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Insights on raft behavior from minimal phenomenological models

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Abstract
We construct a simple phenomenological theory of phase separation in ternary mixtures of cholesterol and saturated and unsaturated lipids. Such separation is relevant to the formation of 'rafts' in the plasma membrane. We also show how simple cross-linking of proteins which prefer one form of lipid to the other can trigger raft-formation, the first step in a signaling pathway.

1. Introduction

The suggestion that the plasma membrane which encloses a cell is not homogeneous but consists of regions in which agglomerations of cholesterol and saturated lipids, like sphingomyelin, float like rafts in a sea of mostly unsaturated lipids has occasioned an enormous amount of research and almost an equal amount of controversy. The latter arises because rafts have never been observed directly *in vivo* whereas many processes ascribed to them have been. A recent review, 'Lipid rafts as a membrane-organizing principle', which makes the case for the existence of such agglomerates, at least on the scale from 10 to 120 nm, is provided by Lingwood and Simons [1]. An earlier, less sanguine review, 'Lipid rafts: elusive or illusive?', is provided by Munro [2]. In contrast to the uncertainty *in vivo*, the behavior *in vitro* of ternary mixtures of cholesterol and saturated and unsaturated lipids is clear: over a wide range of compositions, such mixtures separate into coexisting liquid phases, one enhanced in saturated lipids and cholesterol, the other enhanced in unsaturated lipids. The former was long ago [3] denoted the 'liquid-ordered phase' because the hydrocarbon chains of its constituents are relatively well-ordered, having fewer thermally-excited gauche bonds than do those of the 'liquid-disordered' phase. Were the same phase behavior to occur *in vivo* as *in vitro*, rafts would be identified with regions of liquid-ordered phase coexisting with the liquid-disordered phase. A review of the phase behavior of model ternary systems is provided by Veatch and Keller [4].

One of the most unusual, and illuminating, phase behaviors was observed [5] in a ternary mixture of cholesterol, a saturated lipid, dipalmitoylphosphatidylcholine (or DPPC), and diphytanoylphosphatidylcholine (or diPhyPC). The last of these has branched hydrocarbon tails which make it difficult to pack with the more ordered tails of the DPPC, just as the *cis* double bond in the tails of an unsaturated lipid make such packing difficult. The experiment was carried out at a temperature above the transition of the DPPC to its gel phase, in which its chains are very well-ordered. Consequently, the DPPC exhibited no phase separation when mixed with cholesterol or with diPhyPC. Further, no phase separation occurred in the binary diPhyPC/cholesterol system. Thus, none of the three binary systems exhibited phase separation. The surprising feature was that, over a wide range of compositions, the ternary system did separate into two coexisting fluids.

This result is difficult to understand if, as is commonly done, one tries to describe the system only in terms of the concentrations of its three components which are characterized by binary interactions between them. A 'closed-loop' phase diagram such as this would require that two of its components attract one another very strongly [6, 7], much more so than one could reasonably account for. One way out of this is to postulate that the cholesterol and the saturated lipids form a bound 'complex' which repels the third component, unsaturated lipid or diPhyPC [8]. In this way one introduces a fourth component, the three original ones and the complex. If there is enough saturated lipid and cholesterol in complexes and enough unsaturated lipid to repel the complexes, the system will phase separate. A description solely in terms of the concentrations of these four components and binary interactions suffices. It would seem, however, that if this were a correct picture, one should be able to start with the original ternary system and such binary interactions which would lead to the formation of complexes and thereby derive the closed-loop phase diagram. As noted above, this would require much stronger binary attractions between cholesterol and unsaturated lipid than can be understood. Besides this theoretical caveat,

there is also little experimental evidence for the existence of such complexes.

2. Phenomenological model

We took a different approach to this problem. In our view, the lipids are of sufficient size and complexity that interactions between them cannot simply be encapsulated by their concentrations, but the order of their tails has also to be considered, as in earlier models [3, 9]. We reasoned that the essential physics of the system is as follows. First, the driving force of the phase separation is the fact that the disordered tails of the unsaturated lipid (or the diPhyPC) do not pack well with the relatively well-ordered tails of the saturated lipids, which leads to an effective repulsion between them. The strength of this repulsion should increase with increasing order of the saturated tails. At the temperature of the experiments, however, this repulsion is insufficient to drive phase separation in the binary system without cholesterol. The effect of adding cholesterol to a system of lipids is well-known [10–12]: because of its relatively rigid planar rings, cholesterol packs well with saturated chains and, in the liquid phase, increases their order. This increase in order of the saturated chains will increase the repulsion between them and the unsaturated chains, and will lead to phase separation. These ideas were encapsulated in a phenomenological model [13] which reproduces the closed-loop phase diagram seen experimentally. The point of the present paper is to describe a simpler version of this model in order to further highlight the basic physics.

