Organometallics and Quaternary Ammonium Salts Affect Calcium Ion Desorption from Lecithin Liposome Membranes

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Organometallics, Quaternary Ammonium Salts, Cooperative Effect

The objective of the present work was to compare the effects of groups of tin and lead organometallic compounds and their mixtures with amphiphilic quaternary ammonium salts (QAS) on the process of calcium ion desorption from lecithin liposome membranes, as dependent on the properties of the hydrophilic and hydrophobic parts of QAS. In the investigations the method of radioactive labels was applied. Synergism and antagonism in the action of both groups of compounds were found. The effectiveness of the cooperation depended more on chain length of QAS compounds than on the size and polarity of their hydrophobic parts. The most effective of all compounds studied was a mixture of benzylidimethylammonium chloride in a mixture with tripropylditin. Since the rate of calcium desorption proved to be a good measure of efficacy of biologically active surfactants, it seems that the conclusions reached in this paper may be useful for choosing compounds which are able to decontaminate the environment polluted with heavy metals.

Introduction

This study is concerned with the effect of some organometallic compounds on liposomes a model biological membranes, or a mixture of these compounds and cationic surfactants, amphiphilic quaternary ammonium salts (QAS). Both groups of compounds have a wide range of industrial application (Blunden et al., 1985; Crowe, 1987; Lindstedt et al., 1990; Rubingh and Holland, 1991). Their biological effects have attracted much attention since both groups of compounds may contribute to an environmental pollution (Craig, 1982; Davis and Jordan, 1989; Fent, 1996).

The molecular nature of the organometallic – membrane interactions is not yet fully clear; not clear is also the nature of the joint action of a mixture of both above mentioned groups of compounds, so we thought it useful to carry out an investigation on this topic.

Our preliminary investigations of some organotin compounds and only one mixture of tripropylditin with the cationic surfactant trimethyldeacylammonium bromide showed that the organometallic compounds studied had an effect on calcium ion desorption from lecithin liposomes, and the cationic surfactant inhibited the effectivity of tripropylditin (Kuczera et al., 1997). This effect seemed interesting enough to undertake further studies with a greater group of organotins and also with organoleads, and with mixtures of both groups of organometallics with a group of cationic surfactants with different properties of their hydrophilic and hydrophobic parts.

Materials and Methods

Materials. Egg lecithin (PC) was prepared according to Singleton et al. (Singleton et al., 1965). The compounds used, i.e. organometallic compounds and amphiphilic quaternary ammonium chlorides are presented in Table I. All chemicals were of analytical grade.

Radioactive tracer experiments. Small unilamellar liposomes (SUV) were prepared from yolk lecithin by the sodium cholate method in Liposmat (DIANORM) (Wedder and Zumbuhl, 1984). The solution used to form vesicles contained veronal-acetate buffer of pH 7.5 and 0.3 mM CaCl₂ labelled with radioactive Ca-45. During vesicle for-
Table I. Compounds studied.

<table>
<thead>
<tr>
<th>Code</th>
<th>Chemical structure</th>
<th>Chemical name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDDA</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>N-benzyl-N,N-dimethyl-N-dodecylammonium chloride</td>
<td>FLUKA</td>
</tr>
<tr>
<td>BDTA</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>N-benzyl-N,N-dimethyl-N-tetradecylammonium chloride</td>
<td>ALDRICH</td>
</tr>
<tr>
<td>BDHA</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>N-benzyl-N,N-dimethyl-N-hexadecylammonium chloride</td>
<td>FLUKA</td>
</tr>
<tr>
<td>MTM</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>N-methyl-N-tetradecylmorpholinium bromide</td>
<td>*</td>
</tr>
<tr>
<td>TMDA</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>N,N,N-trimethyl-N-dodecylammonium bromide</td>
<td>SERVA</td>
</tr>
<tr>
<td>TMTA</td>
<td><img src="image6" alt="Chemical structure" /></td>
<td>N,N,N-trimethyl-N-tetradecylammonium bromide</td>
<td>FLUKA</td>
</tr>
<tr>
<td>TMHA</td>
<td><img src="image7" alt="Chemical structure" /></td>
<td>N,N,N-trimethyl-N-hexadecylammonium bromide</td>
<td>FLUKA</td>
</tr>
<tr>
<td>TAT</td>
<td><img src="image8" alt="Chemical structure" /></td>
<td>trimethyltin chloride triethyltin chloride tripropyltin chloride triphenyltin chloride</td>
<td>MERCK FLUKA</td>
</tr>
<tr>
<td>TAL</td>
<td><img src="image9" alt="Chemical structure" /></td>
<td>trimethyllead chloride triethyllead chloride tripropylead chloride triphenylead chloride</td>
<td>ACRC FLUKA</td>
</tr>
</tbody>
</table>

