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Effect of Hydrodynamic Interactions on the Diffusion of Integral Membrane Proteins: Diffusion in Plasma Membranes

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ABSTRACT Tracer diffusion coefficients of integral membrane proteins (IMPs) in intact plasma membranes are often much lower than those found in blebbed, organelle, and reconstituted membranes. We calculate the contribution of hydrodynamic interactions to the tracer, gradient, and rotational diffusion of IMPs in plasma membranes. Because of the presence of immobile IMPs, Brinkman's equation governs the hydrodynamics in plasma membranes. Solutions of Brinkman's equation enable the calculation of short-time diffusion coefficients of IMPs. There is a large reduction in particle mobilities when a fraction of them is immobile, and as the fraction increases, the mobilities of the mobile particles continue to decrease. Combination of the hydrodynamic mobilities with Monte Carlo simulation results, which incorporate excluded area effects, enable the calculation of long-time diffusion coefficients. We use our calculations to analyze results for tracer diffusivities in several different systems. In erythrocytes, we find that the hydrodynamic theory, when combined with excluded area effects, closes the gap between existing theory and experiment for the mobility of band 3, with the remaining discrepancy likely due to direct obstruction of band 3 lateral mobility by the spectrin network. In lymphocytes, the combined hydrodynamic-excluded area theory provides a plausible explanation for the reduced mobility of sIg molecules induced by binding concanavalin A-coated platelets. However, the theory does not explain all reported cases of "anchorage modulation" in all cell types in which receptor mobilities are reduced after binding by concanavalin A-coated platelets. The hydrodynamic theory provides an explanation of why protein lateral mobilities are restricted in plasma membranes and why, in many systems, deletion of the cytoplasmic tail of a receptor has little effect on diffusion rates. However, much more data are needed to test the theory definitively. We also predict that gradient and tracer diffusivities are the same to leading order. Finally, we have calculated rotational diffusion coefficients in plasma membranes. They decrease less rapidly than translational diffusion coefficients with increasing protein immobilization, and the results agree qualitatively with the limited experimental data available.

INTRODUCTION

Plasma membranes of eucaryotic cells are lipid fluid suspensions containing integral membrane proteins (IMPs) and other proteinaceous and glycosylated species. Many IMPs are receptors that bind extracellular ligand molecules with high specificity to stimulate a variety of cellular functions such as growth, motility, secretion, and nutrient uptake (Alberts et al., 1989). Stimulation often requires the interaction of occupied receptors either with each other or with other cell surface structures. For example, multivalent ligands can stimulate basophil and mast cells to secrete histamine and serotonin by cross-linking IgE-occupied Fc_ϵ receptors (Metzger et al., 1986; Erickson et al., 1991). Also, extracellular low density lipoprotein (LDL) binds the LDL receptor, causing the complex to aggregate in coated pits—structures designed for internalization (Goldstein et al., 1981). In each of these cases, and many others, receptor diffusion in the plane of the plasma membrane is essential for proper cellular physiological responses.

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Lateral diffusivities of IMPs at infinite dilution in reconstituted membranes are described adequately by the hydrodynamic theory of Saffman and Delbruck (1975; Peters and Cherry, 1982; Vaz et al., 1984). However, diffusion of IMPs in plasma membranes is significantly slower than predicted by Saffman-Delbruck theory. There are several possible reasons for this reduced mobility: 1) The high concentration of proteins restricts lateral motion due to crowding. We call these *excluded area* or *thermodynamic* effects. 2) A significant fraction of IMPs (~30% of the IMPs in unstimulated cells (Edidin, 1987; Tank et al., 1982)) is immobilized. As a result, there are *hydrodynamic* effects on the remaining mobile proteins. 3) The lateral motion of proteins may be *obstructed* by cytoskeletal structures on the cytoplasmic face of the membrane. 4) Mobile protein may *bind directly* with immobile structures, either proteins or cytoskeleton, inside or outside the membrane.

In this paper, we calculate tracer, gradient, and rotational diffusivities of IMPs in membranes containing immobile particles. We calculate how hydrodynamic interactions influence the mobilities of IMPs when a fraction of them is immobile (item 2 above). Brownian motion of tracer IMPs within a macroscopically homogeneous environment is driven by thermal energy, k_bT. Tracer diffusion is defined as the ratio of the mean-square displacement of a tracer particle in a homogeneous environment divided by $2nt$, where n is the dimensionality and t is time. In addition to tracer diffusion, IMPs also undergo gradient (mutual) and rotational diffusion. The

coefficient of proportionality that relates concentration gradients of mobile IMPs to their fluxes is the gradient diffusion coefficient. In general, gradient diffusion coefficients differ from tracer diffusion coefficients. Finally, rotational diffusivities of IMPs characterize the redistribution of their angular orientation with time.

There have been many experimental measurements of various diffusivities of IMPs in plasma membranes. Fluorescence photobleaching and recovery (FPR), also known as fluorescence recovery after photobleaching (FRAP), is the most frequently used technique to measure tracer diffusivities (Axelrod et al., 1976). The fluorescence emission of labeled IMPs is irreversibly bleached in a small spot by a flash from a laser, and the subsequent recovery of fluorescence into the bleached spot is monitored by an attenuated laser beam that excites the fluorescent emission from the mobile, labeled probe. The technique measures long-time tracer diffusion coefficients, D_l, of proteins in the absence of concentration gradients. Typically, D_l for IMPs in plasma membranes are one or more orders of magnitude less than D_l for IMPs at similar concentrations in organelle or reconstituted membranes (Edidin, 1987). Therefore, the reduction is not attributable entirely to effects of IMP concentration. Moreover, this difference diminishes or disappears when plasma membranes are treated so that they separate from the underlying cytoskeletal components (Tank et al., 1982; Sheetz et al., 1980; Thomas et al., 1992). In these abnormal membranes, tracer diffusivities are greatly enhanced. Hence, cytoskeletal interactions are likely responsible for some of the reduction in lateral mobilities. These cytoskeletal interactions may be due to hydrodynamics, obstruction, or direct binding.

Another experimental technique, post-electrophoresis relaxation (PER), has been used to measure gradient diffusion coefficients, D_g, of IMPs (Young et al., 1984). In these experiments, an electric field induces migration and accumulation of IMPs that depends on the charges of both the proteins and phospholipid molecules. Analysis of the relaxation of established concentration gradients after reduction or elimination of the electric fields enables the determination of IMPs' gradient diffusivities. Some experiments indicate that D_g is much greater than D_l (Zagyansky and Jard, 1979), whereas others indicate that they are nearly equal (McCloskey et al., 1984).

Finally, two techniques, phosphorescence anisotropy (Zidovetzki et al., 1986; Myers et al., 1992) and polarized fluorescence depletion (Rahman et al., 1992), measure rotational diffusion times of IMPs. Both techniques show that as levels of IMP immobilization increase, average times for protein rotation increase.

In this paper, we calculate the hydrodynamic interactions in plasma membranes and try to interpret observations of the diffusion of IMPs in plasma membranes using our theory. Previous theories have neglected hydrodynamics and only considered the effect of hard-core repulsions and interparticle forces (excluded area effects) on diffusion rates. For example, Saxton (1990b) performed Monte Carlo simulations of the diffusion of mobile IMPs in the presence of immobile ones at various concentrations of both populations. He concluded that the effects of obstructions by integral membrane proteins were unable to account for the large difference between the diffusion of band 3 in intact and spectrin deficient erythrocytes (Saxton, 1990b). Here, we include hydrodynamic interactions and consider whether a combined thermodynamic/hydrodynamic theory agrees better with experimental observations. Also, we extend the comparison to other receptor and cellular systems to determine the extent that hydrodynamic interactions influence mobilities in plasma membranes.

We focus on the strong influence of immobile IMPs on the hydrodynamics and drag of the remaining fraction of mobile IMPs. In earlier papers, we explored the effect of hydrodynamic interactions on the tracer and gradient diffusivities of IMPs in organelle and reconstituted membranes that lack immobile IMPs (Bussell et al., 1992, 1994, 1995).

