

The Effect of Solutes on the Temperature of Miscibility Transitions in Multicomponent Membranes

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ABSTRACT We address questions posed by experiments that show small-chain alcohols reduce the miscibility transition temperature when added to giant plasma membrane vesicles (GPMVs), but increase that temperature when added to giant unilamellar vesicles. In giant unilamellar vesicles the change in temperature displays a definite minimum, between decanol and tetradecanol, as a function of alcohol chain length; in GPMVs there is no such minimum. To emphasize the competition between internal entropies of the components and the interactions between them, we model the system as consisting of three different linear polymers. Two of them are the constituents of a liquid, one that can undergo a miscibility transition. To this liquid is added the third polymer component, which represents the short-chain alcohol. We show that, within Flory-Huggins theory, the addition of alcohol causes an increase or decrease of the miscibility transition temperature depending upon the competition of two effects. The first is the dilution of the interactions between the two components of the liquid caused by the introduction of the alcohol. This tends to lower the transition temperature. The second effect is the preferential partitioning of the alcohol into one phase of the liquid or the other. This tends to raise the transition temperature irrespective of which phase the alcohol prefers. This second effect is the smallest, and the decrease in transition temperature the largest, when the alcohol partitions equally between the two phases. Such equal partitioning occurs when the effect of the entropic excluded volume interactions (which cause the alcohol to prefer one phase) just balances the effect of the direct interactions, which cause it to prefer the other. These results allow us to make several predictions, and to propose an explanation for the different behavior of the transition temperature in GPMVs and giant unilamellar vesicles that results from the addition of alcohols.

INTRODUCTION

A long-accepted means of interrogating the properties of a bilayer membrane is to add to it a molecule whose properties are well understood. In this spirit, a series of experiments were carried out in which *n*-alcohols, with chain length $2 \leq n \leq 16$, were introduced into giant plasma membrane vesicles (GPMVs) (1,2). These cell-derived vesicles consist of a large number of different lipid components as well as proteins. They undergo a miscibility transition (3) into two distinct liquid phases: one phase of larger areal density and rich in well-ordered, saturated lipids, the liquid-ordered (lo) phase; and the other of smaller areal density and rich in less-ordered, unsaturated lipids, the liquid-disordered (ld) phase. The addition of alcohols with $n \leq 10$ caused a decrease in the miscibility transition temperature, as shown in Fig. 1.

However, when these same alcohols were introduced into giant unilamellar vesicles (GUVs) composed of only three components, the unsaturated lipid dioleoylphosphatidylcholine (DOPC), the saturated lipid dipalmitoylphosphatidylcholine (DPPC), and cholesterol, the transition temperature increased (4,5) for all of them except $n = 10$ and 14, as shown in Fig. 2. Furthermore, in the GUVs, the dependence of the transition temperature on alcohol chain length showed a definite minimum between decanol, $n = 10$, and tetradecanol, $n = 14$. In the GPMVs no such minimum occurs on increasing the alcohol chain length. In both systems, the longest chain alcohol, hexadecanol $n = 16$, caused the transition temperature to increase. We would like to understand the difference in behavior between the two systems. Following the nomenclature of Landau and Lifshitz (6), we shall refer to the alcohol as a “solute,” and the multicomponent fluid membrane to which it is added as the “solvent.”

It is a rather old problem to predict the effects of an additional component on a system that can, by itself, undergo a

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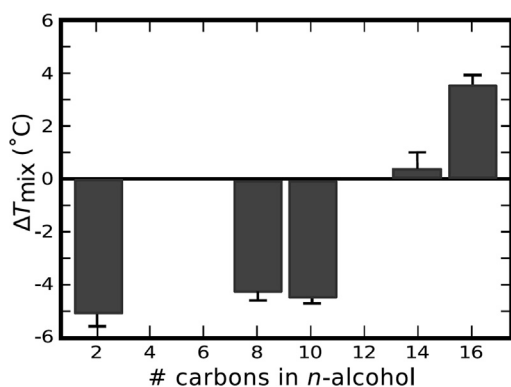


FIGURE 1 Change in transition temperature upon the addition of *n*-alcohol to GPMVs. Concentrations of solute are all equal to a fiducial given in Pringle et al. (23). Figure is from Cornell et al. (4).

miscibility transition. Over 50 years ago, Prigogine and Defay devoted a chapter to it in their volume “Chemical Thermodynamics” (7). By examining a regular solution of two components without any internal structure to which a third structureless component was added, they concluded the following: 1) “Hence the introduction of a third component which is equally soluble in the first two components will lower the critical solution temperature...” and 2) “Hence the addition of a third component which is much less soluble in one of the first two components than in the other, will always raise the critical solution temperature....”

