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Dipyrenylphosphatidylcholine as a Probe of Bilayer Pressures

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We have used dipyrenylphosphatidylcholines (dipyPCs) to study the pressure in the fluid lamellar phase formed by mixtures of fully hydrated dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE). As we increase the DOPE mole fraction at 25°C we observe a linear increase in the ratio of the excimer-to-monomer signal (E/M1). We argue that this observation can be understood in terms of an increase in the lateral pressure in the chain region, i.e., in the bilayer plane. This change itself is driven by the decrease in lateral pressure between headgroups as we add DOPE. We expect the lateral pressure to vary in magnitude as we probe the bilayer at different depths [1]. We have confirmed this by recording E/M1 using di[10-(pyren-1-yl)decanoyl]phosphatidylcholine (10dipyPC) and di[4-(pyren-1-yl)butanoyl]phosphatidylcholine (4dipyPC). We find that in 100% DOPC the E/M1 for 4dipyPC is 2.5 times greater than that for 10dipyPC. The above observations can all be rationalized in terms of changes in the lateral pressure profile. An inverse hexagonal liquid crystalline phase is found in the range 100–83% DOPE [2]. In this region of the phase diagram we observe a quadratic variation in E/M1, with a minimum at 95% DOPE. We hypothesize that this variation reflects the chain stretching that is necessitated by the geometrical packing constraints of the hexagonal phase [3]. Again, we find that the E/M1 for 4dipyPC is greater than that for 10dipyPC, but in this phase only by a factor of two.

KEY WORDS: Dipyrenylphosphatidylcholine; bilayer pressure; excimer-to-monomer signal.

INTRODUCTION

The dipyrenylphosphatidylcholines (dipyPCs) are often used to study phospholipids bilayers. They have been used mostly to study gel-to-liquid crystalline and lamellar-to-inverse hexagonal phase transitions [4,5]. Excimer-to-monomer intensity ratios have generally been interpreted in terms of intralipid free volume and/or rotational dynamics of the lipid acyl chains. We interpret variations in the ratio of the excimer-to-monomer signal (E/M1) as being due to changes in the average pressure in the chain region. Increasing the pressure increases the average number of collisions per second and hence enhances the probability of excimer formation. The chain pressure can be calculated using statistical mechanical methods [1] and has been shown to vary in magnitude at different depths through the lipid monolayer (Fig. 1). The positive chain pressure is balanced by the tension at the polar–nonpolar interface and, additionally, by interactions between headgroups [6] (Fig. 2). The latter may result in either positive or negative pressures. In this regard the experimental data indicate that the phosphatidylethanolamine (PE) headgroup has a significant propensity for direct hydrogen bonding between headgroups, i.e., a negative pressure, while the PC headgroup prefers to bind water, i.e., a positive pressure. We can see this from the following experimental data for the headgroup area of dioleoylphosphatidylethanolamine (DOPE) and dioleoylphosphatidylcholine (DOPC)

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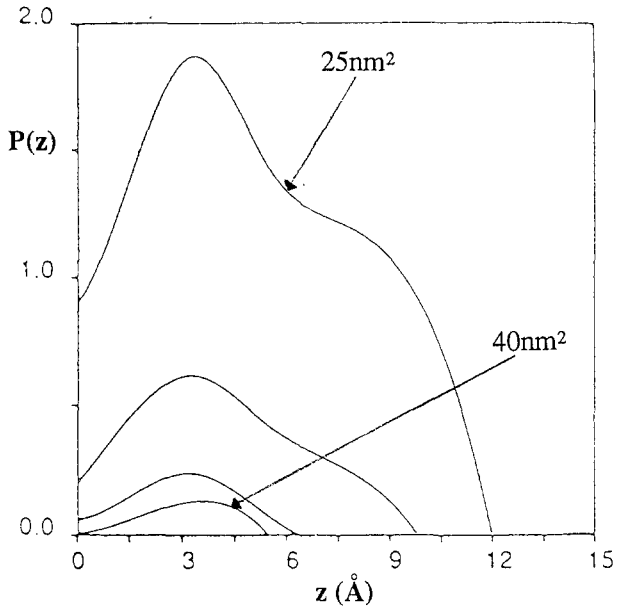

Fig. 1. Calculated chain pressures (kT/v) for saturated $(CH)_{12}$ chains at 25, 30, 35, and 40 nm² (from Ref. 1).

