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A ^{31}P- and ^{2}H-NMR study on the structural perturbations induced by charged detergents in the headgroup region of phosphatidylcholine bilayers

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The effect of cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB) on the structure and mobility of phosphatidylcholine membranes was studied by ^{31}P- and ^{2}H-nuclear magnetic resonance. CTAB and DTAB induced a strong increase in the effective phosphorus chemical shift anisotropy of the phospholipid. In phosphatidylcholine which had been specifically labeled by deuterium in the headgroup segments, the detergents caused an increase in the quadrupole splittings from the *N*-methyl deuterons whereas the splittings from both methylene segments collapsed to a singlet signal at a ratio of about 2 mol of phosphatidylcholine per mol of detergent. The effect of DTAB on the headgroup spectral parameters was largely compensated by the negatively charged detergent sodium dodecylsulfate (SDS). In contrast to the phospholipid headgroup the hydrophobic region of the membrane was almost unaffected by CTAB or DTAB. The trimethylammonium headgroups of membrane-bound detergent molecules were found to be motionally much more restricted than the corresponding headgroup segments of the host phospholipid. From these observations it is concluded that the detergent headgroups are located close to the water/hydrocarbon interface of the phospholipid membrane thereby tilting the phospholipid headgroup dipole from the membrane parallel into a more upright orientation.

Introduction

The investigation of phospholipid structure in model membranes by various physical methods revealed that phosphatidylcholines and phosphatidylethanolamines assume a distinct flexible conformation in the liquid-crystalline state of the membrane. The most important features of this conformation are the orientation of the glycerol backbone perpendicular to the membrane surface and the almost membrane-parallel arrangement of the hydrophilic headgroups [1]. It has been shown by NMR that the spectroscopic parameters of the phospholipid headgroup are scarcely influenced by the nature of the fatty acyl chains [2]. Likewise, cholesterol induces little change in the average conformation of zwitterionic phospholipid headgroups [3]. In contrast, the interaction of charged species such as polyvalent metal ions [4,5], local anesthetics [6–9] or anionic lipids [10,11] lead to distinct changes in the phospholipid headgroup structure as judged from ^{2}H- and ^{31}P-NMR spectroscopy.

In biological membranes local cation charges producing local or transmembrane electric fields are expected to cause a strain upon the phos-

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; egg PC, phosphatidylcholine from egg yolk; DTAB, dodecyltrimethylammonium bromide; CTAB, cetyltrimethylammonium bromide; SDS, sodium dodecylsulfate; TLC, thin-layer chromatography.

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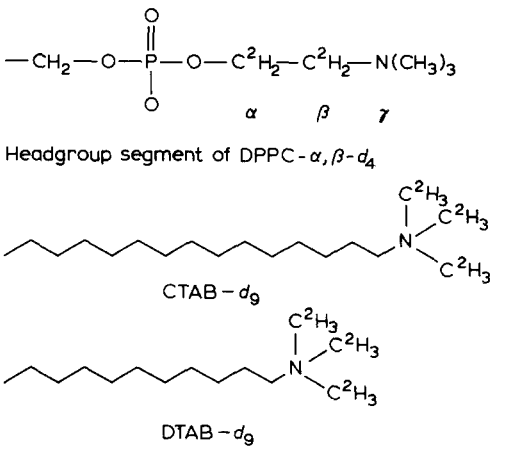
pholipid headgroup dipoles. Also the presence of charged amino acids in membrane proteins may lead to a change in the headgroup conformation of interacting phospholipids. This effect would be difficult to assess by NMR, since the residence time of membrane phospholipids at the surface of membrane proteins is usually short on the NMR time scale. Nevertheless, charge-induced changes in the headgroup conformation may contribute to the proper matching between phospholipids and protein surfaces.

In the present study the effects of positive charges on phospholipid structure have been investigated in membranous mixtures of phosphatidylcholine with *n*-alkyltrimethylammonium bromides. In these model membranes the charged detergent headgroups seem to be located close to the hydrophilic/hydrophobic interface of the membrane. This contrasts with divalent metal ions or local anesthetics which are bound more loosely in the framework of the phospholipid headgroups.



Scheme I.

Experimental procedures

Materials

DTAB, CTAB and SDS were obtained from Sigma and checked for purity by TLC. Also DPPC and DPPE were from Sigma. Egg PC and egg PE were prepared as described previously [12]. Deuterated iodomethane was from Sharp & Dohme and deuterated palmitic acid (palmitic acid-d_{31}) from Serdary Research Lab., Inc., London, Ontario. Deuterium depleted water was obtained from Sigma. (γ-$^2H_9$)-labeled egg PC (cf. Scheme I) was prepared by methylation of egg PE with deuterated iodomethane [13]. DPPC-α,β-d_4 was obtained accordingly from DPPE-α,β-d_4 using CH_3I instead of C^2H_3I for alkylation.

