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A comparison of the translational diffusion of a normal and a membrane-spanning lipid in L_α phase 1-palmitoyl-2-oleoylphosphatidylcholine bilayers

W. L. C. Vaz[1]*, D. Hallmann[1], R. M. Clegg[1], A. Gambacorta[2], and M. De Rosa[2]

[1] Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, Federal Republic of Germany, and
[2] Istituto per la Chimica di Molecole di Interesse Biologico, C. N. R., Via Toiano, 6, Arco Felice (Napoli), Italy

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Abstract. We have used the fluorescence recovery after photobleaching technique to study the translational diffusion, in L_α phase multibilayers of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), of fluorescent derivatives of 1-palmitoyl-2-oleoylphosphatidylethanolamine (NBD-POPE) and a membrane-spanning phosphatidylethanolamine (NBD-MSPE). The latter derivative was prepared from a membrane-spanning glycerol-dialkyl-glycerol tetraether lipid isolated from the thermophilic and acidophilic archaebacterium *Sulfolobus solfataricus*. The translational diffusion was examined between about 15° and 45 °C. It is shown that over this temperature range the translational diffusion coefficient for NBD-MSPE is ~2/3 that for NBD-POPE which spans only one monolayer of the bilayer. The result is interpreted in terms of existing models for translational diffusion in lipid membranes.

Key words: Membrane-spanning lipid, translational diffusion, phospholipid bilayers, fluorescence recovery after photobleaching, diffusion models

Introduction

In the recent literature (for reviews see Vaz et al. 1984; Clegg and Vaz 1985a) it has been shown that the translational diffusion of integral membrane proteins in liquid crystalline phase phospholipid bilayers can be adequately described by continuum fluid hydrodynamic models for diffusion in thin viscous fluid sheets (Saffman 1976; Saffman and Delbrueck 1975; Hughes et al. 1981, 1982). From experimental data for diffusion of membrane-spanning proteins in lipid bilayers (Vaz et al. 1981, 1982; Chang et al. 1981; Criado et al. 1982; Peters and Cherry 1982; Vaz and Criado 1985), and making the assumption that the hydrodynamic model is applicable to the problem, it can be concluded that the viscosity of the L_α phase lipid bilayer is between 1 and 2 poise. We (Vaz and Hallmann 1983; Vaz et al. 1985) have recently shown that the hydrodynamic model is not applicable to the case of lipid diffusion in a lipid bilayer and have proposed that this case may be better described by a free-volume (free-area) model for diffusion which takes into account the viscous drag forces exerted upon the diffusing lipid particle at the membrane-water interface and the lipid bilayer midplane (Clegg and Vaz, 1985b). In that study, only those lipids that spanned one monolayer of the bilayer membrane were considered. In the present paper we describe our results for the translational diffusion of a membrane-spanning lipid which has two equivalent hydrophilic head groups and hydrophobic chains that span both monolayers of the lipid bilayer.

The membrane-spanning lipid was obtained from the thermophilic and acidophilic archaebacterium *Sulfolobus solfataricus* which has also formerly been referred to as *Caldariella acidophila*. The chemical characterization of these lipids and their arrangement in lipid bilayers has been described (De Rosa et al. 1983a, b; Gliozzi et al. 1983). In this study we used a mixture of lipids (for structures, see Figs. 1 and 2 of De Rosa et al. 1983b), the principal components of which are shown in Fig. 1. This derivative was then labeled with the fluorescent NBD group and its translational diffusion in L_α phase bilayers of POPC was examined by the FRAP technique. The translation diffusion coefficient for the membrane-spanning lipid derivative was compared, between about 15° and 45 °C, with that for a lipid derivative which spans only one monolayer of the lipid bilayer.

