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THE LACK OF RELATIONSHIP BETWEEN FLUORESCENCE POLARIZATION AND LATERAL DIFFUSION IN BIOLOGICAL MEMBRANES

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An investigation has been carried out of the relationship between changes in the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and concomittant changes in the lateral diffusion of proteins and lipid probes in membranes. Plasma membranes from lymphocytes and a CH1 mouse lymphoma line were treated with up to 70 mol% (relative to the total membrane phospholipid) of oleic or linoleic fatty acids. Under these conditions the fluorescence polarization of DPH decreased by between 8 and 15% which, in the framework of the microviscosity approach, suggests a membrane fluidity change of between 20 and 50%. The lateral diffusion coefficients of surface immunoglobin and the lipid probes 3,3′-dioctadecylindocarbocyanine and pyrene were also measured in these membranes using the fluorescence photobleaching recovery technique and the rate of pyrene excimer formation. The diffusion rates were found to be unaffected by the presence of free fatty acids. Hence despite large 'microviscosity' changes as reported by depolarization of DPH fluorescence, lateral diffusion coefficients are essentially unchanged. This finding is consistent with the idea that perturbing agents such as free fatty acids do not cause a general fluidization of the membrane but act locally to alter, for example, protein function. It is also consistent with the suggestion that lateral mobility of membrane proteins is not modulated by the lipid viscosity.

Introduction

The formulation of the fluid mosaic model of biological membranes was a significant advance in our understanding of membrane structure and function. The central features of this model are: (1) the lipid bilayer constitutes a homogeneous two-dimensional fluid matrix that is the basic structural unit of the membrane, and (2) integral membrane proteins are inserted into the lipid matrix and are generally free to diffuse laterally in the fluid lipid 'sea' limited only by the viscous drag of the lipids [1]. Lateral movement of membrane lipids and proteins has been demonstrated directly in a number of laboratories [2–14]. Evidence that lateral motion of proteins in the cell membrane plays at least a permissive role in physiological processes is provided, for example, by the apparent requirement for receptor aggregation in mast cell degranulation and the action of some peptide hormones [15–20].

Some researchers have gone a step farther in suggesting that control of the lateral diffusion rate of membrane proteins can be used to modulate a cellular response, such as with a diffusion-linked coupling of a membrane receptor with adenyl cyclase or regulatory

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proteins [20,21]. Such observations and theories have led to a more detailed and quantitative approach to diffusion in the plane of the membrane. While techniques do exist for the direct measurement of lateral diffusion coefficients of some membrane proteins, they are not generally available to most researchers and are not immediately applicable to all cell protein species. As a alternative, many researchers have attempted to measure some 'fluidity' parameter of the lipid phase. From such measurements, effects on two-dimensional protein diffusion are deduced by assuming that it is controlled by lipid viscosity. A theory giving a quantitative relation between viscosity and diffusion coefficients in two dimensions has been developed [22]. Serious reservations exist, however, about the applicability of such a hydrodynamic approach, since membranes are highly anisotropic three-dimensional systems, one of whose dimensions is of molecular size. In addition, the probes used for membrane 'fluidity' measurements are generally single molecules of a size similar to the 'solvent', so that macroscopic hydrodynamic theories are not explicitly applicable.

Nevertheless, the concept of a quantitative parameter of membrane structure termed 'fluidity' has taken firm hold in the literature. If we wish, despite the difficulties mentioned above, to measure an operationally defined fluidity parameter, it is obviously important how such a parameter is measured. In bulk, isotropic fluids, fluidity refers to the reciprocal of the viscosity, which in turn derives from the drag exerted on an object as the fluid translates past it. The most common measure of membrane fluidity has been derived from measurements of fluorescence polarization of lipophilic probes such as the widely used 1,6-diphenyl-1,3,5-hexatriene (DPH) [23]. However, since the polarization of this type of probe undoubtedly reflects more the local properties of its microenvironment rather than resistance to macroscopic flow, the operational term 'microviscosity' has been introduced to describe 'viscous-like' forces retarding the probe's tumbling motion. The hope is that microviscosity values, which are relatively easily measured, are related at least semi-quantitatively to true hydrodynamic viscosity.