Ethanolamine-d_4 was prepared according to Taylor and Smith [14] and DPPE-α,β-d_4 was obtained from 1,2-dipalmitoyl-*sn*-glycerol and ethanolamine-d_4 as described by Eibl [15]. DPPC-2-d_{31} was obtained using the method of Boss et al. [16]. CTAB-d_9 and DTAB-d_9 were made by methylation of the corresponding amines with deuterated iodomethane in analogy to the methylation of egg PE. The crude products were precipitated from the reaction mixture by diethyl ether and stored overnight at 0°C. The precipitates were filtered, washed with a small portion of diethyl ether and carefully dried. The residues were dissolved in 7 ml of water and stirred for 1 h with stoichiometric amounts of Ag_2O. After removal of the precipitated AgI by centrifugation the supernatants were neutralized by 0.9 M HBr. The products were recrystallized from ethanol and checked for purity by TLC and ^{1}H-NMR.

Sample preparation

For NMR experiments in aqueous suspension phospholipids or phospholipid-detergent mixtures were deposited from chloroform solution in 50 ml flasks and thoroughly dried at reduced pressure. The lipid films containing about 100 mg of phosphatidylcholine and the appropriate amounts of detergent were suspended in 0.9 ml of water or deuterium-depleted water. Samples containing DPPC-d_{31} or DPPC-α,β-d_4 were carefully dried from chloroform solution in 6 mm o.d. Duran tubes. After addition of an equal amount by weight of deuterium depleted water the tubes were flame sealed and centrifuged several times forth and back at 50°C. For the preparation of oriented membranes the lipid samples were first hydrated in the same way except for lower water addition. 20–30 mg of the mixtures were spotted onto microscope cover slides. Typically 12 to 13 slides were stacked and mounted to the lower end of the axis of a NMR goniometer. The goniometer axis was inserted into an 8 mm o.d. quartz tube which was fixed coaxially in a 10 mm o.d. NMR sample

tube containing ^2H_2O for field/frequency stabilization. The goniometer was rotated in steps of 10°. The ^{31}P chemical shift anisotropy ($\Delta\nu^{CSA}$) was evaluated by a linear least-square fit of the observed line positions vs. $3\cos^2\theta - 1$, where θ is the angle between the normal to the membranes and the magnetic field direction.

NMR-measurements

All NMR-measurements were carried out with a Bruker SXP4-100 spectrometer operating at a field strength of 2.115 T. ^{31}P-NMR spectra were broadband decoupled at a decoupling field strength of about 150 μT. ^2H-NMR spectra were obtained by the quadrupole echo method [17]. The delay between the first and second pulses was 100 μs. Spectra exhibiting large quadrupole splittings were acquired on resonance which results in an enhancement of the signal to noise ratio by a factor $2^{1/2}$. The temperature was controlled with a digital thermometer.

Results

^{31}P-NMR

^{31}P-NMR spectra of egg PC and of mixture of egg PC with CTAB are displayed in Fig. 1A. The line shape in both spectra indicates that the phospholipids form a bilayer membrane [18]. In the presence of CTAB the absolute value of the effective chemical shift anisotropy ($\Delta\nu^{CSA}$) as defined by the distance of the low and high field edges of the ^{31}P spectrum is strongly enhanced. Obviously mixed CTAB-egg PC micelles do not form at the CTAB concentration given in Fig. 1A as can be concluded from the absence of a narrow peak in the magic-angle region of the ^{31}P powder pattern. Most probably CTAB is incorporated in the lamellar phospholipid membrane, the free concentration outside the membrane being below the critical micelle concentration (0.92 mM).

The determination of $\Delta\nu^{CSA}$ directly from the ^{31}P-NMR powder pattern is not very accurate. Moreover, computer simulation of the ^{31}P powder pattern is hampered since the line width of the component signals are strongly angular dependent [18,19]. To overcome these difficulties, $\Delta\nu^{CSA}$ was measured in planar oriented membranes by evaluating the angular dependence of the ^{31}P resonance frequency (see Methods). It must be noted that planar membrane alignment is progressively lost when the water/phospholipid ratio exceeds 22

