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Estimation of the Equilibrium Lateral Pressure in Liposomes of 1-Palmitoyl-2-[10-(pyren-1-yl)-10-ketodecanoyl]-*sn*-glycero-3-phosphocholine and the Effect of Phospholipid Phase Transition

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ABSTRACT: A phosphatidylcholine analogue, 1-palmitoyl-2-[10-(pyren-1-yl)-10-ketodecanoyl]-*sn*-glycero-3-phosphocholine (k-PPDPC), which contains a chromophore covalently attached to one of the acyl chains, was characterized spectroscopically as liposomes and monolayers on an air/water interface. Differential scanning calorimetry of k-PPDPC liposomes showed two endothermic transitions with peaks at 13.7 and 23.8 °C, having enthalpies of 0.6 and 1.7 kcal/mol, respectively. Liposomes of k-PPDPC also exhibit thermotropic changes in the absorption spectra so that the ratio of the peaks at 289 and 356 nm changes from 2.15 to 1.60 in the temperature range of 20–26 °C, coinciding with the second calorimetric transition. These bands appear in the reflectance spectra of k-PPDPC monolayers on water and reveal a strong surface pressure dependency. Neglecting any possible effects due to coupling of the two leaflets of liposomal bilayers on the changes in absorption spectra and assuming the spectroscopic changes as a function of lateral pressure in monolayers to be equivalent to those in liposomes, we estimated the equilibrium lateral pressure (π) of k-PPDPC liposomes to be approximately 39 and 17 mN/m below and above the transition at 23 °C, respectively. The exact nature of the transition of k-PPDPC is uncertain, and thus a direct comparison with phospholipids such as dipalmitoylphosphatidylcholine is ambiquous. However, as far as we know this is the first relatively direct observation of a change in π accompanying a phase transition in liposomal membranes.

While it is accepted that the equilibrium lateral pressure (π)[1] of cell membranes influences a number of important cellular phenomena involving membranes [for references, see Thurén et al. (1986)], it is unfortunate that there is no technique currently available to allow the direct assessment of this parameter. On the basis of thermodynamic calculations, Nagle (1976, 1980) estimated approximately 50 mN/m for π in DPPC liposomes. Only a few experimental approaches have been published so far. A value of 20 mN/m was deduced from NMR order parameter calculations for liquid-crystalline bilayers (Marcelja, 1974). Use of phospholipases A with dif-

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; k-PPDPC, 1-palmitoyl-2-[10-(pyren-1-yl)-10-ketodecanoyl]-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; PPDPC, 1-palmitoyl-2-[10-(pyren-1-yl)-decanoyl]-*sn*-glycero-3-phosphocholine; π, equilibrium lateral pressure.

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ferent surface pressure optima gave approximately 33–34 mN/m for π in the outer monolayer of the erythrocyte membrane (Demel et al., 1975). Based on isotherms and isobars recorded for DPPC on an air/water interface, assessment of liposomal π yielded a value of 12.5 mN/m (Albrect et al., 1978). An electron diffraction study gave 47 mN/m as the external lateral pressure under which a DPPC monolayer would have the same transition temperature as a hydrated bilayer in the absence of external lateral pressure (Hui et al., 1975). Notably, fluorescence anisotropy data obtained by using diphenylhexatriene as a membrane probe have been analyzed with a model revealing liposomal π (Fulford & Peel, 1980). Their calculations gave a decrease of approximately 40 mN/m at the transition of DPPC, to an equilibrium lateral pressure of about 25 mN/m in the liquid-crystalline state immediately above the transition.