There has been to date no experimental justification that microviscosity values measured in membranes are related to viscous resistance to translational motion. Hare and Lussan [24] have, in fact, demonstrated that microviscosities measured in bulk aliphatic oils do not uniquely reflect bulk viscosity. The connection may be expected to be even more tenuous in the extremely anisotropic environment of a membrane, since the relationship between local lipid acyl chain packing, which should be reflected in polarization measurements, and the mechanism by which lipid molecules slip by one another (lateral diffusion) is far from obvious. It is therefore also not clear how the lateral diffusion of membrane proteins is affected by so-called fluidizing agents which perturb the lipid phase. Indeed the cummulative evidence from photobleaching measurements [25] is that the diffusion coefficients of all proteins studied, with the exceptions of rhodopsin [3,10], band 3 protein in spectrin-free spherocytic mouse erythrocytes [26] and in red cell ghosts treated with high concentrations of phosphate [27] are on the order of 10^{-10} cm^2/s or less. On the other hand, the theory of Saffman and Delbruck [23] suggests that the protein lateral diffusion coefficient should be similar to the lipid ($5-15 \cdot 10^{-9}$ cm^2/s). It may therefore be that the lateral motion of most membrane proteins is not governed by the viscosity of the lipid bilayer.

In this paper we present a direct test of whether microviscosity measurements necessarily reflect lateral translational diffusion rates of lipids or proteins in cell membranes. We measured lateral diffusion coefficients of lipophilic probe molecules using two different techniques, fluorescence photobleaching recovery and pyrene excimer formation, and also the lateral diffusion coefficient of an integral membrane protein using the fluorescence photobleaching recovery technique. We compared the effects of membrane perturbation by free fatty acids on these measurements with their effects on DPH depolarization measurements. Our results show that, despite large 'microviscosity' changes as reported by depolarization of DPH fluorescence, lateral translational diffusion coefficients are essentially unaffected. Finally, we discuss the available evidence relating to the control of protein diffusion in cell plasma membranes.

Materials and Methods

Stock solutions of 5 mM pyrene (Aldrich Co., Milwaukee, WI) were prepared in either acetone (Aldrich

'Gold Label') or dimethylformamide (J.T. Baker, 'Photrex', Phillipsburg, NJ). DPH (Aldrich Co.,) was made up in tetrahydrofuran (J.T. Baker, 'Photrex') at concentrations between 500 μM and 5 mM. Solutions of 1 mg/ml or 10 mg/ml free fatty acids (Nu Chek, Elysian, MN) were prepared in ethanol (U.S.I. New York, NY). Acrylamide was obtained from Biorad (Richmond, CA) and recrystalized twice from water.

Lymphocytes were prepared fresh by mechanical disruption of A/J mouse spleens. Measurements were performed using between $5 \cdot 10^5$ and $5 \cdot 10^6$ cells/ml. The lower concentrations were used for polarization measurements in which the A at 360 nm is less than 0.3. Lymphoma cells were obtained from the peritoneum of B6AF1/J mice (Jackson Labs., Bar Harbor, ME) which had been inoculated with a CH1 lymphoma cell line. Plasma membranes were prepared from these cells essentially by the method of Lemonnier et al. [28]. The phospholipid concentration of the membranes varied between 50 and 170 μM. Small sonicated single walled vesicles were prepared by the method of Huang and Thompson [29]. Phospholipid concentrations were determined by the method of Bartlett [30]. Both cells and membranes were suspended in buffer containing 150 mM NaCl/10 mM Tris-HCl titrated to pH 7.4 at 25°C.

Pyrene fluorescence intensities were determined using a Perkin Elmer model MPF2A fluorimeter with a 4 nm excitation and emission band width and 338 nm excitation wavelength. DPH polarization and lifetime measurements were carried out as described in Klausner et al. [31] using an SLM Instruments model 4800 fluorimeter (SLM Instruments Urbana, IL) for which the excitation was 360 nm and emission was observed through a Corning 3144 filter. Absorption measurements were performed using a Cary model 210 spectrophotometer (Varian, Palo Alto, CA).

DPH in tetrahydrofuran was added directly to the membranes or cells while gently vortex mixing and allowed to incubate for 30 min at room temperature. The total molar DPH concentration was always less than 0.01 of the total molar phospholipid concentration. Aliquots of pyrene were added to membranes while vigorously vortex mixing. This procedure minimized pyrene excimer fluorescence in water which was observed even at concentrations below 1 μM. Two procedures were used to incorporate free fatty acids into cells or membranes. In the first, a membrane or cell suspension was treated with pyrene as described above until the ratio of excimer to monomer intensities (D/M) was large enough (>0.2) to allow changes to be monitored easily. At this pyrene concentration, between 10 and 30 mol% free fatty acid as a concentrated solution in ethanol were added to the mixture and D/M was monitored as a function of time of incubation. In the second method, membranes were allowed to incubated with as much as 70 mol% free fatty acid for 30 min at room temperature. The samples were then washed two times by centrifugation at $100\,000 \times g$ for 30 min and resuspended in buffer. Pyrene was then added to each sample as described above and the D/M ratio as a function of pyrene concentration was determined for control and free fatty acid-containing membranes.

