Critical pressures in multicomponent lipid monolayers

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Abstract

Epifluorescence microscopy has been used previously to study coexisting liquid phases in lipid monolayers of dihydrocholesterol and dimyristoylphosphatidylcholine at the air/water interface. This binary mixture has a critical point at room temperature (22°C), a monolayer pressure of approx. 10 mN/m, and a composition in the vicinity of 20–30 mol% dihydrocholesterol. It is reported here that this critical pressure can be lowered, raised, or maintained constant by systematically replacing molecules of this phosphatidylcholine with molecules of a phosphatidylethanolamine, or an unsaturated phosphatidylcholine, or mixtures of the two, while maintaining the dihydrocholesterol concentration at 20 mol%. Thus, even complex mixtures of lipids may be characterized by a single, well-defined second-order phase transition. In principle, such transitions might be found in biological membranes.

Lipids form monolayers when spread at the air/water interface. Under certain conditions of composition, temperature, and pressure these monolayers can exhibit two coexisting liquid phases. Fluorescence microscopy has been used to observe the shapes, sizes, and movements of domains of one such liquid phase in the background of the other liquid phase [1–4]. Monolayers composed of cholesterol (or cholesterol analogs such as dihydrocholesterol) and phospholipids are of special interest as eukaryotic cell membranes are composed of such mixtures. There have been many previous studies of these lipid mixtures in bilayers for the purpose of describing coexisting liquid phases [5–8]. Although the existence of liquid phases in these binary systems is now well established, there has been relatively little published work seeking to discover whether or not these phases might be present in more complex lipid systems [9]. Such studies are of relevance to long-standing questions concerning the possible occurrence of phase transitions in biological membranes [10–17]. The present study is most conveniently discussed in terms of the approximate phase diagram for the binary mixture of dihydrocholesterol and DMPC shown in Fig. 1. (Dihydrocholesterol, abbreviated DChol, is used because it appears to oxidize less rapidly than cholesterol. The oxidation products are line active and so can strongly affect domain morphology [18].) At low pressures this binary mixture forms two coexisting liquid phases. As the pressure is raised, the system crosses a phase boundary and becomes homogeneous. These phase boundaries were located using epifluorescence microscopy [1–3,18]. In the present study the same method is used to locate the phase boundaries in monolayers of multicomponent lipid mixtures containing 20 mol% dihydrocholesterol. Although the complete phase diagram for such multicomponent systems would require a multidimensional representation, we find that the systems studied behave in some respects as quasi-binary mixtures. That is, the critical composition with respect to dihydrocholesterol remains between 20 and 30 mol% in these mixtures, as judged by the appearance of a stripe phase near the critical pressure [3].

Materials and methods. The phospholipids dimyristoyl-L-α-phosphatidylcholine (DMPC), dimyristoyl-L-α-phosphatidylethanolamine (DMPE), dimyrystoleoyl-L-α-phosphatidylcholine (DMoPC), and the fluorescent lipid probe N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dipalmitoyl-L-α-phosphatidylethanolamine (NBD-DPPE) were purchased...
from Avanti Polar Lipids. The fluorescent lipid probe N-(Texas Red sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DPPE) was purchased from Molecular Probes. The synthetic cholesterol analog dihydrocholesterol (DChol) was purchased from Sigma. All chemicals were used without further purification. All experiments were done at 20–22°C. The sub-phase was distilled, deionized water. Compression and expansion of the monolayer were carried out with a movable barrier and surface pressure was measured with a Wilhelmy plate. The monolayer was viewed with a Zeiss epifluorescence microscope fitted with a Cohu low-level video camera. The monolayer images were recorded on a JVC BR601MU video recorder.

The lipids were spread from 1 mM chloroform solution containing 0.3 mol% of the probe TR-DPPE or 1 mol% of the probe NBD-DPPE. The experiments with DMoPC were done using TR-DPPE. Experiments with DMPE were done first with TR-DPPE and then repeated with NBD-DPPE. After spreading, the monolayers were compressed while under observation. The pressure at which the two phases became homogeneous was noted.

Results and discussion. Note once again the phase diagram for the binary mixture of DChol and DMPC shown in Fig. 1. This diagram is adapted from earlier work; the solid curve is taken from Lee and McConnell [19]. The shaded region is adapted from Seul [20], and is only schematic. This shows a region of composition where the two phases form stripe domains, rather than the more commonly seen circular domains. This ‘superstructure’ stripe phase is only observed in the vicinity of a critical point [3]. Note that the phase boundary between the stripe phase and the homogeneous region is relatively flat. Consequently, the mixing-demixing pressure of the stripe phase provides a measurement of the critical pressure of the system. Further, measurements of critical pressure at the boundary of the stripe phase are not expected to be sensitive to small changes in DChol critical composition.

