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Free volume model for lipid lateral diffusion coefficients. Assessment of the temperature dependence in phosphatidylcholine and phosphatidylethanolamine bilayers

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The lipid lateral diffusion coefficients, D_T, in fluid-phase phosphatidylcholine and phosphatidylethanolamine bilayers have been analysed in terms of the free-volume diffusion model by fitting the expression: $D_T = AT \exp[-B/(T - T_0)]$ to the observed temperature dependence, where A, B and T_0 are the parameters to be optimized. Application of an unconstrained optimization procedure to data obtained from excimer formation (Galla et al. (1979) J. Membrane Biol. 48, 215–236) and from fluorescence photobleaching (Vaz et al. (1985) Biochemistry 24, 781–786) provides statistical evidence for a free-volume model as opposed to a simple Stokes-Einstein model ($T_0 = 0$), only in certain cases. In the instances for which the parameter T_0 can be determined with a reasonable degree of accuracy, it is found that this characteristic temperature at which the free volume extrapolates to zero lies below the bilayer gel-to-fluid phase transition temperature and does not coincide with the pre-transition temperature for phosphatidylcholines.

Lateral diffusion coefficients of lipid molecules in phospholipid bilayers and membranes have generally been interpreted in terms of one of two different models (see e.g. Ref. 1). In the first model, the translational diffusion coefficient is given in terms of the Stokes-Einstein equation

$$D_T = kT/f_T \quad (1)$$

where the frictional coefficient, f_T, is directly related to the effective membrane viscosity, η. This model includes, among others, that of Saffman and Delbrück [5], which has found considerable success in interpreting the translational diffusion coefficients of integral membrane proteins. The temperature dependence of the diffusion coefficient in this model is then determined essentially by the activation energy, E_a, associated with the effective viscosity

$$D_T = AT \exp(-E_a/RT) \quad (2)$$

where A is a temperature-independent constant. Detailed studies of the temperature dependence of the lateral diffusion in phosphatidylcholine and phosphatidylethanolamine bilayers by pyrene excimer formation [2] and fluorescence photobleaching recovery measurements [3] have indicated that this does not conform to the simple Arrhenius law

Abbreviations: NBD-DLPE, -DMPE, -DPPE and -POPC: *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dilauroyl, -dimyristoyl, -dipalmitoyl and -palmitoyloleoyl phosphatidylethanolamine; PDA, ω-pyrenedecanoic acid; PL, 1-palmitoyl-2-ω-pyrenedecanoyl-*sn*-glycero-3-phosphocholine; DLPC, DMPC, DPPC and DOPC: 1,2-dilauroyl-, 1,2-dimyristoyl-, 1,2-dipalmitoyl- and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine.

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of Eqn. 2. For this reason, Galla et al. [2] proposed that the free-volume model of Cohen and Turnbull [4] is more appropriate to the lateral diffusion of lipid molecules in bilayers and membranes. Vaz et al. [3] have also demonstrated that their data can be described by this model.

In the free-volume model, the diffusion coefficient is given by

$$D_T = AT\exp(-\gamma v^*/v_f) \tag{3}$$

where v^* is the critical free volume which is necessary to create a void large enough for a diffusive lipid displacement, and γ is a constant. The free volume, v_f, required in Eqn. 3 depends on the temperature, T, and is given by

$$v_f = \alpha'\bar{v}_m(T - T_0) \tag{4}$$

where α' is the thermal expansion coefficient and $\bar{v}_m$ is the mean molecular volume over the temperature range, $T - T_0$. T_0 is the temperature at which the free volume becomes zero. There is some lack of agreement on the appropriate values for T_0. For liquids T_0 is found to correspond experimentally with the glass transition temperature [4]. For phosphatidylcholine bilayers, Galla et al. [2] have suggested that the pre-transition temperature corresponds to the temperature at which the free volume extrapolates to zero. On the other hand, Vaz et al. [3] have chosen the main gel-to-fluid transition as their reference temperature.

