Effect of Counterions on the Influence of Dodecyltrimethylammonium Halides on Thermotropic Phase Behaviour of Phosphatidylcholine Bilayers

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Effects of dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB) and dodecyltrimethylammonium iodide (DTAI) on thermotropic phase behaviour of phosphatidylcholine bilayers as well as on ^{1}H NMR spectra were studied. In order to enhance the effect of counterions on water structure two series of experiments were performed. In the first one the surfactants were added to the water phase and in the other one directly to lipid phase (a mixed film was formed). The effects of particular surfactants on the main phase-transition temperature were more pronounced when they were added to the water phase (1st method) instead of the lipid phase (2nd method). Furthermore, in the case of the first method the transitions were found asymmetrical while in the second method nearly symmetrical. It is suggested that surfactant-poor and surfactant-rich domains are formed when surfactants are added to the water phase.

Introduction

The type of counterion proved to be an important factor influencing the interaction of amphiphilic compounds with both erythrocytes and plant lipid membranes. Bromide compounds were found to be more efficient in destabilising model membranes than the corresponding chloride (Sarapuk *et al.*, 1998; Kleszczyńska *et al.*, 1998; Kleszczyńska and Sarapuk 1998; Sarapuk *et al.*, 1999).

The greater ability of bromides to destabilize model membranes in comparison with chlorides was attributed to the greater mobility and the smaller radius of the hydrated bromide ion, and consequently to a greater ability to modify the surface potential of model membranes. Similar results pointing to stronger modifying possibilities of bromides were also found in monolayer studies of cationic and anionic surfactants and their mixtures (Góralczyk, 1993; Góralczyk, 1994).

The destabilising effect should also be reflected in the phase transitions of phosphatidylcholine bilayers. The objective of this paper was to study the influence of chloride, bromide and also iodide as counterions on the effect of amphiphilic compound on thermotropic phase behaviour of phosphatidylcholine bilayers. Accordingly, we studied the commonly used surfactants like dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB) and dodecyltrimethylammonium iodide (DTAI).

Two series of experiments were performed. In the first one the surfactants were added to the water phase while in the other directly to the lipid phase (a mixed film was formed). We applied differential scanning calorimetry (DSC), which is widely used to study the effects of cholesterol (McMullen *et al.*, 1993), anesthetics, drugs, proteins (McElhaney, 1986), surfactants (Eliasz *et al.*, 1976; Otten *et al.*, 1995; Grau *et al.*, 1999) and various small molecules on phase transitions of phospholipids. Additionally we used the ^{1}H NMR method; which, like other nuclear magnetic resonance methods, is one of the most powerful techniques (Fenske , 1993; Wu , 1996,) that have been applied to study biological and model membranes.

Materials and Methods

Chemicals

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

Dodecyltrimethylammonium chloride (DTAC) and dodecyltrimethylammonium bromide (DTAB) were purchased from Fluka.

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Dodecyltrimethylammonium iodide (DTAI) was prepared by mixing a concentrated aqueous NaI solution with an aqueous dodecyltrimethylammonium chloride solution 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. 99.9 D_2O was purchased from Dr. Glaser AG Basel.

Sample preparation for differential scanning calorimetry (DSC)

Samples for DSC were performed on multilamellar vesicles (MLVs) which were prepared using two methods.

1st method. DPPC dissolved in chloroform was evaporated. Traces of chloroform were removed with a stream of dry nitrogen under vacuum for two hours. The lipid film formed was dispersed by adding water solution of DTAC, DTAB or DTAI, of appropriate concentration, and agitating the flask on a vortex mixer to give a milky suspension of liposomes. The mixture was heated at about 60 °C for 15 min and finally cooled down to room temperature. The final lipid concentration was 25 mg/ml. Lipid suspension was loaded into the sample cell of a DSC microcalorimeter of Mettler Toledo Thermal Analysis System D. S. C. 821^e. Employed scan rates were 2 °C/min, while time of incubation, performed at 4 °C was 48 hours.

