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Phase changes and mosaic formation in single and mixed phospholipid monolayers at the oil–water interface

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SUMMARY

Insoluble monolayers of phosphatidylcholines and ethanolamines show first-order phase transitions at hydrocarbon–water interfaces, depending on the temperature and chain length. In mixed monolayers of two phosphatidylcholines of different chain lengths, or of phosphatidylcholines and ethanolamines of the same chain length, demixing occurs at the phase transition.

There has been considerable interest in recent years in the state of aggregation of the constituents of biomembranes. A range of studies based principally on X-ray scattering[1–3], calorimetry[4–6] and the fluorescence[7,8], electron spin[9,10] and nuclear magnetic resonance spectroscopy[11] of membranes and model systems have encouraged the view that changes in phase state, particularly of membrane lipids, are important in defining biomembrane function[12,13]. It was suggested by Segerman[1] from X-ray data that the lipids in the erythrocyte membrane exist in small patches of some tens of molecules. Other authors have developed similar ideas of mosaics of molecular clusters in membranes, and of transitions between various forms of these structures[14,15]. Phillips *et al.*[16] give good evidence from the thermal analysis of aqueous dispersions of mixed phospholipids that the constituents of the mixture can separate in the bilayer structures at appropriate temperatures.

We have recently made an extensive collaborative study in parallel experiments in Britain and the U.S.A. of the interfacial properties of pure synthetic phospholipids

with a view to clarifying the interactions between these molecules. Following the development of a technique of spreading, compression and surface pressure measurement with insoluble monolayers at oil–water interfaces by Brooks and Pethica[17] and Taylor and Mingins (Taylor, J.A.G. and Mingins, J., unpublished), the experiments have been made at hydrocarbon oil–water interfaces where the chain–chain interactions in the monolayers are smaller than at the more frequently studied air–water interface. As a result, detailed thermodynamic data over a wide range of surface densities have been obtained. The experimental details and results will be reported elsewhere but we here wish to draw attention to certain of the results which show remarkable phase changes by pure and mixed phospholipid monolayers. These observations are of clear interest for theories of biomembrane structure.

At low surface densities of saturated phosphatidylethanolamines and phosphatidylcholines, the surface pressure–area isotherms are expanded and are only slightly dependent on chain length or head group or salt concentration for a given temperature. At areas below about 2 nM^2/molecule, phase changes occur at pressures and areas dependent on head group, chain length and temperature. The longer the chain and the lower the temperature, the more the changes approximate to a classical first-order phase

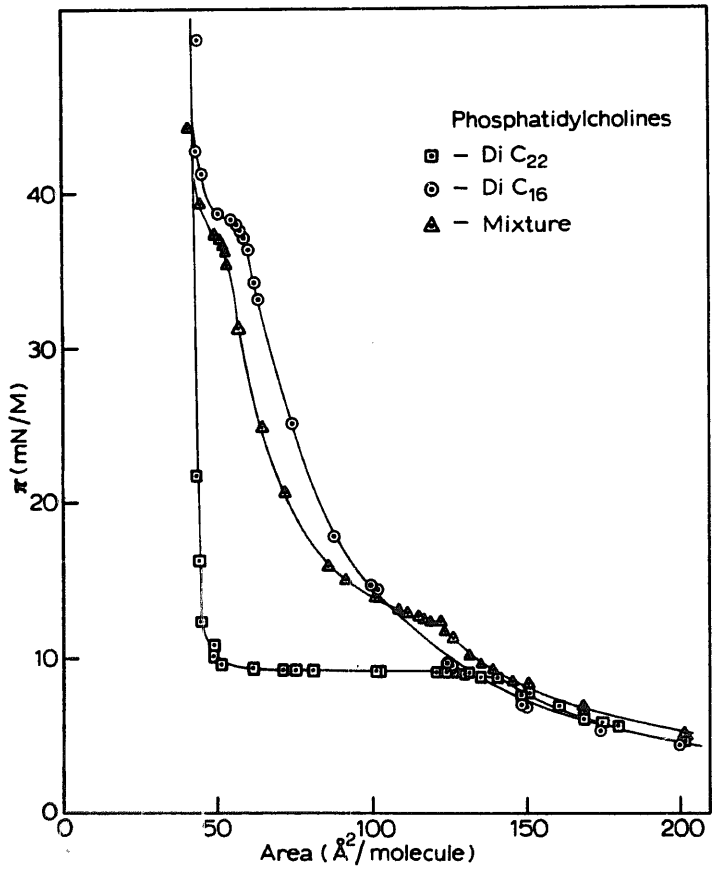

Fig. 1. Surface pressure–area isotherms for two phosphatidylcholines and their mixture at the heptane–water interface. Temperature, 20 ± 0.1 ° C; NaCl concentration, 0.1 M; pH, 5–6. Monolayers are spread from dilute solutions in heptane–ethanol (90:10, v/v). □, Didocosanoylphosphatidylcholine; ⊙, dihexadecanoylphosphatidylcholine; △, equimolar mixture.

