Coexisting liquid phases in lipid monolayers and bilayers

2002 J. Phys.: Condens. Matter 14 4763

(http://iopscience.iop.org/0953-8984/14/19/302)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **14** (2002) 4763–4766 PII: S0953-8984(02)31064-6

Coexisting liquid phases in lipid monolayers and bilayers

Sarah L Keller

Department of Chemistry 351700, University of Washington, Seattle WA 98195, USA

Received 21 November 2001
Published 2 May 2002
Online at stacks.iop.org/JPhysCM/14/4763

Abstract

Several mixtures of phospholipids and cholesterol produce coexisting liquid phases when deposited as a monolayer at an air-water interface. For model mixtures near the miscibility critical point, distinctive 'stripe' phases are observed. Stripe phases are also observed in monolayers with lipid compositions approximating the inner or outer leaflets of human red blood cells. For monolayers containing cholesterol and lipids with high melting temperature, the phase diagram can exhibit two upper miscibility critical points rather than only one.

Lipids which are deposited in low concentrations at the air–water interface of a Langmuir trough align with their hydrophilic headgroups toward the water. The change in surface tension or 'surface pressure', Π, caused by the lipids varies over roughly 0–50 mN m^{-1}. Below $\sim$1 mN m^{-1} a two-dimensional gas phase of lipids exists. As surface pressure is increased (or, equivalently, as the area per molecule is decreased), the monolayer of lipids becomes entirely liquid. For some lipid mixtures, this liquid is composed of two coexisting phases of different lipid composition [1]. As shown in figure 1(a), the two phases merge into one uniform liquid phase as surface pressure is increased. In a binary mixture, the highest pressure at which this miscibility phase transition occurs is the critical point or upper consolute point. If the surface pressure were to increase still further, a solid phase would be formed [1] and would eventually buckle and collapse.

As depicted in figure 1(b), when two liquid phases coexist, domains of different lipid composition are observed. For lipid mixtures containing cholesterol, the domains are typically of the order of 10 μm and are visualized by epifluorescence microscopy where the bright phase is cholesterol-poor and the dark phase is cholesterol-rich [2, 3]. The shape of the domains depends on the lipid mixture's proximity to the miscibility critical point. Experimentally, a stripe phase is only observed close to the critical point [4, 5]. Far from the critical point, domains are circular. If the miscibility phase transition is crossed near the critical point, stripe phases are seen. If it is crossed far from the critical point, stripes are not seen. Instead, circular domains simply diffuse and blur to become one uniform phase [4].

0953-8984/02/194763+04$30.00

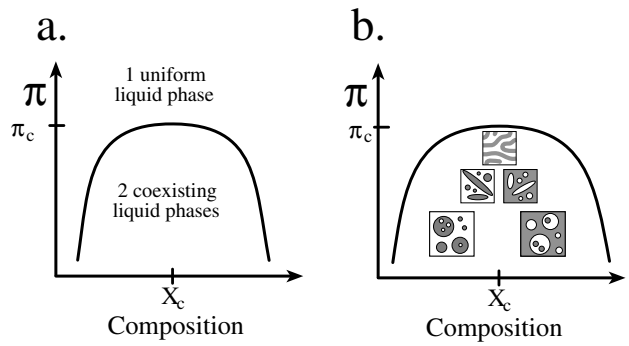

Figure 1. (*a*) The miscibility phase boundary between regions of two coexisting liquid phases and one uniform phase. (*b*) Experimentally observed shapes of domains.

The only region of the miscibility phase diagram in which theory and experiment completely agree is close to the critical point, in the 'stripe phase'. Theoretically, the stripe phase should persist to low surface pressures as long as the lipid composition has an intermediate value [6]. This disagreement is not general for all monolayer systems or thin films. The lack of agreement in the lipid system is a disadvantage for experiments that test the theory's parameters [4], but it is an advantage in other experiments. For example, since stripe phases are only observed near critical points, then if stripe phases are seen, it can be surmised that the lipid system is near a critical point [7, 8]. This is an important time-saving tool when studying biologically relevant lipid mixtures with many components. Mapping the entire phase diagram for these mixtures would be tedious.

