EXTRACTION OF MEMBRANE MICROVISCOSITY FROM TRANSLATIONAL AND ROTATIONAL DIFFUSION COEFFICIENTS

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ABSTRACT We report the exact calculation of the Stokes drag for cylindrical particles diffusing in Saffman's model membrane. Simultaneous prediction of lateral and rotational diffusion coefficients suggests that microviscosities may not be as large as previously thought and implicates the bathing viscosities.

INTRODUCTION

There are now a variety of techniques that measure the diffusion of protein and lipid components in a membrane (1). Fluorescence recovery after photobleaching measures translational diffusion over ~1 μM (2, 3); rotational diffusion can be studied by absorption dichroism, phosphorescence depolarization, and saturation transfer electron paramagnetic resonance (EPR) (4, 5). Recent studies have yielded a wealth of experimental data on the lateral and translational diffusion of a variety of membrane proteins and lipids (1). However, the seemingly complex relationship between translational and rotational diffusion constants has yet to be elucidated, and highlights the need for a more detailed theoretical analysis. The derivation of membrane "microviscosities" from experimentally determined diffusion coefficients has relied mostly on the classic Stokes law (6) for the drag on a sphere in a three-dimensional isotropic medium. However, the membrane is a three-dimensional anisotropic medium, and we report here a solution of the Stokes problem for both translational and rotational motion in membranes. This shows that the viscosities of the fluids bathing the membrane, as well as that of the lipid phase itself, determine protein and lipid diffusion.

Saffman (7, 8) has introduced one possible theoretical model in which an intrinsic membrane protein is represented as a cylinder of radius a spanning a fluid membrane of thickness h and viscosity η (Fig. 1). This is bathed by fluids of viscosities μ_1 and μ_2. The system is characterized by a dimensionless parameter $\epsilon = (\mu_1 + \mu_2)a/\eta h$. The model assumes that the viscosities of the bathing solutions are very much smaller than the viscosity of the membrane, $(\mu_1 + \mu_2) \ll \eta$. Moreover, a is usually less than h, so that the resultant $\epsilon \ll 1$. A similar study by Anderson and Mazo (9) has considered the rotation of spheres and cylinder in a thin liquid film bounded by planar walls; they do not explicitly consider viscous coupling to the external (aqueous) medium and find only a small effect. Motion normal to the plane of the membrane is excluded in the Saffman model, consistent with the anisotropic ordering of membrane lipids and proteins. The effects of proteins protruding from the membrane, which will significantly increase the drag, are ignored. Saffman extracted, using a singular perturbation technique, the lateral drag coefficient in the limit of a very viscous membrane ($\epsilon \ll 1$). This model has recently enjoyed some application to experimental data but with only limited success (1). In particular, the failure of the model to simultaneously describe lateral and rotational diffusion coefficients has led to speculation on the possible role of cytoskeletal elements in the control of lateral motion (10, 11). Other reports have suggested that lateral diffusion may be more akin to diffusion through a polymer network (12, 13).

We have recently solved the Stokes problems for translational and rotational motion (14) in Saffman's model for all values of ϵ. The translational diffusion coefficient for $\epsilon \lesssim 1$ is

$$D_T = \frac{kT}{4\pi(\mu_1 + \mu_2)a} \cdot \epsilon\,[\ln(2/\epsilon) - \gamma + 4\epsilon/\pi - (\epsilon^2/2)\ln(2/\epsilon) + 0(\epsilon^2)], \quad (1)$$

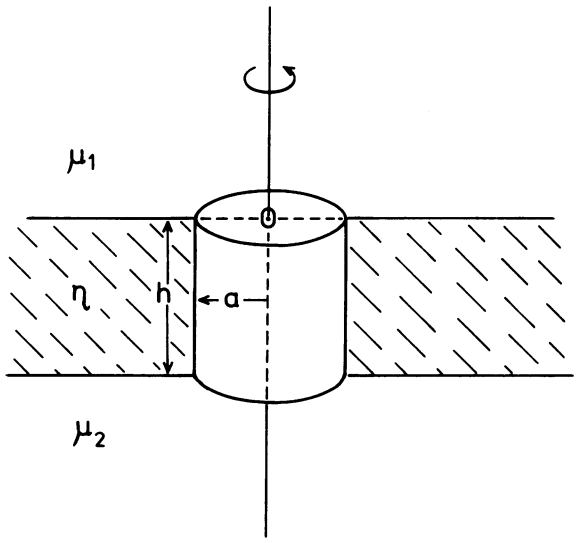

FIGURE 1 Model membrane system introduced by Saffman (7, 8) and comprising a cylindrical object of radius a embedded in a film of thickness h and viscosity η and surrounded by fluids of viscosities μ_1 and μ_2. The system is characterized by the dimensionless constant $\epsilon = (\mu_1 + \mu_2)\, a/\eta h$. The cylinder is constrained to move laterally in the membrane and to rotate about its axis normal to the membrane plane.

