary mixture of TAT compounds and an anionic surfactant performs better than either of its components, may be useful in the pesticidal action of the compound, but should be perceived as an increased environmental threat. It must be stressed that the synergism of the mixture of TAT compounds and a cationic surfactant—two groups of compounds which when acting alone are a threat to the environment—may result in a protective action.

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