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A study on the interaction between 1-decyloxymethyl-3-carbamoylpyridinium salts and model membranes—the effect of counterions

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Abstract

The interaction between 1-decyloxymethyl-3-carbamoylpyridinium salts (PS-X) and two types of vesicles (multilamellar vesicle and sonicated vesicle) was investigated. Vesicles were formed from two classes of phospholipids: 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine (DPPE). The PS-X salts used had nitrate, perchlorate, tetrafluoroborate and halides as counterions. Measurements were carried out using differential scanning calorimetry and ^{1}H NMR. All studied compounds decreased the main phase transition temperatures of both DPPC and DPPE bilayers. All of them also decreased the transition enthalpy of DPPC bilayers, however they had a dual effect on the transition enthalpy of DPPE. Namely, at low concentrations the PS-X salts studied significantly increased the main transition enthalpy of DPPE (perchlorate and tetrafluoroborate the least among them) and decreased it at higher concentrations. We have suggested that surfactant rich and pure domains form on the DPPE bilayer in the presence of PS-ClO_4, PS-BF_4 and PS-NO_3, whereas they form on DPPC bilayer only in the presence of PS-ClO_4. Results are discussed in terms of counterion molecular geometry and the ability of amide group to form hydrogen bonds with lipids.

Keywords: Counterions; 1-Decyloxymethyl-3-carbamoylpyridinium salts; 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine; 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylethanolamine; Differential scanning calorimetry; ^{1}H NMR

1. Introduction

We have been interested in the significance of counterions for the interactions between amphiphilic compounds and model membranes for some time. Previously, we have studied the interaction of dodecyltrimethylammonium halides (DTAX) with 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) liposomes [1], *N*-dodecyl-*N*,*N*-dimethyl-*N*-benzylammonium halides (DBeAX) with DPPC liposomes [2] and DTAX with DPPC/chol. liposomes [3]. It follows from the results we obtained during these studies that the character of the interaction between DTAX (or DBeAX) and liposomes, and the subsequent changes in the

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phospholipid bilayer organization, depend on the kind of counterion. The objective of this study is to better understand the role of counterions in the interactions of surfactants with model membranes. Accordingly, we used 1-decyloxymethyl-3-carbamoylpyridinium salts (PS-X) with various counterions, namely nitrate (PS-NO_3), perchlorate (PS-ClO_4), tetrafluoroborate (PS-BF_4) and halides (PS-Cl, PS-Br and PS-I). In addition, we have tested two classes of phospholipids (DPPC and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine (DPPE)).

The compounds presently studied are interesting and show a wide array of possible applications. Belonging to the group of functionalised surfactants, they contain reactive functions covalently bound to them. PS-X compounds exhibit strong anti-microbial activity [4] similar to that of benzalkonium chloride, which is an antiseptic widely used in pharmaceutical preparations [5,6]. Moreover, PS-X salts can be used as model compounds of Nicotinamide adenine dinucleotide (NAD^+). NAD^+ is an important cofactor in numerous cellular processes [7]. The reactive part of NAD^+ is its nicotinamide ring, a pyridine derivative. The conversion of NAD^+ to NADH and the inverse reaction in the coenzyme is a topic still being discussed [8,9].

We have studied the interaction of PS-X salts with phosphatidylcholine and phosphatidylethanolamine bilayers. As before [2], we have investigated the influence of PS-X compounds on both the thermotropic phase behaviour (using differential scanning calorimetry (DSC)) and ^1H NMR spectra of DPPC and DPPE bilayers.

2. Materials and methods

2.1. Chemicals

DPPC was purchased from Avanti Polar Lipids, Birmingham, Alabama. DPPE was purchased from Sigma-Aldrich Chemie (Germany).

1-Decyloxymethyl-3-carbamoylpyridinium chloride was prepared in very good yield by the nucleophilic attack of nicotinamide on chloromethyl alkyl ether, according to the S_N1 mechanism. 1-Decyloxymethyl-3-carbamoylpyridinium salts (Fig. 1) were obtained via a metathesis reaction from 1-decyloxymethyl-3-carbamoylpyridinium chloride in aqueous solution [4].