The ratio of excimer to monomer intensities (D/M) were determined by averaging the ratio of three peak intensities at 373, 385 and 394 nm to that at 465 nm. If the rate of excimer formation is diffusion limited, D/M is directly proportional to the pyrene diffusion coefficient. Since we were interested only in relative changes in viscosity, we did not attempt to calculate absolute diffusion coefficients, which require a determination of the pyrene lifetime and water/membrane partition coefficient. However, if we assume that the partition coefficient and fluorescence lifetime of the excimer are similar to those in red cell membranes (for which Dembo et al. [32], obtained $7 \cdot 10^4$ and $34 \cdot 10^6$ s^{-1}, respectively), then typical values for the diffusion coefficient (evaluated using the method of Dembo et al.) were found to be about $7 \cdot 10^{-8}$ cm^2/s.

In the studies performed using fluorescence photobleaching recovery, labeling with the fluorescent lipid analogue 3,3'-dioctadecylindocarbocyanine iodide (a gift from Dr. Alan Waggoner) was accomplished by adding 1 μl 10 mg/ml ethanol stock solution of the dye to the cells in 1 ml buffer. Goat anti-mouse IgG antibodies were obtained from Cappel Laboratories, and Fab fragments were prepared by papain digestion as described in Dragsten et al. [33]. Gel filtration on Sephadex G-100 was used to ensure against the presence of undigested antibody. All proteins were labeled with tetramethyl rhodamine isothiocyanate (Research Organics) by 2 h incubation at room temperature in borate buffer, pH 9.0, with 50 μg dye/mg protein. Free rhodamine was removed by exten-

sive dialysis. Aggregated protein was removed from the antibody preps by a preliminary centrifugation at 100 000 × g for 30 min in an airfuge (Beckman Instruments). Lymphocytes from B10 Br mice (Jackson Laboratories) were labeled with Fab anti-IgG by incubation with the antibodies for 20 min on ice. Fluorescence photobleaching recovery measurements of lateral diffusion of 3,3′-dioctadecylindocarbocyanine iodide and the protein probes were made as previously described [33]

Results

As Fig. 1a demonstrates, addition of pyrene to water, even at sub-micromolar concentrations, results in the formation of excimer fluorescence. This indicates that some form of pyrene is soluble in water, fluoresces, and forms excimers at a rate which is not diffusion limited. Upon addition of membranes to the pyrene-water mixture, there is an approx. 3-fold increase in monomer and an even greater decrease in excimer fluorescence. To demonstrate directly that the rate of excimer formation in membranes is diffusion limited, an aqueous suspension of membranes was titrated with pyrene. The results of this study, which are shown in Fig. 2, demonstrate that the rate of excimer formation in membranes follows Stern-Volmer kinetics, strongly suggesting that the reaction is diffusion limited [34].

Exposure of lymphoma plasma membranes and whole lymphocytes to oleic or linoleic acid, as Table I demonstrates, decreases the DPH polarization by 8 to 15%. The effect of free fatty acids on the decay of DPH was also studied and the results are shown in Table II. As discussed previously, a two component decay is expected for a membrane exhibiting lipid phase heterogeneity [31]. Our results demonstrate a small (similar results were obtained from three separate preparations) shortening of both lifetime components. This decrease in lifetime is consistent with a reduction in lipid packing induced by the free fatty acid since, as our previous study demonstrated [31], the decay times of DPH are appreciably longer in solid as compared to liquid phase liposomes. In any case, if the polarization of DPH were governed by an isotropic microviscosity, a decrease in the lifetime of DPH should lead to an increase in polarization. Hence, within the framework of the microviscosity