* – Compound synthesised in the Institute of Organic Chemistry and Polymer Technology of the Technical University of Wroclaw.

...mation calcium cations were adsorbed at the outer and inner liposome membranes (Kuczera and Żyłka, 1979). 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 co-axially mounted inner cylindrical chamber with cellophane side walls. The chambers were kept at 25 °C. The inner chamber was filled with the lipo-
some suspension (with Ca$^{2+}$ to lecithin concentration 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 QAS surfactants, defined amounts of stock solution of those compounds were added at first of both chambers. After one hour incubation, to the liposomes with surfactants proper amounts of organometallic compounds were added to both compartments in equimolar concentrations of the surfactants studied. Aliquots were taken at chosen time intervals and their radioactivity was measured. The experiments were repeated 4–6 times for each compound studied. Standard error was below 10%.

The theoretical work-out of the transport and desorption measurements as previously described (Mazgis and Kuczera, 1981) was used with minor modifications. Briefly, in order to determine the rate constant of the ion desorption process, a three-compartment analysis was used. Calcium ions released from the liposome membrane (first compartment) were present in the inner chamber (second chamber) and from there they passed through a cellophane membrane to the outer chamber (third compartment). The ion flux 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 (Kuczera and Żylka, 1979).

Solving a system of kinetic equations of balance for the amount of radiotracer present in each compartment, the following solution for relative radioactivity, $U$, is obtained:

$$U = (A_{\infty} - A)/A_{\infty} = \left[\beta/\beta - \alpha\right] e^{-\alpha t} - \left[\alpha/\beta - \alpha\right] e^{-\beta t}$$  \hspace{1cm} (1)

with:

$A_{\infty}$ - equilibrium radioactivity (in cpm), determined as $A_{\infty} = [V_0/(V_0+V_1)] A_i + [V_1/(V_0+V_1)] A_o$; $A_i$ and $A_o$ - radioactivity of samples taken from the inner and outer chamber, respectively; $V_i$ and $V_0$ - volumes of the inner and outer chamber; $t$ - time, $\alpha$ - rate constant of calcium ion desorption process from the liposome membrane, $\beta$ - rate constant of calcium ion transport through the cellophane membrane ($\beta$ 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 equation (1) were fitted to them using a computer-programmed Newton iteration method that allows to determine the optimal value of the rate constant $\alpha$ (Kubica et al., 1994).

**Experimental conditions**

In order to determine the effect of hydrophobic groups in the organometallic compounds on the calcium ion desorption process, the organotin and organolead compounds presented in Table I were used. The organometallic compounds and one of the cationic surfactants, namely TMDA, in equimolar mixtures were used to determine the effect on the calcium ion desorption process of mixtures of organometallic compounds and cationic surfactants as dependent on the number of hydrophobic groups in organometallics. The influence of chain length of surfactants was determined with two groups of cationic surfactants: TMDA, TMTA, TMHA and BDDA, BDTA, BDHA in equimolar mixtures with tripropyltin or tripropyllead. The effect of electric and steric properties of the polar parts of surfactants was determined with three compounds with the same alkyl chain and different polar parts (BDTA, MTM and TMTA) in equimolar mixtures with tripropyltin and tripropyllead.