Two schematic phase diagrams illustrating the two possible behaviors of the transition temperature upon the addition of a solute, its increase or decrease, are shown in Fig. 3, *a* and *b*. Here the solvent consists of two components, *A* and *B*, and a solute *S* is added to it. In the absence of the solute, the solvent undergoes a miscibility transition which is everywhere of first order except at the critical point at temperature T_c . The addition of solute causes the critical point to be drawn out into a line of critical transitions in which the critical temperature depends upon the amount

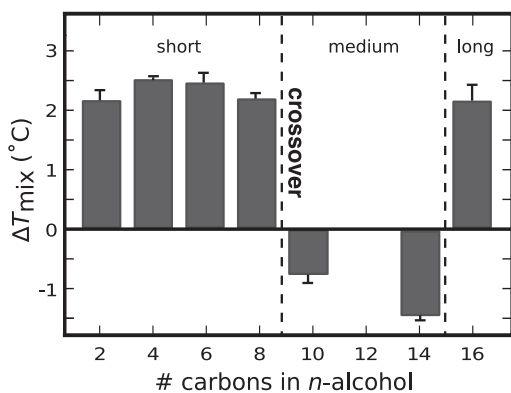


FIGURE 2 Change in transition temperature upon the addition of *n*-alcohol to a GUV composed of mol fractions 35:35:30 DOPC/PPC/cholesterol. Concentrations of solute are all three times a fiducial given in Pringle et al. (23). Figure is from Cornell et al. (4).

of solute. Fig. 3 *a* depicts the case in which the initial addition of solute causes the transition temperatures to increase, and Fig. 3 *b* the case in which the transition temperature decreases.

The statements of Prigogine and Defay (7) were recently made more quantitative by the simulation of a simple Ising, or lattice-fluid, model of a liquid (8). It was composed of two components without internal structure, a liquid displaying a miscibility transition. To this two-component solvent was added a solute, a third structureless component. Because of the simplicity of the Ising lattice-fluid model, the behavior of the transition temperature depended upon a single parameter, the relative strength of the difference of the interactions between solute and solvent components compared to the interaction between solvent components themselves. Because of the symmetry of the lattice-fluid model, the entropies of the two coexisting phases are equal. The lack of internal entropy of the components, and the equality of the entropies of the coexisting phases, are not characteristic of biological lipid components nor of their phases (9,10). In particular, the *ld* phase, rich in unsaturated lipids, is more disordered than the *lo* phase, and therefore has a larger entropy per particle (10). That the difference in entropy of the coexisting phases is of importance was noted by one of us (11). It was observed that in a one-component system, it is well known (6) that the temperature of a transition, such as between gel and fluid, is decreased if a solute partitions preferentially into the phase with greater entropy. The behavior of this transition temperature upon the entropy difference of the two phases is in marked contrast to that of the miscibility transition temperature of the lattice fluid. The behavior of that transition temperature depends only on whether the solute interactions prefer one phase or the other, but not on which phase it prefers.

Note that the simplicity of a lattice-fluid model does not permit it to address the effects of the internal entropy of components of the system. Likewise, the simplicity of a transition in a model system of a one-component solvent does not permit it to address the effects of the difference of interactions between solute and different components of a solvent.

It is the purpose of this article to illuminate the combined effects of the internal entropies of all components, membrane and solute, and of their mutual interactions on the behavior of a miscibility transition temperature of a multi-component solvent as solute is added. We do this by considering the undiluted membrane as composed of two components. Component *A* represents the unsaturated lipids and those constituents of the experimental systems that prefer the *ld* phase, whereas component *B* represents the saturated lipids and constituents that prefer the *lo* phase. We treat components *A* and *B* as linear polymer chains with polymerization indices N_A and N_B . The solute is considered as a third component of the diluted membrane and is treated as a polymer chain of index N_S . The physical interpretation

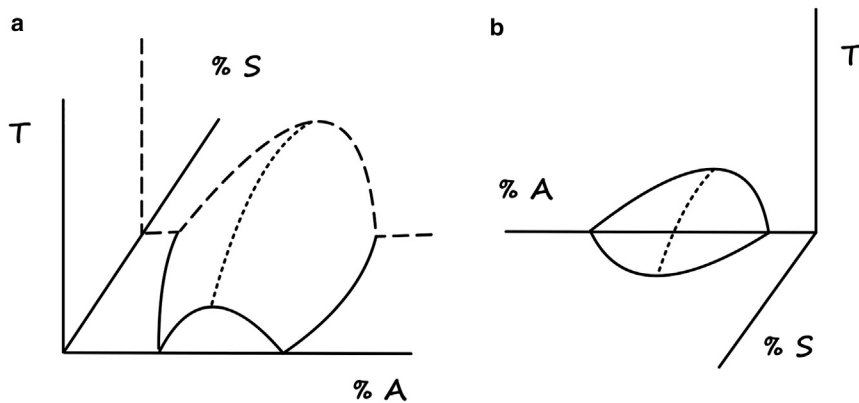


FIGURE 3 Schematic phase diagram in the space of temperature, T , and compositions of the solvent component A and solute S . (a) Given here is an illustration of the case in which addition of solute raises the miscibility transition temperature. One is looking in the direction of increasing solute concentration. The line of critical points is shown with short dashes. The nearest plane to the viewer is the plane of zero solute fraction. The longer dashed lines indicate a plane at some nonzero solute concentration. (b) The case in which addition of solute lowers the transition temperature is shown. One is looking in the direction of decreasing solute concentration. The vertical plane is that of zero solute concentration.

