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Interaction of DPPC monolayers with soluble surfactants: electrostatic effects of membrane perturbants

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Abstract

We studied the effects of four soluble surfactants on DPPC monolayers to elucidate the action of these membrane perturbants. The presence of nonionic N-9 and amphoteric C31G strongly affected the pure DPPC isotherm, while anionic SDS and cationic DTAB had little effect. The impact of surfactant on DPPC domain shape in the liquid condensed-liquid expanded coexistence region showed the opposite result. Neutral surfactants had minimal effect on the shape of DPPC domains; charged surfactants, on the other hand, induced a new shape transition at high surface pressures previously unreported for DPPC domains. All of these results are discussed with particular attention given to electrostatic effects at the interface. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Air/water monolayer; Soluble surfactant; Domain shape; Isotherm; Electrostatics

1. Introduction

The disruption of biological membranes by amphiphilic molecules has been a frequent focus for study because of its applicability to basic science as well as widespread human use. Membrane-perturbing surfactants are commonly used to lyse cells for study of their contents as well as to solubilize their membrane proteins [1,2]. In clinical applications, membrane perturbants can serve in several capacities, such as emulsions for cosmetic and dermatologic use [3], microbicides [4–6], oral anti-microbial applications [7–9] and spermicides [10–12]. Targetting the cell's membrane as opposed to its nucleus or cytoplasm has two distinct advantages: cells are less able to develop a resistance to this type of attack, and the effects are more likely to be broad spectrum. Agents studied have ranged from surfactants [13–17] and amphiphilic proteins [18–21] to lysophospholipids [22,23], the single chain analog to the membrane phospholipid.

Although membrane perturbants are commonly used and frequently studied, their mechanism of action is still poorly understood. The action of amphiphiles as perturbants has been examined using several membrane models, including liposomes [24,25], bilayer lipid membranes [16] and monolayers [26]. Because their perturbation is directed by physicochemical processes, model membrane systems can provide valuable information that would be difficult to attain in vivo. In assessing a particular pertur-

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bant, the pivotal issues lie in its efficacy towards the cell under study [27], its selectivity to specific membrane composition and structural characteristics [28–30], and its irritation of surrounding tissues. A better understanding of membrane disruption will aid in the design of specific perturbants to address these issues.

We have studied membrane perturbation using an insoluble phospholipid monolayer as a model membrane and several soluble surfactants as perturbants. Monolayers offer several key advantages over other membrane models. A monolayer can be carefully controlled by defining molecular density on a Langmuir film balance. In addition, the planar geometry of the monolayer makes it accessible to several optical techniques; in particular, both fluorescence microscopy [31–33] and Brewster angle microscopy [34,35] have been used to image phase transitions in monolayers. The penetration of insoluble monolayers by soluble amphilphiles has been extensively studied from a thermodynamic perspective [29,36–45], providing a valuable tool to examine the interaction of a membrane perturbant with the phospholipid monolayer.

Phospholipid monolayers can exhibit behavior which provides a unique window to observe interactions at the interface. Several phospholipids undergo phase transitions when compressed or cooled, entering a state of phase coexistence; the resulting heterogeneity can be imaged using microscopy. Images display 'domains' of one phase dispersed in another. The shape of these domains is unique to the phospholipid and ultimately related to molecular interactions within the monolayer. Theories have been developed to predict domain shape based on the competition between line tension and electrostatic repulsion (from oriented dipoles in the phospholipid head groups) [46–50]. These theories thus tie molecular phenomena (line tension, electrostatics) to a macroscopic effect (shape of the domain). Domain shape has also been shown to be very sensitive to the presence of a second component at the interface [51–53]. We take advantage of this sensitivity to conditions at the interface by using domain shape analysis to probe the interaction between phospholipids and soluble surfactants.

In this study, we use monolayers of dipalmitoylphosphatidylcholine (DPPC) at the air/water interface and four soluble surfactants: nonoxynol-9 (N-9), C31G (an amphoteric mixture of alkyl betaine and alkyl amine oxide), sodium dodecyl sulfate (SDS), and dodecyltrimethylammonium bromide (DTAB). The selection of the phospholipid and surfactants was based both on their biological significance and their electrostatic properties. Phosphocholines are a major component of cell membranes, and monolayers of DPPC are well characterized both with respect to pressure-molecular area isotherm behavior [54] and domain shape [55]. N-9 is a nonionic surfactant that exhibits microbicidal activity and is commonly used as a spermicide [10,11,56]. Amphoteric C31G has applications including wound healing, vaginal microbicides, and spermicides [4,7,8,12,57]. SDS is anionic and a frequent choice for cell lysis and suspension of membrane proteins as well as for microbicide applications [6,25]. Finally, cationic DTAB is representative of a class of quaternary ammonium compounds that have been also studied for their membrane perturbative properties [6,13,16,28]. In this paper, we report isotherms of DPPC/surfactant mixtures as well as changes in DPPC phase behavior due to the presence of soluble surfactant.

2. Materials and methods

L-α-1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids (Birmingham, AL), as was the fluorescent probe, 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC). Both were at purities $>99\%$. Nonoxynol-9 (N-9) was obtained from Rhone-Poulenc as Igepal CO-630 Special at a purity of 95%. C31G (equimolar mixture of C14 amine oxide and C16 alkyl betaine) was obtained from Biosyn, (Philadelphia, PA) as a 10% aqueous stock solution. Sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium bromide were obtained from Sigma with purities $>99\%$. All were used without further purification. The subphase for all experiments was Millipore water (18.2 MΩ cm resistivity), maintained at 20°C by a Neslab circulating unit with an accuracy of 0.1°C. The pH of the subphase was between 5.0 and 5.5 at which both amphoteric components of C31G are deprotonated, thus rendering them electrostatically neutral.

