Monolayers with One Component of Variable Solubility: Studies of Lysophosphocholine/DPPC Mixtures

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We have examined the effects of lysophosphocholines on dipalmitoylphosphocholine (DPPC) monolayers compressed through the liquid-expanded/liquid-condensed coexistence region. Isotherms and the shapes of liquid-condensed domains revealed three consistent behavioral regimes based on lysolipid chain length. Group I lysolipids (C_8 through C_{12}) desorb readily from the interface upon compression, as shown by isotherm results, and produce little effect on DPPC domain shape. Group II lysolipids (C_{14} and C_{16}) remain kinetically trapped at the interface upon compression and show hints of line activity toward DPPC domains. Both group II and group I lysolipids exhibit a high-pressure transition in domain shape reported previously for mixtures of DPPC and charged surfactants. Finally, group III lysolipids (C_{18} through C_{22}), which are insoluble, strongly affect DPPC isotherms as well as domain shapes; the lysolipid exhibits significant line activity, resulting in noncompact, extended domains. All of these results are discussed with respect to the solubility of the lysolipid component as well as the impact of intermolecular forces within the monolayer.

Introduction

Insoluble monolayers at the air/water interface have long been a focus for examination, and in particular, multicomponent monolayers have been of interest both in theoretical and experimental studies. Theoretical treatments have focused on prediction of multicomponent monolayer properties particularly when one component is soluble in the bulk subphase.[1−10] Studies have examined both equilibrium and dynamic effects when a soluble component comes into contact with an insoluble monolayer. Experimental studies have been largely motivated by biological interest because a phospholipid monolayer can serve as a model for a membrane bilayer.[11] To elucidate information about membrane physical chemistry under certain conditions, multicomponent monolayers have been employed. In particular, the effects of lipid mixtures,[12−16] cholesterol,[17−23] proteins,[16,24−28] and membrane-disrupting surfactants[29] have been studied.

Mixtures of phospholipid and a single-chain lysophospholipid provide an intriguing multicomponent monolayer, both from physicochemical and biological perspectives. By choosing a double-chained phospholipid and a single-chained lysophospholipid with the same headgroup, the effect of hydrophobicity can be isolated. More specifically, by using different chain lengths of lysolipid, one can tune the solubility of the lysolipid while maintaining a constant electrostatic environment at the interface. In addition, the single-chain lysolipid forms a very different volumetric profile in a monolayer when compared to that of the phospholipid; this has implications in packing at the interface. From the biological perspective, lysolipids are a key player in membrane structure and functionality. Because they have a more conical shape than double-chained phospholipids, lysolipids can minimize the mechanical strain associated with protein incorporation in a membrane.[30] Lysolipids are one product and an activator of phospholipid hydrolysis by phospholipase A_2,[31−33] and

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finally, they carry implications in membrane structure and fluidity, including permeability,[34−38] fusion,[39−41] solubilization,[42] and release of Ca^{2+} from mitochondria.[43,44]

Previous work examining the effects of lysolipids on model membrane systems have focused on the impact that lysolipid has on membrane physical properties. Lysolipids induce spontaneous vesiculation of phospholipid bilayers[38] and increase the permeability of existing vesicles.[37] In other vesicle studies, lysolipids were shown to experience free exchange between an aqueous phase and incorporation into a phospholipid bilayer;[45,46] this process was shown to be inhibited in membranes containing PEG-lipid.[47] Ternary mixed monolayers of phospholipid/lysolipid/fatty acid were studied both for their intrinsic behavior[48] and for effect on phospholipase A_2 activity.[49]

We have studied the effects of lysophosphocholines on dipalmitoylphosphocholine (DPPC) monolayers. DPPC was chosen because its monolayer characteristics are well established and because phosphocholines are prevalent in biological membranes. We examined the effects of lysolipid on DPPC surface pressure/mean molecular area isotherm behavior as well as on the shapes of domains that form in the DPPC liquid-expanded/liquid-condensed coexistence region. Lysolipid chain length and interfacial concentration were varied to establish trends in behavior, taking advantage of the fact that both isotherms and domain shapes are very sensitive to the presence of a second component in the monolayer.

Experimental Section

L-α-1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1-caprlyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_8PC), 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{12}PC), 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{14}PC), 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{16}PC), 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{18}PC), 1-arachidoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{20}PC), 1-behenoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (C_{22}PC), and the fluorescent probe1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]-dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC) were all obtained from Avanti Polar Lipids (Birmingham, Alabama). All were at purities > 99% and used without further purification. The subphase for all experiments was Millipore filtered water (18.2 MΩ cm resistivity), maintained at 20 °C by a Neslab circulating unit with an accuracy of 0.1 °C. The film balance and fluorescence microscope have been described previously.[50] DPPC was prepared in a solution with choroform (Fisher, HPLC grade).