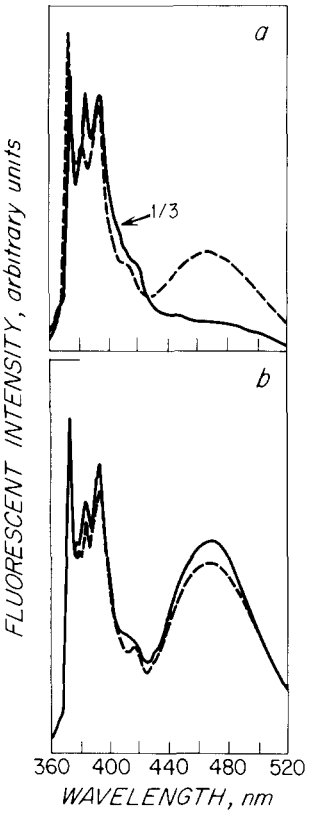

Fig. 1. (a) Pyrene fluorescence emission spectra. The - - - - - - curve was obtained by adding 0.6 μM pyrene to buffer, and demonstrates that pyrene excimers can be formed by a non-diffusion limited mechanism. The solid curve was obtained by adding lymphoma membranes (phospholipid concentration = 50 μM) to the pyrene-buffer suspension The disappearance of the excimer peak indicates that the pyrene partitions into the membrane and at these concentrations exists largely as monomers in the excited state (b) The - - - - - - curve was obtained by adding 1 μM pyrene to aqueous buffer, and the solid curve after adding 12 μM oleic acid to this same solution. The observed increase in the excimer intensity demonstrates the formation of excimers in fatty acid micelles.

approach [23] the polarization changes correspond to a decrease in the microviscosity of between 20 and 50%.

On the other hand, as is seen in Fig. 2 and Table I, the introduction of free fatty acids into membranes or cells exhibiting appreciable pyrene excimer formation causes at most a 2–3% increase in the D/M ratio. Even this small increase in D/M can be accounted for

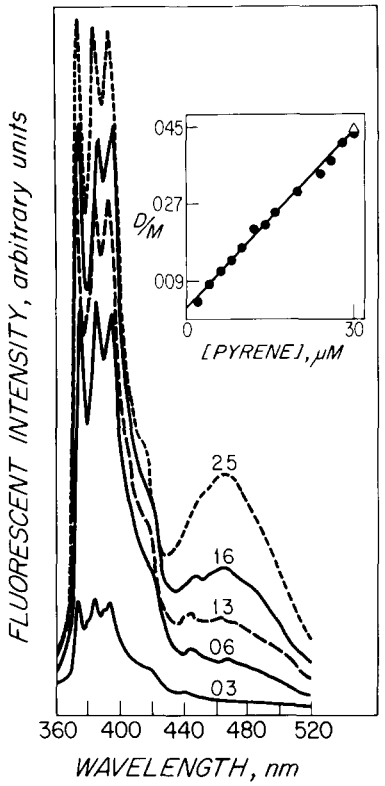

Fig. 2. Pyrene fluorescent intensities in lymphoma plasma membranes. Spectra were obtained at the indicated pyrene concentration (in μM). The insert depicts the D(Excimer)/M ratio as a function of total pyrene concentration. The solid circles represent values obtained in the control membranes, while the open triangle is the value obtained after adding 20 mol% oleic acid. Details of the procedures are giving in Materials and Methods.

without recourse to membrane fluidization. As shown in Fig. 1b, the partition of pyrene into fatty acid micelles is accompanied by an increase in excimer fluorescence. To eliminate the contribution of aqueous and micelle-associated pyrene, 320 mM acrylamide were added to the membrane-pyrene system. In a separate experiment we found that 300 mM acrylamide will quench the aqueous plus micell-associated pyrene fluorescence by about 80%, while this same concentration of acrylamide had essentially no effect on pyrene fluorescence in egg phosphatidylcholine vesicles. As expected, in the presence of acrylamide no increase in D/M was observed upon addition of oleic acid (Table I).

To further ensure that the presence of excess fatty acid in the aqueous phase could not influence excimer fluorescence, pyrene fluorescence was studied with membranes which were cleansed of external fatty acid as described in Materials and Methods. Pyrene titrations were performed with both the control and fatty acid-treated membranes. Although the dependence the of D/M vs. pyrene concentration was linear for both, the slope of the fatty acid-treated membranes was 29% greater than the untreated. However, the ratio of treated to untreated monomer intensities also increased by about 29%. This suggests that, although the fatty acids do not fluidize the membrane, they do increase the membrane solubility of pyrene. Therefore, the slope of the D/M curve, which is proportional to the product of the diffusion coefficient and the membrane phase concentration of pyrene, increases.

TABLE I

Sample	Polarization of DPH		Pyrene excimer formation (D/M)	
	Control	+ free fatty acid	Control	+ free fatty acid
Lymphocytes	0.289 ± 0 008	0.250 ± 0.008 [a]	0.349 ± 0.008	0.345 ± 0.009
Lymphocyte plasma membrane	0.302 ± 0.004	0.256 ± 0.009 [a]	n.d.	n d.
Lymphoma plasma membrane	0.313 ± 0.004	0.280 ± 0.022 [b]	0.428 ± 0 005	0 439 ± 0.005 [b]
Lymphoma plasms membrane + 320 mM acrylamide	0.300 ± 0.003	0.271 ± 0 005 [b]	0.492 ± 0.005	0.486 ± 0 005 [b]

[a] Linoleic acid (see text for details)
[b] Oleic acid.