2nd method. DPPC and appropriate amounts of DTAC, DTAB or DTAI were dissolved in chloro-

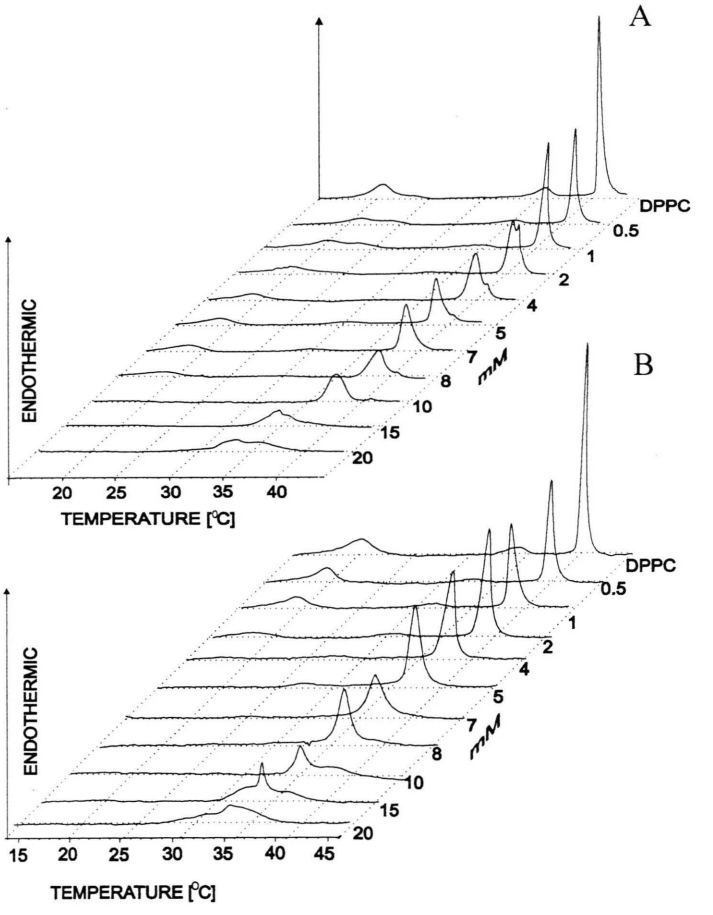

Fig. 1. DSC heating curves of MLVs with increasing concentrations of dodecyltrimethylammonium chloride (DTAC). The curves were normalized for the amount of DPPC; (A) 1st method, (B) 2nd method.

form and evaporated to form a thin mixed film on the flask wall. After that distilled water was added and the rest of the procedure was analogous to the 1st method.

Sample preparation for ^1H NMR

Measurements were performed on sonicated vesicles (SUVs) which were prepared also using two methods:

1st method. Egg lecithin dissolved in chloroform was evaporated. Traces of chloroform were removed with a stream of dry nitrogen under vacuum for two hours. The formed lipid film was dispersed by adding heavy water solution of DTAC, DTAB or DTAI of appropriate concentration and agitating the flask on a vortex mixer to give a milky suspension of liposomes. The obtained liposome suspension was sonicated for ½ hour with a 20 kHz sonicator. Samples were enclosed in 5 mm NMR tubes.

^1H-NMR spectra were recorded on an Avance Bruker DRX 300 Spectrometer at 300.13 MHz. Signals were acquired using a 6173 Hz spectral windows, 10,7 us pulse and 2.65 s acquisition time. Digital resolution was 40.9268 Hz/cm or 0.1364 ppm/cm. The residual heavy water signal was used as a chemical shift reference.

2nd method. Egg yolk lecithin and appropriate amounts of DTAC, DTAB or DTAI were dissolved in chloroform and then the mixture was evaporated to form a mixed film on the flask wall. Traces of chloroform were removed with a stream of dry nitrogen by evaporation under vacuum. Then D_2O water was added and the rest of procedure was analogous to the 1st method.

