Effect of Amphiphilic Cationic Compounds on Calcium Ion Desorption from Lecithin Liposome Membranes. Kinetic Studies and Quantum Chemical Calculations

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The effect of four amphiphilic cationic compounds that differ in their polar head properties on the kinetics of calcium ion desorption from unilamellar lecithin liposome membranes has been studied. The compounds under investigation were: N-benzyl-N,N-dimethyl-N-tetradecylammonium chloride (BDTA), N-methyl-N-tetradecylmorpholinium bromide (MTM), N-methyl-N-dodecyloxymethylenemorpholinium chloride (MDOM) and N,N,N-trimethyl-N-tetradecylammonium bromide (TMTA). Kinetic constants were determined using the three-compartmental analysis for isotopic labels.

For the above mentioned compounds electronic structure calculations were performed and point charges and dipole moments of the molecules determined by using quantum chemistry methods.

It was found that the rate of desorption increases with increasing concentration of the compounds studied, and effectiveness of the compounds follows the sequence: BDTA>MTM>TMTA>MDOM. For dipole moments the sequence obtained is somewhat different, namely: BDTA>MTM>MDOM>TMTA. Apparently, the differences in the effectiveness of action of the compounds in the calcium desorption process follow not only from the values of dipole moments of their polar heads but also from the values of point charges on some atoms and from steric properties.

Introduction

The role of membrane as the first target for the surface active substances has been studied for many years (Isomaa *et al.,* 1989; Lindstaed *et al.,* 1990; Devinsky *et al.,* 1991; Przestalski and Kuczera, 1992), as well as their influence on model phospholipid membranes (Grupe *et al.,* 1978; Gabrielska *et al.,* 1981; Kuczera *et al.,* 1983, 1988, 1989; Przestalski *et al.,* 1983; Frischleder *et al.,* 1984; Sarapuk *et al.,* 1984; Subczyński *et al.,* 1988; Gallova *et al.,* 1990). To determine the molecular mechanism of amphiphiles action on membranes, the role of different physico-chemical properties of the substances in their effect on membranes were studied (Gabrielska *et al.,* 1979, 1981; Kuczera *et al.,* 1983, 1985, 1987; Subczyński *et al.,* 1988; Balgavy and Devinsky, 1994).

The calcium ion plays an important and very known role in living organisms, and is investigated very extensively in biological and model systems (Wallis *et al.,* 1993; Mittlerneher and Knoll, 1993; Isomaa *et al.,* 1994). It adsorbs very well to the surface of phospholipid bilayer (Kuczera and Żyłka, 1979; MacDonald and Seelig, 1987) and its permeability throught the lipid bilayer is very low, which allows to neglect the flux of permeating ions in the desorption measurements (Kuczera and Żyłka, 1979). For this reason we choose the calcium ion desorption process as the phenomenon which can give information not only about the desorption process, but also about the properties of membrane surface. The desorption of calcium ions can also be used as a measure of the biological activity of particular surfactants (Kuczera *et al.,* 1988).

In our earlier studies (Gabrielska *et al.,* 1979; Kuczera *et al.,* 1983, 1987, 1988) the role of the electric field distribution in the polar head of the amphiphilic ammonium salts in calcium ion de-

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sorption process was discussed, but no additional, experimental or theoretical, studies were performed. In the present paper the calcium ion desorption process from the liposome membrane modified with four cationic amphiphiles, that differ in their polar head properties, has been investigated. The quantum chemical methods were used for the electronic structure studies on the four chosen cationic surfactants.

Materials and Methods

Amphiphiles used are presented in Table I. The compounds MTM and MDOM were synthesized in the Institute of Organic Chemistry and Polymer Technology of the Technical University of Wrocław. Elemental and spectral analysis confirmed the identity of compounds structures shown in Table I. Purity of the compounds was not less than 98%.

