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Bounding fluid viscosity and translational diffusion in a fluid lipid bilayer

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Abstract. Fluorescence recovery after photobleaching was used to investigate the translational diffusion of a fluorescent derivative of a membrane-spanning lipid in L_α phase multibilayers of 1-palmitoyl-2-oleoylphosphatidylcholine prepared in water and in glycerol. The translational diffusion coefficient in hydrated bilayers (D_w) ranged between 2 and 5×10^{-8} cm^2/s and in glycerinated bilayers (D_g) the range was between 3 and 24×10^{-10} cm^2/s between 10° and 40 °C. These results are discussed in terms of models for diffusion in membranes.

Key words: Translational diffusion, lipid bilayers, bipolar lipids, fluorescence recovery after photobleaching, models for diffusion

Introduction

The translational diffusion of lipids and proteins in L_α phase lipid bilayers has been investigated in many laboratories and some attempts have been made to compare experimental results with theoretical models for diffusion in membranes (for recent reviews see Vaz et al. 1984; Clegg and Vaz 1985). While it seems clear that protein diffusion is well described by models, based upon continuum fluid hydrodynamic considerations, for diffusion in thin viscous fluid sheets (Saffman 1976; Saffman and Delbrück 1975; Hughes et al. 1981, 1982), there is some uncertainty whether lipid diffusion in lipid bilayers can be equally well described by these models (Galla et al. 1979; Vaz and Hallmann 1983; Vaz et al. 1985a). Further, the applicability of any model to diffusion in membranes bounded by fluids with viscosities equal to or greater than the viscosity of the membrane has never been experimentally examined although this problem has been theoretically described (Hughes et al. 1981, 1982). This is important since natural membranes are usually bounded by structures such as the glycocalix and cytoskeleton which have important effects on their mechanochemical properties (see, for example, Stokke et al. 1986a, b).

In a recent paper (Vaz et al. 1985b) we have described a fluorescent derivative of a membrane-spanning phospholipid (NBD-MSPE), derived from archaebacterial glycerol-dialkyl-glycerol tetraether lipids, and the study of its translational diffusion in L_α phase 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) multibilayers using the fluorescence recovery after photobleaching (FRAP) technique. This molecule has some advantages as a probe of diffusion in lipid bilayers. First, it has about the same radius as a typical lipid molecule. Second, since its height is the same as the thickness of the lipid bilayer, it is the type of particle whose diffusion Saffman's model attempts to describe.

The viscosity of the bounding fluid in a multibilayer phospholipid-water system can be altered by temperature or by addition of solutes such as sucrose to the aqueous phase. The change in the aqueous phase viscosity caused by changing the temperature is too small to be of practical use and it has been recently shown (Stümpel et al. 1985) that high concentrations of sucrose dehydrate the lipid-water multilamellar system. This may have effects upon the chain density of the bilayers and upon the interbilayer interactions in multilamellar systems and could result in artifacts in the diffusion measurements. With this in view, we have opted to change the bounding fluid viscosity by replacing the water with glycerol. McDaniel et al. (1983) have shown that there are no structural differences between L_α phase lipid-water and lipid-glycerol multilamellar systems. We have confirmed that this is also the case for the system reported in this paper.

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The differences in the viscosity of water and glycerol at the same temperatures, within the temperature range of interest, however, are at least a factor of 10^2.

Materials and methods

NBD-MSPE was prepared as described earlier (Vaz et al. 1985 b). POPC was purchased from Fluka Feinbiochemica, Buchs, Switzerland, and used as received from the supplier. Glycerol (≧95%) was from J. T. Baker Chemicals, Deventer, The Netherlands. Two separate lots of glycerol were used: 96% and 97% as judged by comparison of the measured refractive indices with values in published tables (CRC Handbook of Chemistry and Physics 1983). Preparations for FRAP experiments were done as previously described (Vaz et al. 1985 b) using either water or glycerol as the solvating medium. FRAP experiments were performed as described earlier (Vaz and Hallmann 1983). The radius of the uniform circular spot was 7.5 μm using a Zeiss Plan 10/0.30 objective. Diffusion coefficients were determined from half times for complete fluorescence recovery after having ascertained that the recovery curves could be well described as being due to diffusion of one fluorescent species only (Axelrod et al. 1976).