The film balance, fluorescence microscope, and Brewster angle microscope have been described previously [55]. DPPC doped with 0.5% fluorescent probe was spread from a stock chloroform solution (Fisher HPLC grade) on the film balance with no measured increase in surface pressure. At least 10 min was allowed for solvent evaporation from the interface. Surfactants were injected under the monolayer so as to yield an equilibrium surface pressure between 1 and 2 mN/m. Two to three hours was allowed for equilibration, during which the interface came to a constant surface pressure. We note that different bulk concentrations are required to achieve the desired pressure range: 2.85×10^{-7} M (N-9), 5.62×10^{-7} M (C31G), 1.27×10^{-4} M (SDS), and 1.67×10^{-4} M (DTAB). All concentrations fall well below the respective critical micelle concentrations (CMC) for each surfactant: 7.3×10^{-5} M (N-9), 4.1×10^{-5} M (C31G), 5×10^{-4} M (SDS), and 1.44×10^{-2} M (DTAB).

Monolayer compression and compression/expansion cycles were carried out at a rate of 0.86 Å^2/molecule/min (with respect to DPPC) unless otherwise noted. 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.

3. Results

3.1. Isotherm data

Surface pressure-mean molecular area isotherms were taken for pure DPPC and DPPC/surfactant mixtures. Fig. 1 shows a pure DPPC isotherm with its characteristic kink at 3.6–3.8 mN/m, signifying the onset of the phase transition between the so-called liquid expanded (LE) and liquid condensed (LC) phases. All surfactant concentrations were chosen to yield an equilibrium surface pressure below this kink so that the presence of surfactant would not induce domain formation. As such, this study presents results in the dilute limit of subphase surfactant concentrations.

Fig. 1 also shows isotherms of DPPC monolayers compressed in the presence of each of the four surfactants. All isotherms begin at the equilibrium pressure of the soluble surfactant (1–2 mN/m), and end in a region between 35 and 40 mN/m where the slope of the isotherm approaches that of pure DPPC. Monolayers containing ionic surfactants (SDS and DTAB) have isotherms resembling that of pure DPPC; the characteristic plateau is preserved. Isotherms with neutral surfactants (N-9 and C31G), however, exhibit an immediate surface pressure increase at the onset of compression and no evidence of a plateau, despite subphase concentrations three orders of magnitude less than those with charged surfactants.

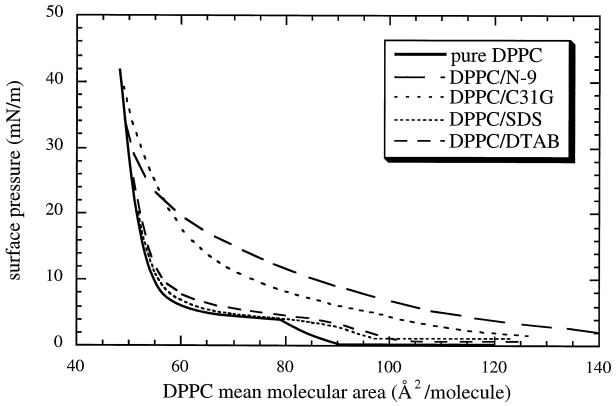

Fig. 1. DPPC/surfactant mixed isotherms at 20°C. Subphase concentrations were as follows: 2.85×10^{-7} M (N-9), 5.62×10^{-7} M (C31G), 1.27×10^{-4} M (SDS), and 1.67×10^{-4} M (DTAB). Compression rate was 0.86 Å^2/molecule/min (referenced to pure DPPC molecular area).

The distinction between the effects of charged and neutral surfactants is further highlighted when the

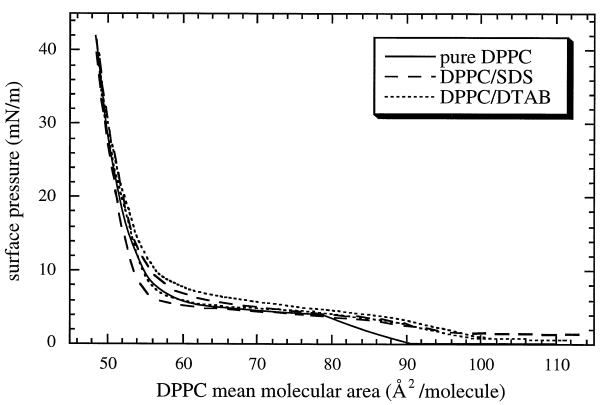

Fig. 2. DPPC/charged surfactant compression-expansion isotherms at 20°C.

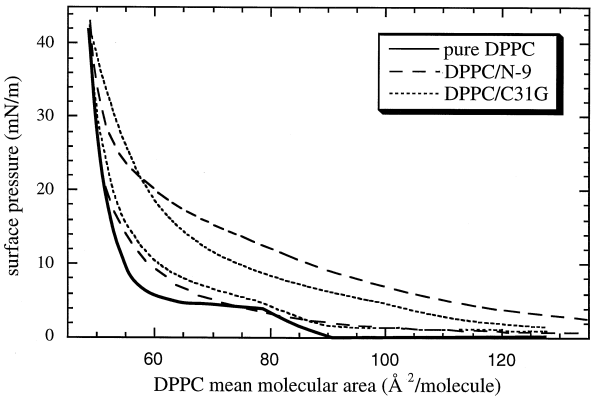

Fig. 3. DPPC/neutral surfactant compression-expansion isotherms at 20°C.

compressed monolayer is subsequently expanded. Pure DPPC monolayers show no hysteresis when compressed to 40 mN/m and then expanded. As shown in Fig. 2, DPPC monolayers in the presence of SDS and DTAB are mildly hysteretic, recovering to match the original compression isotherm by the end of the expansion. Fig. 3 shows the effects of N-9 and C31G; these isotherms display more pronounced hysteresis and an inability to recover the original shape of the compression isotherm. The impact of these results will be addressed in Section 4.

