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The lateral distribution of cholesterol in the plane of lipid multibilayers

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We consider three models of cholesterol distribution in the plane of a bilayer of DMPC. We analyse recent ^{2}H-NMR data obtained from deuterated fluorescent probes and show that, on the characteristic time-scale of ^{2}H-NMR, it is in accord with a random distribution of cholesterol in a fluid-like DMPC bilayer in a single phase at least for $T \gtrsim 35\,^\circ$C and for $0 \leqq c \leqq 0.42$

In the last 15 years considerable effort has gone into studying the thermodynamics of cholesterol in lipid bilayer membranes (see, for example, Refs. 1–11), and attempts have been made to deduce the distribution of cholesterol in the plane of the bilayer from an analysis of some of these measurements. Some of the conclusions have been only semi-convincing because to the complexity of what was being measured. Thus, for example the question of the resolution of differential scanning calorimetry measurements into two or more peaks [4,5,12] together with improved measuring capability has not shed substantial light on the proposals regarding lipid-cholesterol complexes [2,10,13–15] while the analysis of Hoffman et al. [16] is complicated, in the case of cholesterol, by questions concerning the dependence of lifetimes upon cholesterol concentration.

Recently, ^{2}H-NMR spectra were obtained for a ^{2}H-labeled fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) reconstituted in dimyristoylphosphatidylcholine (DMPC) multilamellar bilayers containing cholesterol [17]. These spectra were analysed in terms of an ordering matrix as a function of cholesterol concentration, c, for various temperatures, T, greater than the pure lipid main transition temperature, $T_m = 23\,^\circ$C. Results were reported for a wide range of cholesterol concentration ($0 \leqq c \leqq 0.5$), and it is our intention in this report to analyse these results by considering different models of cholesterol distribution in the plane of the bilayer. We will show that the experimental data for the order parameters offers support for the view that cholesterol is randomly distributed in a single homogeneous phase for the range of temperatures considered here.

The distribution of cholesterol in the plane of the bilayer can be characterized by a set of probabilities $\{p_A(T, c)\}$ for finding a lipid hydrocarbon chain in an environment labelled A. The first question to consider is how many phases there might be for $T > T_m$. All recent evidence suggests that for $0 \leqq c \lesssim 0.42$ and for $T \gtrsim 35\,^\circ$C the DMPC-cholesterol bilayer is in a single phase [6,17–19] and that this single phase may extend to lower values of $T > T_m$ especially at lower values of c. The strongest evidence for this comes from the work of Knoll et al. [19] using the inverse contrast variation technique. They find that at $T = 35\,^\circ$C, $c = 0.42$ there is only a slight heterogeneity. It is possible that this heterogeneity is masked by fast lateral diffusion of the DPH mole-

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cules on the time-scale of ^{2}H-NMR measurements [17]. That these measurements [17] can be fitted by a line-shape appropriate to a single phase is in accord with the existence of an apparent single phase, on the ^{2}H-NMR time-scale, for $0 \leqq c \leqq 0.5$ and $T \gtrsim 25\,^\circ$C. The second broad (high-temperature) peak in DSC measurements is at a maximum at $c \approx 0.16$ and is not significant above about $28\,^\circ$C. We therefore assume that we are dealing with a single phase, at least for $T \geqq 35\,^\circ$C.

Accordingly, if we denote by $S_{\alpha\beta}(T, c)$ the measured average value of the α,β-component of an order parameter tensor at temperature T and cholesterol concentration c, then we can write

$$S_{\alpha\beta}(T, c) = \sum_A p_A(T, c) S_{\alpha\beta}(A, T, c) \qquad (1)$$

where $S_{\alpha\beta}(A, T, c)$ is the average value of that component of the order parameter in environment A. Such an averaging is justified by the fact that the characteristic time-scale of ^{2}H-NMR is approx. 10^{-5} s during which each DPH molecule should experience most environments. Clearly, without a knowledge of $S_{\alpha\beta}(A, T, c)$ it is not possible to decide which distribution best describes the case of cholesterol. We shall consider three models: (a) the Random model in which the cholesterol molecules are randomly distributed in the bilayer; (b) the Repulsive model in which no two cholesterols may be adjacent; (c) the 1 : 1 Complex model in which one lipid molecule is bound, in some sense, to each cholesterol [10,14]. The complexes are then randomly distributed in the plane of the bilayer. We assume that the DPH molecules are distributed like those lipid hydrocarbon chains in the plane of the bilayer which are not part of a complex. Although they are shorter than chains and are more rigid, they have approximately the same cross sectional area (perpendicular to the long axis) as hydrocarbon chains in their fluid state in a bilayer.

