Adhesion induced by mobile binders: Dynamics

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Contributed by P. G. de Gennes, April 12, 2002

We consider a vesicle bilayer loaded with molecules that can bind (upon contact) with a solid surface, following the classical model of Bell, Dembo, and Bongrand. We are interested in situations where the contact area varies with time: we assume that binders can then migrate via diffusion. The resulting dissipation and lag create a retarded force on the contact line, which could be significant in squeezing or rolling experiments. However, there are two cases where we expect the lag force to be ineffective: (i) separation by shrinking of an adhesive patch (where the Evans “tear out” process turns out to be less costly) and (ii) spontaneous growth of a patch from a point contact. In this last case, the lag force is weak, and we give detailed predictions for the growth laws.

Cell adhesion is based on a set of bridging molecules (“binders”) that can attach to specific ligands on the opposite surface. The density Γ of binders per unit area is originally rather small. But, when facing a surface with enough ligands, the binders converge towards this surface and build up a more concentrated adhesive region (Fig. 1).

The equilibrium picture for this process has been described long ago by Bell, Dembo, and Bongrand (1). They showed that the effective work of adhesion G is equal to the difference between the two-dimensional (2D) osmotic pressures of the binder, inside and outside of the sticky region. (In practice, the osmotic pressure inside is largely dominant.)

However, the nature of this osmotic pressure is delicate: ref. 1 was mainly based on an ideal gas behavior of the binders in two dimensions. But there are strong proofs of cooperativity in binding, as discussed in ref. 2: a nominal adhesive zone is often fragmented into smaller patches of high binder density.

The explanation provided in ref. 2 is essentially the following: in the absence of binder, the protective glycocalix forces the two opposed surfaces to remain rather distant (say 3 nm away). When one binder molecule adheres, it forces the two sides to become locally closer; the resulting thinned region acts as an attractor for other binders.

The net result is a phase transition between a 2D “gas phase” of binders, extending over unbound regions, and a “liquid phase” (much more concentrated) occupying the sticky regions.

Our aim, in the present work, is to extend some of these ideas to the *dynamics*, i.e., to situations where the contact area is time-dependent.

If (for instance) we tend to decrease the contact area, we are faced with two possible types of behavior: (i) a *diffusion* response where the binders remain coupled to the surface but move and become more crowded inside the patch, and (ii) a *reaction* response, where some binders decouple from the surface.

The “reaction” dynamics has been analyzed on some typical cases by Evans and coworkers (3). They showed that the bonds appear strong if they are loaded fast, and weaker if they are loaded slowly.

In the present work, we analyze the diffusion mode, and the resulting lag force, opposing the motion. The final question for separation experiments will be to compare the lag force to the Evans force (we give some comments on this point in *Competition Between Tear Out and Diffusion*).

For simplicity, we begin by assuming that the contact is made of a *single patch*, rather than the structure of micropatches plus blisters, which is often observed in practice (2). We shall

incorporate the multipatch systems in *Spontaneous Growth of a Patch*. From an experimental point of view, it may be possible to achieve a single patch by very slow expansion of a single contact, with relatively high numbers of binders/vesicle.

On the other hand, we can lump many features of the binder–binder interaction into the osmotic pressure, which is then higher than the ideal gas value.

Our starting point is shown on Fig. 1: a vesicle establishes contact with a solid wall via a certain number of “binder” molecules. In the contact area, each binder experiences an attractive potential U . In dilute conditions, the surface concentrations inside (Γ_i) and outside (Γ_0) of binders are related by

$$\Gamma_i = \exp(U/kT)\Gamma_0 \equiv f\Gamma_0, \quad [1]$$

where f is large. To each concentration is associated an osmotic pressure $\Pi(\Gamma_i)$ and an osmotic “rigidity” $K \equiv \Gamma_i d\Pi/d\Gamma_i$.

We now modify the contact area either by squeezing the vesicle (Fig. 1*a*) or by rolling it (Fig. 1*b*). For instance, if we decrease the contact area during a time t , a number of binders move inward to stay in the attractive region. This means that the concentration Γ is increased in an annulus of size $(Dt)^{1/2}$ near the contact line. The result is an excess osmotic pressure Π , or a lag force F , opposing the motion.