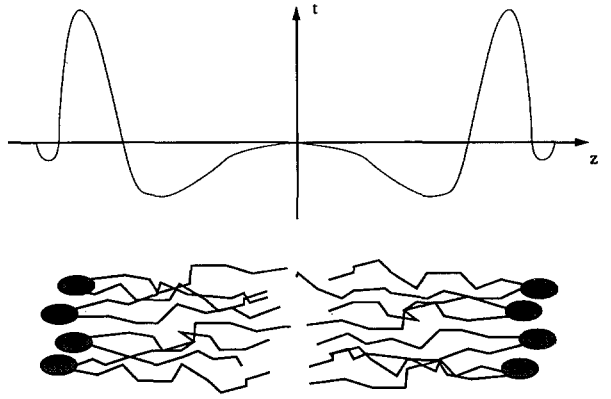

Fig. 2. The lateral stress profile in the fluid lamellar phase.

in excess water at 25°C. In the case of DOPE it is 0.48 nm², whereas for DOPC it is 0.82 nm².

Since the lateral pressure profile must balance, i.e., the integrated lateral pressure at equilibrium must be zero, it is clear that the addition of DOPE to DOPC will reduce the average molecular cross section and therefore lead to an increase in the positive chain pressure. The first moment of the lateral pressure tells us how strong the desire for a curved interface is. This moment increases with the addition of DOPE until a phase transition to the hexagonal phase occurs. In the lamellar phase we need only consider lateral stresses, since expansion of the headgroup area can be accommodated by an orthogonal reduction in the thickness of the lipid monolayer. In the hexagonal phase this is not the case. Here the hexagonal packing of cylindrical lipid monolayers forces some chains to stretch and others to compress (Fig. 3). In other words there is mixing between lateral and transverse pressure due to the geometry of the phase and its packing constraints.

Our measurements of E/M1 follow these variations in average hydrocarbon pressures at different depths through the monolayer.

EXPERIMENTAL

DOPC and DOPE were purchased from Avanti lipids; di[10-(pyren-1-yl)decanoyl]phosphatidylcholine (10dipyPC) and di[4-(pyren-1-yl)butanoyl] phosphatidylcholine (4dipyPC) were purchased from Molecular Probes, and these were all used without further purification. All preparation was performed in a room illuminated by dim, red safelights. The required lipid mixtures were prepared by weighing the nonfluorescent lipids into a vessel and adding fluorescent lipid from a stock chloroform solution. The fluorescent lipid was present at 0.01 mol% of the total lipid, a concentration at which there is negligible intermolecular excimer formation. The chloroform was removed under a stream of nitrogen, and the lipid mixture redissolved in

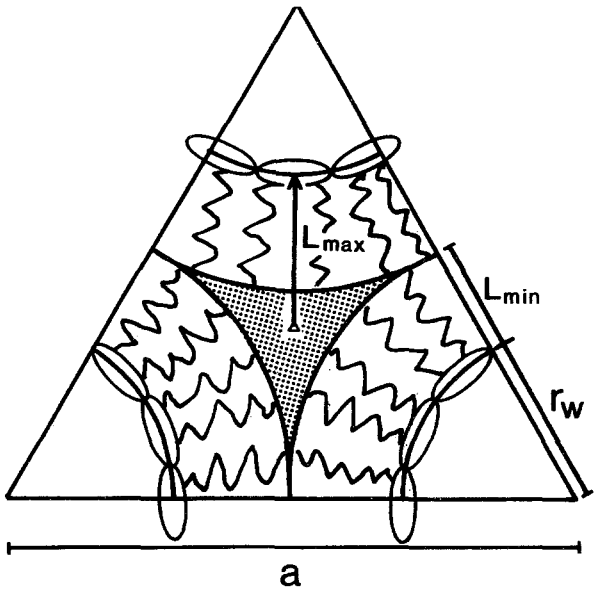

Fig. 3. Geometrical frustration in the hexagonal phase.

cyclohexane. Portions of this solution were transfered into 2-mm glass X-ray capillary tubes (wall thickness, 10 μm), and then lyophilized to remove all solvent. The samples were hydrated with excess water and allowed to equilibrate at 25°C for 1 h. After this time the samples were repeatedly centrifuged, so that the lipid and water phase was at the bottom of the capillary tube. The excess water on top of the bulk solution was removed with a thin strip of filter paper. When no more excess water was visible, the sample tubes were flame-sealed. Steady-state fluorescence measurements were made on a Perkin–Elmer LS50 spectrometer, with the sample tube held centrally in the beam, at a sample temperature of 25.0 ± 0.2°C. The excitation beam was 345 nm, with excitation and emission slit widths of 5 nm. Comparison with samples in quartz tubes shows no significant glass absorption.

