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PHASE TRANSITIONS IN 1,2- AND 1,3-DIACYL-*sn*-GLYCERO-3-PHOSPHOCHOLINE MONOLAYERS AT THE OIL/WATER INTERFACE

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Summary

Moving the phosphatidylcholine group from the 3- to the 2-position in monolayers of distearoyl-*sn*-glycero-3-phosphocholine at the oil/water interface expands the surface pressure-area isotherm and markedly increases the surface pressure at which phase separation occurs with only a slight change in the monolayer surface density at the onset of the transition. This is interpreted in terms of a change in an ordering parameter in the solid-condensed state.

Following Demel's early observations here (unpublished results) that dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine monolayers spread at the oil/water interface show pronounced phase changes further work on oil/water monolayers was instituted on a wide range of pure synthetic glycerophospholipids kindly supplied by the laboratories of Chapman and Van Deenen. Phase changes were seen to be more ubiquitous with phospholipids and by studying variables such as temperature, chain length, unsaturation, head group type, ionic strength, pH and the nature of the oil phase, phase properties became better defined (unpublished results). From this work it became clear that increase of chain length or decrease of temperature favour the phase transition, that ionic strength and moderate pH changes have only a marginal effect, whereas olefinic bonds cause complete degeneration As at the air/water interface [1] ethanolamines are more condensed than cholines of the same chain length. The phospholipids de-mix from different solvents at different surface coverages and surface energies, and from other phospholipids [2] at areas and pressures which depend on head group and chain length differences All the above studies on phase transitions were limited to synthetic, 1,2-diacyl compounds — the

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naturally occurring isomer The availability of a small sample of the 1,3-isomer* gave us the exciting possibility of investigating the phase change with head group and chain length held constant but with packing the phase change with place in any solid-condensed phase

Thin-layer chromatography runs on the sample of 1,3-distearoyl *sn*-glycero-3-phosphocholine showed traces of lysophosphotidylcholine and a less polar compound ($\approx$ 0 5%), these levels were judged low enough not to affect the position of the phase transition point or the surface pressure (π), area per molecule (A) isotherms (π-A isotherms) at the area above the phase change The sample was therefore used without further purification although it was appreciated that the slope of the isotherms at the area below the phase change could, in principle, be affected The purification of materials and techniques for obtaining accurate π-A isotherms at the oil/water interface were the same as described elsewhere [3]

1,3-distearoyl-*sn*-glycero-3-phosphocholine gives the distinct two dimensional phase transition which is shown in Fig 1 Although the π-A isotherm for the 1,3-isomer is such more expanded than the one for the 1,2-isomer there is no significant difference in the areas at the phase transition (A_T) In contrast the surface pressures at the kink point (π_T) are markedly different

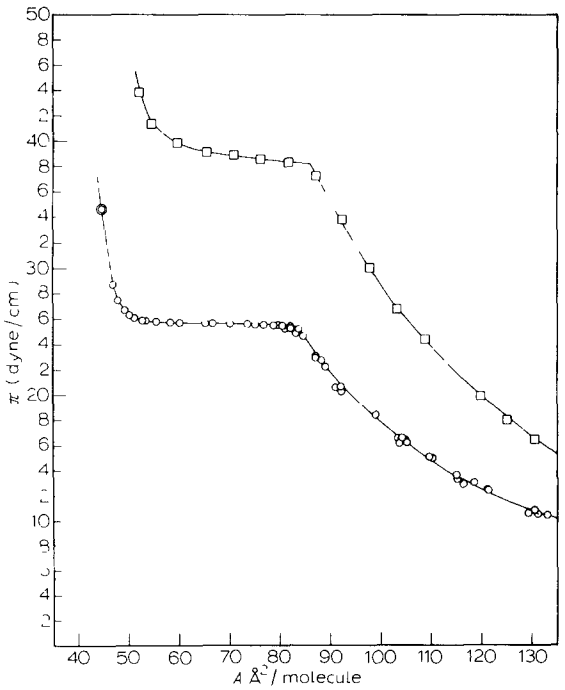

Fig 1 Surface pressure versus area per molecule isotherms at the heptane/0 01 M NaCl interface □, 1,3-distearoyl-*sn*-glycero-3-phosphocholine Temperature = 20 ± 0 3°C ○, 1,2-distearoyl-*sn*-glycero-3-phosphocholine data from [1]. Temperature = 20 ± 0 1°C 1,3-distearoyl-*sn*-glycero-3-phosphocholine monolayers spread from solution (0 3 mg/ml) in a mixture of heptane/ethanol (9 1, v/v) The curve fitting routine of ref 3 was used to correct for spreading errors