The major process (for $\Gamma_i \gg \Gamma_0$) is therefore taking place at a fixed number of bonds; thus, we do not depend on the complex (multistep) bonding processes discussed in ref. 4.

At first sight, we might hope to describe the movement through a velocity-dependent separation energy $G(V)$, as is usually done in adhesion science (5–7). But this approach does not make sense here. For instance, if we increase persistently the contact area, the system has to bring in binders from very distant regions, and one cannot generate a steady-state solution for a moving line at constant velocity V .

We present here two (equivalent) ways of reformulating the problem. The first way is based on a very compact scaling argument, described in *Scaling Structure of the Lag Force*. The second way makes use of a complete analysis for small sinusoidal perturbations (*Small Oscillations*). In *The Rolling Problem*, we discuss an extension of these results to certain rolling motions. In *Spontaneous Growth of a Patch*, using the same ideas, we analyze the spontaneous growth of a single patch after contact. Here, we find that for this growth problem, in most realistic cases, the lag force is negligible, because the motions are very slow. In *Competition Between Tear Out and Diffusion*, we compare the tear out process and the diffusion process for separation experiments.

Scaling Structure of the Lag Force

As shown in Fig. 2*a*, we consider a single contact line and restrict our attention to time scales t , such that the diffusion length $\sqrt{Dt}$ is small when compared to the size of the contact zone. D is the diffusion coefficient (for realistic binders, it is expected to be very small, of order 10^{-11} to 10^{-9} cm²/sec).

Abbreviation: 2D, two-dimensional.

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Fig. 1. Typical modifications of the contact between a vesicle and a binding flat surface. The vesicle wall is represented by a single line. The binder molecules are represented by a head + tail system, (a) squeezing and (b) rolling by a hydrodynamic flow $V(z)$.

The line moves with a small velocity $V(t)$, and we want to compute the opposing force $F(t)$ to first order in V , as explained after Eq. 1. This force will be proportional to the internal osmotic rigidity at equilibrium $K = \Gamma_i d\Pi/d\Gamma_i$. Then, its most general structure is

$$F(t) = K \int_{-\infty}^t dt' V(t') R(t - t'), \quad [2]$$

where R is a certain response function, which dimensionally must be an inverse length. The only available ingredients to define a length are the diffusion constant D and the time interval $t - t'$. Thus, we must have

$$R(t) = \alpha [D(t - t')]^{-1/2}, \quad [3]$$

where α is a numerical constant.

We see, in Eqs. 2 and 3, why the situation of constant velocity is not acceptable: at fixed V , the integral over t' diverges (physically it would be cut off by the finite size of our specimen; Eq. 2 holds only for sizes $\gg \sqrt{Dt}$).

It is also important to notice that the force F has a mixture of reactive and viscous behaviors. Indeed, if we Fourier transform Eq. 2, we find that, at a given frequency ω , $F_\omega/v_\omega \sim (i\omega)^{-1/2}$ has both a real and an imaginary component. This will appear naturally in the next section.

Small Oscillations

We assume here that the speed of the line V is modulated sinusoidally. This situation is described in Fig. 2b:

$$V = V_0 e^{i\omega t}. \quad [4]$$

This creates, at point x , a small deviation $\delta\Gamma(x,t)$ from the initial (local equilibrium) value. This is ruled by a diffusion equation:

$$\frac{\partial}{\partial t} \delta\Gamma \equiv i\omega \delta\Gamma \equiv D \frac{\partial^2 \delta\Gamma}{\partial x^2}. \quad [5]$$

The perturbations then decay exponentially from the unperturbed line position:

$$\begin{aligned} \delta\Gamma(x) &= \delta\Gamma_0 \exp(-\kappa x) \quad (x > 0) \\ \delta\Gamma(x) &= \delta\Gamma_i \exp(+\kappa x) \quad (x < 0) \end{aligned} \quad [6]$$

where the characteristic length κ^{-1} is complex and is defined by

$$\kappa^2 = i\omega/D. \quad [7]$$

Fig. 2. Concentration profiles of the binder $\Gamma(x)$ for a line oscillating back and forth (a) general aspect (b) profiles at an instant where the line moves to the right: more attractive sites are presented, and the binders diffuse towards this region. The main effect is a strong drop of the 2D osmotic pressure at the inner side ($x = 0^-$) opposing the motion.