Results and discussion

Figure 1 shows a typical fluorescence recovery curve for NBD-MSPE in glycerinated POPC multibilayers at 20 °C. It is seen that the agreement of the theoretical recovery curve for one diffusing component with the experimental curve is quite good. In previous work (Vaz et al. 1985 b) we had shown this to be the case for NBD-MSPE in hydrated POPC multibilayers.

In Fig. 2 we show the temperature-dependence of the translational diffusion coefficient for NBD-MSPE in multibilayers of hydrated (D_w) and glycerinated (D_g) POPC between 10° and 40 °C. Typical standard deviations were ≦ ± 12% of the mean for the hydrated bilayers and ≦ ± 20% of the mean for the glycerinated bilayers. The glycerinated multibilayers were also more difficult to prepare and there was a greater variability in the microscopic appearance of the preparations which may explain the greater experimental error in measurements performed on these samples.

In Fig. 3 we attempt to understand the values of D_g obtained experimentally on the basis of the continuum fluid hydrodynamic model of Hughes et al.

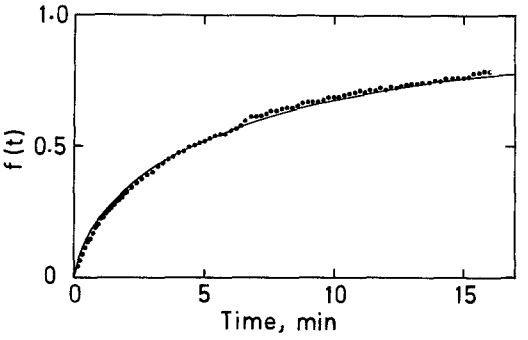

Fig. 1. Comparison of the experimental FRAP curves (*points*) at 20 °C for NBD-MSPE in POPC/glycerol multibilayers with the theoretical fluorescence recovery (*line*) expected for a single fluorescent diffusing component

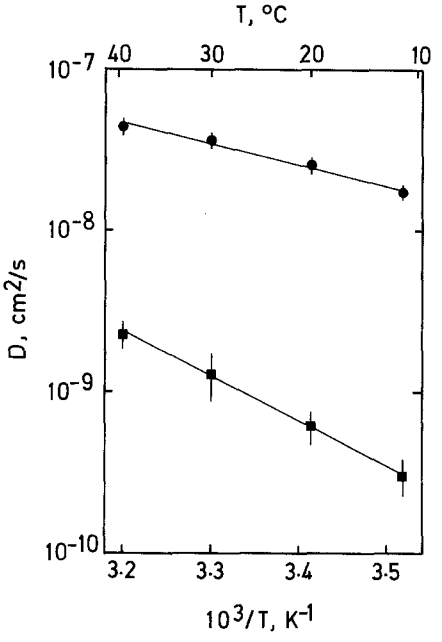

Fig. 2. Temperature dependence of the translational diffusion coefficient for NBD-MSPE in multibilayers of hydrated (*circles*) and glycerinated (*squares*) POPC multibilayers. Data represent the mean ± standard deviation of at least three FRAP experiments on different multibilayer domains of each of at least three separately prepared slides (nine FRAP experiments per point)

(1981). We have had to use the solution given by these authors for $1 < \varepsilon < 10$ where $\varepsilon = (2\eta_g a/\eta h)$. Here η_g and η are the viscosities of glycerol and the lipid bilayer, respectively, a is the radius of the test particle (0.5 nm), and h is the thickness of the fluid bilayer sheet and the height of the particle (5.0 nm). In the calculation of the theoretical curve (solid line) in Fig. 3 we have used the membrane viscosities derived from earlier measurements of D_w for NBD-MSPE in hydrated POPC bilayers (Vaz et al. 1985 b), and taken glycerol viscosities from tables (Landolt-

