Polymer-Induced Membrane Contraction, Phase Separation, and Fusion via Marangoni Flow

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ABSTRACT Experiments have shown that the depletion of polymer in the region between two apposed (contacting or nearly contacting) bilayer membranes leads to fusion. In this paper we show theoretically that the addition of nonadsorbing polymer in solution can promote lateral contraction and phase separation of the lipids in the outer monolayers of the membranes exposed to the polymer solution, i.e., outside the contact zone. This initial phase coexistence of higher- and lower-density lipid domains in the outer monolayer results in surface tension gradients in the outer monolayer. Initially, the inner layer lipids are not exposed to the polymer solution and remain in their original "unstressed" state. The differential stresses on the bilayers give rise to a Marangoni flow of lipid from the outer monolayers in the "contact zone" (where there is little polymer and hence a uniform phase) to the outer monolayers in the "reservoir" (where initially the surface tension gradients are large due to the polymer-induced phase separation). As a result, the low-density domains of the outer monolayers in the contact zone expose their hydrophobic chains, and those of the inner monolayers, to the solvent and to each other across the narrow water gap, allowing fusion to occur via a hydrophobic interaction. More generally, this type of mechanism suggests that fusion and other intermembrane interactions may be triggered by Marangoni flows induced by surface tension gradients that provide "action at a distance" far from the fusion or interaction zone.

INTRODUCTION

A fundamental understanding of the processes involved in bilayer fusion is important for the analysis of both synthetic (e.g., vesicular) dispersions and biological systems (e.g., cell adhesion and fusion, and tissue formation) (Arnold, 1995). Recent measurements of the interactions of two bilayers attached to mica surfaces have shown (Kuhl et al., 1996) that the presence of an aqueous solution of nonadsorbing polymer can promote fusion of the bilayers when they are in close proximity. Although it might be tempting to interpret this result in simple terms, as arising from the attractive, depletion force (Israelachvili, 1992) induced by the "crowding" of the polymer in solution as the distance between the two surfaces is decreased, one has to remember that adhesion does not automatically imply fusion. The fusion of two bilayers into one requires not only an attraction between the surfaces, but also the removal of the outer monolayers of each bilayer. In other words, fusion occurs between the two *inner* monolayers of each bilayer and there must therefore be a driving force for the *outer* monolayers to flow or diffuse away from the fusion zone. Once the density of the outer monolayer lipids is reduced (Helm et al., 1989) hydrophobic interactions are enhanced. Hydrophobic interactions between the inner monolayers in the contact zone along with any other attractive interactions such as depletion forces and van der Waals attractions can then complete the fusion process. In biological systems, one can imagine that this process can be repeated (via chemical gradients) on the inner monolayer, leading to the eventual removal of the fused inner monolayers and the complete unification of two cells.

In this paper we focus on the first, but crucial, step in the fusion process (sometimes denoted as hemifusion or semifusion (Arnold, 1995)) in which the outer monolayers flow away from the fusion zone. We explain theoretically how phase separation in the outer monolayers, induced by their interaction with the polymer, results in surface tension gradients in regions far from the fusion zone. The resulting Marangoni flow functions as a mechanism by which the outer monolayers of each bilayer are forced to leave the fusion zone. The theory is motivated by the polymer solution experiments (Kuhl et al., 1996); however, this is only one way to generate a Marangoni flow and our suggested fusion mechanism is universal in nature. Indeed, previous work (Chanturiya et al., 2000) on the effects of calcium in inducing vesicle fusion has shown that the fusion is sensitive to the rate of calcium addition and requires an asymmetry in the calcium concentration in the inner and outer monolayers; these experimental results led to the suggestion (Chanturiya et al., 2000) that an important contribution to membrane fusion is the change in tension in the outer monolayer of lipid vesicles (Leckband et al., 1993).

For now, we consider the specific case where the presence of polymer in solution induces phase separation and surface tension gradients in the outer monolayer that leads to Marangoni flow and eventually fusion. We consider two curved bilayers separated by the water/polymer solution.

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Each bilayer is composed of an inner and an outer monolayer; the outer monolayer faces the water/polymer solution, as shown in Fig. 1. The two bilayers are in close proximity in region B that is macroscopic in its lateral extent, and are macroscopically separated in region A. This is the relevant geometry in many biological situations and in the surface forces experiments where the spacing between the two bilayers, corresponding to region B, is controllable and can range from microns to several angstroms. If the interaction of the lipids with the polymer is repulsive (nonadhesive), this will cause the lipid headgroups to minimize their contact area with the solution and, in turn, to increase the lipid packing density. If both the area and the total number of lipid molecules are fixed, this effect can result in phase separation and in the coexistence of higher and lower density regions of lipid.

