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Rapid report

Two fatty acids can replace one phospholipid in condensed complexes with cholesterol

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Abstract

We report that the monolayer phase diagram for binary mixtures of dimyristoylphosphatidylethanolamine (DMPE) and dihydrocholesterol (DChol) is largely unchanged when each phospholipid molecule is replaced by two myristic acid (MA) molecules or various mixtures of the lysophospholipid and myristic acid. The corresponding phase diagrams all show the formation of ''condensed complexes'' of DChol and lipid. The condensed complex stoichiometry is thus largely determined by the C14 fatty acid acyl chains, in this case about 4–4.6 per DChol molecule.

Keywords: Membrane structure; Glycerol backbone; Monolayer; Phase diagram; Lysophospholipid; Acyl chain

Phase diagrams of phospholipid–cholesterol mixtures in monolayers at the air–water interface can be determined using epifluorescence microscopy [1]. These diagrams provide evidence for the formation of ''condensed complexes'' having specific compositions of phospholipid and cholesterol [2–4]. Phase diagrams of lipid mixtures forming condensed complexes are unique in that they show two upper miscibility critical points with an intervening cusp. The membrane composition at this cusp gives the stoichiometry in terms of the relative proportions of cholesterol and phospholipid in the condensed complex (see Fig. 2) [2,4]. Such phase diagrams are interpreted using a mean field thermodynamic model [5] in which condensed complexes form between cholesterol, C, and phospholipid, P:

$$nqC + npP \leftrightarrow C_{nq}P_{np} \quad (1)$$

Here p and q are stoichiometry integers, and n is an oligomerization parameter reflecting the cooperativity of the condensed complex formation. The complexes are referred to as ''condensed complexes'' because the observed average molecular area at the stoichiometric composition is typically much less than that expected for ideal mixing. In mixtures studied to date, the stoichiometry of the condensed complex is in the range 25–60 mol% cholesterol (or dihydrocholesterol, DChol) [4].

While much is known about condensed cholesterol–phospholipid complex compositions in monolayers and the conditions under which they form, little is known about the factors that determine their composition. Extensive earlier modeling of these mixtures by others has considered molecular areas of cholesterol and the fatty acid chains [6–13]. However, the structural role of the glycerol linkage of the two fatty acid chains has received little attention [14–16]. In the present work, we have sought to discover if the glycerol linkage in phospholipids is essential in the formation of condensed complexes, and in determining their stoichiometries.

The structures of the lipids studied, 14:0 lysophosphatidylethanolamine (LPE), free myristic acid (MA), dimyristoylphosphatidylethanolamine (DMPE) and DChol, are illustrated in Fig. 1. The experiments were carried out with DChol instead of cholesterol to minimize artifacts due to cholesterol oxidation. Previous work has shown that the phase behavior of both cholesterol and DChol in mixtures with phospholipid are closely similar to one another [17].

LPE and DMPE were purchased from Avanti Polar Lipids (Alabaster, AL) and DChol and MA were obtained from Sigma (St. Louis, MO). The fluorescent lipid, Texas Red dihexanoylphosphatidylethanolamine (TR–DHPE, Molecular Probes, Eugene, OR), is preferentially excluded from the DChol-rich phase and provides contrast between the liquid

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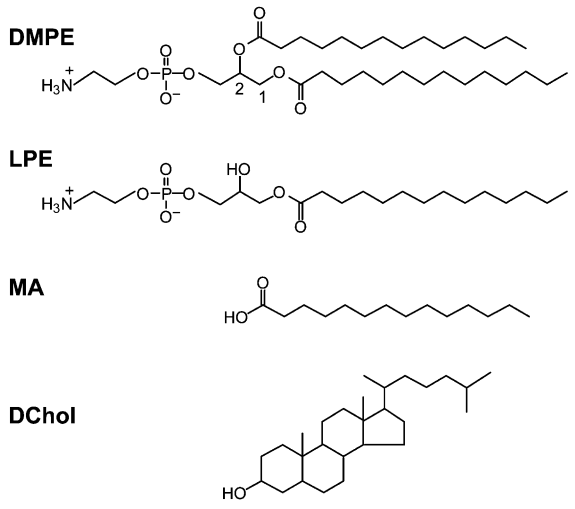

Fig. 1. The structures of DMPE, 14:0 LPE, free MA and the saturated cholesterol analog DChol. The *sn*1 and *sn*2 carbons of the glycerol backbone are labeled in DMPE.

phases. Lipid mixtures including 0.2 mol% TR–DHPE were spread from 1 mg/ml chloroform solutions onto the air–water interface of a Teflon trough, which had a movable barrier to change the surface pressure between 0 and 40 dyn/cm (mN/m). Experiments were performed at 24.5 ± 0.5 °C. All samples were examined within 2 weeks of preparation and stored at − 20 °C. Epifluorescence microscopy methods described previously were used for the phase diagram measurements [4,18]. Under our experimental conditions, MA is not fully protonated. To test for a pH effect on condensed complex formation, the pH of the subphase was lowered from approximately 5 to 2.6 in order to approach full protonation of the MA. At the lower pH, the monolayer had a higher collapse pressure and the domains were about 25% smaller, but the critical pressures did not change.