We assume that the fraction of IMPs immobilized by cytoskeletal components remains immobile on a time scale, t_i, that is long compared with the time-scale necessary to establish diffusion. This assumption enables us to treat the plasma membrane as a suspension containing separate populations of mobile and immobile proteins, and its strict validity depends on the mode of diffusive interaction we consider. PER experiments show that when gradients are established for times less than 5 min in unstimulated cells, they relax fully after the electric field is removed. For times of 30 min or more, the gradients do not relax fully (Poo, 1981). This suggests that the time scale for rearrangement of the structures that immobilize IMPs is 5 min $< t_i <$ 30 min, which is sufficiently long to establish diffusion in all modes we shall consider. Although an objective of this paper is to calculate tracer diffusivities observed over *long times*, the role of hydrodynamics can be incorporated by calculating tracer diffusivities over *short times*, as we show. Because the time-scale to establish short-time diffusion is $O(10^{-5}$ s), it appears that our assumption concerning the time-scale for immobilization is quite reasonable for tracer diffusion. Furthermore, the theory that we develop does not require that the immobilized IMPs are completely fixed; instead, the immobile IMPs must exert net forces on, and resist the flow of, lipid molecules while exerting equal and opposite forces on cellular structures like the cytoskeleton.

In this paper, we explain that if a fraction of IMPs is immobile, hydrodynamic interactions between the immobile proteins and the remaining mobile ones greatly reduce their diffusivities. This fact alone helps explain why tracer diffusivities in a plasma membrane are greatly reduced compared with those in blebbed or reconstituted membranes. Several experimental studies on erythrocytes and lymphocytes provide some support for the theory. However, lateral mobilities in most systems cannot be explained completely by the simple addition of hydrodynamics to the thermodynamic theory. Discrepancies are attributed to obstruction and direct binding during protein lateral motion.

GENERAL HYDRODYNAMIC THEORY OF FIXED BEDS

The fluid dynamics of suspensions with immobile particles, called fixed beds in the engineering literature, are well known, and we rely heavily upon fundamentals already elucidated in the study of these suspensions. The governing equations for a particle in an incompressible Newtonian fluid undergoing creeping flow are the N-body Stokes equation and the equation of continuity. These equations are based on the assumption that the intervening lipid fluid is a continuum, which is strictly true only if the protein radius is significantly greater than the radius of lipid molecules. These equations are ensemble-averaged to formulate equations that can be solved for effective quantities, such as the effective viscosity or permeability (Hinch, 1977).

The averaged equations for a fixed bed were first studied by Brinkman (1947) for spheres, and later fully solved by Howells (1974) and Hinch (1977). Based on physical reasoning, Brinkman characterized a medium with a fixed array of obstacles by a permeability and added an additional term, $\mu k^{-1}\boldsymbol{u}$, to the governing Stokes equation to account for the drag exerted by fixed particles. The Brinkman equation is

$$-\nabla p + \mu\nabla^2\boldsymbol{u} + \mu k^{-1}\boldsymbol{u} = 0, \tag{1}$$

where p is pressure, μ is membrane viscosity, $\boldsymbol{u}$ is velocity, and k is the isotropic permeability. Brinkman's equation, as we will show later, is the governing fluid dynamic equation for membranes with immobile proteins. $k^{1/2}$ is the length-scale of hydrodynamic interactions in the membrane, and we use it to make important insights into the physics of hydrodynamic interactions in plasma membranes. Brinkman derived this equation based on heuristic arguments, and it was formally derived later by Hinch using ensemble averaging (Hinch, 1977). A self-consistent approximation matches the pressure drop through a fixed bed with the viscous drag on the particles to determine the value of the permeability for a given area fraction of fixed particles, ϕ_i. The definition of ϕ_i is

$$\phi_i = \frac{\pi a^2 N_i}{A}, \tag{2}$$

where N_i is the number of immobile particles in the membrane, a is their radius, and A is the membrane area.

The results for the permeability and mobility of a single cylindrical particle moving in a planar membrane based on the solution of the Brinkman equation are (Howells, 1974)

$$a^2k^{-1} = \frac{4\phi_i}{4\pi\mu h m_s} \tag{3}$$

$$\frac{1}{4\pi\mu h m_s} = \frac{1}{2}a^2k^{-1} + ak^{-1/2}\frac{K_1(ak^{-1/2})}{K_0(ak^{-1/2})},$$

where the mobility, m_s, is defined as the ratio of the disk velocity to the force the disk imparts to the fluid, h is the membrane thickness, and K_0 and K_1 are modified Bessel functions of order 0 and 1, respectively. The results for k and m_s are given in Fig. 1 and 2, respectively. As ϕ_i increases, both k and m_s decrease. Wiegel (1979) used similar equations to describe the effect of cross-linking on the mobility of small, permeable receptor aggregates.

We must determine whether the dynamics and stresses in the aqueous fluid surrounding the membrane influence the diffusion of IMPs. A plasma membrane is not merely a planar fixed bed as modeled by Brinkman's equation: it is a suspension of IMPs in a viscous lipid bilayer sandwiched between less viscous aqueous phases.

The aqueous phases have been shown to have an effect on the mobility of an isolated IMP, m_0 (Saffman, 1976; Saffman and Delbruck, 1975). In a membrane in which there are no immobile proteins,

$$m_0 = \frac{1}{4\pi\mu h}[\ln(\lambda) - \gamma], \tag{4}$$

where $\lambda = \mu h/\mu' a$, μ is the membrane viscosity, h is its thickness, and μ' is the viscosity of the aqueous phases surrounding the membrane. A typical value for λ in biological membranes is 250 (Saffman, 1976). The derivation of (4) is

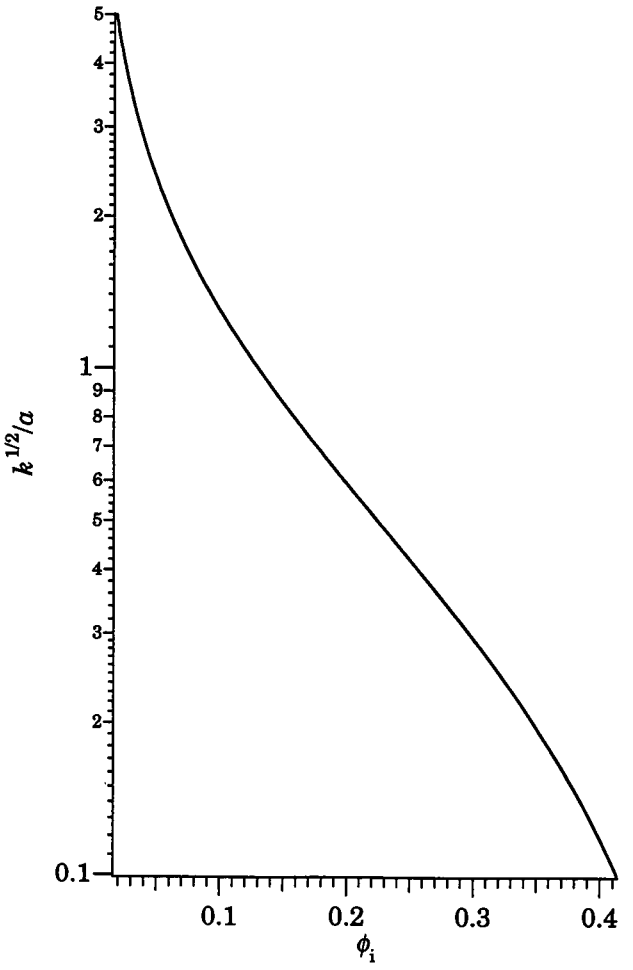

FIGURE 1 The dimensionless screening length, $k^{1/2}/a$, as a function of ϕ_i, the area fraction of immobile IMPs. The screening length is an indication of the length scale for hydrodynamic interactions.

based on an asymptotic analysis (Saffman, 1976). In an inner domain, $r \ll O(\lambda a)$, where r is the distance from the IMP, the IMP is treated as a disk in an isolated planar membrane. The viscous drag of the aqueous phase is insignificant compared with the drag in the membrane because $\mu' \ll \mu$. The solution for the velocity of the lipid molecules in the inner domain has a logarithmic divergence (Stokes paradox) and, consequently, does not decay. This necessitates the formulation of an outer solution.

At $r \sim O(\lambda a)$, viscous dissipation in both the aqueous phases and the membrane are equally important. Accordingly, an outer solution that accounts for flows in all phases but treats the IMP as a point force can be formulated. The outer solution decays to zero as required by the boundary conditions, and the two solutions are matched in the intermediate regime, where both are valid. The distance $O(\lambda a)$ is a screening length at which interactions in the membrane decay (Saffman, 1976). The distance λa is also the length-scale over which IMPs interact in lipid bilayers that contain no immobile IMPs (Bussell et al., 1992, 1994, 1995).

