Lateral Diffusion in Planar Lipid Bilayers

Abstract. *Direct measurements by fluorescence correlation spectroscopy of lateral diffusion coefficients of fluorescent lipid analogs in lipid bilayer membranes indicate self-diffusion coefficients* $D \gtrsim 10^{-7}$ *square centimeters per second for various lipid systems above their reported transition temperatures. Cholesterol in egg lecithin at mole ratio of 1 : 2 reduces* D *by about twofold, while retained hydrocarbon solvent can increase it by two- to threefold.*

The fluidity of lipid molecular bilayer membranes is well established (*1*) and has become a prominent feature of cell membrane models (*2*). We have now measured lateral diffusion in planar lipid bilayer membranes (BLM's) for the first time and have obtained values of the diffusion coefficients (D) of two lipid probes. We have appraised the effects of cholesterol, residual solvent, temperature, and the possibility of convective transport.

Lateral diffusion coefficients in small phospholipid bilayer vesicles and liquid crystal multilayers have been estimated from energy exchange interactions by nuclear magnetic resonance (NMR) (*3–5*), electron spin resonance (ESR) (*6*), and fluorescence (*7*). Reported values of D range upward from 10^{-8} cm²/sec (*1*). However, model assumptions, effects of high label concentration, membrane curvature, and multilayer interactions limit comparisons with these data. Brûlet and McConnell (*4*) and Kroon *et al.* (*5*) have analyzed the problems of deriving lateral diffusion coefficients from NMR line shapes. Measurements of the lateral transport of proteins, receptors for antibodies and lectins, and a lipid probe on cell surfaces confirm the fluidity of cell membranes and invite comparison with lipid bilayer diffusion measurements (*1, 8–10*).

We have applied fluorescence correlation spectroscopy (FCS) (*11*) to BLM systems. This technique analyzes the stochastic temporal fluctuations around equilibrium of the number of fluorescent molecules within a small volume defined by a focused laser beam. The characteristic time dependence of the number fluctuations provides a measure of the diffusion coefficient, D. Our fluorescent probes were 3,3′-dioctadecylindocarbocyanine iodide (diI) (*12*), which has two 18-carbon fatty acid tails, and rhodamine-labeled dipalmitoylphosphatidylethanolamine (DPPE-Rh). Because of their structure and amphipathic nature, these probes should diffuse in the BLM's very much like their constituent phospholipids.

We have studied lipid BLM's formed by both the Mueller-Rudin (MR) technique (*13*), and the Montal-Mueller (MM) technique (*14*) as discussed by White *et al.* (*15*). The MR membranes are formed by partition from an excess of alkane solvent (hexane, octane, or hexadecane), while the MM membranes are formed by uniting layers spread on a water surface after solvent evaporation. We estimated solvent retention in the membranes by measurements of specific membrane capacitance (*16*). For diffusion measurements, stable membranes of both types are formed on electron microscope grids of 100 or 200 mesh in a buffer of pH 7.0 (0.1M KH_2PO_4 and 0.0058M NaOH). The materials were all of the highest certified purity: n-alkane solvents were obtained from Fisher Scientific Co.; egg phosphatidylcholine (EPC), dipalmitoyl-PC (DPPC), and glycerol monooleate (GMO) were obtained from Applied Science Laboratories, Inc.; and 7-dehydrocholesterol was obtained from Sigma Chemical Co. The lipids were assayed for purity (> 98 percent) by thin-layer chromatography and were usually used without further purification.

Fluorescence was excited by an attenuated 520.8-nm krypton laser line ($\sim 10^{-5}$ watt) focused on the membrane through the vertical illuminator of a fluorescence microscope to a spot of radial gaussian intensity profile $I(r) = I_0\exp(-2r^2/w^2)$; $w = 1.2$ μm. The excited fluorescence was collected through the microscope optics, filtered, detected by photon counting, corrected for laser fluctuations, and processed on line by a PDP-11 computer to calculate the normalized photocount correlation function, $g(\tau)$ (*17*).