(We choose to define κ as the root of Eq. 7, with a positive real part.) We must now supplement Eq. 5 by two boundary conditions at the interface.

(i) There is a rapid equilibrium at the interface, implying

$$(\Gamma_i + \delta\Gamma_i)/(\Gamma_0 + \delta\Gamma_0) = e^{\beta V} = f, \quad [8]$$

and giving, by comparison with Eq. 2,

$$\delta\Gamma_i/\delta\Gamma_0 = f. \quad [9]$$

(ii) We must match the currents at the boundary; in the reference frame moving with the contact line, at velocity V , the current is

$$J = -D d\Gamma/dx - V\Gamma. \quad [10]$$

It must be continuous at the line, and this gives

$$\begin{aligned} -D[d\Gamma/dx|_{0^-} - d\Gamma/dx|_{0^+}] &= V[(\Gamma_i + \delta\Gamma|_{0^-}) - (\Gamma_0 + \delta\Gamma|_{0^+})] \\ -D[d\Gamma/dx|_{0^-} - d\Gamma/dx|_{0^+}] &\equiv V[\Gamma_i - \Gamma_0] \end{aligned} \quad [11]$$

to first order in V . Making use of Eq. 6, this gives

$$-D\kappa[\delta\Gamma_i + \delta\Gamma_0] = V(\Gamma_i - \Gamma_0). \quad [12]$$

Eqs. 12 and 8 give us the complete solution. But we may simplify things in our limit $f \gg 1$: then $\delta\Gamma_0 \ll \delta\Gamma_i$, and the contribution to the lag force due to the external region is negligible. We replace Eq. 12 by

$$\delta\Gamma_i/\Gamma_i = V/(D\kappa). \quad [13]$$

The main role of the lag force F is then the increase of osmotic pressure just inside the contact line:

$$F = K\delta\Gamma_i/\Gamma_i = KV/(D\kappa) = K(i\omega D)^{-1/2}V. \quad [14]$$

This has exactly the form required by Eqs. 2 and 3, with $\alpha = \pi^{-1/2}$.

Starting from Eq. 3, we choose to use time intervals of order 1 sec, a diffusion constant, $D = 10^{-9}$ cm²/sec, and a velocity V of order 1 μ m/sec. For the surface concentration Γ_i , we assume an area per binder in the contact zone of order 1000 Å², and we use the perfect gas law for an estimate of K . This gives forces of order 1 mJ/m². Thus, the lag force is not negligible.

How would we measure F ? We might possibly use an Evans *et al.* (8) set up, where the pressure in a micropipette allows us to modulate the surface tension γ at some low frequency. We would then measure the modulation of the contact angle θ and of the contact radius (giving V). Then we would write a dynamic form of the Young equation:

$$G + F(V) = \tilde{\gamma}(1 - \cos \theta). \quad [15]$$

The Rolling Problem

In the last two sections, we considered a single contact line, moving over times t (or at frequencies $\omega \sim t^{-1}$), such that the diffusion length κ^{-1} is much smaller than the sample size. This is adequate for squeezing experiments at small amplitudes of drive.

However, it may be tempting to measure the lag force differently, through a rolling experiment, as shown in Fig. 1b.

How would we drive the rolling? The first, naive, idea is to impose a density difference between the vesicle and the surrounding water (via a passive solute) and to tilt the support plane: the vesicle should roll under the Archimedes force, as observed in ref. 9. But, in our case of strong adhesion, this force is much too small for our purposes. A better approach would be to impose a tangential flow on the vesicle, with a certain velocity V_0 at the level of the vesicle center. Then, we might write a rough balance of force (ignoring hydrodynamic wall effects and rotation effects):

$$6\pi\eta L_0(V_0 - V) = F_{\text{lag}}(V), \quad [16]$$

where η is the viscosity, and L_0 is the vesicle diameter. We shall now derive F_{lag} , for a contact area of diameter L , in the limit $\kappa L \ll 1$. The equations of the last section are not valid here.