In contrast with IMPs in constraint-free membranes, proteins interact strongly in plasma membranes because of the presence of immobile particles. At radial separations larger than the screening length, $k^{1/2}$, velocity disturbances caused by mobile proteins decay because of the drag exerted by the fixed proteins. If $k^{1/2} \ll \lambda a$, particle interactions dominate the interactions between the aqueous phases and IMPs. In this case, the aqueous phases will have a negligible effect on the dynamics of the membrane. For example, if $\phi_i = 0.02$, $k^{1/2}/a \sim 5 \ll \lambda(\lambda$ typically equals 250 or more). At appreciable ϕ_i, values for m_s resulting from (3) are less than the value of m_s from Saffman-Delbruck theory (Saffman and Delbruck, 1975). In fact, the two values for m_s approach each other only when ϕ_i is as low as 10^{-5}, at which $k^{1/2}/a \sim 300 \sim O(\lambda)$. This implies that the immobilization of a very small fraction of proteins in a membrane will alter greatly the hydrodynamic interactions and the rate of protein diffusion in the membrane. It also implies that the hydrodynamics of the aqueous phases can be neglected and Brinkman's equation is applicable when studying protein dynamics in plasma membranes.

Equation 1 is an ensemble-averaged equation and, independent of the neglect of the aqueous phase surrounding the membrane, only approximates the hydrodynamics occurring in a two-dimensional fixed bed. Howells (1974) evaluated the errors inherent in using (1) to generate a solution for the mobility, m_s, of a particle in a fixed bed by evaluating additional particle interactions that are not included in Brinkman's equation. He carried out this analysis for three-dimensional suspensions of spheres as well as planar arrays of disks. Although (3) is strictly valid in the limit $\phi_i \to 0$, the errors are small unless the area fraction of immobile particles in the medium exceeds 0.3 (Howells, 1974). Consequently, predictions based on m_s obtained from solutions of Brinkman's equation are accurate over a range of immobile fractions relevant to describe diffusion in plasma membranes.

The asymptotic behavior for k and m_s as $\phi_i \to 0$ in (3) is important because it identifies the strength of the interactions between the mobile and immobile particles. After taking the limit of $K_0(x)$ and $K_1(x)$ as $x \to 0$, we find

$$\frac{k}{a^2} = \frac{\pi\mu h m_s}{\phi_i} = \frac{\ln(1/4\phi_i)}{4\phi_i}. \tag{5}$$

Equation 5 is valid for $\lambda^{-2} \ll \phi_i \ll 1$. Therefore, hydrodynamic interactions between IMPs have a large effect on particle mobilities even as $\phi_i \to 0$. Even though the fixed bed results are only valid when $k^{1/2}$ is much less than λ, the result given by (5) is indicative of the strong effect of the fixed bed on the mobilities of mobile IMPs.

The scaling of m_s with ϕ_i in (5) also indicates that interactions between mobile IMPs are weaker than their interactions with the fixed bed. The strength of hydrodynamic interactions between mobile IMPs scales like $O(\phi_m)$ (Bussell et al., 1994), where ϕ_m is the area fraction of mobile proteins. Consequently, hydrodynamic interactions of mobile particles with each other are asymptotically smaller than those between mobile IMPs and the fixed bed at small area fractions, and we assume they can be ignored to leading order.

CALCULATION OF DIFFUSION COEFFICIENTS

Having determined that the lipid motions induced by mobile IMPs are governed by Brinkman's equation and assumed that interactions between mobile IMPs are small, we now relate the solutions of Brinkman's equation to the various diffusion coefficients.

For time periods $t_s \sim t \ll a^2/D$, where a^2/D is $O(10^{-5}$ s) for tracer diffusion coefficients, D, $\sim 10^{-9}$ cm^2/s and protein radii, a, ~ 2 nm, IMPs move small distances relative to a, and their relative positions remain nearly constant. t_s is the shortest time for which a translational diffusion constant can be defined. Accordingly, the diffusion coefficients are short-time tracer coefficients, D_s, and they are characterized by the viscous drag on the IMPs as they move through the suspension. The drag implicitly includes only the hydrodynamic interactions occurring between all IMPs in the membrane whether they are mobile or immobile. However, for $t \gg a^2/D$, relative motions between particles are large compared with a, and IMPs intermingle. Their diffusion is hindered by excluded area effects as well as hydrodynamic interactions. Diffusion coefficients for $t \gg a^2/D$ are characterized by long-time tracer diffusion coefficients, D_l, and are not necessarily the same as the short-time diffusion coefficients.

Diffusion coefficients are related to mobilities that are calculated from solutions to Brinkman's equation. Both translational and rotational diffusion coefficients and motions of IMPs in response to forces and torques are directly proportional to mobility coefficients. The mobilities are defined as

$$m_i = \frac{V}{z}, \tag{6}$$

where IMPs have mobilities m_i, and V is either their velocity or rotation rate in response to the respective force or torque, z. Brinkman's equation enables us to determine V given z, and thus we can calculate m_i from (6). The diffusion coef-

ficients are related to the mobilities by

$$\begin{aligned} \boldsymbol{D}_s &= k_b T m_s \boldsymbol{I} = D_s \boldsymbol{I} \text{ for short-time tracer diffusion,} \\ \boldsymbol{D}_l &= k_b T m_l \boldsymbol{I} = D_l \boldsymbol{I} \text{ for long-time tracer diffusion,} \\ -\boldsymbol{D}_g \cdot \nabla n_m &= n f^* m_g \text{ for short-time gradient diffusion, and} \\ D_r &= k_b T m_r \text{ for rotational diffusion,} \end{aligned} \tag{7}$$

where n_m is the number density of the mobile IMPs, and f^* is the thermodynamic driving force arising from concentration gradients.

TRACER DIFFUSION

The tracer diffusion of IMPs in plasma membranes is distinguished by the time scale over which the diffusion occurs. For $t \sim t_s \ll a^2/D$, mobilities, and hence diffusivities, are inversely proportional to viscous drag coefficients that are elucidated by the solution to Brinkman's equation, (1). For $t \gg a^2/D$, diffusion coefficients include the effects of obstructed particle motions as well as hydrodynamic interactions.

Short-time tracer diffusion

Short-time diffusion coefficients can be calculated readily from the results for m_s in Fig. 2 and (7). The dependence of D_s on ϕ_i also appears in Fig. 2. The results indicate that as ϕ_i increases from zero, D_s is different from D_0, the tracer diffusion coefficient of an isolated protein in a lipid bilayer of infinite extent given by Saffman-Delbruck theory. Thus, Saffman-Delbruck theory is not an appropriate description for short-time diffusion coefficients of IMPs in plasma membranes even as $\phi_m \to 0$ when $\phi_i \neq 0$.

Because of the strong effect of particle interactions, D_s decreases with increasing ϕ_i. Without considering long-time effects, we already see that immobilization of a fraction of IMPs has a profound effect on the mobilities and diffusivities of mobile ones. Because D_s depends solely on hydrodynamic effects, this trend is not seen if hydrodynamic interactions are excluded.

The short time scale for D_s, $t \ll 10^{-5}$ s, precludes its measurement by FPR or PER. Techniques fast enough to measure D_s exist (Konig et al., 1992; Esmann and Marsh, 1992), and Esmann and Marsh have measured the short-time diffusivity of the Na^+,K^+-ATPase in a protein-lipid dispersion (Esmann and Marsh, 1992). To our knowledge, the short time diffusivity of a protein has not been measured in a bilayer phospholipid membrane. If such an experiment were performed, it would be important that these proteins have a large transmembrane radius, or be cross-linked smaller proteins, for the continuum approximation to be valid. In addition, for full analysis of concentration effects, D_s would have to be measured at several protein-lipid ratios with varying immobile fractions.

