Counterion Effects on Interaction of Amphiphilic Quaternary Ammonium Salts with Model Membranes

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The micellization as well as the interaction with model membranes of dodecyltrimethylammonium halides (DTAX) and N-dodecyl-N,N-dimethyl-N-benzylammonium halides (DBeAX) were studied at 298K and 313K by means of titration calorimetry. The calorimetric curves reflect both the counterion and benzyl group effects on the interaction of the surfactants studied with the lipid bilayer. Bromide as counterion enhanced the interactions more than chloride of both DTAX and DBeAX compounds with model membranes.

Further, we studied the influence of DTAX and DBeAX on calcium ion desorption from the liposome membrane using a radioactive tracer method. DBeAX proved more efficient in desorption of calcium than DTAX. Iodides of these compounds enhanced this process more than bromides and chlorides.

Introduction

It has been known that counterions effect micellization (De Lisi. *et al.,* 1988) and the interaction of amphiphilic compounds with biological and model membranes (Sarapuk *et al.*, 1998; Kleszczyńska *et al.*, 1998; Kleszczyńska and Sarapuk 1998; Sarapuk *et al.*, 1999).

However, the role of the counterion in these processes is not quite clear. In order to elucidate the role played by a counterion we studied the hydration of dodecyltrimethylammonium halides (DTAX) at 298K and 313K (Różycka-Roszak *et al.,* 2000; Różycka-Roszak *et al.,* 2000) as well as the influence of DTAX on phase transitions of phosphatidylcholine bilayer and phosphatidylcholine/cholesterol bilayer (Różycka-Roszak and Pruchnik, 2000). This time we studied the micellization of N-dodecyl-N,N-dimethyl-N-benzylammonium halides (DBeAX) and the interaction of DTAX and DBeAX with model membranes.

DBeAX differ from DTAX in replacement of the methyl group by benzyl group. Thus, formally, DBeAX can be treated as derivative of DTAX. Micellization studies (Różycka-Roszak, 1990) suggest that the benzyl group of N-dodecyl-N,N-dimethyl-N-benzylammonium chloride (DBeAC) shows a hydrophobic character and behaves as if it were a second hydrocarbon chain. Besides, thermochemical studies (Rózycka-Roszak and Fisicaro, 1992) showed that the benzyl group of N-dodecyl-N-benzylmorpholinium chloride (DBeMC) can be treated as equivalent to a chain of five carbon atoms. Recently, experimental evidence (Różycka-Roszak and Cierpicki, 1999) was provided that a benzyl group of DBeAC changes its position during the micellization and, as a consequence, locates inside the micelle. Apparently, the benzyl group of DBeAX may also incorporate into the phospholipid bilayer and DBeAX can be treated as double chain compounds.

The objective of this paper was a calorimetric comparison of the influence of counterions on the interaction of DBeAX and DTAX with model membranes. We applied a calorimetric titration method widely used to study micellization of various amphiphilic quaternary ammonium salts (Kresheck and Hargraves, 1974; Różycka-Roszak *et al.,* 1988; Różycka-Roszak *et al.,* 1988; Różycka-Roszak, 1990) as well as the interaction with membranes (Kale *et al.,* 1978; Kresheck *et al.,* 1980; Kresheck and Long, 1988). The experiments were

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done at two temperatures, 298K and 313K that is below and above phase transition temperature of phosphatidylcholine (DPPC).

Besides, we studied the influence of DTAX and DBeAX on calcium ion desorption process from the liposome membrane using the radioactive tracer method.

Materials and Methods

Chemicals

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and egg yolk lecithin were purchased from Avanti Polar Lipids, Birmingham, Alabama, USA.

Dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB), N-dodecyl-N,N-dimethyl-N-benzylammonium chloride (DBeAC) and N-dodecyl-N,N-dimethyl-N-benzylammonium bromide (DBeAB) were purchased from Fluka, Buchs, Switzerland.