transition, implying the formation of highly aggregated clusters in the condensation process. As the chain shortens and the temperature rises, the transition becomes one of degenerate first order, indicating the formation of smaller clusters during the transition. These findings are typified in Fig. 1 for didocosanoyl- and dihexadecanoyllecithins at the heptane–water interface at 20 °C. A similar phase change was first observed at Port Sunlight by Demel (Demel, R.A., unpublished) for dihexadecanoylphosphatidylethanolamine. For the fully condensed pure monolayers, the clusters will undoubtedly merge into large homogeneous sheets of molecules. In a mixed film of these two phospholipids a further striking phenomenon is that each component condenses out of the expanded state separately, as shown in Fig. 1. The clusters formed in the initial stages of condensation are probably smaller than the corresponding clusters from the one-component monolayers, and may well contain a minor fraction of the second component in each case. The implication is, however, that in the fully condensed close-packed state of the mixed phospholipids studied, the monolayer will be a mosaic of two kinds of small cluster each rich in one or other of the two components. This same conclusion follows from the further example shown in Fig. 2, in which a phosphatidylethanolamine and a phosphatidylcholine of the same chain length (dihexadecanoyl) exhibit clear demixing at the isooctane–water interface at 1 °C in a two-

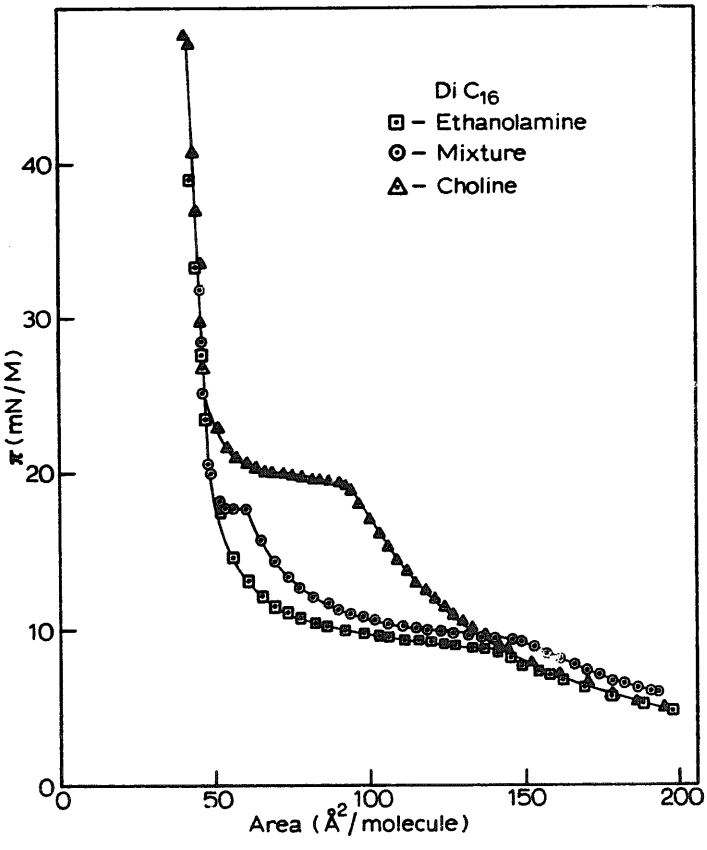

Fig. 2. Surface pressure–area isotherms for a phosphatidylethanolamine, a phosphatidylcholine and their mixture at the isooctane–water interface. Temperature, 1 °C; NaCl concentration, 0.1 M; pH, 5–6. Monolayers are spread from dilute solutions in various isooctane–ethanol mixtures. ⊡, dihexadecanoylphosphatidylethanolamine; △, dihexadecanoylphosphatidylcholine; ⊙, a 1–1.4 mixture of the phosphatidylcholine and phosphatidylethanolamine.

stage phase change during compression. We conclude that in close packed arrays of mixed saturated phospholipids, as occur in bilayer smectic systems and perhaps in biomembranes, the individual components may, dependent on the temperature, show substantial demixing into clusters of differing chain length and head group. The situation for unsaturated chain systems is under investigation.

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