TABLE II

EFFECT OF FREE FATTY ACIDS ON THE FLUORESCENCE DECAY OF DPH

Measurements were performed by the phase-modulation method at 6, 18 and 30 MHz. $\tau_p(f)$ and $\tau_m(f)$ are the experimental phase and modulation lifetimes determined at modulation frequency f. A two component analysis was performed according to the methods outlined in Klausner et al. [31] This analysis yields the fractional intensity (α_i) and lifetime (τ_i) of the ith components as well as the normalized chi square value (χ^2). χ^2 values less than approx. 1.8 are deemed acceptable.

Sample	Measured values (ns)						Heterogeneity analysis				
	$\tau_m(6)$	$\tau_p(6)$	$\tau_m(18)$	$\tau_p(18)$	$\tau_m(30)$	$\tau_p(30)$	α_1	τ_1(ns)	α_2	τ_2(ns)	χ^2
Control	9.2 ± 0 8	8 1 ± 0.7	8.7 ± 0.2	7.1 ± 0.6	7 9 ± 0 1	6 9 ± 0.3	0 5 ± 0.2	12 2 ± 0 2	0.5 ± 0 2	5 3 ± 0.3	0.4
+25 mol% oleic acid	8.7 ± 0 7	7 8 ± 0 7	8 2 ± 0.2	6 7 ± 0 6	7 3 ± 0.1	6.0 ± 0.2	0.5 ± 0.1	11 5 ± 0 2	0.5 ± 0 1	4.4 ± 0 2	0 3

TABLE III

DIFFUSION COEFFICIENTS IN LYMPHOCYTE PLASMA MEMBRANES

Data are given as mean ± S.E. with the number of cells measured in parenthesis. Data are presented as cm^2/s ($\times 10^{10}$).

Probe	Control	+Linoleic acid
3,3'-dioctadecylindocarbocyanine iodide	128 ± 8 (13)	112 ± 7 (12)
(Fab) Anti IgG	2 33 ± 0.18 (16)	2 06 ± 0 23 (12)

Table III shows the results of the fluorescence photobleaching recovery measurements of lateral diffusion of 3,3'-dioctadecylindocarbocyanine iodide and surface immunoglobulin. The mean diffusion coefficient of 3,3'-dioctadecylindocarbocyanine iodide and surface immunoglobulin are lower after linoleic acid treatment, rather than 20–50% higher as predicted from the microviscosity measurements. The hypothesis that the diffusion coefficients in the fatty acid-treated membranes are at least 20% higher can be rejected by the Student's t test criteria for 3,3'-dioctadecylindocarbocyanine iodide ($P < 0.0005$) and for surface immunoglobulin ($P < 0.01$).

Agents such as free fatty acids may alter the shape of cells and this may artifactually either result in or mask lateral diffusion rate changes as measured by fluorescence photobleaching recovery. In fact, fluorescence photobleaching recovery reveals no difference in lipid diffusion rates when measured in 'flat' as apposed to ruffled areas of lymphocytes [33]. Recent theoretical calculations also support these results [51]. Furthermore, such surface shape changes should not alter pyrene excimer formation and therefore it is likely that the lack of change in the diffusion rates reported here correctly reflects a lack of change in membrane lateral mobility.

Discussion

Our results clearly show that large changes in the fluorescence polarization of DPH in biological membranes do not imply similar changes in lateral diffusion rates of other membrane constituents. Incubation of whole cells or isolated plasma membrane fragments with linoleic or oleic acid produces a marked decrease in DPH polarization, corresponding roughly to a 20–50% decrease in membrane microviscosity calculated according to the method of Shinitzky and Barenholz [23]. If microviscosity were the controlling factor in lateral diffusion rates, one would thus expect lateral diffusion coefficients to increase by 20–50% according to either three- or two-dimensional hydrodynamic theories of diffusion [22]. As is apparent in Table I, lateral diffusion coefficients of two lipophilic probes and an integral membrane protein were not measurably affected by incorporation of free fatty acid.

It is not surprising that the polarization of DPH does not predict the lateral diffusion rates of molecules in the membrane. The problem of making a simple or reliable connection between microviscosity and DPH polarization has been dealt with by several authors [24,35–41,53]. These studies have demonstrated the lack of correlation of DPH polarization with macroscopic viscosity [24] and have shown that DPH decay anisotropy does not support the supposition of unhindered, isotropic rotation [35–41].