We have recently described a method for the estimation of π in liposomes of 1-palmitoyl-2-[6-(pyren-1-yl)-hexanoyl]-*sn*-glycero-3-phospholipids, i.e., -choline, -ethanolamine, -glycerol, -serine, and -monomethyl ester (Thurén et al., 1986). Briefly, compression isotherms were recorded for these lipids on an argon/water interface. Thereafter, pyrene excimer/monomer fluorescence intensity ratio was recorded for liposomes of the same lipids. The assessment of π from these data was based on the following assumptions: (i) Pyrene excimer/monomer fluorescence intensity ratio for the above lipids is linearly dependent on the reciprocal of the mean molecular area of these lipids in liposomes, (ii) equilibrium surface pressure is the same in liposomes of these pyrene-labeled phospholipids regardless of the head-group structure, and (iii) possible head group dependent differencies in the orientation of the pyrene moiety are neglected. We then sought for that surface pressure value in the compression isotherms where the correlation of the reciprocal of the mean molecular area in monolayers to the excimer/monomer fluorescence intensity ratios observed in liposomes was maximal. The value obtained in this study, 12 mN/m, is rather low and is likely to reflect the rather loose packing of the membranes due to the bulky pyrene moiety brought in the vicinity of the head-group region.

The present paper describes a somewhat more direct technique for the estimation of π in liposomes. Like in our previous study, a phospholipid analogue with a covalently linked chromophore in one of the acyl chains was employed. Therefore, direct conclusions on membranes consisting of straight-chain phospholipids are still ambiquous. However, the present study reveals additional evidence for a drastic change in π associated with the thermotropic phase transition of phospholipids. Although the biological significance of the latter membrane process is by and large not understood, there is evidence for the involvement of an isothermal phase transition of phospholipids in the molecular mechanism of nerve excitability (Kinnunen & Virtanen, 1986). Accordingly, understanding of changes produced by the phase transition in biomembrane properties can be considered to be of importance.

Materials and Methods

The phospholipid analogue 1-palmitoyl-2-[10-(pyren-1-yl)-10-ketodecanoyl]-*sn*-glycero-3-phosphocholine (k-PPDPC; M_r 893.11) was from the Department of Chemistry of Liquid Crystals of KSV Chemical Corp. and was supplied as a chloroform solution.

By use of a KSV 2200 LB system (KSV Chemical Corp., Helsinki, Finland) equipped with the DFC unit and a Langmuir trough made of Teflon, monolayers of k-PPDPC were spread in a chloroform solution (1 mg/mL) with a Hamilton microsyringe on Milli-RO/Milli-Q (Millipore) filtered water

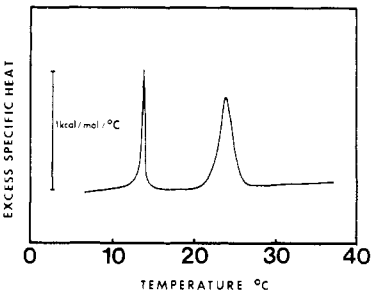

FIGURE 1: DSC scan of k-PPDPC liposomes (0.5 mg/mL) in water recorded at a heating rate of 0.5 °C/min.

of pH 7.0. Temperature of the subphase was ≈23 °C. Isotherms were recorded by using a constant compression rate of 4 Å^2/(min·molecule). Reflectance spectra of k-PPDPC monolayers on water as a function of surface pressure were subsequently recorded with a Photal MCPD-100 spectral analyzer (Otsuka, Osaka, Japan) equipped with quartz fiber optics and interfaced to a NEC-PC. Identical results were obtained by using either an air/water or an argon/water interface. Accordingly, due to easier experimentation the former was routinely employed.

For the preparation of liposomes aliquots of k-PPDPC were dried under nitrogen flow in a glass test tube. Residual solvent was then removed by maintaining the lipid under reduced pressure overnight. The dry lipid was hydrated with ultrafiltered water to yield the indicated final lipid concentrations whereafter the suspension was exposed to ultrasound for 2 min with a Bransonic 220 bath type sonicator at ≈45 °C.