For simplicity, we assume, as in the surface forces experiments (Kuhl et al., 1996), that the area occupied by the inner lipid monolayer is fixed. [The area occupied by the outer layer of lipid in region A is fixed in the surface forces experiment because it is constrained by the underlying inner layer of lipid attached to the mica surfaces. In a closed vesicle, the area of the outer layer of lipid is similarly constrained by the closed, inner layer. The number of lipid molecules in region A is regarded as being fixed only at early times, before Marangoni flow occurs; once the resulting Marangoni flow begins, the total number of molecules is no longer fixed because lipid is transported from region B to region A. In addition, we assume that the amount of free lipid in solution is very small and that adsorption to the bilayer occurs only on very long time scales. This is reasonable, given the extremely small values of the CMC for lipids in water (Israelachvili, 1992). Finally, fluctuating vesicles can decrease the interfacial tension gradients by reducing their thermal undulations. However, this is not relevant to the surface force experiments or to systems with enough tension so that fluctuations are negligible.]

Initially, the system is prepared with no polymer, and the outer lipid monolayer is at a density that is determined by the chain packing and the lipid head/water interaction. When polymer is added, the outer monolayer of lipid will tend to become more dense due to its interaction with the polymer. The condensation of lipids by polyethylene glycol (PEG) has been extensively demonstrated in previous work with lipid monolayers and membranes (Tilcok and Fisher, 1979; Bartucci et al., 1996; Maggio and Lucy, 1978). As the concentration of PEG is increased, the main transition temperature increases, suggesting an increase in the lateral packing of the lipids. Initially, this will happen at a fixed number of lipids and could (if the polymer/lipid interaction is large enough) result in a phase separation into domains of higher and lower lipid density (compared with the initial, homogeneous density) in the outer monolayer in region A. In the lower-density domains, the hydrocarbon chains will be exposed to the water/polymer solution, significantly increasing their interfacial tension. The abrupt change in interfacial tensions at the boundary between the higher- and lower-density lipid domains in region A will lead to a Marangoni flow (Oron et al., 1997) of lipids that will tend to reduce the interfacial tension in this region. The additional lipid that can be used to reduce the area occupied by the lower-density lipid domains can come from the outer monolayer lipids in region B, as shown in Fig. 2.

When the two lipid bilayers are in close contact (region B), the polymer, whose free energy would be raised by confinement, can "escape" to region A, where the interlayer spacing is large. Thus, in region B there will be relatively fewer polymers in contact with the lipid bilayers, and phase separation is not expected to occur. There is therefore little tendency for the lipids in region B to phase-separate and therefore no counterbalancing flows; the lipids in region B move only in response to surface tension gradients induced in region A, as shown in Fig. 2. This Marangoni flow drives the outer monolayer lipids from region B to join the lipids

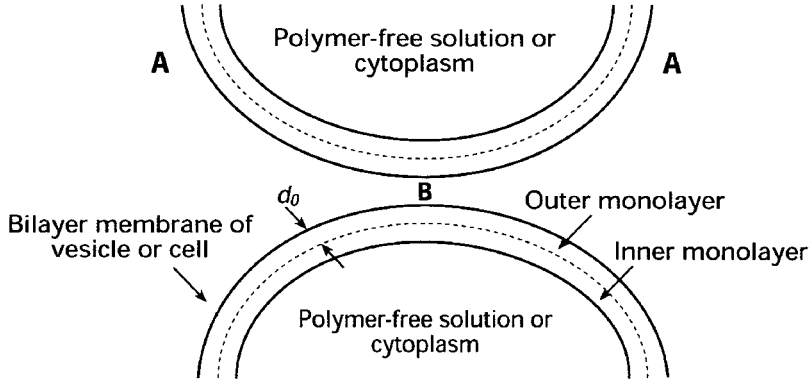

FIGURE 1 Initially, before phase separation and Marangoni flow occur, the monolayers have the same packing density (number per unit area), σ_0, and thickness, d_0. The polymer solution is in regions A and B, although one expects fewer polymer molecules in region B when the spacing between the bilayers is small.