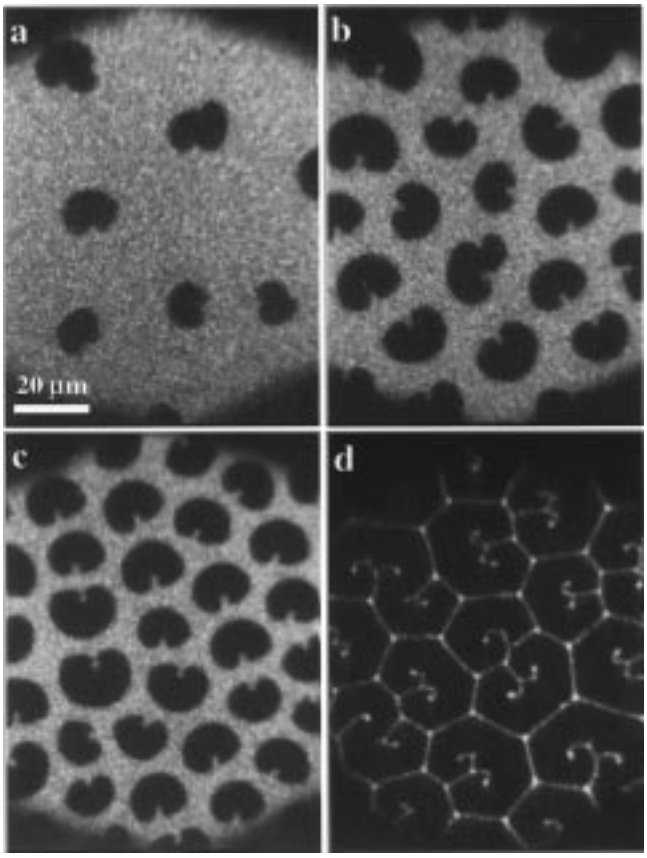

Figure 1. Pure DPPC domains formed at 20 °C by a compression at 0.86 Å^2 molecule^{-1} min^{-1}. Surface pressures are as follows: (a) 3.9 mN/m; (b) 4.2 mN/m; (c) 4.3 mN/m; (d) 7.5 mN/m.

All lysolipids were similarly prepared, either in choroform alone or in a 16:1 chloroform to methanol solution to enhance their solubility. Mixtures were prepared before each experiment to yield 20, 40, or 60 mol % lysolipid in solution, with 0.5 mol % fluorescent probe. The mixture was spread at the interface, and 10 min was allowed for adequate solvent evaporation.

Monolayer compression and compression/expansion cycles were carried out at a rate of 0.86 Å^2 molecule^{-1} min^{-1} (with respect to the total lipid spread at the interface). Surface pressures were measured with a platinum Wilhelmy plate accurate to within 0.1 mN/m. Fluorescence images were gathered throughout the compression and are presented without image enhancement.

Results and Discussion

To determine how lysolipid affects phospholipid behavior, an understanding of pure DPPC behavior is a prerequisite. A DPPC isotherm will be presented for comparison with each of the mixtures, so we do not present it here; it is characterized by a kink and a subsequent plateau, indicating a liquid-expanded/liquid-condensed (LE/LC) coexistence region. A detailed study of pure DPPC domains has been reported previously.[50] Figure 1 tracks a pure DPPC monolayer compressed through the LE/LC coexistence region, as imaged using fluorescence microscopy. The resulting condensed phase domains resemble "beans", with a distinct cavity and one flattened edge. Higher order shapes (bilobes and trilobes) are also possible by subtly changing experimental conditions such as compression rate. All such shapes, however, transform to beans over time, signifying that the bean is the stable shape for pure DPPC. Domains are visibly repulsive at higher surface pressures, as they deform to fill all available space and yield polygons. These are the expected be-

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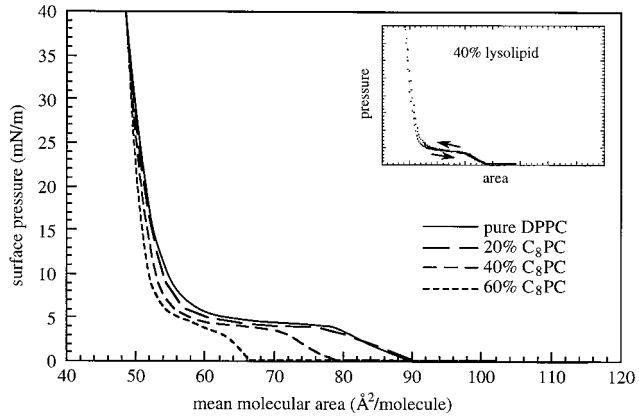

Figure 2. DPPC/C_8PC isotherms at 20 °C. Isotherms were displaced in molecular area to match those of pure DPPC at 35 mN/m. Displacement values were as follows: −6 Å²/molecule (20% C_8 PC); +4 Å² /molecule (40% C_8 PC); +16 Å²/molecule (60% C_8 PC).

haviors for pure DPPC and will serve as a bench mark for each of the following mixtures containing lysolipid.

