Experimental evidence against the applicability of the Saffman-Delbrück model to the translational diffusion of lipids in phosphatidylcholine bilayer membranes

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Received 28 December 1982

The translational diffusion coefficient (D_t) of the fluorescent lipid derivative, *N*-(4-nitrobenz-2-oxa-1,3-diazole)-phosphatidylethanolamine (NBD-PE), with acyl chains of 6, 12, and 18 carbon atoms was measured in multibilayers of 1-palmitoyl-2-oleoyl-, dilauroyl-, and distearoyl-phosphatidylcholines using the fluorescence recovery after photobleaching technique. In a given bilayer, in the liquid crystalline phase, D_t was found to be independent of the height (length in a direction parallel to the bilayer normal) of the diffusing species. This is in disagreement with fluid hydrodynamic models for diffusion in bilayers which predict an almost linear dependence of D_t upon the height of the diffusing species in the bilayer.

Lipid diffusion *Phosphatidylcholine* *Fluorescence recovery after photobleaching* *Model membrane*

1. INTRODUCTION

Artificial phospholipid bilayer membranes are convenient and simple model systems for the highly complex biological membranes. Studies on model membranes have provided much of our understanding of the basic physico-chemical principles underlying the structural and functional organization of biomembranes. Recent studies on the translation diffusion of membrane components in artificial phospholipid or protein-containing phospholipid bilayers [1–5] have shown that proteins and lipids diffuse quite rapidly in the plane of the bilayer with typical translational diffusion coefficients (D_t) between 10^{-7} and 10^{-8} cm^2/s in the liquid crystalline phase. It has, further, been experimentally verified [2,3] that the diffusion of large proteins (molecular radii in the membrane plane $\gtrsim$ 15 Å) may be reasonably well modelled by the continuum fluid hydrodynamic model of Saffman [6,7] for diffusion of cylindrical particles in thin viscous fluid sheets. The validity of this model for the diffusion of lipid molecules in the bilayer has, however, not been experimentally clearly shown [2].

Here, we present a preliminary account of our studies on the translational diffusion of the fluorescent lipid derivative, *N*-(4-nitrobenz-2-oxa-1,3-diazole)-phosphatidylethanolamine (NBD-PE), in bilayers of phosphatidylcholines using the fluorescence recovery after photobleaching (FRAP) method [8,9]. D_t was measured for the diffusion of NBD-PEs with *n*-acyl chains having 6, 12, and 18 carbon atoms incorporated at a molar ratio (NBD-PE/PC) of 5×10^{-4} in multibilayers of dilauroyl phosphatidylcholine (DLPC) and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) over the range 15–45°C and in distearoyl phosphatidylcholine (DSPC) at 60°C. In all cases, the value of D_t obtained at a given temperature in a given phosphatidylcholine bilayer was independent of the chain length of the NBD-PE. The model in [6,7] predicts that D_t should have an almost linear dependence upon the reciprocal height, $1/h$, of the diffusant (treated here as a

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

cylinder). Our result, therefore, provides experimental evidence that the model of Saffman does not adequately describe the diffusion of lipids in lipid bilayers.

2. MATERIALS AND METHODS

DLPC, DSPC, POPC, dilauroyl phosphatidylethanolamine (DLPE), distearoyl phosphatidylethanolamine (DSPE), and 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE) were purchased from Fluka AG (Buchs). Dihexanoyl phosphatidylethanolamine (DHPE) was synthesized essentially as in [10]. NBD-PEs were synthesized by the reaction of NBD-chloride (Aldrich-Europe Division, Nettetal) with the phosphatidylethanolamines in chloroform solution in the presence of a slight excess of sodium carbonate as a base. The reaction product was purified by preparative thin-layer chromatography on Silica gel plates (E. Merck, Darmstadt). Slides for FRAP experiments were prepared as described in [11] and experiments were performed on these slides not earlier than 18 h, and not later than 7 days after hydration. In control experiments, it was found that the measured value of D_t remained unchanged over at least 3 weeks after hydration of the lipid (storage at 37°C). FRAP experiments were done as in [4] except that an uniform circular disk profile with an 8.8 μm radius (10× objective) was used. This was achieved by insertion of a pinhole in the path of the illuminating laser beam as in [12]. FRAP curves were evaluated from the half-times for complete recovery ($D_t = 0.22\ \omega^2/t_{1/2}$) as in [7]. Some fluorescence recovery curves (taken at random) were compared to theoretical curves for recovery due to one diffusing component and the agreement between experiment and theory was found to be good. Fluorescence recovery was complete in all cases.