of the parameters N_A , N_B , and N_S is that they each represent the hydrophobic volume of an average molecule of the respective component. Note that these parameters involve a variety of factors including the lengths of the chains of the lipids, the degree of order of the acyl chains of the lipids, and an average over all the molecular species that the component represents. We do not attempt to refine the model by developing formulae relating these factors to the polymerization indices, but rather treat them as empirical parameters. To be definite, we shall take $N_A > N_B$. We show in [Appendix I: Relative Entropies of the Coexisting Phases](#) that, as a consequence, near the critical transition temperature, the A -rich phase II has a greater entropy per volume than the B -rich phase I. Therefore, the A -rich phase can be thought of as representing the experimental l_d phase, and the B -rich as the l_o phase.

To illuminate the behavior of the temperature of the general miscibility transition, we consider the behavior of the critical temperature, which is calculated within mean-field theory as a function of the solute volume fraction. For small volume fractions, comparable to those in the experiments noted above, we find that the behavior of the miscibility transition temperature as a function of solute volume fraction results from a competition between two tendencies. The first is simply that the introduction of any solute reduces the number of solvent-solvent interactions, and therefore lowers the transition temperature. The second tendency is essentially that noted by Prigogine and Defay (7): if the solute prefers one phase to the other, that preference will tend to increase the transition temperature. However, the magnitude of this increase depends not only upon the difference in the direct interactions between the solute and the solvent components, but also upon the intrinsic entropies of the membrane components and of the solute—that is, on excluded volume interactions. This tendency to increase the transition temperature is minimal, in general, when the solute partitions equally between the two coexisting phases. This occurs when the solute interactions favoring one phase just balance the entropic, or the excluded volume interactions, which favor the other. With the aid of our results,

we are able to propose an explanation for the difference in the behavior of the transition temperature in GPMVs and GUVs. Further, by combining them with previous results for the partitioning of single, saturated, chains in l_o and l_d phases (12), we are able to provide an explanation for the pronounced minimum in the miscibility transition temperature of the GUVs as a function of alcohol chain length.

METHODS

The model

We consider an incompressible membrane at temperature T composed of n_A molecules of component A and n_B molecules of component B . We treat the components as linear polymers with polymerization indices N_A and N_B . To this membrane, we add n_S molecules of a solute, also treated as a linear polymer with polymerization index N_S . Because the system is incompressible, its volume, Ω , is not a thermodynamically independent variable, but is related to the number of molecules of the components according to

$$\Omega(n_A, n_B, n_S) = v_0(n_A N_A + n_B N_B + n_S N_S), \quad (1)$$

where v_0 is the monomer volume of A , B , or S chains, volumes that are assumed to be equal.

In mean-field, or Flory-Huggins, approximation (13), the Helmholtz free energy of the system, F , can be written

$$\begin{aligned} F(T, n_A, n_B, n_S) = & V_{AB} n_A N_A \frac{n_B N_B v_0}{\Omega} + V_{AS} n_A N_A \frac{n_S N_S v_0}{\Omega} \\ & + V_{BS} n_B N_B \frac{n_S N_S v_0}{\Omega} \\ & + k_B T \left[n_A \ln \left(\frac{n_A N_A v_0}{\Omega} \right) \right. \\ & \left. + n_B \ln \left(\frac{n_B N_B v_0}{\Omega} \right) + n_S \ln \left(\frac{n_S N_S v_0}{\Omega} \right) \right], \end{aligned} \quad (2)$$

where k_B is Boltzmann's constant. The energy V_{AB} is related to the interaction energy between pairs of A monomers, \tilde{V}_{AA} , pairs of B monomers, \tilde{V}_{BB} , and AB pairs, \tilde{V}_{AB} , according to

$$V_{AB} = \tilde{V}_{AB} - \frac{1}{2}(\tilde{V}_{AA} + \tilde{V}_{BB}), \quad (3)$$

and similarly for V_{AS} and V_{BS} . From the free energy, the chemical potentials of the three components are obtained:

$$\mu_\alpha = \frac{\partial F(T, n_A, n_B, n_S)}{\partial n_\alpha}, \quad \alpha = A, B, S. \quad (4)$$

The chemical potentials are functions of temperature and two other intensive quantities. It is convenient to take them to be the volume fractions of the A and S components

$$\begin{aligned} \Phi_A &\equiv \frac{n_A N_A}{n_A N_A + n_B N_B + n_S N_S}, \\ \Phi_S &\equiv \frac{n_S N_S}{n_A N_A + n_B N_B + n_S N_S}. \end{aligned} \quad (5)$$

The volume fraction of the B component is then $\Phi_B = 1 - \Phi_A - \Phi_S$.