Liposomes. Small unilamellar liposomes (SUV) were prepared from egg yolk lecithin by using sodium cholate in Liposomat (Dianorm) (Weder and Zumbuhl, 1984). Lecithin was prepared according to the technique described by Singleton (Singleton *et al.,* 1965). The solution used to form vesicles contained a veronal-acetate buffer, pH 7.5 and 0.3 mmol/dm^3 $CaCl^2$ labelled with radioactive tracer, Ca-45. During the vesicle formation calcium cations were adsorbed at the outer and inner liposomes membranes. The radioactive tracers were removed from the external medium during liposomes preparation.

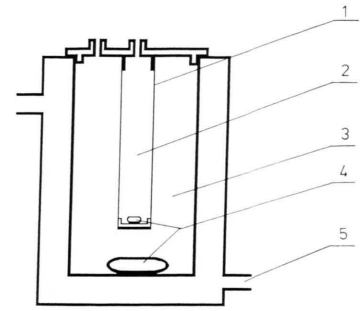
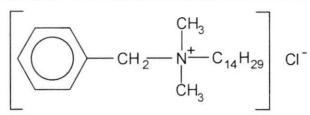
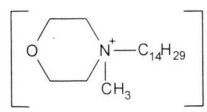
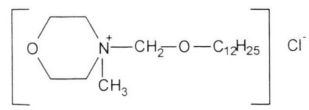
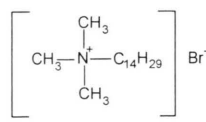


Fig. 1. Measuring set-up. 1 = cellophane membrane, 2 = inner chamber, 3 = outer chamber, 4 = stirrers, 5 = outlet to the thermostat.

Measurements of calcium ion desorption. The measuring set-up was composed of 16 vessels. A simplified scheme of a vessel is presented in Fig. 1. Each vessel contains 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, and the outer one with the solution alone. Defined amounts of the stock solution of amphiphiles studied were added to both com-

Table I. Compounds studied.

Code	Chemical structure	Name	Company
BDTA		N-benzyl-N,N-dimethyl-N-tetradecylammonium chloride	FLUKA
MTM		N-methyl-N-tetradecyl-morpholinium bromide	*
MDOM		N-methyl-N-dodecyl-oxymethylenemorpholinium chloride	*
TMTA		N,N-trimethyl-N-tetradecylammonium bromide	SERVA

* Compounds were synthesized in the Institute of Organic Chemistry and Polymer Technology, Technical University of Wrocław.

partments to give an identical concentrations in both sides of the cellophane wall. The final detergent concentrations ranged between 0.5 and 7.0 mmol/dm^3 Aliquots were taken at chosen time intervals and their radioactivity was measured with a liquid scintillation counter. These data allowed to calculate relative radioactivity of samples, U defined as: $U = (A_\infty - A)/A_\infty$, where A_∞-radioactivity at infinitive time, i.e. equilibrium radioactivity (in cpm), determined as $A_\infty = [V_0/(V_0 + V_i)]A + [V_i/(V_0 + V_i)]A_i$; A_i and A-radioactivity of samples taken from the inner and outer chamber, respectively; V_i and V_0-volume of the inner and outer chamber, respectively. The experiments were repeated 4–6 times for each compound studied.

The theoretical work-out of the transport and desorption measurements described previously (Mazgis and Kuczera, 1981) was used, with minor modifications. In order to determine the rate constant α of ion desorption, the three-compartmental analysis was used. Calcium ions released from the liposome membrane (first compartment) are in the inner chamber (second compartment) from there 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 the very low concentration of Ca^{2+} in the bulk inner medium and its very low permeability through the lipid bilayer (Kuczera and Żyłka, 1979).

Solving the system of kinetic equations of balance for the amount of radiotracer present in each compartment, one obtains the following solution for relative radioactivity, U

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

where: t-time, α-rate constant of the calcium ion desorption process from liposome membrane, β-rate constant of calcium ion transport through cellophane membrane (β was determined in a separate experiment).

Plots of logarithm of the relative radioactivity, lnU, against time were constructed from the 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 α (Fig. 2).