Fluctuations of fluorophore concentration from equilibrium (δc) evolve in accord with the diffusion equation $\partial\delta c/\partial t = D\nabla^2\delta c$ in two dimensions (*18*) leading to

$$g(\tau) \equiv [<n(t)n(t+\tau)> - <n(t)>^2]/<n(t)>^2 = [<N>(1+\tau/\tau_c)]^{-1}$$

where $n(t)$ is the number of photocounts in an interval at time t; the angular brackets signify long-time average; $<N>$ is the effective average number of fluorophore molecules within the laser beam (*11*). Here $\tau_c = w^2/4D$ is the characteristic correlation time for diffusion over a distance w. Notice that a linear fit of $[g(\tau)]^{-1}$ as a function of τ yields intercept $<N>$ and slope $<N>/\tau_c$. In these experiments $<N> \sim 2 \times 10^3$ (see Fig. 1).

To evaluate effects of driven flow processes, such as convection, we analyzed our correlation function data (*11, 19*) with respect to shape and to scaling with beam size, and we found no evidence of nondiffusive transport. Moreover, visual observations of the motion of individual fluorescent membrane inclusions also excluded convective flow. The laser power absorbed by the bilayers was $< 10^{-10}$ watt; local heating and fluorophore bleaching were thus negligible. Temperatures were determined to ± 0.2°C by a thermocouple immersed adjacent to the membrane.

Our results are summarized in Table 1. Probe concentration effects should be negligible at our probe levels, $\sim 5 \times 10^{-4}$ mole fraction. The reported diffusion coefficients should approximate self-diffusion coefficients of the phospholipids in these membranes. The corresponding diffusion coefficients of diI and DPPE-Rh differ only slightly with DPPE-Rh diffusing about 1.5 times faster.

We have measured the effects on D of solvent retention (*20*) by comparing membranes with various solvent content (Fig. 1). Specific membrane capacitance increases as membrane thickness and, presumably, solvent content decrease (*16*). We have found that elimination of solvent from phospholipid membranes either by forming them with the MM technique or by using hexadecane as the solvent in the MR technique reduced the diffusion constant by about twofold and increased the membrane capacitance to the value expected for solvent-free phospholipid bilayers. Data thought to be perturbed by solvent effects are identified in Table 1 by parentheses. The capacitances of our GMO membranes (formed either by the MM technique or by the MR technique from long-chain hydrocarbon solvent) suggest that they are also nearly free of solvent (*15*). Furthermore, the measured amplitudes of the correlation functions $G(0) = <N>^{-1}$ show that the number density of diffusing objects equals the expected concentration of individual fluorophore molecules in membranes formed by the MM technique or from hexadecane solvent by the MR technique. Thus, contributions to diffusion by fluorophore clusters or microlenses seem to be excluded. In membranes formed from short-chain alkanes, observed values of $<N>$ were typically one-fourth to one-half the expected values.

The addition of cholesterol to EPC reduces the probe diffusion coefficient by about twofold at a 1 : 2 mole ratio, but

we found little or no effect at a 1 : 1 mole ratio. However, our compositions are uncertain because variable partition of cholesterol, phospholipid, and residual solvent cannot be excluded. There is evidence that cholesterol complexes with EPC around a mole ratio of 1 : 2 to 1 : 1 (*21*).

Only weak temperature dependence of the diffusion coefficients ($dD/dT \lesssim 2 \times 10^{-9}$ cm^2/sec °C) has been observed over the range of 50° to 35°C in those EPC and ECP + C bilayers that were free of solvent effects. Larger effects appeared ($dD/dT \sim 7 \times 10^{-9}$ cm^2/sec °C) in those EPC systems affected by retained solvents. The dil diffusion in pure DPPC is slightly slower at 47°C, above the expected hydrocarbon tail ordering temperature, than in EPC at 25°C. Solvent effects are comparable in these two phospholipids.

Our results in these membranes are remarkably independent of membrane composition and temperature. Over the entire range of composition the diffusion coefficients for solvent-free membranes vary by less than a factor of 3. Thus, pending measurements on extracted membrane phospholipids, we might expect uninhibited diffusion of our lipid analog and labeled lipids in cells to occur with $D \gtrsim 10^{-7}$ cm^2/sec. Schlessinger *et al.* (*9, 10*) have found D(dil) $\lesssim 10^{-8}$ cm^2/sec in rat myoblasts, a difference yet to be resolved. We anticipate useful applications of direct lateral transport measurements on both planar lipid bilayers and cells now that these measurements are tractable.