3.2. Domain formation and shape analysis

Before examining the effects of surfactant on DPPC domain shape, a basic understanding of pure DPPC behavior is necessary. A study of pure DPPC domain formation was published previously [55] and Fig. 4 summarizes the results relevant to this work. The fundamental shape for a pure DPPC domain is shown in Fig. 4a: an asymmetric 'bean' with a flattened left

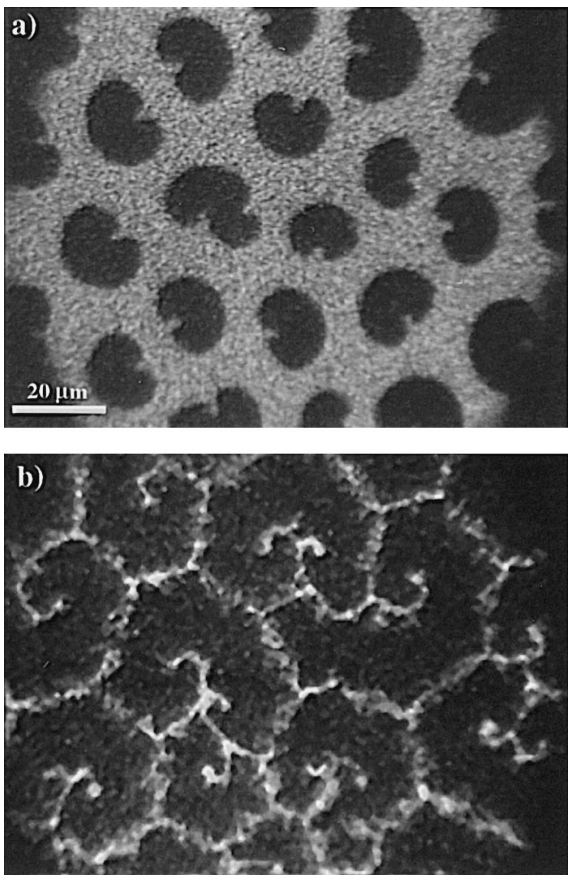

Fig. 4. Pure DPPC domains formed by compression at 0.86 Å^2/molecule/min at 20°C. (a) 4.2 mN/m. (b) 12.3 mN/m.

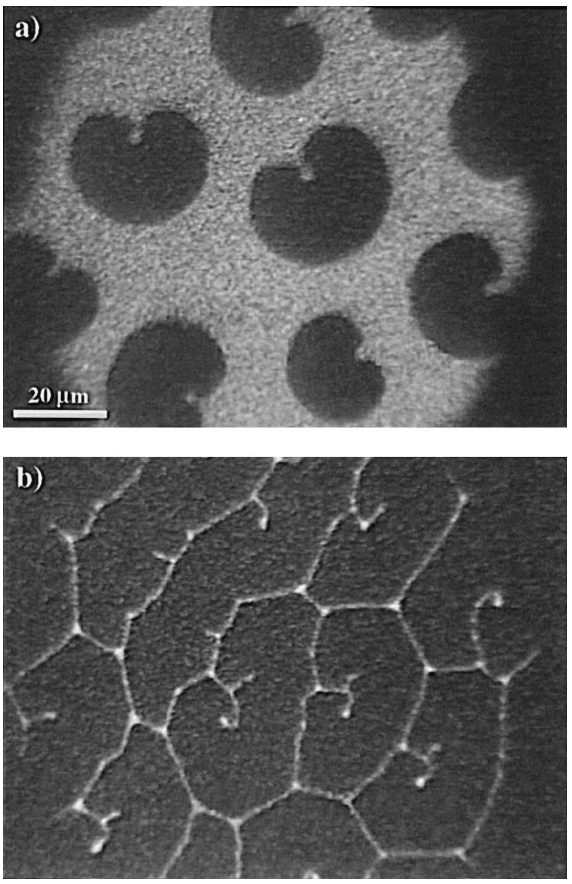

Fig. 5. DPPC/N-9 mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min at 20°C. (a) 5.0 mN/m. (b) 21.9 mN/m.

edge and a distinct cavity. Multilobed domains can also form, but transform to beans over time. As the monolayer is compressed, domains grow and display their repulsive nature (arising from their oriented dipoles) by deforming to fill all available space, thus transforming them into polygons. At surface pressures between 11 and 15 mN/m, we reported a shape instability resulting in the 'cutting' of the domain along intrinsic chiral paths as shown in Fig. 4b. This transition is attributed to the presence of the fluorescent probe because no such effect is seen using Brewster angle microscopy (which requires no probe). In addition, the transition is completely suppressed at higher compression rates, suggesting a kinetic rather than a thermodynamic origin.

There are several common characteristics of domain formation among the DPPC/surfactant mixtures. DPPC exhibits a LE/LC phase transition in the presence of each surfactant (which cannot be taken for granted in the case of the neutral surfactant isotherms since they exhibit no plateau). Nucleation occurs at surface pressures between 3.5 and 4.0 mN/m regardless of where this pressure falls along the DPPC mean molecular area axis. The presence of the surfactant thus 'artificially' compresses the DPPC monolayer, promoting nucleation at a higher DPPC molecular area than possible in a pure monolayer. This is similar to results seen in phospholipid/polymer mixtures [58]. If a surfactant concentration is chosen which results in an equilibrium surface pressure greater than 4.0 mN/m, domains nucleate and grow as the surfactant adsorbs to the interface. Surfactant-induced domain formation is uncontrolled and unpredictable because our injection procedure does not allow for uniform adsorption of surfactant to the interface. We thus restricted our surfactant concentrations to values that yield surface pressures less than 4.0 mN/m. However, we found no dependence of domain shape on surfactant concentration.