Solution of the model

Given a net repulsive interaction, $V_{AB} > 0$, between the solvent components, there will be a transition from one uniform phase to two coexisting phases, I and II, below some critical temperature. The equations that determine the volume fractions of the components in the coexisting phases are

$$\begin{aligned} \mu_A(T, \Phi_A^I, \Phi_S^I) &= \mu_A(T, \Phi_A^{II}, \Phi_S^{II}), \\ \mu_B(T, \Phi_A^I, \Phi_S^I) &= \mu_B(T, \Phi_A^{II}, \Phi_S^{II}), \\ \mu_S(T, \Phi_A^I, \Phi_S^I) &= \mu_S(T, \Phi_A^{II}, \Phi_S^{II}). \end{aligned} \quad (6)$$

These three equations in five unknowns determine the surface of coexistence $T(\Phi_A^I, \Phi_S)$, $\Phi_A^{II}(\Phi_A^I, \Phi_S^I)$, and $\Phi_S^{II}(\Phi_A^I, \Phi_S^I)$. From this surface, we obtain the slope of the tie lines

$$M \equiv \frac{\Phi_S^{II} - \Phi_S^I}{\Phi_A^{II} - \Phi_A^I}, \quad (7)$$

which gives the partitioning of the solute into the coexisting phases normalized by the difference in volume fraction of component A in the two phases. As a purely notational choice, we arbitrarily let phase II be such that $\Phi_A^{II} \geq \Phi_A^I$, i.e., phase II is the A -rich phase.

Having obtained the surface of coexistence, we have arrived, in principle, at the answer to our question of how the addition of a solute affects the miscibility transition of a multicomponent membrane. But it is certainly clearer, and more informative, to consider not the entire surface of coexistence, at which the transitions are generally of first order, but rather to focus on the critical line in this surface at which the transitions become continuous (see Fig. 3, a and b). The locus of the critical line is a function of the temperature and solute volume fraction: $T_c(\Phi_S)$ and $\Phi_{A,c}(\Phi_S)$. Although experimental paths do not, in general, pass through the critical line, and do so only if great care is taken to that end (14), nevertheless the behavior of the critical line with solute fraction illustrates the general behavior of the coexistence surface on the addition of solute. Furthermore, the causes of its behavior are far more transparent.

The critical volume fractions, $\Phi_{A,c}(\Phi_S)$, the values at which the difference in the properties of the two coexisting phases just vanish, are obtained from the nontrivial solution of the two homogeneous equations in two unknowns:

$$\lim_{\Phi_A^I \rightarrow \Phi_{A,c}, \Phi_S^I \rightarrow \Phi_S} \Phi_A^{II}(\Phi_A^I, \Phi_S^I) - \Phi_A^I = 0, \quad (8)$$

$$\lim_{\Phi_A^I \rightarrow \Phi_{A,c}, \Phi_S^I \rightarrow \Phi_S} \Phi_S^{II}(\Phi_A^I, \Phi_S^I) - \Phi_S^I = 0. \quad (9)$$

From this line of critical volume fractions, the critical temperature is obtained as a function of solute volume fraction, $T_c(\Phi_S) = T(\Phi_{A,c}(\Phi_S), \Phi_S)$.

To obtain the properties of the critical line, we expand Eq. 6 in the small parameters $\Phi_A^{II} - \Phi_A^I$ and $\Phi_S^{II} - \Phi_S^I$. If we keep only terms in linear order, the resulting equations are not independent, but yield the two linear homogeneous equations, Eqs. 8 and 9, noted above. Setting the determinant of these equations to zero, we obtain the following expression for the critical temperature in terms of the unknown critical volume fraction, $\Phi_{A,c}$, and the given solute volume fraction Φ_S ,

$$\begin{aligned} k_B T_c &= V_{AB} \frac{\beta + (\beta^2 + 4\alpha\gamma)^{1/2}}{2\alpha}, \quad \text{where} \\ \alpha &\equiv \frac{1}{N_A \Phi_{A,c}} \left[1 + \frac{N_S \Phi_S}{N_B (1 - \Phi_S)} \right] + \frac{1}{N_B (1 - \Phi_{A,c} - \Phi_S)} \\ &\times \left[1 + \frac{N_S \Phi_S}{N_A (1 - \Phi_S)} \right], \\ \beta &\equiv 2 \left[1 + \frac{N_S \Phi_S V_{BS}}{N_A \Phi_{A,c} V_{AB}} + \frac{N_S \Phi_S V_{AS}}{N_B (1 - \Phi_{A,c} - \Phi_S) V_{AB}} \right], \\ \gamma &\equiv N_S \Phi_S \left[1 - 2 \frac{(V_{AS} + V_{BS})}{V_{AB}} + \left(\frac{V_{AS} - V_{BS}}{V_{AB}} \right)^2 \right]. \end{aligned} \quad (10)$$