Abbreviations: D_t, translational diffusion coefficient; FRAP, fluorescence recovery after photobleaching; MSPE, a membrane-spanning phosphatidylethanolamine derived from a glycerol-dialkyl-glycerol tetraether lipid isolated from *Sulfolobus solfataricus;* NBD, 4-nitrobenz-2-oxa-1,3-diazolyl; PE, phosphatidylethanolamine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine

* To whom offprint requests should be sent

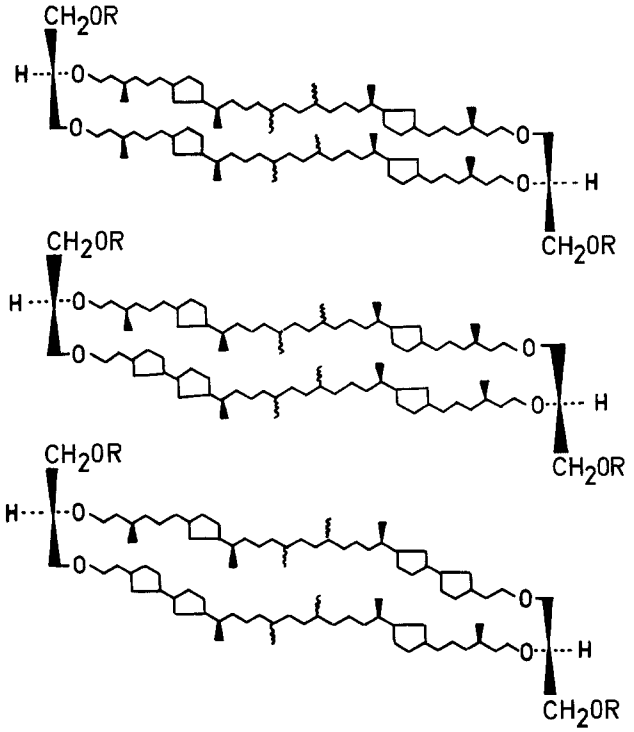

Fig. 1. Structures of the principal components in NBD-MSPE. *R* is the *N*-(NBD)-phosphoethanolamine group

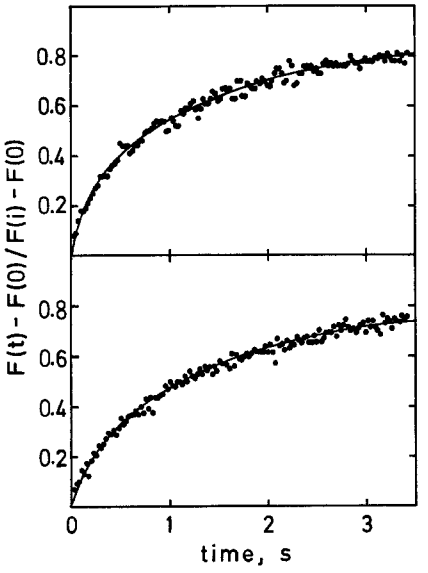

Fig. 2. Comparison of the experimental FRAP curves at ~35 °C for NBD-POPE *(upper panel)* and NBD-MSPE *(lower panel)* in POPC multibilayers with the theoretical fluorescence recovery for one diffusing fluorescent component *(lines)*

Materials and methods

POPC and POPE were from Fluka AG, Buchs, Switzerland, and were used as received without further purification. NBD-chloride was from Aldrich Europe Division, Nettetal, F.R.G. NBD-PEs were prepared as described earlier (Vaz and Hallmann 1983). All other chemicals were commercial reagent grades.

Preparation of the diPE derivative of glycerol-dialkyl-glycerol tetraethers obtained from Sulfolobus solfataricus: Glycerol-dialkyl-glycerol tetraethers (Fig. 1, R = H), backbone of the complex lipids of *Sulfolobus solfataricus,* were recovered from an acid hydrolysate of *Sulfolobus solfataricus* complex lipids as previously described by De Rosa et al. (1983b). The diPE derivative of the glycerol-dialkyl-glycerol tetraether mixture was prepared according to Eibl (1978) via the diphosphoric acid dichloride which in turn was converted into the corresponding diPE. The diPE derivative (about 50% of reaction mixture) has an R_F of 0.5 when chromatographed on silica gel thin layer plates developed with chloroform:methanol:water (65:25:4, by volume).