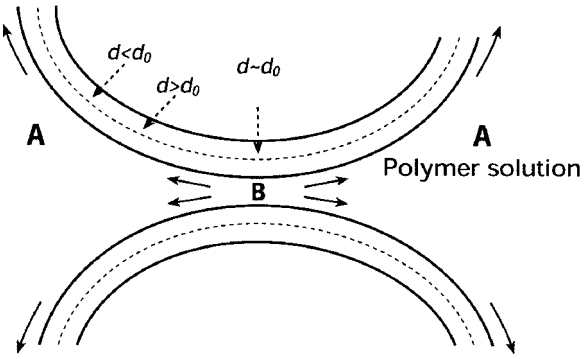

FIGURE 2 After the polymer is added, the reservoir in region A shows phase separation with coexistence of higher density ($\sigma > \sigma_0$, $d > d_0$) and lower density ($\sigma < \sigma_0$, $d < d_0$) domains, whereas initially, the contact region B shows little change. The dashed arrows schematically show regions of different thicknesses with adjacent domains of $d > d_0$ and $d < d_0$ in region A and the domain of $d \sim d_0$ in region B. The density differences give rise to tension gradients and to Marangoni flow of lipids from the contact region B to the reservoir A. After this flow begins, the thickness of the contact region begins to thin as the outer monolayers in this region become depleted. In the experiments (Kuhl et al., 1996), a thinning of 3 Å was observed prior to fusion. The flow lines are shown by solid arrows.

of the outer monolayer of region A, decreasing the extent of the lower-density (and higher-tension) lipid domains in region A, and possibly reversing the phase separation and tension gradients altogether. However, in doing so, the outer monolayer lipids leaving region B expose the hydrophobic chains of the lipid monolayers remaining in region B to water, which is energetically highly unfavorable. However, this can be avoided if the *inner* monolayers of the two adjacent surfaces in region B fuse, as shown in Fig. 3. This fusion can be aided by attractive interactions such as depletion or van der Waals effects, and is likely to occur if the two surfaces in region B are close enough; if not, no fusion can occur and the outer monolayer of lipids in region B cannot be used to reduce the number or size of low-density domains in region A.

In what follows, we show theoretically how the lipid/polymer interaction can result in phase separation in region A during the period before the lipids in regions A and B have had the time to exchange (i.e., fully equilibrate) via the Marangoni flows. Some scaling arguments and other mechanisms for inducing such flows are discussed at the end. [The hydrodynamics of Marangoni flows of surfactants or lipids at the interface between high- and low-tension surfaces has been treated in the literature (see references in Oron et al., 1997). One cannot consider only the surfactant flow because any motion of the surfactant carries with it motion of the surrounding fluid (the water/polymer solution). It is important to note that the viscous dissipation in our situation will come from the lipid/water motion in region B, where the two surfaces are close together and the water monolayer between these surfaces is thin.]

THEORETICAL MODEL

We now estimate the polymer concentration near the surfaces in regions A and B and show how the larger polymer concentration in region A can modify the lipid packing density and result in phase separation. The treatment of inhomogeneous polymer solutions near surfaces is well known (De Gennes, 1979). We apply these results to the geometry considered here and review the calculation for the polymer density as a function of the spacing between the bilayers for completeness.

We consider the case where the system is initially prepared with no polymer added and the lipid density, σ, in each monolayer in both region A and region B is the equilibrium density, $\sigma = \sigma_0$, in the presence of the pure water solvent. Because to a good approximation, the volume of the bilayer must be conserved, an *increase* in the local area density of the lipids will result in an *increase* in the bilayer thickness, from the initial value of $d_0 \sim \sigma_0$ to $d \sim \sigma$, as depicted in Fig. 2. These thickness variations are experimentally observable (Kuhl et al., 1996). We assume that the polymer in solution can freely exchange between regions A and B. Polymers in these two regions must therefore have the same chemical potential. The polymer chemical potential, μ, is fixed by the reservoir (the bulk solution in region A far from the surfaces) with a polymer concentration c_0 (c_0 multiplied by the monomer volume is

