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INCORPORATION OF THERMOTROPIC LIQUID CRYSTALS IN PHOSPHOLIPID MONOLAYERS: NECESSARY CONDITION OF HOMEOTROPIC ANCHORING

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Abstract We have studied the monolayer phase behavior of various mixtures of dipalmitoylphosphatidylcholine (DPPC) with 4-cyano-4'-n-pentylbiphenyl (5CB) at the air/water interface by means of Langmuir technique and Brewster angle microscopy. The phase behavior is compared with the ability of 5CB to anchor homeotropically in liquid crystal cells (LC cells) whose surfaces were covered with DPPC monolayers of various molecular area using Langmuir-Blodgett technique. In the present investigations DPPC cells orient 5CB perfectly homeotropically, if the monolayers were transferred to the display surfaces as tilted phases. In case of a transferred non-tilted phase the orientation of bulk 5CB was no more homeotropical.
We show that an essential prerequisite for the homeotropic anchoring is the incorporation of 5CB in the orienting surface monolayer and thus the formation of new phases. Our conclusions are supported by DSC and x-ray experiments on fully hydrated DPPC bulk specimens (bilayer systems) mixed in various concentrations with 5CB.

INTRODUCTION

A homeotropic alignment of a low molecular weight liquid crystal (LC) in an LC sandwich cell (direction of LC bulk director normal to the cell surface) is generally produced by covering the display surfaces with amphiphilic molecules. The transfer of amphiphilic compounds onto the substrates is usually realized by self assembling, rubbing or by means of Langmuir-Blodgett technique (LB technique)[1,2,3,4].
In general, it is widely accepted that the homeotropic anchoring of liquid crystals in displays performed by amphiphilic mono- or multilayers has a sterical nature like the planar anchoring. It was reported on brush-like surfaces or small holes existing in the aligned layers where the LC molecules can enter and orient the LC bulk homeotropically[5,6].
However, this statement contradicts the following facts: On the same surface conditions, only certain groups of LC molecules orient homeotropically[7]. Furthermore, lipid surfaces prepared by means of LB technique still orient the LC homeotropically, although the aligned lipid monolayers have been deposited as tilted phases[8,9]. In addition, the changing of homeotropic orientation to a planar or tilted orientation of some LC's at a temperature slightly below the nematic-isotropic transition

temperature[10,11,12] is not completely understood yet.
We will report on differences between monolayer phase behavior of pure dipalmitoylphospatidylcholine (DPPC) and mixtures of DPPC with 5CB (4-cyano-4'-n-pentylbiphenyl) on the water/air interface detected by means of Langmuir technique and Brewster angle microscopy (BAM). Our results concerning monolayer phase behavior are supported by differential scanning calorimetric (DSC) and x-ray experiments on bulk samples. Finally, we will summarize the ability of 5CB to anchor homeotropically in LC cells whose surfaces were prepared with DPPC of various molecular areas using LB technique.