Results of fluorescence experiments are presented for the two pressure regimes in which behavior was notable: relatively low pressure (4–10 mN/m) and relatively high pressure (11–15 mN/m). The low pressure regime is characterized by domains that are well separated; in the high pressure regime, domains are compressed and closely packed.

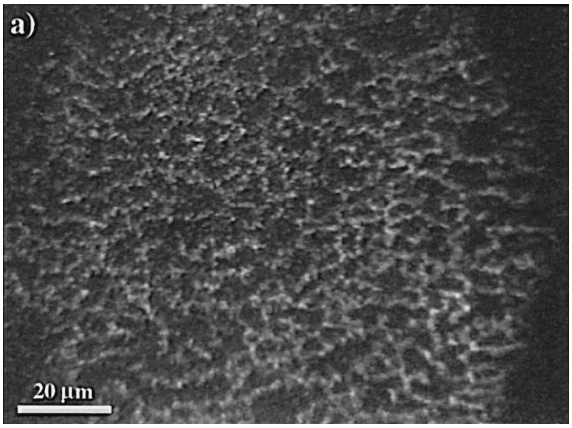

Fig. 7. DPPC/C31G mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min to 14 mN/m and subsequently expanded at the same rate to 6.5 mN/m.

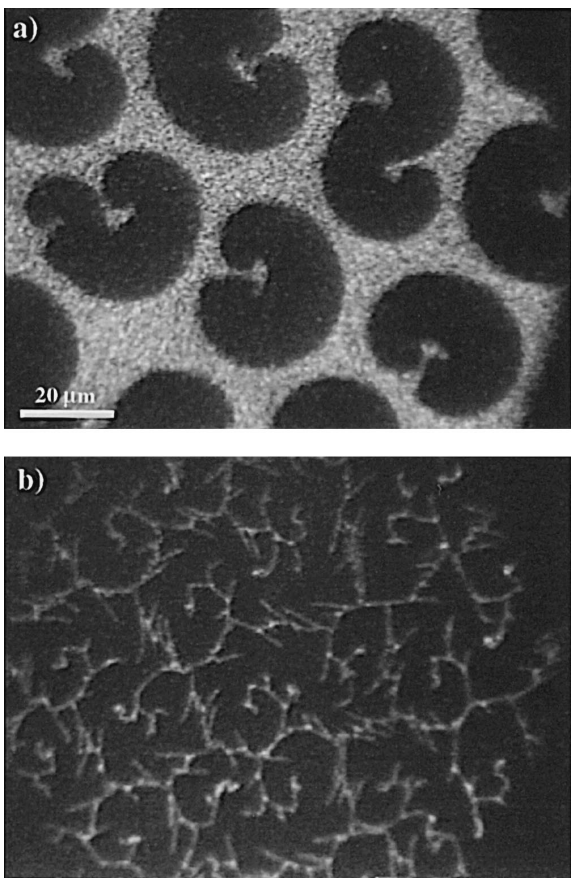

Fig. 6. DPPC/C31G mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min at 20°C. (a) 4.6 mN/m. (b) 12.8 mN/m.

Fig. 5 shows domains formed with the DPPC/N-9 mixture. At low surface pressures, the domain shape matches domains formed with pure DPPC. The fundamental bean shape is preserved as is the flattened edge and the cavity. Interestingly, deviation from pure DPPC behavior occurs at higher surface pressures, where we do not see the probe-induced shape instability common to the pure film. In the presence of N-9, domains remain intact (unaffected by the probe) at pressures well above 20 mN/m.

With regard to domain formation, the behavior of the DPPC/C31G mixed monolayer is virtually indistinguishable from that of a pure DPPC monolayer. As seen in Fig. 6, domain shape is identical at low surface pressures, and the probe-induced shape instability persists at higher surface pressures. Fig. 7 shows a DPPC/C31G mixed monolayer that had undergone the probe-induced transition and been subsequently expanded. In this case, the 'cutting' of the domains during the transition was complete, leaving only pieces of the original domains (the same expansion behavior is seen for a pure DPPC monolayer).

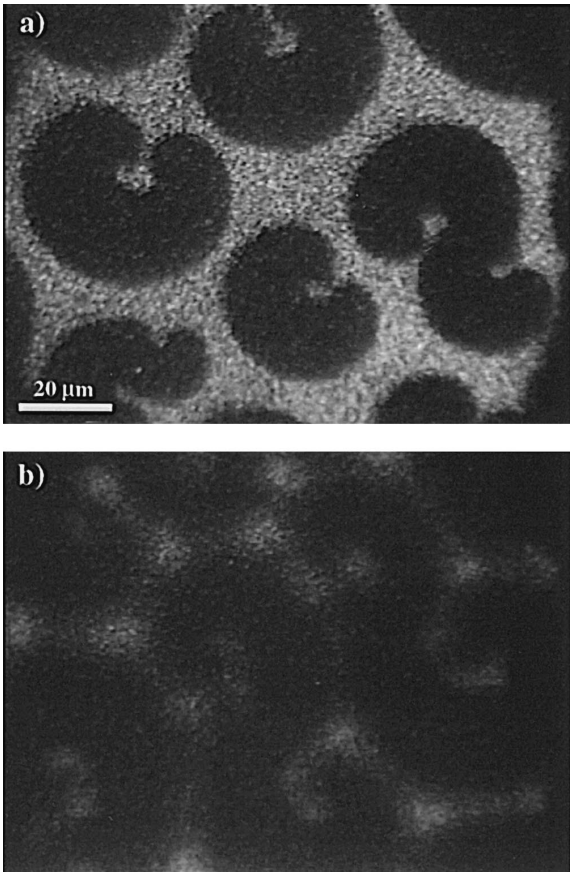

Fig. 8. DPPC/SDS mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min at 20°C. (a) 5.6 mN/m. (b) 14.5 mN/m.