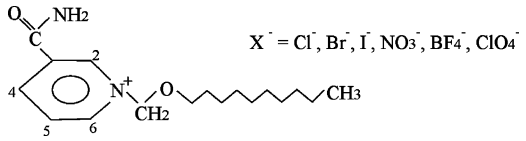

Fig. 1. Chemical structure of 1-decyloxymethyl-3-carbamoylpyridinium salts with the indicated positions of aromatic protons.

2.2. Sample preparation for DSC

Samples for DSC were performed on multilamellar vesicles (MLVs). DPPC or DPPE, with appropriate amounts of 1-decyloxymethyl-3-carbamoylpyridinium salt, was dissolved in chloroform. Chloroform was evaporated and a thin mixed film formed on the flask wall. After this, distilled water was added and the mixed film was intensively shaken at 60 °C (for DPPC) or at 80 °C (for DPPE) until a milky suspension of liposomes was obtained. Final phosphatidylcholine and phosphatidylethanolamine concentrations were 25 mg/cm^3. The lipid suspension was then loaded into the sample cell of a DSC microcalorimeter (Mettler Toledo Thermal Analysis System D.S.C. 821^e). Scan rate was 2 °C/min, and incubation (performed at 4 °C) lasted 5 days. The estimated experimental error for T_m and ΔH_m were ± 0.13 °C and ± 0.28 kJ/mol, respectively.

2.3. Sample preparation for ^1H NMR

Measurements were performed on sonicated vesicles. DPPC or DPPE, with appropriate amounts of PS-X salt, was dissolved in chloroform. Chloroform was evaporated and a thin mixed film formed on the flask wall. Traces of chloroform were evaporated with a stream of dry nitrogen under vacuum. This mixed lipid film was dispersed by adding heavy water and agitating the flask on a vortex mixer, which resulted in a milky suspension of liposomes. This liposome suspension was sonicated for half an hour above main phase

transition temperature (~50 °C for DPPC and 70 °C for DPPE) with a 20-kHz sonicator. Samples were then enclosed in 5-mm NMR tubes. Final phosphatidylcholine and phosphatidylethanolamine concentrations were 22.5 and 5.55 mg/cm^3, respectively. Unfortunately, due to the low solubility of PS-I, it was not possible to prepare liposomes containing this salt with the same procedure.

^{1}H NMR spectra were recorded at 25 and 45 °C (DPPC) or 25 and 65 °C (DPPE) on an Avance Bruker DRX 300 Spectrometer at 300.13 MHz. Signals were acquired using a 6173 Hz spectral window, 10.7 μs pulse and an acquisition time of 2.65 s. Digital resolution was 40.9268 Hz/cm, which equals 0.1364 ppm/cm. Chemical shifts were referenced to external HMDSO. Samples were incubated for 5 days at 4 °C, and additionally equilibrated in the NMR spectrometer for at least 30 min before reading the spectra at higher temperature (45 °C for DPPC and 65 °C for DPPE).

3. Results

3.1. DSC

The influence of PS-NO_3, PS-ClO_4 and PS-BF_4 on the thermotropic phase behaviour of DPPC and DPPE liposomes is presented in Figs. 2 and 3, respectively. Other compounds exhibited a significantly lower effect on DSC thermograms, therefore they are not presented.

Main phase transition for DPPC and DPPE broadens and shifts progressively towards lower temperatures with increasing PS-X concentration. At higher concentrations of PS-ClO_4, the transition separates into two peaks for both DPPC and DPPE liposomes. At higher concentrations of PS-NO_3 and PS-BF_4 this happens only for DPPE liposomes.

As seen in Figs. 4 and 5, the influence of PS-X salts on main phase transition temperature (T_m) and transition enthalpy (ΔH_m) depends on both the kind of counterion and lipid. In the case of separated peaks, the one corresponding to a lower temperature strongly depends on concentration, while the other to a lesser extent. PS-X compounds decreased both T_m and ΔH_m for DPPC and only T_m for DPPE liposomes. They showed a dual effect on the ΔH_m of DPPE liposomes: transition