We also obtain from the nontrivial solution of the homogeneous equations the limiting slope, M_c , of the tie line at the critical point. It, too, is expressed in terms of the unknown critical volume fraction, $\Phi_{A,c}$ and the given solute volume fraction Φ_S :

$$\begin{aligned} M_c &= -N_S \Phi_S \\ &\times \frac{k_B T_c - N_B (1 - \Phi_{A,c} - \Phi_S) (V_{AB} + V_{BS} - V_{AS})}{[k_B T_c - 2N_S \Phi_S V_{BS}] N_B (1 - \Phi_{A,c} - \Phi_S) + k_B T_c N_S \Phi_S}. \end{aligned} \quad (11)$$

Finally, if we include in our expansion of Eq. 6 the terms of third order in the small expansion parameters, we recover a third independent equation,

$$0 = \frac{1}{N_A \Phi_{A,c}^2} - \frac{(1 + M_c)^3}{N_B (1 - \Phi_{A,c} - \Phi_S)^2} + \frac{M_c^3}{N_S \Phi_S^2}. \quad (12)$$

Equations 10–12 determine $\Phi_{A,c}(\Phi_S)$ and $M_c(\Phi_S)$, and $T_c(\Phi_S)$.

RESULTS

We first consider the critical concentration, $\Phi_{A,c}(0)$ and critical temperature, $T_c(0)$, in the limit of no solute, $\Phi_S \rightarrow 0$. Clearly, $M_c(0) = 0$. Following the above procedure, we obtain for the critical concentration of the A component, the result (13)

$$\Phi_{A,c}(0) = \frac{\sqrt{N_B}}{\sqrt{N_A} + \sqrt{N_B}}, \quad (13)$$

and find the critical temperature to be given by

$$2 \frac{V_{AB}}{k_B T_c(0)} = \left(\frac{1}{\sqrt{N_A}} + \frac{1}{\sqrt{N_B}} \right)^2. \quad (14)$$

In the symmetric case for which $N_A = N_B \equiv N$, these results reduce to $\Phi_A = \Phi_B = 1/2$ and $NV_{AB}/k_B T_c(0) = 2$. (In the polymer literature, $V_{AB}/k_B T$ is denoted χ so that this relation is written $\chi_c N = 2$, a well-known result (13).)

Now let the solute be introduced. With the interactions between solute and the solvent components being nonzero, we find it convenient to characterize them by the average interaction, normalized by V_{AB} and the difference in the interactions, again normalized:

$$\begin{aligned} r &\equiv \frac{V_{AS} + V_{BS}}{2V_{AB}}, \\ \delta r &\equiv \frac{V_{AS} - V_{BS}}{V_{AB}}. \end{aligned} \quad (15)$$

We also introduce

$$\delta\nu \equiv \frac{\sqrt{N_A} - \sqrt{N_B}}{\sqrt{N_A} + \sqrt{N_B}}. \quad (16)$$

We now solve the equations for the critical temperature in a power series in the solute volume fraction and obtain

$$M_c = -\frac{N_S V_{AB}}{k_B T_c(0)} (\delta\nu + \delta r) \Phi_S + O(\Phi_S^2), \quad (17)$$

$$\begin{aligned} \frac{\Phi_{A,c}(\Phi_S) - \Phi_{A,c}(0)}{\Phi_{A,c}(0)} &= -\left\{ 1 - \frac{N_S}{4} \left(\frac{1}{N_B} + \frac{1}{\sqrt{N_A N_B}} \right) \right. \\ &\quad \times (\delta\nu + \delta r) \left[3 - \frac{N_S V_{AB}}{2k_B T_c(0)} \right. \\ &\quad \left. \left. \times (\delta\nu + \delta r)^2 \right] \right\} \Phi_S + O(\Phi_S^2), \end{aligned} \quad (18)$$

$$\begin{aligned} \frac{T_c(\Phi_S) - T_c(0)}{T_c(0)} &= c_1 \Phi_S + c_2 \Phi_S^2 + O(\Phi_S^3), \\ c_1 &= -1 + \frac{N_S V_{AB}}{2k_B T_c(0)} (\delta\nu + \delta r)^2, \\ c_2 &= -\frac{N_S^2}{4} \left(\frac{V_{AB}}{k_B T_c(0)} \right)^2 (\delta\nu + \delta r)^2 \left\{ [1 - 4r + (\delta r)^2] \right. \\ &\quad \left. + \frac{1 - (\delta\nu)^2}{4} c_1^2 \right\}. \end{aligned} \quad (19)$$

These three equations present the shift, on the addition of solute, of three observable quantities at criticality: the slope of the tie line, the volume fraction of the A component, and the transition temperature. From the result of Eq. 17 for the first of these quantities, we see that if $\delta\nu + \delta r$ is positive, then M_c will be negative. But M , from its definition in