The temperature dependence of the diffusion coefficient in both models can be expressed in the form

$$D_T = AT\exp[-B/(T - T_0)] \tag{5}$$

where B is a temperature-independent constant and for the Stokes-Einstein model $T_0 = 0$. We have attempted to fit the temperature dependence of the diffusion coefficient data of both Galla et al. [2] and Vaz et al. [3] to Eqn. 5 using a sum of squares minimization procedure in which the parameters A, B and T_0 are optimized. In this way it is possible to decide whether the free volume model fits the data significantly better than the Stokes-Einstein model, and to obtain values for the parameters of the appropriate model.

Routine E04FCF of the NAG (Numerical Algorithms Group, Oxford, U.K.) library was used for the optimization, which is based on a modified Gauss-Newton procedure. The values of the parameters A, B and T_0 at the least-squares solution, and their 90% confidence limits, are given in Table I for various phospholipid bilayers in the fluid phase. The $100(1-\beta)\%$ confidence limits for the parameter, x_i, are defined by: $\bar{x}_i - \sqrt{\sigma}_i^2 \cdot t_{(\beta/2,m-n)} < x_i^* < \bar{x}_i + \sqrt{\sigma}_i^2 \cdot t_{(\beta/2,m-n)}$, where x_i^* is the true solution, $\bar{x}_i$ is the value at the least squares solution, σ_i^2 the variance of x_i and $t_{(\beta/2,m-n)}$ is the $(\beta/2)$ point in the t-distribution with $(m-n)$ degrees of freedom. It is immediately seen from Table I that in many cases the variance in the optimized parameters is very large. It should

TABLE I

PARAMETERS FROM THE NON-LINEAR LEAST-SQUARES OPTIMIZATION OF THE EQUATION: $D_T = AT\exp[-B/(T-T_0)]$ TO THE TEMPERATURE DEPENDENCE OF THE TRANSLATIONAL DIFFUSION COEFFICIENTS, D_T, OF NBD-PE, PDA AND PL IN PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE BILAYERS

System [a]	A (10^{-8} cm$^2\cdot$s$^{-1}\cdot$K^{-1})	B (K)	T_0 (K)
NBD-DLPE/DLPC	0.41 ± 1.11	234 ± 444	221 ± 78
NBD-DMPE/DMPC	0.21 ± 0.12	113 ± 64	255 ± 16
NBD-DPPE/DPPC	0.62 ± 8.83	283 ± 2967	222 ± 541
NBD-POPE/POPC	6.7 ± 27.4	1079 ± 1495	123 ± 126
PDA/DPPC	1.8 ± 11.1	402 ± 1406	217 ± 200
PDA/DLPE	0.33 ± 0.10	82 ± 35	266 ± 12
PDA/DOPC	0.45 ± 0.95	253 ± 412	217 ± 79
PL/DPPC	2.8 ± 14.2	555 ± 1274	206 ± 144
PL/DLPE	0.12 ± 0.01	12.6 ± 5.6	290 ± 5

[a] Data for NBD-PE are from Ref. 3 and for PDA and PL are from Ref. 4.

be emphasized that the calculated temperature dependence of the diffusion coefficients, using the parameters at the least-squares solution, provides a very good fit to the experimental data points. In particular, the are no systematic deviations between experimental and calculated values, which could suggest that some other theory might better describe the temperature dependence.

Of primary interest are the values of T_0, since these can in principle be used to distinguish between the two models. Although none of the values of T_0 are close to 0 K, in several cases the 90% confidence limits are very large and embrace $T_0 = 0$ K. Thus in these latter cases, NBD-DPPE in DPPC, NBD-DOPE in DOPC and PDA in DPPC, it is not possible to decide between the two models. In the other cases, the temperature dependence of the diffusion coefficient is significantly better fitted by the free-volume model, although for half of these cases T_0 is still not determined with any reasonable degree of accuracy. Since the free volume model is definitely favoured in these latter cases, there is little doubt that this is also the more appropriate model for the ambiguous cases.

