Elementary theory of Brownian motion of trapped domains in lipid monolayers

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ABSTRACT An elementary theoretical model is described for the Brownian motion of circular lipid domains electrostatically trapped within larger circular lipid domains in monolayers at the air–water interface. Earlier work is briefly reviewed, in which it is shown that the r.m.s. amplitude of the trapped Brownian motion follows a simple Maxwell–Boltzmann distribution, and can be used to determine the absolute value of the difference of dipole densities in the two co-existing phases $|\mu|$. A comparison of this dipole density difference with that obtained from surface potential measurements provides a critical test of the theoretical model. It is also shown that the kinetics of Brownian motion can be analyzed to provide information on monolayer fluid mechanics and to provide a further test of the model.

It is pointed out that the ease with which domain movements can be observed in the fluorescence microscope, coupled with the fact that the energies involved are only of the order of magnitude of kT, suggests that lipid monolayers can be used to detect weak specific intermolecular bonds between ligands incorporated in the lipid monolayer, and receptors fixed in the aqueous subphase.

INTRODUCTION

Fluorescence microscopy has been used for many studies of co-existing monolayer lipid phases at the air–water interface (1–3). This technique permits the study of not only the sizes and shapes of lipid domains, but also their motions. Recent work has described the r.m.s. amplitudes of Brownian motion of circular lipid domains that are electrostatically trapped within larger circular domains (4). Such measurements of Brownian motion can provide critical tests of an electrostatic model that has been used to describe the behavior of monolayers (2). Measurements of the kinetics of Brownian motion provide tests of hydrodynamic models of domain motion. Finally, in principle the modulation of Brownian motion of lipid domains by intermolecular forces offers the possibility of detecting weak, specific, biologically significant intermolecular bonds.

BACKGROUND

We consider co-existing monolayer liquid phases such as those present in binary mixtures of cholesterol and dimyristoylphosphatidylcholine (DMPC) at the air–water interface (4). In treating the electrostatics it is assumed that because the individual phases are liquid, the molecular dipoles on average are oriented vertically, so that only average forces between the vertical components of these dipoles need be considered. Fig. 1 depicts two phases: a light phase and a dark phase. This arrangement of phases is seen frequently in binary mixtures of cholesterol and DMPC (4). The cholesterol-rich phase is dark since it preferentially excludes the fluorescent lipid probe. The small light domain is electrostatically trapped within the large dark domain and undergoes Brownian motion about the center. As shown elsewhere, that part of the dipole–dipole electrostatic energy that depends on the location of the small domain within the larger domain is given by the expression (2)

$$F_{el} = -\mu^2 \oint\oint \frac{d\vec{l} \cdot d\vec{l}'}{r} \qquad (1)$$

As discussed below, μ is the difference in dipole density in the two phases, and $d\vec{l}$ and $d\vec{l}'$ are elements of perimeter around the inner and outer circular boundaries, having the separation r. (See Fig. 1) As shown previously, the component of F_{el} that is quadratic in the displacement ρ is (4)

$$F_{el}^{(2)} = (3/2)\mu^2\pi^2(a^2\rho^2/A^3). \qquad (2)$$

As shown in Fig. 1, a is the radius of the inner domain, A is the radius of the outer domain, and ρ is the displacement of the center of the inner domain from the center of the outer domain.

The corresponding harmonic force constant is K,

$$K = 3\mu^2\pi^2a^2/A^3. \qquad (3)$$

Assuming equipartition of energy, the time average potential energy is

$$\langle F_{el}^{(2)} \rangle = kT. \qquad (4)$$

Earlier work (4) employed the parameter η,

$$\eta = a^2\rho^2/A^3 \qquad (5)$$

Experimental values of η, $\bar{\eta}$ and $\langle \bar{\eta} \rangle$ have been determined, where η refers to the result of a single measurement, $\langle \eta \rangle$ refers to a time average over the domains of a given a, and A, and $\langle \bar{\eta} \rangle$ refer to an ensemble average over monolayers with domains of different radii, a and A. From Eqs. 2 and 3 it follows that the experimental values of $\langle \eta \rangle$ should be constant, independent of a and A, and equal to $\langle \bar{\eta} \rangle$. For the cholesterol–DMPC mixtures studied, this was indeed found to be the case to