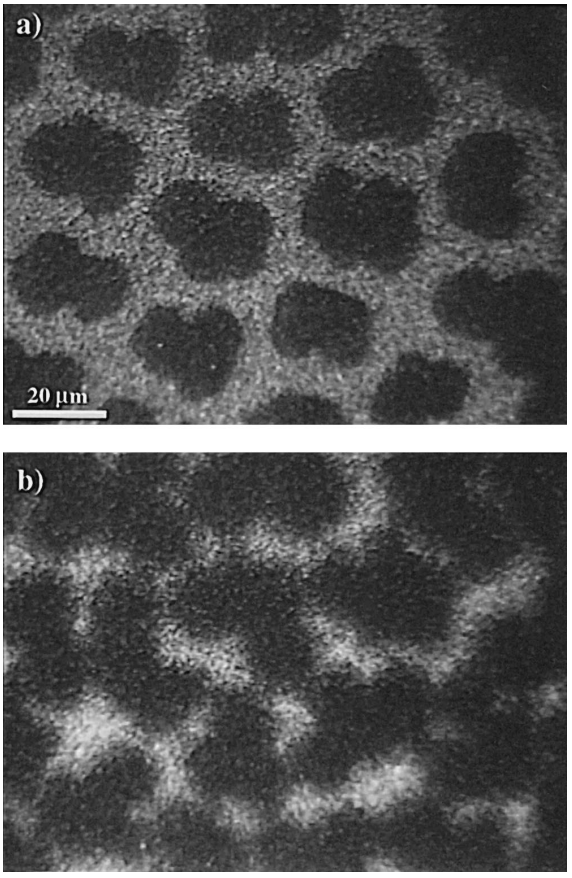

Fig. 9. DPPC/DTAB mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min at 20°C. (a) 6.5 mN/m. (b) 12.6 mN/m.

DPPC domains formed in the presence of the anionic SDS are shown in Fig. 8. Again at lower surface pressures, no clear difference exists between domains formed in the mixed monolayer and those formed by pure DPPC. However, at surface pressures corresponding to the probe-induced shape instability (11–15 mN/m), a new transition is seen as shown in Fig. 8b. Domains, rather than cut inward, are uniformly dispersed about their boundaries. This transition is distinct from the probe-induced shape instability not only in its appearance, but also because we find evidence for it using Brewster angle microscopy. In addition, the transition is identical at higher com-

pression rates (4.3 Å^2/molecule/min) unlike the probe-induced transition (which is suppressed at compression rates above 2.6 Å^2/molecule/min).

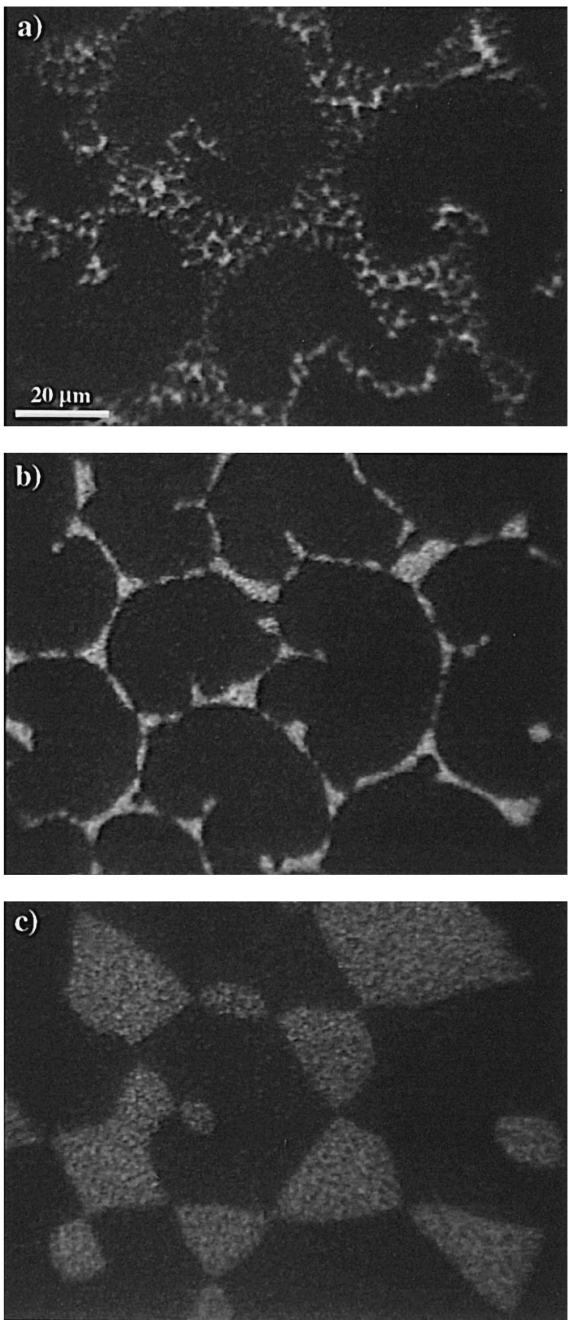

Fig. 10. DPPC/SDS mixed monolayer domains formed by compression at 0.86 Å^2/molecule/min to 15 mN/m and subsequently expanded at the same rate. (a) 10.5 mN/m. (b) 8.5 mN/m. (c) 5.8 mN/m.