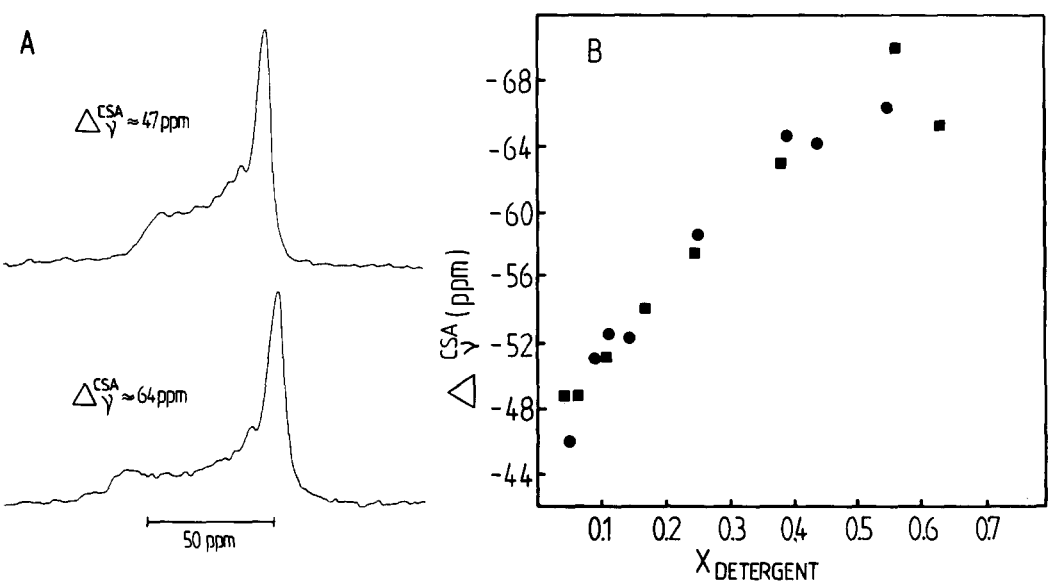


Fig. 1. (A) ^{31}P-NMR spectra of an egg PC dispersion in water (upper trace) and of a mixture of egg PC with CTAB, molar ratio 2:1 (lower trace). (B) Change in ^{31}P chemical shift anisotropy in egg PC with increasing mole fraction of DTAB (●) or CTAB (■) as determined in planar oriented membranes. Temperature 11°C.

mol/mol. Thus in all samples the water content was adjusted to about 20 mol per mol of egg PC. In parallel experiments it was found, that above 16 mol water per mol phosphatidylcholine $\Delta\nu^{CSA}$ is essentially independent of water concentration.

Both CTAB and DTAB cause a considerable change in $\Delta\nu^{CSA}$ as shown in Fig. 1B. The values decrease from −46 ppm in egg PC membranes to about −66 ppm in equimolar mixtures of egg PC with CTAB or DTAB. Obviously the effect does not depend on the aliphatic chain length of the detergent. The scatter in $\Delta\nu^{CSA}$ at detergent mol fractions above 0.5 may be traced to the onset of membrane micellization which becomes evident as a sharp signal superimposed to the powder pattern in ^{31}P-NMR of these mixtures in excess water (spectra not shown). Due to this uncertainty, it cannot be judged from the data in Fig. 1B whether $\Delta\nu^{CSA}$ approaches a limiting value at high concentrations of CTAB or DTAB.

^{2}H-NMR

The interaction of CTAB or DTAB with PC membranes was studied in more detail by ^{2}H-NMR after introduction of deuterium into several positions of the phosphatidylcholine molecule and into the detergent headgroup. Incorporation of CTAB into bilayers of egg PC-γ-d_9 leads to an increase in the ^{2}H-quadrupole splitting (Fig. 2A). At the highest detergent concentration attainable without membrane solubilization the quadrupole splitting is about 50% larger than the original value in the unperturbed egg PC-γ-d_9 membrane. Again the effect seems to be independent of whether CTAB or DTAB was added (Fig. 2B).

It is conceivable that electrostatic repulsion between the *N*-trimethylammonium headgroups of egg PC-γ-d_9 and CTAB contributes to the observed change in ^{2}H-quadrupole splitting. Thus it is to be expected that increasing ionic strength in the bulk aqueous medium would diminish the charge interaction in the headgroup region of egg PC.

However, the addition of 500 mM NaCl led only to a slight line broadening in the ^{2}H-spectra of CTAB-d_9-egg PC mixtures (spectra not shown), the ^{2}H-quadrupole splitting being almost identical to the value obtained in salt free solution. Charge screening as predicted by the Gouy-Chapman model is likely to be an oversimplification here, since the molecular dimensions of the interacting headgroup segments as well as the radii of the ions

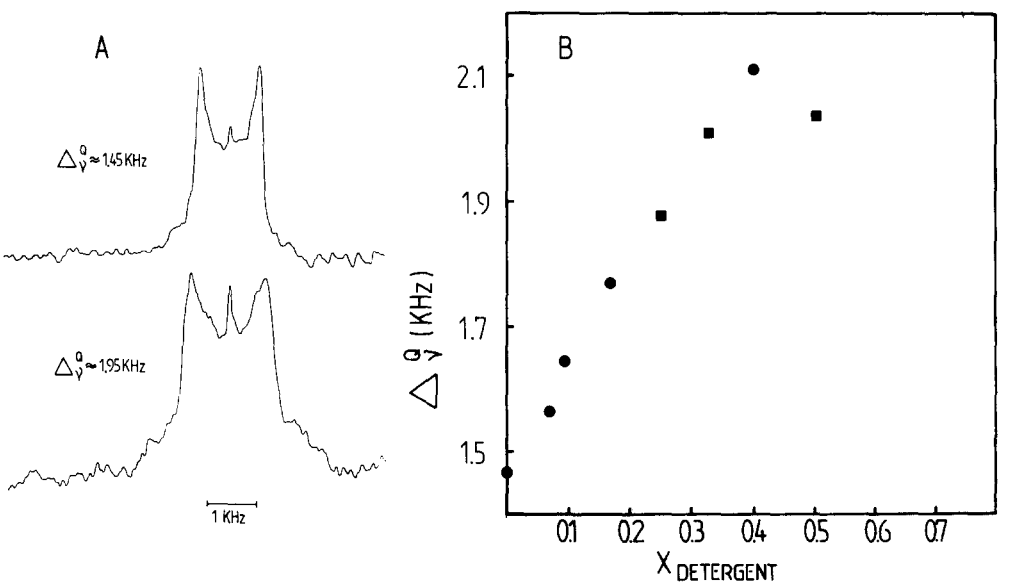


Fig. 2. (A) ^{2}H-NMR spectra of egg PC-γ-d_9 without (upper trace) and with DTAB (lower trace). Molar ratio egg PC-γ-d_9/DTAB 2:1. (B) Effect of increasing mole fractions of DTAB (●) and CTAB (■) on the ^{2}H-quadrupole splitting of egg PC-γ-d_9. Temperature 11°C.