FRAP experiments: Samples for FRAP experiments were prepared as described earlier (Vaz et al. 1985). The molar ratio of NBD-PE/POPC was 5×10^{-4} in all cases. FRAP experiments were done on multibilayer domains of the samples as described earlier (Vaz and Hallmann, 1983). D_t was evaluated from the fluorescence recovery curves using the half-time for complete recovery of fluorescence in a uniform circular spot as described by Axelrod et al. (1976). The fluorescence recovery was complete in all cases. A few experimental FRAP curves were compared with the theoretical fluorescence recovery expected from one fluorescent diffusing component and the agreement between experiment and theory was found to be good. The FRAP data reported here is the result of at least five experiments on different multibilayer domains of each of at least five separately prepared samples for each case investigated. The standard deviation was $\leqslant \pm 12\%$ of the mean.

Results

Figure 2 compares experimental FRAP curves for NBD-POPE and NBD-MSPE in POPC multibilayers with the theoretical fluorescence recovery expected for one fluorescent diffusing component. It is seen that the agreement between experiment and theory is good. Figure 3 shows the temperature dependence of D_t for NBD-POPE and NBD-MSPE in L_α phase POPC bilayers between about 15° and 45 °C. The data in Fig. 3 are plotted as an Arrhenius plot from which an "activation energy" of ~28 kJ/mole, in both cases, can be deduced. It is also seen that throughout the temperature range examined D_t for NBD-MSPE is about 2/3 of the D_t for NBD-POPE.

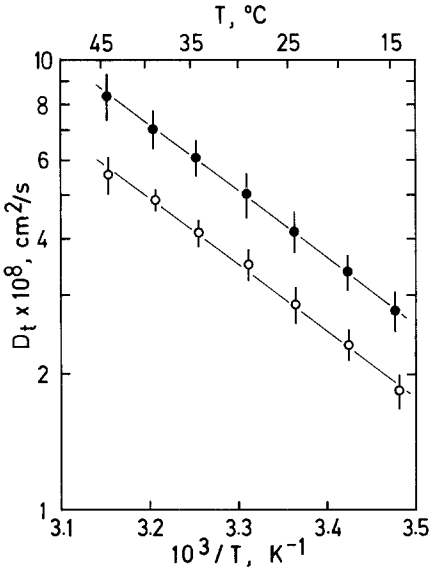

Fig. 3. Temperature-dependence of D_t for NBD-POPE (●), and NBD-MSPE (○) in L_α phase POPC multibilayers. The data represent the mean ± standard deviation of at least five FRAP experiments on different multibilayer domains of each of at least five separately prepared slides (25 FRAP experiments per point). The data are plotted as Arrhenius plots. The lines are linear regression leastsquares analysis of the experimental points with coefficients of correlation ≥ 0.995

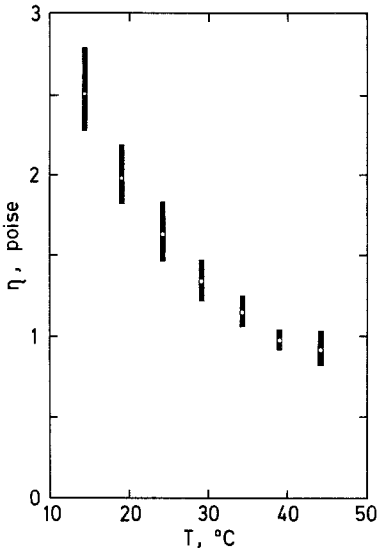

Fig. 4. Temperature-dependence of the viscosity, η, of the L_α phase POPC bilayer deduced from the translation diffusion data for NBD-MSPE in POPC bilayers. It has been assumed that the continuum hydrodynamic model for diffusion in thin viscous fluid sheets (Eq. (1), taken from Saffman 1976; Saffman and Delbrueck 1975) can be applied to the translational diffusion of NBD-MSPE in POPC bilayers. The bars represent the values of the η calculated from the experimentally obtained mean values of D_t *(open circle)* and its extremes given by the standard deviation