Fluorescence polarization is also sensitive to the probe lifetime. This is significant since recent studies have demonstrated that the lifetime of DPH is a function of membrane structure [31]. In this respect, it should be commented that a serious deficiency in many 'microviscosity' measurements is the failure to perform lifetime measurements. In addition, Klausner et al. [31] have shown that DPH exhibits more than one fluorescence lifetime when incorporated into either mixed phase lipid vesicles or plasma membranes of many cells, indicating partitioning of the probe into more than one microenvironment.

Even if the difficulties alluded to above are ignored, it is not clear how restrictions on the rotation of a lipid probe in a highly anisotropic membrane are related to restrictions imposed upon translational diffusion of membrane components. This caveat is all the more important since most intuitive ideas relating membrane function and structure to fluidity are more directly connected to translational diffusion. Moreover, it has been found that the lateral diffusion coefficient of lipids or lipophilic probes in model bilayers undergoes a dramatic change at the phase transition temperature, or upon incorporation

of cholesterol [42–44]; although, the change in rotational rate of DPH is not significant [37,40]. Fluorescence polarization of lipid probes more likely reflects local lipid packing constraints, and this may be expected to depend on the specific location of the probe in the bilayer.

The question remains as to whether membrane protein diffusion is in fact, controlled by the viscous drag of the lipid bilayer. The fact that direct measurements of lateral diffusion of cell membrane proteins have consistently detected a fraction that is immobile suggests that this notion may not be entirely correct. Whether it is applicable even to the observed mobile fraction is more properly addressed by directly comparing lateral diffusion rates of lipid and protein probes. Such measurements have been carried out using a number of different probes in a variety of cell types, and a representative survey of the results of such measurements are given in Ref. 25. In general, the results can be summarized as follows: (1) protein diffusion coefficients are all around $5 \cdot 10^{-10}$ cm^2/s or smaller (with three exceptions), (2) lipid diffusion coefficients are all of the order of 10^{-8} cm^2/s. A hydrodynamic theory for diffusion in membranes has been worked out by Saffman and Delbrück [23]. They derived an expression for the lateral diffusion coefficient of a cylindrical particle of radius a embedded in a membrane of thickness h.

$$D = \frac{kT}{4\pi\eta h}\left(\ln\frac{\eta h}{\eta' a} - \gamma\right) \qquad (3)$$

where η and η' are the viscosities of the membrane and surrounding aqueous phases respectively, k is Boltzmann's constant and γ is Euler's constant. If protein and lipid diffusion rates are both determined by the same viscous forces, the ratio of their lateral diffusion coefficients depends primarily on their relative molecular sizes. Using a lipid radius of 5 Å, a protein radius of 50 Å, h = 50 Å, and $\eta/\eta' = 100$, Eqn. 3 gives a ratio of lipid to protein diffusion coefficients of 1.6. If anything this is an overestimate; increasing η/η' and decreasing h both decrease the diffusion coefficient ratio, and the 10-fold difference in molecular radii is probably an upper limit. Using representative diffusion coefficients of $D_{\text{lipid}} = 10^{-8}$, $D_{\text{protein}} = 1\text{–}3 \cdot 10^{-10}$ we calculate a ratio of between 33 and 100, clearly incompatible with the theoretical estimate.

A possible explanation for this discrepancy is that the logarithmic size dependence of the diffusion coefficient given by Eqn. 3 is simply not correct. The Stokes-Einstein formula for the diffusion coefficient of a sphere in an isotropic three-dimensional fluid, for example, varies inversely with the first power of the particle radius. However, even if we use the Stokes-Einstein formula with its much stronger size dependence to calculate the ratio of diffusion coefficients (for which there is admittedly no theoretical justification) we still get at most only a ratio of 10. It therefore seems unlikely that the same viscous forces determine lipid and protein diffusion in cell plasma membranes. Consistent with lack of correlation of DPH polarization changes with protein lateral diffusion is the work of Schindler et al. [45] in which no correlation was found between the polyphosphate-stimulated increase in lateral diffusion of glycoprotein, and DPH polarization.

What then determines the rate of protein diffusion. One attractive candidate is the interaction of membrane proteins with the cell's cytoskeletal architecture. Cytoskeleton-membrane protein interactions are well documented as, for example, in the case of capping of surface antigens where the cytoskeleton appears to play a dominant role [45,46]. Smith et al. [47], using a periodic pattern photobleaching technique, have also reported that diffusion of succinyl-concanavalin-A-labeled membrane glycoproteins is asymmetric in adherent mouse fibroblasts and in fact, correlates with the orientation of cytoskeletal stress fibers: diffusion parallel to the stress fibers is between 2- and 10-fold faster than diffusion perpendicular to the fibers.

