phorylation sites by these antibodies and/or by inhibiting the binding of the kinase to rhodopsin due to the large size of the antibody. Support for the latter, i.e., steric inaccessibility of the kinase due to antibody binding, comes from an experiment in which another monoclonal antibody, rho 4B4, which is directed against the F_1–F_2 loop of rhodopsin (MacKenzie & Molday, 1982), was also found to inhibit phosphorylation as well as rho 3A6 antibody binding at the C-terminus of rhodopsin (unpublished results).

In conclusion, these monoclonal antibodies and specific proteases can be used to distinguish phosphorylated from nonphosphorylated rhodopsin, and therefore, they can serve as valuable probes for both in vitro and in vivo studies directed toward analyzing the molecular properties of the carboxyl-terminal segment of rhodopsin and elucidating the role of light-induced phosphorylation in vision and other cellular processes in ROS.

Registry No. Rhodopsin kinase, 54004-64-7; L-threonine, 72-19-5; L-serine, 56-45-1; trypsin, 9002-07-7; *Staphylococcus aureus* V-8 protease, 66676-43-5.

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Translational Diffusion of Lipids in Liquid Crystalline Phase Phosphatidylcholine Multibilayers. A Comparison of Experiment with Theory

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Received March 27, 1984

ABSTRACT: A systematic study of the translational diffusion of the phospholipid derivative *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) has been undertaken in liquid crystalline phase phosphatidylcholine bilayers by using the fluorescence recovery after photobleaching technique. This work was done with the intention of comparing the experimental results with the predictions of theoretical models for diffusion in membranes. The following is shown. (1) For NBD-PE, the dependence of the translational diffusion coefficient (D_t) upon the acyl chain length of the diffusant is not that predicted by continuum fluid hydrodynamic models for diffusion in membranes [Saffman, P. G., & Delbrueck, M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3111–3113; Hughes, B. D., Pailthorpe, B. A., & White, L. R. (1981) *J. Fluid Mech. 110*, 349–372]. (2) Plots of D_t vs. $1/T$ (Arrhenius plots) are nonlinear in dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayers where the acyl chain composition of the NBD-PE is matched with that of the host bilayer lipid. This suggests that a "free volume" model may be appropriate for the description of lipid diffusion in lipid bilayers. (3) In bilayers of phosphatidylcholines with saturated acyl chains at the same "reduced temperature", the magnitude of D_t follows the order distearoylphosphatidylcholine > DPPC > DMPC > DLPC. This is the inverse of what may be expected from the hydrodynamic model but is in agreement with the free volume in these bilayers. (4) A free volume model that takes into account the frictional drag forces acting upon the diffusing NBD-PE at the membrane–water interface and also at the bilayer midplane is shown to adequately describe the diffusion results for NBD-PEs in DLPC, DMPC, DPPC, and POPC bilayers in the liquid-crystalline phase.

The translational diffusion of lipids and lipid-like probe molecules in phospholipid bilayer membranes has been examined in several laboratories [for a review, see Vaz et al. (1982b)]. It has been experimentally verified that continuum

0006-2960/85/0424-0781$01.50/0

fluid hydrodynamic models (Saffman, 1976; Saffman & Delbrueck, 1975; Hughes et al., 1981, 1982) adequately describe the translational diffusion of large integral membrane proteins with radii in the plane of the membrane of 1.0 nm or greater (Vaz et al., 1982a). However, the applicability of these models to the translational diffusion of lipids in lipid bilayers has been questioned (Galla et al., 1979; Vaz et al., 1982a,b; Vaz & Hallmann, 1983). We have recently provided some experimental evidence that the diffusion of the lipid derivative NBD-PE[1] in liquid crystalline phase phosphatidylcholine bilayers is not adequately described by the continuum fluid hydrodynamic models (Vaz & Hallmann, 1983). We have chosen this lipid derivative for our studies because the FRAP technique we use requires a fluorescent diffusing species and because the structural similarity of this probe molecule to the host lipid is likely to cause the least perturbation of the host bilayer packing characteristics (Vaz et al., 1982b; W. L. C. Vaz, unpublished results). In this paper we provide further evidence that the translational diffusion of NBD-PE in liquid crystalline phase phosphatidylcholine bilayers is not accurately described by the model formulated by Saffman (1976) and Saffman & Delbrueck (1975) or later modifications of it (Hughes et al., 1981, 1982). Our results indicate that the diffusion of lipids in the bilayer is closely related to the free volume (or, rather, the free area) of the bilayer. Further support of this comes from the fact that our results are in reasonable agreement with an interfacial viscosity-limited "free volume" model, the details of which are described in the Appendix. In short, this model combines the free volume model of Cohen & Turnbull [1959; see also Galla et al. (1979)] and hydrodynamic considerations at the membrane–water interface and the bilayer midplane.