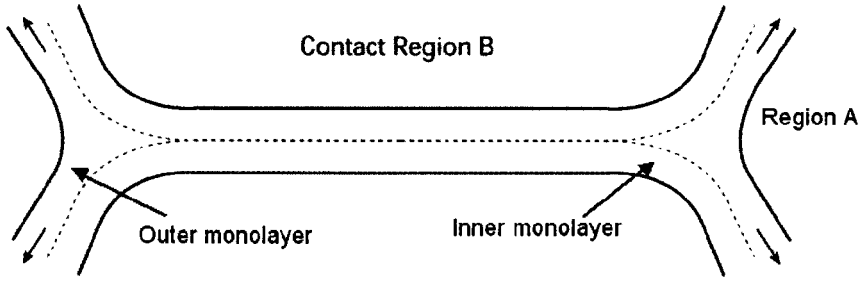

FIGURE 3 Eventually, enough of the outer layer lipids in the contact region (region B) flow to the reservoir (region A) and hemifusion occurs. The dashed lines show the center of the bilayers, and the solid arrows indicate the flow directions.

the volume fraction of polymer in solution). The thermodynamic grand potential of the polymer solution (per unit volume and in units of k_BT) in the reservoir is (De Gennes, 1979):

$$g_r = \tfrac{1}{2}\, v\psi_0^4 - \mu\psi_0^2 \qquad (1)$$

where v is the excluded volume (we shall write $v = a^3$, where a is the monomer size) and $\psi_0 = \sqrt{c_0}$ is a related to the probability density of finding a monomer (De Gennes, 1979). The chemical potential is determined by minimizing g_r with respect to ψ_0 and one finds $\mu = v\psi_0^2 = vc_0$.

When the effect of the surfaces is considered, the concentration depends on the distance, z, from the surfaces located at $z = \pm D$. The free energy is written in terms of the variable $\psi(z) = \sqrt{c(z)}$, where $c(z)$ is the *local* polymer concentration in the system. The free energy for polymers in the semi-dilute regime accounts for both the excluded volume interaction between the polymer segments and a gradient energy, $(a^2/2)(\partial\psi(z)/\partial z)^2$, that arises from the nonuniform polymer concentration in the region between the two membranes, where a is the monomer size. This approximation is valid (De Gennes, 1979) when the changes in the polymer concentration are confined to a distance much smaller than the polymer radius of gyration, R_g. We shall consider the case of two locally flat surfaces separated by a distance $2D$; in the region between the surfaces we have the polymer/water solution. In region A, D is effectively infinite, while in region B, D is finite and is of the order of a few nanometers ($D < R_g$). We denote the midpoint between the two bilayers (located at $z = \pm D$) by $z = 0$; in our mean-field approximation, the polymer concentration varies only in the z direction. Including a chemical potential term, $-\mu\psi^2(z)$, to form the grand potential, expanding for small deviations of ψ from ψ_0, and subtracting the reservoir grand potential, we find that the grand potential of the bulk solution (per unit area and in units of k_BT), relative to that of the reservoir is:

$$\Delta g_b = \int_{-D}^{D} dz \left[4v\psi_0^2(\psi - \psi_0)^2 + \frac{a^2}{2}\left(\frac{\partial\psi(z)}{\partial z}\right)^2 \right] \qquad (2)$$

A more accurate theory would take into account terms proportional to ψ^4; this can be done analytically for region A where $D \to \infty$. For simplicity, we have considered the case where the polymer concentration is close to the bulk value; the qualitative results are similar whether or not the fourth-order term is included.

The simplest model of the polymer/surface interaction considers the surface as a hard wall that excludes the polymer; this allows us to model the system with fewer phenomenological parameters. We take this surface to be the surface dividing the lipid headgroups from the chains; the water/polymer solution cannot penetrate the lipid chains. The grand potential of Eq. 2 is minimized with the boundary condition that the polymer density (and hence ψ) is zero at the surface and that by symmetry, $\partial\psi(z)/\partial z = 0$ at the midplane, $z = 0$. Of course there is some polymer in contact with the lipid headgroups and we estimate it by finding the amount of polymer at some molecular distance from the surface defined by the lipid chains. Minimization of the bulk grand potential with respect to $\psi(z)$ with the boundary conditions discussed above yields:

$$\psi(z) = \psi_0\left(1 - \frac{\cosh(z/\xi)}{\cosh(D/\xi)}\right) \qquad (3)$$

where the polymer correlation length is $\xi = a/(\sqrt{8v\psi_0^2}) = a/\sqrt{8vc_0}$. We note that this correlation length can be large with respect to the monomer size, when the concentration of polymer is small. In addition, for the mean-field theory to be valid, we require that $\xi \gg R_g$, where R_g is the radius of gyration the chain; this can be satisfied for a wide range of concentrations if the molecular weight of the polymer is large enough. The polymer concentration at the lipid headgroup surface which is a microscopic distance from the lipid chains is estimated by calculating $c_s = c(D - a) = \psi^2(D - a)$, where $a \ll D$. From Eq. 3 we estimate this to be:

$$c_s = \psi^2(D - a) \approx c_0\left(\frac{a}{\xi}\right)^2 \tanh^2\left(\frac{D}{\xi}\right) \qquad (4)$$

where c_s is the polymer concentration at the lipid headgroup surface; we denote this as the surface polymer concentration.