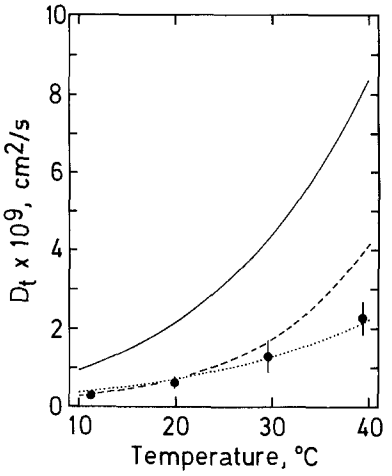

Fig. 3. Comparison of the experimental results for the temperature dependence of NBD-MSPE translational diffusion in POPC/glycerol multibilayers with the predictions of the continuum fluid hydrodynamic model (Hughes et al. 1981) for diffusion in thin viscous fluid sheets. The theoretical equation valid for $1 < \varepsilon < 10$ was used in the computation of all theoretical curves. ●, experimental results; (——), theoretical curve assuming membrane viscosities to be the same in hydrated (Vaz et al. 1985b) and glycerinated POPC bilayers. The viscosities of glycerol were obtained from tables (Landolt-Börnstein 1969; CRC Handbook of Chemistry and Physics 1983). (....), theoretical temperature dependence of D_g obtained when the membrane viscosity in glycerinated POPC bilayers is assumed to be 10-fold higher than the membrane viscosity in hydrated POPC bilayers (Vaz et al. 1985b) over the entire temperature range; (– – –), theoretical temperature dependence of D_g obtained by assuming the interfacial glycerol viscosity at 20 °C to be 54.4 poise and that the temperature dependence of the interfacial glycerol viscosity is the same as that of bulk glycerol viscosity

Börnstein 1969; CRC Handbook of Chemistry and Physics 1983). Under these conditions it is seen that the theory predicts values of D_g which are about 3.5 fold higher than those obtained experimentally. The theoretical values would, in principle, be reduced if either the viscosity of glycerol at the interface were considerably higher than that of bulk glycerol or if the viscosity of the bilayer was higher for glycerinated bilayers than it is for hydrated bilayers or a combination of both.

The theoretical curve obtained when only a higher membrane viscosity is assumed is shown by the dotted line in Fig. 3. Throughout the temperature range examined, a ten fold higher membrane viscosity has to be assumed for the glycerinated bilayers as compared to the hydrated ones to obtain a good agreement between the theoretical curve and the experimental results. This difference in membrane viscosities is similar to that between the gel and liquid crystalline phases of saturated acyl chain phosphatidylcholine-water systems (Lentz et al. 1976). A direct measurement of bilayer viscosity was not possible using polarization of fluorescence of diphenylhexatriene since this probe is significantly soluble in glycerol. An indirect indication that the bilayer viscosities in hydrated and glycerinated POPC bilayers are not significantly different was obtained from X-ray diffraction studies on the multilamellar systems. In both cases the reflexes in the small angle (interlamellar lattice) and wide angle (chain lattice) regions were almost identical. The same result has been obtained by other workers (McDaniel et al. 1983; T. J. McIntosh, personal communication). This would suggest that the density (and, we infer, the viscosity) of the POPC bilayer is very nearly the same whether the solvating medium is water or glycerol. In any case, we doubt that the glycerinated bilayer is ten fold more viscous than the hydrated one.

The possibility that the viscosity of glycerol is lower in the bulk than it is at the membrane-glycerol interface was also considered (Fig. 3, broken line). At 20 °C we have to assume η_g to be 54.4 poise, about 3.7 fold higher than bulk glycerol viscosity at the same temperature in order to obtain a coincidence of experimental results and theoretical expectations. In Fig. 3 (broken line) we have assumed that the interfacial glycerol viscosity has the same dependence on temperature as bulk glycerol viscosity over the entire temperature range studied. If this assumption is made, it is seen that the temperature-dependence of the theoretical curve obtained is different from that of the experimental result.

A comparison of the experimental results with a model for diffusion based upon a free volume theory (Cohen and Turnbull 1959; Galla et al. 1979; Vaz et al. 1985a) is undertaken below. This model gives the observed translational diffusion coefficient as a product of the probability of the test particle being in the immediate neighbourhood of a sufficiently large free space and the diffusion coefficient within this free space, i.e. $D_t = D(v_f) \cdot p(v_f)$. For the case of translational diffusion of lipids in lipid bilayers it was proposed (Vaz et al. 1985a) that the diffusion coefficient within the free space could be given by $D(v_f) = kT/f$ where k is Boltzmann's constant. T is the temperature in degrees Kelvin and f is the translational frictional coefficient of the NBD-MSPE particle in the POPC bilayer. In this model f is the result of interactions of our test particle with the bounding fluid layer at the membrane interface and with the relatively disordered layer at the bilayer midplane. Figure 4 shows how the model describes the temperature dependence of D_g for NBD-MSPE in glycerinated L_α phase POPC bilayers. In obtaining the theoretical curve, we have assumed that the probability of having a free space of adequate size in the vicinity of the test particle,