Materials and Methods

All phospholipids were purchased from Fluka AG, Buchs, Switzerland. NBD-chloride was from Aldrich Europe Division, Nettetal, FRG. DHPE was synthesized according to the method of Eibl (1978). NBD-PEs were synthesized as described by Vaz & Hallmann (1983).

FRAP Experiments. In the preparation of slides for FRAP experiments, all steps were done in a nitrogen atmosphere. The NBD-PE and phosphatidylcholine desired were mixed in a molar ratio of 1:2000 in a chloroform–methanol (1:1 by volume) solution. The mixture was allowed to stand at room temperature for ~1 h and then deposited on an approximately 1-cm^2 area of a clean glass slide. The solvent was evaporated under a stream of dry nitrogen at ~35 °C. To ensure complete removal of the solvent, the slide was then heated for ~5 min at 80 °C. The total amount of lipid deposited on a slide was typically 3 mg. Hydration was done by dropping a cover glass with a hanging drop of 10 mM sodium phosphate, pH 7.5, containing 0.02% sodium azide over the residue and sealing with paraffin wax to avoid loss of water due to evaporation. Hydration temperatures were 35 °C for POPC, DLPC, and DMPC, 50 °C for DPPC, and 60 °C for DSPC samples. The samples were stored at temperatures above the phase transition temperature of the lipid for a minimum of 16 h between hydration and the beginning of the FRAP experiments.

FRAP experiments were done as described by Vaz & Hallmann (1983). A uniform circular beam profile with a radius of 5.5 μm (Zeiss "Plan" 16/0.35 objective) was used. Typical bilayer domain sizes were at least 100 μm across and usually considerably larger. The fluorescence recovery was complete in all cases. D_t was calculated from the half-times ($t_{1/2}$) for complete fluorescence recovery, which in the case of a uniform circular beam profile is given (Axelrod et al., 1976) by

$$D_t = 0.22\omega^2/t_{1/2} \quad (1)$$

where ω is the radius of the bleached spot. Some fluorescence recovery curves (taken at random) were compared with theoretical fluorescence recovery curves due to one diffusing component, and the agreement between experiment and theory was found to be good.

Theory. (*a*) *Continuum Fluid Hydrodynamic Model.* This model was proposed by Saffman (1976; Saffman & Delbrueck, 1975) for the diffusion of cylindrical particles in thin viscous fluid sheets such as membranes. In an extension of this model by Hughes et al. (1981, 1982), D_t for the cylindrical particle diffusing in the viscous sheet with its long axis perpendicular to the plane of the sheet is given by

$$D_t = (kT/4\pi\eta h)[\ln(2/\epsilon) - \gamma + 4\epsilon/\pi - (\epsilon^2/2)\ln(2/\epsilon)] \quad (2)$$

In this solution the "stick" assumption has been made. k is Boltzmann's constant, T is the temperature in kelvin, η is the viscosity of the sheet (membrane), h is the thickness of the viscous sheet and the height of the particle, γ is Euler's constant (0.5772), and $\epsilon = (\eta_1' + \eta_2')a/(\eta h)$ where η_1' and η_2' are the viscosities of the bounding fluids on both sides of the sheet and a is the radius of the particle. It is assumed that the cylinder is completely embedded in the viscous sheet with its ends exposed to the bounding fluids on both sides of it. In this treatment η is usually considered to be a three-dimensional fluid viscosity. ηh is then a product of this three-dimensional viscosity and the thickness of the membrane or the height of the diffusing particle. Evans & Hochmuth (1978) have argued that the mobility in membranes should be discussed in terms of a surface viscosity, η_s, given in poise per centimeter. In their treatment D_t can still be given by eq 2 provided that ηh is replaced by η_s throughout. It must be emphasized, however, that although ηh and η_s have the same dimensions, the two are not necessarily equivalent.