Our results are assembled in three sections, each presenting and discussing the impact of a series of lysolipids on DPPC behavior. Presentation of data in this format comes naturally from the results, as each lysolipid group modifies DPPC monolayers in the same fashion. Each group corresponds to a series of chain lengths. Group I consists of lysolipid components C_8 through C_{12}, group II includes C_{14} and C_{16}, and group III comprises C_{18} through C_{22}. Boundaries between each group are consistent in both isotherm and domain shape behavior. In our presentation of the experimental data, we show for each group a set of isotherms for one lysolipid and a set of domain shapes for a different lysolipid. Indeed, the key result of this study is that the behavior of any lysolipid typifies behavior generic to the group as a whole.

Throughout the study, we examined the effects of 20, 40, and 60 mol % lysolipid on DPPC monolayers. Because each lysolipid varies in its solubility, accurate assessment (and comparison) of total lipid area per molecule at the interface is impossible. We have thus shifted the isotherm data so that the area per molecule of each mixture matches that of pure DPPC at 35 mN/m. Each mixed isotherm is thus displaced in molecular area relative to that for pure DPPC; the displacement value for each isotherm is noted in the figure caption. This normalization diminishes the usefulness of the molecular area axis in the graphs but aids the comparison of isotherm shape between systems—a primary result of this study.

Group I—C_8 through C_{12}. Figure 2 shows the series of pressure/area isotherms for DPPC mixed with varying concentrations of C_8PC. With each concentration, the general shape of the isotherm closely matches that of pure DPPC. The kink in the isotherm corresponding to the onset of LE/LC coexistence for DPPC is followed by a flat plateau region. This plateau becomes shorter (and increases in slope) as the amount of DPPC in the monolayer decreases. In addition, the isotherms exhibit minimal hysteresis upon reexpansion, as shown in the figure inset.

Before presenting results for domain shapes, we establish a convenient means of describing the state of the monolayer. We choose to use the language "low/high surface pressure" to indicate a monolayer early/late in the coexistence region. These are not indications of absolute pressure, as this varies with the concentration of lysolipid in the monolayer.

We show in Figure 3 DPPC domains formed in the presence of C_{12}PC. At relatively low concentrations (20 and 40 mol %), there is effectively no change in the domain shapes that form as compared to those for pure DPPC (a—d and e—h). There are more multilobed shapes present than are common with a pure DPPC monolayer, but the general shape characteristics still match those of pure DPPC. This is also true for 60 mol % lysolipid at low surface pressures (i—k); however, at higher surface pressures (l), a shape change is evident. We find a transition in which the domains are dispersed uniformly about their boundaries, resulting in domains that appear to be solubilized. This effect is common to all lysolipids in group I at higher concentrations.

With relatively short chain lengths, group I lysolipids are the most soluble of the three groups we examined. Consequently, group I lysolipids desorb from the interface in response to the interfacial stress caused by compression of the monolayer. The isotherm results and the domain shape data both support this assertion. The compression isotherms indicate that lysolipid desorbs readily from the interface because lysolipid concentration has little effect on isotherm shape. This result is confirmed by the lack of hysteresis in the expansion isotherm. These results are consistent for all lysolipids with chain lengths of C_{12} and shorter.

Our observations of DPPC domain shapes in the presence of group I lysolipids support the conclusions drawn from the isotherm analysis. Under most conditions, there is effectively no difference between the shapes of domains formed in pure DPPC monolayers and those formed in the presence of lysolipid. There are several contributing factors to explain why group I lysolipids have little effect on domain shape. They have the same electrostatic properties as DPPC (because of the identical headgroup). Their short chain length produces minimal van der Waals attractive forces with adjacent DPPC chains. And finally, lysolipid concentration is modulated during monolayer compression by their desorption from the interface. Concentration does play a role at higher surface pressures, however, as shown in the case with 60 mol % lysolipid. In this state, the combination of high lysolipid concentration and high surface pressure creates a driving force to induce the dispersive transition seen in this monolayer. These results match domain shapes formed using monolayers of DPPC mixed with charged surfactants.[29] In that study, surfactant desorbed readily from the interface (as shown by isotherm data), yet the dispersive transition appeared at high surface pressure.