Absorption spectra of liposomes of k-PPDPC in water were measured at a lipid concentration of 10 nmol/mL with a Hitachi U-3200 spectrophotometer with a cuvette holder thermostated with a Lauda RC 6 circulating water bath. Scattering was corrected by using DPPC liposomes of equal phospholipid concentration as a reference and subtracting this as a background. Excitation and emission spectra of k-PPDPC liposomes were recorded with an SLM 4800S fluorescence spectrometer with a cuvette holder thermostated with a Lauda circulating water bath and a Lauda PM 350 temperature programmer. The fluorometer is interfaced to a Hewlett-Packard 85 microcomputer.

DSC measurements were performed with a Microcal MC-2 instrument (Microcal, Amherst, MA interfaced to a Sperry AT computer. A scanning rate of 0.5 °C/min was used.

Results and Discussion

DSC revealed two endotherms for k-PPDPC liposomes at 13.7 and 23.8 °C, with enthalpies of 0.60 and 1.7 kcal/mol, respectively (Figure 1). Comparing these data with the synthetic, structurally very similar lipid PPDPC which shows a single endotherm at 17.1 °C with an enthalpy of 9.3 kcal/mol (Lotta et al., 1987), it is somewhat unexpected that the introduction of the keto group at C_{10} in the decanoyl chain is capable of causing such a pronounced difference.

The main spectral features in the excitation spectra measured for the k-PPDPC excimer fluorescence at 10, 19, and 32 °C were essentially similar whereas emission spectra show an increase in I_e with increasing temperature while I_m decreases (Figure 2). There is also a slight shift to longer wavelengths in the excimer emission maximum at temperatures above the first calorimetric transition. The changes in I_e/I_m recorded as a function of temperature occur abruptly with half-maximal changes at 13.5 and 23 °C, thus comparing favorably to the calorimetric data (Figure 3). Temperature-induced changes were also seen in the absorption spectra of k-PPDPC liposomes

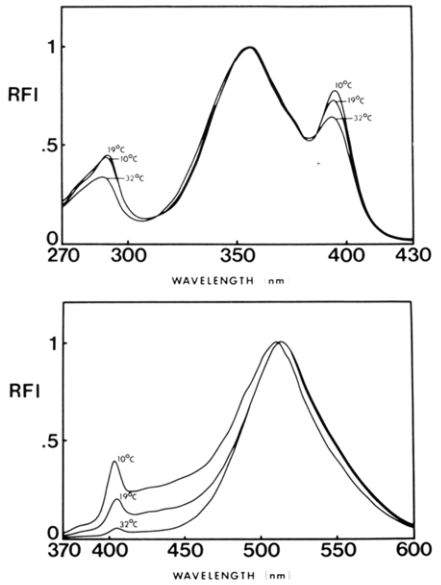

FIGURE 2: Excitation (top panel) and emission (bottom panel) spectra of k-PPDPC liposomes (100 μg/2.5 mL of water) at 10, 19, and 32 °C. Excitation spectra were obtained for the excimer fluorescence at 510 nm. Emission was monitored by using 355-nm light for excitation. The recorded spectra have been normalized to the same peak intensity.

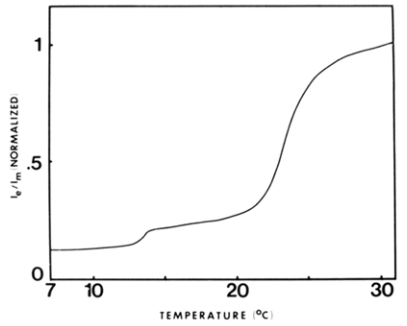

FIGURE 3: I_e/I_m of k-PPDPC liposomes (100 μg/mL of water) as a function of temperature. Half-maximal changes occur at 13.5 and 23 °C. Scanning rate was 0.6 °C/min.