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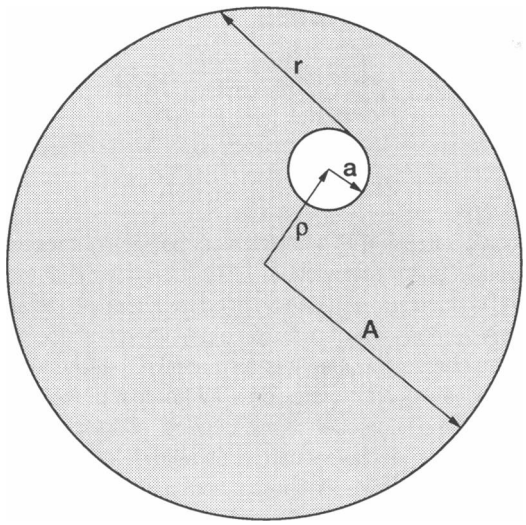

FIGURE 1 Electrostatically trapped lipid domain. Circular domains of two co-existing monolayer lipid phases are represented by white and dark regions. The central domain of radius a is electrostatically trapped within the dark domains of radius A. Displacements of distance ρ from the center arise from thermal excitation, corresponding to Brownian motion. (This figure is taken from reference 4).

within the experimental error (4). The experimental distribution of the observed values corresponds quite well to the expected distribution, $\exp(-\eta/\langle\bar{\eta}\rangle)$. See Fig. 2. (For lipid mixtures of DMPC and dihydrocholesterol, where μ is smaller, it is necessary to include terms in ρ^4 in Eq. 2.—Benvegnu, N., and H. M. McConnell, manuscript submitted for publication.)

The approximate constancy of the experiment values of $\langle\bar{\eta}\rangle$, and the conformity of the observed values to the expected exponential distribution are evidence in support of the theoretical model, and the view that the motion is Brownian. Since

$$\mu^2 = 2kT/3\pi^2\langle\bar{\eta}\rangle \tag{6}$$

these measurements provide a determination of μ^2. An important additional test of the theoretical model can be obtained from measurements of surface potentials of each of the two liquid phases, V_l and V_d:

$$V_l = (\mu_l/4\pi) + \text{const.} \tag{7}$$

$$V_d = (\mu_d/4\pi) + \text{const.} \tag{8}$$

where

$$\mu = \mu_l - \mu_d$$

Such measurements require a knowledge of the compositions of the individual phases. Experimental results obtained thus far give values of $|\mu|$ by the two methods that agree with one another to better than a factor of two. In view of a number of sources of experimental error, this degree of agreement is considered satisfactory. (Benvegnu, N., and H. M. McConnell, manuscript submitted for publication.)

KINETICS OF BROWNIAN MOTION

In considering the kinetics of Brownian motion of trapped lipid domains, we need to consider two experimental limiting cases. In one case, the domain radii and dipole density differences are such that the trapped domain is tightly bound to the center of the trap, and undergoes excursions of small amplitude. This motion is most easily analyzed in terms of the position correlation function described below. In the other limiting case, the trapped domain undergoes excursions of large amplitude, much larger than the radius of the trapped domain. We suggest that this later motion is most easily analyzed in terms of the Smoluchowski equation of Brownian motion. Both limiting cases are observed experimentally. As mentioned earlier, one objective of analyzing the Brownian motion is to test the theoretical model of the electrostatic interactions, and to measure the hydrodynamic drag on this motion.

The electrostatic potential energy of the trapped domain is cylindrically symmetric, and the equations of motion are separable into x- and y-components. In order to calculate the correlation function for motion in the x-direction, $\langle x(t)x(t+\tau)\rangle$, we assume that the motion of the trapped domain follows the Langevin equation (5–7).

$$m\ddot{x} = R(t) - \gamma\dot{x} - kx. \tag{9}$$

Here $R(t)$ is the Brownian random force acting on the trapped domain, assumed to be independent of x. The correlation function corresponding to this motion is known (5–7),

$$\langle x(t)x(t+\tau)\rangle = \frac{D}{\lambda\omega_0^2}\, e^{-\lambda\tau/2}\left(\cos\omega_1\tau + \frac{\lambda}{2\omega_1}\sin\omega_1\tau\right) \tag{10}$$

where D is the domain diffusion coefficient, $\lambda = \gamma/m$, $\omega_0^2 = K/m$, and

$$\omega_1^2 = \omega_0^2 - (\lambda^2/4). \tag{11}$$

This result is valid for both underdamped motion ($\omega_1^2 > 0$) and overdamped motion ($\omega_1^2 < 0$). It is likely that both cases can be realized experimentally.