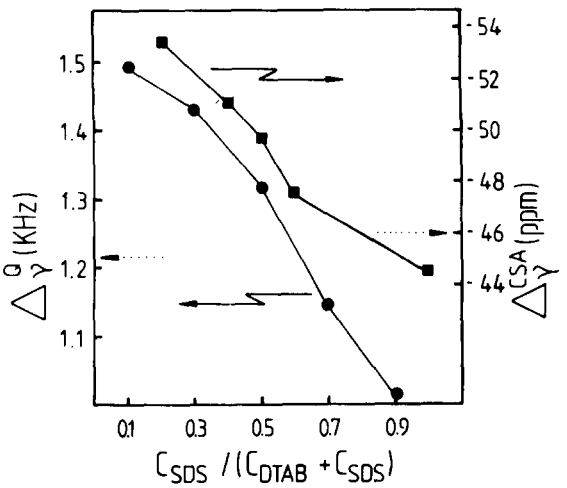


Fig. 3. ^{2}H-quadrupole splittings of egg PC-γ-d_9 at 28°C (●) and ^{31}P-chemical shift anisotropy of egg PC at 11°C (■) in the presence of varying proportions of DTAB and SDS. Total ratio of PC to detergent 6 mol/mol. The dotted arrows indicate the respective values of $\Delta\nu^Q$ and $\Delta\nu^{CSA}$ without detergent.

in solution must be taken into account.

The enhancement of $\Delta\nu^Q$ and $\Delta\nu^{CSA}$ caused by DTAB can be compensated by addition of the negatively charged detergent sodium dodecylsulfate (SDS) as shown in Fig. 3. SDS and DTAB were mixed with egg PC-γ-d_9 or egg PC at a fixed PC to detergent molar ratio of 6 : 1. ^{2}H spectra of the egg PC-γ-d_9 samples (not shown) exhibit a single quadrupole splitting in the whole range of SDS/DTAB ratios. The magnitude of the splitting decreases monotonically with increasing SDS concentration. At an equimolar ratio of the two oppositely charged detergents $\Delta\nu^Q$ is only slightly larger than in unperturbed egg PC-γ-d_9 membranes. Higher proportions of SDS lead to a reversal of the effect, i.e. $\Delta\nu^Q$ becomes smaller than the value observed in detergent-free egg PC-γ-d_9 (1.2 kHz). Analogously, $\Delta\nu^{CSA}$ decreases with increasing SDS concentration (see Fig. 3). The neutralization of the DTAB-induced spectral changes by a detergent of the same length but of opposite headgroup charge corroborates the assumption that the effect upon $\Delta\nu^Q$ and $\Delta\nu^{CSA}$ is primarily due to charge-charge interaction.

The observed increase in $\Delta\nu^Q$ is most probably due to a change in the average orientation of the phosphatidylcholine headgroup. Alternatively, restriction in the configurational space available to

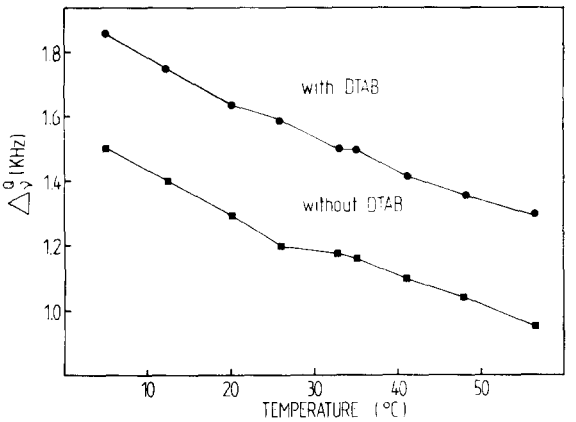


Fig. 4. Temperature dependence of the ^{2}H-quadrupole splittings of egg PC-γ-d_9 and of a mixture of egg PC-γ-d_9 with DTAB. Molar ratio egg PC-γ-d_9/DTAB 5 : 1.

the $N(C^2H_3)_3$-moiety of egg PC-γ-d_9 could result in an increased quadrupole splitting. However, the temperature dependence of $\Delta\nu^Q$ argues against restriction in motional freedom. As depicted in Fig. 4, the change in $\Delta\nu^Q$ with temperature is