Results for the cationic DTAB are presented in Fig. 9. In this case, clear deviations from pure DPPC behavior are evident even at low surface pressures. Domains lack the characteristic features of pure DPPC. At higher surface pressures, domains undergo a similar dispersive transition to that seen with the DPPC/SDS mixture. In particular, at pressures between 11 and 15 mN/m, the domain boundaries in concert become blurred around the entire perimeter.

The uniqueness of the dispersive transition seen in the presence of ionic surfactants extends beyond the initial compression. Fig. 10 shows the results of a DPPC/SDS monolayer when dispersed domains are subsequently expanded. Many small, new domains surrounding the originals come into view, which, upon further expansion, coalesce with each other and with the parent domains to create a domain network. This network persists despite expansion, and domains deform in order to maintain contact with their neighbors (Fig. 10c). This expansion behavior, unlike that after the probe-induced transition, alters only the boundary of a domain but leaves the core intact.

4. Discussion

4.1. Isotherm data

There are important similarities among all of the DPPC/surfactant isotherms. In each case, the compressibility of the film decreases throughout the compression, ultimately approaching that of pure DPPC. This suggests that soluble surfactant is continuously 'squeezed out' of the monolayer by the film compression, leaving a film with properties approaching those of pure DPPC. In fact, the shape of the compression isotherm shows the extent to which the surfactant is forced into the bulk. If the surfactant desorbed completely as the film was compressed, the resulting isotherm would match that of pure DPPC. Thus, any deviation from the pure DPPC isotherm can be attributed to incomplete desorption of soluble surfactant.

Desorption behavior can be used to contrast the effects of charged and neutral surfactants on DPPC isotherms. DPPC/charged surfactant isotherms follow closely that of pure DPPC; hence desorption

from the interface appears quite efficient. In contrast, the neutral isotherms deviate strongly from that of pure DPPC, indicating that desorption is less efficient. This can be explained using a kinetic model for desorption. When molecules are packed at an interface, a cohesive force develops between their hydrocarbon chains due to van der Waals attraction [59]. The cohesive force grows as the chains are brought closer 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. Neutral amphiphiles are particularly susceptible to this effect due to low headgroup repulsion; charged headgroups provide repulsion to counter the van der Waals attraction. In addition, a charged surfactant will be more soluble in the subphase as compared to a neutral surfactant because of increased solvation of its head group. The neutral surfactant isotherms thus deviate more from pure DPPC behavior both because of the kinetic barrier and because of lower solvation in the subphase.

This explanation of surfactant behavior in the presence of DPPC is supported by examining compression isotherms of the pure surfactants. Fig. 11 shows compression isotherms for each of the four surfactants in this study. The charged surfactants exhibit no surface pressure increase upon compression, independent of compression rate. Both neutral surfactants, however, show significant pressure increases which are dependent on compression rate. This indicates that the kinetic desorption barrier can be overcome by slower perturbations of the interface.

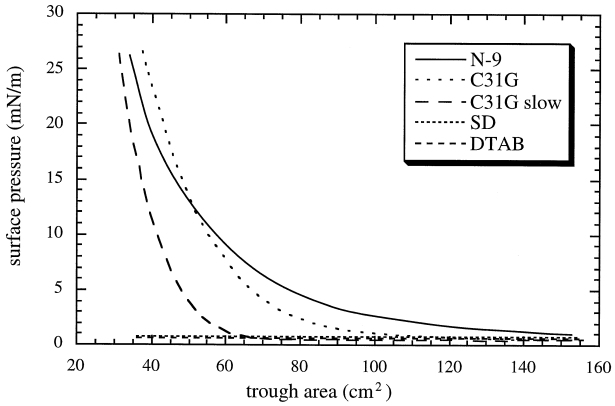

Fig. 11. Pure surfactant compression isotherms at 20°C. Compression rate was 1.2 cm^2/min except in the case of the slow C31G isotherm, which was 0.24 cm^2/min.

The concepts of desorption upon compression and a barrier for desorption can be combined to understand the hysteresis of the DPPC/surfactant isotherms. With neutral surfactants, a higher surface pressure is kinetically maintained upon compression due to slow desorption of surfactant from the interface. Desorption, however, does occur as indicated by the hysteresis in the isotherm. The charged surfactants experience a lower barrier to desorption, keeping the compression isotherms at lower surface pressures and not enhancing the appearance of hysteresis.

The shape of the expansion isotherm provides information about the composition of the expanding film. As stated above, a film from which all surfactant had been squeezed would resemble a pure DPPC film. None of the expansion isotherms follow the DPPC curve exactly, and the neutral surfactants deviate more than the charged surfactants. This is likely due to residual surfactant within the film, as the desorption barrier allows less neutral surfactant to leave the interface than charged surfactant. This observation is borne out when comparing the compressibilities of the films at 35 mN/m. DPPC monolayers with N-9 and C31G have compressibilities of 5.38 m/N and 7.03 m/N, respectively. In contrast, the compressibilities of SDS and DTAB mixed monolayers are 2.98 m/N and 3.21 m/N, approaching more closely the compressibility of a pure DPPC film (2.61 m/N). The difference in compressibility indicates that more neutral surfactant remains trapped in the film at this pressure.

4.2. Domain formation and shape analysis

A domain shape analysis reinforces the differences between the effects of charged and neutral surfactants on DPPC monolayers. The demarcation lies in the high pressure behavior where two different shape transitions are evident. We know that this variance in shape transition is not a result of differences in surfactant concentration because each transition occurs independently of concentration. Further examination of domain shape reveals differences even within the classes of charged and neutral surfactant. We examine the implications in the following.