In region A, where $D \to \infty$, this determines the concentration of polymer at the surface as $c_s = c_A \approx 8vc_0^2$, where v is the polymer excluded volume (roughly the volume of a monomer) and c_0 is the bulk polymer concentration (far from the surface, in region A). In region B, where we shall assume $D/\xi \ll 1$ (but D can still be much larger than the monomer size, a), we find the concentration of polymer at the surface, $c_s = c_B \approx 64v^2c_0^3(D/a)^2$. The ratio $c_B/c_A = (D/\xi)^2$ and in region B, $D/\xi \ll 1$. Region B thus has a negligible amount of polymer near the surface compared with region A, because the confinement of the polymer between the surfaces in this region induces the chains to "escape" to the reservoir in region A.

We shall now show that the polymer-lipid interaction can induce phase separation (Tilcok and Fisher, 1979; Maggio and Lucy, 1978; Bartucci et al., 1996; Chatellier and Andelman, 1995) in region A. This is only a local equilibrium state and is applicable to early times (i.e., transiently) before the lipids in region A and region B can fully equalize their chemical potentials via Marangoni flow. In this initial period we can regard the number of lipids in region A as being fixed. Although our discussion focuses on the lipid packing area, σ, it is important to note that it may be more convenient in experiments to measure the changes in the lipid layer thickness, d, which is proportional to the lipid density, σ. We assume that in the absence of polymer the lipids are

in a single, homogeneous phase with packing density (number per unit area), $\sigma = \sigma_0$, which is the packing density in the presence of water. For simplicity, we expand the free energy as a function of the lipid density about the homogeneous liquid-phase area density, σ_0 (Safran, 1994). As the temperature is lowered there is, in general, a first-order transition to a more densly packed (usually ordered) phase. In the absence of polymer, the thermodynamic grand potential per unit area of the lipid layer, $g_l(\sigma) = [f_l(\sigma) - f_l(\sigma_0)] - \mu_l(\sigma - \sigma_0)$, with $f_l(\sigma)$ the lipid free energy per unit area and $\mu_l = \partial f_l/\partial\sigma$ the lipid chemical potential. For small variations in the lipid density, the grand potential can be written (Safran, 1994)

$$g_l(\sigma) = \frac{p}{2}(T - T_c)(\sigma - \sigma_0)^2 + \frac{q}{3}T(\sigma - \sigma_0)^3 + \frac{r}{4}T(\sigma - \sigma_0)^4 \qquad (5)$$

where T is the temperature (measured in energy units) and T_c is a characteristic energy scale, which is determined by the strength of the attractive interactions between the lipids. [If the system showed a spinodal or second-order transition, T_c would be the critical temperature, which is determined by the strength of the interactions in the system.] The coefficient $p > 0$ has dimensions of an area of order a^2, where a is a microscopic length, comparable to the maximum lipid packing area, while q is of order a^4, and $r > 0$ is of order a^6. At temperatures higher than the transition temperature, $T_0 = T_c/[1 - 2q^2/(9pr)]$, there is only a single minimum of the thermodynamic potential at the lipid density σ_0, while for lower temperatures the system phase-separates into two coexisting phases: one whose surface density is higher than σ_0 and one whose surface density is lower than σ_0. The high-density phases may also be ordered, but that is not of particular importance here. The spatial extent of the higher- and lower-density domains is determined by the overall lipid density, σ_0, and the domain size is kinetically determined; in equilibrium there are two domains with a single interface between them. In accord with the experimental (Kuhl et al., 1996) situation where the temperature is only 2°C higher than the transition temperature of the lipids, we consider the case where the temperature $T > T_0$ and the system is in a single, fluid phase in the absence of added polymer. [In the surface force apparatus experiments (SFA), the bilayer system is prepared by Langmuir-Blodgett deposition with no polymer in the aqueous medium. An inner, "fixed" monolayer of dipalmitoyl phosphatidylethanolamine (DPPE) is transferred onto molecularly smooth mica sheets at 25°C at a nominal surface pressure of 37 mN/m (the area per molecule is 42 Å^2). The DPPE layer is assumed to be "fixed" at the mica surface due to the strong physical binding of the molecules through electrostatic interactions between the negatively charged mica surface and the zwitterionic lipid headgroup. An outer monolayer of dimyristoyl phosphatidylethanolamine (DMPC) is subsequently deposited at a surface pressure of 31 mN/m (the area per molecule is 55 Å^2). After construction of the bilayers the surfaces are transferred into the measuring device (SFA), which contains the polymer polyethylene glycol (MW = 6–10 k) at a fixed concentration on the order of 10%.] We next show how the addition of polymer can drive the system to phase-separate by shifting the value of the critical temperature.