Eq. 7, gives the partitioning of the solute. In particular, we see that if M is negative, the solute partitions preferentially into phase I, the B -rich phase. It is clear that if $\delta\nu$ is positive, as we have taken it to be, then the excluded volume interactions favor the solute to leave the A -rich phase of greater entropy and favor the B -rich phase where there are fewer excluded volume interactions. The combination $\delta\nu + \delta r$ appears in our calculation as a natural extension of the difference in direct interactions to a difference in direct and excluded volume interactions:

$$\begin{aligned} \delta\nu + \delta r &= \frac{\sqrt{N_A} - \sqrt{N_B}}{\sqrt{N_A} + \sqrt{N_B}} + \delta r \\ &= \frac{(\sqrt{N_A} - \sqrt{N_S}) - (\sqrt{N_B} - \sqrt{N_S})}{\sqrt{N_A} + \sqrt{N_B}} + \frac{V_{AS} - V_{BS}}{V_{AB}}. \end{aligned} \quad (20)$$

The change in $\Phi_{A,c}$, the volume fraction of component A at criticality, is a result of two effects. The first term in Eq. 3 is simply the effect of dilution; that is, as a volume fraction of solute is introduced, the volume fraction of the other components must decrease. The second term shows that if the solute prefers the B -rich phase, this will tend to increase the volume fraction of the A component at the critical point.

For the shift in the critical temperature due to the addition of solute, which is the principal result of this article, we note that for the concentrations employed in the experiments of interest, estimated on the order of 0.06 mol fraction (4), it is sufficient to keep in Eq. 3 only the term linear in Φ_S ,

$$\frac{T_c(\Phi_S) - T_c(0)}{T_c(0)} = \left[-1 + \frac{N_S V_{AB}}{2k_B T_c(0)} (\delta\nu + \delta r)^2 \right] \Phi_S. \quad (21)$$

From this expression, one sees that the effect of the solute on the temperature at criticality, just as on the concentration of component A at criticality, results from a competition between two terms. The first, $-\Phi_S$, is simply the reduction in the transition temperature due to the dilution of the number of AB interactions resulting from the introduction of the solute. This effect would be present no matter the nature of the solute. The second term, being positive, always tends to increase the transition temperature. It reflects, but modifies, the dictum of Prigogine and Defay (7) that "...the addition of a third component which is much less soluble in one of the first two components than in the other, will always raise the critical solution temperature..." Indeed if $\delta\nu + \delta r$ is sufficiently large in magnitude, and of either sign, then this term will cause an increase of the transition temperature. It is interesting that this effect depends on the magnitude of the difference, δr , between the solute interactions, but is independent of the strength of these interactions, encapsulated in the parameter r .

We note that the largest reduction of the critical transition temperature occurs when

$$\delta r + \delta\nu = 0, \quad (22)$$

that is, when the total interactions, direct and excluded volume, between solute and solvent components are equal in magnitude but opposite in sign. In other words, the entropic, excluded-volume, preference of the solute for one phase is just balanced by an energetic preference of the solute for the other. Furthermore, a comparison of this equation with Eq. 17 shows, as one expects, that when the total interactions of the solute, direct and excluded volume, is the same with the two components of the solvent, then the solute partitions equally between the coexisting phases. As a consequence, we conclude that the largest reduction in transition temperature occurs when the solute partitions equally between the coexisting phases. This is an extension of the result of Prigogine and Defay (7) obtained for structureless components.

In sum, we have shown that as a result of the addition of a small amount of solute into a two-component solvent, one which undergoes a miscibility transition, the effect of the solute on the critical transition temperature is the result of a competition between two tendencies. The first is that the addition of the solute simply dilutes the number of solvent-solvent interactions, and this tends to lower the transition temperature. The second arises from an unequal partitioning of the solute between the coexisting phases. Any such preference of the solute for one phase or the other tends to increase the transition temperature. Irrespective of whether the net result of the two effects is to increase or decrease the transition temperature, we have shown that the critical transition temperature depends quadratically upon the partitioning of the solute, attaining its minimum value when the solute partitions equally between the two phases. This equal partitioning occurs when the effect of the excluded volume interactions that favor the phase of lesser entropy is just balanced by the direct interactions favoring the other phase. Although our results have been directed to the critical transition temperature for clarity, we expect its behavior to also be exhibited by the temperature of the more commonly encountered first-order transitions.

DISCUSSION

Utilizing our results, we address the two questions raised by the experiments discussed earlier. 1) Why is the shift in temperature more often negative in the GPMVs and more often positive in the GUVs? 2) What is the reason for the pronounced minimum in the shift of transition temperature with alcohol chain length in the model-membrane GUVs of three components (Fig. 2), and why is there no such minimum in the cell-derived GPMVs of many lipid components (Fig. 1)?