In the case of nonionic N-9, surfactant at the interface is electrostatically invisible, having no interaction with DPPC. N-9 artificially compresses the monolayer as shown by the isotherm but does not alter DPPC domain shape. This result is striking because of the sensitivity of domain shape to a second component at the interface. At higher surface pressures, the probe-induced shape instability is inhibited by the presence of N-9. As DPPC condenses and domain size increases, fluorescent probe is left behind in the expanded phase, thus enriching that phase with probe. In the mixed monolayer experiment, N-9 is also insoluble in the condensed phase and thus enriched along with the probe, 'diluting' it in the expanded phase and delaying the onset of the transition.

This contrasts with results of the amphoteric C31G, which electrostatically most closely resembles the zwitterionic DPPC. In this case, behavior in the presence of C31G matches that of pure DPPC, both in the low pressure and high pressure regimes. Domain shapes are identical to pure DPPC at low surface pressure. At high pressures, C31G is enriched in the expanded phase in the same fashion as N-9, but because it carries a similarly-oriented dipole to that of the DPPC molecule, it is not electrostatically transparent to the fluorescent probe. Instead, the oriented dipole repels the probe, mimicking the electrostatic environment in a pure DPPC film. Instead of suppressing the probe-induced transition, then, the presence of C31G in the monolayer does not change the pure DPPC behavior.

Negatively-charged SDS displays an interesting mix of interactions with DPPC. At low surface pressures, the presence of SDS has surprisingly little effect; DPPC domains retain their characteristic shape despite the presence of a strongly electrostatic molecule at the interface. At higher surface pressures, however, the relative enrichment of the surfactant becomes evident as the monolayer undergoes a new transition, namely the dispersion of domains into the expanded phase. We distinguish this transition from the probe-induced transition in several ways. Our results suggest that this is a thermodynamic rather than kinetic effect, supported by its insensitivity to compression rate. It also appears to be independent of the fluorescent probe, as the renucleation upon expansion is also seen using Brewster angle microscopy. Thus, at some critical point where the surface fraction of surfactant has been sufficiently enriched in the expanded phase (due to condensation of DPPC), the surfactant effectively solubilizes the domain. Then, upon expansion, the expanded phase which is now 'supersaturated' in DPPC spontaneously condenses, forming the tiny domains seen in Fig. 10.

The solubilization of domains at high pressures appears intrinsic to the presence of a charged surfactant as this is the result seen with cationic DTAB. DTAB, however, has a more far-reaching effect on the monolayer, illustrated by mutated domain shapes throughout the coexistence region. One might expect that interaction of an ion with a zwitterion would yield similar cooperative effects, regardless of the charge of the lone ion. In a monolayer, however, the zwitterion is constrained at the interface, thus requiring a specific electrostatic interaction with an ion also pinned at the interface. Our results reflect this specificity, namely in the difference between the effects of SDS and DTAB at low surface pressures.

5. Conclusions

We have examined the effects of soluble surfactants with different electrostatic properties on DPPC monolayers. The impact of surfactant on isotherm shape highlighted the demarcation between charged and neutral surfactants, the latter having a significant effect on the DPPC isotherm as compared to their charged counterparts. This can be explained via a kinetic barrier for desorption of surfactant from the interface, where electrostatically neutral surfactants are more susceptible to this desorption barrier.

The differences between the effects of charged and neutral surfactants are borne out in studies of DPPC domain shape, but subtle differences within each class of surfactant are also evident. DPPC monolayers containing neutral surfactants yield domains that closely resemble those seen in pure films. Nonionic N-9, however, suppresses a high pressure shape transition seen in pure DPPC monolayers using fluorescence microscopy. DPPC monolayers in the presence of charged surfactant exhibit a new high pressure transition resulting in the dispersion of domains about their boundaries. Among all of the surfactants stud-

ied, cationic DTAB was the only one to affect domain shape at relatively low surface pressure.

Our results with this model system confirm that electrostatic effects will dominate interactions of perturbants with real membranes. This may ultimately be used to design surfactants with specific perturbative properties.