Group II—C_{14} and C_{16}. Isotherm data for group II lysolipids are represented by C_{14}PC in Figure 4. In this case, clear deviations from the pure DPPC isotherm are evident with the kink and plateau of the isotherm shifting to higher surface pressure with increasing lysolipid concentration. The plateau also increases in slope with concentration. Each isotherm exhibits strong hysteresis upon expansion, as shown in the inset.

We present domain shape data for C_{16}PC in Figure 5. At low concentrations and surface pressures (a and b), domains appear similar to those of pure DPPC. Increases in lysolipid concentration yield slightly abnormal DPPC domains (e—f and i—j). At higher surface pressures, regardless of the concentration (c, g, and k), domain boundaries develop flattened regions and points. This mutation quickly disappears as the domains undergo the same dispersive transition seen with group I lysolipids. The dispersive transition occurs at all concentrations but appears more prominently with 40 and 60 mol % lysolipid (h and l).

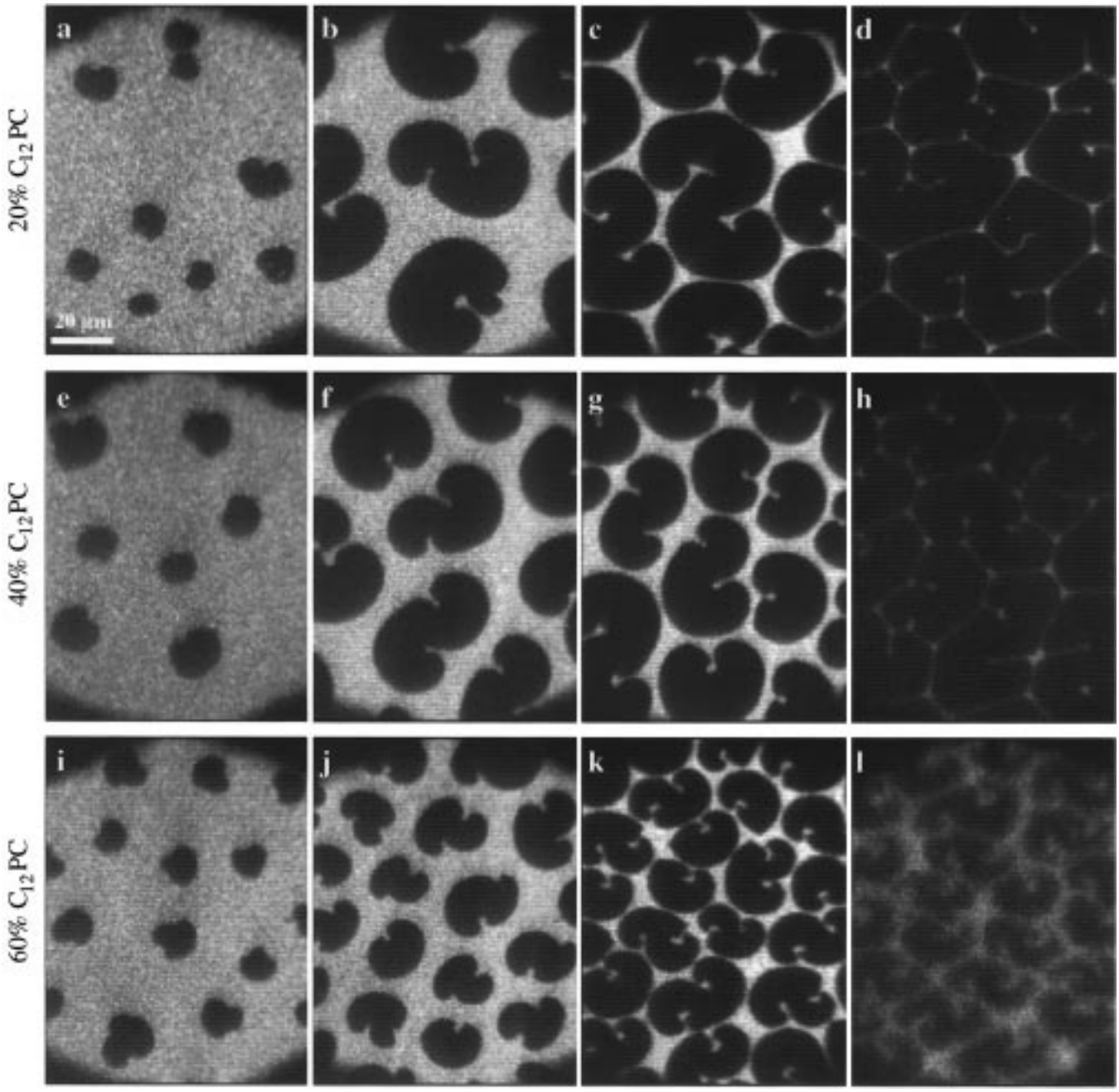