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

99.98 D_2O was purchased from Dr. Glaser AG Basle, Switzerland.

Calorimetric measurements were done by a titration method with a home-made calorimeter at 298K and 313K (Różycka-Roszak *et al.*, 1988). The titrant containing an appropriate amount of a compound studied was delivered to 20 ml of bidistilled water (micellization studies) or 0.06% DPPC dispersion at a constant speed (0.125 ml min^{-1}). The temperature was measured continuously. The experimental curves were corrected as described earlier and adiabatic curves of heat of dilution (Q) were obtained (Różycka-Roszak *et al.*, 1988).

Radioactive tracer experiments

Small unilamellar liposomes (SUV) were prepared from egg yolk lecithin (EYL) by using sodium cholate in a Liposomat (DIANORM, München, Germany) (Weder and Zumbuhl, 1984). Lecithin was prepared according to the technique based on Singleton *et al.*, (1964). The solution used to form vesicles contained a veronal-acetate buffer, pH 7.5, and 0.3 mmol/l $CaCl_2$ labelled with the radioactive tracer ^{45}Ca. During vesicle formation calcium cations were absorbed at the outer and inner liposome membranes. The radioactive tracers were removed from external medium during liposome preparation. The theoretical workout of the transport and desorption measurements was used as described previously (Kuczera and Żyłka, 1979; Mazgis and Kuczera, 1981) with minor modifications.

Results

Calorimetric studies

For calorimetric studies it was not possible to use DTAI and DBeAI due to their low solubility.

Micellization of DBeAX

Examples of corrected adiabatic curves obtained by titration of DBeAC and DBeAB to water at 298 and 313K are compared in Fig. 1. Like it was in the case of DTAX (Rózycka-Roszak *et al.,* 2000), significant differences in the shape of the titration curves for DBeAC and DBeAB are visible at 298K, while at 313K the curves became similar to each other. At 298K the initial slope of the dilution curve of DBeAC was positive (exothermic process) while that of DBeAB is negative (endothermic process). At 313K in both cases the initial slope in negative (endothermic process). The initial slope of the dilution curve corresponds to micelle dissociation (Różycka-Roszak *et al.*, 1988). As a first approximation we can assume that the endothermic process of micelle dissociation implies that the energy of breaking the hydrocarbon contact in the micelle interior is greater than the hydration energy released by the attachment of water molecules to the hydrocarbon chain. The opposite is due for the endothermic process. The enthalpies of micellization (ΔH_m) and critical micelle concentrations (CMC) were

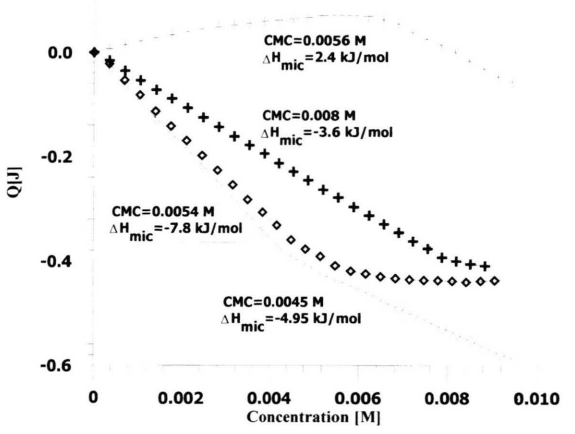

Fig. 1. Calorimetric titration curves of N-dodecyl-N,N-dimethyl-N-benzylammonium chloride (DBeAC) (+,✚) and N-dodecyl-N,N-dimethyl-N-benzylammonium bromide (DBeAB) (◇,◆) to water at 298K and 313K, respectively.
ΔH_{mic} – enthalpy of micellization, CMC – critical molecular concentration.