The interaction of the lipid and polymer is proportional to the surface polymer concentration c_s and depends on the in-plane contacts between the lipid polar heads and the polymer solution. We write the lipid headgroup/polymer (which we shall abbreviate as lipid/polymer) interaction energy as βTac_s per lipid molecule; the interaction is linear in the polymer density adjacent to the surface of the lipid headgroups in a layer of thickness a. The parameter β (whose dimensions are those of an area) represents the difference between the interaction of a single lipid headgroup with the polymer compared to its interaction with the water. When $\beta > 0$, the lipid/polymer interaction is more repulsive than the lipid/water energy and when $\beta < 0$, lipid/polymer contact is more favorable than that of the lipid/water. In what follows we shall assume a repulsive lipid/polymer interaction so that $\beta > 0$. If the interaction were attractive ($\beta < 0$), the polymer would adsorb to the surface even in region B and prevent fusion.

The interaction energy per unit area between the polymer solution and a layer of lipid headgroups in the non-interacting ($\sigma \to 0$) limit is written as $f_p(\sigma \to 0) = \frac{1}{2}\beta Tac_s\sigma$. As other lipid molecules are added, this repulsive interaction is reduced because the presence of the other adjacent lipids locally reduces the in-plane contact of the headgroups with the polymer solution. We model this effect by reducing the energy f_p by an amount proportional to the local density of other lipid molecules; when the lipids are locally close-packed (defined by $\sigma = 1/a^2$) the polymer solution cannot penetrate the lipid headgroup layer and the repulsive interaction with the polymer vanishes. For illustrative purposes we shall assume that at close packing there are only lipid polar groups at the water surface and that the lipid chain/polymer interaction thus vanishes. We thus write

$$f_p = \tfrac{1}{2}\beta Tac_s\sigma(1 - \sigma a^2) \qquad (6)$$

The first term just renormalizes the lipid chemical potential by an amount proportional to the polymer surface concentration. However, the second term, linear in c_s and quadratic in σ, behaves like an effective, attractive interaction between the lipid molecules, and its introduction in the free energy modifies the term proportional to p (see Eq. 5). The physical origin of this term is the reduction of the surface area by the neighboring lipid molecules; this prevents contact between the lipid headgroups and the polymer solution.

A more sophisticated treatment of the lipid/polymer interactions that allows the polymer concentration to adjust to the local lipid density can be formulated (Chatellier and Andelman, 1995; G. Hed and S. A. Safran, to be published]. These models involve a larger number of phenomenological parameters; they generally predict an effective attraction between lipids mediated by the polymers, consistent with the simpler model described above.

Finally, several studies have shown that fusion can be induced in vesicle dispersions by PEG that is physically separated from the vesicle solution by a semipermeable membrane (Wu and Lentz, 1991; MacDonald, 1985). Although such osmotic effects bring the vesicles closer together and make fusion more likely, they may also act to increase the local, lateral packing of the lipid molecules by making it less favorable for water to enter the lipid headgroup surface, resulting in an effective, lipid headgroup interaction, similar to that discussed above (Arroyo et al., 1998). However, this mechanism may only apply at relatively high polymer concentration.