Acknowledgements

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References

[1] R. Nararrete, R. Serrano, Biochim. Biophys. Acta 728 (1983) 403.
[2] G.W. Stubbs, B.J. Littman, Biochemistry 17 (1978) 215.
[3] M.K. Polano, J. Soc. Cosmetic Chemists 19 (1968) 3.
[4] E.B. Michaels, E.C. Hahn, A. Kenyon, J. Am. J. Vet. Res. 44 (1983) 1378.
[5] C.J. Elias, C. Coggins, AIDS 10 (1996) S43.
[6] T.R. Neu, Microbiol. Rev. 60 (1996) 151.
[7] E.B. Michaels, E.C. Hahn, A.J. Kenyon, Am. J. Vet. Res. 44 (1983) 1977.
[8] A.M. Corner, M.M. Dolan, S.L. Yankell, D. Malamud, Antimicrob. Agents Chemother. 32 (1988) 350.
[9] A.M. Corner, V.J. Brightman, S. Cooper, S.L. Yankell, D.J. Malamud, Clin. Dent. 2 (1990) 234.
[10] S.C. Stone, F. Cardinale, Am. J. Obstet. Gynecol. 133 (1979) 635.
[11] D. Sharman, E. Chantler, M. Dukes, F.G. Hutchinson, M. Elstein, Fert. Steril. 45 (1986) 259.
[12] K.A. Thompson, D. Malamud, B.T. Storey, Contraception 53 (1996) 313.
[13] B. Isomaa, G. Paatero, C. Lönnqvist, Acta Pharmacol. Toxicol. 44 (1979) 208.
[14] N. Jayasuriya, S. Bosak, S.L. Regen, J. Am. Chem. Soc. 112 (1990) 5851.
[15] D. Ing, F. Devínsky, I. Lacko, D. Mlynarčík, V. Račanský, Ľ. Krasnec, Tenside Detergents 22 (1985) 10.
[16] L. Ter Minassian-Saraga, J. Wietzerbin, Res. Commun. Biochem. Biophys. 41 (1970) 1231.
[17] B. Zaslavsky, N. Ossipov, A.Y. Lisichkin, Y.A. Davidovich, S. Rogozhin, Coll. Polym. Sci. 257 (1979) 953.
[18] K.S. Birdi, J. Coll. Int. Sci. 57 (1976) 228.
[19] R.E. Jacobs, S.H. White, Biochemistry 26 (1987) 6127.
[20] D.P. Clark, S. Durell, W.L. Malloy, M.J. Zasloff, Biol. Chem. 269 (1994) 10849.
[21] K.S. Moore, S. Wehrli, H. Roder, M. Rogers, J.N. Forrest, D. McCrimmon, M. Zasloff, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 1354.
[22] A.D. Bangham, R.W. Horne, J. Mol. Biol. 8 (1964) 660.
[23] D. Needham, D.V. Zhelev, Ann. Biomed. Eng. 23 (1995) 287.
[24] E.A.J. Keukens, T. de Vrije, C. van den Boom, P. de Waard, H.H. Plasman, F. Thiel, V. Chupin, W.M.F. Jongen, B. de Kruijff, Biochim. Biophys. Acta 1240 (1995) 216.
[25] A. de la Maza, J.L. Parra, Arch. Biochem. Biophys. 322 (1995) 167.
[26] G. Colacicco, Lipids 5 (1968) 636.
[27] R.W. Egan, M.A. Jones, A.L. Lehninger, J. Biol. Chem. 251 (1976) 4442.
[28] S. Watanabe, S.L. Regen, J. Am. Chem. Soc. 116 (1994) 5762.
[29] Y. Nagawa, S.L. Regen, J. Am. Chem. Soc. 113 (1991) 7237.
[30] K. Naka, A. Sadownik, S.L. Regen, J. Am. Chem. Soc. 115 (1993) 2278.
[31] M. Lösche, H. Möhwald, Rev. Sci. Instrum. 55 (1984) 1968.
[32] R. Peters, K. Beck, Proc. Natl. Acad. Sci. U.S.A. 80 (1983) 7183.
[33] V. von Tscharner, H.M. McConnell, Biophys. J. 36 (1981) 409.
[34] D. Hönig, D. Möbius, Thin Solid Films 210–211 (1992) 64–68.
[35] S. Hénon, J. Meunier, Rev. Sci. Instrum. 62 (1991) 936.
[36] B.A. Pethica, Trans. Faraday Soc. 51 (1955) 1402.
[37] P.J. Anderson, B.A. Pethica, Trans. Faraday Soc. 52 (1956) 1080.
[38] I.I. Panaiotov, L. Ter Minassian-Saraga, G. Albrecht, Langmuir 1 (1985) 395.
[39] D.G. Hall, Langmuir 2 (1986) 809.
[40] S. Sundaram, K.J. Stebe, Langmuir 12 (1996) 2028.
[41] S. Sundaram, K.J. Stebe, Langmuir 13 (1997) 1729.
[42] Q. Jiang, C.J. O'Lenick, J.E. Valentini, Y.C. Chiew, Langmuir 11 (1995) 1138.
[43] M.A. McGregor, G.T. Barnes, J. Coll. Int. Sci. 49 (1974) 362.
[44] K. Motomura, Y. Hayami, M. Aratono, R. Matuura, J. Coll. Int. Sci. 87 (1982) 333.
[45] D.M. Alexander, G.T. Barnes, J.C.S. Faraday I 76 (1980) 118.
[46] M.A. Mayer, T.K. Vanderlick, J. Chem. Phys. 103 (1995) 1.
[47] M.A. Mayer, T.K. Vanderlick, J. Chem. Phys. 100 (1994) 8399.
[48] D.J. Benvegnu, H.M. McConnell, J. Phys. Chem. 96 (1992) 6820.
[49] R. de Koker, H.M. McConnell, J. Phys. Chem. 97 (1993) 13419.
[50] K.Y.C. Lee, H.M. McConnell, J. Phys. Chem. 97 (1993) 9532.

[51] P.A. Rice, H.M. McConnell, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 6445.
[52] H.M. McConnell, D. Keller, H. Gaub, J. Phys. Chem. 90 (1986) 1717.
[53] R.M. Weis, H.M. McConnell, J. Phys. Chem. 89 (1985) 4453.
[54] A. Jyoti, R.M. Prokop, J. Li, D. Vollhardt, D.Y. Kwok, R. Miller, H. Möhwald, A.W. Neumann, Coll. Surf. A 116 (1996) 173.
[55] C.W. McConlogue, T.K. Vanderlick, Langmuir 13 (1997) 7158.
[56] S. Roy, E. Ruckenstein, P. Stroeve, J. Coll. Int. Sci. 92 (1983) 383.
[57] P.B. Wyrick, S.T. Knight, D.G. Gerbig, J.E. Raulston, C.H. Davis, T.R. Paul, D. Malamud, Antimicrob. Agents Chemother. 41 (1997) 1335.
[58] J.R. Charron, R.D. Tilton, J. Phys. Chem. 100 (1996) 3179.
[59] S.Y. Lin, K. McKeigue, C. Maldarelli, Langmuir 10 (1994) 3443.