EXPERIMENTAL

DPPC was obtained by Fluka and 5CB by Merck with 99.5% purity. 5CB was checked by its clearing temperature and the phospholipid was tested by its surface pressure/area isotherms on aqueous subphase[13,14]. The amphiphilics were dissolved in chloroform which also was checked by its isotherm. The isotherms were taken on a commercial Langmuir balance (Riegler & Kirsten, Wiesbaden) equipped with a continuous measuring system and thermostat. The speed of compression for the measurements was on an average 3 $Å^2$ per molecule per minute. Below this limit a change in compression speed did not affect the isotherms. The used subphase was demineralized, twice distilled water. Small amounts of amphiphilic or ionic contamination were removed by a filtering system (Elgastat UHQ II) which links five purification technologies: reverse osmosis, adsorption, deionisation, microfiltration and photo-oxidation. The specific resistance of water was over 18 MΩcm.
For the LC sandwich cells we used B270 glass plates which were cleaned for 25 minutes with a glass cleaning mixture (Hellmanex) at 80°C without ultrasonic treatment. Subsequently, the substrates were rinsed with pure water and once more cleaned for 25 minutes in saturated chromosulfuric acid at 80°C. Finally, the glasses were washed by highly purified water and dried with nitrogen. By means of dynamic surface tensiometer, advancing and receding angles of less than 4 degrees were obtained for water at these plates. The speed of monolayer deposition was 3 mm/min. Two plates were mounted antiparallely to an LC cell using spacer of 25 μm.
The bulk specimens were prepared as follows. DPPC and 5CB were weighed in the desired molar ratio into glass tubes, dissolved in chloroform/methanol and evaporated under high vacuum (10^{-6} Torr) for 24 hours. Afterwards, they were dispersed in deionized water (Millipore) and homogenized above the main transition of DPPC from gel to L_α (T>42°C). At the chosen water concentrations of R_W=50** and R_W=5000 DPPC forms dispersions of multilamellar vesicles[15,16]. DSC measurements on the bulk systems were performed with the Perkin Elmer DSC-7 (R_W=50) and a Privalov calorimeter DASM-4 (R_W=5000) applying cooling and heating scan rates of 0.5K/min. Wide (WAXS) and small angle x-ray scattering (SAXS) at 20°C were carried out on the synchrotron beam line X13 of the EMBL outstation at DESY (Hamburg, Germany) operating at a wavelength of 0.15nm [17]. The WAXS and SAXS diffraction patterns were recorded simultaneously with two sealed linear detectors[21]. The samples with R_W=50, contained in capillaries of 1mm diameter, were exposed 10 times for 5s followed by a 25s period where they were protected from synchrotron radiation by a shutter.

**$R_W = n_{Water}/(n_{DPPC} + n_{5CB})$, n_x= mole number of component x

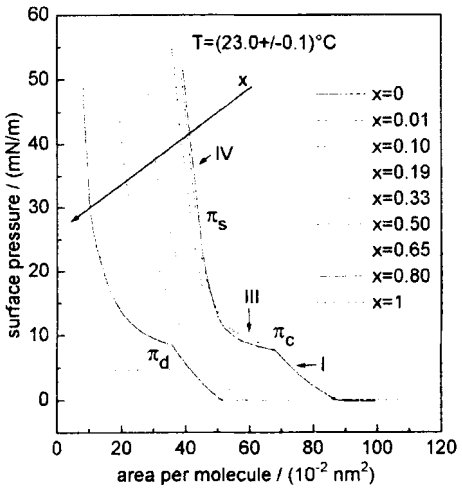

Figure 1a Isotherms of DPPC/5CB monolayers for various molar ratios x. For pure DPPC (x=0), three monolayer phases can be distinguished: an isotropic fluid lamellar (tilted) phase I, a tilted crystalline lamellar phase III and a non-tilted crystalline lamellar phase IV.

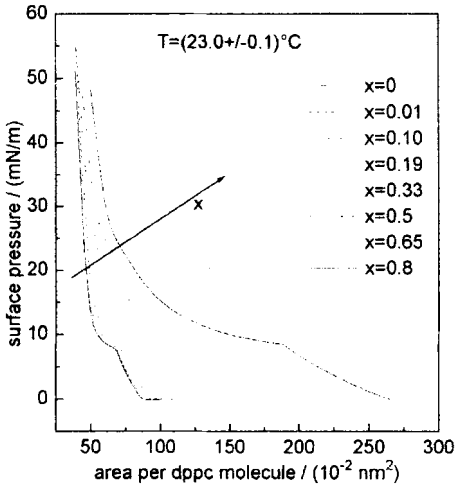

Figure 1b The same isotherms as in Fig. 1a but only the number of DPPC molecules was taken into account in the calculation of the molecular area.

RESULTS AND DISCUSSION

Isotherms of pure DPPC and 5CB. In Fig. 1a, the isotherms of pure DPPC (x=0; solid line) and pure 5CB (x=1; dashed line) are depicted. At a temperature of 23°C and in dependence on the available area per molecule, DPPC forms three monolayer phases (I, III and IV) on the air/water interface[13]. Starting from higher molecular area, phases I and III are separated by a first order phase transition at pressure π_c. Whereas the isotropic lamellar phase I is a fluid phase, III and IV are crystalline phases which are separated by a transition of second kind at pressure π_s [18]. In phases I and III the alkyl chains are tilted, in phase IV the chains are non-tilted, respectively. Using BAM, the crystalline tilted phase is easily observable because monolayers form domains of various tilt directions inside a matrix of the fluid phase[18]. The domains can be extended over $70\mu m$. The BAM reflections of the fluid phase I and the non-tilted crystalline phases IV are homogeneous.
Pure 5CB (x=1) collapses at π_c=5.7 mN/m to trilayers and multilayers on compression[19]. By means of BAM, the formation of such multilayers on the water surface is visible by formation of interference rings.