(*b*) *Interfacial Viscosity Limited Free Volume Model.* This model is based upon the free volume model proposed by Cohen & Turnbull (1959) for diffusion in glasses and extended by Galla et al. (1979) to describe diffusion in the plane of a membrane. The model has been further extended by us (R. M. Clegg and W. L. C. Vaz, unpublished results) to take into consideration the viscous drag forces experienced by a particle in a membrane due to its contact with water at the aqueous interface and with the ends of the lipid molecules of the opposing monolayer at the bilayer midplane. In this model D_t is given (see Appendix) by

$$D_t = (kT/f)\exp[-\gamma a^*/[a_0[\beta + \alpha_a(T - T_m)]]] \quad (3)$$

where k is Boltzmann's constant, T is the temperature in kelvin, f is the translational friction coefficient resulting from drag forces at the membrane–water interface and the bilayer midplane, T_m is the lipid bilayer main phase transition tem-

[1] Abbreviations: NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine; FRAP, fluorescence recovery after photobleaching; D_t, translational diffusion coefficient; DHPE, dihexanoylphosphatidylethanolamine; DLPE, dilauroylphosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; DSPE, distearoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; DLPC, dilauroylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; T_m, main lipid bilayer phase transition temperature.

Table I: Dependence of Radius, Height, and Volume per Acyl Chain $-CH_2-$ Segment of Phosphatidylcholine Molecules in Bilayers at about 20 °C above Their Phase Transition Temperatures Calculated from the Data of Cornell & Separovic (1983)

no. of acyl chain carbons	height (nm)	radius[a] (nm)	volume per acyl chain $-CH_2-$ segment[b] (nm^3)
6[c]	1.13	0.38	0.0258
12	1.33	0.44	0.0246
14	1.40	0.45	0.0250
16	1.46	0.47	0.0253
17[d]	1.50	0.48	0.0257
18	1.53	0.49	0.0259

[a] Calculated by assuming that the lipid molecules are cylindrical. [b] Assuming the glycerophosphocholine group to have a volume of 0.204 nm^3 (Small, 1967). [c] Calculated by assuming that the linearity demonstrated by Cornell & Separovic (1983) extends to lipids with acyl chains of six carbon atoms. [d] About the length of a POPC molecule.

perature in kelvin, γ is a numerical factor that accounts for the overlap of free area (γ has values between 0.5 and 1.0), a^* is the critical free area, i.e., the minimum area surrounding a molecule that will allow a translocation of this molecule, a_0 is the van der Waals area per lipid molecule, $a_0\beta$ is the free area at T_m, and α_a is the lateral thermal expansion coefficient in the liquid-crystalline phase.

Results

Acyl Chain Length Dependence of NBD-PE Diffusion in Liquid Crystalline Phase Phosphatidylcholine Bilayers. A preliminary report of this section has been published (Vaz & Hallmann, 1983). In order to compare our results with the theoretical models described briefly in the preceding section, we consider the lipid molecules (in our case NBD-PE) to be cylindrical particles whose radii, heights, and volumes per acyl chain $-CH_2-$ segment depend upon the acyl chain length as discussed by Cornell & Separovic (1983) and listed in Table I. For purposes of comparison with the hydrodynamic model, the NBD-PE molecules are assumed to be embedded in a single monolayer of the membrane with a viscosity, η, between 1 and 2 P (Vaz et al., 1982a). The viscosities of the bounding fluids are taken to be that of water, η_1', on one side of the monolayer and that of the bilayer midplane, η_2', on the other. There is no good estimate of η_2'. We have, therefore, used our experimental results and the information in Table I to estimate the values of η_2' from eq 2 assuming that η = 1 P and η_1' = 0.01 P. Theoretical dependencies of D_t upon acyl chain length were calculated for the case of diffusion in POPC bilayers at 25 °C and DLPC bilayers at 18.2 °C, both temperatures being 20 °C above the phase transition temperatures of the respective lipids. For estimation of the value of η_2', the diffusants considered were NBD-POPE in POPC and NBD-DLPE in DLPC bilayers. In both cases the apolar portion of the NBD-PE is matched to that of the host bilayer lipid. As seen in Figure 1, eq 2 predicts that D_t will decrease monotonically with increasing chain length of the diffusing lipid particle. We attempted to verify this experimentally. NBD-PEs were synthesized with acyl chains of 6 (NBD-DHPE), 12 (NBD-DLPE), and 18 (NBD-DSPE) carbon atoms. The diffusion of these probes was examined in liquid crystalline phase bilayers of POPC, DLPC, and DSPC. The results at different temperatures are given in Tables II–IV. It is seen that, in all cases, at a given temperature and in a given host lipid bilayer D_t is considerably less dependent upon acyl chain length than eq 2 would predict by using a constant three-dimensional η. This is clearly seen in Figure 1 for POPC and DLPC bilayers at temperatures that are about 20 °C above their respective phase transition temperatures. If eq 2 is expressed in terms of a surface viscosity, η_s, this result would mean that η_s is not strongly dependent upon the height of the diffusing lipid particle. It is also noteworthy that in bilayers of DLPC (Table III) D_t for NBD-DSPE is about the same