3. RESULTS

Table 1 shows the results for diffusion of NBD-DHPE, NBD-DLPE, NBD-DSPE and NBD-POPE in multibilayers of POPC at 4 temperatures between 15°C and 45°C. It is seen that the values of D_t are similar regardless of the chain length of the probes. Also, there appears to be no difference between D_t for the probes with fully saturated acyl chains and NBD-POPE. A similar result is seen in table 2 for the diffusion of NBD-DHPE, NBD-DLPE and NBD-DSPE in multibilayers of DLPC. In this case even the values of D_t for the probe with acyl chains which are longer than those of the host lipid (DLPC) are similar to those seen for NBD-DHPE and NBD-DLPE. Comparing the data in tables 1 and 2, it is seen that the values of D_t for all the probes are about 30–40% lower in POPC than in DLPC. If the data are plotted as Arrhenius

Table 1

Diffusion results for different NBD-PEs in multibilayers of POPC

NBD-PE	$(D_t \pm SD) \times 10^8$, cm^2/s[a]				E_a[b] kJ/mol
	15°C	25°C	35°C	45°C	
NBD-POPE Sample 1	2.6 ± 0.1	4.1 ± 0.3	6.4 ± 0.7	8.9 ± 0.5	32.2
Sample 2	3.0 ± 0.3	4.3 ± 0.2	6.5 ± 0.4	9.1 ± 1.4	28.5
NBD-DHPE Sample 1	3.3 ± 0.3	4.7 ± 0.2	7.2 ± 0.6	9.9 ± 0.4	27.6
Sample 2	2.8 ± 0.2	4.5 ± 0.3	7.0 ± 0.5	9.8 ± 0.4	31.8
NBD-DLPE Sample 1	2.6 ± 0.1	4.2 ± 0.3	6.5 ± 0.2	8.6 ± 0.5	30.5
Sample 2	2.6 ± 0.1	4.3 ± 0.2	6.1 ± 0.4	8.3 ± 0.5	29.3
NBD-DSPE Sample 1	2.7 ± 0.2	4.3 ± 0.6	6.2 ± 0.4	8.6 ± 0.6	29.7
Sample 2	2.5 ± 0.1	4.2 ± 0.3	6.0 ± 0.4	8.4 ± 0.2	30.1

[a] The values given here are the mean ± standard deviation (SD) of at least 5 FRAP expts on different multibilayer domains on each slide

[b] The apparent Arrhenius activation energies were calculated from the slopes of plots of $\log(D_t)$ *vs* $1/T$. (1 kJ = 0.2390 kcal)

Table 2

Diffusion results for different NBD-PEs in multibilayers of DLPC

NBD-PE	$(D_t \pm SD) \times 10^8$, cm^2/s[a]				E_a[b] kJ/mol
	15°C	25°C	35°C	45°C	
NBD-DHPE Sample 1	3.8 ± 0.3	6.2 ± 0.3	9.9 ± 0.8	13.2 ± 1.0	32.2
Sample 2	3.6 ± 0.3	6.7 ± 0.5	9.8 ± 1.0	13.2 ± 1.0	32.2
NBD-DLPE Sample 1	3.7 ± 0.1	5.8 ± 0.3	8.2 ± 0.9	10.0 ± 0.9	25.9
Sample 2	3.5 ± 0.1	5.5 ± 0.3	8.6 ± 0.7	12.6 ± 0.8	33.1
NBD-DSPE Sample 1	3.1 ± 0.3	5.6 ± 0.3	8.5 ± 0.8	12.3 ± 1.0	33.9
Sample 2	3.4 ± 0.2	5.3 ± 0.2	8.3 ± 0.2	12.1 ± 0.7	31.4