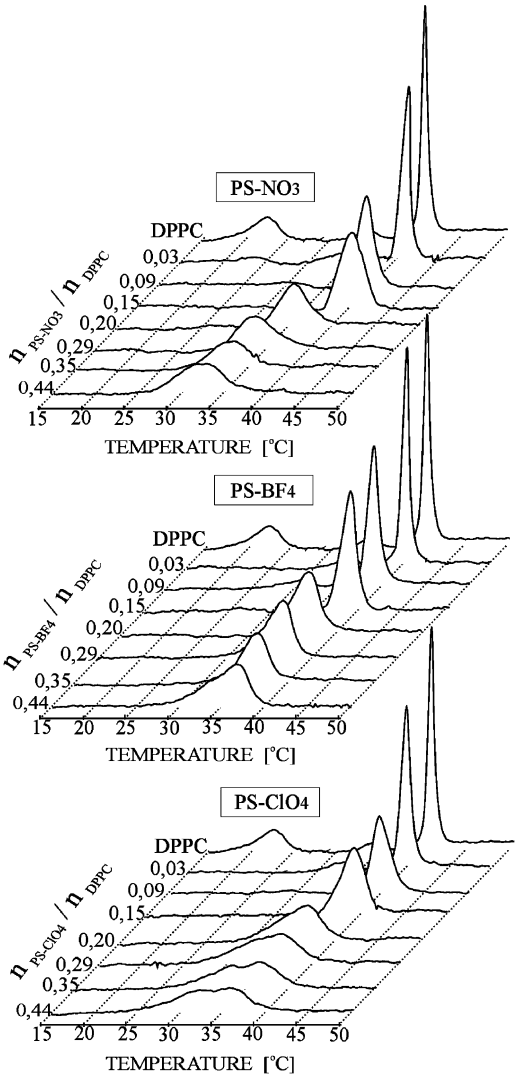

Fig. 2. DSC heating curves of MLVs with increasing molar ratios of PS-NO_3, PS-BF_4 and PS-ClO_4 vs. DPPC. The curves were normalized with regard to the amount of DPPC.

enthalpy increased at low PS-X concentrations and decreased at high concentrations.

In the case of DPPC liposomes, the extent to which PS-X compounds decreased T_m and ΔH_m was similar, except for PS-Cl (Fig. 4). The latter decreased T_m the most (at least at low concentrations) and ΔH_m the least. In the case of DPPE liposomes, T_m was decreased the most by PS-ClO_4 and PS-BF_4 (considering the lower temper-

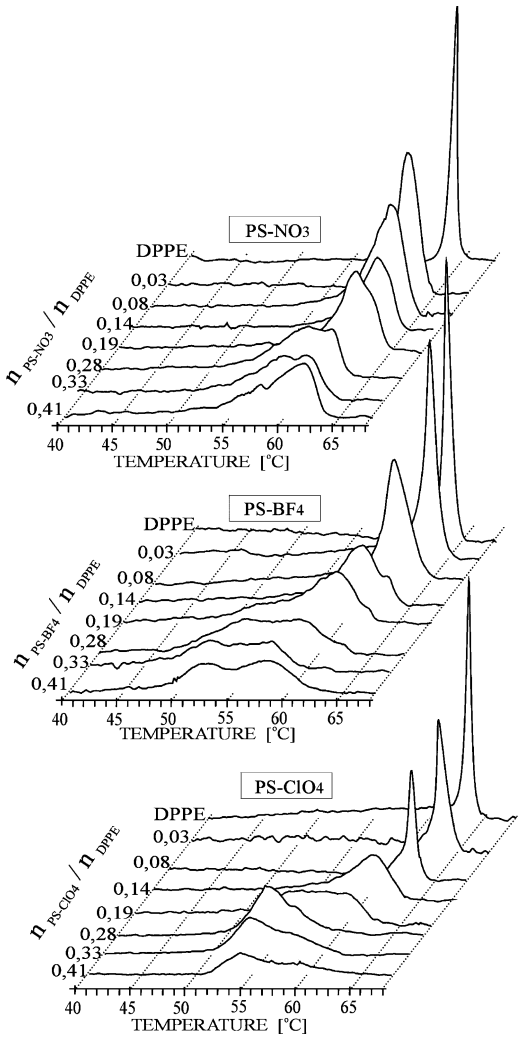

Fig. 3. DSC heating curves of MLVs with increasing molar ratios of PS-NO_3, PS-BF_4 and PS-ClO_4 vs. DPPE. The curves were normalized with regard to the amount of DPPE.

ature peak), while ΔH_m was increased the most by PS-Cl (Fig. 5).