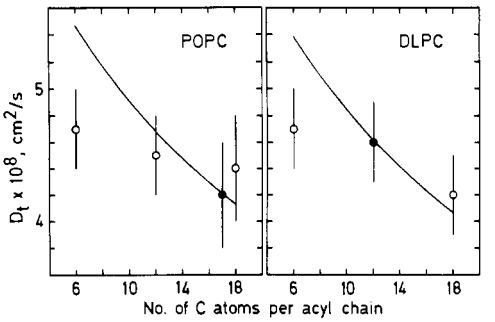

FIGURE 1: Dependence of D_t upon acyl chain length of NBD-PE in liquid crystalline phase multibilayers of POPC at 25 °C and DLPC at 20 °C. The lines are theoretical dependencies calculated from eq 2 and by using the lipid size data in Table I. The points are experimental and are shown with their standard deviations. Each point represents the mean ± standard deviation of at least five FRAP experiments on different multibilayer domains on each of five separately prepared slides. The solid circles represent the experimental points for the case where the NBD-PE acyl chain composition was the same as that of the host bilayer lipid. In obtaining the theoretical curves, we used this experimental point to arrive at a value for η_2' as described in the text.

Table II: Diffusion Results for Different NBD-PEs in Liquid Crystalline Phase POPC Multibilayers

	$(D_t \pm SD) \times 10^8$ (cm^2/s)[a]						
NBD-PE	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	45 °C
NBD-DHPE	3.1 ± 0.3	3.7 ± 0.3	4.7 ± 0.3	5.8 ± 0.3	7.0 ± 0.6	8.1 ± 0.7	9.8 ± 0.7
NBD-DLPE	2.7 ± 0.2	3.4 ± 0.3	4.5 ± 0.3	5.4 ± 0.5	6.6 ± 0.6	7.6 ± 0.6	9.1 ± 1.0
NBD-DSPE	2.8 ± 0.3	3.5 ± 0.5	4.4 ± 0.4	5.4 ± 0.6	6.6 ± 0.7	7.7 ± 0.6	9.0 ± 1.2
NBD-POPE	2.8 ± 0.3	3.4 ± 0.3	4.2 ± 0.4	5.0 ± 0.6	6.1 ± 0.6	7.1 ± 0.7	8.4 ± 1.0

[a] The results are reported as the mean ± standard deviation (SD) of at least five FRAP experiments on different multibilayer domains on each of five separately prepared slides.

Table III: Diffusion Results for Different NBD-PEs in Liquid Crystalline Phase DLPC Multibilayers

	$(D_t \pm SD) \times 10^8$ (cm^2/s)[a]						
NBD-PE	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	45 °C
NBD-DHPE	3.7 ± 0.4	4.7 ± 0.3	6.0 ± 0.6	7.4 ± 1.0	9.1 ± 1.1	10.1 ± 1.5	12.9 ± 1.4
NBD-DLPE	3.6 ± 0.2	4.6 ± 0.3	5.7 ± 0.6	6.8 ± 0.7	8.8 ± 0.9	10.1 ± 1.2	11.5 ± 1.2
NBD-DSPE	3.3 ± 0.4	4.2 ± 0.3	5.4 ± 0.3	6.6 ± 0.7	8.2 ± 0.7	9.6 ± 1.1	11.1 ± 1.5

[a] The results are reported as the mean ± standard deviation (SD) of at least five FRAP experiments on different multibilayer domains on each of five separately prepared slides.