Quantum chemical calculations. Electronic structures of the systems under consideration have been calculated using the non-empirical molecular orbital method. In all cases the molecular geometries of the cationic surfactants were optimized by using the PM3 all-valence approach (Stewart,

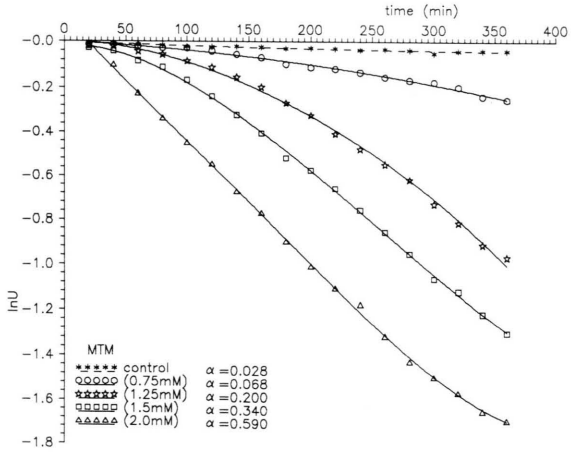


Fig. 2. A representative relationship between logarithm of relative radioactivity, ln$U$, and time for several concentrations of the MTM modifier. $U = (A_\infty - A)/A_\infty$, where A – is radioactivity of sample taken from the outer chamber, A_∞ – radioactivity of sample at infinite time. The theoretical 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.

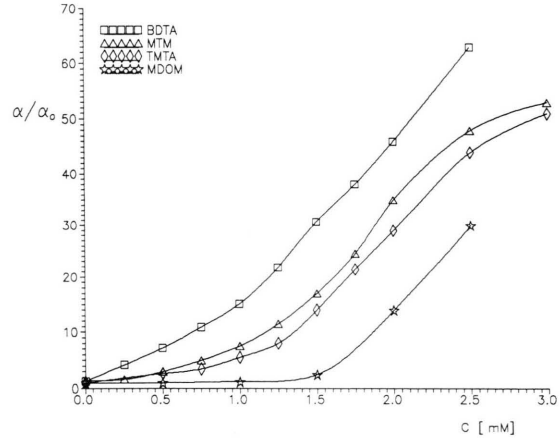


Fig. 3. Relative rate constant, α/α_o, of calcium ion desorption from liposome membranes against concentration of the compounds studied. α and α_o are kinetic constants for modified and unmodified membrane, respectively.