Consider the first question. Our theory states that the tendency of the transition temperature to increase clearly depends upon the degree of preferential partitioning of the alcohol into one of the coexisting phases. Thus we interpret

the experimental data as indicating that the difference in the partitioning of the alcohol between phases is small in the cell-derived GPMVs. This in turn indicates that the difference between the coexisting phases in GPMVs is smaller than in GUVs. This agrees with what we know about the composition of the plasma membrane as compared to that of the model membranes. The inner leaf of the plasma membrane differs significantly in composition from the outer leaf, having only a small fraction of saturated sphingomyelin (15,16). Bilayers whose two leaves mimic those of the inner leaf of the plasma membrane do not undergo phase separation (17). Hence the compositions of the inner leaves of the coexisting phases in GPMVs are expected to differ only slightly. As the alcohol, with its small headgroup is expected to undergo rapid flip-flop between leaves just as cholesterol does (18), this lack of differentiation between the composition of the inner leaves will affect the preferential partitioning of alcohol between phases, reducing it from the value observed in model membranes with identical inner and outer leaves.

We now consider the second question, concerning the dependence of the transition temperature shift on alcohol chain length. There is a clear prediction from the theory that follows from Eqs. 17 and 21. If the shift in transition temperature were measured at the same solute concentration for a series of n -alcohols, then that shift would depend quadratically on the partitioning of the solute into the coexisting phases. Specifically, denoting the relative shift in transition temperature by $\Delta T \equiv [T_c(\Phi_s) - T_c(0)]/T_c(0)$, then a plot of $[1 + (\Delta T/\Phi_s)]^{1/2}$ versus the partitioning, M , would be linear. As ΔT , Φ_s , and the relative partitioning, M , are all measurable quantities, this prediction can be verified or falsified. In the cited experiments, however, the partitioning of the different alcohols was not measured. Nevertheless, the partitioning has been calculated for a closely related system, a series of acyl chains in a membrane composed of DPPC, DOPC, and cholesterol (10). The chains were described in detail by the rotational isomeric states model (19), but the headgroups were generic, not detailed. Thus the calculation does not describe n -alcohols specifically, but the results should indicate the effect on the partitioning of the differing chain lengths. The results for saturated chains, as well as for various unsaturated chains, are shown in Fig. 4. Here, if we neglect the difference between mol fractions and volume fractions, M must be proportional to $1 - X^{lo}/X^{ld}$.

From Fig. 4, we see that short, saturated, acyl chains with $n < 14$, partition preferentially into the ld phase. The partitioning, M , from its definition, Eq. 7, is positive when the solute partitions preferentially into the A-rich phase II, identified with the ld phase. As M is positive, we infer from Eq. 17 that $\delta\nu + \delta r$ must be negative. Because $\delta\nu$ is positive, δr must be negative and of a sufficient magnitude, i.e., $-\delta r > \delta\nu$. That δr is negative means direct solute-solvent interactions cause the solute to prefer the phase with

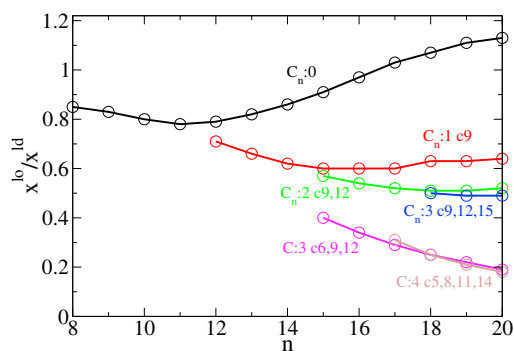


FIGURE 4 Ratio of the mol fractions in the lo phase to ld phase, $X^{\text{lo}}/X^{\text{ld}}$, for several kinds of single chains of length n in a system of DOPC/DPPC/cholesterol. $C_n:0$ denotes a chain of length n and no double bonds, whereas $C_n:1c9$ denotes a chain of length n with one double bond at the ninth position from the head of the chain, etc. The temperature is 290 K. Illustration is from Uline et al. (10). To see this figure in color, go online.

greater entropy, i.e., the A-rich phase II. Presumably this preference for the ld phase is due to the larger area per lipid headgroup in that phase providing more room for the added acyl chain.

As the length of the chain increases from 8 to 12, the partitioning into the lo phase decreases slightly, and increases slightly into the ld phase. It is plausible that this is due to the increasing disorder down the chains of the ld phase allowing additional room for the added acyl chain. For these values of n for which there is a definite preference of the acyl chain for the ld phase, we expect $\delta\nu + \delta r$ to be large and negative, i.e., $-\delta r \gg \delta\nu$. With increasing n , the excluded volume interactions between the added acyl chain and the DOPC chain increases due to the double bond of the latter. The favorable energy of interaction with the ordered chains of the DPPC also increases, so δr increases, i.e., becomes less negative. Insertion of the additional acyl chain into the lo phase is opposed, however, by the cholesterol, as some of the cholesterol would be displaced by the alcohol acyl chain (20,21). As a consequence of these various factors, there will be a range of n for which the alcohol partitions roughly equally into the two phases. In this range, $\delta\nu + \delta r$ is small. Eventually for sufficiently large n , on the order of 18, the added acyl chain partitions predominantly into the lo phase. For such values of n , $\delta\nu + \delta r$ is once again large, but now is positive.