Table IV: Diffusion Results for Different NBD-PEs in Liquid Crystalline Phase DSPC Bilayers at 61 °C

NBD-PE	$(D_t \pm SD) \times 10^8$ $(cm^2/s)^a$
NBD-DHPE	14.1 ± 1.8
NBD-DLPE	13.2 ± 1.2
NBD-DSPE	14.5 ± 1.6

[a] The results are reported as the mean ± standard deviation (SD) of at least five FRAP experiments on different multibilayer domains on each of three separately prepared slides.

as D_t for NBD-DHPE and NBD-DLPE. The acyl chains of NBD-DSPE are somewhat longer than those of the host bilayer lipid (DLPC) and may penetrate into the second monolayer of the host bilayer. If the probe does not penetrate the second monolayer, it will effectively have the same value of h as the host bilayer lipid but a larger radius. In this case the effect will be felt on the ϵ term in eq 2. NBD-DHPE and NBD-DLPE have acyl chains that are respectively shorter and equal in length to the acyl chains of the host bilayer lipid. A similar result was obtained for the diffusion of indocarbocyanine and oxacarbocyanine dyes with different alkyl chains embedded in liquid crystalline phase phosphatidylcholine bilayers by Derzko & Jacobson (1980).

Dependence of D_t upon the Acyl Chain Length of the Host Bilayer Lipid in Liquid Crystalline Phase Saturated Acyl Chain Phosphatidylcholine Bilayers. We have also examined the dependence of D_t upon the acyl chain length of the host bilayer lipid in saturated acyl chain phosphatidylcholine bilayers in the liquid-crystalline phase. The bilayers examined were from DLPC, DMPC, DPPC, and DSPC. The diffusants were respectively NBD-DLPE, NBD-DMPE, NBD-DPPE, and NBD-DSPE. Thus, the apolar portions of the NBD-PEs and the host bilayers were matched. We have shown (Vaz et al., 1982b; W. L. C. Vaz, unpublished results) that there is no detectable perturbation of the host bilayer structure by the probe, particularly at the dilutions used in this work (NBD-PE:PC molar ratios of 1:2000). The results for diffusion in DLPC, DMPC, DPPC, and DSPC bilayers are compared in Figure 2. Within experimental error there is no difference between the values of D_t in the four bilayer systems examined when the data are plotted on an absolute temperature scale (Figure 2A). It is also seen in Figure 2A that the temperature dependence of D_t does not give linear Arrhenius plots. Such nonlinear Arrhenius plots have been obtained for translational diffusion in some liquid-crystal systems (Krueger, 1982). In this case the behavior was attributed to the nonapplicability of continuum fluid hydrodynamic models and the better applicability of free volume models to the translational diffusion in these liquid crystals (Krueger, 1982). The diffusion data in Figure 2 may also be plotted on a "reduced temperature" scale where the reduced temperature is defined as $T_r = (T - T_m)/T_m$, all temperatures being given in kelvin. A plot of D_t vs. T_r is shown in Figure 2B. It is evident that D_t at the same T_r has an inverse relationship to the acyl chain length, i.e., diffusion in this series is fastest in DSPC and slowest in DLPC bilayers at the same reduced temperature. This result is in disagreement with what might have been expected if the fluid hydrodynamic model were applicable to lipid diffusion in these bilayers. However, the result agrees well with the fact that the volume per lipid acyl chain $-CH_2-$ segment increases with increasing chain length if the lipids are compared on a reduced temperature scale [see Table I; also see Cornell & Separovic (1983), Janiak et al. (1979), and Nagle & Wilkinson (1978)]. This correlation makes us propose that a free volume model may be better able to describe the translational diffusion of lipids in lipid bilayers than the continuum fluid hydrodynamic model.