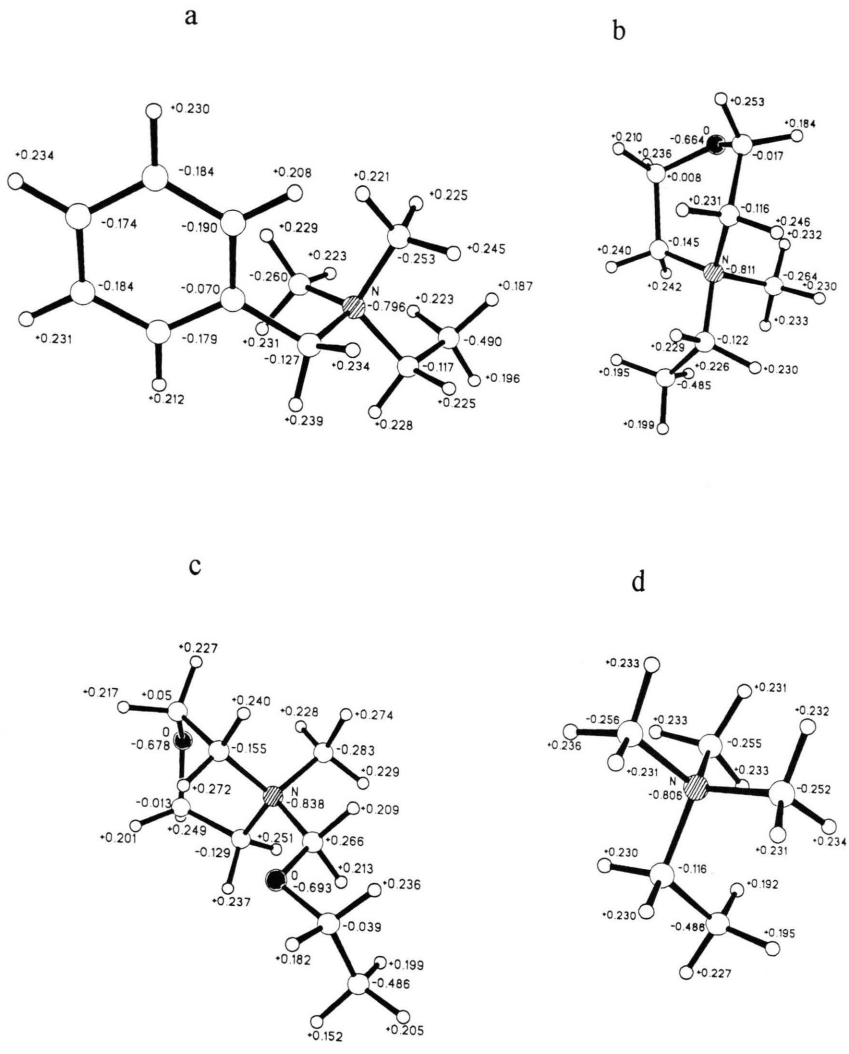


Fig. 4. Optimized structure and respective atomic net charges for: BDTA (a), MTM (b), TMTA (c), MDOM (d). The mark denote: ▨–N, ●–O, ◯–C, ○–H.

1989). In the next step of ab initio calculations the GAMESS package of computer programs (Schmidt *et al.*, 1993) was used. This calculation has been performed for standard choice of coordinates, i.e. with their origin at the centre of molecular mass. In order to simplify the computations, the alkyl chains of the systems were limited to two carbon residues. All results presented here were obtained at the Hartree-Fock level of the MO LCAO SCF formalism.

Based on the SCF molecular orbitals, the electron densities, point atomic charges and the relevant dipole moments were evaluated according to the Mulliken population analysis.

Results

The results of kinetic studies on the calcium ion desorption process are presented in Fig. 3, where the relative rate constants are plotted against concentration of the compounds studied. The relative rate constant α/α_o has been defined as the ratio of the rate constant of the calcium ion desorption process in the presence of a compound studied, α,

Table II. The calculated dipole moment values*.

Compound	Dipole moment [D]
BDTA	5.539
MTM	5.037
MDOM	2.874
TMTA	1.291

* Evaluated for the coordinate origin at the centre of mass.

to that, α_o, measured in the absence of the modifier. The standard error was below 10%.

It can be seen that for all the compouds studied an increase in concentration results in an increase in the relative rate constant. The most effective is compound BDTA; at a concentration of 2.5 mM BDTA causes near 60-fold enhancement in the desorption rate constant. The effectiveness of MTM compoud is smaller than that for BDTA and a little bit greater than for TMTA. MDOM acted much weaker than all the other compouds studied. MDOM causes measurable effect at a concentration of 1.5 mM, and its effectiveness increases almost 32-fold at a concentration of 2.5 mM.

Fig. 4 shows the results of the atomic net charges for analogs of all the compounds studied. Tab. II presents dipole moments calculated under the assumption that the dipole moment for alkanes is equal to zero and the origin of coordinate systems was taken at the mass center in all cases. It can be seen in Table II that the dipole moment value of BDTA is greatest, smaller is that for MTM; two times smaller than for BDTA is the dipole moment for MDOM, and the smallest one is for TMTA.

Discussion

Our results, in agreement with those obtained previously in our laboratory with similar amphiphilic ammonium salts (Gabrielska *et al.*, 1979, 1981; Kuczera *et al.*, 1983, 1987, 1988, 1989; Sarapuk *et al.*, 1984; Subczyński *et al.*, 1988), and also those reported by other authors (Eliasz *et al.*, 1976; Grupe *et al.*, 1978; Bangham and Lea, 1978; Gallowa *et al.*, 1990) suggest that adding such compounds to liposome dispersion results in incorporation of the compounds into the lipid membrane.

As we have mentioned earlier, the membrane of liposomes used in our experiments is loaded by positively charged calcium ions. The positively charged fragments of the compounds interacting with the liposome membrane, because of the competition between calcium ions and the incorporated ammonium ions, weaken the strength of the bond between the adsorbed calcium ions and the polar head of the lecithin molecules, thus facilitating the release of calcium ion from the membrane.