[a] The values given here are the mean ± standard deviation (SD) of at least 5 FRAP expts on different multibilayer domains on each slide

[b] The apparent Arrhenius activation energies were calculated from the slopes of plots of $\log(D_t)$ *vs* 1/T. (1 kJ = 0.2390 kcal)

Table 3

Diffusion results for different NBD-PEs in multibilayers of DSPC at 60°C

NBD-PE	$(D_t \pm SD)$, cm^2/s[a]
NBD-DHPE Sample 1	$(12.8 \pm 0.9) \times 10^{-8}$
Sample 2	$(15.5 \pm 1.3) \times 10^{-8}$
NBD-DLPE Sample 1	$(12.7 \pm 1.1) \times 10^{-8}$
Sample 2	$(13.7 \pm 1.2) \times 10^{-8}$
NBD-DSPE Sample 1	$(13.2 \pm 0.5) \times 10^{-8}$
Sample 2	$(15.7 \pm 1.5) \times 10^{-8}$

[a] Values reported are the mean ± standard deviation (SD) from 5 FRAP expts on different multibilayer domains on each slide

plots, an apparent activation energy (E_a) of about 30 kJ/mol is calculated from the slopes. In table 3 we show the result for diffusion of NBD-DHPE, NBD-DLPE and NBD-DSPE in multibilayers of DSPC. Due to the high temperatures involved in measurements in the liquid crystalline phase of this lipid (above 56°C) and melting problems with the paraffin wax sealant for the slides (m.p. about 68°C), we have measured D_t in DSPC only at 60°C. Here again, D_t is found to be similar for all 3 probes examined.

4. DISCUSSION

Recently, we have shown [2] that, while the diffusion of large integral membrane proteins (radius in the plane of the membrane ≥ 15 Å) in liquid crystalline phase phospholipid bilayers may be adequately modelled by fluid hydrodynamic considerations [6,7], the diffusion of lipids in these bilayers can only be modelled on this basis if:

(i) the 'stick' boundary condition is assumed, the bilayer 'viscosity' is about 1 poise, and the bilayer midplane viscosity is close to 0 poise; or

(ii) the lateral viscous drag forces experienced by the diffusing lipid molecule in the bilayer are lower than those experienced by the diffusing protein molecules.

Alternatively, we have suggested [2] that hydrodynamic models may be inadequate to describe the diffusion of entities as small as lipid molecules in a bilayer. Non-fluid hydrodynamic models have been proposed [13,14] to describe lipid diffusion in bilayers. An essential difference between the hydrodynamic and non-hydrodynamic models is that the former predict an almost linear dependence of D_t upon the height (h), or length of the lipid molecule in a direction parallel to the bilayer normal, whereas the latter give D_t as independent of h. A direct way to verify which of the models proposed is more adequate to describe lipid diffusion in bilayers is, therefore, to examine the dependence of D_t upon diffusant height. We have done this here by measuring D_t for NBD-PEs with acyl chain lengths from 6–18 carbon atoms (a variation of about 2-fold in h) in bilayers formed from POPC, DLPC and DSPC. As seen in tables

1–3, D_t is insensitive to h in all cases examined. This is clearly in disagreement with the model of Saffman and Delbrück [6,7].

Diffusion models for lipids in lipid bilayers, in which D_t is independent of h, have been proposed [13,14]. The results presented here are in agreement with either of these models. It may also be argued that the values of D_t obtained for the different NBD-PEs simply reflect the self diffusion rate of the host lipid molecules in the bilayer. In further work (W.L.C.V. and D.H., in preparation) we have examined the diffusion of NBD-PEs, whose acyl chain lengths are matched to those of the host lipid, in liquid crystalline phase phosphatidylcholine bilayers over large temperature ranges (e.g., NBD-DLPE in DLPC bilayers between 10°C and 60°C). We note that the diffusion behaviour does not obey the Arrhenius law. This fact is in contradiction to the expectations of the models in [6,7] and in [13].

ACKNOWLEDGEMENTS

We gratefully acknowledge the encouragement and continued support of this work by Dr Thomas M. Jovin, and useful discussions of it with Drs Robert Clegg, Edmund Matayoshi, Hagen Füldner and Jürgen Stümpel.

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