We restrict the order parameter describing the chains of the saturated lipid to two values only, as in earlier work of others [3], so that the saturated lipids can be divided into those which are more ordered, with a concentration s_o, and less ordered, with a concentration s_d. This is in contrast to our previous formulation [13] in which the order parameter could take on a continuum of values. The concentrations of cholesterol and unsaturated lipids are denoted c and u with $u = 1 - s_o - s_d - c$. The free energy per particle of the system, in units of $k_B T$, is

$$\begin{aligned} f(s_d, s_o, c) &= s_o \ln s_o + s_d \ln s_d + c \ln c \\ &+ (1 - s_o - s_d - c) \ln(1 - s_o - s_d - c) \\ &+ J_d s_d (1 - s_o - s_d - c) + J_o s_o (1 - s_o - s_d - c) \\ &- J_{sc} s_o c. \end{aligned} \tag{1}$$

The first four terms are simply the entropy of mixing of the components. The next two terms, with $J_d < J_o$, express the idea that the repulsion between the unsaturated lipids and saturated lipids is greater if the tails of the latter are more ordered. Finally the last term expresses the tendency of cholesterol to increase the number of saturated lipids with relatively well-ordered tails. Because the two kinds of saturated lipid can be converted into one another, their chemical potentials must be equal:

$$\frac{\partial f}{\partial s_o} = \frac{\partial f}{\partial s_d}.$$

This is an equation which, together with $s_o + s_d = s$, the total concentration of saturated lipids, determines s_o and s_d:

$$s_d(s, c) = \frac{1}{1 + \exp(-\{(J_o - J_d)(1 - s - c) - J_{sc} c\})} s, \tag{2}$$

$$s_o(s, c) = \frac{\exp(-\{(J_o - J_d)(1 - s - c) - J_{sc} c\})}{1 + \exp(-\{(J_o - J_d)(1 - s - c) - J_{sc} c\})} s. \tag{3}$$

These expressions encapsulate the expected effects of the various interactions. As the strengths of the unfavorable interactions between saturated and unsaturated lipids, J_o and J_d, increase, with $J_d < J_o$, the fraction of saturated lipids which are ordered decreases. Further, as the strength of the attractive interaction between cholesterol and those saturated lipids which are ordered, J_{sc}, increases, the fraction of saturated lipids which are ordered increases. Note that when these expressions are substituted in equation (1) for the free energy, the ostensibly binary interactions between saturated lipids and other components become effective multi-particle interactions. In particular, when s_o and u are both small, equation (3) yields

$$s_o \approx \frac{1}{2} s + \frac{J_{cs}}{4} cs$$

so that the interaction term $J_o s_o u$ in the free energy becomes

$$\frac{J_o}{2} su + \frac{J_o J_{sc}}{4} csu.$$

The second term is an effective three-particle repulsive interaction which encourages phase separation in the central part of the Gibbs triangle where the three components have equal concentrations. It has often been noted that this is precisely the region where phase separation is most often observed to occur. Note also that in this theory the strength of this effective three-particle interaction depends on the product of the strengths of the repulsion between unsaturated and ordered saturated lipids, and the attraction between cholesterol and ordered saturated lipids. This is an explicit realization of the basic ideas of the theory. A phase diagram resulting from this theory is shown in figure 1.

3. The effect of cross-linking

The advantage of simple, phenomenological, theories, such as the one we formulated earlier [13] and the even simpler one presented above, is that they can often be applied easily to related problems of the same system. This is the case here. The observations are as follows.

It is a commonplace in biology that a complex series of interactions—that is, a pathway—is triggered by the cross-linking of proteins. Several examples of signaling processes which are triggered this way are presented by Simons and Toomre [14]. The question arises as to whether this cross-linking plays any role in the formation of rafts which, to quote the above article, provide ‘a new micro-environment, where the phosphorylation state can be modified by local kinases and phosphatases, resulting in downstream signaling’.