It can be seen from Table I that the BDTA and MDOM compounds have chloride as counter-ion, and MTM and TMTA have bromide as counter-ion. We have checked earlier (results not published) that the bromide cationic amphiphiles act a little bit stronger, but only about 3–4 units of α/α_o, than the chloride amphiphiles. So, we assumed that in the studies presented the differences are negligibly small.

Comparing the effectiveness of action of the respective compounds on the desorption process (Fig. 3) one may conclude that they can be arranged in the following sequence: BDTA>MTM>TMTA>MDOM. The values of dipole moments (Tab.II.) are, instead, according to the sequence: BDTA>MTM>MDOM>TMTA. Thus there is no full correlation between the two factors; the MDOM compound has more than twice larger dipole moment though its effect on desorption is weaker than that of TMTA. If, moreover, we compare differences of their dipole moments with effectiveness, it is apparent that also for the remaining compounds the correlation is not complete. As follows from Table III, the slight differences in the relative kinetic constants of compounds BDTA and MTM as well as BDTA and TMTA correspond to very large differences in dipole moments.

BDTA as an isolated molecule has a large dipole moment (Table II). Interacting with phospholipid molecules which possess also dipole moments, it is attracted by the membrane and, because of the flat aromatic ring with delocalized π electrons, may assume parallel position to the membrane (Gabrielska *et al.*, 1979). The forces cooperate with the hydrophobic interaction and this results in a yet deeper penetration of the compound into the bilayer, causing a loosening of the phospholipid membrane structure and a resultant

Table III.

Compounds	The dipole moment differences [D]	The relative rate constant difference* (the range of concentrations 1.5 mM–2.5 mM)
BDTA and MTM	0.5	~13
BDTA and TMTA	4.25	~17

* The difference between relative rate constants was determined as a distance, in α/α_0 units, between the parallely shifted, almost linear parts of the curves in Fig. 3, in the mentioned range of concentrations.

increase in the kinetic constants of the studied process. It cannot also be excluded that the aromatic ring that has hydrophobic properties positions itself in the upper layer of the hydrophobic part of the membrane (Różycka-Roszak and Fisicaro, 1992) and due to the strong cone-like shape of the entire molecule it causes a local deformation of the membrane and weakening of the calcium ion – membrane bond. In the case of the two molecular mechanisms of action, the effect of BDTA molecules on calcium ion desorption may be strong.

The polar part of compound MTM has a morpholinium ring with a chair-like shape and considerable partial negative charge at the oxygen atom (Fig. 4). The ring may constitute a steric hindrance in the interaction with phospholipid membrane, and is embedded not too deep into the membrane. Thus, though the dipole moment is only slightly smaller, its effect on the desorption process of calcium ions may be markedly weaker than that of BDTA.

Compound TMTA, in spite of the small value of its dipole moment, may incorporate deeper into the membrane due to the small size of its polar part, and hence its effect is comparable with the MTM compound.

The weaker effect, when compared to the remaining compounds, of compound MDOM and the almost twice lower value of its dipole moment than that of MTM can seemingly be explained by the presence of an oxymethylene group. The large negative charge at the oxygen atom of the group (Fig.4), situated on the opposite side of the nitrogen atom with respect to the oxygen atom of the morpholinium group, may considerably weaken the dipole moment of the molecule. The effectiveness of amphiphilic compounds strongly depends on alkyl chain lengths of the molecules. Elongation of the alkyl chain by two methylene groups may at times increase the effectiveness 10-fold (Gabrielska *et al.*, 1981). Comparing the effectiveness of compounds MTM and MDOM that differ only in the presence of oxymethylene group, which substituted for two methylene groups in the MDOM compound, one should expect that this group affects the hydrophilic properties of the compound and does not constitute elongation of the alkyl chain.

It can be concluded that the differences in the effectivenes of action of the compounds in the calcium desorption process follow not only from the values of dipole moments of their polar heads but also from the values of point charges on some atoms and from steric properties.

Acknowledgements

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