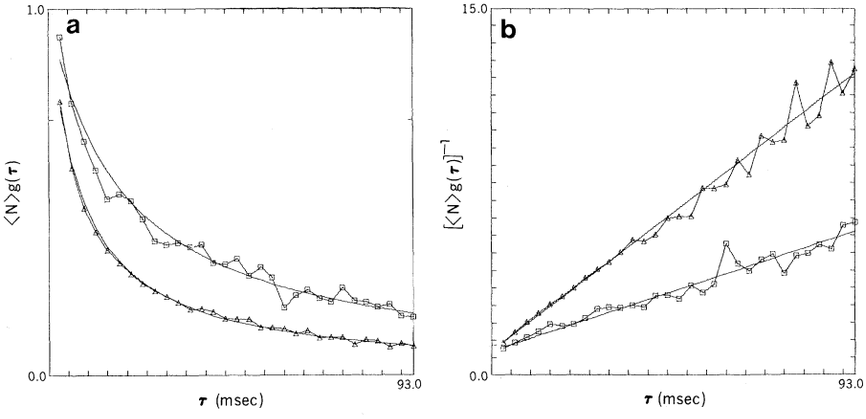

Fig. 1. Correlation function and its reciprocal for several lipid bilayer membranes (a) $N\,g(\tau)$ and (b) $[N\,g(\tau)]^{-1}$ versus τ for (□) EPC, MM technique and (Δ) EPC, MR technique with octane solvent. The points are experimental data and the smooth curves are least squares fits. The slopes in (b) are proportional to the diffusion coefficients.

Table 1. Diffusion coefficients of lipid membrane preparations formed by the Montal-Mueller (MM) and Mueller-Rudin (MR) techniques.

Membrane preparation	Probe	D* ($\times 10^7$ cm^2/sec)	Temperature (°C)	Specific capacitance (μf/cm^2)
		Glycerol monooleate (GMO)		
MM	dil	2.4 ± 0.2	24.5	0.57 ± 0.04
MR, hexadecane†	dil	2.2	24.0	0.52 ± 0.03
		Egg phosphatidylcholine		
MM	dil	1.7 ± 0.2	26.0	0.62 ± 0.04
MR, hexadecane	dil	1.8	24.2	0.61 ± 0.02
MR, octane	dil	(4.4)	24.2	0.40 ± 0.03
MM	DPPE-Rh	2.4	24.0	
		Egg phosphatidylcholine : cholesterol (2 : 1)		
MM	dil	1.0 ± 0.1	24.0	
MR, hexadecane	dil	1.0 ± 0.2	24.0	
MR, hexane	dil	(4.0)‡ (1.8)§	24.0	
MM	DPPE-Rh	1.6	24.0	
		Egg phosphatidylcholine : cholesterol (1 : 1)		
MR, hexadecane	dil	1.6 ± 0.2	24.0	
		Dipalmitoylphosphatidylcholine		
MM	dil	1.1 ± 0.3	47.0	
MR, hexane	dil	(2.8 ± 0.6)	48.0	

*Relative uncertainty in D is less than ±20 percent unless otherwise stated; the uncertainty of absolute values is ±40 percent. Parentheses indicate data affected by retained solvent. †Generated as a mixed monolayer (of 0.4 GMO and 0.6 mole fraction of hexadecane) by the MM technique. ‡Forming solution contained 2.5 mg of lipid per milliliter of hexane. §Forming solution contained 10 mg of lipid per milliliter of hexane.

P. F. FAHEY,* D. E. KOPPEL
L. S. BARAK,† D. E. WOLF
E. L. ELSON, W. W. WEBB
School of Applied and Engineering Physics and *Department of Chemistry, Cornell University, Ithaca, New York 14853*