With the inclusion of the lipid/polymer interaction of Eq. 6 the total thermodynamic potential has the form given in Eq. 5, but with the transition temperature *increased* by an amount $\beta a^3 c_s/p$; if the reduction is large enough, the system will phase-separate at temperatures higher than the transition temperature, T_0, in the absence of polymer. Indeed, such increases in T_0 have been directly measured using spectroscopic techniques (Bartucci et al., 1996) and calorimetry (Tilcok and Fisher, 1979; Yamazaki et al., 1992). If we take both β and p to be of order a^2, the relative change in the critical temperature is an increase of the order of the surface polymer volume fraction, $a^3 c_s$. We first focus on the behavior of the system in region A on time scales where the lipids in the regions A and B have not yet equilibrated. Using the value for $c_A \approx 8vc_0^2$, and taking the excluded volume, $v \sim a^3$, we estimate that the critical temperature is increased by $8\phi_b^2$, where $\phi_b = a^3 c_0$ is the bulk polymer volume fraction. The effect of the polymer on the lipid packing is relatively small and arises from the fact that the polymer concentration at the surface is proportional to the square of the bulk polymer concentration. For volume fractions of order 0.1 this predicts an upward shift of the transition temperature, T_0, by a maximum of $\sim$10%. [In general, we might expect that $\beta/p < T_c$ because T_c is governed by the van der Waals interactions of the lipids, while β is determined by the difference between the lipid chain/polymer interaction and the lipid chain/water interaction. This will reduce the estimates from their maximal values.] Thus, if the temperature of the system is within a few percent of the phase transition temperature, the addition of polymer could induce phase separation in the lipid layer. The lipid packing in the domains of different density can be calculated from the minimum of the thermodynamic potential with respect to σ and depends on both the difference between the actual temperature and the transition temperature in the absence of polymer, as well as the polymer-induced shift of the critical temperature.

This effect should only be significant in region A. In region B, where the surfaces are closely spaced, the polymer is mostly excluded; the surface polymer density is much smaller and the shift in the critical temperature is negligible. We therefore generally expect the lipids in region B to remain in a single, homogeneous phase. Thus, before the lipids in regions A and B begin to equilibrate, region B is almost completely covered by lipid, as it was initially, while in region A the phase separation induced by the polymer can result in the coexistence of higher- and lower-density lipid domains if the shift in the transition temperature is large enough. In the lower-density domains more of the chains of the inner lipid monolayer are exposed to the water; this represents a high interfacial tension region.

We now recall that the system described thus far is not in true, global equilibrium; the lipid chemical potential is not equal in regions A and B. The Marangoni flow is established to equalize this chemical potential and decrease the number or the size of low-density lipid domains in region A, thus reducing the high interfacial tension portion of region A. This flow pulls the lipids in the outer monolayer in region B toward region A to create more higher-density domains or higher-density domains of larger extent (possibly reversing the phase separation altogether). This tends to reduce the overall interfacial tension and eliminate surface tension gradients. This can only happen if the inner lipid monolayers in region B on the two surfaces fuse and expel any water between them. Otherwise, the flow of the outer monolayer of lipid from region B to region A would expose the chains of the inner monolayer of lipid of region B to water, costing considerable hydrophobic energy. Of course, this scenario is only likely when the two surfaces in region B are close enough to be pulled together by the long-range hydrophobic interaction, which will "switch on" as soon as the outer layer of lipids have started to flow out of the contact zone. The outer monolayers in the contact zone need not be completely removed; a relatively small reduction in their surface density may sufficiently enhance the hydrophobic interactions for fusion to occur (Helm et al., 1989).

DISCUSSION

What is new in this picture is that the driving force for fusion is not simply a vertical "push" due to a direct attraction between the bilayers or outer monolayers in region B, but rather a lateral "pull" of the lipids in region A on the outer monolayer of lipids in region B. This pull arises from the Marangoni force in region A initiated at the boundary between the higher-tension, lower-density domains and the lower-tension, higher-density domains that result from the initial, polymer-induced phase separation. The lateral pull on the outer monolayer in region B results in exposure of the chains of both the outer and inner monolayers of region

B to the hydrophilic solvent and that effect, along with any direct attractions, can result in fusion when the surfaces are close enough.

The Marangoni flow of the lipids of region B to region A can be induced by a variety of mechanisms, and our suggestion is universal in nature. Any perturbation of the outer monolayer of lipid in region A whose energy is sufficiently reduced by an increase in packing (that leads to an effective increase in the critical temperature for phase separation) can lead to surface tension gradients and a fusion-enhancing Marangoni flow. For example, a local increase in salinity or decrease in pH in the water in region A results in more effective electrostatic screening and reduced repulsion between the charged lipid groups. This effectively acts like an increase in the lipid attractive interaction and increases the value of the transition temperature, T_0, making phase separation more likely.