We will consider models of cholesterol distribution in which we distinguish only two environments: Environment 0 where a DPH molecule has only lipid chains as its nearest neighbours and environment 1 where it has at least one cholesterol or one complex as its nearest neighbour. We assume that because of the extended nature of DPH only one adjacent cholesterol or complex is sufficient to determine $S_{\alpha\beta}(1, T, c)$ and that so strong is the effect of cholesterol or complex that this quantity is independent of c. We than have

$$S_{\alpha\beta}(T, c) = S_{\alpha\beta}(0, T, c) p_0(c) + S_{\alpha\beta}(1, T)(1 - p_0(c)) \qquad (2)$$

where $p_0(c)$ is the probability that a DPH molecule has zero cholesterols or complexes adjacent to it. This kind of model has been successfully used to study protein lateral distribution in lipid bilayers [16,20,21].

We have used a lattice model to evaluate the probabilities [20,21]. We represent the plane of one half of the bilayer by a triangular lattice each site of which can be occupied by a lipid hydrocarbon chain, a cholesterol molecule or a DPH molecule. A 1 : 1 complex occupies three such sites. In the case of the Random model we have

$$p_0^{\mathrm{ran}}(c) = (1 - x)^6, \quad x = c/(2 - c) \qquad (3)$$

In the case of the Repulsive model we have been unable to obtain an analytic expression for $p_0^{\mathrm{rep}}(c)$ and have evaluated it using computer simulation as described elsewhere [20]. In the case of the 1 : 1 Complex model we assume that DPH molecules may not replace either of the lipid chains which are bound to a cholesterol. This does not necessarily imply that there cannot be exchange of lipids which are bound to a cholesterol. We then have, to at least a very good approximation, [20].

$$p_0^{\mathrm{com}}(c) = (1 - x)^9, \quad x = c/(2 - 3c) \qquad (4)$$

Before presenting our results it is appropriate to comment on the models. Although earlier results were analysed in terms of the Random model [16] it was necessary to make assumptions about the behaviour of various quantities and, from an inspection of the figure showing the analysis (Fig. 2A in Ref. 16), there are suggestions that the random distribution does not account for the cholesterol data as well as it does for the data of other molecules (Figs. 2B, C in Ref. 16). The Repulsive model will have six lipid hydrocarbon chains adjacent to each cholesterol which is similar to the model of Engelman and Rothman [2]. It can be argued that lipid hydrocarbon chains in a fluid phase, even at high cholesterol concentrations will project an area onto the plane of the