We try to understand this result in terms of the various models available for translational diffusion in lipid bilayer membranes. First, we consider the continuum fluid hydrodynamic model presented by Saffman (1976; Saffman and Delbrueck 1975). These authors give D_t for a cylindrical particle with a radius, a, and height, h, diffusing in an infinite viscous continuum fluid sheet of viscosity, η, and thickness, h, bounded on both sides by a fluid of viscosity, η', (where $\eta' \ll \eta$) by

$$D_t = (kT/4\pi\eta h)\{\ln(\eta h/\eta' a) - \gamma_E\}. \quad (1)$$

Here the assumption of a "stick" boundary condition has been made. k is Boltzmann's constant, T is the temperature in degress Kelvin, and γ_E is Euler's constant (0.5772). Applying Eq. (1) to the experimental results for the translational diffusion of NBD-MSPE in POPC bilayers allows us to calculate η for the POPC bilayers as a function of temperature. The result is shown in Fig. 4. NBD-MSPE is the first membrane-spanning lipid whose translational diffusion in lipid bilayers has been examined, and for the first time the diffusion of a lipid derivative in a membrane can be directly compared to that of membrane-spanning integral proteins. Several such proteins have been examined in L_α phase lipid bilayers (for recent reviews see Vaz et al. 1984; Clegg and Vaz, 1985a). The essential conclusion of these studies has been that the translational diffusion of integral membrane-spanning proteins can be well described by Saffman's model. The membrane viscosity, η, compatible with the protein translational diffusion results is estimated at between 1 and 2 poise at ~35°C (Vaz et al. 1982, 1984). The lower value is in agreement with the values in Fig. 4. It must be pointed out, however, that the values of D_t for lipid translational diffusion in L_α phase POPC bilayers are somewhat lower than the values seen in L_α phase saturated acyl chain phosphatidylcholine bilayers at comparable temperatures (Vaz et al. 1985).

In recent work we have provided arguments and experimental evidence against the applicability of the continuum fluid hydrodynamic model to translational diffusion in lipid bilayers of lipids which only extend into one of the monolayers (Vaz and Hallmann 1983; Vaz et al. 1982, 1984, 1985; Clegg and Vaz 1985a). We emphasize that the objection to the hydrodynamic model referred only to the case of normal lipid molecules, not the membrane-spanning species. A free area model for diffusion of lipid-like particles in lipid bilayers was presented (Clegg and Vaz 1985b). This model proposes that diffusive motion of a lipid molecule in a bilayer is determined by the probability of having a vacant area larger than a critical free area, a^*, in the neighbourhood of the diffusing particle and the rate at which the diffusant can move into the vacant

space. The latter is, in this model, determined primarily by the translational frictional coefficient, f, which is a result of the friction interactions of the particle with the aqueous interface and the bilayer midplane. D_t is then given (Clegg and Vaz, 1985b) by

$$D_t = (kT/f) \cdot \exp\{-\gamma a^*/a_0[\beta + \alpha_a(T-T_m)]\}, \quad (2)$$