3.2. ^1H NMR

^{1}H NMR spectra of DPPC and DPPE liposomes were performed at temperatures below (25 °C) and above (45 °C for DPPC, 65 °C for DPPE) the gel to liquid-crystalline phase transition. Only spectra at 25 °C are presented.

3.2.1. Aromatic protons

Aromatic proton resonance originating from the pyridine group of PS-X salts (Fig. 1) in water solution gives four signals (Fig. 6), which can be assigned to particular protons in the positions 2, 4, 5 and 6. In the presence of DPPC and DPPE liposome dispersions, the lineshapes and linewidths of these protons are significantly changed. In particular, they are significantly broader (signals from the aromatic protons in positions 4 and 6 even overlap) indicating a decreased mobility of these protons.

NMR lineshape and intensity depend on the kind of counterion, especially at 25 °C. In the lipid gel state, signals originating from the aromatic protons of only PS-Cl, PS-Br and PS-NO_3 are

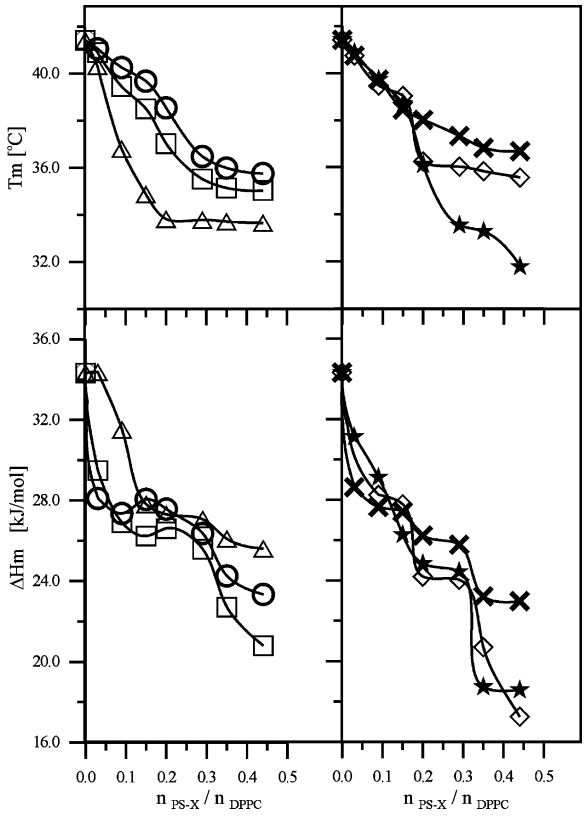

Fig. 4. Changes in temperature and enthalpy for the gel to liquid-crystalline phase transition of DPPC, as a function of surfactant/lipid molar ratio: PS-Cl (△); PS-Br (□); PS-I (○); PS-NO_3 (★); PS-BF_4 (×); PS-ClO_4 (◇).