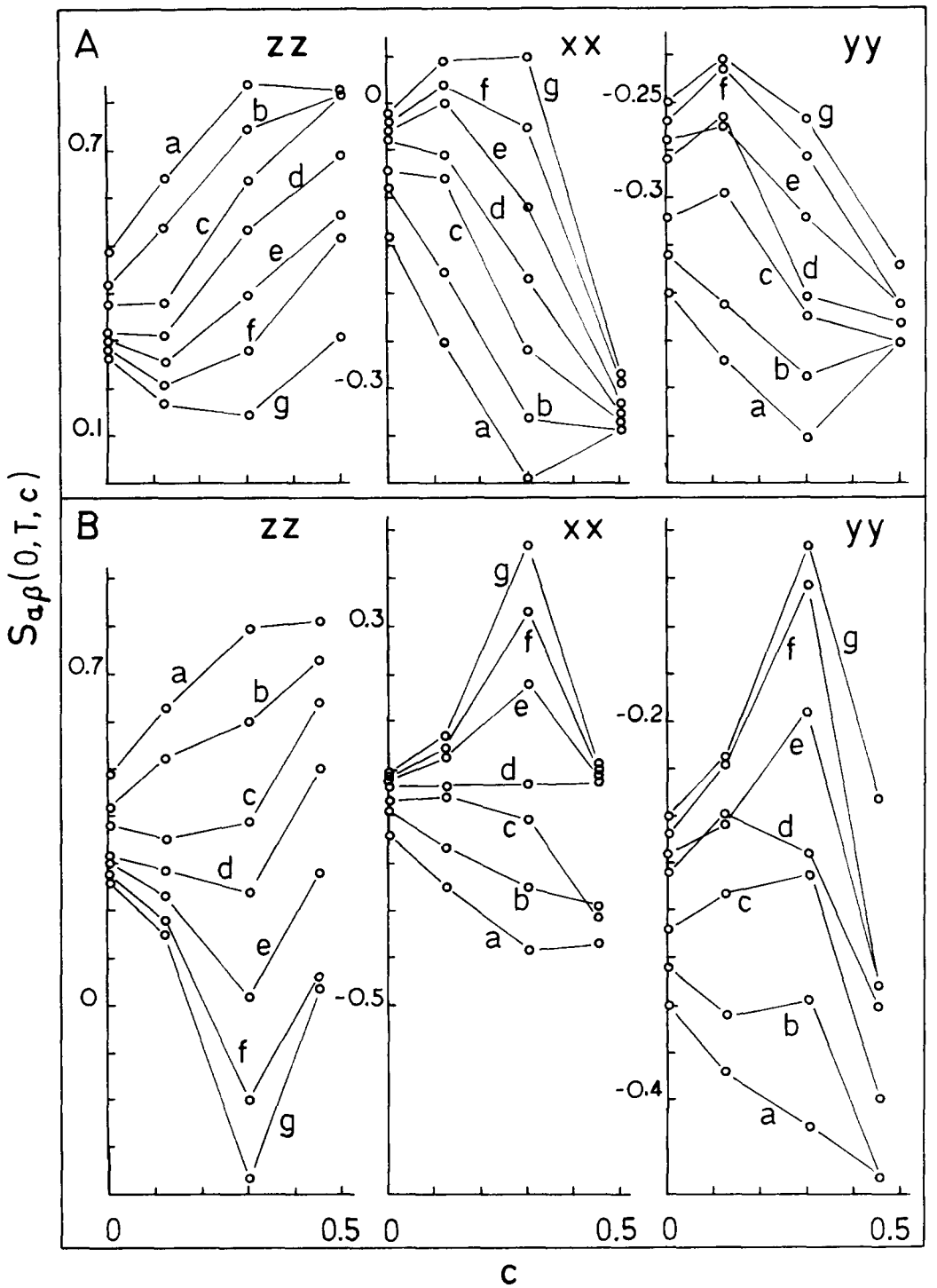

Fig. 1. $S_{\alpha\beta}(0, T, c)$ (Equation 2) for the Random model (A) and the Repulsive model (B) as a function of cholesterol concentration, c. $\alpha\beta = zz$, xx and yy as shown. The temperatures are: (a) 25 °C, (b) 30 °C, (c) 35 °C, (d) 40 °C, (e) 45 °C, (f) 50 °C, (g) 55 °C.

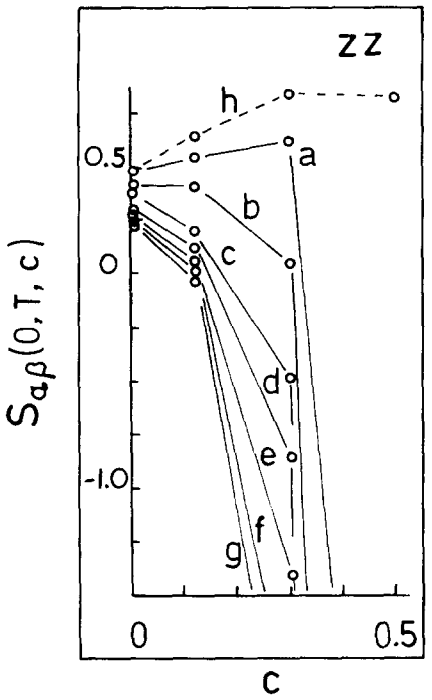

Fig. 2. $S_{zz}(0, T, c)$ for the 1 : 1 Complex model. The temperatures are (a) 25 °C, (b) 30 °C, (c) 35 °C, (d) 40 °C, (e) 45 °C, (f) 50 °C, (g) 55 °C. For comparison, $S_{zz}(0,T,c)$ for the Random model is shown for 25 °C (h).

bilayer greater than approx. 18 Å^2, the all-*trans* area, assumed in Ref. 2 so that about six chains is a more appropriate number than seven [2]. An appealing aspect of the Repulsive model arises from the fact that we are not aware of any reports suggesting that substantially more than 50 mol% ($c = 0.5$) of cholesterol can be incorporated into a DMPC bilayer. If no two cholesterols can be adjacent then the maximum number of cholesterols which can be thus placed on a triangular lattice corresponds to $c = 0.5$. The approximate maximum concentration can, thus, be accounted for by this single assumption though there are suggestions that the maximum may be $c \approx 0.43$ [19]. There is little point in studying models in which the cholesterols are constrained to be further apart because cholesterol concentrations up to 50 mol% would not be permitted in such models, contrary to observation.