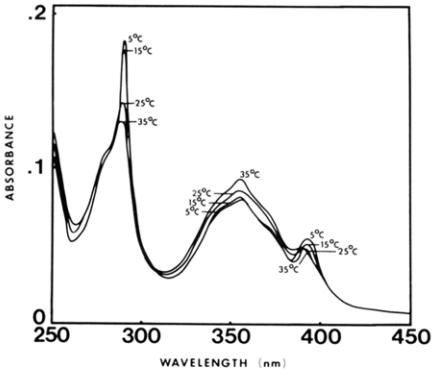

FIGURE 4: Absorption spectra for k-PPDPC liposomes (10 nmol/mL of water) recorded at 5, 15, 25, and 35 °C, as indicated.

(Figure 4). Notably, the absorption peak at 356 nm increased with an increasing temperature in the range of 5–35 °C, while the intensity of the peak at 289 nm revealed the opposite behavior. These data are shown as a T vs I_{289}/I_{356} plot in Figure 5. In accordance with the DSC and I_e/I_m measurements, the ratio of the absorption intensities I_{289}/I_{356} upon heating exhibited a sudden decrease in the temperature range of 20–26 °C and centered at approximately 23 °C.

Unlike PPDPC, monolayers of k-PPDPC on an air/water interface do exhibit a smooth phase transition like behavior,

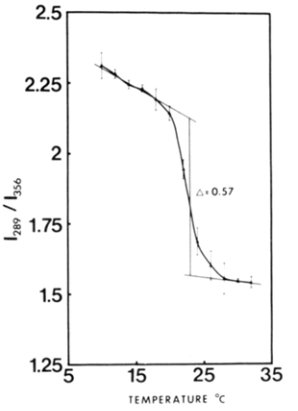

FIGURE 5: Ratio of the absorption intensities at 289 and 356 nm for k-PPDPC liposomes as a function of temperature. Each data point is the mean from three separate experiments, with the bars indicating the variation range. Accompanying the transition at 23 °C the ratio I_{289}/I_{356} changes from 2.13 to 1.56, thus corresponding to a decrease in surface pressure from approximately 39 to 17 mN/m, respectively, as calculated from the best fit curve illustrated in Figure 8.

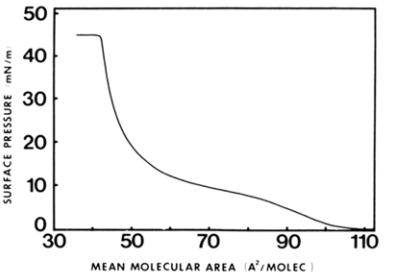

FIGURE 6: Compression isotherm at 23 °C for a monolayer of k-PPDPC on an air/water interface.

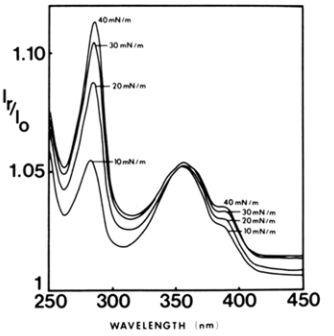

FIGURE 7: Reflectance spectra for a k-PPDPC monolayer on an air/water interface recorded at surface pressures of 10, 20, 30, and 40 mN/m, as shown. I_0 is the reference signal collected for a pure water surface, and I_r is the signal from a lipid monolayer on water. The angle of incidence of both illumination and light collection with respect to the measured surface was 45°.

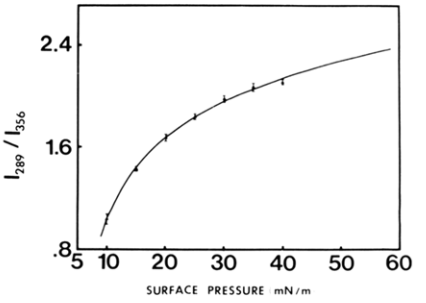

FIGURE 8: Ratio of the reflection intensities at 289 and 356 nm for a k-PPDPC monolayer on water as a function of surface pressure. Each data point represents the mean from three to five separate experiments, and the bars show the range of variation. Also shown (solid line) is the best fit curve $I_{289}/I_{356} = A \ln(\pi + B) + C$, where $A = 0.536$, $B = -5.670$, and $C = 0.248$.