Figure 3. DPPC/C_{12}PC domains formed by compression at 0.86 Å^2 molecule^{-1} min^{-1} at 20 °C: (a) 3.9 mN/m; (b) 4.6 mN/m; (c) 6.2 mN/m; (d) 13.4 mN/m; (e) 4.7 mN/m; (f) 5.1 mN/m; (g) 6.0 mN/m; (h) 15.9 mN/m; (i) 4.8 mN/m; (j) 5.7 mN/m; (k) 9.9 mN/m; (l) 16.6 mN/m.

Group II lysolipids exist in a middle range of neither complete solubility nor true insolubility. They can indeed desorb upon compression into the subphase but are hydrophobic enough to remain present at the interface despite stress caused by compression. This is supported by the isotherm data, where isotherm shapes deviate significantly from those of pure DPPC. In addition, the hysteretic nature of the isotherms suggests that material is irreversibly desorbed from the interface upon compression. This behavior can be explained using a kinetic model for desorption of lysolipid from the interface. When molecules are packed at an interface, a cohesive force develops between their hydrocarbon chains due to van der Waals attraction.[51] The cohesive force grows as the chains increase in length and as they are brought closer

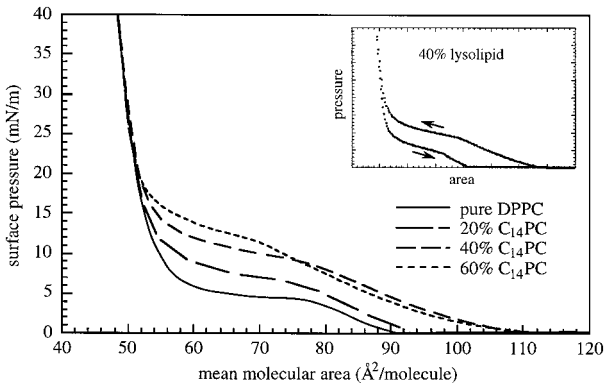

Figure 4. DPPC/C_{14}PC isotherms at 20 °C. Displacement values were as follows: −1 Å^2/molecule (20% C_{14}PC); +4 Å^2/molecule (40% C_{14}PC); +13 Å^2/molecule (60% C_{14}PC).