The five monolayer phase diagrams, (1) DMPE/DChol, (2) (1:1 LPE/MA)/DChol, (3) MA/DChol, (4) (1:1:1 LPE/MA/DMPE)/DChol and (5) (1:2 LPE/MA)/DChol, are shown in Fig. 2A–E. These diagrams feature two regions of two coexisting liquid phases separated at a cusp, characteristic of condensed complex formation. Although the two immiscibility regions (called α and β, see Fig. 2B) are visually quite distinct in terms of domain size and dye contrast, mixtures near the cusp may display characteristics of both regions due to compositional inhomogeneities in the monolayer. These inhomogeneities, in most cases, disappear at larger mixing times, of the order of an hour. The phase diagram for DMPE/DChol (Fig. 2A) has a cusp at about 0.30 mol fraction DChol, which corresponds to a condensed complex stoichiometry of nearly 2 DMPE:1 DChol. In terms of acyl chains, the stoichiometry is 4.6 acyl chains:1 DChol. Table 1 lists the experimental DChol cusp composition and number of acyl chains involved in condensed complex formation with DChol for each of the five lipid mixtures studied. LPE and MA each have a single chain and DMPE has two chains.

In all phase diagrams shown (Fig. 2A–E), the two phases in the α region are DChol-poor and condensed complex-rich. The two phases in the β region are DChol-rich and condensed complex-rich. Immiscibility in the β region persists to much higher pressures than in the α region. Critical points are expected theoretically at the peaks of both the α and the β two-phase regions. In monolayers, proximity to a critical point is often characterized by the formation of stripes. However, the domains in the β two-phase region are so small, of the order of a few microns, that stripe formation is not easily discernable. Stripes are invariably observed near the critical points in the α two-phase region.

The phase diagram for (1:1 LPE/MA)/DChol (Fig. 2B) has a cusp at about 0.18 mol fraction DChol. This cusp composition corresponds to a ratio of 4.6 chains:1 DChol. Some of the phase diagrams are modeled as psuedobinary mixtures where LPE and MA together act as an average phospholipid. LPE and MA must structurally mimic DMPE in the condensed complex. The phase diagram for MA/DChol in Fig. 2C has a cusp at 0.20 mol fraction DChol and a 4 chains:1 DChol ratio. DChol forms condensed complex with MA in the same acyl chain/DChol ratio as with DMPE and LPE/MA. This suggests that the acyl chains in a phospholipid act independently and that condensed complex formation does not require the glycerol backbone or the phosphatidylethanolamine headgroup. These moieties still likely play a role in the energetics of condensed complex formation as discussed later. Based on the above results, one would expect that (1:1:1 LPE/MA/DMPE)/DChol and (1:2 LPE/MA)/DChol would also form condensed complex in about a 4 acyl chains:1 DChol ratio. Fig. 2D,E show that this is indeed the case (and see Table 1).

Recall that the two phases in the α region are DChol-poor and condensed complex-rich and that the two phases in the β region are condensed complex-rich and DChol-rich (see Fig. 2B). The transition pressures in the β regions in all five diagrams (Fig. 2A–E) are nearly the same (11–13 dyn/cm). If the condensed complex is similar in all five systems (as suggested by the common 4:1 ratio), then it is not surprising that their critical pressures with DChol would also be similar. In contrast, the non-DChol components in the five systems are not the same, and therefore the DChol-poor phases and their miscibility with the condensed complex-rich phase should differ. Accordingly, the transition pressures in the α region for these systems range from 0.5 to 7 dyn/cm. The trend in these transition pressures is as follows: (1:1 LPE/MA)/DChol > (1:2 LPE/MA)/DChol> (1:1:1 LPE/MA/DMPE)/DChol>MA/DChol>DMPE/DChol. LPE increases the immiscibility of the DChol-poor and condensed complex-rich phases.