The theory presented here can thus be used to quantify the observations of the role of calcium asymmetry and the rate of calcium addition as discussed in Chanturiya et al. (2000). Indeed, experiments on mixtures of charged and neutral lipids showed (Leckband et al., 1993) that Ca^{2+} promotes the condensation of the anionic lipids into Ca^{2+}-containing domains. The condensation of the charged lipids in these domains causes an expansion of the uncharged lipid regions, exposing the hydrophobic chains across the water gap and thus leading to fusion. Again, in that work it was concluded that the fusion site need not be the site of initial calcium binding; in fact, the fusion site is related to the expansion of the uncharged lipid in the outer monolayer. This effect is another example of the "action at a distance" of lipid densification far from the fusion zone.

Another example where compositional changes in one part of the membrane may trigger fusion in another region is the action of SNARE proteins in mediating fusion (Pelham, 1999); while the SNARE proteins bring the membranes together, other mechanisms trigger the actual fusion event (Peters et al., 2001). In addition to their role in docking the membranes, the SNARE proteins may also cause a change in the lateral tension on the outer monolayers that results in Marangoni flow and fusion, consistent with our model. Although the control of chemical species such as salt or macromolecules in solution might be used by biological cells to regulate the fusion process, laboratory experiments on vesicles can also use additional methods of inducing Marangoni flows to locally increase the lipid-packing density in region A far from the fusion zone, such as laser tweezers that can generate tension in vesicles (Bar-Ziv and Moses, 1994; Moroz et al., 1997) or nanoelectrodes. [Indeed, the observed (Moroz et al., 1997) fusion and eventual expulsion of a lipid vesicle initially contained in a larger vesicle may be related to the mechanism suggested here arising from changes in interfacial tension induced by the optical tweezers in the larger vesicle in a region far from the fusion and expulsion zone.]

The picture presented here could be more quantitatively tested in two ways. First, one can check the temperature dependence of the effect. Because the flow is induced by an initial phase separation in region A, its properties should be sensitive to the difference between the temperature, T, and the transition temperature for phase separation, T_0, in the absence of added polymer or charge. When the temperature is far from the transition temperature, the perturbation due to the polymer will be less effective at inducing phase separation and fusion may not occur; conversely, closer to the transition, the tension gradients should be larger and the Marangoni flows faster, leading to a speedier fusion process. The experiments of Kuhl et al. (1996) were performed at 2°C above the gel transition temperature of the lipid tails, where one might expect phase separation to naturally occur; it was only necessary for the polymer to cause a small shift in the transition temperature to induce phase separation and the resulting Marangoni flow and fusion.

Another type of dynamical experiment would be to apply a highly localized perturbation of the outer monolayer in region A (in a small region and far from the fusion zone) at time $t = 0$ while monitoring the time for fusion to occur in region B. If that time is smaller than the time for the perturbation to diffuse from region A to region B, it would suggest that the fusion observed in region B is not due to the direct effect of the perturbation, but rather to the induced lipid flows.

In simple geometries (Oron et al., 1997; Borgas and Grotberg, 1988), a scaling argument shows that the time, $\tau(L)$, for an amphiphilic layer bounding a film of thickness D, with viscosity η, to be driven a distance L due to a Marangoni flow scales as

$$\tau(L) \sim \eta \frac{L^2}{\Delta\gamma D} \tag{7}$$

where $\Delta\gamma$ is the relevant interfacial tension difference between the high- and low-density domains. The inverse dependence on thickness is due to the larger hydrodynamic dissipation in thin films, while the dependence on the tension shows the connection between the flow and the driving force due to the tension gradients: larger tension gradients provide larger driving forces and faster flows. For an interbilayer spacing of $D = 10$ Å, and a modest surface tension difference of 1 dyne/cm, this naive scaling argument predicts that the Marangoni flow will advance a macroscopic distance of $L = 50$ μm in a time of ~4 min and a microscopic distance of $L = 30$ nm in a time of 0.1 ms. The estimated distance of 30 nm covered in 0.1 ms should be relevant to microscopic fusion events occurring, for example, at synaptic junctions. Although this scaling estimate is a simple order of magnitude estimate, it is consistent with the measured time scales for macroscopic fusion observed in (Kuhl et al., 1996). Further experimental studies of the dependence of the fusion time on the thickness and surface

tension gradients (controlled by the polymer concentration) can provide additional evidence for the importance of the Marangoni flow in the fusion process. In general, if the diffusion time of the molecule used to perturb the lipid packing in region A a large distance from region B is much longer than $\tau(L)$, one can demonstrate that it is the "action at a distance" of the phase separation in region A that is responsible for the Marangoni flow of the lipid in region B and the resulting fusion of the bilayers.

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