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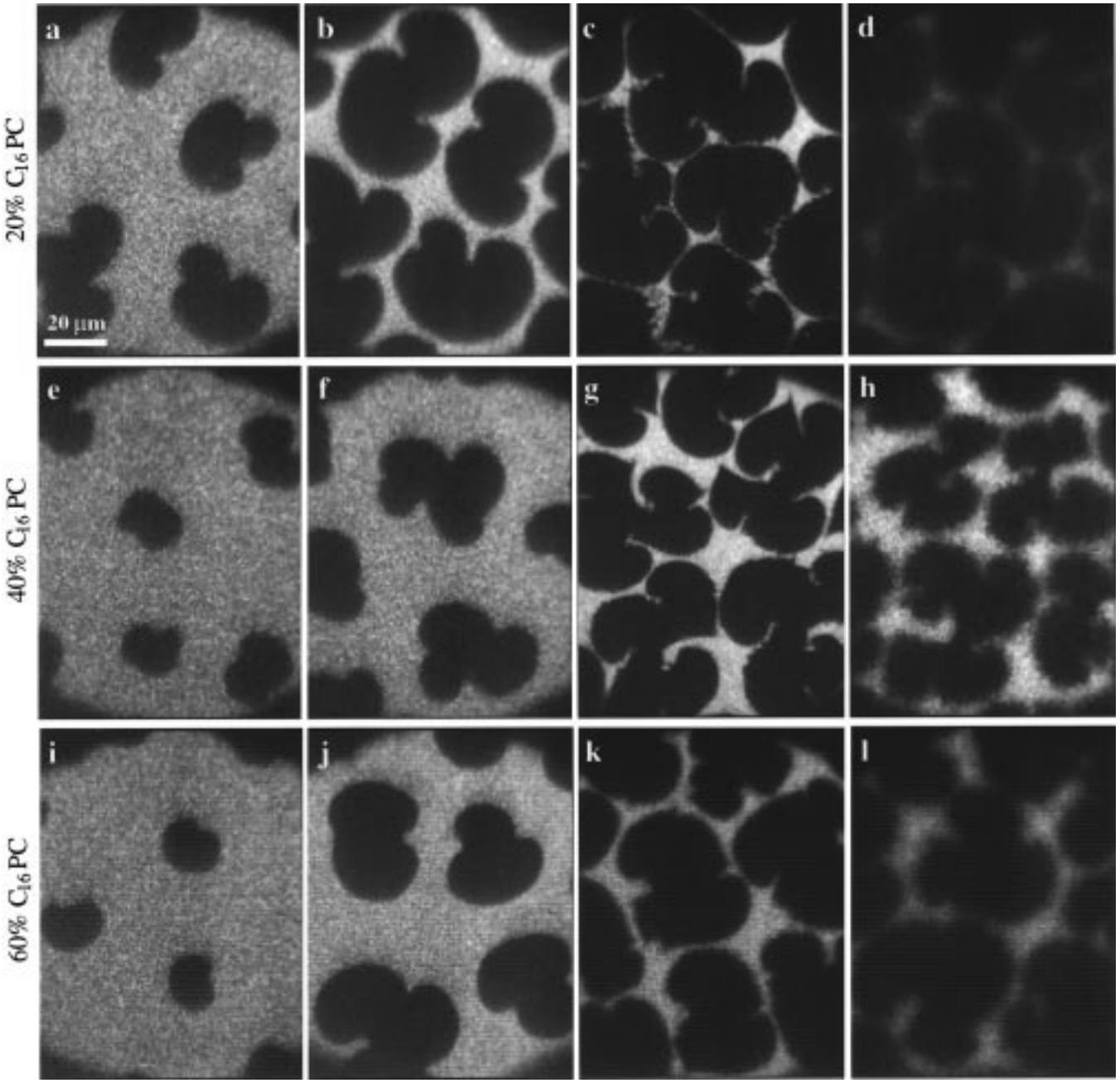

Figure 5. DPPC/C_{16}PC domains formed by compression at 0.86 Å^2 molecule^{-1} min^{-1} at 20 °C: (a) 9.7 mN/m; (b) 19.3 mN/m; (c) 27.0 mN/m; (d) 31.2 mN/m; (e) 14.9 mN/m; (f) 19.6 mN/m; (g) 31.3 mN/m; (h) 33.2 mN/m; (i) 10.8 mN/m; (j) 17.3 mN/m; (k) 30.7 mN/m; (l) 33.1 mN/m.

together (as in a film compression). This force sets up a barrier for desorption from the interface, as soluble molecules are stabilized by the cohesive force. In the case of group II lysolipids, the lysolipid component not only is less soluble than the group I lysolipid but also experiences a larger kinetic barrier for desorption due to its longer hydrocarbon chain. The resulting isotherms thus show larger surface pressures than pure DPPC isotherms because of trapped lysolipid at the interface. This effect was previously reported for neutral surfactants mixed with DPPC at an interface.

Group II lysolipids also show an impact on DPPC domain shapes. Unlike results shown for group I, group II lysolipids influence domain shape at low surface pressure. Further compression at all concentrations yields domains that become more flat and pointed on their boundaries. This phenomenon hints that the lysolipid component exhibits line activity, where lysolipid is adsorbed to the

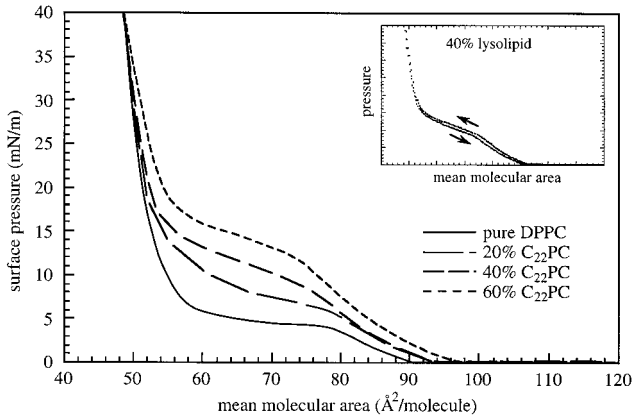

Figure 6. DPPC/C_{22}PC isotherms at 20 °C. Displacement values were as follows: +1 Å^2/molecule (20% C_{22}PC); +6 Å^2/molecule (40% C_{22}PC); +8 Å^2/molecule (60% C_{22}PC).