For large amplitude motion it is convenient to analyze the motion in terms of the Smoluchowski equation,

$$\frac{\partial P}{\partial t} = \vec{\nabla}\{D\vec{\nabla}P + \tau_0^{-1}P\} \tag{12}$$

where $P = P(\vec{\rho}, t)$ is the probability that the domain can be found at position $\vec{\rho}$ at time t, and $\tau_0 = \gamma/K$. Equation 12 is readily solved for special cases. Consider the normalized probability distribution

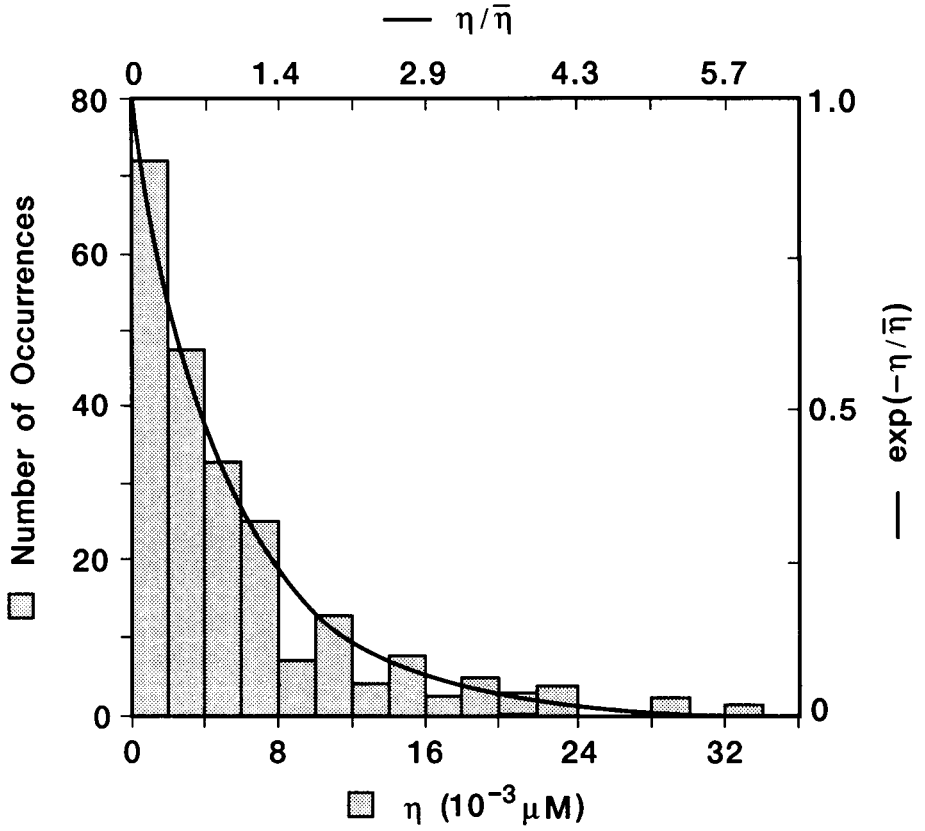

FIGURE 2 Maxwell–Boltzmann distribution of domain displacements. A Maxwell–Boltzmann distribution of domain positions corresponds to a probability distribution proportional to $\exp(-\eta/\langle\bar{\eta}\rangle)$ where $\eta = a^2\rho^2/A^3$ and $\langle\bar{\eta}\rangle$ is a time and ensemble average of η. Bars give the results of experimental observations, at different times, and with circular domains of different radii, a and A. Solid line is the Boltzmann probability distribution. See text. (This figure is taken from reference 4).

$$P = (\beta/\pi)\exp(-\beta\rho^2) \tag{13}$$

where $\beta = \beta(t)$ depends on the time. The differential equation for β is

$$\frac{d\beta}{dt} = 2\beta\left[-2\beta D + \frac{1}{\tau_0}\right] \tag{14}$$

for which a particular solution is

$$\beta = [2\tau_0 D\{1 - \exp(-2t/\tau_0)\}]^{-1}. \tag{15}$$

The solution in Eqs. 13 and 15 is appropriate for describing a domain that starts out at time $t = 0$ at the origin, $\rho = 0$. At time $t = \infty$, $P(\vec{\rho}, \infty)$ is a Boltzmann equilibrium distribution. Other solutions can be obtained for other starting conditions. In particular Eq. 12 is separable in cylindrical coordinates (ρ, φ). Diffusion in the φ-direction depends only on D, not on τ_0. Thus experimental measurements of the φ-dependence of the diffusion can yield D, independent of τ_0. The diffusion coefficient D is simply related to the friction coefficient γ, $D = kT/\gamma$.

Thus, in principle from the kinetics of Brownian motion, one can determine the diffusion coefficient (and thus the drag γ) as well as test the model by measurements that involve an interplay of the drag and electrostatic forces. At present, the best available theoretical approximation for the fluid mechanics for calculating γ is that of a thin solid circular disk of radius a moving in an infinite two-dimensional monolayer on the surface of water. See Hughes et al. (8).