Results

DSC

The effects of DTAC, DTAB and DTAI on the phase transitions of DPPC liposomes prepared by

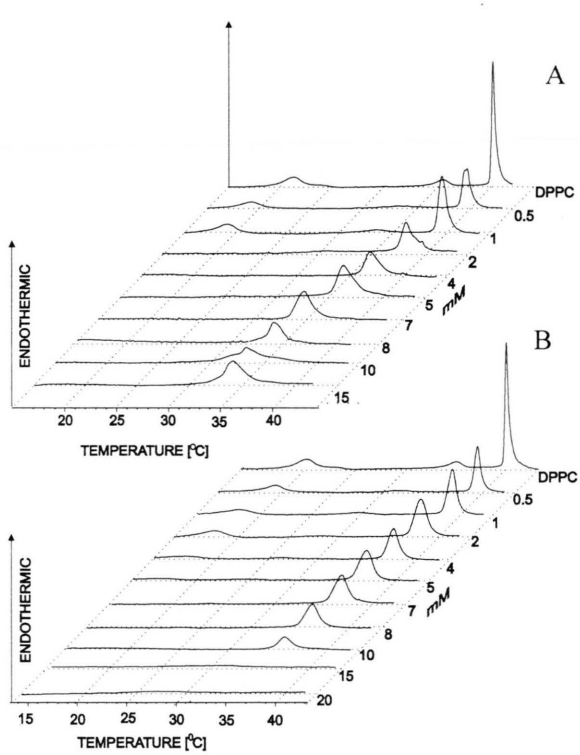

Fig. 2. DSC heating curves of MLVs with increasing concentrations of dodecyltrimethylammonium bromide (DTAB). The curves were normalized for the amount of DPPC; (A) 1st method, (B) 2nd method.

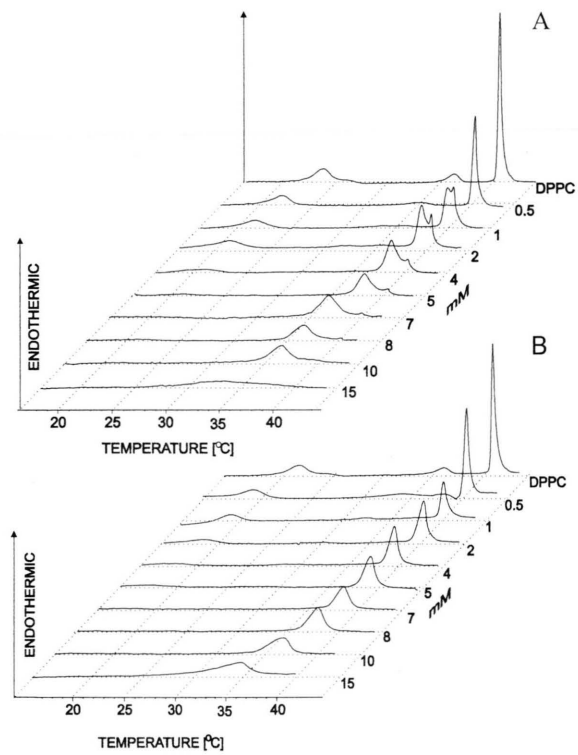

Fig. 3. DSC heating curves of MLVs with increasing concentrations of dodecyltrimethylammonium iodide (DTAI). The curves were normalized for the amount of DPPC; (A) 1st method, (B) 2nd method.

means of the first method and second method are compared in Figs. 1, 2 and 3, respectively. In both cases the main phase transition temperature (T_m) shifts progressively to lower temperatures with increasing concentration of the surfactants (Fig. 4). In the case of the 1st method the transition peaks are more broadened and asymmetrical, while in the second method they are less broad and almost symmetrical. After addition of DTAI to liposomes according to the 1st method the main phase transition of DPPC is not only asymmetrical but even separates into two peaks. The high temperature peak can be ascribed to pure DPPC.

The effects of particular surfactants on T_m were more pronounced when they were added to the water phase than to the lipid phase. DTAC shows stronger effect on T_m in the case of the 2nd method, DTAB in the case of the 1st method and DTAI approximately the same in both case.