Evidence from cytoskeleton-free systems supports this hypothesis. Smith et al. [47] have measured lateral diffusion rates of the M-B phage coat protein incorporated into synthetic dimyristoylphosphatidylcholine bilayers. In fluid phase lipids, its diffusion coefficient was only about 2-fold lower than that of a lipid probe. Since this is a relatively small protein (molecular weight 5260), it does not afford a rigorous test of the theory of Saffman and Delbruck. Recently, however, Wu et al. [42] have incorporated cytochrome *P*-450 and Vaz et al. [52] have incorporated glycophorin into synthetic multibilayers and measured diffusion coefficients of around $5 \cdot 10^{-9}$ cm^2/s and $2 \cdot 10^{-8}$ cm^2/s, respectively, in fluid phase

lipids. Equally interesting is the fact that rhodopsin in frog rod outer segments, which have no cytoskeletal elements, has a lateral diffusion coefficient of around $5 \cdot 10^{-9}$ cm^2/s, [3,11], close to lipid probe diffusion values in other cell membranes. Recent measurements on mutant (spectrin-depleted) erythrocytes show protein diffusion values much larger than on normal spectrin-containing cells [26]. Moreover, the work of Golan and Veatch, [27], on the effect of phosphate on the mobility of band 3 proteins of human erythrocytes points again to the role of cytoskeletal modulation on protein movement.

In view of this evidence it appears that protein lateral diffusion rates are governed by lipid viscosity in model membrane systems, but generally not in cell plasma membranes. Therefore, a direct connection between changes in lipid dynamics and protein lateral movement is unlikely. Lipid structure can, nevertheless, modulate membrane protein function (see, for example, the review of Sandermann, [48]). We feel these effects more likely reflect perturbation of protein conformation in the membrane. For example, it has been suggested that addition of cholesterol can cause a vertical displacement of membrane proteins [49] and that inhibition of capping in lymphocytes by free fatty acids involves a conformational change in a membrane protein brought about by selectively perturbing the lipid packing in domains surrounding the protein [50].

In summary, we believe that fluorescence polarization measurements of lipophilic probes can be sensitive indicators of local lipid packing. Changes in polarization parameters cannot, however, be generalized to imply changes in rates of lateral movement of either lipids or proteins in cell plasma membranes. Current evidence also strongly suggests that the rate of lateral diffusion of most integral membrane proteins is not determined by a viscous drag due to the membrane lipid matrix. Direct control of cell functions via lipid matrix modulation of protein lateral diffusion is therefore highly unlikely. The lipid composition of cell membranes is nevertheless under strict cellular control and has been shown to modulate cell function. We feel such specific effects are mediated through local lipid packing constraints which can affect membrane protein conformation. Finally, it is clear that the simplest depiction of the fluid mosaic model for cell plasma membranes in terms of membrane proteins freely floating in a homogenous fluid lipid sea is inadequate. The roles of protein-protein interactions, particularly involving the cytoskeleton, of local lipid packing constraints on protein function, and the long range organization of the membrane are clearly areas of central importance in furthering our understanding of membrane structure and function.

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References

1 Singer, S.J. and Nicolson, G L (1972) Science 175, 720–731
2 Frye, L.D. and Edidin, M (1970) J Cell Sci. 7, 319–335
3 Poo, M.M and Cone, R A. (1974) Nature (London) 247, 438–441
4 Taylor, R.B., Duffus, W.P H., Ratt, M C. and DePetris, S. (1971) Nature New Biol. 233, 225–229
5 Schlessinger, J., Koppel, D.E., Axelrod, D., Jacobson, K , Webb, W W and Elson, E.L (1976) Proc. Natl. Acad Sic. U S.A. 73, 2409–2413
6 Jacobson, K., Derzko, A., Wu, E.-S., Hou, Y. and Poste, G. (1976) J. Supramol. Struct. 5, 565–576
7 Edidin, M and Fambrough, D. (1973) J. Cell Biol 57, 27–37
8 Pinto da Silva, P. (1972) J. Cell Biol 53, 777–787
9 Speth, A. and Wunderlich, F. (1973) Biochim. Biophys. Acta 291, 621–628
10 Liebmann, P.A. and Entine, G. (1974) Science 185, 457–459
11 Edidin, M (1974) Annu. Rev. Biophys. Bioeng. 3, 179–201
12 Cherry, R.J. (1976) Biol. Memb. (Chapman and Wallach, eds.), pp. 47–97, Academic Press, New York
13 Scandella, C.J., Deveux, P. and McConnell, H.M. (1972) Proc. Natl. Acad Sci. U.S.A. 69, 2056–2060
14 Sackmann, E., Trauble, H , Galla, H.-J. and Ovarath, P. (1973) Biochemistry 12, 5360–5364
15 Kahn, C.R., Raird, K., Flier, J S. and Jarrett, D.B. (1977) J Clin. Invest. 60, 1094–1106
16 Ishizaka, K. and Ishizaka, T. (1971) Annu. N.Y Acad. Sci. 190, 443–456
17 Jacobs, S., Chang, K -J. and Cuatrecasas, P. (1978) Science 200, 1283–1284
18 Siraganian, R.P , Hook, W.A. and Levine, B.B (1975) Immunochemistry 12, 149–157
19 Isersky, H., Taurog, J., Poy, G. and Metzger, H (1978) J Immunology 12, 549–558