To test whether a biological mixture of lipids might be near a miscibility critical point, lipid monolayers with compositions approximating that of either the inner or the outer leaflet of an adult human red blood cell were assembled. Each monolayer exhibited behaviour similar to that in model systems as follows: at low surface pressures liquid domains were circular and as surface pressure increased the domains elongated to become stripes before the transition to one uniform phase [8]. The miscibility transition pressures were high and occurred at an area per lipid close to that of a lipid in a red-blood-cell membrane. This result implies that the red-blood-cell lipid bilayer may be near a critical point since the two systems are at comparable molecular densities [8].

When the monolayer lipid compositions were altered such that they no longer approximated red-cell leaflets, stripes were no longer observed, although the surface pressure at the transition from circular domains to one uniform phase was still high. If the lipid compositions were altered only slightly, stripes were often still observed. Because of the complexity of the mixtures, the compositions of the two coexisting phases are not known. However, as in the model system, the percentage of dark phase increases with overall cholesterol concentration [8].

As discussed above, the area per lipid in a bilayer corresponds to a high surface pressure in a monolayer. An 'equivalent bilayer pressure' of a monolayer is usually assumed to be in the range of 30–35 mN m^{-1} [9, 10], although some theoretical work suggests it should be higher [11, 12]. Few lipid monolayer mixtures have miscibility phase transitions above 30 mN m^{-1}. In general, higher transition pressures are found in monolayers for binary mixtures of cholesterol and phospholipids with either shorter acyl chain lengths or higher unsaturation [13]. Miscibility transition pressures as high as 40 mN m^{-1} have been found in ternary mixtures of cholesterol and phospholipids with different acyl chain lengths [7]. For this case the mechanisms which produce high-miscibility transition pressures in monolayers

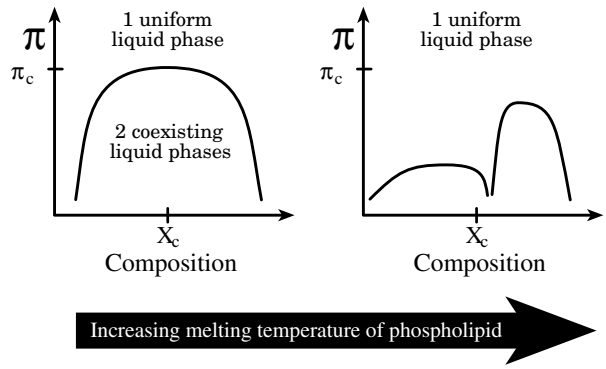

Figure 2. Miscibility phase diagrams for binary mixtures of cholesterol and lipids with either low melting temperatures (left) or high melting temperatures (right).

would probably not result in phase separation in bilayers of the same lipid composition.

Does the separation of cholesterol-rich and cholesterol-poor phases in monolayers correspond to the formation of 'rafts' in bilayers? Rafts are thought to be regions in cell membranes that are rich in cholesterol, sphingolipids, and long-chained lipids and particular proteins, including GPI-linked proteins and palmitoylated proteins [14]. In cell membranes, raft regions are usually sub-micron and are thought to be important for cell functions such as signalling [14].

In some model bilayer systems, coexisting liquid domains are large enough to observe by epifluorescence microscopy [15, 16]. All of these model systems contain cholesterol and a lipid with a high melting temperature. These lipids are often those with long, saturated acyl chains or sphingolipids. When monolayers containing cholesterol and lipids with high melting temperature are examined, the miscibility phase diagram exhibits two distinct regions of coexisting liquid phases as in figure 2 [17]. This phase diagram has been attributed to a three-component system consisting of (1) cholesterol, (2) a high-melting-temperature lipid, and (3) a complex formed between the cholesterol and phospholipid which acts as a separate entity [18, 19]. When similar high-melting-temperature phospholipids are mixed with cholesterol in a bilayer, high fractions of detergent-resistant membranes and liquid-ordered phases result, apparently because the molecules pack tightly [20, 21]. The study of coexisting lipid domains in bilayers, the elucidation of why domains form, and the application to biological membranes promises to be a field of intense activity in the near future.