^1H NMR

The effect of DTAB, DTAC and DTAI on ^1H NMR spectra of liposome dispersion prepared according to the 1st method and to the 2nd method are compared in Fig. 5. ^1H NMR resonance of the trimethylammonium group of lecithin, $(N\text{-}CH_3)_L$, remains almost unchanged after addition of a surfactant. ^1H NMR resonance of the trimethylammonium group of the surfactant, $N\text{-}CH_3$, is downfield shifted in liposomes dispersion in comparison to pure water. The lowest shift is in the case of DTAC and is greatest in the case of DTAI. Besides, when lipids were added to the lipid phase the $N(CH_3)_3$ signals coming from the surfactant headgroups were more downfield shifted and superimposed the choline $N(CH_3)_3$ headgroup signal. This suggest that more surfactants are embedded into the lipid phase upon addition according to 2nd method. Besides, in the case of both methods the strongest effect is for DTAI and the lowest for DTAC.

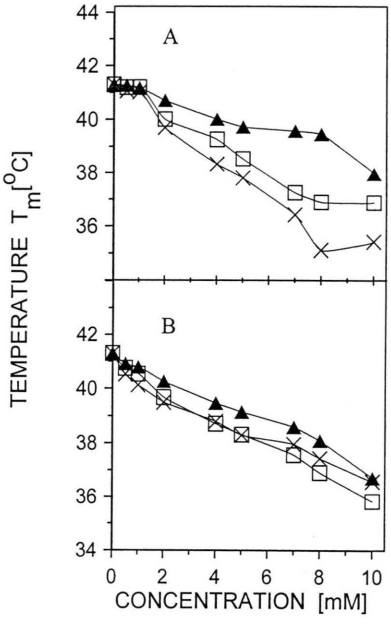

Fig. 4. Main phase transition temperatures (T_m) as a function of dodecyltrimethylammonium halide concentration: ▲ DTAC; × – DTAB; □ – DTAI. (A) 1st method, (B) 2nd method.

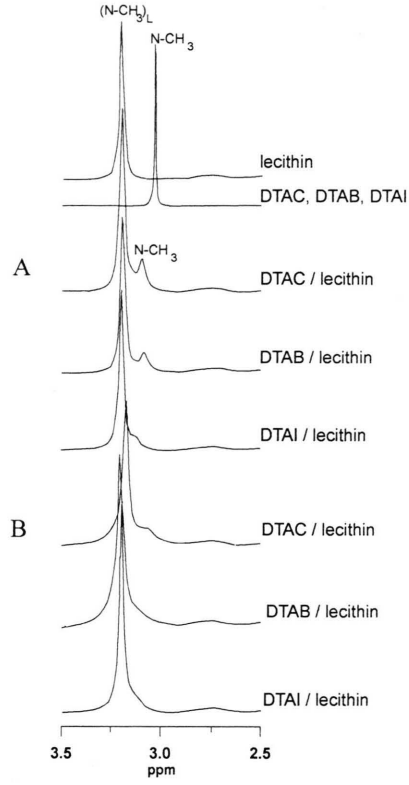

Fig. 5. ^1H NMR spectra of: egg yolk lecithin liposome suspension in the presence and absence of surfactants. Lecithin concentration 10 mg ml^{-1}, surfactant concentration 2 mM.
$(N\text{-}CH_3)_L$ – ^1H NMR resonanse of the trimethylammonium group of lecithin.
$N\text{-}CH_3$ – ^1H NMR resonanse of the trimethylammonium group of a surfactant.
(A) 1st method, (B) 2nd method.

Discussion

The effects of counterions studied on the influence of dodecyltrimethylammonium halides on phase transitions of DPPC as well as on ^{1}H NMR spectra depend on the way a surfactant was introduced to liposomes. In the case of the 1st method of preparation the transitions were asymmetrical, while in the 2nd method nearly symmetrical. Asymmetrical transitions may suggest that domains are formed.

Liposome preparation of 2nd method cause that surfactants are probably uniformly located in the liposome, probably due to the symmetrical transitions. As it follows from ^{1}H NMR results more surfactants are embedded into liposomes upon their addition to the lipid phase. This may explain why DTAC shows a stronger effect on T_m in the case of 2nd method than 1st method. Anyway, DTAB shows an opposite effect, that is a stronger change T_m in the case of 1st method than the 2nd method. This may be due to water- counterion interaction.