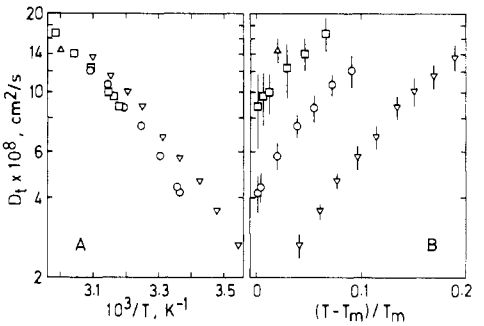

FIGURE 2: Translational diffusion of NBD-PE in liquid crystalline phase saturated acyl chain phosphatidylcholine multibilayers. The data are plotted as an Arrhenius plot using an absolute temperature scale (A) and as a semilogarithmic plot using a reduced temperature scale (B). The acyl chain compositions of the NBD-PEs and the host bilayer lipid were matched. Data are for the translational diffusion of NBD-DLPE in DLPC bilayers (▽), NBD-DMPE in DMPC bilayers (○), NBD-DPPE in DPPC bilayers (□), and NBD-DSPE in DSPC bilayers (△). The bars represent the standard deviations obtained from at least five FRAP experiments on different multibilayer domains on each of at least five slides. The phase transition temperatures used in the calculation of the reduced temperatures were −1.8 °C for DLPC, 23.9 °C for DMPC, 41.4 °C for DPPC, and 54.9 °C for DSPC (Mabrey & Sturtevant, 1976).

Discussion

A priori there is no good reason to expect that the continuum fluid hydrodynamic treatment would adequately describe the diffusion behavior of lipid molecules in a fluid sheet composed of other lipid molecules. The assumption of a continuum fluid implies that the particles composing the fluid are small in comparison with the size of the diffusing particle (Landau & Lifshitz, 1959). This criterion is obviously not satisfied in the case under consideration. It is, therefore, not surprising that the hydrodynamic models (Saffman, 1976; Saffman & Delbrueck, 1975; Hughes et al., 1981, 1982) do not adequately describe lipid diffusion in membranes. The experimental results of Peters & Cherry (1982) had previously been interpreted by these authors to indicate that lipid diffusion in lipid bilayers could be described by the model of Saffman and Delbrueck. More recently, however, Peters & Beck (1983) have demonstrated that a free volume model can be used to describe the diffusion of lipids in monolayers even at lateral surface pressures normally encountered in bilayers. The results reported in this paper demonstrate quite clearly that the hydrodynamic model is not applicable to the self-diffusion of lipids in phosphatidylcholine bilayers in the liquid-crystalline phase. In contrast, the diffusion of large integral proteins in lipid bilayers (Vaz et al., 1982a; Criado et al., 1982; Peters & Cherry, 1982) is adequately described by Saffman's equations (Saffman, 1976; Saffman & Delbrueck, 1975). It may be possible to describe the lipid diffusion by the hydrodynamic model if a free volume component could be incorporated into this treatment. The contention of such a treatment is that a diffusing lipid particle in a lipid bilayer effectively senses the free volume of the "solvent" more than a larger diffusant, which leads to a somewhat lower viscosity being felt by the lipid than would be sensed by a larger particle such as a protein in the same bilayer. Such a reduction of the frictional drag for particles that are approximately the size of the solvent particles has been proposed for simple liquids in the "generalized hydrodynamic theory" (Alder & Alley, 1984). To our knowledge no such treatment for lipid diffusion in membranes exists in the literature so that the dependence of

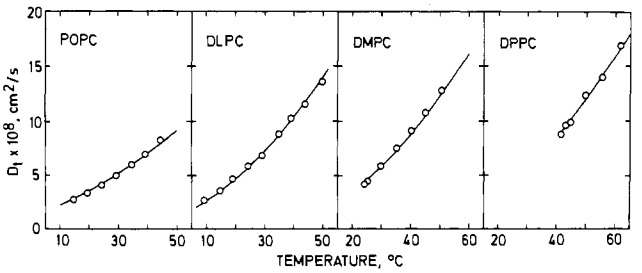

FIGURE 3: A comparison of the temperature dependence of D_t in different phosphatidylcholine bilayers obtained experimentally with the theoretical predictions of the modified free area model given by eq 3. The lines are theoretical and the points are experimental. The parameters used to obtain the theoretical curves are discussed in the text.

the bilayer viscosity upon the frequency of the dynamic events cannot be predicted.