*We are grateful to Professor van Dorp and his colleagues (Unilever Research Laboratory, Vlaardingen) for their generous gift of 1,3-distearoyl-*sn*-glycero-3-phosphocholine

From molecular models it can be seen that the phosphatidylcholine group on the 1,2-isomer can curl under the glycerol backbone to give a head group cross-sectional area no greater than that given by the two alkyl chains and still preserve an arrangement of the zwitterion co-planar with an interface taken perpendicular to the cahins This is not possible with the 1,3-isomer and either the head group increases the cross-sectional area of the molecule or the positive choline group moves away from the interface An increase in area would explain the expansion of the solid-condensed portions of the isotherms in Fig 1 but does not explain the expansion in the dilute region ($A > A_T$ in Fig 1) if the classical Volmer term, $kT/(A-Ao)$, with the co-area A_o taken as the cross-sectional area of a vertically oriented molecule is applied There is no long range repulsive electrostatic interaction from out-of-plane zwitterions

In preliminary studies with a molecualr field theory of two-dimensional critical phenomena the form of some of the phospholipid phase transitions was obtained using both ordering and cohesive terms (Bell, G.M and Mingins, J., to be published). The chain length dependence of the cohesive term is shown by the marked dependence of A_T on chain length for saturated 1,2-diacyl phosphatidylcholines and ethanolamines (unpublished results), but because the isotherms in each class superpose at $A > A_T$ the long-range contributions are either zero or chain length independent. Dispersion interactions among the identical chains for the two isomers here ought to be similar and the near-correspondence of the A_T values would indicate that this is the case. The difference in π_T and the expansion of the rest of the isotherms must therefore be attributed to changes in ordering terms brought about by changes in head group size or orientation or chain packing near the glycerol backbone

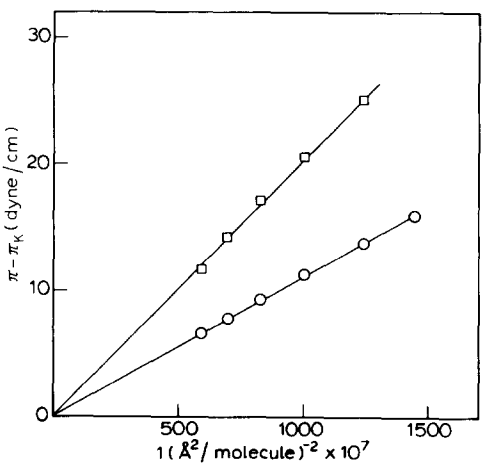

Fig 2 Plot of $\pi-\pi_K$ versus $1/A^2$ for distearoyl-*sn*-glycero-3-phosphocholine at the heptane/0.01M NaCl interface $\pi_K = kT/(A-A_o)$, where A_o is a co-area and kT = 404 6·10^{-16} ergs/molecule at 20°C □, 1,3-distearoyl-*sn*-glycero-3-phosphocholine, A_o = 50 Å^2/molecule, ○, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, A_o = 40 Å^2/molecule

The plot in Fig. 2 shows that, provided some allowance is made for the difference in A_o, then in the range of $A > A_T$ in Fig. 1 any residual repulsive

term π_R can be written in the form

$\pi_R = K_1/A^2$

The value of K_1 for 1,3-distearoyl-*sn*-glycero-3-phosphocholine is almost double that for the 1,2 molecule up to the kink point Strong cohesive forces then come into play at approximately the same molecular separations to induce phase separation The near-zero slope of the isotherm for 1,2-distearoyl-*sn*-glycero-phosphocholine at areas just below A_T is not held for the 1,3-isomer and if the effect of trace impurities can be discounted this would correspond to a decrease in the number density of molecules in the aggregates With the evidence presented here it would seem that the properties of the whole isotherm are intimately connected with short range order and that a detailed knowledge of the lattice energy of the *n*-mers at the transition is needed before any useful formulation of an equation of state for phospholipid monolayers at the oil/water interface can be considered

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