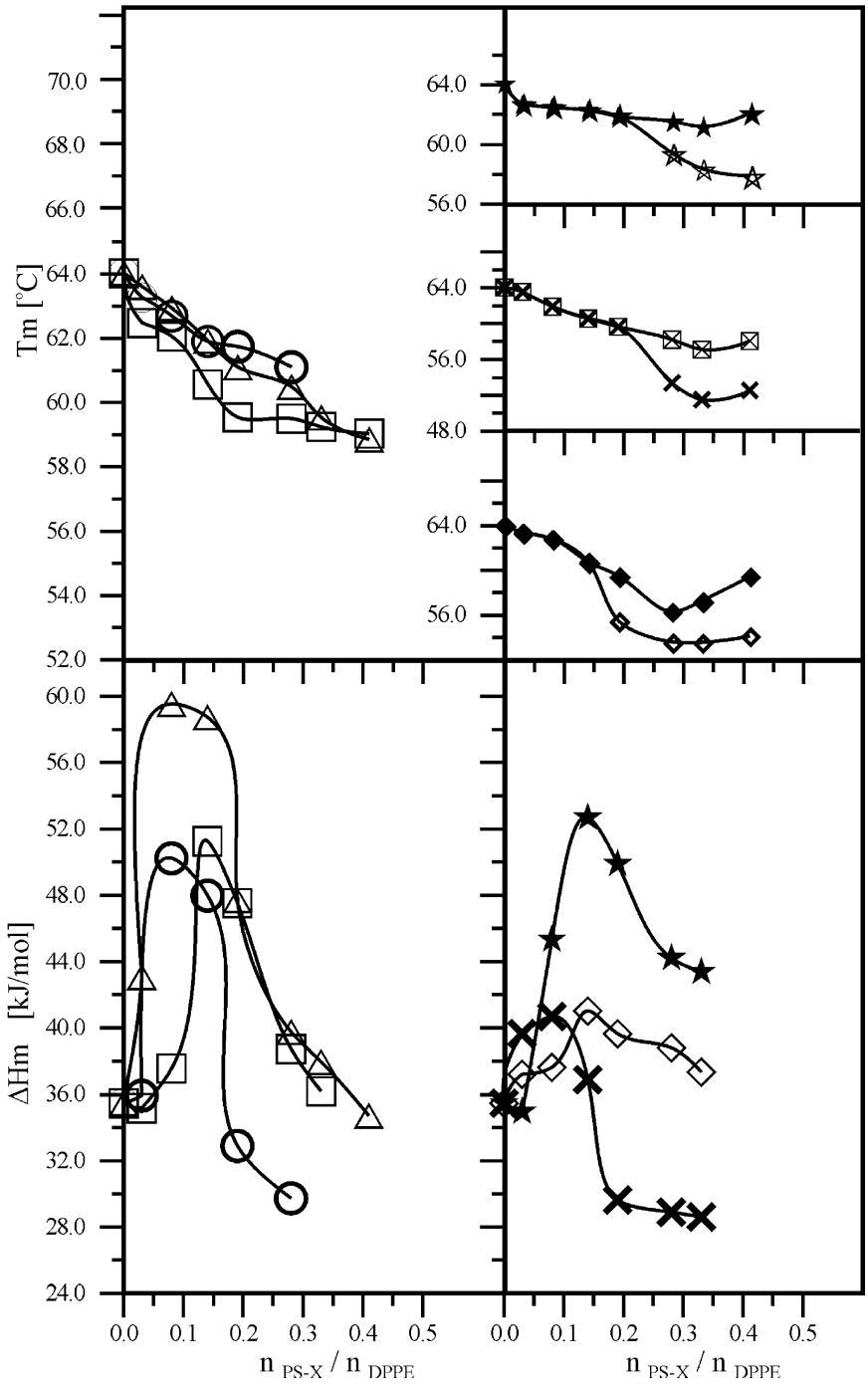

Fig. 5. The temperature and enthalpy change for the gel to liquid-crystalline phase transition of DPPE, as a function of surfactant/lipid molar ratio: PS-Cl (△); PS-Br (□); PS-I (○); PS-NO_3 (★); PS-BF_4 (×); PS-ClO_4 (◇).