20 Hanski, B., Rimon, G and Levitzky, A (1979) Biochemistry 18, 846–853
21 Hirata, F , Strittmatter, W J. and Axelrod, J. (1979) Proc. Natl Acad Sci. U.S A 76, 368–372
22 Saffman, P G. and Delbruck, M (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3111–3113
23 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657
24 Hare, F. and Lussan, C (1977) Biochim. Biophys. Acta 467, 262–272
25 Cherry, R J. (1979) Biochim. Biophys. Acta 559, 289–327
26 Sheetz, M P , Schindler, M. and Koppel, D E. (1980) Nature 285, 510–512
27 Goland, D.E. and Veatch, W.R. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2537–2541
28 Lemonnier, F , Mescher, M , Sherman, L and Burakoff, S (1978) J. Immunol. 120, 1114–1120
29 Huang, C. and Thompson, T.E (1974) Methods Enzymol 32, 485–501
30 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
31 Klausner, R.D., Kleinfeld, A., Hoover, R. and Karnovsky, M.J. (1980) J. Biol Chem. 255, 1286–1295
32 Dembo, M , Glushko, V , Aberlin, M E. and Sonenberg, M. (1979) Biochim Biophys. Acta 522, 201–211
33 Dragsten, P., Henkart, P., Blumenthal, R., Weinstein, J and Schlessinger, J (1979) Proc Natl. Acad. Sci. U S A 76, 5163–5167
34 Birks, J.B. (1970) Photophysics of Aromatic Molecules, pp. 301–371, John Wiley and Sons, New York, NY
35 Chen, L.A , Dale, R E., Roth, S and Brand, L (1977) J Biol Chem. 252, 2163–2169
36 Dale, R E., Chen, L A. and Brand, L (1977) J Biol. Chem. 252, 7500–7510
37 Kawato, S , Kinosita, K., Jr. and Ikegami, A. (1977) Biochemistry 16, 2319–2324
38 Kawato, S , Kinosita, K., Jr and Ikegami, A (1978) Biochemistry 17, 5026–5031
39 Veatch, W.R and Stryer, L (1977) J Mol Biol. 117, 1109–1113
40 Lakowicz, J.R , Prendergast, F G. and Hogan, D. (1979) Biochemistry 18, 508–519
41 Jahnig, F (1979) Proc. Natl Acad. Sci. U.S.A. 76, 6361–6365
42 Wu, E.-S., Jacobson, K and Papahadjopoulos, D. (1977) Biochemistry 16, 3936–3941
43 Fahey, P F. and Webb, W W (1978) Biochemistry 17, 2046–3053
44 Rubenstein, J.L.R., Smith, B A. and McConnell, H.M (1979) Proc Natl Acad. Sci. U S.A 76, 15–18
45 Schindler, M , Koppel, D E and Sheetz, M P (1980) Proc. Natl. Acad Sci U.S.A 77, 1457–1461
46 Nicolson, G.L (1976) Biochim. Biophys Acta 457, 57–108
47 Smith, B.A., Clark, W R and McConnell, H.M. (1979) Proc Natl Acad. Sci. U S A 76, 5641–5644
48 Sandermann, H., Jr. (1978) Biochim. Biophys Acta 515, 209–237
49 Borochov, H and Shinitzky, M (1976) Proc Natl Acad Sci, U S A 73, 4526–4530
50 Klausner, R.D , Bhalla, D K , Dragsten, P , Hoover, R.L and Karnovsky, M J. (1980) Proc. Natl. Acad. Sci U.S A. 77, 437–441
51 Aizenbud, B and Gershon, N D. (1980) Fed. Proc. 39, 1990
52 Vaz, W.L C., Kapitza, H G , Stumpel, J , Sackmann, E and Jovin, T.M. (1981) Biochemistry, in the press
53 Heyn, M.P (1979) FEBS Lett. 108, 359–364