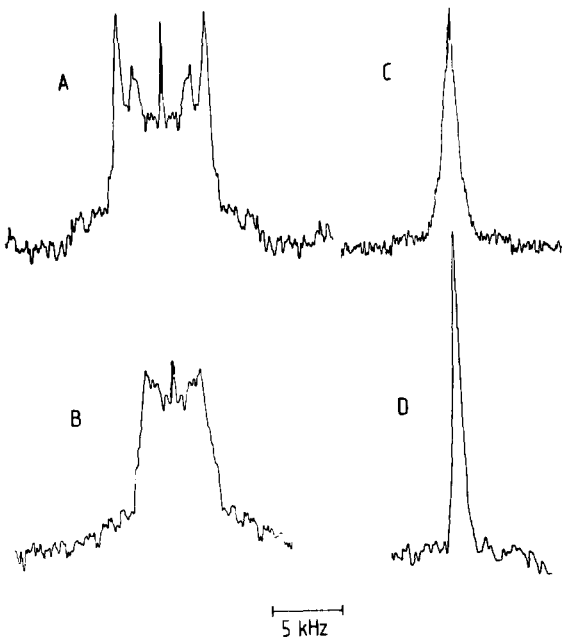


Fig. 5. ^{2}H-NMR spectra of DPPC-α,β-d_4 in the absence (A) and in the presence (B–D) of CTAB. Molar ratios phospholipid/CTAB 12 (B), 6 (C) and 2 (D), respectively. Temperature 45°C. The different intensities of the superimposed powder patterns are due to different deuterium incorporation into the α- and β-positions (> 90% vs. ≃ 50–60%).

quite similar with and without DTAB. The absolute difference in the splitting decreases by about 10% on going from 5°C to 55°C. Qualitatively it can be expected that hindrance of thermal oscillations in the PC headgroup by the bulky headgroup of the detergents would lead to a reduced slope in the $\Delta\nu^Q$ vs. temperature plot and, eventually, to intersection of the curves at low temperature. On the other hand, a slight change in the average oritentation of the PC headgroup without reduction of the vibrational amplitude is likely to yield a similar temperature dependence of $\Delta\nu^Q$ as in the original membrane, in agreement with the experimental result. The slight decrease in the enhancement of $\Delta\nu^Q$ with increasing temperature suggests that thermal fluctuations tend to outweigh the DTAB-induced conformational change in the choline headgroup.

A conformational change in the phospholipid headgroup became also evident when CTAB was added to dipalmitoylphosphatidylcholine labeled with deuterium in the α- and β-positions of the choline moiety (DPPC-α,β-d_4). Without CTAB the two C^2H_2-groups exhibit well resolved quadrupole splitting of about 5.8 and 4.4 kHz, respectively *, at 45°C (Fig. 5). Increasing concentrations of CTAB in the membrane lead to progressive reduction of the splitting from both C^2H_2-segments and, at a molar ratio of DPPC-d_4 to CTAB of 2:1, to coalescence into a single resonance signal. ^{31}P-NMR in the same sample clearly revealed the bilayer state (cf. Fig. 1A), indicating that the narrow ^{2}H-signal is not due to isotropic motion of the phospholipid molecules. Also decomposition of the phospholipid after the prolonged ^{2}H-NMR measurements was excluded by thin-layer chromatography.

Below 44°C the intensity of the narrow signal in Fig. 5D decreased with decreasing temperature (see Fig. 6). At 33°C the signal was no longer observable, suggesting that the phospholipid underwent the thermotropic phase transition. The broad temperature range over which the transition

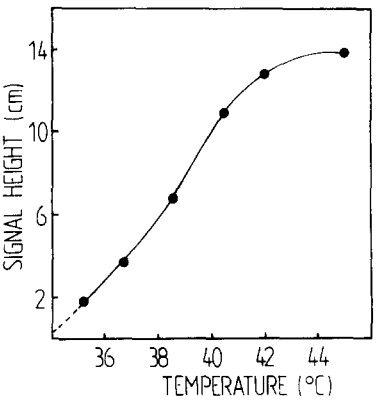


Fig. 6. Temperature dependence of the signal height of the singlet signal obtained in a mixture of DPPC-α,β-d_4 with CTAB at a molar ratio 2:1 (cf. Fig. 5D).

occurs is most probably due to phase separation in the membrane [20].

The effect of CTAB insertion upon the hydrophobic region of the membrane was investigated in dipalmitoylphosphatidylcholine perdeuterated in the *sn*-2 fatty acid chain (DPPC-*sn*-2-d_{31}). ^{2}H-spectra obtained with and without CTAB (Fig. 7) show the characteristic superposition of large quadrupole splittings from the first 9–10 C^2H_2 groups (except for the C^2H_2 in the 2-position of the *sn*-2 chain) [21]. Obviously, the perturbation caused by CTAB in this region is negligible. Also the narrow quadrupole splitting at the center of the spectrum, which is due to the terminal fatty acid methyl group seems to be unaffected. Subtle changes at intermediate splittings are not resolvable due to poor signal to noise ratio and base line distortions. The basic conclusion of negligible disordering over the total length of the *sn*-2 chain seems however to be justified since perturbation on the extreme ends of the chain are barely detectable.

The ordering of the *n*-alkyltrimethylammonium bromide detergents when intercalated into egg PC membranes was studied at the level of the detergent headgroup in the specifically labeled derivatives CTAB-d_9 and DTAB-d_9, respectively (cf. Scheme I). ^{2}H-quadrupole splittings at different temperatures and detergent concentrations are summarized in Fig. 8. Obviously, as anticipated above, the two detergents behave quite similar in the temperature range investigated when judged

* These quadrupole splittings differ slightly from literature values obtained in selectively α- or β-labeled phosphatidylcholines [4], presumably due to the presence of deuterium in both positions.