where k is Boltzmann's constant, T is the absolute temperature, and γ is Euler's constant (0.577). The exact result for arbitrary ϵ is available (14). Saffman's result is accurate for $\epsilon \lesssim 0.1$.

The asymptotic result for the rotational diffusion coefficient is given by

$$D_R \simeq \frac{kT}{4\pi(\mu_1 + \mu_2)a^3} \cdot \left[\frac{\epsilon + 0(\epsilon^2)}{1 + 8\epsilon/3\pi + 0(\epsilon^2)}\right] \qquad (2)$$

and is acceptable for $\epsilon \lesssim 2$. The exact result for larger ϵ is also available (14).

RESULTS AND DISCUSSION

Typical results of our approach are given in Table I. For lipids, the choices η = 2 poise (P) and $\bar{\mu} = (\mu_1 + \mu_2)/2$ = 0.01 – 2P give values of D_T that are in reasonable agreement with experiment ($\sim 10^{-8}$ cm^2/s). However, only the choice $\bar{\mu}$ = 1P and η = 0.01 P allows D_R to approach experimentally observed values ($\sim 10^8$ s^{-1}) while keeping D_T reasonably bounded. Generally our predictions of D_R are smaller than experimental values. This is not surprising since the motion of fluorescent probes commonly used to estimate rotational relaxation times is not equivalent to the motion of phospholipid molecules themselves. For proteins, it is only by choosing $\bar{\mu}$ = 1–2P and η = 0.1–0.2P that D_T and D_R approach the experimentally determined range of values ($D_T = 10^{-9}$–10^{-8} cm^2/s; $D_R = 10^3$–10^5 s^{-1}) (1).

The results in Table I indicate that D_T and D_R are almost equally sensitive to membrane microviscosity, η. This appears at first sight to be at variance with the observation (15) that 50 mol % (mol/100 mol) cholesterol in the bilayer significantly decreases D_T for a membrane protein but leaves D_R unaffected (16). However, our hydrodynamic study (14) indicates that the rotational velocity field is considerably shorter ranged than that associated with translational motions. Thus rotational diffusion samples only the local lipid environment, whereas translational motions sample more of the bulk lipid. Given an annulus of boundary lipid around membrane proteins (17) we may expect a differential partitioning of cholesterol and other membrane lipids between the bulk and the region-neighboring proteins. The "taper-fray" accommodation of lecithin and cholesterol (18, 19) in mixed-lipid vesicles further suggests that cholesterol may not contribute to the local environment of membrane proteins and so may not affect protein rotational motions. Translational motions will, however, sample a cholesterol-rich environment.

The further result that the ratio $D_T/a^2 D_R$ is a function only of ϵ (Fig. 2) now facilitates an experimental determination of ϵ. Given simultaneous measurements of D_T and D_R, an estimate of the radius a (typically 5–10 Å for lipid

TABLE I
PREDICTED VALUES OF MEMBRANE DIFFUSION COEFFICIENTS*

Lipids (a = 5 Å, h = 50 Å)			$10^8 D_T$(cm^2/s)	$10^{-8} D_R$
$\bar{\mu}$	η	ϵ		
(P)	(P)			(s^{-1})
0.01	1	0.002	4.2	0.03
	0.1	0.02	27	0.26
0.1	1	0.02	2.7	0.03
	0.1	0.2	13	0.23
	0.01	2	47	1.1
1	1	0.2	1.3	0.03
	0.1	2	4.7	0.11
	0.01	20	5.1	0.25
2	0.1	4	2.0	0.08
	0.01	40	2.6	0.13
Proteins (a = 50 Å, h = 75 Å)			**$10^9 D_T$**	**$10^{-4} D_R$**
$\bar{\mu}$	η	ϵ		
(P)	(P)			
0.01	1	0.013	20	1.3
	0.1	0.13	84	15
0.1	1	0.13	9	1.5
	0.1	1.3	28	9.4
1	0.1	13.3	5	2.6
2	0.2	13.3	2.0	1.9