where γ is a numerical factor ($0.5 \leq \gamma \leq 1.0$) which accounts for the overlap of free area; a_0 is the van der Waals cross-sectional area for a lipid molecule; β is the fractional free area at the phase transition temperature, T_m, and α_a is the area expansion coefficient in the L_α phase. We have previously shown (Vaz et al. 1985) that the experimentally observed temperature-dependence of D_t for NBD-POPE in POPC bilayers is well described by Eq. (2) if $\gamma a^*/a_0 = 0.4$, $\alpha = 2.3 \times 10^{-3}$ K^{-1}, $\beta = 0.142$. The value of f, for NBD-POPE diffusion, was given by a sum of translational frictional coefficients, $f_1 + f_2$, where f_1 is due to the interactions with the aqueous interface and f_2 is due to the interactions at the bilayer midplane. As discussed elsewhere (Vaz et al. 1985), $f_2 = 3.7 \times 10^{-8}$ ergs-s/cm^2 and is independent of temperature whereas $f_1 \propto \eta_1$, where η_1 is the aqueous viscosity and is dependent upon the temperature. For NBD-MSPE diffusion, $f = 2f_1 + f_2'$; $2f_1$ since the lipid particle under consideration here is in contact with the aqueous phase on both sides of the membrane, and f_2' since the frictional coefficient at the midplane may be somewhat different than for a lipid which extends only halfway through the membrane. The theoretical curve for the temperature-dependence of D_t for NBD-MSPE shown in Fig. 4 was obtained by setting $f_2' = 1.15 \cdot f_2$. A possible reason for this could be the fact that NBD-MSPE presents a larger volume at the bilayer midplane than does NBD-POPE. T_m for POPC is 5 °C. Equation (2) equally well describes the temperature-dependence of D_t for NBD-MSPE in POPC with the same values for $\gamma a^*/a_0$, α, β, and f_1 as given for NBD-POPE translational diffusion. This is shown in Fig. 5 where the theoretical predictions of Eq. (2) are compared with the experimental results for NBD-MSPE and NBD-POPE in POPC bilayers.

Very recently, Nadler et al. (1985) have presented a theoretical model which compares the diffusion of a membrane-spanning rod-like particle in a lipid bilayer with that of a rod-like particle that spans only one monolayer of the bilayer. The model assumes that random diffusive steps in each of the two monolayers (labeled 1 and 2, respectively) which constitute the lipid bilayer are independent of each other. Thus a bilayer-spanning diffusant such as NBD-MSPE diffuses, in this picture, by a series of tilt-untilt processes. Tilting occurs when the diffusant moves into a neighbouring free volume in monolayer 1, this step being not necessarily coupled to an equivalent (in direction and magnitude) step in monolayer 2. According to Nadler et al. (1985) the experimentally observed ratio $D_{t(\text{NBD-MSPE})}/D_{t(\text{NBD-POPE})} = 2/3$ can only be explained within this model if NBD-MSPE behaves as a "stiff" rod with a strong tendency to remain in an untilted state, i.e. with its long axis perpendicular to the plane of the membrane. This would effectively force the "creation" of an adequate free volume in the proper place in monolayer 2 to complete a step, or in monolayer 1 to revert to the starting position. Considering that NBD-MSPE has two alkyl chains linked to the same head groups at both ends, it is not surprising that this molecule would behave as a "stiff" rod.

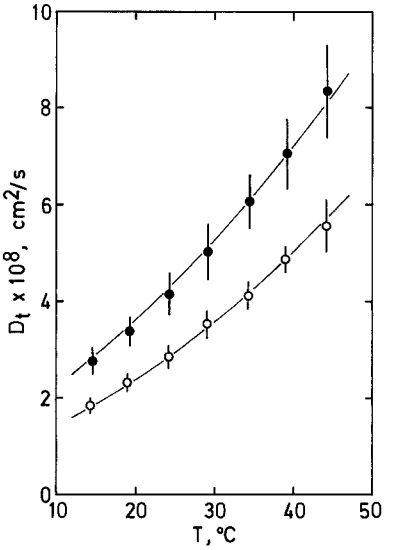

Fig. 5. Comparsion of the experimentally obtained temperature-dependence of D_t for NBD-POPE (●), and for NBD-MSPE (○) in L_α phase POPC bilayers with the theoretical temperature-dependence predicted by the free area theory (Eq. (2), taken from Clegg and Vaz, 1985b). The theoretical curve was obtained by placing $\gamma a^*/a_0 = 0.4$, $\alpha = 2.3 \times 10^{-3}$ K^{-3}, and $\beta = 0.142$ for both cases. The values of f for NBD-POPE and NBD-MSPE were obtained as described in the text