While maintaining a dihydrocholesterol composition of 20 mol%, we have systematically replaced DMPC with DMPE. Fig. 2a shows the effect on the critical pressure of replacing DMPC with DMPE. There is an approximate linear decrease in critical pressure with increasing mole fraction of DMPE.

We then examined the effect of replacing DMPC with DMoPC. We once again maintained a dihydrocholesterol composition of 20 mol%, but now systematically replaced DMPC with DMoPC. Fig. 2b shows the effect on the critical pressure. There is an approximate linear increase in critical pressure with increasing mole fraction of DMoPC.

Several quartenary mixtures of DChol, DMPC, DMPE, and DMoPC were examined and their critical pressures noted. These results and those of the ternary mixtures are summarized in Table 1. The theoretical values of critical pressure calculated using the model discussed below as a guide are also listed.

The results in Table 1 can be accounted for qualitatively in terms of a simplified thermodynamic model. Consider lipid mixtures of N components, where $X_1, \ldots, X_{N-1}$ are...
Table 1
Critical pressure for different monolayer compositions

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<tr>
<th>%DChol</th>
<th>%DMPC</th>
<th>%DMPE</th>
<th>%DMoPC</th>
<th>Pressure (mN/m)</th>
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the phospholipid mole fractions, and $X_0$ is the mole fraction of DChol. Let $\bar{G}$ be the molar Gibbs free energy of this mixture:

$$\bar{G} = \sum_{i=0}^{N-1} (X_i \mu_i^0 + RT_i \ln X_i) + \sum_{i<j} \alpha_{ij} X_i X_j$$  \hspace{1cm} (1)$$

In this equation $\mu_i^0$ are the chemical potentials of the pure components, and $\alpha_{ij} = 2RT_{ij}$ where $T_{ij}$ is the is the critical temperature of a binary mixture of $i$ and $j$. The parameter $\alpha_{ij}$ is the non-ideal interaction energy coefficient for components $i$ and $j$. This assumes for simplicity that the critical composition for each two-component mixture is $X_i = X_j = 0.5$. If we further assume that (a) all the lipid mixtures other than those involving DChol are ideal, then $\alpha_{ij} = 0$ except when $j = 0$ (that is, all the binary phospholipid-phospholipid mixtures behave ideally), and (b) the fraction of each phospholipid $f_i = X_i/(1 - X_0)$ is constant in both phases, then Eq. (1) can be rewritten:

$$\bar{G} = X_0 \mu_0^0 + (1 - X_0) \sum_{i=1}^{N-1} (f_i \mu_i^0 + RT_i \ln f_i)$$

$$+ RT(X_0 \ln X_0 + (1 - X_0) \ln (1 - X_0))$$

$$+ X_0(1 - X_0) \bar{\alpha}$$  \hspace{1cm} (2)$$

Here the average non-ideal interaction $\bar{\alpha}$ is given by:

$$\bar{\alpha} = \sum_{i=1}^{N-1} f_i \alpha_{0,i}$$  \hspace{1cm} (3)$$

Eq. (2) has the form of the chemical potential of a two-component binary mixture, so $T_c$ is determined only by the magnitude of $\bar{\alpha}$. The equation relating the critical pressures found in the present work,

$$\pi_c = \sum_{i=1}^{N-1} f_i \pi_{0,i}$$  \hspace{1cm} (4)$$

follows from Eq. (3) if the pressure dependence of all of the $\alpha_{0,i}$ is the same. This situation can arise if the area contraction that takes place on mixing each of the phospholipids with cholesterol is approximately the same. Eq. (4) is examined in Fig. 3 using the data in Table 1. It is clear from these data that a given critical pressure can be achieved by means of a variety of lipid mixtures.

A question of long standing is how the lipid composition of biological membranes is regulated. One possibility that has been considered is that some physical property of the bilayer provides a control signal for enzymes that modulate lipid composition. The present work potentially bears on this subject in the following way.

We have shown here that certain complex lipid mixtures can exhibit well-defined critical points, and that the critical pressures (and hence temperatures) can be regulated in a systematic way through variations in composition. This conclusion can be extrapolated to even more complex mixtures. Enzyme activities that are affected by the phase boundaries of lipid domains may provide a means whereby lipid composition itself is regulated through enzyme activity. Although this possibility has not been proposed previously to the best of our knowledge, published experimental work [21–26] on the action of enzymes on lipid membranes is not inconsistent with this hypothesis.

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References