Then, instead of having exponential decays in the concentration profile, we go to a constant concentration gradient inside. From the diffusion equation in steady state we get

$$d\Gamma/dx = VT_i/D. \quad [17]$$

Integrating the pressures over the circular contact line, we arrive at a total lag force:

$$F_{\text{lag}} = \frac{\pi}{4} L^2 \frac{K}{D} V (V < D/L). \quad [18]$$

A number of remarks are useful at this point:

(i) Note the difference in dimension between Eqs. 18 and 15: Eq. 15 gives a force per unit length, while Eq. 18 is the total force.

(ii) Eq. 18 holds when $\kappa^{-1} > L$, where the effective modulation frequency $\omega \sim V/L$. Thus, we must have $V < D/L$.

(iii) For the single line problem (with $\kappa^{-1} < L$), we never reached a steady-state regime at constant V . But we reach it here when Eq. 17 holds.

Returning to Eq. 16, we can now compare the drift velocity V to the applied velocity V_0 . It turns out that V/V_0 is very small:

$$V/V_0 = 6\pi\eta D/(KL). \quad [19]$$

Even with anomalously high values of D (10^{-6} cm²/sec), we get $V/V_0 \sim 10^{-3}$. The difficulty is that, at these high contrasts, the vesicle is probably very strongly distorted by the flow; then the hydrodynamic friction is not properly estimated by Eq. 16.

Fig. 3. Growth of an adhesive patch in idealized conditions. At $t = 0$, the vesicle enters into contact with the surface and is under 0 surface tension. At $t > 0$, an adhesive patch of radius r builds up by migration of binder molecules. The contact angle θ increases with time.

Spontaneous Growth of a Patch

The problem is described in Fig. 3. We start from a spherical vesicle under a small initial tension $\gamma_0 > 0$. (The case $\gamma_0 = 0$ would lead to large fluctuations and the possibility of more than one contact).

After a time t , we assume that a single patch has grown, with a radius $R(t) = R_v\theta(t)$, where R_v is the vesicle radius, and θ (assumed small) is the external contact angle. Experiments of this type have been performed in particular in Paris (10, 11) and Munich (12). In the following sections, we present our (naive) theoretical views on this problem.

The Surface Tension γ . The contact has imposed an increase of area ΔA for the vesicle. The relative increase is

$$\Delta A/A = \theta^4/16. \quad [20]$$

The classic formula for the surface tension γ superposes fluctuation effects (of small γ) and intrinsic elasticity (for large γ). It is

$$\Delta A/A = f(\gamma) - f(\gamma_0)$$

$$f(\gamma) = \frac{kT}{8\pi K_b} \ln\left(1 + \frac{R_v^2 \gamma}{6K_b}\right) + \frac{\gamma}{E_2}. \quad [21]$$

Here, K_b is the bending modulus of the bilayer ($K_b \sim 10kT$ in typical situations). The logarithmic term in Eq. 21 describes the smoothing-out of fluctuations by the tension γ . The last term corresponds to the intrinsic elasticity of the membrane, with a large elastic modulus E_2 .

The fluctuation regime holds whenever

$$\gamma < E_2 kT/(8\pi K_b), \quad [22]$$

and this is well satisfied for our purposes. We may also safely assume that $\gamma > K_b/6R_v^2$, and rewrite Eq. 21 in the following compact form:

$$\frac{\Delta A}{A} = \frac{kT}{8\pi K_b} \ln \frac{\gamma}{\gamma_0} = \frac{1}{16} \left(\frac{R}{R_v}\right)^4, \quad [23]$$

or equivalently:

$$\frac{\gamma}{\gamma_0} = \exp\left[\frac{\pi K_b}{2 kT} \left(\frac{R}{R_v}\right)^4\right]. \quad [24]$$

Taking $K_b/kT = 10$ and $\theta = R/R_v = 0.1$, we see that the argument in the exponential is of order 10^{-3} . Thus, $\gamma = \gamma_0$. The surface tension should remain constant during the growth of the patch.