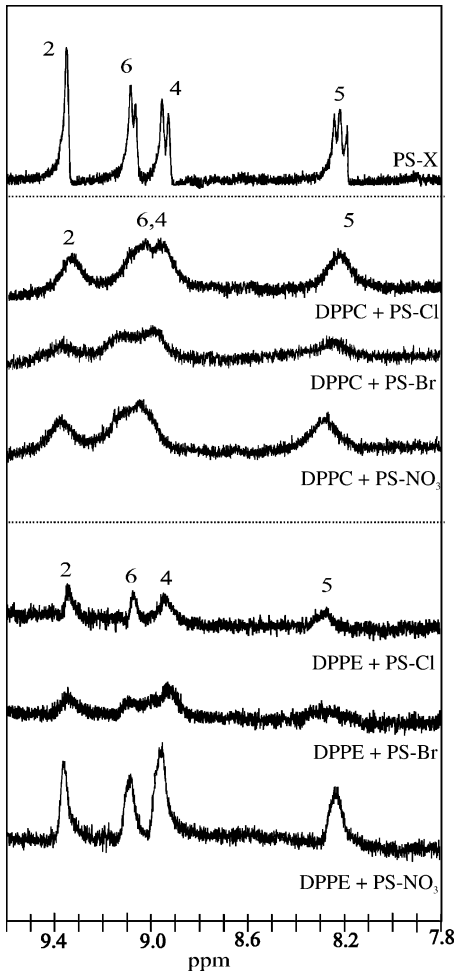

Fig. 6. ^{1}H NMR spectra of PS-X at 25 °C. The figure shows changes in the aromatic signals of PS-X in the absence (the upper most curve) and presence of DPPC liposomes (upper curves) and also in the presence of DPPE liposomes (lower curves). Surfactant/DPPC molar ratio was equal 0.33 and surfactant/DPPE molar ratio was equal 0.32.

detectable for both DPPC and DPPE liposomes. In the liquid-crystalline phase of both lipids, signals from the aromatic protons of all compounds are visible. For DPPC, the lineshapes of these aromatic protons are considerably narrower in the liquid-crystalline state than in the gel state. For DPPE, on the other hand, signals from PS-Cl and PS-Br in the liquid-crystalline state are of approximately the same intensity as those in the liquid and gel phases. For PS-NO_3, they exhibit lower intensity in the liquid crystalline phase than in the lipid gel phase, and for PS-ClO_4 and PS-BF_4 little intensity whatsoever in the liquid crystalline phase (and none detectable in the gel phase).

3.2.2. Trimethylammonium group protons of lecithin

The effects of various PS-X salts in liposome dispersions on the ^{1}H NMR resonance of trimethylammonium group protons in lecithin, $-[N^+(CH_3)_3]_L$, are compared in Fig. 7. In the lipid gel phase, adding PS-X results in considerable line narrowing. Besides, PS-ClO_4 and PS-BF_4 induce a shoulder in the upper field side of the $-[N^+(CH_3)_3]_L$ signal.

In the liquid-crystalline state, only minor line narrowing (for all counterions) was observed after adding PS-X.

3.2.3. Alkyl chain protons

The addition of a PS-X compound leads to a significant narrowing in the $(CH_2)_n$ proton signals of lipid alkyl chains, which indicates their increased mobility. In the gel state, PS-Cl, PS-Br and PS-NO_3 cause significant narrowing in the $(CH_2)_n$ proton signals of both DPPC and DPPE acyl chains. PS-ClO_4 and PS-BF_4 cause only minor narrowing in the $(CH_2)_n$ proton signals for DPPC acyl chains (Fig. 7).

In the liquid-crystalline state, approximately the same extent of signal narrowing was observed for all compounds and for both DPPC and DPPE liposomes.

4. Discussion

It seems more difficult to incorporate PS-X salts with large counterions into the lipid bilayer (especially in the gel phase) than ones with small counterions. Furthermore, insertion is deeper for small counterions. Therefore, compounds with large counterions may be expected to show low miscibility in the gel phase. Probably for this reason signals originating from the aromatic protons of PS-BF_4 and PS-ClO_4 are not detectable in the gel phase for both DPPC and DPPE liposomes (Fig. 6). In this phase, the narrowing of $(CH_2)_n$