Long-time tracer diffusion

Long-time diffusion coefficients are difficult to calculate analytically because motions of IMPs in plasma membranes

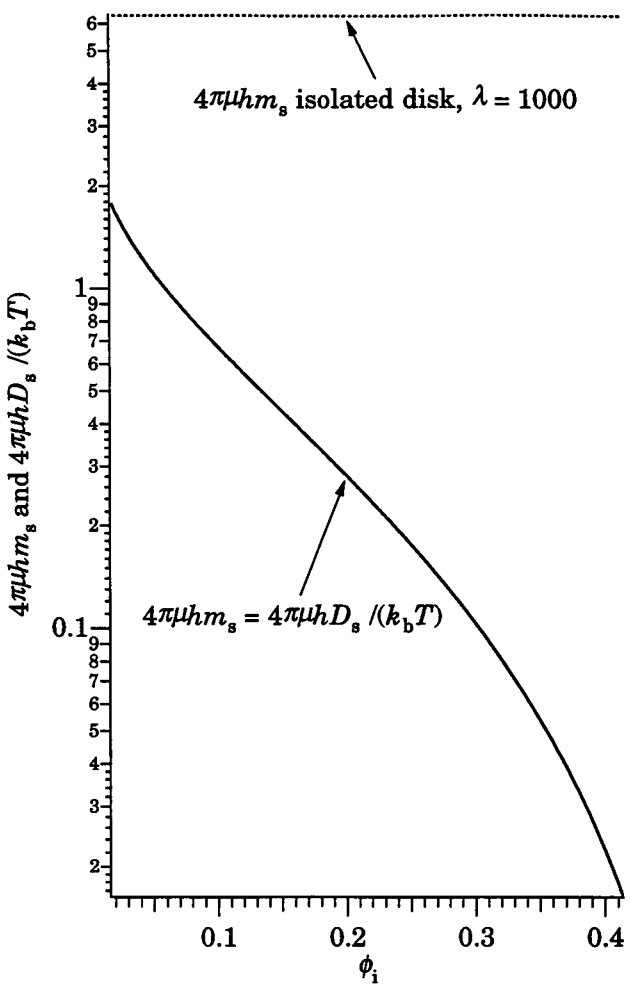

FIGURE 2 The mobility, m_s, and short-time diffusion coefficient, D_s, of IMPs in plasma membranes as a function of immobile area fraction, ϕ_i. Even at the lowest area fractions shown, the mobility of IMPs are significantly less than predicted by Saffman-Delbruck theory (· · · · ·).

are hindered by both mobile and immobile proteins. Furthermore, corrections for long-time effects are $O(\phi_t)$, the probability that a diffusing protein encounters a neighboring IMP, where ϕ_t is the total area fraction of all IMPs. The $O(\phi_t)$ correction is much smaller than the correction for the short-time diffusion, at least in the limit of low ϕ. Strictly speaking, it is inconsistent to calculate D_l to $O(\phi_t)$ because the calculated values for D_s are already more imprecise than this, having $O(1/\ln(\phi_i))$ errors. However, the work of Howells (1974) demonstrates that the results from Brinkman's equation for D_s are more accurate at high ϕ_i than can be expected based on asymptotic analyses. Moreover, a number of previous studies that calculate D_l exist, and we use these to complement our hydrodynamic theory for D_s to construct an approximate description for D_l for IMPs in plasma membranes.

A number of techniques have been used to calculate D_l. In Monte Carlo simulations, diffusivities calculated in computer time are related to real time using a reference diffusivity, D_{ref} (Saxton, 1990b). D_{ref} is the diffusivity that would

be experienced by a single particle over the shortest time a diffusivity can be defined. Hence, D_l is usually reported in relation to this reference diffusivity, D_l/D_{ref}. Because hydrodynamic interactions have been excluded from these calculations, D_{ref} is usually assumed to be D_o. However, hydrodynamic interactions influence the reference diffusivity, and we interpret D_{ref} to be the short time diffusivity, D_s, which includes hydrodynamic interactions. To calculate D_l, one may multiply D_l/D_{ref} and D_s,

$$D_l = \left(\frac{D_l}{D_{ref}}\right) D_s. \tag{8}$$

This technique has been used to calculate the long-time tracer diffusivity in concentrated, three-dimensional colloidal dispersions (Medina-Noyala, 1988; Brady, 1994), and it gives excellent agreement with measured diffusivities in three dimensions for which both short- and long-time diffusivity data are available.[1]

The calculations of D_l/D_{ref} most relevant to plasma membranes are those of Saxton (1990b), in which simulations were performed for different fractions of mobile and immobile IMPs. These techniques consider the effects of excluded area while neglecting the effects of hydrodynamic interactions in order to evaluate perturbations of D_l from the sterically unhindered reference diffusivity, D_{ref}. In the next section, we use Saxton's values for D_l/D_{ref}, which incorporate thermodynamic effects, with our calculations of D_s, which incorporate hydrodynamic effects, to calculate D_l using (8). We proceed to compare the resulting values for D_l with a variety of experimental observations on long-time diffusivities.

Analysis of experiments

Having developed a way of incorporating hydrodynamic effects into existing theories for long-time tracer diffusivity, we wish to compare the theory with the large body of experimental results on tracer diffusivities. In making our comparisons with published data, we assume that there is no difference between the radius of the portion of the IMP that is external to the membrane to that internal to the membrane. This assumption leads to a slight underestimate of excluded area effects and, hence, an overestimate of D_l/D_o.

We have mentioned that four factors affect tracer mobilities: 1) excluded area or thermodynamic interactions; 2) hydrodynamics; 3) obstruction; and 4) direct binding. The excluded area interactions have been calculated by Saxton (1990b) using Monte Carlo simulation. We are presenting the solution for hydrodynamic interactions in this paper. Now, we combine thermodynamic and hydrodynamic interactions into a combined theory and compare the results with experiments. Any deviations between this theory and experiments are likely to be attributable to cytoskeletal obstruction or direct receptor binding (items 3 and 4).

Our choices of which systems to analyze from the expansive literature on protein diffusion in plasma membranes are based on selection criteria. First, our hydrodynamic theories assume that the lipid is a continuum fluid, and that the protein radius is larger than the lipid radius. So, we can only analyze data on receptors with multiple transmembrane chains. This excludes some intensely studied systems, such as MHC class I antigens and GPI-linked proteins. Second, we must have data on diffusivity as a function of total or mobile protein concentration; presentation of an isolated datum point at a single concentration has little value. Third, we avoid systems in which the receptor is heterogeneous (e.g., concavalin A (conA) receptors) or in which the receptors are labeled heterogeneously (e.g., polyclonal antibodies). Fourth, it is unwise to compare the behavior of a receptor between cell types, because receptor-cytoskeletal interactions might be different in different cells. Fifth, we avoid systems in which labels fully stimulate cells and for which ϕ_i are unreported. Although we will not analyze data falling outside our selection criteria, we may mention such systems (mostly at the request of referees).

Erythrocytes

There have been many experiments on the diffusion of band 3 in plasma membranes of erythrocyte membranes. Band 3 and the glycophorins are known integral membrane proteins, with band 3 being the predominant protein present in 10^6 copies (Mohandas, 1991). The cytoskeleton of the erythrocyte consists of spectrin tetramers linked to actin oligomers at nodes. Roughly six tetramers may be bound to the nodes (Saxton, 1990b). Spectrin may dissociate from tetramer to dimer, and this dissociation depends on ionic strength, pH, temperature, and the presence of chemical agents such as amines (Tsuji and Ohnishi, 1986). Ankyrin binds spectrin to band 3, and band 4.1 links spectrin to glycophorin (Goodman et al., 1988; Bennett, 1989; Lux and Becker, 1989; Tsuji and Ohnishi, 1986; Saxton, 1990b).

Saxton (1990b) estimated the area fraction of band 3 to be roughly 0.1. Golan et al. (1984) estimated its area fraction to be 0.17. Both estimates are based solely on measurements of band 3 and ignore glycophorin and other proteins that might be present. Because 2-D SDS-PAGE with isoelectric focusing has identified many other erythrocyte proteins that have not been characterized (Mohandas, 1991), and because recently discovered erythrocyte integral membrane proteins such as aquaporin (Agre et al., 1993) are not included in this total, the area fraction of 0.1 is a conservative estimate. However, we restrict our detailed comparison to the range $0.1 \leq \phi_t \leq 0.17$, where ϕ_t is the total area fraction.

In normal erythrocytes, ϕ_i, the area fraction of immobile protein can be estimated from stoichiometry (Saxton, 1990b)

[1] Some readers might prefer to have diffusivity "referred" to the Saffman-Delbruck diffusivity, D_o, of a single tracer at infinite dilution. This is easily achieved by dividing both sides of (8) by D_o. In such a way, D_l can be referred to any diffusivity the reader wishes. Our main point is that Monte Carlo simulations of protein lateral mobility must be referred to the appropriate short-time diffusivity of the system.

from the relative numbers of band 3 and ankyrin molecules. Saxton (1990b) estimated that 40% of band 3 would be immobile.