Establishment of a Nonspecific Contact. At early times, the binders cannot move. They maintain a concentration near Γ_0 at all

points. Their contribution to the adhesion energy G (and to the lag force) is negligible. We can set G equal to G_0 , a small value due to nonspecific interactions (e.g., van der Waals) between bilayer and wall. This assumption would not be valid for the experiments of ref. 12, where a peptide analog of the glycocalix is present and suppresses G_0 . The corresponding contact angle at equilibrium is θ_0 , defined by:

$$G_0 = \gamma_0(1 - \cos \theta_0) \cong \gamma_0 \theta_0^2/2. \quad [25]$$

The only force opposing the growth of θ from $\theta = 0$ to $\theta = \theta_0$ is the classical force due to viscous flow in the wedge of angle $\theta[t]$. This force has already been used in this context by di Meglio and coworkers (10).

The basic balance between Young force and viscous force reads (13):

$$3\ell\eta\theta^{-1}dR/dt = \gamma_0(\theta_0^2 - \theta^2)/2, \quad [26]$$

where ℓ is a logarithmic factor of order 10, and η is the viscosity of water. This may be rewritten as

$$d\theta/dt = V^*R_\eta^{-1}\theta(\theta_0^2 - \theta^2), \quad [27]$$

with $V^* = \gamma_0/(6\ell\eta)$. Thus, the rise time for the nonspecific contact is

$$\tau = R_v/V^*\theta_0^3. \quad [28]$$

and is of order 1 min for $\theta_0 = 0.1$.

The diffusion length over the time τ is a fraction of microns, while the final radius $R_0 = \theta_0 R_v$ is of order 1 μm . Thus, indeed diffusion was weak during this first stage.

Accumulation of Binders: The Perfect Gas Regime. We now redefine the time t as starting at the end of the first step. At $t > 0$, the accumulation of binders becomes important. There is a nearly uniform concentration Γ_i in the patch. The binders come from a region of radius $\sqrt{Dt}$ outside. (We assume now that this region is large, $\sqrt{Dt} > R$.) Then the number conservation of binders imposes:

$$(\Gamma_i - \Gamma_0) = k\Gamma_0 \frac{Dt}{R^2}. \quad [29]$$

One can derive the factor k from the solution for steady-state diffusion in two dimensions, in quasi-static conditions ($Dt \gg R^2$). The result is (for $f \gg 1$)

$$k = \frac{2}{\ln(R_v/R)} \sim 1, \quad [30]$$

and we shall set $k = 1$, for simplicity, in what follows.

Eq. 29 must be supplemented by a balance of forces at the contact line. Here again, we assume quasi-static conditions.

The lag force is negligible when diffusion is fast ($Dt > R^2$), and we may write

$$\frac{1}{2} \gamma_0 \theta^2 = G = \frac{1}{2} \gamma_0 \theta_0^2 + \Pi(\Gamma_i). \quad [31]$$

For the moment, let us assume a perfect gas law for the 2D gas of binders $\Pi(\Gamma_i) = kT\Gamma_i$. Comparing Eqs. 29 and 31, we arrive at the growth law:

$$\theta^2(\theta^2 - \theta_0^2) = \varepsilon Dt/R_v^{-2}, \quad [32]$$

where

Fig. 4. Spontaneous growth laws for a patch, in the presence of a cooperative phase transition of the binders, giving a plateau in osmotic pressure within an interval (Γ_-, Γ_+) of concentrations. (a) Relation between time t and patch concentration $\Gamma_i(t)$ as derived from Eq. 36. (b) Value of the contact angle as a function of the internal concentration Γ_i .

$$\varepsilon = 2kT\Gamma_0/\gamma_0 \quad [33]$$

is a small dimensionless parameter.

Eq. 32 is our final answer for perfect gas conditions. Note first that the quasi-static assumption ($R^2 < Dt$) makes sense. Indeed, from Eq. 29 we see that

$$y \equiv R^2/(Dt) = \Gamma_0/(\Gamma_i - \Gamma_0).$$

Thus, whenever we have reached interesting values of Γ_i (much larger than Γ_0), we do expect $y \ll 1$.