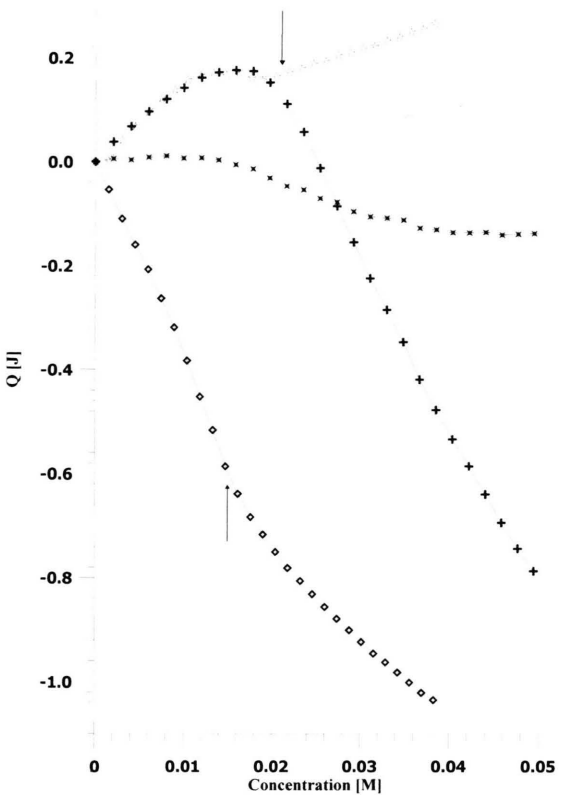

Fig. 2. Calorimetric titration curves of dodecyltrimethylammonium chloride (DTAC) (✚,✶) and dodecyltrimethylammonium bromide (DTAB) (◆,☆) to lipid dispersion at 298K and the difference curves for the above and those taken from the paper (Różycka-Roszak *et al.*, 2000). The arrows on the titration curves to lipid dispersion indicate the CMC of DTAC and DTAB in pure water.

calculated according to (Różycka-Roszak, 1990). At 298K, ΔH_m and CMC were 2.4 kJ/mol and 0.0056 mol/l for DBeAC while for DBeAB they were −4,95 kJ/mol and 0.0045 mol/l, respectivly. At 315K, ΔH_m and CMC were −3,6 kJ/mol and 0.008 mol/l for DBeAC and for DBeAB −7,8 kJ/mol and 0.0054 mol/l, respectivly. Previously, the values obtained for DBeAC at 298K were 0.004 mol/l (CMC) and 1.1 kJ/mol (ΔH_m (Różycka-Roszak, 1990).

Interaction with DPPC

The results for the titration of DPPC in multilamellar state (MLV) with DTAX and DBeAX at 298K and 313K are shown in Figs. 2, 3, 4 and 5. We have used MLV because the heat effect associated with solubilization of MLV is greater than that for small unillamellar vesicles (SUV) (Kresheck and Long, 1988). According to the solubilization theory (Lichtemberg, 1985) the incorporation of a surfactant into the bilayer structure (first step of solubilization) occurs until CMC point is reached. Approximately at CMC (in pure water) the curves show a break. Then the solubilization of the phospholipid begins. So, the initial parts of the curves refer to incorporation of the surfactant into the bilayer. Since the demicellization process precedes the incorporation of a surfactant in the liposome the curves represent the enthalpy change due to both these processes. Subtraction of the curves presented in Fig. 1 (in the case of DBeAX) or the corresponding ones taken from the previous paper (Różycka-Roszak *et al.*, 2000) gave difference curves in the case of DTAX, also shown in Figs. 2, 3 and 4. The difference curves give the effect connected with the interaction of a surfactant with liposomes only. The heat effects of the interaction differ between the compounds with respect not only to magnitude but also sign. At 298K the heat effect of the interaction of DTAC is approximately zero, that of DTAB is positive (exothermic process) (Fig. 2), while those of DBeAC and DBeAB are negative (endothermic processes)