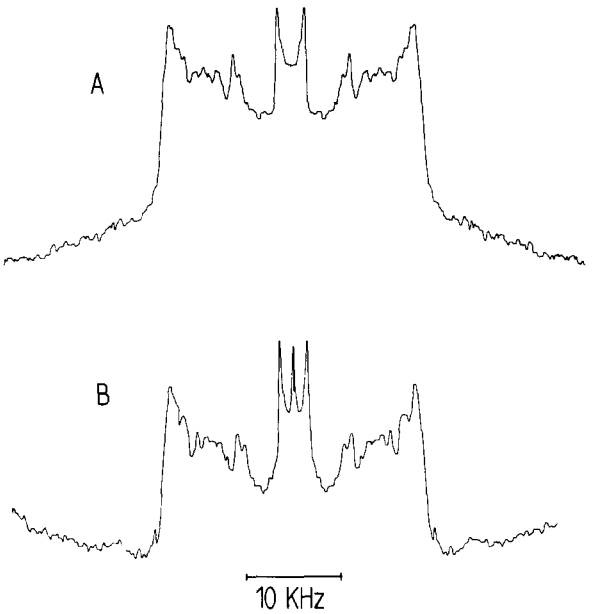


Fig. 7. ^{2}H-NMR spectra of DPPC-*sn*2-d_{31} (A) and DPPC-*sn*2-d_{31} in the presence of CTAB (B). Molar ratio phospholipid/CTAB 4:1, temperature 45°C.

by their respective $\Delta\nu^Q$ values. Slightly enhanced splittings are observed at very high detergent to phospholipid ratios, presumably due to mutual steric hindrance of the detergent $N(C^2H_3)_3$-groups in the membrane plane. Note that the magnitude of $\Delta\nu^Q$ is about 3–4-fold larger than the corresponding values obtained from the $N(C^2H_3)_3$ group in PC-d_9.

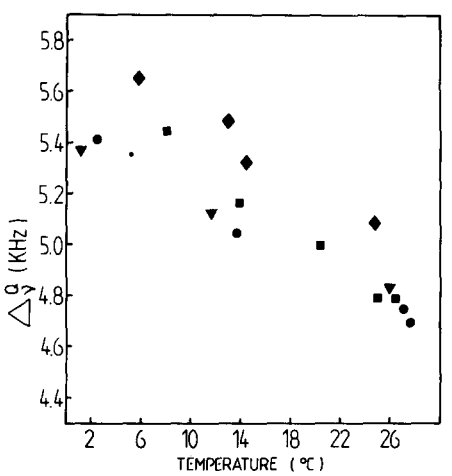


Fig. 8. ^{2}H-quadrupole splittings of CTAB-d_9 and DTAB-d_9 in egg PC membranes. Egg PC + CTAB, molar ratio 6:1 (●); egg PC + DTAB, molar ratio 5:1 (▼); egg PC + CTAB, molar ratio 4:1 (■); egg PC + CTAB, molar ratio 2:1 (◆).

^{31}P- and ^{2}H-spin lattice relaxation

The motional properties of the PC and CTAB headgroups in the mixed membrane were determined by measuring ^{31}P- and ^{2}H-spin lattice relaxation times. For the $N(C^2H_3)_3$ deuterons in the PC-d_9 headgroup very similar T_1 values were found over a large temperature range in the presence and absence of CTAB or DTAB (see Fig. 9). Thus the spectral density function relevant to T_1 relaxation as well as the thermal activation of the T_1 process seem to be unaffected by the presence of CTAB. Also ^{31}P-T_1 relaxation times without detergent and at egg PC/CTAB molar ratios of 4 and 2, respectively, were nearly identical (1.2–1.3 s at 9°C). This corroborates the tentative conclusions drawn from the temperature dependence of the ^{2}H-quadrupole splittings in PC-d_9 (cf. Fig. 4).

When ^{2}H-T_1 values of the $N(C^2H_3)_3$-deuterons in egg PC-γ-d_9 and in membrane bound CTAB-d_9 or DTAB-d_9 are compared, it turns out that the detergent headgroups have considerably less motional freedom than the corresponding headgroup segment of the host phospholipid. At 24°C T_1 was 19 ± 3 ms in membrane bound CTAB-d_9 and 47

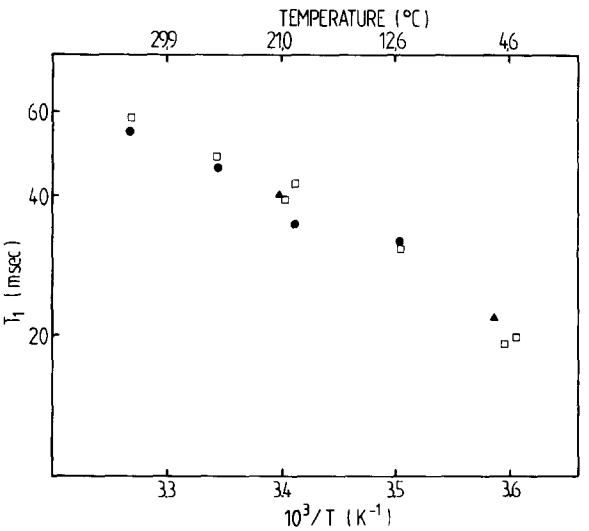


Fig. 9. ^{2}H-spin lattice relaxation times of egg PC-γ-d_9 (□), egg PC-γ-d_9 + DTAB (●) and CTAB (▲). Molar ratio phospholipid/detergent 4.7:1.