Ultimately, at $\theta \gg \theta_0$, Eq. 32 reduces to a simple power law:

$$R^4 = \varepsilon Dt R_v^2. \quad [34]$$

This is not far from the observations of refs. 10 and 11.

Modification Due to a Phase Transition of the Binders. Eqs. 32–34 assumed an ideal gas behavior for the binders. But, in many cases, the binders attract each other as explained in ref. 2. The osmotic pressure rises linearly at small Γ , and then reaches a 2D gas/2D liquid coexistence plateau in an interval $\Gamma_- < \Gamma < \Gamma_+$. The lower end Γ_- is conditioned by the Bruinsma interactions (2). The upper end Γ_+ is due to the finite number of receptor sites, available on the support surface. For $\Gamma > \Gamma_+$, the osmotic pressure rises very high. Note incidentally that the equilibrium condition (1) is modified and becomes

$$\int_{\Gamma_0}^{\Gamma_i} \Gamma_i^{-1} d\Pi = U \equiv kT \ln f. \quad [35]$$

But this modification will not play a major role in what follows. If we return to Eqs. 31 and 29 (with $k = 1$), we arrive at an implicit equation for $\Gamma_i(t)$:

$$Dt/R_v^2 = 2\Pi(\Gamma_i)(\Gamma_i - \Gamma_0)/\gamma_0\Gamma_0. \quad [36]$$

The general aspect of this relation is shown on Fig. 4. At relatively low concentrations ($\Gamma_i < \Gamma_-$), we essentially retain the perfect gas behavior. For $\Gamma_- < \Gamma_i < \Gamma_+$, the contact region will contain islands of the dense phase, with a well defined osmotic pressure (the plateau value Π_p). In this region, the concentration $\Gamma_i(t)$ increases linearly with time and rather fast. Ultimately, we reach Γ_+ , and beyond this point, the growth is very slow.

These effects also show up in the contact angle $\theta(t)$. In the dilute regime ($\Gamma < \Gamma_-$), Eq. 32 still holds. When we enter the plateau region, the contact angle should be locked by Eq. 31 at a constant value. Ultimately, at $\Gamma > \Gamma_+$, we expect a very slow growth of $\theta(t)$ and $R(t)$.

Competition Between Tear Out and Diffusion

Here we start from an adhesive patch at equilibrium and (by some external means) we tend to *decrease* the contact area. As mentioned in the introduction, we can think of two scenarios: *tear out*, where some bridges are broken, and diffusion, where the binders migrate, but the number of bridges is constant. Clearly, the diffusion scenario is limited in time: if the patch becomes very small, Γ_i reaches a saturation value $\Gamma_{\max}$, where all binders are side by side. Beyond this point, tear out must prevail. We assume here $\Gamma_i < \Gamma_{\max}$.

We want to compare the horizontal forces corresponding to both scenarios: F for the diffusion mode, and F_E (where E stands for Evans) for the tear out process. We consider a contact line moving at a prescribed velocity V and first construct a simple estimate for $F_E(V)$ based on the model of ref. 3.

At a microscopic scale, we consider one couple binder/receptor and assume that this couple begins to be separated by a vertical distance z . In the simplest case, with a single barrier of activation energy B , we expect a rate equation of the form:

$$\frac{dz}{dt} = V_0 \exp\left[-\frac{1}{kT}(B - \varphi a)\right]. \quad [37]$$

Here $dz/dt = V dz/dx$, where V is the line velocity and x defines the horizontal location of the binder, while φ is the pull out force on one binder, a is a molecular length, and V_0 is a typical thermal velocity (of order 10 m/sec). Eq. 37 may be rewritten in the form:

$$a\varphi = B + kT\left[\ln\left(\frac{V}{V_0}\right) + \ln\left(\frac{dz}{dx}\right)\right]. \quad [38]$$

We can now construct the entropy loss due to the motion as an integral over all sites near the line that are partially detached. We call this $T\dot{S}$ (per unit length of line in the y direction):

$$T\dot{S} = \Gamma_i \int dx \varphi dz/dt = V \Gamma_i z_m a^{-1} \left[B + kT \ln\left(\frac{V}{V_0}\right) - \ell \right], \quad [39]$$

where z_m is the overall distance required for separation (~ 1 nm), and

$$\ell = -z_m^{-1} \int dx \ln(dz/dx) dz/dx \quad [40]$$

is a constant of order unity.