There are two sets of erythrocyte experiments that we can compare with our theoretical predictions. The first compares the lateral mobilities of band 3 on normal erythrocytes and spherocytes, which are deficient in spectrin, ankyrin, and band 4.1 (Sheetz et al., 1980). For band 3 in erythrocytes, $D = 4.5 \times 10^{-11}$ cm^2/s. The experimental diffusivity on spherocytes is 2.5×10^{-9} cm^2/s, or a factor of 56 faster. We assume, as has Saxton (1990b), that band 3 is free of all lateral constraints on spherocytes. Even though these data do not strictly meet our selection criteria (data collected on more than one cell line and no directly determined information about ϕ_i), we analyze them to compare our theoretical results with previous theoretical treatments (Saxton, 1990b).

In Table 1 we illustrate, for the two cases where $\phi_t = 0.1$ and $\phi_t = 0.17$, the extent of lateral reduction for both cell lines expected from the thermodynamic theory, D_l/D_s (Saxton, 1990b), the hydrodynamic theory, D_s/D_o, or the combined thermodynamic/hydrodynamic theory, D_l/D_o.

Saxton (1990b) performed Monte Carlo simulations of protein mobility in mixtures of mobile and immobile proteins. For hexagons of radius 1 on a triangular lattice, the dependence of D_l/D_s on ϕ_m (the mobile area fraction) can be estimated from Fig. 1 of Saxton (1990b) as

$$\begin{aligned} D_l/D_s &= 0.6 + 1.9\phi_m \qquad \phi_t = 0.1 \\ D_l/D_s &= 0.33 + 1.9\phi_m \qquad \phi_t = 0.17. \end{aligned} \tag{9}$$

In Table 1, we show the magnitude of reduction due to thermodynamic interactions alone. If $\phi_t = 0.1$ and 40% of the integral membrane proteins are immobile, the degree of reduction due to thermodynamic effects is $D_l/D_s = 0.71$, a 29% reduction. If $\phi_t = 0.17$, D_l/D_s decreases to 0.52, a 48% reduction. The column in Table 1 marked D_s/D_o gives the reduction due to hydrodynamic interactions alone, as determined from Fig. 2. This column is generated by comparing the line marked $4\pi\mu hm_s$ with that of an isolated disk with $\lambda = 1000$. For $\phi_t = 0.1$, $D_s/D_o = 0.2$, a 80% reduction; for $\phi_t = 0.17$, $D_s/D_o = 0.16$, an 84% reduction. The combined hydrodynamic/thermodynamic theory at best gives a reduction in mobility, D_l/D_o of 0.08 (a factor of 13), which falls short of the factor of 56 reduction seen in experiments. Assuming that estimates of protein area fraction are accurate, the remaining discrepancy is likely due to lateral obstructions between the tail of band 3 and the spectrin network, because band 3 is a freely rotating molecule (Corbett et al., 1994). However, including the hydrodynamic interactions does bring the theory much closer to the experimental data than could be achieved with a solely thermodynamic model. We have calculated that if the total area fraction were 0.3, our combined thermodynamic/hydrodynamic results would be in good agreement with the experimental data (data not shown). This is another way of quantifying the discrepancy between our theory and experimental data. However, there is no evidence that the integral protein concentration in the erythrocyte membrane is this high.

A second set of relevant data was obtained by Golan and Veatch (1982) on the diffusion of band 3 in erythrocyte ghosts. They measured both D_l and ϕ_i using different conditions of ionic strength and temperature of the medium surrounding the membrane to vary ϕ_i (Golan and Veatch, 1982). These factors change the state of spectrin association and, hence, the degree of band 3 immobilization. The results for the long-time diffusion coefficients of band 3 are $D_l = 5 \times 10^{-10}$ cm^2/s for $\phi_i/\phi_t = 0.2$ and $D_l = 10^{-11}$ cm^2/s for $\phi_i/\phi_t = 0.9$. This represents a 50-fold variation in lateral mobility. Because band 3 is the most abundant IMP in erythrocyte membranes (Gennis, 1989), to make qualitative comparisons, we assume that its immobilization is representative of all the proteins in erythrocyte plasma membranes.

Again, we present our theoretical results as the magnitude reduction in D_l expected from thermodynamic, hydrodynamic, and combined thermodynamic/hydrodynamic theories. The results are given in Table 2. Again, we restrict ourselves to the two limits, $\phi_t = 0.1$ and $\phi_t = 0.17$. The main goal is to determine the level of reduction of lateral mobility due to the increase in immobilization from $\phi_i/\phi_t = 0.2$ to $\phi_i/\phi_t = 0.9$. Of the two reported values for ϕ_t, we see the best agreement at $\phi_t = 0.17$. Based on thermodynamics only, D_l decreases by a factor of 1.6 (D_l/D_s goes from 0.6 to 0.37; 0.6/0.37 = 1.6). Based on hydrodynamics only, we expect D_l to decrease by a factor of 2.9 (D_s/D_o goes from 0.21 to 0.073; 0.21/0.073 = 2.9). The overall reduction is a factor of 4.8, compared with a factor of 50 seen in the experiment. The combined thermodynamic/hydrodynamic theory shows the proper qualitative trend — that increasing immobilization substantially decreases the mobility — but still falls short by an order of magnitude in quantitatively accounting for the reduction in mobility. This discrepancy would narrow if our protein concentrations are underestimates (Bussell,

TABLE 1 Comparison between theory and experiment for the diffusion of band 3 in erythrocytes and spherocytes

ϕ_t	ϕ_i	ϕ_m	D_s/D_0*	D_l/D_s‡	D_l/D_0
0.1	0.04	0.06	0.2	0.71	0.14
0.17	0.068	0.102	0.16	0.52	0.08

Experimental data from Sheetz et al. (1980) shows $D_l/D_0 = 0.018$ (comparing erythrocytes to spherocytes).
*See Fig. 2 for D_s/D_0.
‡We used (9) to calculate D_l/D_s based on the work of Saxton (1990b).

TABLE 2 Comparison between theory and experiment for the diffusion of band 3 in erythrocytes ghost membranes

ϕ_t	ϕ_i/ϕ_t	D_s/D_0*	D_l/D_s‡	D_l/D_0	Reductions§
0.1	0.2	0.26	0.75	0.20	
0.1	0.9	0.12	0.62	0.074	2.7
0.17	0.2	0.21	0.60	0.13	
0.17	0.9	0.073	0.37	0.027	4.8

*See Fig. 2.
‡Calculated from (9) based on the work by Saxton (1990b).
§Factor reduction in going from $\phi_i/\phi_t = 0.2$ to $\phi_i/\phi_t = 0.9$.

1992), but for the moment it appears that the remaining reduction in mobilities is due to cytoskeletal obstruction of band 3.

Lymphocytes

We next apply the theory to systems in which lateral mobilities are altered by local binding of conA-coated platelets to surfaces of cells. These cells have receptors, likely heterogeneous, that bind conA. In many cell types, the mobilities of the remaining proteins can drop substantially after a certain threshold coverage of the cell surface with platelets. This reduction has been ascribed to the transient association of the "mobile" proteins with the cytoskeleton—either obstruction or binding. These experiments have been performed on several cell types (lymphocytes, fibroblasts, and macrophages) and examined the mobility of several different receptors (conA receptors; surface immunoglobulins, sIg; and the major histocompatibility class I antigen, H-2K^k) (Henis and Elson, 1981a, b; Henis, 1984; Henis and Gutman, 1983; Schlessinger et al., 1977). Of these experiments, the only set that satisfies our preset selection criteria are those involving sIg on lymphocytes (Henis and Elson, 1981a). We therefore explore these data in detail, and later mention the relevance of the hydrodynamic theory to other systems.

Henis and Elson (1981a) monitored the diffusion of surface immunoglobulins (sIg) labeled with monovalent fragments of rabbit anti-mouse IgG in mouse lymphocytes using FPR. The results for sIg show that as conA platelets are bound to the cell, the immobile fraction of sIg increases, and the diffusion coefficient of the remaining mobile sIg decreases.