± 2 ms in PC-γ-d_9 (molar ratio egg PC/TAB 5 : 1).

The large difference in the quadrupole splittings of the two deuterated headgroups probably reflecting different ordering may provide a contribution to the observed differences in T_1. Brown et al. [22] derived an equation pertinent to the interrelationship between the C-^{2}H order parameter (S_{C^2H}) and T_1:

$$\frac{1}{T_1} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \left(1 + \tfrac{1}{2}S_{C^2H} - \tfrac{3}{2}S^2_{C^2H}\right)\tau_c$$

where e^2qQ/h denotes the static quadrupole coupling constant for C-^{2}H bonds (170 kHz) and τ_c the motional correlation time. It follows from this equation that the contribution of S_{C^2H} to the spin-lattice relaxation is 1.7% and 0.5% for CTAB-d_9 and egg PC-γ-d_9, respectively. Thus the difference in T_1 relaxation rate is mainly due to the restricted mobility of the CTAB headgroup in the mixed bilayer membrane.

Discussion

The ^{31}P and ^{2}H data presented here indicate that the incorporation of positively charged alkyltrimethylammonium bromide detergents into the phospholipid bilayer induces distinct conformational changes in the phospholipid headgroups but not in the fatty acyl chains. Obviously the electrostatic and steric interactions in the membrane surface are not carried over to the hydrophobic core of the membrane as witnessed by ^{2}H-NMR in mixed bilayers containing deuterated phospholipid fatty acyl chains (cf. Fig. 7).

The large quadrupole splittings and the reduced spin lattice relaxation time in the headgroup deuterated detergents suggest that the detergent $N(C^2H_3)_3$ groups are quite immobilized. The motional restriction of the detergent headgroup becomes even more obvious when the quadrupole splittings are evaluated in terms of order parameters [23]. An order parameter S_{CN} with respect to the membrane normal (the director axis) can easily be derived for the bond between the N-terminal methylene carbon and the trimethyl ammonium group. The observed quadrupole splitting $\Delta\nu^Q$ is related to the order parameter for the C-^{2}H bond, S_{C^2H}, by (Ref. 21):

$$\Delta\nu^Q = \frac{3}{4}\frac{e^2qQ}{h}S_{C^2H}$$

The evaluation of S_{CN} from S_{C^2H} is quite simple, when the motion about the bond axes of the C^2H_3 groups and of the whole $N(C^2H_3)_3$ group is rapid on the NMR time scale. Transformation from the C^2H bond direction into the direction of the C-$N(C^2H_3)_3$ bond results in:

$$S_{CN} = S_{C^2H}/\tfrac{1}{2}(3\cos^2\alpha - 1)\tfrac{1}{2}(3\cos^2\beta - 1)$$

where α and β denote successive rotations through the bond angles ^{2}H-C-N and C-N-C, respectively.

An additional rotation γ may be performed, with γ being the angle between the C-$N(C^2H_3)_3$ bond vector and the long axis of the detergent molecule in the all-*trans* conformation. From this rotation a molecular order parameter S_{mol} may be derived assuming an extended orientation of the detergent molecule with the long molecular axis running on the average parallel to the membrane normal:

$$S_{mol} = S_{CN}/\tfrac{1}{2}(3\cos^2\gamma - 1)$$

With $\alpha = 109°$, $\beta = 113°$ [24] and $\gamma = 35°$ (using 109° for the C-C-N bond angle) rather large values of S_{mol} are obtained. It follows from the data summarized in Fig. 8, that at low detergent concentrations S_{mol} increases from 0.80 to 0.89 when the temperature decreases from 25°C to 6°C. In an equimolar CTAB-egg PC mixture the values of S_{mol} are even close to unity (0.85 at 25°C and 0.95 at 6°C). It cannot be excluded, however, that the tentative assumptions discussed above are not valid notably at very high detergent concentrations in the membrane.

The interpretation of the NMR data from the phospholipid headgroup in terms of a unique conformational change is yet more difficult. The location of positive detergent charges in the membrane surface suggests, that the trimethylammonium group of PC will be pushed away from the membrane plane. The continuing increase in quadrupole splitting with detergent concentration indicates that the phospholipid C-$N(C^2H_3)_3$ bond is far from reaching a completely upright conforma-

tion. Fixation of this bond in an upright, director parallel direction ($S_{mol} = 1$) would result in a quadrupole splitting of about 12 kHz following an analogous reasoning as above. Thus the experimentally observed maximal increase in $\Delta\nu^Q$ from 1.4 kHz to 2.1 kHz (Fig. 2) may be due to a rather small change in the average orientation of the headgroup. Most probably the C-N$(CH_3)_3$ segment is slightly tilted away from the magic angle towards the membrane director.