We now derive from Eq. 39 the horizontal friction force F_E :

$$F_E = \frac{\partial}{\partial V} T\dot{S} = \Gamma_i \frac{z_m}{a} \left[B + kT \ln\left(\frac{V}{V_0}\right) - (\ell - 1)kt \right]. \quad [41]$$

(In what follows, for our rough estimates, we shall set $\ell - 1 = 0$).

We can now compare this Evans force to the lag force F derived in *Scaling Structure of the Lag Force*: for a duration t (or a frequency t^{-1}), we replace Eq. 2 by the simplified form:

$$F = KV(D/t)^{-1/2}. \quad [42]$$

From Eqs. 41 and 42, we get the ratio:

$$r \equiv \frac{F}{F_E} = \frac{V}{V_1} \frac{1}{\ln(V/V^*)}, \quad [43]$$

with

$$V_1 = \left(\frac{D}{t}\right)^{1/2} \frac{\Gamma_i kT}{K} \quad [44]$$

$$V^* = V_0 \exp(-B/kT). \quad [45]$$

The plot of $r(V)$ shows a minimum at $V = eV^* = 2.7V^*$ and $r = 2.7V^*/V_1$.

(i) If $V_1 < 2.7V^*$, the ratio r is always larger than unity: the reaction process demands less force and dominates the separation.

(ii) If $V_1 > 2.7V^*$, there is an interval (around $3V^*$) where $r < 1$, and, in this interval, the lag force may be dominant.

Thus, the crucial parameter is

$$y = eV^*/V_1. \quad [46]$$

Let us make a rough estimate of y , using Eqs. 44 and 45, taking $\Gamma_i kT/K \cong 1$, and assuming that the diffusion constant D is controlled by the *same barrier* B , which opposes separation. Hopping inside the adhesion patch demands a separation binder/receptor:

$$D = aV_0 \exp(-B/kT), \quad [47]$$

where a is a molecular diameter.

We choose $V_0 = 10$ m/sec, $t = 100$ sec, and $a = 1$ nm. Then,

$$y = (V_0 t/a)^{1/2} \exp(-B/2kT) \cong 10^6 \exp(-B/2kT), \quad [48]$$

and the lag force plays a role only if

$$B/kT \gtrsim 28. \quad [49]$$

The conclusion is that for most practical separation experiments ($B/kT \sim 15$), tear out should dominate over diffusion.

Discussion

Our calculations of the patch growth in the diffusion regime are crude for a number of reasons. (i) We treated the outer region as a large reservoir of binders. But, in reality, the total amount of binders available in our vesicle is fixed, and the growth of the patch may stop trivially, because all binders have been used. (ii) We ignored the complexity of the contact line: on the outer side of the line, the angle θ shows up only after a certain distance $\lambda = (K_b/\gamma)^{1/2}$. All our discussion assumes $R > \lambda$. (iii) The diffusion constants may be very different in the unbound/bound regions. The bound binders must break out from their receptor site to be able to move, and the diffusion constant D_i , inside the adhesive patch, should thus be small. On the other hand (and especially for vesicles without any cytoskeleton), the diffusion D_0 in the unbound region may be much faster.

There is, however, a certain rule of the thumb: in the squeezing and rolling problems of previous sections, it is the internal diffusion D_i that controls the force lag, and we can put $D = D_i$. On the other hand, in the growth problem of the last section, what limits the growth is the external diffusion D_0 towards the patch, and we should put $D = D_0$.

Summary

We expect the lag forces to be important in certain (not all) squeezing or rolling experiments. But their observation is delicate: the simplest procedure may be to use a modulated squeezing and to monitor simultaneously the modulations of the radius R and of the contact angle θ . (θ is an independent variable in this

case: Eq. 20 does not hold?) The angle θ gives us the force $\gamma(1 - \cos\theta)$, and we could end up with an experimental relation $F(V)$. However, we may face a complex situation where the lag force acts upon squeezing, while the tear out process dominates in the other half period.

We have benefited from the helpful advice of R. Bruinsma.

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