Combining the results shown in Fig. 4 with those of our theory, we would expect that, for a given small amount of n -alcohol, the miscibility transition temperature would attain its minimum value as a function of n near 16. At the minimum value, we would predict that the alcohol decreases the transition temperature. The temperature will increase from that minimum for n either larger or smaller than 16. This behavior is qualitatively correct for GUVs as shown in Fig. 2. At the minimum, the shift in transition

is indeed negative. The minimum occurs near $n = 16$. Even the small maximum in the GUV transition temperatures for values of n between 2 and 8 is reflected in the small minimum of the partitioning of acyl chains at n of 11 in Fig. 4. (Recall that the change in transition temperature is proportional to the square of the relative partitioning, so that the minimum in partitioning leads to a maximum in temperature shift.) Again, the reason for the shift in partitioning of the alcohol from ld to lo phase with increasing alcohol chain length is the increasing repulsive interaction between the alcohol and unsaturated chains. This is the reasonable explanation proposed by Cornell and colleagues (4). Our results support it.

Why is this minimum in temperature shift with chain length not observed in GPMVs? As just noted, the largest decrease in transition temperature for a given solute concentration occurs when the solute partitions equally into the coexisting phases. This occurs in our theory when $-\delta r = \delta\nu$. For there to be a minimum in the temperature shift as a function of chain length, there must be short chains for which the solute partitions preferentially into the ld phase, i.e., $\delta r + \delta\nu < 0$, or $-\delta r > \delta\nu$. But δr is a measure of the difference between the interactions of solute with the constituents of the lo phase and those of the ld phase. As we observed above, the composition of the inner leaves in the lo and ld phases must be very similar in the GPMVs. The magnitude of δr is therefore expected to be much smaller in GPMVs than in GUVs. Hence, it is plausible that the condition $-\delta r > \delta\nu$ is not satisfied for any chain length, and the shift in transition temperature simply varies monotonically with chain length.

We note again that our proposed explanations for the experimental results could be confirmed, or not, if the partitioning of the alcohols into the coexisting phases exhibited by the GPMVs and GUVs were to be measured.

CONCLUSIONS

Based on our analysis encapsulated in Eq. 21, we can make several predictions concerning the effect of an additional component on the miscibility transition temperature of a multicomponent membrane. For example, we would predict that the addition of a given amount of any solute at all, independent of its nature, that partitions equally into the coexisting phases will reduce the miscibility transition temperature by the same fraction. We would predict, from Fig. 4, that acyl chains with double bonds would be much less effective in reducing the transition temperature than are n -alcohols as their preference for the ld phase is so much greater. Of course, any molecule that strongly prefers one phase to the other will tend to increase the transition temperature. There are many peptides that do this (22). If their partitioning were known, the same quadratic dependence of the transition temperature on that partitioning should be observed.

APPENDIX I: RELATIVE ENTROPIES OF THE COEXISTING PHASES

Let $N_A > N_B$. We note that, from Eqs. 1 and 2, in the absence of solute, the entropy per unit volume, S/Ω , is given by

$$\frac{Sv_0}{\Omega k_B} = - \left[\frac{\Phi_A}{N_A} \ln(\Phi_A) + \frac{(1 - \Phi_A)}{N_B} \ln(1 - \Phi_A) \right]. \quad (23)$$

The volume fraction Φ_A in the A-rich phase, phase II, can be written $\Phi_A = \Phi_{A,c} + \delta\Phi_A$ with $\delta\Phi_A > 0$ and $\Phi_{A,c}$ given by Eq. 13. Near the critical point, $\delta\Phi_A/\Phi_{A,c}$ is small, so the above can be expanded in this parameter. We obtain

$$\frac{(S^{II} - S^I)v_0}{\Omega k_B} \approx -2 \left[\frac{\ln(\Phi_{A,c}) + 1}{N_A} - \frac{\ln(1 - \Phi_{A,c}) + 1}{N_B} \right] \delta\Phi_A. \quad (24)$$

Finally setting $N_A = N + \delta N$, $N_B = N - \delta N$ with $\delta N > 0$, and expanding in $\delta N/N$, we obtain

$$\frac{(S^{II} - S^I)v_0}{\Omega k_B} \approx 2(3 - 2\ln 2) \frac{\delta N}{N^2} \delta\Phi_A \approx 3 \frac{\delta N}{N^2} \delta\Phi_A, \quad (25)$$

which is positive.

AUTHOR CONTRIBUTIONS

D.W.A. and M.S. contributed equally to this work.

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