The change in ^{31}P chemical shift anisotropy may be evaluated tentatively assuming, that the orientation of the shielding tensor as obtained earlier in barium diethylphosphate [25] applies to the phosphodiester moiety of phosphatidylcholines. In barium diethylphosphate the most negative value of the shielding tensor, σ_{11} (-76 ppm) is perpendicular to the plane O-P-O, containing the nonesterified oxygens, whereas σ_{22} (-18 ppm) bisects the O-P-O angle. On the basis of this tensor orientation it has been inferred [26], that in hydrated PC membranes the O-P-O plane is tilted by about 50° with respect to the bilayer normal. A slight increase in this tilt angle would lead to a more negative shielding value $\sigma_{\parallel}$ (bilayer normal parallel to the magnetic field direction) and correspondingly to a more positive $\sigma_{\perp}$ or rather to an enhanced $\Delta\nu^{CSA}$. This is in qualitative agreement with the results of extensive computer modelling by Skarjune and Oldfield [27]. Although this interpretation is not unique, it seems reasonable to assume that steric interaction with the bulky trimethylammonium groups of the membrane bound detergent leads to a tilt in the orientation of the phosphodiester moiety.

The drastic decrease of the ^2H-quadrupole splittings from the methylene deuterons of the phospholipid headgroup (α- and β-C^2H_2; cf. Scheme I) is also indicative of a conformational change. The most convincing evidence of a conformational change follows from the observation that at a high CTAB concentration in the membrane the quadrupole splitting collapses completely, whereas the ^{31}P spectrum exhibits a bilayer line shape and an even enhanced absolute value of $\Delta\nu^{CSA}$. It is, however, impossible to draw any conclusion on the 'average' values of the torsion angle about the α-β bond on the basis of the available data.

It has been shown previously by NMR, that the addition of positively charged amphiphilic drugs such as local anesthetics [7–9] and phenothiazines [6] leads to structural perturbations in the phospholipid membrane. However, the changes in NMR parameters caused by CTAB and DTAB are distinctly different from the effects reported earlier for the drug molecules.

Binding of tetracaine leads to a decrease in $\Delta\nu^Q$ from the α-C^2H_2 segment and to an enhanced $\Delta\nu^Q$ from the β-C^2H_2 group in selectively labeled egg PC [8]. This behavior is quite similar to the change in quadrupole splittings caused by di- and trivalent metal ions [4]. The addition of CTAB, however, results in reduced $\Delta\nu^Q$ values from both methylene segments (cf. Fig. 5). On the other hand, an enhancement of the ^{31}P chemical shift anisotropy and of the quadrupole splitting from PC-γ-d_9, though smaller than in the presence of the detergents, was also obtained with tetracaine [8]. A similar increase of $\Delta\nu^{CSA}$ was found upon addition of chlorpromazine [6]. Thus it seems not unreasonable to assume that positively charged amphiphilic molecules induce generally similar conformational changes in the phospholipid headgroup. The most important difference probably resides in the average orientation of the α- and β-methylene groups (cf. Scheme I) leading eventually in the case of the trimethylammonium bromide detergents to fluctuations of these C^2H_2-segments about the magic angle.

Also the finding, that in the presence of large amounts of the detergents the ordering profile of the lipid acyl chains is maintained (Fig. 7) may be contrasted to the disordering induced by the anesthetics [6,8,9]. Thus it can be assumed that the alkyl chains of the detergents fit quite qell into the fatty acid region of the bilayer. This agrees with the observation that the onset of the thermotropic phase transition in a mixture of DPPC-d_4 and CTAB is very close to the transition temperature of pure DPPC (cf. Fig. 6). Mabrey and Sturtevant [28] have shown by differential scanning calorimetry that the phase transition temperature of DPPC was even enhanced upon addition of palmitic acid suggesting that the fatty acid reduces steric repulsion of the phospholipid headgroups without affecting the lateral packing of the hydrocarbon chains.

The quadrupole splittings observed in membrane-bound CTAB-d_9 and DTAB-d_9 are much larger than the splittings of correspondingly headgroup-deuterated tetracaine (4.9 kHz vs. 1.9 kHz at 20°C). Moreover, with procaine specifically ^{2}H-labeled in the methylene groups of the *N,N*-diethyl headgroup, no quadrupole splitting was obtained [8,9]. Thus, in the membrane the local anesthetics have much more motional freedom than the trimethylammonium detergents at the level of their respective charged headgroups. The same conclusion follows from spin lattice relaxation times of headgroup deuterated tetracaine at pH 5.5 obtained in bilayers of phosphatidylethanolamine [9] and of CTAB-d_9 bound to PC membranes (51 ms vs. 19 ms at 20°C). These observations suggest, that the detergent headgroups are fixed close to the membrane surface. In this molecular arrangement the high ordering of the phospholipid backbone as determined by ^{2}H-NMR (cf. Fig. 7) may be conveyed to the first CH_2 segments of the membrane bound detergent and thereby to the detergents $N(CH_3)_3$-group.

In summary, the spectral changes observed in the presence of CTAB and DTAB can be satisfactorily explained by a small tilt of the entire phospholipid headgroup dipole from the membrane-parallel into a somewhat more upright position. The immobilization of the CTAB- and DTAB-trimethylammonium groups suggests, that the detergent headgroup is deeply embedded in the phospholipid headgroup layer thereby minimizing the water-hydrocarbon contact. This may explain the unique change in the orientation of the phospholipid methylene segments.

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