References and Notes

1. M. Edidin, *Annu. Rev. Biophys. Bioeng.* **3**, 179 (1974).
2. S. J. Singer and G. L. Nicolson, *Science* **175**, 720 (1972).
3. A. G. Lee, N. J. M. Birdsall, J. C. Metcalfe, *Biochemistry* **12**, 1650 (1973).
4. P. Brûlet and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1451 (1975).
5. P. A. Kroon, M. Kainosho, S. I. Chan, *Biochim. Biophys. Acta* **433**, 282 (1976).
6. E. Sackman and H. Trauble, *J. Am. Chem. Soc.* **94**, 4482 (1972); P. Devaux and H. M. McConnell, *ibid.*, p. 4475.
7. K. Razi Naqvi, J. P. Behr, D. Chapman, *Chem. Phys. Lett.* **29**, 705, 440 (1974).
8. M. M. Poo and R. A. Cone, *Nature (London)* **247**, 438 (1974); M. Edidin and D. Fambrough, *J. Cell Biol.* **57**, 27 (1973); M. Edidin, Y. Zagyansky, T. J. Lardner, *Science* **191**, 466 (1976).
9. J. Schlessinger, D. E. Koppel, D. Axelrod, K. Jacobson, W. W. Webb, E. L. Elson, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2409 (1976).
10. J. Schlessinger, D. Axelrod, D. E. Koppel, W. W. Webb, E. L. Elson, *Science* **195**, 307 (1977).
11. D. Magde, E. Elson, W. W. Webb, *Phys. Rev. Lett.* **29**, 705 (1972); E. Elson and D. Magde, *Biopolymers* **13**, 1 (1974); D. Magde, E. L. Elson, W. W. Webb, *ibid.* **13**, 29 (1974); D. Koppel, *Phys. Rev. Sect. A* **10**, 1938 (1974).
12. P. J. Sims, A. S. Waggoner, C. H. Wang, J. F. Hoffman, *Biochemistry* **13**, 3315 (1974). The dil was a gift from A. S. Waggoner. The DPPE-Rh was a gift from J. Reidler.
13. P. Mueller, D. O. Rudin, H. T. Tien, W. C. Westcott, *Nature (London)* **194**, 979 (1962).
14. M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3561 (1972).
15. S. H. White, D. C. Petersen, S. Simon, M. Yafuso, *Biophys. J.*, in press.
16. R. Berry, O. Frölich, P. Laüger, M. Montal, *Biochim. Biophys. Acta* **394**, 323 (1975); R. Fettiplace, D. M. Andrews, D. A. Haydon, *J. Membr. Biol.* **5**, 277 (1971); S. H. White, *Biophys. J.* **15**, 95 (1975).
17. D. E. Koppel, D. Axelrod, J. Schlessinger, E. L. Elson, W. W. Webb, *Biophys. J.* **16**, 1315 (1976).
18. The analysis of molecular diffusion in two dimensions suffers from several difficulties [B. J. Alder and T. E. Wainwright, *Phys. Rev. Sect. A* **1**, 18 (1970); T. Keyes and I. Oppenheim, *ibid.* **8**, 937 (1973); J. C. Lewis, *Phys. Lett.* **44A**, 245 (1973)]. However, P. G. Saffman and M. DelBruck [*Proc. Natl. Acad. Sci. U.S.A.* **72**, 3111 (1975)] and P. G. Saffman [*J. Fluid Mech.* **73**, 593 (1976)] have provided a practical solution for the case of a rod-shaped molecule whose axis is perpendicular to the plane of a membrane. Their results suggest the diffusion equation does apply and that the two-dimensional translational diffusion coefficient is adequately described by a modified Stokes-Einstein relation.
19. B. J. Berne and R. Pecora, in *Dynamic Light Scattering* (Wiley, New York, 1976), chap. 6.
20. R. E. Pagano, J. M. Ruysschart, I. R. Miller, *J. Membr. Biol.* **10**, 11 (1972).
21. Evidence for and against PC:C (phosphatidylcholine:cholesterol) = 2 : 1 and 1 : 1 complexes is reviewed by D. Chapman, *Quart. Rev. Biophys.* **8**, 185 (1975).

22. Supported by NIH grant GM21661, the NSF Division of Condensed Matter, an NSF faculty science fellowship (P.F.F.), a Guggenheim fellowship, a grant from the Research Corporation (W.W.W.), and an NIH career development award (E.E.).

* On leave from Department of Physics, University of Scranton, Scranton, Pa. 18510.
† Present address: University of Michigan Medical School, Ann Arbor 48104.

20 May 1976; revised 3 August 1976