RESULTS

The ratio E/M1, the excimer intensity measured at 475 nm divided by the first monomer peak's intensity at 377 nm, was measured for 10dipyPC in DOPC/DOPE mixed bilayers at a range of compositions (Fig. 4). Examining the lamellar-phase region the excimer-to-monomer ratio decreases with increasing DOPC. The line on the graph in Fig. 4 is a linear least-squares fit with slope $(-2.2 \pm 0.7) \times 10^{-3}$/%DOPC and intercept 1.08 ± 0.04. We would expect a linear decrease with increasing DOPC since this should increase the average headgroup pressure, thereby reducing the lateral pressure in the chain region (see Fig. 1). The length of 10dipyPC is such that the average lateral pressure is, in broad terms, being sensed between the terminal methyl group of the DOPE/DOPC hydrocarbon chain and the C10 methylene group. According to the calculations of Szleifer *et al.* [1], we should find an increase in E/M1 for shorter-chain dipyPC if it is indeed sensing the average lateral pressure. To do this we have used 4dipyPC, which senses the lateral pressure somewhere between the fourth and the twelfth carbon. In Fig. 5 we show the difference in the emission spectra for 4- and 10dipyPC in pure DOPC. The difference is large, a factor of 2.5, and is in qualitative agreement with the theoretical expectations. We believe that this is persuasive evidence that the E/M1 and, indeed, the excimer kinetics are set not by the available molecular cross-sectional area, as has been argued elsewhere [4,5], but by the average lateral pressure.

Within the inverse hexagonal phase the excimer-to-monomer ratio varies by a far greater amount than in the lamellar phase. As far as we are aware, there is as yet no theoretical description of the pressure distribution in the chain region for such phases. As a first approximation we have modeled the response with a quadratic and obtain a least-squares fit of $E/M1 \times 10^3 = (4 \pm 1)x^2 - (46 \pm 18)x + (828 \pm 62)$, where x is the mole percentage of DOPC. A proper model remains to be developed, but we believe that it may be related to the geometrical need for chains to stretch to L_{max} (Fig. 3). In stretching at constant volume the pressure in the chain region must increase, since the average cross-sectional area decreases. Whether or not the behavior should be quadratic remains to be determined. Measurements on 4dipyPC once again show an increase in E/M1, which we might expect (Fig. 6), although here the signal is only twice that for 10dipyPC.

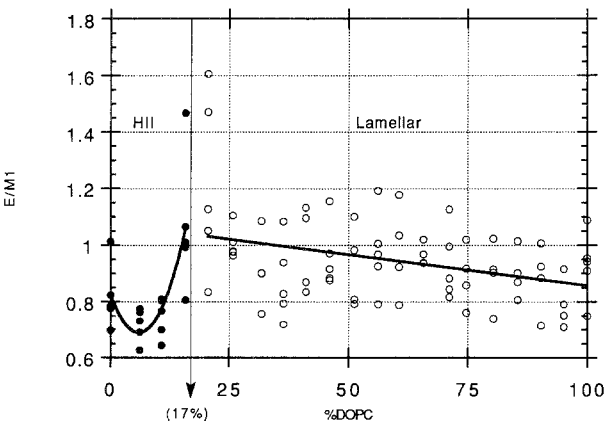

Fig. 4. Excimer-to-monomer ratio for 10dipyPC in mixed DOPC–DOPE systems.

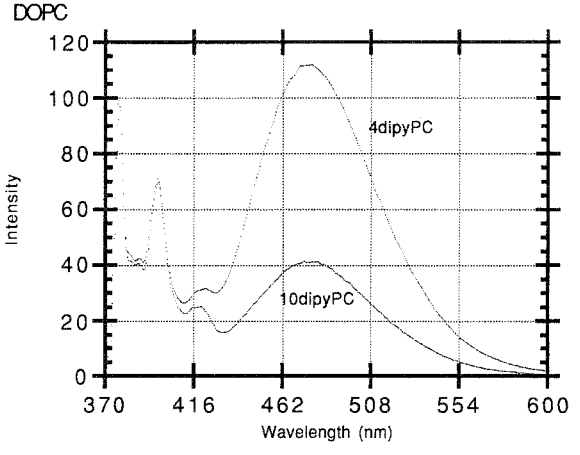

Fig. 5. Normalized spectra (M1 = 100) of 4dipyPC and 10dipyPC in DOPC.

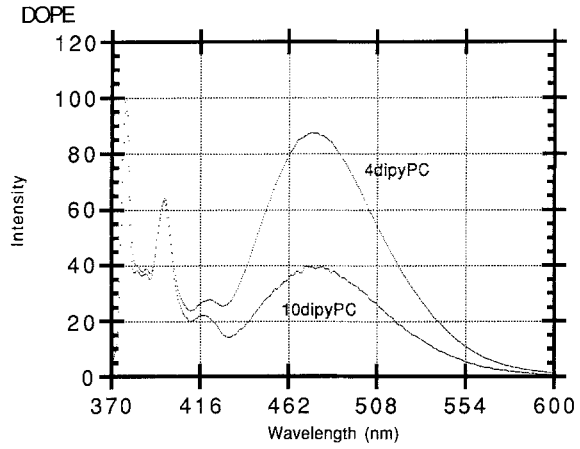

Fig. 6. Normalized spectra (M1 = 100) of 4dipyPC and 10dipyPC in DOPE.

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