**Results and Discussion**

The results of kinetic studies on the calcium ion desorption process are presented in Figs 1 and 2, where the relative rate constants are plotted against concentration of the compounds when present in the solutions alone and in mixtures with cationic surfactants. In Tables II and III, the values of the relative rate constant are presented for chosen concentrations. The relative rate constant $\alpha/\alpha_0$, being a measure of compound’s efficiency, is defined as the ratio of the rate constant of calcium ion desorption in the presence of the compounds studied to that measured in the absence of modifiers. As follows from Fig. 1a, the coefficient $\alpha/\alpha_0$...
Table II. Relative rate constant, \( \alpha/\alpha_0 \), of calcium ion desorption from liposome membranes for using a 2 mM concentration of the TMDA compound and its mixtures with organotins.

<table>
<thead>
<tr>
<th>Compounds and mixtures</th>
<th>( \alpha/\alpha_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMDA</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>TMT</td>
<td>1.33 ± 0.3</td>
</tr>
<tr>
<td>TMT+TMDA</td>
<td>1.22 ± 0.4</td>
</tr>
<tr>
<td>TET</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>TET+TMDA</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>TPT</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>TPT+TMDA</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>TPhT</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>TPhT+TMDA</td>
<td>1.2 ± 0.4</td>
</tr>
</tbody>
</table>

Table III. Relative rate constant, \( \alpha/\alpha_0 \), of calcium ion desorption from liposome membranes for a chosen concentration of tripropylin and tripropyplead compounds and their mixtures with alkylbenzylammonium chlorides and alkyltrimethylammonium chlorides.

<table>
<thead>
<tr>
<th>Compounds and mixtures</th>
<th>Concentration [mM]</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPT</td>
<td>3.5 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>TPL</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>BDDA</td>
<td>5.0 ± 0.5</td>
<td>12 ± 0.8</td>
<td>27 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>BDTA</td>
<td>16.0 ± 1.0</td>
<td>30.0 ± 1.0</td>
<td>46 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>BDHA</td>
<td>12.5 ± 1.0</td>
<td>17.0 ± 1.0</td>
<td>16.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>BDDA+TPT</td>
<td>4.0 ± 0.6</td>
<td>7.5 ± 0.6</td>
<td>12.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>BDTA+TPT</td>
<td>10.0 ± 1.0</td>
<td>14.0 ± 1.0</td>
<td>15.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>BDHA+TPT</td>
<td>12.0 ± 1.0</td>
<td>14.5 ± 1.0</td>
<td>16.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>BDDA+TPL</td>
<td>6.5 ± 0.7</td>
<td>9.5 ± 0.7</td>
<td>13.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>BDTA+TPL</td>
<td>8.0 ± 0.8</td>
<td>14.0 ± 0.8</td>
<td>17.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>BDHA+TPL</td>
<td>3.5 ± 0.6</td>
<td>8.5 ± 0.6</td>
<td>14.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>BMDA</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>TMDA</td>
<td>7.0 ± 0.8</td>
<td>16.0 ± 0.8</td>
<td>28.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>TMDA</td>
<td>6.0 ± 0.9</td>
<td>13.0 ± 0.9</td>
<td>22.0 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>2.0 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>5.0 ± 0.7</td>
<td>11.0 ± 0.7</td>
<td>16.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>6.5 ± 0.8</td>
<td>14.0 ± 0.8</td>
<td>20.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>1.5 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>3.5 ± 0.6</td>
<td>8.5 ± 0.6</td>
<td>14.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>TMDA+TPT</td>
<td>5.5 ± 0.7</td>
<td>10.0 ± 0.7</td>
<td>14.5 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

increased with increasing concentration of alkyl organotins (TAT) until a concentration of about 3 mM, the increase became stronger with longer alkyl chains. For the most effective compound TPT the increase was 5-fold. Above 3 mM concentration \( \alpha/\alpha_0 \) did not change, even decreased for TPT. The curve describing the action of TPhT runs between the curves for TET and TPT. In the case of organolead compounds (TAL) as follows from
Fig. 1b, the relative rate constant increased with concentration only for TPL, and the effect was weaker than for TPT.