For PDA in DLPE, $T_0 = -7 \pm 12$°C, and for PL in DLPE, $T_0 = +17 \pm 5$°C. These two values are not consistent for the same lipid host, and may reflect to some extent the smaller free volume required for the single-chain pyrene decanoic acid than for the two-chain pyrene phosphatidylcholine. Both values are significantly lower than the gel-to-fluid phase transition temperature of DLPE, $T_t = 30.5$°C, indicating that the free volume in the fluid phase does not extrapolate to zero at the chain-melting transition temperature. (For phosphatidylethanolamines there is no pre-transition.) For NBD-DMPE in DMPC, $T_0 = -18 \pm 15$°C, indicating that the free volume in the fluid phase extrapolates to zero at a temperature below that of the pre-transition for DMPC bilayers, $T_p = +14$°C. The temperature T_0 lies $T_t - T_0 = 41 \pm 15$ K below the gel-to-fluid transition for DMPC, and $T_t - T_0 = 13.5 \pm 5$ K below the gel-to-fluid transition for PL in DLPE. These two values are not equal to within the confidence limits, perhaps suggesting that there is no uniform behaviour on a reduced temperature scale. However, the range of uncertainty is rather large, the data rather limited, and in addition DLPE is subject to metastable phase behaviour even at maximum hydration [9].

The other two parameters, A and B, are only determined to any degree of accuracy for NBD-DMPE in DMPC, PDA in DLPE and PL in DLPE, and in several of these cases the 90% confidence limits are even so $\pm 50\%$ of the optimal value. The parameter B is given by: $B = \gamma v_f^* / (\alpha' v_m)$. For lipid bilayers in the fluid phase, the volume expansion coefficient is $\alpha' \approx 10^{-3}$ K^{-1} [6,7] and the area expansion coefficient is approx. 3-times greater [8]. In previous work on the free-volume model for lipid bilayers it has been taken that $\gamma v^*/v_m \approx 0.4$ [2,3] compared with a value of $\gamma v^*/v_m \approx 0.6$ for simple liquids [4]. Hence one expects values for the parameter B in the range 133–400 K, depending on whether the appropriate expansion coefficient is that for area or for volume. The values of B in Table I lie within this range (taking into account in some cases the large confidence limits), with the exception of PL in DLPE. There seems at the moment no simple explanation for the extremely small values shown in Table I for the latter case, except perhaps the previously mentioned metastability of hydrated DLPE bilayers [9].

The parameter A in the free-volume model is determined by the diffusion coefficient of the lipid in the void, $D_T(v^*) = AT$. For free diffusion within the void $D_T(v^*) = \frac{1}{4}\lambda^* \sqrt{(2kT/m)}$, where λ^* is the mean distance of free travel within the volume, v^*, and m is the mass of the lipid molecule, as in gas kinetic theory [2]. Taking $\lambda^* \approx 8$ Å, corresponding to the lipid intermolecular spacing, yields $D_T(v^*) \approx 1.7 \cdot 10^{-4}$ cm$^2 \cdot$ s^{-1} at 30°C, which is to be compared with the values of $A \cdot T$ in Table I. From the latter, $D_T(v^*)$ varies between $0.4 \cdot 10^{-7}$ and $1 \cdot 10^{-7}$ cm$^2 \cdot$ s^{-1} at 30°C, for the values of A which are determined to within any degree of accuracy. This indicates that the lipid diffusion in the void is strongly restricted by an effective viscous drag at the headgroups and chain ends, as pointed out previously by Vaz et al. [3].

In summary, it can be stated that only in certain cases do the temperature dependences of the lipid diffusion coefficients reported in Refs. 2 and 3 provide strong evidence for a free volume as opposed to a Stokes-Einstein diffusion model. The

values of the parameters are in rough accord with the free-volume model, but mostly are not rigorously determined with any high degree of accuracy. It is hoped that the present analysis will stimulate further measurements, over the widest possible temperature range, to improve the statistics so that the parameters of the free-volume model can be compared accurately between the different systems. Such measurements would then yield a more detailed description of the free volume mechanism of lipid diffusion.

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