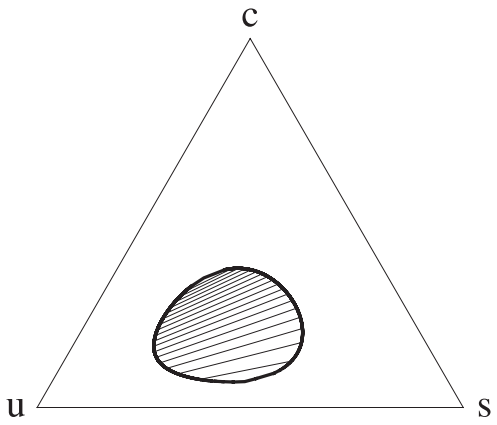

Figure 1. Phase diagram of the ternary mixture at a temperature above that of the gel transition of the pure saturated lipid. The interaction parameters which produce this diagram are $J_d = 1.6$, $J_o = 2.2$ and $J_{sc} = 4.5$.

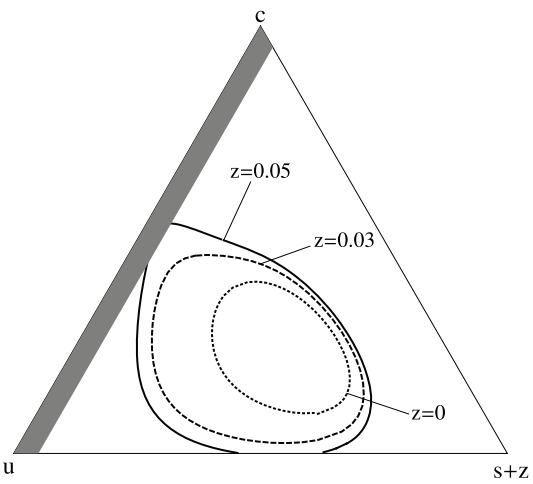

Figure 2. Effect of different concentrations of the same linker, which binds five saturated lipids, on the boundary of liquid–liquid coexistence. The dotted line shows this boundary in the absence of any cross-linkers, while the dashed and solid lines show the boundaries for concentrations of saturated lipid within linked aggregates, denoted z, of 0.03 and 0.05. In the latter case, the total composition of all saturated lipids, $z + s$ where s is the concentration of those not in aggregates, cannot be less than 0.05, so that region is shown shaded. Reproduced from [17].

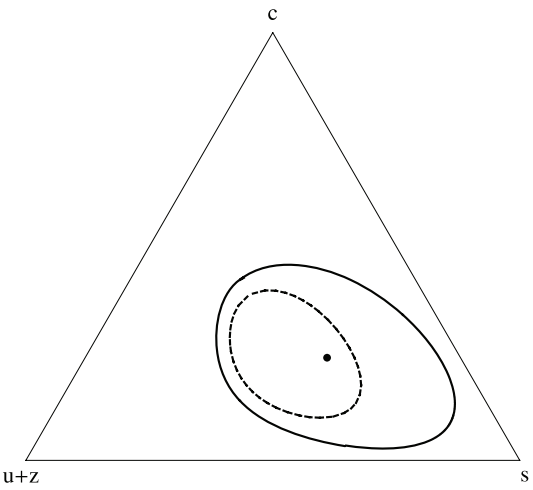

Figure 3. Effect of dimerizing unsaturated lipids on the phase boundary of the system. The dashed line shows the boundary of one-phase stability for no dimerizers, while the solid line is for a concentration of $z = 0.03$ of dimerized unsaturated lipids. The particular composition of $s = 0.49$, $u = 0.27$ and $c = 0.24$ noted in the text is shown with a dot. Reproduced from [17].

To investigate the effect of cross-linking in the absence of proteins, experiments were carried out in which lipids themselves were cross-linked. In one [15], lipids which are usually thought to be components of a raft (those which we have denoted 'saturated' before), such as monosialotetrahexosylganglioside (GM_1), were cross-linked into groups of five by cholera toxin. In another [16], phosphatidylinositol 4,5 biphosphate (PIP2), a lipid which is not thought to be a raft component (like those which we have denoted 'unsaturated' before), was cross-linked into oligomers by actin. In both cases, phase separation into liquid-ordered and -disordered phases was induced at compositions which formerly exhibited a single phase.