*Calculated translational and rotational diffusion coefficients for the model membrane system defined in Fig. 1. Particle radius a and membrane thickness h are taken to be (a) a = 5 Å, h = 50 Å (lipids), and (b) a = 50 Å, h = 75 Å (proteins). The average outer bathing viscosity is $\bar{\mu}$ = ½ ($\mu_1 + \mu_2$) (in Poise) and the membrane microviscosity is η; the dimensionless membrane parameter is $\epsilon = 2\,\bar{\mu}a/\eta h$.

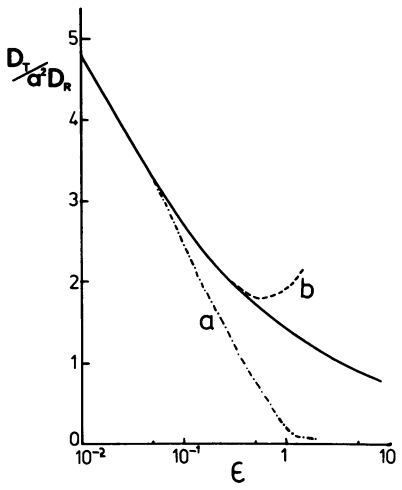

FIGURE 2 Ratio of translational to rotational diffusion coefficients D_T/a^2D_R as a function of ϵ for the system defined in Fig. 1. The solid curve is the exact (12) result, and the dashed curves are (*a*) predicted by Saffman (7, 8) and (*b*) calculated from our approximate Eqs. 1 and 2.

and 30–80 Å for protein) and membrane thickness h, we directly calculate ϵ and the viscosities $\bar{\mu} = (\mu_1 + \mu_2)/2$ and η. Complete data are only available in a few instances: rhodopsin (20, 21), erythrocyte band 3 protein (11, 16), and some lipid analogues (22, 23). Our preliminary calculations indicate $\epsilon \gtrsim 1$, a high bathing viscosity ($\bar{\mu} \simeq 1$ P), and relatively low microviscosity ($\eta \simeq 0.2$P). This is contrary to the conventional wisdom that a membrane is highly viscous ($\eta \gtrsim 1$ P) and is bathed by a low viscosity, typically aqueous medium ($\bar{\mu} = 0.01$ P). Saffman's pioneering study (8) has explicitly assumed these conditions and so has been restricted to $\epsilon \ll 1$.

It is clear that failure of a particular choice of a, h, $\bar{\mu}$, and η to describe D_T and D_R simultaneously argues against the applicability of the present model. In particular, we expect protrusions of proteins beyond the membrane interface into the aqueous phase to significantly increase the drag forces when ϵ is large.

It is now apparent that membrane diffusion experiments cannot be interpreted in terms of a microviscosity (η) alone. The bathing viscosities (μ_1, μ_2) also play an important role. Several points arise from these calculations. In the case of lipids, there is an encouraging agreement between the experiment and theoretical values of D_T. The disagreement in the case of D_R may be due to the experimental method of its determination. Most values come from fluorescence depolarization measurements that are influenced by an order component (24) as well as by the motion of the fluorophore, which may be different from the motion of the lipid about the bilayer normal (Fig. 1). In addition, there is the difficulty of using the Perrin equation to determine the relaxation time of an asymmetric fluorophore in an anisotropic environment (25). Estimates of D_R might well be improved by taking into account the flexible nature of the diffusing species. In the case of membrane proteins, the results suggest that the bathing viscosities are much greater than is usually envisaged. Such an effect would be provided by the glycocalyx, or, in the case of whole cells, by the significant viscosity of the cytoplasm.

Interpretation of lateral motion in terms of diffusion through a polymer network provided by cytoskeletal elements can be recast to explain the apparently large $\bar{\mu}$ indicated in the present study. Future experiments that vary the bathing viscosity (e.g., by the addition of appropriate polymeric solutes) should be of interest. Finally, we urge simultaneous measurement of D_T and D_R, to facilitate calculation of both $\bar{\mu}$ and η.

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