It is surprising that (1:1 LPE/MA)/DChol and MA/DChol mixtures form condensed complexes with acyl chains/DChol ratio similar to DMPE/DChol. This means that the minimum phospholipid structural requirement for condensed complex formation with cholesterol is the acyl

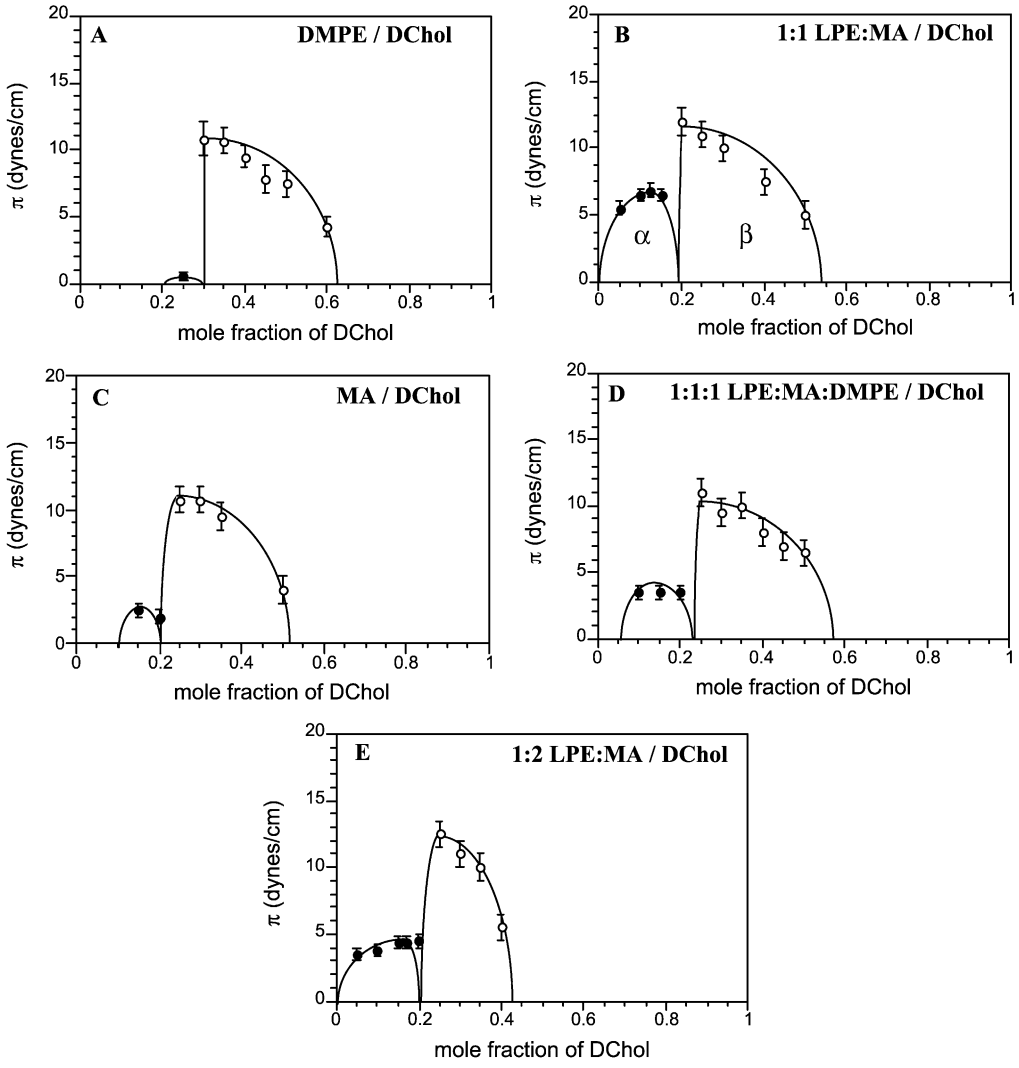

Fig. 2. Monolayer pressure–composition phase diagrams at room temperature. The curves do not constitute a fit but are drawn simply to guide the eye. There are two phases below each curve and one phase above. Closed circles represent stripe domains that are indicative of proximity to a critical point. Open circles represent a phase boundary not involving stripes. The two two-phase regions are labeled α and β. Error bars on the transition pressures in the α region are ± 0.5 dyn/cm and ± 1 dyn/cm in the β region. The error in the *x*-axis is ± 0.01 mol fraction DChol.

chain moiety and that the phospholipid's acyl chains act independently and under certain circumstances, equally. These results are supported by the fact that other mixtures of DMPE, LPE and MA with DChol (Fig. 2D,E) also formed condensed complex with the same acyl chain/DChol ratio. Physical models involving chain–sterol interactions have been used in interpreting a wide variety of bilayer and monolayer data. There have been a number of suggestions for complexes with different stoichiometries [9,11,12] or superlattices that form at specific cholesterol compositions [6–8]. The experimental conditions used in our phase diagram measurements clearly favor the formation of a single stoichiometry.