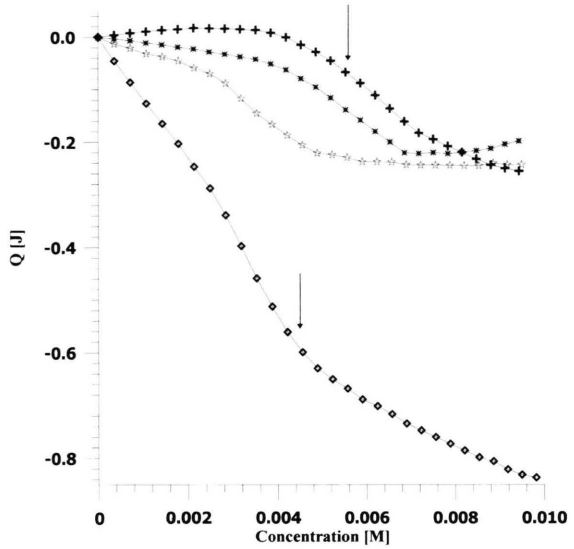

Fig. 3. Calorimetric titration curves of DBeAC (✚,✶) and DBeAB (◇,☆) to lipid dispersion at 298K and the difference curves for the above curves and those taken from Fig. 1. The arrows on the titration curves to lipid dispersion indicate the CMC of DBeAC and DBeAB in pure water.

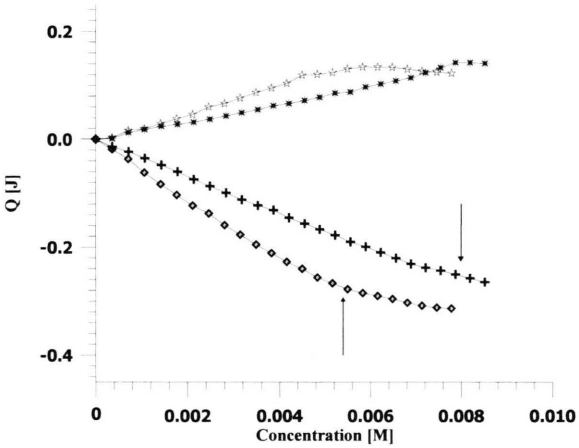

Fig. 4. Calorimetric titration curves of DBeAC (✚,✶) and DBeAB (◇,☆) to lipid dispersion at 315K and the difference curves for the above and those taken from Fig. 1. The arrows on the titration curves to lipid dispersion indicate CMC of DBeAC and DBeAB in pure water.

(Fig. 3). At 315K the interactions of DBeAX with the lipid dispersion are exothermic and more exothermic for DBeAC than DBeAB (Fig. 4), while the interaction of DTAX with a lipid dispersion did not give measurable heat effect. At 315K the curves for titration of the lipid dispersion with DTAX were practically indistinguishable from those for titration with water (therefore the curves are not documented). The same was reported by Kresheck and Long (1988) for titration of 0.1% DPPC samples with DTAC.

Radioactive tracer experiments

The results of kinetic studies on the calcium ion desorption process are presented in Fig. 5, where the relative rate constants are plotted against concentration of the DTAC and DBeX, respectively. The relative rate constant α/α_0 is defined as the ratio of the rate constant of calcium ion desorption in the presence of compounds studied to that measured in the absence of modifiers. The standard error was below 10%. All compounds studied induce a multiple increase in the rate constant, compared with unmodified membrane. Effectiveness of the compounds increases with concentration.