Instead of trying to model the behaviour of $S_{\alpha\beta}(0, T, c)$ we have simply inserted the observed experimental value for $S_{\alpha\beta}(T, c)$ [17] on the left-hand side of Eqn. 2 and, using our calculated probabilities, solved for $S_{\alpha\beta}(0, T, c)$. We have interpolated the published measurements as best we could to obtain values at $c = 0.4$ and $c = 0.45$, and extrapolated to $c = 1.0$ to obtain $S_{\alpha\beta}(1, T, 1.0)$ for the Random model. There are not substantial errors in our estimates. The results of choosing $S_{\alpha\beta}(T, c)$ to be $S^{P}_{xx}(T, c)$, $S^{P}_{yy}(T, c)$ or $S^{P}_{zz}(T, c)$ (Table II in Ref. 17) for two models are shown in Fig. 1A (Random) and Fig. 1B (Repulsive) where $S^{P}_{xx}(0, T, c)$, $S^{P}_{yy}(0, T, c)$ and $S^{P}_{zz}(0, T, c)$ are plotted. Fig. 2 shows a plot of $S^{P}_{zz}(0, T, c)$ for the 1 : 1 Complex model. It will be recalled that these are the order parameters of a DPH molecule which is adjacent only to lipid hydrocarbon chains. The fact that the hydrocarbon chains can be 'stiffened' by the presence of cholesterol leads to the c dependence.

It is clear that only the Random model gives reasonable value for the quantities plotted. That an increasing concentration of cholesterol would cause these quantities to decrease (or increase) to the extent that the Repulsive or the 1 : 1 Complex models yield, is implausible. It is for this reason that we have plotted only one component in the case of the Complex model. In the case of the Random model, note that for $T \geqq 35$ °C $S^{P}_{zz}(0, T, c)$ is approximately constant (except at 55 °C) for a range of c, as c increases from 0, and that this range gets larger as T increases. This is followed by an increase in that order parameter. Analogous behaviour is seen in the other two components. This behaviour is similar to that reported for lateral diffusion coefficients [8] and viscosity [22]. Although for higher temperatures, the DPH order parameters for the Random model appear to decrease (or increase) for $0 \leqq c \lesssim 0.3$, we are not convinced that this should be taken too seriously. Our models in which there are only two environments considered must be the simplest possible. On the other hand it is difficult to imagine how a refining of the Repulsive or 1 : 1 Complex models could change the results where such extreme decreases of the order parameters are manifested.

We conclude, then, that the results of recent ^{2}H-NMR studies [17] are in favour of cholesterol being randomly distributed in the plane of a DMPC bilayer above $T = T_m$, at least for $T \gtrsim$

35 °C and $0 \leqq c \leqq 0.42$. For $T = 25$ °C, where there may be phase separation, we are not willing to commit ourselves to a conclusion.

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References

1 Hinz, H.J. and Sturtevant, J.M. (1972) J. Biol. Chem. 247, 3697–3700.
2 Engelman, D.M. and Rothman, J.E. (1972) J. Biol. Chem. 247, 3694–3697.
3 Shimshick, E.J. and McConnell, H.M. (1973) Biochem. Biophys. Res. Commun. 53, 446–451.
4 Estep, T.N., Mountcastle, D.B., Biltonen, R.L. and Thompson, T.E. (1978) Biochemistry 17, 1984–1989.
5 Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464–2468.
6 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2740.
7 Gershfeld, N.L. (1978) Biophys. J. 22, 469–488.
8 Rubenstein, J.L.R., Smith, B.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. USA 76, 15–18.
9 Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954.
10 Presti, F.T., Pace, R.J. and Chan, S.I. (1982) Biochemistry 21, 3831–3835.
11 Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287.
12 Imaizumi, S. and Hatta, I. (1984) J. Phys. Soc. Japan 53, 4476–4487.
13 Cadenhead, D.A. and Muller-Landau, F. (1984) Can. J. Biochem. Cell Biol. 62, 732–737.
14 Phillips, M.C. and Finer, E.G. (1974) Biochim. Biophys. Acta 356, 199–206.
15 Martin, R.B. and Yeagle, P.L. (1978) Lipids 13, 594–597.
16 Hoffmann, W., Pink, D.A., Restall, C.J. and Chapman, D. (1981) Eur. J. Biochem. 114, 585–589.
17 Kintanar, A., Kunwar, A.C. and Oldfield, E. (1986) Biochemistry 25, 6517–6524.
18 Kinosita, K. and Ikegami, A. (1984) Biochim. Biophys. Acta 769, 523–527.
19 Knoll, W., Schmidt, G., Ibel, K. and Sackmann, E. (1985) Biochemistry 24, 5240–5246.
20 Pink, D.A., Chapman, D., Laidlaw, D.J. and Wiedmer, T. (1984) Biochemistry 23, 4051–4058.
21 Pink, D.A., Quinn, B. and Laidlaw, D.J. (1987) Eur. Biophys. J., in press.
22 El-Sayed, M.Y., Guion, T.A. and Fayer, M.D. (1986) Biochemistry 25, 4825–4832.