Table 1
Condensed complex stoichiometries corresponding to the cusp position (DChol mole fraction) and the stoichiometric ratio (acyl chain/DChol) in various lipid mixtures

Lipid mixtures	Cusp position (DChol mole fraction)	Putative complex composition (acyl chain/DChol)
DMPE/DChol	0.30 ± 0.02	4.6∓0.6:1
(1:1 LPE/MA)/DChol	0.18 ± 0.02	4.6∓0.6:1
MA/DChol	0.20 ± 0.02	4.0∓0.5:1
(1:1:1 LPE/MA/DMPE)/DChol	0.23 ± 0.02	4.6∓0.6:1
(1:2 LPE/MA)/DChol	0.20 ± 0.02	4.0∓0.5:1

In our experiments with DMPE and DMPE hydrolysis products, 4(C14 acyl chains):1DChol is the common stoichiometry, whether the phosphate headgroup and glycerol backbone are present (DMPE and LPE/MA) or not (MA). The effect of chain length on condensed complex formation and stoichiometry was not pursued here. Previous studies with phosphatidylcholine (PC) have shown that at room temperature, di 15:0 PC forms condensed complexes with

DChol, whereas di 14:0 PC does not [3]. Here we show that di 14:0 PE (DMPE) does form condensed complexes with DChol. The larger PC headgroup may interfere with condensed complex formation by disrupting C14 acyl chain/sterol contacts but this can be overcome by increasing the number of carbons in the acyl chains. Although the glycerol linkage in DMPE does not appear critical to condensed complex stoichiometry when compared to mixtures where the phospholipid is replaced by fatty acids, earlier work has shown that the glycerol linkage is important in cases where the *sn*1 and *sn*2 fatty acid chains have different lengths (see Fig. 1 for *sn*1 and *sn*2 positions) [3]. For example, 14:0–16:0 PC and 16:0–14:0 PC each formed condensed complexes with DChol at significantly different stoichiometries. Our work leaves open the question of the behavior of mixtures of DChol with fatty acids with different chain lengths.

There is much indirect evidence for the presence of condensed complexes in bilayers (see Ref. [19] for leading references). The recent demonstration of liquid–liquid immiscibility in cholesterol–PC bilayers opens the possibility of experiments analogous to those used to study complexes in monolayers [20,21]. It is not evident, however, that bilayers based on cholesterol and fatty acids will be stable.

Acknowledgements

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References

[1] H.M. McConnell, Annu. Rev. Phys. Chem. 42 (1991) 171–195.
[2] A. Radhakrishnan, H.M. McConnell, J. Am. Chem. Soc. 121 (1999) 486–487.
[3] S.L. Keller, A. Radhakrishnan, H.M. McConnell, J. Phys. Chem., B 104 (2000) 7522–7527.
[4] A. Radhakrishnan, H.M. McConnell, Biophys. J. 77 (1999) 1507–1517.
[5] L.R. Corrales, J.C. Wheeler, J. Chem. Phys. 91 (1989) 7097–7112.
[6] J.A. Virtanen, M. Ruonala, M. Vauhkonen, P. Somerharju, Biochemistry 34 (1995) 11568–11581.
[7] J. Huang, G.W. Feigenson, Biophys. J. 76 (1999) 2142–2157.
[8] P.L.-G. Chong, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10069–10073.
[9] D.A. Cadenhead, F. Müller-Landau, Can. J. Biochem. Cell Biol. 62 (1984) 732–737.
[10] F.T. Presti, R.J. Pace, S.I. Chan, Biochemistry 21 (1982) 3831–3835.
[11] B.R. Martin, P.L. Yeagle, Lipids 13 (1978) 594–597.
[12] D.M. Engelman, J.E. Rothman, J. Biol. Chem. 247 (1972) 3694–3697.
[13] J.B. Finean, Experientia 9 (1953) 17–19.
[14] R. Bittman, in: R. Bittman (Ed.), Subcellular Biochemistry, vol. 28, Plenum, New York, 1997, pp. 145–171.
[15] R.E. Brown, J. Cell Sci. 111 (1998) 1–9.
[16] P. Mattjus, R. Bittman, P. Slotte, Langmuir 12 (1996) 1284–1290.
[17] A. Radhakrishnan, T.G. Anderson, H.M. McConnell, PNAS 97 (2000) 12422–12427.
[18] S. Subramaniam, H.M. McConnell, J. Phys. Chem. 91 (1987) 1715–1718.
[19] T.G. Anderson, H.M. McConnell, Biophys. J. 81 (2001) 2774–2785.
[20] A.V. Samsonov, I. Mihalyov, F.S. Cohen, Biophys. J. 81 (2001) 1486–1500.
[21] S.L. Veatch, S.L. Keller, Biophys. J. 82 (2002) 521a.