Discussion

We have shown here that the translational diffusion of a membrane-spanning lipid embedded in a liquid crystalline phase POPC bilayer can be equally well described by a continuum fluid hydrodynamic model for diffusion in thin fluid sheets (Saffman 1976; Saffman and Delbrueck 1975) and by a free volume model which takes into account the frictional interactions of the diffusing particle with the aqueous interface and the bilayer midplane (Vaz et al. 1985; Clegg and Vaz 1985b). We have not considered the polymer diffusion model adapted for lipid bilayers by Pace and Chan (1982) since this model is not applicable to the diffusion of membrane-spanning lipids in its present form.

Further, as discussed earlier, the diffusion results indicate that the NBD-MSPE particle may behave in the bilayer as a "stiff" rod. As we have discussed elsewhere (Vaz et al. 1985; Clegg and Vaz, 1985a), experimental results and theoretical considerations make it questionable whether continuum fluid hydrodynamic considerations can be applied when the diffusing particle is about the same size as the particles composing the fluid in which it is diffusing. This is the case of NBD-MSPE in POPC bilayers. In simple fluids a frequency-dependent viscosity can be used to extend the hydrodynamic considerations to a smaller dimensional scale (Alder et al. 1981). In this treatment, the effective translational friction coefficient is smaller for smaller particles than it is for larger ones. If this argument is extended to our case, we would expect that NBD-MSPE would experience a smaller effective membrane viscosity than the considerably larger integral membrane proteins. From the diffusion results for large proteins in fluid bilayers the membrane viscosity is estimated to be between 1 and 2 poise at ~35°C (for reviews see Vaz et al. 1984; Clegg and Vaz, 1985a). It also appears that the larger proteins experience a membrane viscosity closer to 2 poise while the smaller ones experience a membrane viscosity closer to 1 poise. The viscosity of POPC bilayers at ~35°C felt by NBD-MSPE in its translational diffusion is ~1 poise (see Fig. 4) which we interpret to mean that the hydrodynamic model may be applicable to the case in question and that the concept of a frequency-dependent viscosity may also be applicable to lipid bilayers in their liquid crystalline phase. This observation is indicated by the data obtained to date but more experimental results are required to confirm this unambiguously. It is also of interest that a free volume diffusion model describes the temperature dependence of NBD-MSPE diffusion quite well (see Fig. 5). This model has been frequently invoked in the recent literature to interpret diffusion in liquid crystals and lipid bilayers (for a review see Clegg and Vaz (1985a).

Thus, two disparate but complementary models for translational diffusion in lipid bilayer membranes describe the diffusion of a membrane-spanning lipid within liquid crystalline phase lipid bilayers. The hydrodynamic model, which has only been derived for a membrane-spanning particle, treats the membrane as a continuum fluid and disregards the particle-like property of this two-dimensional fluid. Despite the fact that the radius of the transmembrane lipid is equal to that of the surrounding lipids, this model accounts for its translational diffusion. On the other hand, the free-area model is expected to apply to particles with radii similar to that of the particles composing the fluid in which diffusion occurs but it was not originally proposed for membrane-spanning particles. Our extension of the model is not only consistent with the present results for NBD-MSPE, but all the parameters of the model (except the slightly larger midplane friction coefficient) are identical whether a normal or a membrane-spanning lipid is being considered. It is impossible, from the data presented here, to decide which of the above models is more correctly used in the interpretation of diffusion of membrane-spanning particles of small radii in lipid bilayers. Further studies will have to decide this.

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