Cohen & Turnbull (1959) first incorporated the concept of a "critical free volume" and proposed a free volume theory for diffusion in three-dimensional fluids and glasses. Galla et al. (1979) adapted the model of Cohen and Turnbull to describe the diffusion of lipid-like molecules in lipid bilayer membranes. More recently, MacCarthy & Kozak (1982) have directly derived an expression for the two-dimensional diffusion of lipids in bilayers and monolayers using a free volume approach. In these treatments, however, the contacts of the lipids with the bounding fluids at the membrane–water interface and at the midplane of the membrane have not been taken into account. If this is considered, the rate of diffusion of a lipid in a lipid bilayer will be determined not only by the probability of having a free volume of a certain size in the bilayer but also by the viscous drag forces felt by the diffusing particle at the aqueous interface and at the bilayer midplane. We have modified the Cohen–Turnbull model to take this fact into consideration (R. M. Clegg and W. L. C. Vaz, unpublished results). The details are briefly described in the Appendix. The agreement between the experimental results and the modified free volume theory is shown in Figure 3 for four phosphatidylcholine bilayer systems. In computing the theoretical curves in Figure 3, we assumed $\gamma a^*/a_0 = 0.4$, which is in agreement with the results of previous workers (Galla et al., 1979). Since γ can have values between 0.5 and 1.0, a^* must have a value between $0.8a_0$ and $0.4a_0$. The value of α_a used by us was 2.3×10^{-3} K^{-1} (Galla et al., 1979; Albrecht et al., 1978). The values of T_m used were −1.8 °C for DLPC, 23.9 °C for DMPC, 41.4 °C for DPPC (Mabrey & Sturtevant, 1976), and 5 °C for POPC (H. Eibl, personal communication). The friction coefficient in the preexponential term of eq 3 was considered to be due to two terms, i.e., $f = f_1 + f_2$. f_1 is due to the interaction of the lipid polar head group with the aqueous phase at the bilayer–water interface, and f_2 is due to the interaction of the acyl chain ends of the lipid with the other half of the bilayer and the effective drag of the chain ends of the same monolayer. A good fit of the theoretical curve with the experimental data was achieved by setting $f_2 = 3.7 \times 10^{-8}$ erg s/cm^2 and $f_1 \propto \eta_1'$ where η_1' is the viscosity of water and is a function of temperature [for tables, see, for example, *CRC Handbook of Chemistry and Physics* (1972)]. If $\eta_1' = 0.0132$ P, the effective sphere defined by the Stokes relation, $f_1 = 6\pi\eta a$, has a radius, a, of 1.4 nm. This is larger than the radius of the lipids (see Table I). If a is fixed at a value of 0.5 nm, $\eta_1' = 0.037$ P at 283 K. Thus, we could consider that the viscosity of water at the bilayer interface is higher than that of bulk water. The temperature dependence of the theoretical curve agreed well with the experimentally obtained temperature dependence of D_t when f_2 was assumed to be invariant with temperature and η_1' was assumed to have the same temperature dependence as that of bulk water. In the theoretical curves in Figure 3, the same values of the above parameters were used for all the saturated chain lipids. For the unsaturated acyl chain lipid, POPC, the only difference was that η_1' had to be given a value of 0.0654 P with $f_2 = 6.6 \times 10^{-8}$ erg s/cm^2. Finally, the values of β for the different bilayers were 0.142 for POPC, 0.105 for DLPC, 0.148 for DMPC, and 0.185 for DPPC. These values represent the free area at T_m in terms of a fraction of the van der Waals molecular area of these lipids. It is of interest to note that for the saturated chain lipids the free area at their respective T_m increases linearly with the increase in chain length. This is in agreement with the data of other workers (Nagle & Wilkinson, 1978; Janiak et al., 1979).

In summary, we have shown here that the translational diffusion of lipids in liquid crystalline phase phosphatidylcholine bilayers is not adequately described by the continuum fluid hydrodynamic models for diffusion in membranes. It may be possible that modifications of the existing continuum fluid hydrodynamic models to include a free volume term would result in a better description of lipid diffusion in membranes by these models. We have shown that an alternative model based upon the free volume theory for diffusion describes the diffusion of lipids in liquid crystalline phase phosphatidylcholine bilayers quite well.