although not as pronounced as, for instance, DPPC (Figure 6). We also measured reflectance spectra for k-PPDPC at different surface pressures (Figure 7). The most significant finding in these spectra is the pressure dependency of I_{289}/I_{356} (Figure 8). This is likely to arise from a packing-dependent torsion of the bond between the decanoyl chain C_{10} and the pyrene ring, which influences the orbital overlap for the keto group and pyrene, thus leading to changes in the energies of both ground- and excited-state bands of the chromophore. Assuming the packing density in monolayers and liposomes to influence the electronic excitation of k-PPDPC in the same manner and further assuming any effect due to the coupling of the two leaflets in the phospholipid bilayer on the transition to be negligible (Sillerud & Barnett, 1982), we can estimate the equilibrium lateral pressure in liposomes of k-PPDPC. We can also obtain an estimate on the influence of the thermal transition at 23 °C on π. This analysis indicates that, for k-PPDPC at temperatures >26 °C (i.e., above the transition), π is <17 mN/m. Below 23 °C there should be a dramatic increase in π by approximately 22 mN/m, to an equilibrium lateral pressure of approximately 39 mN/m. These results are qualitatively as well as quantitatively consistent with those obtained by other investigators (Fulford & Peel, 1980). It should probably also be noted that the curve shown in Figure 5 is identical in shape with that calculated by Fulford and Peel on the basis of diphenylhexatriene fluorescence polarization data for DPPC liposomes and illustrating the dependency of π on temperature. Our results on k-PPDPC liposomes also compare favorably to the absorption spectra of k-PPDPC measured at room temperature in ethanol at a lipid concentration of 20 μg/mL which gives I_{289}/I_{356} = 1.14; i.e., at approximately 11 mN/m the rotation of the keto group with respect to the pyrene should be rather unrestricted (data not shown).

An increase in π accompanying chain crystallization in liposomes upon cooling is logical as the average distance between the acyl chains decreases, therefore leading to enhanced associating interactions between the phospholipid molecules. Interestingly, the obtained values are reasonably close to those forwarded on the basis of theoretical considerations (Hui et al., 1975; Nagle, 1986, 1980) as well as to those obtained from NMR order parameter calculations (Marcelja, 1974), and from the analysis of fluorescence data (Fulford & Peel, 1980).

It must be stressed that the correlation between the applied pressure in monolayers and the intrinsic pressure prevailing in liposomal bilayers has not been defined unambiquously [see review by Nagle (1980)]. Therefore, the numerical values obtained in the present study must be treated with caution. What then remains to be claimed is that the probe responds to changes in its physical environment (lateral pressure in monolayers) by altered spectral characteristics and that assuming similar changes in the absorption spectra of liposomes to be caused by analoguous changes in the environment of the probe (i.e., intrinsic pressure in bilayers), we can estimate the magnitude of π in the latter type of membrane. Another pitfall concerning the present study is that the thermotropic transitions exhibited by k-PPDPC are as yet poorly characterized. Accordingly, the correlation, if any, between the main-chain melting transition of phospholipids such as DPPC and the transition at 23.8 °C for k-PPDPC is uncertain. Our preliminary infrared spectroscopy studies on k-PPDPC indicate, however, that the thermotropic changes in the acyl chain ester bond C=O stretching accompanying the transition at 23.8 °C are similar to those observed for DPPC at the main transition of the latter.[2]

Although one should be cautious in extrapolating from the present results to biomembranes and liposomes of straight-chain phospholipids, we may nevertheless consider these data to indicate that the influence of phase transition on equilibrium lateral pressure should perhaps be taken into account when physical explanations for changes in membrane properties upon transition are sought. Finally, the present results may also provide additional rationale for the design of optical probes, allowing direct measurement of π in biomembranes.

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