DISCUSSION

Reconstituted membranes composed of lipid monolayers and bilayers together with specific proteins and lipids have been used in a wide range of biophysical and biochemical studies. For examples involving immune recognition, see (9). Lipid monolayers at the air–water interface have well-known advantages, as well as disadvantages for such studies. It is our conjecture that a quantitative understanding of lipid phase behavior, and domain structure and dynamics can significantly increase the utility of lipid monolayers at the air–water interface for such studies.

The immediate purpose of this brief communication has been to indicate how a quantitative analysis of Brownian motion of electrostatically trapped lipid domains can serve to test a phenomenological theory that has been developed for lipid domains (2). Thus, two entirely independent methods are described for the mea-

surement of the square of the dipole density difference, μ^2. One method uses the r.m.s. amplitude of the Brownian motion, and the other uses surface potential measurements. Measurements of the kinetics of Brownian domain motion can also be used to test the electrostatic model as well as assumptions concerning hydrodynamic drag on lipid domains (10). Of course, other experimental arrangements can be used to provide additional tests of models, and determinations of experimental parameters.

A special intriguing feature of the Brownian motion of lipid domains in monolayers is that such motion can be easily monitored experimentally in a fluorescence microscope, and the motion reflects energies that are only of the order of kT. It is also true that a wide variety of molecules of biological significance can be attached to lipid monolayers. Thus, if the lipid monolayer has attached ligands, and the aqueous subphase contains receptors for these ligands, it should be possible to detect specific interactions between ligands and receptors, even when the interactions are intrinsically weak, or few in number, or both. The effects will be particularly large if the receptors are attached to a massive support, such as a biological cell, or solid support. The great potential advantage of monolayer membrane systems for such studies is that their hydrophilic surfaces can be selected or designed to mimic closely biological membranes, and thus minimize non-specific interactions.

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Notes added in proof:

(*a*) In recent work the dipole density difference μ has been determined from measurements of the field gradient electrophoretic mobility of lipid domains. (Klingler, J., and H. M. McConnell. *J. Phys. Chem.* In press.)

(*b*) For binary mixtures of DMPC and dihydrocholesterol the values of μ obtained from surface potential measurements, and field gradient electrophoretic mobility measurements, are in good agreement with values of $|\mu|$ obtained from measurements of the r.m.s. amplitudes of Brownian motion of electrostatically trapped lipid domains. (Benvegnu, N., and H. M. McConnell. *J. Phys. Chem.* submitted).

(*c*) Measurements of the Brownian motion of essentially free lipid domains have now been carried out, verifying theoretical calculations given in reference 8 for the hydrodynamic drag on solid circular lipid domains in contact with a two-dimensional liquid lipid monolayer, and with water. (Klingler, J., and H. M. McConnell, to be published.)

(*d*) Suppression of Brownian and other motions of lipid domains by submerged potential probes has been detected. (Lee, K.-Y., and H. M. McConnell, unpublished observation.)

REFERENCES

1. Möhwald, H. 1990. Phospholipid and phospholipid-protein monolayers at the air/water interface. *Annu. Rev. Phys. Chem.* 41:441–476.
2. McConnell, H. M. 1991. Structures and transitions in lipid monolayers at the air-water interface. *Annu. Rev. Phys. Chem.* 42:171–195.
3. Knobler, C. M. 1990. Seeing phenomena in flatland: studies of monolayers by fluorescence microscopy. *Science (Wash. DC).* 249:870–874.
4. McConnell, H. M., P. A. Rice, and D. J. Benvegnu. 1990. Brownian motion of lipid domains in monolayers. *J. Phys. Chem.* 94:8965–8968.
5. Kubo, R., M. Toda, and N. Hishitsume. 1991. Statistical Physics II, Springer-Verlag, Berlin. 14–21.
6. Chandrasekhar, S. 1943. Stochastic problems in physics and astronomy. *Rev. Mod. Physics.* 15:1–91.
7. Wang, M. C., and G. E. Uhlenbeck. 1945. On the theory of the Brownian motion II. *Rev. Mod. Physics.* 17:323–342.
8. Hughes, B. D., B. A. Pailthorpe, and R. L. White. 1981. The translational and rotational drag on a cylinder moving in a membrane. *J. Fluid Mech.* 110:349–372.
9. McConnell, H. M., T. H. Watts, R. M. Weis, and A. A. Brian. 1986. Supported planar membranes in studies of cell-cell recognition in the immune system. *Biochem. Rev. Biochemica et Biophysica Acta.* 864:95–106.
10. Benvegnu, D. J., and H. M. McConnell. 1992. Line tension between liquid domains in lipid monolayers. *J. Phys. Chem.* 96:6820–6824.