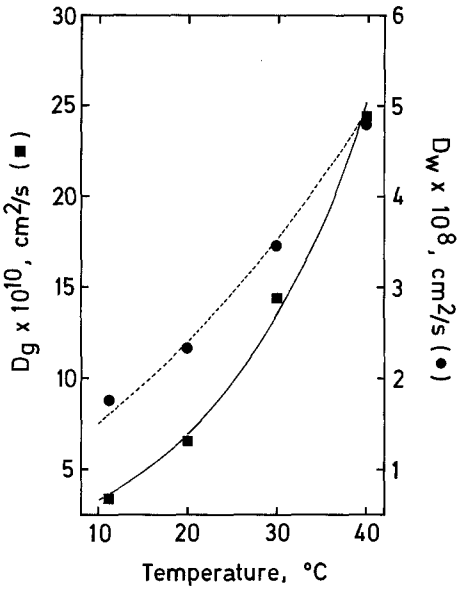

Fig. 4. Comparison of the experimental results for the temperature dependence of NBD-MSPE translational diffusion in POPC/glycerol multibilayers with the predictions of a free-volume (area) model for diffusion in lipid bilayers (Vaz et al. 1985a). All parameters in the theoretical "fit" except the translational friction coefficient, f, and its temperature dependence are the same as previously used (Vaz et al. 1985b) to describe diffusion of NBD-MSPE in POPC/water multibilayers. (●), experimental results in POPC/water; (---), fit for POPC/water with $f = 1.92 \times 10^{-7}$ erg · s/cm² at 10 °C; (■), experimental results for POPC/glycerol; (—), fit for POPC/glycerol with $f = 8.74 \times 10^{-6}$ erg · s/cm² at 10 °C

$p(v_f)$, is the same for glycerinated and hydrated POPC bilayers. Only the value of f was different for the two cases. For hydrated bilayers $f_w = 1.92 \times 10^{-7}$ ergs · s/cm², and for glycerinated bilayers $f_g = 8.74 \times 10^{-6}$ ergs · s/cm², both at 10 °C. As discussed elsewhere (Vaz et al. 1985a), the translational frictional drag upon the particle is a sum of the drag at the membrane-bounding fluid interface and the drag in the relatively disordered bilayer midplane. No independent estimates are available for either of these values and their temperature dependence so that, at this stage, further interpretation of the f values obtained would be too speculative. It may, however, be of interest to compare the temperature-dependencies of f_w and f_g with that of the viscosities, η_w and η_g, of water and glycerol, respectively. Plots of $\ln f_w$ and $\ln f_g$ versus the temperature (between 283 K and 313 K) have slopes of -0.0094 and -0.0371, respectively. The ratio of these slopes is 0.2534. In comparison, plots of $\ln \eta_w$ and $\ln \eta_g$ versus temperature (in the same range) have slopes of -0.0230 and -0.0909, respectively. The ratio of these slopes is 0.2530, a value very similar to that obtained for the temperature dependence of the translational friction coefficients.

The rather good description of the experimental results by the free volume model described above leaves some questions open. The most important among these is the exact nature of f and its dependence upon the viscosities of the bounding fluid at the bilayer surface and of the layer of high disorder at the bilayer midplane. Also, though we have assumed here that $p(v_f)$ is the same for hydrated and glycerinated POPC bilayers this need not necessarily be the case. We are currently working on these problems.

In conclusion, it has been shown that the translational diffusion of a membrane-spanning lipid in a fluid lipid bilayer is very strongly affected by the viscosity of the fluid bounding the bilayer. This is of importance to the understanding of diffusion in cellular membranes where the membrane is bounded by highly structured and/or viscous cellular components. It has also been shown that existing models for the theoretical description of diffusion in membranes or membrane-like thin viscous fluid sheets are either unable to describe this effect or describe it incompletely. It is suggested that new approaches including computer simulation techniques (see, for example, van Gunsteren and Berendsen 1984; Edholm et al. 1984) or the application of the concept of a generalized frequency-dependent viscosity (Alder and Alley 1984) should be attempted for the resolution of the problems, mostly of a theoretical nature, of diffusion of lipids in lipid bilayers.

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