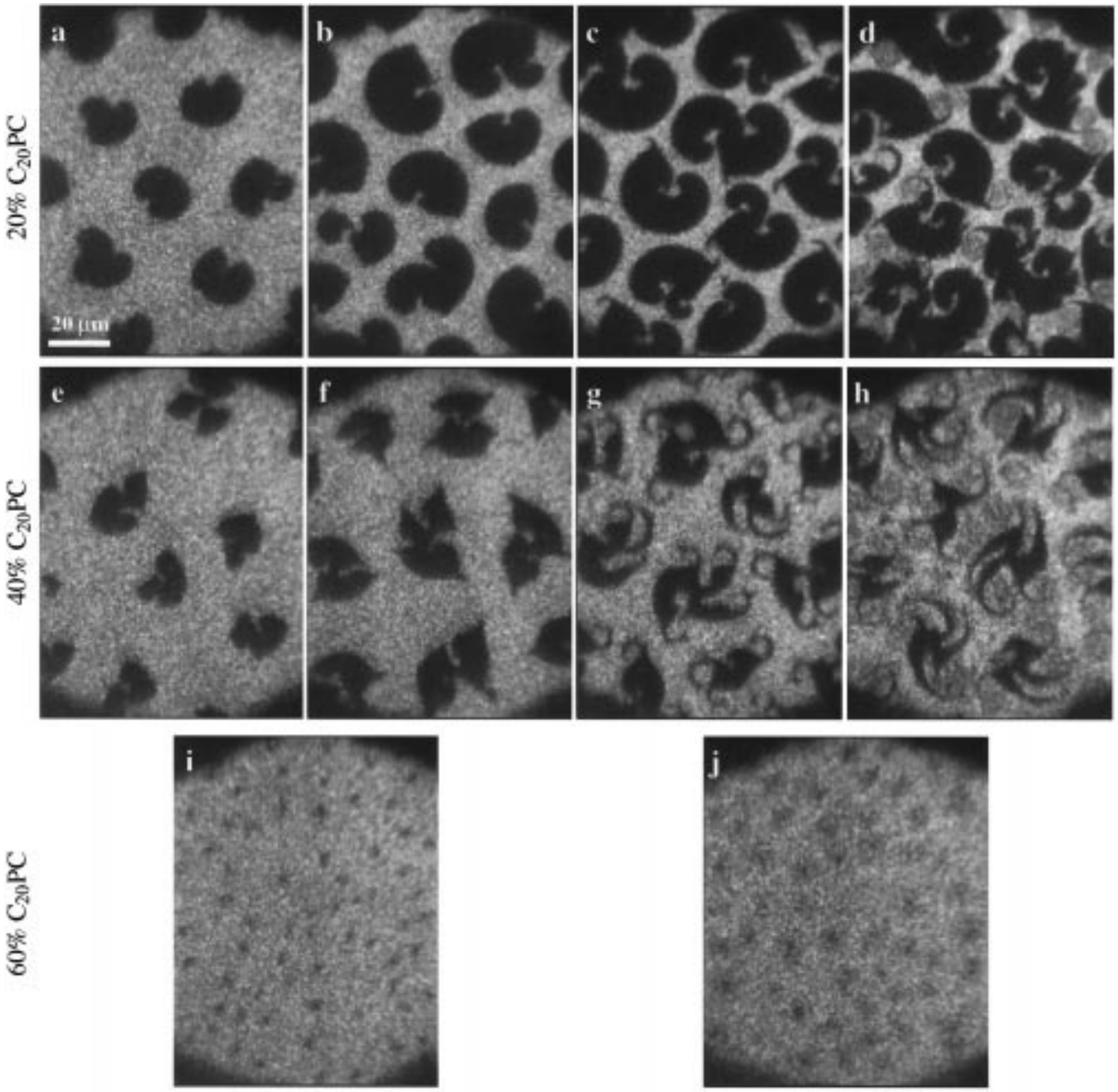

Figure 7. DPPC/C_{18}PC domains formed by compression at 0.86 Å^2 molecule^{-1} min^{-1} at 20 °C: (a) 7.5 mN/m; (b) 9.3 mN/m; (c) 11.6 mN/m; (d) 12.0 mN/m; (e) 10.7 mN/m; (f) 11.7 mN/m; (g) 12.6 mN/m; (h) 13.1 mN/m; (i) 13.5 mN/m; (j) 13.9 mN/m.

exterior of the domain, lowering the line tension and allowing it to "extend" to less compact shapes. This effect will be revisited in the following section. The isotherm data suggest that lysolipid remains kinetically trapped at the interface; the dispersive transition seen at high surface pressures and all concentrations supports this suggestion. As for group I, the dispersive transition for group II lysolipids is concentration dependent, occurring more dramatically at higher lysolipid concentrations.