We make several assumptions to calculate the theoretical results. First, we assume that the immobilization of sIg is representative of receptors as a whole on lymphocytes. We assume that the intrinsic viscosity of the membrane is 4 poise and that the total area fraction of the membrane is 0.25. Using $D_l/D_s = 0.15 + 1.6\ \phi_m$ for $0.07 \leq \phi_m \leq 0.25$, $D_l/D_s = 0.08 + 2.6\ \phi_m$ for $\phi_m \leq 0.07$ (Saxton, 1990b), and the results in Fig. 2 for D_s, we obtain the results in Fig. 3, where $k_bT/4\pi\mu h = 1.66 \times 10^{-9}$ cm^2/s. The dotted line on Fig. 3 represents the long time diffusivity calculated from Monte Carlo calculations for protein diffusion through a membrane with immobile obstacles (Saxton, 1990b). The dashed line represents the purely hydrodynamic results. The solid line represents the combined hydrodynamic/thermodynamic theory, which shows surprisingly good agreement with experiments. Decreasing or increasing the assumed value for ϕ_i would shift the experimental data left or right, respectively, while changing the value of μ from 4 poise to μ_2 poise would shift the theoretical curve up or down by a factor of approximately $4/\mu_2$. We have plotted this data as a function of the immobile fractions reported by the authors. Although it is clear that uncertainty in the immobile fraction will shift the theoretical predictions laterally, it will not affect the conclusion that the principle effect of protein immobilization is to decrease the diffusion to levels that are in qualitative agreement with the data. The correspondence between theory and experiment for D_l over a range of values for ϕ_i supports the idea that the immobile IMPs dominate the hydrodynamics of the suspension.

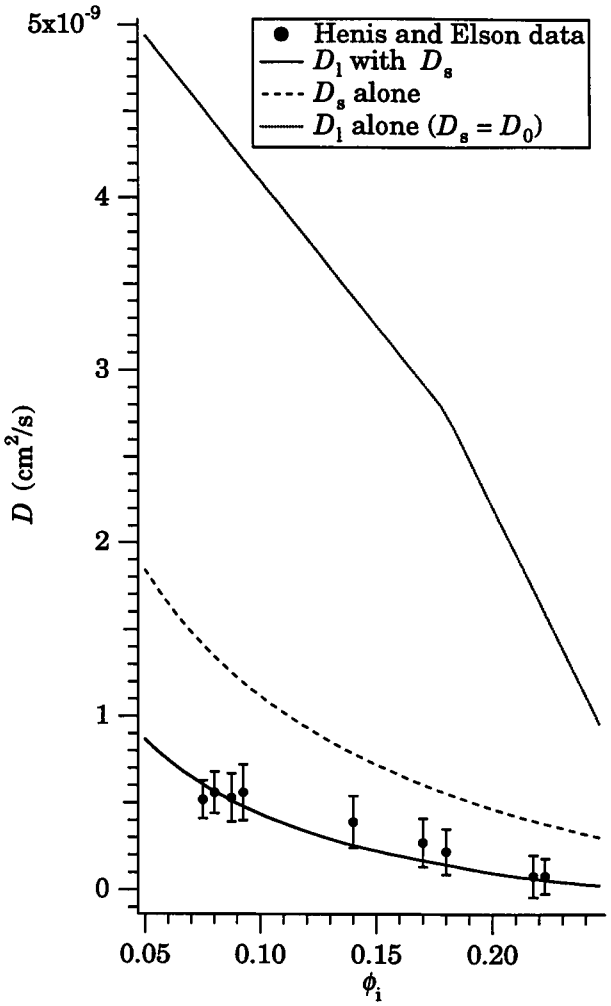

FIGURE 3 Comparison between theoretical prediction for D_l over a range of values with the measured values for sIg on leukocyte plasma membranes. Experimental data are interpreted from Table 1 of Henis and Elson (1981). The agreement between theory and experiment is good only if both short-time hydrodynamic and long-time thermodynamic interactions are considered.

Although the sIg/lymphocyte system shows good agreement with theory, it is clear that other systems in which protein mobilities are influenced by conA platelet binding do not. Henis and Elson (1981b) show enormous differences in behavior among cell types. In fibroblasts, they show that heterogeneous conA receptor mobility may decrease even though the immobile fraction remains unchanged. Schlessinger and co-workers (1977) show similar results, albeit using a polyclonal anti-murine antibody. So, it appears in fibroblasts that the reduced mobility may be due to direct receptor-cytoskeletal binding induced by conA platelet binding, rather than solely hydrodynamic interactions. In macrophages, no reduction in conA receptor mobility is seen, suggesting that this cell type is not stimulated by conA plate-

let binding (Henis and Elson, 1981b). This system sheds no light on the validity of the hydrodynamic model.

Our view is that hydrodynamics provides a reasonable explanation for the modulation of sIg mobility on lymphocytes. If conA platelets induce global immobilization of integral membrane proteins, then hydrodynamics dictates that the diffusivity of the remaining mobile sIg will decrease. The reduced mobility need not be due to obstruction or direct binding between sIg and cytoskeleton. Platelet binding could activate a third molecule that links receptors to the cytoskeleton.

Upon binding of conA-coated platelets in fibroblasts, the mobility of conA receptors decreases, although the immobile fraction of conA receptors remains unchanged (Henis and Elson, 1981b). One explanation is that the immobilization of conA receptors is independent of conA platelet binding in fibroblasts, and that conA platelet binding in fibroblasts induces a change in the overall immobile fraction of other receptors. Therefore, the diffusion coefficients of all remaining mobile proteins, including conA receptors, would decrease, because an increase in the overall immobile fraction would increase the hydrodynamic resistance to motion in the membrane.

An additional set of observations to be considered is the behavior of H-2K^k antigens in conA platelet-bound cells. As a single chain receptor, H-2K^k cannot be analyzed with the hydrodynamic theory unless it is cross-linked to form aggregates with radii significantly larger than the surrounding lipid. Experimentally, Bierer et al. (1987) observed H-2K^k antigens diffusing as fast as lipids when labeled with a monovalent Fab fragment, but diffused almost a factor of 10 more slowly when labeled with and cross-linked by the full antibody. Henis (1984) also observed H-2K^k antigens to diffuse more slowly when labeled (and cross-linked) by a monoclonal antibody. He did not use Fab fragment against H-2K^k antigens in his experiments. He also found that H-2K^k antigen diffusion does not decrease with increasing ϕ_i after labeling lymphocytes with conA platelets, unlike the behavior of sIg. Interpreting the results in light of the hydrodynamic theory, when labeled by the Fab fragment, H-2K^k antigens are single chained and diffuse like lipids. When cross-linked by antibody, the effective radius of H-2K^k molecules is sufficiently large to subject these species to partial hydrodynamic effects. Also, the lack of influence of conA platelet binding on H-2K^k antigen mobility suggests that cross-linked H-2K^k antigens are obstructed by the cytoskeleton, and this interaction dominates its mobility even when conA platelets induce global immobilization. H-2K^k antigens are likely not binding directly with the cytoskeleton, as the molecules freely rotate (Damjamovich et al., 1983).

GRADIENT DIFFUSION

The analysis of tracer diffusion involves IMPs moving within a homogeneous environment. In contrast, the analysis of gradient diffusion involves IMP motions in response to concentration fields.

Theory

All mobile IMPs in a local neighborhood experience the same thermodynamic force while undergoing gradient diffusion. The problem is relatively simple because of the nature of the hydrodynamic interactions in a Brinkman bed. Because the interactions between mobile IMPs are relatively weak, they are dominated by the interactions with the fixed bed. In addition, the thermodynamic driving force also only contributes a relatively weak $O(\phi_i)$ enhancement to the diffusivity (Batchelor, 1976). Therefore, the result for the gradient diffusivity, D_g, is identical to the result for D_l to leading order. This contrasts with the results for D_g in organelle or reconstituted membranes in which the differing physical forces occurring during tracer and gradient diffusion create leading order differences between D_g and D_l (Bussell et al., 1994).

Comparison with experiment

A complication in the comparison between theoretical predictions and experimental results for gradient diffusion coefficients is that some of the experiments used labeling procedures that are known to stimulate cell surfaces, consequently altering the diffusion processes by changing ϕ_i. Our result is consistent with experimental measurements of similar values for D_g and D_l for unoccupied and occupied Fc_ϵ receptors in rat basophilic leukemia (RBL) cells (McCloskey et al., 1984). In these experiments, a monovalent fluorescent antibody is used to label the receptors, thereby avoiding cross-linking between them. Our results also support the suggestion by Young et al. (1984), to explain the discrepancy between D_g and D_l in other systems (for example, see Zagyansky and Jard, 1979) for which the values differ by as much as two orders of magnitude. They hypothesize that the ligand used to label the receptors in the FPR experiments extensively cross-linked and immobilized the receptors, whereas the PER experiments used post-field labeling to tag receptors only after IMP migrations occurred, thereby avoiding cell stimulation. Different levels of immobile receptors in the two experiments would explain the discrepancy in the observed diffusion rates. Large decreases in m_s and D_s as ϕ_i increased as a result of cell stimulation would have caused FPR measured diffusivities to be much lower, as was observed for the diffusion of band 3 in erythrocyte membranes.