The results obtained indicate that the influence of a counterion on the interaction of a surfactant with a lipid bilayer was more enhanced when the surfactant was added to the water phase than to the lipid phase. This, in turn, may indicate at the significance of water-ion interactions and may be due to different abilities of counterions to modify the water structure. All halides studied are chaotropes, although a chloride is only marginally chaotropic (Collins, 1977; Collins, 1997). This may explain why chloride shows the lowest effect on phase transitions as well as on ^{1}H NMR spectra. Chaotropes according to Collins are "pushed" onto weakly hydrated surfaces by strong water-water interactions. This results in their adsorption on the surface of liposomes. The chloride ion is only marginally chaotropic and thus probably less attached to the liposomes surfaces than bromides and iodides. A strong adsorption of a counterion decreases the electrostatic headgroup repulsion, so the incorporation of surfactant in the vicinity of an adsorbed ion should be easier than in other locations. In consequence, this may lead to formation surfactant–poor and surfactant-rich domains.

Acknowledgements

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Sarapuk J., Kleszczyńska H. and Różycka –Roszak B. (1998), The role of counterions in the interaction of bifunctional active compounds with model membranes. Biochem. Mol. Biol. Intl. **44**,1105–1110.

Kleszczyńska H., Sarapuk J. and Różycka –Roszak B. (1998), The role of counterions in the interaction of some cationic surfactants with model membranes. Polish J. Environ. Studies **7,** 347–350.

Kleszczyńska H. and Sarapuk J. (1998), The role of counterions in the protective action of some antioxidans in the process of red cell oxidation. Biochem. Mol. Biol. Intl. **18**, 323–422.

Sarapuk J., Kleszczyńska H., Pernak J., Kalewska J. and Różycka-Roszak B. (1999), Influence of counterions on the interaction of pyridinium salts with model membranes. Z. Naturforsch. **54 c,** 952–955.

Góralczyk D. (1993), Effect of inorganic electrolytes on anionic-cationic adsorption films. Tenside Surf. Det. **30**, 356–358.

Góralczyk D. (1994), Effect of inorganic electrolyte concentration on anionic-cationic adsorption films.4. Dodecylpyridinium bromide + sodium octylsulfonate + sodium bromide. J. Coll. Interface Sci. **167**, 172–180.

McMullen T. P. W., Lewis R. N. A.H. and McElhaney R. N. (1993), Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behaviour a homologous series of linear saturated phosphatidylcholines. Biochemistry **32**, 516–522.

McElhaney R. N. (1986), Differential scanning calorimetric studies of lipid-protein interactions in model membrane systems. Biochim. Biophys. Acta **864** 361–421.

Otten D., Löbbecke L. and Beyer K. (1995), Stages on the bilayer-micelle transition in the system phosphatidylcholine-$C_{12}H_8$ as studied by deuterium- and phosphorous-NMR, light scattering, and calorimetry. Biophysical J. **68**, 584–594.

Eliasz A. W., Chapman D. and Ewing D. F. (1976), Phospholipid phase transition. Effects of on n-alcohols, n-monocarboxylic acids, phenylalkyl alcohols and quaternary ammonium compounds. Biochim. Biophys. Acta **448**, 220–230.

Grau A., Gómez Fernández J. C., Peypoux F. and Ortiz A. (1999), A study on the interactions of surfactin with phospholipid vesicles, Biochim. Biophys. Acta **1418,** 307–319.

Fenske D. (1993), Structural and motional properties of vesicles as revealed by nuclear magnetic resonance, Chem. Phys. Lipids **64,** 143–162.

Wu W. (1996), Sonicated membrane vesicle. In Encyclopedia of NMR. D. M. Grant and R. K. Harris editors, John Wiley & Sons, Ltd., Sussex, England, pp. 4485–4492.

Collins K. D. (1977), Sticky ions in biological systems. Proc. Natl. Acad. Sci. USA **92**, 5553–5557.

Collins K. D. (1997), Charge density-dependent strength of hydration and biological structure. Biophys. J. **72**, 65–76.