The differences in effectiveness between organotins and organoleads in their action on calcium ion desorption from lecithin liposome membranes seem to result from properties of both the groups of compounds and properties of the medium and membrane. Within each group of trialkyl compounds an increase in chain length results in increased hydrophobicity, and hence increased partition coefficient between the membrane and medium. Though the molecular size increases also, imparting lower mobility and partition coefficient, but surely the increased hydrophobicity is the dominant factor. However, in the case of phenyl compounds the dominant factor is their size, which is substantially greater than that of other compounds. The same sequence of effectiveness of organometallics has been observed for processes and objects other than those studied here (Gray et al., 1987; Boyer, 1989).

Under our experimental conditions, pH 7.5, organotins are present in non-ionic forms as \( R_3 SnOH \times H_2 O \), while organoleads exist almost exclusively as \( R_3 Pb(H_2 O)_2 \) \(^+\) (Tobias, 1978). The positively charged membrane, due to calcium ion adsorption, is easier accessed by the non-ionic molecules than their positively charged counterparts. The ionic molecules locate inside membrane, inducing structural changes, weaken the ionic bounds between \( Ca^{2+} \) and membrane with resulting desorption. The ionic molecules locate in the hydrophilic part of the membrane, or in the adjacent hydrophobic layer, so that \( Ca^{2+} \) ions are released as a result of competition for binding sites. Among the organolead ions only TPL with the longest alkyl chain can anchor in the membrane and effectively compete with calcium ions for binding sites.

The following Figures and Tables present the dependence of \( \alpha/\alpha_0 \) on concentration not only for the tested mixtures of the organometallic compounds with QAS compounds but also, for comparison, include curves and data for QAS compounds acting alone (results published previously by Kuczera et al., 1996). Among the QAS compounds chosen for the study of the cooperate effect only TMDA does not exert any action at concentrations up to 3.5 mM. Selection of this compound allowed to differentiate the cooperation of the respective organometallic compounds with long-chain cationic compounds.

In Table II are presented values of the relative rate constant \( \alpha/\alpha_0 \) for a chosen concentration, \( C = 2 \) mM, of organotin compounds, TMDA and their mixture. As documented, a long-chain cationic compound present in the membrane caused similar results for almost all organotins, namely a decreased efficacy of the compounds on the desorption process. The decrease for TMT, TET and TPhT was found at almost the same level, which is slightly lower than the efficacy of TMDA alone. Only for TPT the effectiveness is a little greater than for TMDA alone. From the results obtained for the organoleads it can be concluded that the compounds TML, TEL and TPhL in mixtures with TMDA in liposome dispersion act almost the same as TMDA alone, while TPL was found to be markedly more effective when in mixture with TMDA.

It is evident that for the weaker acting organometallic compounds of tin and lead, i.e. TMT, TET, TPhT and TML, TEL, TPhL, the interaction is dominant between the membrane and TMDA. As a result of incorporation of the cationic compound into the membrane its positive potential on the surface increases, and for the weakly acting cationic compounds the membrane is even more difficult to access. For the stronger acting propyltin, TPT, the cooperation of these molecules with TMDA molecules is sufficient to overcome the calcium-membrane binding forces and increased desorption results for both TPT and TMDA when applied separately. This corresponds to the „threshold effect” observed earlier for TMDA in its separate action (Kuczera et al., 1988), indicating that its effect begins above a certain concentration. The observed lowering of the effectiveness of the TMDA+TPT mixture relative to TPT may result from a more difficult incorporation into the membrane of TPT molecules in ionic and non-ionic forms due to competition with TMDA molecules present in the membrane.

For the most effective of the organometallic compounds, i.e. TPT and TPL, their cooperation with cationic compounds that differ in their hydrophobic and hydrophilic parts has been investigated.