There also exist theoretical calculations of the influence of concentration on gradient diffusion for systems in which all proteins are mobile (Abney et al., 1989). Abney et al. (1989) concluded that there were little experimental data available to make reasonable comparisons with theory in these systems.

ROTATIONAL DIFFUSION

Up to this point, we have concerned ourselves with translational motions. However, Brinkman's equation can also be used to analyze rotational motions.

Theory

We calculate rotational diffusion coefficients, D_r, by solving for rotational mobilities, m_r, of IMPs in fixed beds. The two coefficients are related to each other by (7).

Equation 1 governs the velocity field generated by an IMP with a rotation rate Ω. We assume no slip boundary conditions on the surface of the IMP and require the velocity and pressure fields to decay as $r \to \infty$. The permeability in (1) is the same as shown in Fig. 1. After solving (1) for $\boldsymbol{u}$ and p, we find $p = 0$ and

$$u_i = \frac{a\epsilon_{ijk}\Omega_j x_k K_1(k^{-1/2}r)}{rK_1(k^{-1/2}a)}, \tag{10}$$

where Einstein notation is used in the equation.

The torque, $\boldsymbol{L}$, is related to the velocity field by

$$L_i = -\int_{r=a} \epsilon_{ijk} x_j T_{kl} n_l dS, \tag{11}$$

where $\boldsymbol{n}$ is the unit outward normal and $\boldsymbol{T}$ is the stress tensor. It is defined

$$\boldsymbol{T} = -p\boldsymbol{I} + \mu(\nabla\boldsymbol{u} + (\nabla\boldsymbol{u})^t). \tag{12}$$

Substituting (10) into (12), we find

$$T_{il}n_l = \frac{-\mu a}{r^2K_1(k^{-1/2}a)}\left\{K_1(k^{-1/2}r) + \frac{1}{2}k^{-1/2}a\left[K_0(k^{-1/2}r) + K_2(k^{-1/2}r)\right]\right\}\epsilon_{ijk}\Omega_j x_k. \tag{13}$$

Thus, the torque is

$$\boldsymbol{L} = \frac{2\pi a^2 h\mu\Omega}{K_1(k^{-1/2}a)}\left\{K_1(k^{-1/2}a) + \frac{1}{2}k^{-1/2}a\left[K_0(k^{-1/2}a) + K_2(k^{-1/2}a)\right]\right\}\boldsymbol{e}_3, \tag{14}$$

where $\boldsymbol{e}_3$ is the unit vector perpendicular to the membrane.

Equation 6 for m_r yields

$$m_r = \frac{K_1(k^{-1/2}a)}{2\pi a^2 h\mu}\left\{K_1(k^{-1/2}a) + \frac{1}{2}k^{-1/2}a\left[K_0(k^{-1/2}a) + K_2(k^{-1/2}a)\right]\right\}^{-1}. \tag{15}$$

Equation 15 can be simplified by using the relationships in (3) and the recurrence formulas for Bessel functions. We find that

$$\frac{m_r}{m_{r,0}} = 1 - 2\phi_i, \tag{16}$$

where $m_{r,0}$ is the rotational mobility of an isolated protein in a lipid bilayer of infinite extent, which equals $(4\pi a^2 h\mu)^{-1}$ (Saffman and Delbruck, 1975). The rotational mobility, m_r, is a weaker function of ϕ_i than is the translational mobility, m_s. Although m_r approaches a constant for small ϕ_i, m_s is proportional to $1/\ln(1/\phi_i)$ when $\phi_i \ll 1$ and $k^{1/2}/a \ll \lambda$. These distinctions between the behaviors of the mobilities arise from the different dependencies of the velocity fields produced by isolated rotating and translating disks on radial position. The velocity field produced by a rotating disk decays like $1/r$, but the velocity produced by a translating disk behaves like $\ln r$ for $r \ll k^{1/2}$ and only decays for $r \gg k^{1/2}$. Thus, the surrounding immobile disks have a profound effect on the velocity produced by a translating disk at $\phi_i \ll 1$, whereas only a relatively minor effect on the velocity produced by a rotating disk.

Inserting (16) into (7), we find

$$\frac{D_r}{D_{r,0}} = 1 - 2\phi_i, \tag{17}$$

where $D_{r,0}$ is the rotational diffusion coefficient of an isolated IMP in a lipid bilayer (Saffman and Delbruck, 1975). Equation 17 is only valid in the limit $\phi_i \to 0$. The result shows that decreases in D_r with increasing ϕ_i are much less pronounced than decreases in D_s, D_l, or D_g, which undergo orders of magnitude changes.

Comparison with experiment

Data on rotational motion (Zidovetzki et al., 1986; Myers et al., 1992; Rahman et al., 1992) show that average times for rotation increase by factors less than 10 as ϕ_i increases. This is consistent with our results for D_r but, unfortunately, the experimental data do not enable a more detailed comparison.

DISCUSSION

The lateral motion of proteins in plasma membranes is likely influenced by four factors: 1) excluded area (or thermodynamic) effects; 2) hydrodynamic interactions; 3) obstruction of lateral mobility by cytoskeleton; and 4) direct binding between receptor and immobile structures. In this paper, we have calculated the hydrodynamic interactions in plasma membranes by modeling them as two-dimensional fixed beds of proteins. This theory is based on the continuum hypothesis that the protein is larger than the lipid. Its applicability is restricted to a subset of proteins that are appropriately large. Using the hydrodynamic interactions, we construct a combined thermodynamic/hydrodynamic theory. We use this theory to analyze existing data of lateral mobilities in plasma membranes that satisfy a set of selection criteria. In general, agreement of experimental findings with the combined theory is better than the agreement between the hydrodynamic or thermodynamic theory alone. However, the combined theory fails to account quantitatively for many observations of protein lateral mobility. These discrepancies between theory and experiment are likely due to obstruction or direct binding, neither of which is incorporated into the theory yet.

We have presented physical arguments that Brinkman's equation governs the fluid dynamics in plasma membranes and have used it to solve for mobilities of integral membrane proteins. At appreciable ϕ_i, small hydrodynamic screening lengths dominate membrane dynamics, and ϕ_i is the dominant determinant of the short-time mobilities and diffusion coefficients of IMPs. The main implication is that immobilization of a very small fraction of proteins greatly decreases translational diffusivities. This principle explains why mobilities are greatly reduced in plasma membranes, which generally have a significant fraction of immobile proteins.

Monte Carlo results developed by Saxton (1990b) incorporate excluded area and structural effects into calculations of D_l/D_{ref} for mixtures of mobile and immobile proteins. Hydrodynamic interactions are excluded from these calculations. The combination of hydrodynamic results for D_s/D_o with Saxton's results for D_l/D_{ref} allows us to solve for D_l/D_o that includes both hydrodynamic and thermodynamic interactions.

The dependence of the short-time translational mobilities on ϕ_i is given in Fig. 2. Translational mobilities are proportional to $\ln(1/\phi_i)$ at low ϕ_i and decay with increasing ϕ_i. They decrease by orders of magnitude relative to m_o as ϕ_i approaches 0.3. When $\phi_m \to 0$, ϕ_i finite, values of D_s for IMPs in plasma membranes are much smaller than predicted by Saffman-Delbruck theory. The difference arises because of the large inhibitory effect of the fixed bed on the mobilities of mobile proteins. This is consistent with Saffman-Delbruck theory, which strictly applies only at infinite dilution ($\phi_m \to 0$, $\phi_i \to 0$) (Peters and Cherry, 1982). These hydrodynamic arguments suggest that the Saffman-Delbruck diffusivity is not the appropriate reference diffusivity in plasma membranes containing immobile particles.

The method of FPR has been used to measure D_l in a wide range of IMP-membrane systems. We have compared the combined thermodynamic/hydrodynamic theory with experimental observations. In erythrocytes, the addition of hydrodynamics improves the comparison between theory and data on band 3 lateral mobility. For the best case for reported total area fraction, in which $\phi_t = 0.17$, the combined theory predicts a 13-fold reduction in the lateral mobility between spherocytes and normal erythrocytes, even though a 56-fold reduction was observed (Sheetz et al., 1980). This discrepancy is likely due to obstruction, because band 3 rotates without restriction (Corbett et al., 1994). Similar results were found when comparing the combined theory with data on band 3 lateral mobility in erythrocyte ghosts that had been treated in ways to modify the polymerization of the spectrin network (Golan and Veatch, 1982).