APPENDIX

Extended Free Volume Theory. The three-dimensional free volume model of Cohen & Turnbull (1959) for diffusion in glasses has been redefined by Galla et al. (1979) to apply to the two-dimensional diffusion of lipids within bilayers. Their expression for the diffusion constant is

$$D_t = g l_c u \exp\{-\gamma a^*/[a_0\alpha_a(T - T_0)]\} \qquad \text{(A1)}$$

where g = a geometrical factor, l_c = the diameter of the cage (approximately the molecular diameter), u = the average velocity of the test molecule within the free volume (free area for two dimensions), which is the gas kinetic velocity, γ = a factor, $^1/_2$–1, to account for any overlapping free volumes (areas), a^* = the critical free area per test molecule, i.e., the minimum free area that must exist so that transport occurs, a_0 = the van der Waals molecular area, T_0 = the temperature where there is no free volume, α_a = the lateral thermal expansion coefficient of the membrane, and $a_0\alpha_a(T - T_0) = a_f$, where a_f is the average free volume per molecule.

We propose an extension of this theory to include the influence of the viscous forces in the aqueous phase and at the midplane of the bilayer upon the lateral diffusion constant. A complete description of our extended free area model and a critical discussion of its application will be given elsewhere. Here we only indicate the approach.

The available free volume per molecule for the diffusant is defined to be $v_f = v_c - v_0$, where v_f is the free volume per molecule, v_c is the total cage volume, and v_0 is the van der Waals volume of the test molecule. Cohen & Turnbull (1959) showed that the probability, $p(v_f)$, that for any test molecule the free volume will have the value v_f is

$$p(v_f) = (\gamma/v_f)\exp(-\gamma v^*/v_f) \qquad \text{(A2)}$$

The average diffusion constant they then define by

$$D_t = \int_v^\infty D(v_f)p(v_f)\,dv_f \qquad \text{(A3a)}$$

v_f can be written as

$$v_f = v_0[\beta + \alpha(T - T_m)] \qquad \text{(A3b)}$$

α is the coefficient of volume expansion, T_m is the temperature of the phase transition, and $v_0\beta$ is the free volume *at the phase transition*. This is the original "Doolittle equation" for free volume (Grest & Cohen, 1981).

Within the free area of the bilayer, the diffusant (a lipid molecule) is hindered by the frictional forces of the external aqueous solvent and the midplane of the bilayer. The movement of the molecules can no longer be considered according to the diffusion mechanism of a gas, as was done by Cohen & Turnbull (1959) and Galla et al. (1979); the diffusion constant *within the free volume* (*area*) of eq A3 can be replaced by

$$D(v_f) = kT/f \quad \text{(A4)}$$

which is a constant where f is the translational friction coefficient of the molecule that is free to move without dissipative interactions in the bilayer but is retarded by the viscous drag of the aqueous phase and the lipid bilayer midplane, k is the Boltzmann constant, and T is the temperature in kelvin. Thus, the new expression for D_t is

$$D_t = (kT/f)\exp\{-\gamma a^*/[a_0[\beta + \alpha_a(T - T_m)]]\} \quad \text{(A5)}$$

which should be compared to eq A1. The friction coefficient will have the form

$$f = f_1 + f_2 \quad \text{(A6)}$$

where $f_i = \alpha_i\eta_i$, α_i is a constant, and η_i is the viscosity of the corresponding fluid, either the aqueous phase or the lipid bilayer midplane. The constant, α_i, will be approximately $4\pi R$, where we have to pick some reasonable equivalent radius of a sphere, R, of the protruding portion of the molecule. These are only approximate guidelines for estimating the numerical values of the friction coefficients, but when the measured diffusion coefficients are simulated according to eq A5, good agreement is found between reasonable estimates and the fitted friction coefficients.

Registry No. NBD-DHPE, 85414-15-9; NBD-DLPE, 85414-16-0; NBD-DSPE, 85414-17-1; NBD-POPE, 85414-18-2; POPC, 6753-55-5; DLPC, 18285-71-7; DSPC, 4539-70-2; DMPC, 13699-48-4; DPPC, 2644-64-6.

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