This effect could be explained quite simply, we believed [17]. Phase separation is promoted by energetic considerations, while it is opposed by entropic ones, particularly by the entropy of mixing which is maximized in a one-phase region. Cross-linking reduces the entropy of mixing, and therefore promotes phase separation. To see whether cross-linking some raft-associated lipids into groups of five would have a significant effect on the phase behavior, we utilized the earlier formulation [13] of the phenomenological model and considered the ordered lipids to be of two kinds: those within cross-linked aggregates, and those not. The entropy of the lipids in the aggregates was reduced by a factor of five because, being confined, they are not free to interchange with other lipids. The interactions of the lipids were unaffected. We found the effect of even a small concentration of such aggregates to be quite striking. It is shown in figure 2, which is reproduced from our previous work [17], which the interested reader can consult for specifics of the form of the interactions and their strengths. Note that the effect of cross-linking the raft-associated lipids is greatest in expanding the region of phase-coexistence into what was previously a region of liquid-disordered phase.

The mechanism we have employed should be equally effective were non raft-associated lipids to be cross-linked, and that is indeed the case. We show in figure 3, reproduced from our previous work, the effect of simply dimerizing such lipids. Note the analogous result that the cross-linking of non-raft-associated lipids has the greatest effect in expanding the region of coexistence into what was previously a region of liquid-ordered phase. In particular, consider the composition $s = 0.49$, $u = 0.27$, and $c = 0.24$, a point shown with a dot in figure 3. Before cross-linking, the concentrations of the unsaturated lipid in the liquid-ordered and -disordered

phases were 0.21 and 0.47, respectively, so that the partition coefficient of these lipids into the liquid-ordered phase was $K_u = 0.21/0.47 = 0.45$. After dimerization of an amount of unsaturated lipids corresponding to only 3 mol% of the total mixture, the concentration of all unsaturated lipids, dimerized or not, in these phases is now 0.17 and 0.54, so that the partition coefficient is reduced to $K_u = 0.17/0.54 = 0.31$. Thus the liquid-ordered phase is now poorer in unsaturated lipids and richer in saturated ones and cholesterol.

The picture that emerges is as follows. The membrane can be in a one-phase region. Proteins have associated with them a small amount of lipids. The cross-linking of the proteins, and the effective cross-linking of their lipids, can trigger raft-formation. The difference in areal densities between the rafts and the sea would then promote the aggregation of other proteins into one region or the other thereby affecting the efficacy of the function these proteins fulfill. In this way, simple physical organization leads to functional organization.

There is one further point which we would like to make. The compositions of the cytoplasmic and exoplasmic leaves of the plasma membrane are not at all similar. Unlike the outer leaf, the inner leaf has few saturated lipids. Therefore it is likely that the difference in lipid concentrations in the 'raft' and the 'sea' would be small in this leaf, even if it were large in the outer leaf to which it is coupled [18, 19]. Were this the case, such a raft would not be very useful as the small composition difference in the inner leaf would make it difficult for any chain which anchors there to distinguish one region from another [20]. Further, it might cause the coupled system to be in a one-phase region even if the outer leaf would, by itself, prefer to phase separate [19, 21–23]. As we have seen, however, even the cross-linking of proteins associated with the unsaturated lipids could trigger phase separation, a separation which occurs in both leaves, and thereby could bring about a functionally useful raft where there was none previously.

This problem illustrates the utility of minimal phenomenological models, such as the one we have presented here, in that it is relatively straightforward to extend the model in order to describe the asymmetric bilayer and to determine the effect of such asymmetry on the ability of the system to undergo phase separation when constrained to realistic biological compositions. Additional components, such as proteins or their anchors, can also be incorporated so that the effect of cross-linking such proteins directly can be addressed. Further the addition of a squared gradient term in the usual manner also permits the description of interfaces between the phases and their free energies [24].

Of course some degree of simplicity can always be ceded to a fuller description of the components if that is deemed necessary to address particular issues. For example, the effect of different chain architectures cannot be addressed unless a description of that architecture is included in the model. Several attempts along these lines have been made ranging from those in which the order is described by the usual nematic order parameter, S, averaged over the entire length of the lipid chain [25, 24] to those in which the order is calculated at discrete positions all along the chain [26, 27].

4. Conclusion

In summary, one sees that the fundamental physics that underlies lipid membrane rafts and their formation can be brought into sharp relief by relatively simple theoretical modeling that highlights the principles involved. Because of their simplicity, one can build upon such models to incorporate additional features which might only be obscured by more inclusive, detailed, approaches.

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