LC Cells. The DPPC monolayers were deposited on glass substrates at molecular areas in the range from 42 Å^2 to 111 Å^2 per molecule. In order to obtain higher molecular areas, we used mixtures of DPPC with 5CB (see Fig. 1b). Down to an area of about 48 Å^2 per molecule, the LC sandwich cells orient 5CB perfectly

homeotropically although the aligning lipid monolayers have been deposited as tilted phases I and III. If the lipid monolayers were transferred to the display surfaces as non-tilted phase IV (molecular area smaller than 48 Å^2), then the orientation of bulk 5CB was no more homeotropic.

Isotherms of DPPC/5CB Mixtures. With increasing content of 5CB in the DPPC monolayer (see Fig. 1a), the phase transition pressure π_c is increased and the almost horizontal slope, indicating phase III of pure DPPC, is also increased. From the images of BAM one can observe that the extensions of domains in phase III are decreased with higher portion of 5CB. At a molar fraction** of x=0.33, the domains of the previous phase III of pure DPPC are no more observable.
Starting from the side of pure 5CB in Fig. 1a, with increasing content of DPPC in the 5CB monolayer (x<1) the collapse pressure π_d of 5CB is also continuously increased. For the isotherm x=0.33, the 5CB collapse pressure amounts to about 27 mN/m. For higher pressures above π_d, 5CB collapses continuously until the slope of the isotherm is the same as for pure DPPC. Using BAM, this removal of 5CB from the pressured monolayer is visible in the following manner: At compression above π_d, interference rings appear indicating the pushing out process of 5CB. The number of such interference rings is growing until the slope of the isotherm is the same as for pure DPPC as mentioned above. At decompression, the interference rings disappear gradually until π_d is reached. The isotherms do not show hysteresis

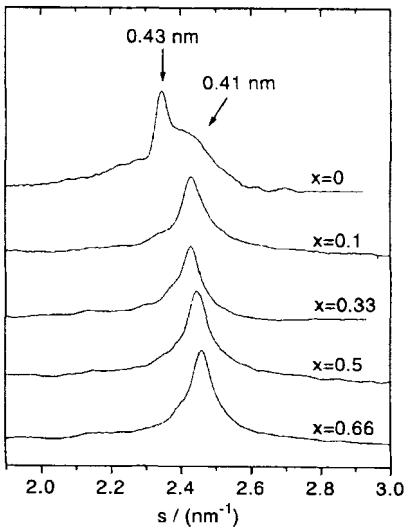

Figure 2a Wide-angle x-ray scattering (WAXS) intensities versus reciprocal spacing s of pure DPPC and various mixtures of DPPC with 5CB at molar fractions x=0.1, 0.33, 0.5 and 0.66 in water (R_W=50) at 20°C.

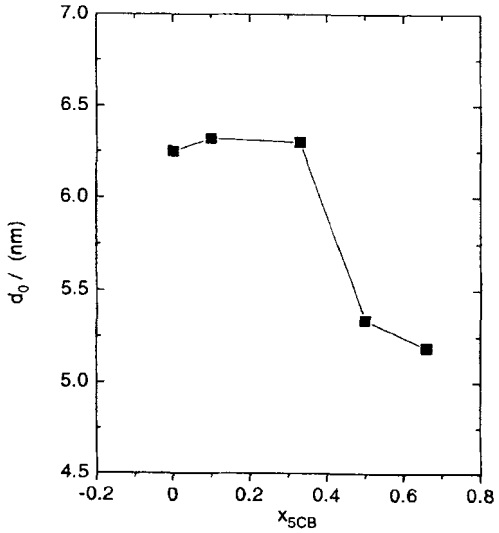

Figure 2b Lamellar repeat distance d_0 (bilayer plus water layer thickness) of DPPC/5CB at 20°C versus molar fraction x determined from small-angle x-ray scattering (SAXS).
$\frac{1}{d_0} = s = \frac{2}{\lambda}\sin\theta \quad 2\theta \ldots$ Bragg angle