Group III—C_{18} through C_{22}. Similar to group II, group III lysolipids have a dramatic impact on DPPC isotherms, as shown in Figure 6 for C_{22}PC. All kinks and plateaus are shifted to higher surface pressure, and the plateau slope increases with lysolipid concentration. The difference between group II and group III appears in the relatively nonhysteretic behavior of the isotherm upon expansion. All group III lysolipid expansions follow the original compression isotherm as shown in the Figure 6 inset.

DPPC domain shapes formed in the presence of group III lysolipids are markedly different from those of the previous two groups. The shapes shown in Figure 7 are for C_{20}PC. At low surface pressures and concentrations, the domain shapes remain similar to those of pure DPPC (a and b). There appears to be a concentration-dependent effect where domains tend toward extension; this extension becomes more prevalent at higher lysolipid concentration. Extension proceeds in a chiral direction, with projections curving counterclockwise from their origination. Upon further compression, domain growth tends to expand its perimeter at the expense of its area, resulting in the smaller, but more extended, domains seen at higher surface pressures (d and h). At 60 mol % lysolipid, the

effect is extreme, and domains begin this extension process immediately upon nucleation. As such, they are unable to grow to an optically resolvable size.

Group III lysolipids are the least soluble of those in this study, an observation supported by the isotherm data. Similar to results seen with group II, strong deviations in the measured isotherm indicate that lysolipid remains at the interface. Unlike the more soluble group, however, group III lysolipids exhibit little hysteresis, indicating that they remain adsorbed at the interface and are not squeezed out upon compression. This is likely due to both their relative insolubility and the kinetic barrier to desorption seen with group II lysolipids.

The dramatic effect of group III lysolipids on DPPC domain shapes also suggests that the lysolipid component remains at the interface. Group II lysolipids display hints of line activity, but with group III, a true line activity is apparent. Domains grow preferentially by lengthening their projections rather than growing uniformly. This effect is concentration dependent. As lysolipid concentration increases, domain boundary extensions occur earlier in the compression and to a greater extent. In addition, the domain extension for a given concentration becomes more prevalent as the monolayer is compressed because lysolipid is enriched in the expanded phase. The energetic cost of extended growth is diminished by a decrease in line tension associated with adsorption of lysolipid to the domain boundary.

Our results indicate that line activity increases with chain length. Van der Waals forces drive molecules to associate with others. A longer chain length increases the ultimate energetic payoff of this interaction. A conically shaped lysolipid, which intrinsically prefers curved microstructures, cannot fully profit from these interactions when confined to a planar environment. However, a heterogeneous monolayer offers opportunities to maximize these interactions by adsorption to the ordered boundary of a phospholipid domain.

In experiments with a high concentration of group III lysolipid, limited phase coexistence suggests that the mixture might be leaving the phase envelope for this system. The mixture may be approaching a critical point or simply experiencing a miscibility effect due to the increased lysolipid concentration. Lysolipid is insoluble in the subphase and in pure form does not exhibit phase coexistence. Hence, a continued increase in the lysolipid concentration will inevitably result in a transition point at which condensed domains no longer form. One must be careful, however, in using domain size to determine the thermodynamic state of the monolayer. Because the proportion of DPPC in the monolayer drops with increased lysolipid concentration, the amount of condensable material is decreasing. As such, the smaller domain size may be the result of a mass rather than a critical phenomenon. An effort to map out the phase envelope for this system would shed considerable light on this particular detail.

Conclusions

We have examined the effects of lysolipid on DPPC monolayer behavior, measuring the influence of both lysolipid concentration and chain length. While lysolipid concentration influences monolayer properties, a key result of this study is that the behavior of the lysolipids enables their assignment by chain length to one of three well-defined groups each with distinct effects on DPPC monolayers. Results are closely tied to the relative solubility of each lysolipid, as indicated by the isotherm data. In addition, intermolecular forces also influence monolayer properties, in both the kinetic barrier for desorption and the line activity of the lysolipids. Group I lysolipids (C_8 through C_{12}) are most soluble and have minimal effect on monolayer properties. Group II lysolipids (C_{14} and C_{16}) can be kinetically trapped at the interface and show hints of line activity. Group III lysolipids (C_{18} through C_{22}) remain at the interface and exhibit significant line activity. Finally, some of the effects we see in this system mirror those of a previously studied system which examined the impact of soluble surfactants on DPPC monolayers.[29] Both the kinetic desorption barrier and the dispersive transition of DPPC domains were evident in that study.

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