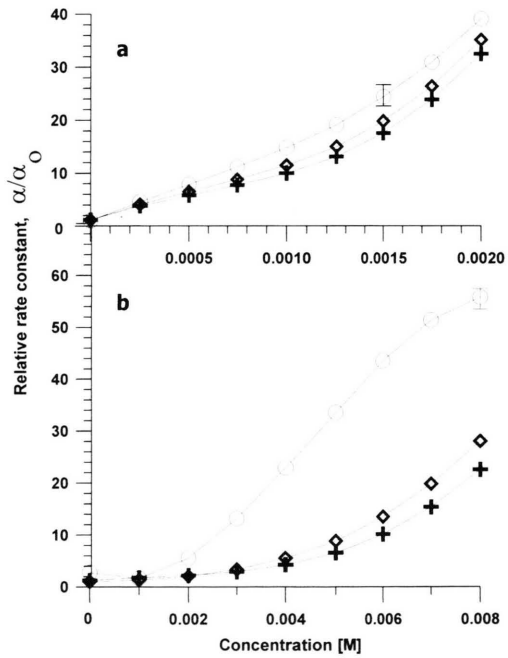

Fig. 5. The relative rate constant, α/α_o, of the calcium ion desorption process from liposome membranes against the concentration of the:
a. DTAX compounds: DTAC (✚); DTAB (◇); DTAJ (○).
b. DBeAX compounds: DBeAC (✚); DBeAB (◇); DBeAJ (○).
α and α_o are kinetic constants for modified and unmodified membrane, respectively.

The observed desorption of Ca^{2+} ions from the liposome membrane in the presence of cationic surfactants is the result of competition between the calcium ion and surfactants for the negatively charged binding sites localised at the polar moieties of the lecithin molecules (Fogt *et al.*, 1994; Kuczera *et al.*, 1996, 1997).

As it follows from Fig. 5, DBeAX are more efficient in calcium ion desorption than DTAX. Besides, in the case of DTAX and DBeAX most efficient are iodides, less bromides and least chlorides.

Discussion

The calorimetric curves reflect both the counterion and benzyl group effects on the interaction of the surfactants studied with the lipid bilayer. At 298K the interaction of DBeAX is endothermic, while that of DTAX either equal zero (DTAC) or exothermic (DTAB). The incorporation of a surfactant molecule into a liposome needs some energy to brake the hydrocarbon contact in the liposome interior. This energy should be greater at 298K than 315K because the lipid bilayer is below gel-to-liquid transition temperature of DPPC. Besides, the energy should be greater for the incorporation a compound with two than one hydrocarbon chain. As a first approximation, we can assume that the endothermic process of surfactant interaction with liposomes implies that the energy needed to break the hydrocarbon contact in the liposome interior is greater than the energy coming from the interaction of DBeAX molecules with DPPC. The opposite is due for the exothermic process. At 298K the incorporation of DBeAX is endothermic while that of DTAX is not. DBeAX differs from DTAX because of the replacement of the methyl group by the benzyl group. So, the endothermic process in the case of DBeAX is probably due to benzyl group and is in agreement with the suggestion that benzyl group of DBeAX may be treated as a second chain. For DTAX (without benzyl group) the energy to break hydrocarbon contacts should be lower, which may explain why the interaction of DTAX with the lipid bilayer is not endothermic; for DTAC equal zero while for DTAB is even exothermic.

The differences between curves for DBeAC and DBeAB, and also DTAC and DTAB, reflect the influence of counterion on the surfactant interaction with the lipid bilayer. In the case of DBeAX as well as DTAX the stronger effect is due to the bromide counterion. At 298K, the interaction of DBeAB is more endothermic than DBeAC, while the interaction of DTAB more exothermic than that of DTAC.

At 315K, above the gel-to-liquid transition temperature of DPPC, less energy is needed to break hydrocarbon contacts in the liposome interior than at 298K. So, the contribution coming from the interaction energy between a surfactant and DPPC must be larger. This may explain why the interaction of DBeAX is exothermic and like that of 298K the stronger effect is for DBeAB than DBeAC. Anyway, the interaction of DTAX occurs without a measurable heat effect although at 298K the interaction of DTAB was exothermic. This may be due to the fact that the phospholipid-surfactant interaction is stronger in the gel phase (Lohner, 1991). The exothermic effect in the case of DBeAX indicates that DBeAX interacts stronger with the lipid bilayer than DTAX. Besides, the more exothermic process in the case of DBeAB than DBeAC reflects, a stronger effect due to the bromide than chloride counterion.

From the calorimetric studies it follows that DBeAX interaction with the phospholipid bilayer is stronger than that of DTAX. Besides, bromide as counterion enhances these interactions more than chloride in the case of both DTAX and DBeAX compounds. This may explain why DBeAX compounds are more efficient in desorption of calcium than DTAX and why bromides of these compounds enhance this process more than chlorides.

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