**$x = \frac{n_{5CB}}{n_{DPPC}+n_{5CB}}$

which indicates that the 5CB molecules are incorporated again in the DPPC monolayer. In Fig. 1b the removal of 5CB molecules from the compressed monolayer at higher surface pressures is more clearly recognizable. Here, in the calculation of molecular area only the number of DPPC molecules was taken into account. At a surface pressure above 30 mN/m, the isotherms of various mixture ratios are nearly identical. That means, 5CB molecules are displaced out of the DPPC monolayer.

X-Ray Scattering. At 20°C and full hydration, DPPC multilayers form the $L_{\beta'}$ phase, a bilayer gel phase with tilted alkyl chains in the hydrophobic core. They are arranged on a two-dimensional disturbed pseudohexagonal lattice[22]. The wide-angle reflection at 0.43nm (s=2.344nm^{-1}) with a broad shoulder at 0.41nm (s=2.427nm^{-1}) (Fig. 2a, x=0) in the $L_{\beta'}$ phase originates from the chain packing in an orthogonal or triangular subcell. Small amounts of 5CB incorporated into the DPPC multilayers lead to only one symmetric WAXS reflex at 0.41nm. Therefore, it can be concluded that the lattice of chain packing in the $L_{\beta'}$ phase is transformed into a regular hexagonal one which is typical for lamellar gel phases with no alkyl chain tilts. Considering also the lamellar repeat distances d_0 of the lamellar stacks, which are provided by the SAXS measurements (cf. Fig. 2b), this gel phase is an L_β phase. Remarkable is the decreasing value of d_0=6.3nm down to 5.3 nm for x>0.33. This supports either the formation of partial chain interdigitation among the alkyl chains of adjacent monolayers which belong to one bilayer or strong dehydration of the bulk system and therefore reduction of the water layer thickness. The last assumption would affect an increase of the main transition temperature, documented for noncomplete hydrated lipid systems[15], and was not observed by our DSC measurements.

DSC Investigations. Small organic molecules, ionic and nonionic tensids in phospholipid membranes are known to exhibit a wide spectrum of physiological or pharmacological effects. Therefore, a great number of studies is devoted to the interaction between these molecules and phospholipid membranes. DSC provides a convenient way to study these influences on phospholipid phase transitions[23]. In Fig. 3, the pure DPPC multilayer system shows two endothermic phase transitions of first order at 34°C and 40.5°C. They are well known as the pre- and main phase transition of DPPC in excess water[16]. The pretransition at 34°C indicates the transformation from a lamellar gel phase with tilted alkyl chains ($L_{\beta'}$) to the ripple phase $P_{\beta'}$, a lamellar gel phase with no chain tilt and a modulation along the membrane normal. Energetically, this is a process of middle energy transfer. The enthalpy amounts 5.5 kJ/mol. At 41°C the lamellar gel phase $P_{\beta'}$ turns over into the lamellar liquid crystalline phase L_α indicating the main transition. This one is associated with trans-gauche isomerization of the hydrocarbon chains. Because of its large enthalpy change of 36kJ/mol it is called "chain melting". With increasing content of 5CB in the DPPC multilayer system several processes are observed. The temperature and enthalpy change of the lipid main transition is decreasing and the DSC transition curves are broadened. This means that 5CB molecules are incorporated in the DPPC membrane in the vicinity of the hydrophobic core of phospholipids. This fact can be concluded by the phase behavior of alcohols or nonionic surfactants in phospholipids. Localized with their alkyl chains in the hydrophobic core of the lipid membrane, they are reducing the size of cooperative units of phospholipids and induce a broadening of transition profile and a decreasing of transition temperature[23]. Above x=0.33, there is no significant change in the DSC pattern. A very broad curve