In lymphocytes, the binding of conA-coated platelets dramatically slows the mobility of sIg molecules (Henis and Elson, 1981a). Henis and Elson reported both the immobile fraction and the mobility of remaining mobile sIg. We find that the dependence of sIg mobilities on immobile fraction is in excellent agreement with the combined hydrodynamic/thermodynamic theory. A critical assumption we make is that the immobilization of sIg is indicative of all receptors on the cell surface. This assumption must be tested. In contrast, the combined model does not explain the results seen on conA platelet-modified fibroblasts (Henis and Elson, 1981b) or of the lack of effect conA platelet binding has on H-2K^k mobilities (Henis, 1984). In fibroblasts, the discrepancy is most easily explained if the immobilization of conA receptors (the probe used to assess mobility) is not indicative of the immobilization of proteins as a whole. The mobilities of H-2K^k antigens are best explained by their small transmembrane radius and by direct receptor-cytoskeletal obstruction when these antigens are cross-linked by antibody. This illustrates that the combined theory does not explain all of the complex interactions that appear in plasma membranes.

The predictions for D_l in this paper are also consistent with nanovideo microscopy experiments on the motion of low density lipoprotein receptors on human skin fibroblasts (Ghosh, 1992). Ghosh observed a number of different modes of motion, only some of which were purely diffusive. We focus on the diffusive modes for comparison to our theory. When cells are treated with metabolic inhibitors that disrupt cytoskeletal constraints, diffusivities increase from 10^{-11} to 10^{-10} cm^2/s. Although this observation might be properly explained by percolation theories and transient constraints, it is also consistent with the hydrodynamic theory advanced in this paper. Moreover, when the proteins are observed to move purely diffusively, not by convection or affected by transient immobilization, in untreated cells, the diffusivities are still small (10^{-11} cm^2/s). Because IMPs are not transiently immobile during these motions, nor are they trapped, explanations of the slow diffusion based upon transient obstruction, direct binding or percolation effects are unsatisfactory. On the other hand, hydrodynamic theories are consistent with this data, predicting that unconstrained and untrapped proteins diffuse slowly because of the hydrodynamic interactions between mobile IMPs and the fixed bed (Ghosh, 1992).

Hydrodynamic theories are also consistent with observations that receptors lacking cytoplasmic tails move identically to those possessing tails (Ghosh, 1992). The membrane-spanning regions of mobile IMPs, not the cytoplasmic tails, are the controlling determinants of the hydrodynamic interactions between mobile IMPs and the fixed bed. Other systems show mobilities that are insensitive to the presence of the cytoplasmic tail, including VSV glycoprotein (Scullion et al., 1987), the EGF receptor (Linveh et al., 1986), and MHC class I molecules (Edidin and Zuniga, 1984). Some systems show a mobility that is sensitive to the cytoplasmic tail, including MHC class II molecules (Wade et al., 1989). This observation suggests the mobilities of these receptors are retarded by obstruction.

Other observed phenomena are qualitatively consistent with the combined hydrodynamic/thermodynamic theory. Tank et al. (1982) used a steroylated dextran to label membrane receptors on L6 myoblast membranes and studied the lateral mobility by FPR on intact and blebbed plasma membranes. They showed increased diffusivity (eightfold) in blebbed membranes compared with normal membranes.

Such a system falls outside our selection criteria because dextran labels a heterogeneous set of surface receptors. However, the dramatic increase in diffusivities is to be expected from the release of immobilized proteins in blebbed plasma membranes, consistent with the hydrodynamic theory. In fact, we analyzed the data quantitatively and were able to obtain excellent agreement between the combined hydrodynamic/thermodynamic theory and experiment (Bussell, 1992).

Another observation from the Webb laboratory is consistent with the hydrodynamic theory. Thomas et al. (1992) measured the lateral mobilities of Fc_ϵ receptors on RBL cells whose receptors have been concentrated at one pole by an electric field. The hydrodynamic theory predicts that lateral mobilities will be dominated by the immobile fraction. In these experiments, the mobilities of mobile proteins were the same in both regions of the cell regardless of the concentration of mobile proteins. Because the immobile concentration is likely unaltered by the electric field and uniform over the cell, the lack of dependence of lateral mobilities on position over the cell is consistent with the hydrodynamic theory. When the membrane is blebbed, mobility of the Fc_ϵ receptor is much faster and does depend on location when an electric field is applied to the cell. The concentration dependence of mobilities in membranes free of constraints was pointed out in the previous paper (Bussell et al., 1995) and is consistent with the combined theory applied to these membranes.

Many FPR experiments have been performed on phosphatidyl inositol-linked proteins (for a review, see Zhang et al., 1993), none of which can be analyzed by the hydrodynamic model in its current form, because phosphatidyl inositol-linked molecules do not satisfy the continuum hypothesis. Analysis of these experiments must await the development of an appropriate noncontinuum theory.

To summarize the comparison of our theory to experiment for tracer diffusivities in fixed beds:

- 1) The immobilization of a small fraction of proteins greatly decreases the mobility of the remaining mobile proteins. A small degree of immobilization has led to large decreases in protein mobilities in several systems (Henis and Elson, 1981a; Tank et al., 1982).
- 2) The combined hydrodynamic/thermodynamic theory is closer to experiment than either the hydrodynamic or thermodynamic theory alone, but discrepancies still exist that are likely due to lateral obstruction by cytoskeleton.
- 3) Tracer diffusivities decrease with increasing protein area fraction, although this decrease is not as sharp as seen when the first proteins are immobilized.

A number of research groups have explored different theories for the observed motions of IMPs in membranes including percolation analyses (Saxton, 1990a), micrometer-scale membrane lipid domains of different phases (Edidin and Stroynowski, 1991), and transient immobilization (Ghosh, 1992). All of these phenomena are likely to occur in biological systems, and our calculations neither prove nor disprove their existence. The relative importance of hydrodynamic interactions, along with all of these phenomena, in each membrane can be assessed by detailed analyses of nanovideo microscopy experiments (Ghosh, 1992) along with detailed simulations that incorporate hydrodynamic and thermodynamic multiparticle effects.

In contrast with long-time diffusion coefficients, there are very few experimental measurements of short-time diffusion coefficients for IMPs in any membrane system for which the continuum hypothesis is valid (protein/lipid radii ratio >1). Such measurements require techniques with temporal resolutions of 10^{-5} s or faster. Techniques with sufficiently rapid temporal resolution are electron spin resonance and NMR, which have resolutions of 10^{-8} and 10^{-5} s, respectively (Gennis, 1989). Esmann and Marsh (1992) measured D_s of Na^+-K^+-ATPase in a lipid dispersion at one concentration, which is not enough for a complete test of the theory.

The method of PER has measured D_g values under a wide range of experimental conditions. The theories advanced in this paper suggest that D_g and D_i in plasma membranes are equal to leading order. This result is consistent with measurements for the diffusion of both vacant and occupied Fc_ϵ receptors using FPR and PER (McCloskey et al., 1984). We also conclude that large discrepancies between other PER and FPR measurements for D_g and D_i, respectively, arise because the FPR experiments stimulate the cells while fluorescently labeling them. The stimulation leads to an increase in ϕ_i that radically reduces m_s. Thus, in such systems, the FPR measurement produce much lower values for D_i than PER measurements produce for D_g.

In contrast with results for tracer and gradient diffusivities, decreases in D_r with ϕ_i are less rapid and are given by the expression $D_r/D_{r,0} = 1 - 2\phi_i$. This result is consistent with experiments, but we are unable to quantitatively test the theory because of a lack of detailed experimental measurements on rotational diffusivities. Current experimental measurements are difficult to resolve into accurate values for m_r and D_r (Zidovetzki et al., 1986; Myers et al., 1992).

A great deal of future work is required in this problem, both theoretical and experimental. In our hydrodynamic derivations, we have assumed that analytic results for $\phi_i \ll 1$ are accurate for $\phi_i < 0.3$, and that hydrodynamic interactions in membranes are dominated by interactions between mobile proteins and fixed proteins, with interactions between mobile proteins negligible. These assumptions must be tested rigorously with computational techniques that solve for hydrodynamic interactions in concentrated membranes (Bossis and Brady, 1984; Sangani and Yao, 1989). In experiments, better measurements of protein diffusivities in systems where membrane viscosity and mobile and immobile area fractions are known are required to verify predictions of the model and to ascertain properly the influence of hydrodynamic interactions in plasma membranes.

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