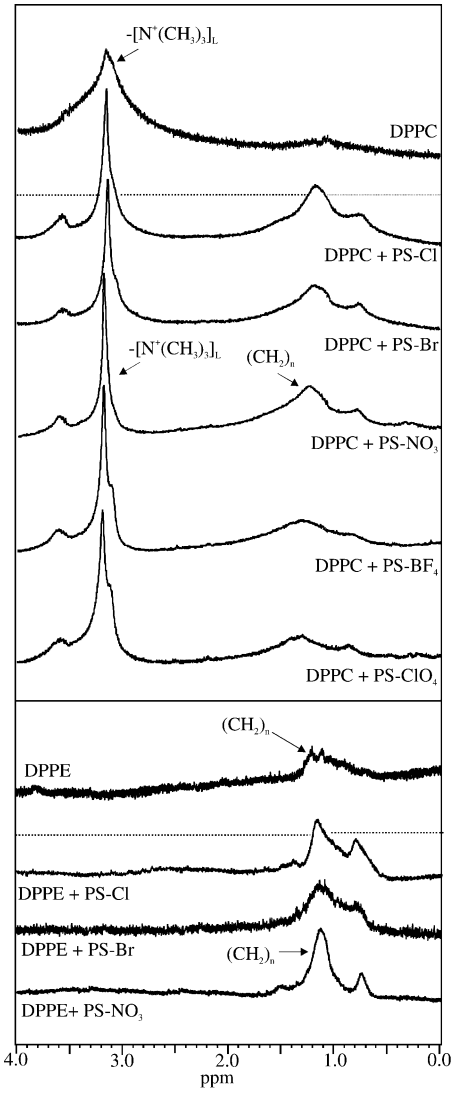

Fig. 7. ^{1}H NMR spectra of DPPC (upper curves) and DPPE (lower curves) liposome suspensions in the presence and absence of surfactants, at 25 °C. Surfactant/DPPC molar ratio was equal 0.33 and surfactant/DPPE molar ratio was equal 0.32. $-[N^+(CH_3)_3]_L$—^{1}H NMR resonance of the trimethylammonium group protons in lecithin. $(CH_2)_n$—^{1}H NMR resonance of alkyl chain protons.

proton signals (Fig. 7) due to PS-PF_4 or PS-ClO_4 insertion is lower than that for other PS-X compounds (DPPC liposomes) or even undetectable (DPPE liposomes). This means that alkyl chain mobility in the gel phase increases to a larger extent after the incorporation of PS-NO_3, PS-Cl or PS-Br. This may explain why these compounds decrease the T_m of DPPC more than the other compounds do (Fig. 4). Chloride and bromide are the smallest among those studied, thus they can easily intercalate between the polar groups of phospholipids. The same seems to be true for nitrate. Although nitrate is rather large, it is a planar ion and its cross section is lower than the diameters of chloride or bromide.

It might be anticipated, similarly to T_m, that ΔH_m is decreased to larger extent by PS-NO_3, PS-Br and PS-Cl than other PS-X compounds. As expected, the greatest decrease in ΔH_m was induced by PS-NO_3. Unexpectedly, PS-ClO_4 (the compound with the largest counterion) also induced such a decrease, while the lowest decrease in ΔH_m was noted, also unexpectedly, by PS-Cl (the compound with the smallest counterion). A large counterion may screen the positive charge on the nitrogen atom of the lecithin trimethylammonium group stronger than a small ion, and by this induce a sharper decrease in the electrostatic interaction between DPPC head groups. Such a process may explain why compounds with large counterions induced the steepest falls in ΔH_m. With respect to its influence on transition enthalpy, NO_3^- behaves like a large counterion, while influencing transition temperature like a small ion. Such a dual behaviour is possibly related to the molecular geometry of nitrate ion. The small cross section of NO_3^- enables PS-NO_3 to intercalate deeply into the bilayer (strong effect on T_m), while its rather large planar area allows it to effectively screen the positive charge on the nitrogen atom of the lecithin trimethylammonium group (strong effect on ΔH_m).

The effects of PS-X compounds on DPPC and DPPE thermotropic phase behaviour are in some ways quite different. These differences may be partly related to a significantly lower area per lipid in DPPE than in DPPC bilayers [10], and the consequential deeper insertion of PS-X molecules into DPPC than DPPE bilayers. Therefore, PS-X compounds should decrease T_m for DPPC to a larger extent than for DPPE. This was indeed observed in the case of PS-Cl, PS-Br, PS-I and PS-NO_3.

The addition of PS-ClO_4, PS-BF_4 or PS-NO_3 induced significant shift, broadening and splitting in the DPPE main phase transition (Figs. 3 and 5). The transition temperatures of these two peaks progressively decreased, especially for the one measured at lower temperature. This indicates that there are two overlapped thermal events, which can be attributed to the lateral phase separation of PS-ClO_4, PS-BF_4 or PS-NO_3 rich and pure domains. This phase separation may be a result of H-bonding between NO_3^-, ClO_4^- and BF_4^- ions and the ammonium group of DPPE. According to a hypothesis by Jain and Wu [11], a compound that specifically interacts with the polar group of DPPC promotes separation. If this hypothesis is extended onto DPPE, then the observed phase separation is a consequence of H-bonding between the oxygen atoms of NO_3^- or ClO_4^- or fluorine atom of BF_4^-, and a proton of the DPPE ammonium group.