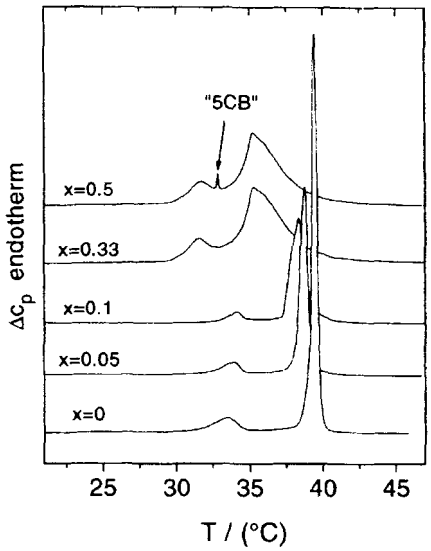

Figure 3 Unnormalized DSC endotherms of mixtures DPPC/5CB in water (R_W=5000) at various molar fractions x. The scans were recorded with a heating rate of 0.5K/min with the Privalov calorimeter DASM-4. There were no discrepancies observed between these experiments and the samples with R_W=50 measured by means of the Perkin Elmer DSC-7. The sharp endotherm peak at 33°C for x=0.5 (labeled with "5CB") is attributed to pure 5CB which is not incorporated into the lamellar matrix.

with two maxima exists and the shape is not modified with increasing 5CB content up to x=0.6. The similar type of DSC curves was observed on lipid/surfactant systems in the region of partially lateral separation or demixing, respectively[23]. At x=0.5 a sharp small DSC peak is observable at 33.5° (Fig. 3, label "5CB"). Comparing to DSC measurements of pure 5CB, this transition can be assigned to the nematic-isotropic phase transition of free 5CB which is not located in the lamellar matrix. Obviously, more than one molecule 5CB per two DPPC molecules (x=0.33) do not incorporate in the DPPC bilayer.
The excess of 5CB for x>0.33 is collected in separate domains or droplets of pure 5CB.

SUMMARY AND CONCLUSION

At the presence of 5CB molecules in DPPC monolayers, the Langmuir and BAM investigations prove that the tilted crystalline phase disappears. Unfortunately, from the shape of isotherms and BAM observations we cannot conclude which phases are formed. But from the x-ray investigation we can exclude that these phases exhibit a chain tilt. This means for the LC cells that if the aligned DPPC monolayer was transferred as a tilted phase on the substrate surfaces, 5CB molecules penetrate into the lipid surface layer and erect the alkyl tails. The model about 'holes' in the aligned interface[20] is right as long as the incorporation of 5CB molecules in the lipid monolayer requires a definite space. 5CB can enter into tilted monolayers of 5CB because tilted alkyl chains exhibit more space inside the hydrophobic part of the layer as in the non-tilted phase. In the crystalline non-tilted phase the alkyl chains are most densely packed and 5CB molecules cannot penetrate into the lipid interface. On the contrary, incorporated 5CB will be displaced out of the lipid membrane at surface densities corresponding to a non-tilted crystalline phase. Consequently, if the lipid monolayer was transferred as non-tilted crystalline phase, the LC molecules cannot enter the layer. They are organized anyway on the top of a closed interface and that leads to disturbed orientation of bulk 5CB.

The mixing isotherms and DSC investigations point out that there is a saturation behavior of 5CB in the lipid mono- and bilayer, respectively. From the present experiments, we cannot determine whether the 5CB molecules are arranged in a subcell within the alkyl chains of DPPC or whether there is a phase separation. The latter possibility is plausible because the typically DSC curves for x=0.33 and x=0.5 were observed in similar systems as mentioned above.

In this contribution we have shown that an essential prerequisite for the homeotropic anchoring is the incorporation of thermotropic liquid crystal in the oriented monolayer. The incorporation induces a phase changing in the interface monolayer and the formation of new phases without tilt. In this way, the bulk director in the LC cell will be oriented normally to the surface.

A more interesting mixture system seems to be DPPC/PCH5. It is known that at higher temperature and smaller molecular area of deposited oriented monolayer there is a reversible transition from homeotropic to tilted anchoring[20]. Preliminary investigations reveal that this transition is based on the same process of pushing the thermotropic LC out of the lipid interface in dependence on surface packing density and additionally on temperature. A complete phase diagram and an extensive discussion will be presented in a further paper.

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