References

[1] McConnell H M 1991 Structures and transitions in lipid monolayers at the air–water interface *Annu. Rev. Phys. Chem.* **42** 171–95

[2] Subramaniam S and McConnell H M 1987 Critical mixing in monolayer mixtures of phospholipid and cholesterol *J. Phys. Chem.* **91** 1715–18

[3] Hirshfeld C L and Seul M 1990 Critical mixing in monomolecular films: pressure–composition phase diagram of a two-dimensional binary mixture *J. Physique* **51** 1537–52

[4] Keller S L and McConnell H M 1999 Stripe phases in lipid monolayers near a miscibility critical point *Phys. Rev. Lett.* **82** 1602–5

[5] Seul M and Chen V S 1993 Isotropic and aligned stripe phases in a monomolecular organic film *Phys. Rev. Lett.* **70** 1658–61

[6] Andelman D, Brochard F and Joanny J-F 1987 *J. Chem. Phys.* **86** 3673–81

[7] Keller S L, Anderson T G and McConnell H M 2000 Miscibility critical pressures in monolayers of ternary lipid mixtures *Biophys. J.* **79** 2033–42
[8] Keller S L, Pitcher W H III, Huestis W H and McConnell H M 1998 Red blood cell lipids form immiscible liquids *Phys. Rev. Lett.* **81** 5019–22
[9] Demel R A, Geurts Van Kessel W S M, Zwaal R F A, Roelofsen B and Van Deenen L L M 1975 Relation between various phospholipase actions on human red cell membranes and the interfacial phospholipid pressure in monolayers *Biochim. Biophys. Acta* **406** 97–107
[10] Marsh D 1996 Lateral pressure in membranes *Biochim. Biophys. Acta* **1286** 183–223
[11] Nagle J F 1986 Theory of lipid monolayer and bilayer chain-melting phase transitions *Faraday Discuss. Chem. Soc.* **81** 151–62
[12] Feng S-S 1999 Interpretation of mechanochemical properties of lipid bilayer vesicles from the equation of state or pressure–area measurement of the monolayer at the air–water or oil–water interface *Langmuir* **15** 998–1010
[13] Hagen J P and McConnell H M 1997 *Biochim. Biophys. Acta* **1329** 7–11
[14] Simons K and Ikonen E 1997 Functional rafts in cell membranes *Nature* **387** 569–72
[15] Dietrich C, Bagatolli L A, Volovyk Z N, Thompson N L, Levi M, Jacobson K and Gratton E 2001 Lipid rafts reconstituted in model membranes *Biophys. J.* **80** 1417–28
[16] Samsonov A V, Mihalyov I and Cohen F S 2001 Characterization of cholesterol–sphingomyelin domains and their dynamics in bilayer membranes *Biophys. J.* **81** 1486–500
[17] Keller S L, Radhakrishnan A and McConnell H M 2000 Saturated phospholipids with high melting temperature form complexes with cholesterol in monolayers *J. Phys. Chem.* B **104** 7522–7
[18] Radhakrishnan A and McConnell H M 1999 Condensed complexes of cholesterol and phospholipids *Biophys. J.* **77** 1507–17
[19] Radhakrishnan A and McConnell H M 1999 Cholesterol–phospholipid complexes in membranes *J. Am. Chem. Soc.* **121** 486–7
[20] Schroeder R, London E and Brown D 1994 Interactions between saturated acyl chains confer detergent resistance on lipids and glycolsylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior *Proc. Natl Acad. Sci. USA* **91** 12 130–4
[21] Xu X and London E 2000 The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation *Biochemistry* **39** 843–9