At first glance, the formation of hydrogen bonds can also be expected between NO_3^-, ClO_4^- and BF_4^- counterions and the trimethylammonium group of DPPC. However, this does not seem to be likely due to the bulky methyl groups of DPPC head groups, which make them exhibit lower hydrogen-bonding possibilities than DPPE head groups (DPPE bears easily available polar hydrogen atoms). The bulky methyl groups are even thought of being unable to form hydrogen bonds at all [12].

PS-ClO_4 and PS-BF_4 probably also induced a phase separation in the DPPC liposomes, which is suggested by the appearance of a shoulder on the ^{1}H NMR resonance of the trimethylammonium group protons of lecithin, $-[N^+(CH_3)_3]_L$, in the presence of these compounds (Fig. 7). The reason for this separation seems to be different from that for DPPE liposomes. In this case, it is probably due to an unequal distribution of PS-ClO_4 and PS-BF_4 (compounds with the largest counterions) between the inner and outer monolayers. Small unilamellar vesicles used in NMR experiments show greater area per lipid molecule in the outer than the inner monolayer, which means that a molecule with a large counterion should incorporate more easily into the outer than the inner layer. In the case of the multilamellar bilayers used in DSC experiments, the difference in area per lipid between the two monolayers was lower. This is probably the reason why the presence of PS-ClO_4 (the compound with the largest counterion, larger even than BF_4^-) induced a small separation in the main phase transition of DPPC (Fig. 2).

The insertion of a molecule into the DPPE bilayer is connected with the disruption of the hydrogen bonds that can be formed between the ammonium group and water molecules or between the ammonium and phosphate groups. The disruption of these hydrogen bonds should lead to the destabilization of phospholipid bilayer structure and to a decrease in ΔH_m, which was indeed observed at higher concentrations (Fig. 5). At low concentrations of PS-X, a significant increase (the lowest effect for PS-ClO_4 and PS-BF_4) instead of an expected decrease in transition enthalpy was observed. This is probably due to the formation of another H-bond between the carbonyl group of PS-X and the ammonium group of DPPE. Due to steric reasons, the formation of these hydrogen bonds seems to be more likely for compounds with small counterions than for those with large counterions, excluding PS-NO_3. NO_3^- cross section is probably so small that the intercalation of the ion between the head groups of DPPE is deep enough to enable hydrogen bond formation. The involvement of the PS-X amide group in hydrogen bonding should restrict the molecular motion of the pyridyne ring. That is why, contrary to DPPC, the intensity of ^{1}H NMR signals originating from the aromatic protons of PS-Cl, PS-Br and PS-NO_3 in the liquid-crystalline state of DPPE (not shown) was not greater than that in gel phase.

Non-lamellar phases (cubic and hexagonal) have been observed when surfactant/egg-yolk DPPC systems [13] melt from the gel to liquid-crystalline phase. Such intermediate phases have also been observed in other cases, for example in fatty acid/DPPC systems [14]. It seems likely that non-lamellar structures will also rise in the studied PS-X/DPPC and PS-X/DPPE systems. Due especially to differences in their molecular geometry, the counterions of the compounds studied will probably influence the effective cross section of

the lipid molecule polar and non-polar regions. Consequently, these counterions may affect the structure assumed by possible PS-X/DPPC and PS-X/DPPE non-lamellar phases in various ways. X-ray diffraction measurements are planned in order to study the influence of counterions on the structural characteristics of the systems considered above. Such studies may essentially contribute to explaining the role of counterions in surfactant biological activity.

In our previous papers [1–3], we have shown that the interaction of surfactants with DPPC model membranes depends on the kind of counterion. Results therein were discussed in terms of the ability of counterions to modify water structure. The results of this paper illustrate the important role of counterions in the interaction of amphiphilic compounds with both DPPC and DPPE (representing a new class of lipids). Furthermore, these results enhance the significance of the molecular geometry of individual counterions in such interactions.

Acknowledgments

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