Fig. 2 presents the results obtained for mixtures of TPT with three amphiphilic cationic compounds
that differ in their polar heads. For comparison the
dependence of \( \alpha/\alpha_0 \) on concentration of the
compounds studied is shown when applied separately.
As follows from Fig. 2, all mixtures operate weaker
than the ammonium salts acting separately, but sev-
teral times stronger than the organometallic com-
ponents. The action of all the mixtures is almost the
same. For mixtures of TPL with a cationic com-
 pound the courses of the relationships are very sim-
ilar to those for mixtures with tripropyltin. The sub-
 substantial weakening of the effectiveness of QAS+
TPT mixtures, seen in Fig. 2, compared with the sin-
gle action of the compounds, may result from struc-
tural changes in the membrane after incorporation
of organometallic compounds. Due to their large
cross-section the organometallic molecules may
displace QAS molecules from the membrane bind-
ing centres, and thus weaken the interaction be-
tween the centres and QAS molecules.

The considerable differences in the effects of
QAS compounds with different polar parts were
reduced when present in mixtures with com-
 pounds TPT and TPL. This seems to be due to the
dominating role of molecular size of the organo-
metallics compared with the structural differences
between the respective QAS molecules.

At variance to the results presented in Table II
for TMDA, TPT compounds and their mixture
TMDA+TPT, for the strongly acting QAS a
stronger mixture effect was observed than with the
organometallic compounds alone. It is thus appar-
ent that the efficacy of QAS compounds is the do-
nominate factor.

Table II presents data for mixtures of com-
 pounds TPT and TPL with two groups of com-
 pounds: alkylbenzylammonium chlorides and al-
kytrimethylammonium chlorides, which differ in
their alkyl chains lengths. Values of the relative
rate constant \( \alpha/\alpha_0 \) for three chosen concentrations
of the compounds studied are documented. Anal-
yzing the results, it can be stated that for mixtures
of the homologous series of quaternary ammoni-
um salts with the two organometallic compounds
the dependence on chain length of QAS gives a
clearer differentiation of the results than the de-
pendence on polar head differences. In all the
cases, aside of the TMDA+TPT and TMDA+TPL
mixtures previously analysed, a lowering in the ef-
cicacy of QAS compounds in the presence of orga-
nometallic compounds is observed, or increased
efficacy of organometallic compounds in the pres-
ence of QAS. The cut-off effect (Gabrielska et al.
1981; Balgavy and Devinsky, 1996), observed for
separately acting QAS compounds, (with a maxi-
mum of effectiveness observed mostly for 14-car-
bon-atom chains), is disturbed by the addition of
organometallic compounds. For the mixture
BDTA+TPL this effect is still observed, although
for the other mixtures a steady increase with QAS
alkyl chain length is observed. As in the cases pre-
sented in Fig. 2, the greatest changes in efficacy of
mixtures in comparison with the compounds alone
are observed for BDTA, which is the most effec-
tive compound for both groups. Apparently the
location of the compound molecules, whose polar
heads are nearest to the membrane active centres
and thus to \( \text{Ca}^{2+} \) ions, apparently is caused by
disturbances of the organometallic compounds
that are incorporated into the membrane.

The results presented in this paper indicate both
synergism and antagonism in the action of com-
 pounds of the two groups. By proper choice of an
organometallic compound and an amphiphilic
quaternary ammonium salt one can obtain either
an increase or decrease in the rate constant of
calcium ion desorption from phospholipid mem-
branes. In view of our previous studies, showing a
far-reaching coincidence between standard tests
on fungicidal compounds and the effect of biolo-
gically active compounds on ion desorption pro-
cess from liposome membranes (Gabrielska et al.
1979; Rucka et al., 1980), one can expect that syn-
ergism of the groups of compounds studied may
be of importance for problems of environment
protection. Thus, if we want to weaken the action
of organometallic compounds, very weakly acting
cationic compounds should be applied in addition.
If, instead, increased efficacy is needed, cationic
amphiphilic compounds of strong action should be
used. In case biological tests confirm the mutual
influence of the groups of compounds studied, the
method may be applied for decontamination of
soils polluted with heavy metals, by cultivating
proper plants (Wierzbicka and Panufnik, 1988).

Acknowledgements

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Boyer I. J. (1989), Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and experimental animals. Toxicology 55, 253–298.


