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MEMBRANE THICKNESS AND ACYL CHAIN LENGTH

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It appears reasonable to expect that the primary result of a change in the length of the acyl chains within a lipid bilayer is a similar change in the bilayer thickness. In the present communication we draw attention to the somewhat more complicated effects which are found experimentally for phosphatidylcholine bilayers as the hydrocarbon chain is varied from twelve to eighteen carbons in length. The major change in dimension which occurs with variation in acyl chain length is the area occupied per molecule rather than the bilayer thickness. The same effect is seen with solute hydrocarbon such as hexane which partition into the membrane and cause only a small variation in membrane thickness but a large increase in the molecular area of the lipid. The origin of this effect arises from the almost isotropic distribution of the additional hydrocarbon to the lipid core of the membrane.

Diffraction techniques [1,2] provide one of the most direct methods for obtaining information on the structural details of ordered material. Many studies have now been reported of the area occupied by phospholipids in multilamellar aqueous dispersions [3,4]. In Fig. 1 we show a summary of the currently available estimates of molecular area based upon the results of X-ray diffraction [5–14].

These areas have been derived using the expression:

$$\frac{S \cdot d_l}{2} = \text{Volume of water per lipid molecule} + \text{volume of lipid per lipid molecule}$$

where S is the molecular area per lipid, and $d_l/2$ is half the experimentally observed long spacing.

The volume of water per lipid molecule

$$= \frac{MW_{water} \cdot (\text{weight of water}) \cdot MW_{lipid}}{N_A \cdot \rho_w \cdot (\text{weight of lipid}) \cdot MW_{water}}$$

where N_A is Avagadro's number and ρ_w is the density of the water.

Likewise the volume of lipid per lipid molecule

$$= \frac{MW_{lipid}}{N_A \cdot \rho_l}$$

where ρ_l is the density of the lipid.

In order to determine the fully hydrated area it is necessary to perform a series of estimates of molecular area at increasing water contents. The limiting area is reached when the addition of further water fails to produce an increase in long spacing.

As part of the present study we have performed complementary estimates from X-ray diffraction of the molecular area of the series of saturated diacyl phosphatidylcholines: dilauroylphosphatidylcholine (DLPC, 12:0), dimyristoylphosphatidylcholine (DMPC, 14:0) and distearoylphosphatidylcholine (DSPC, 18:0). The results of this work are included in Fig. 1. These data have all been obtained for fully hydrated dispersions at temperatures 20°C above the phase transition of

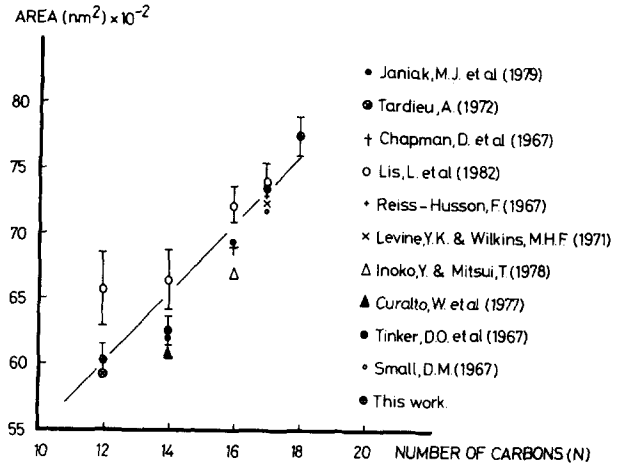

Fig. 1. The area per phosphatidylcholine molecule (S) in nm^2 for dispersions of different chain length (N) diacylphosphatidylcholines. All lipids were fully hydrated and well above their respective phase transitions. The data points plotted at $N = 17$ refer to egg yolk phosphatidylcholine. Error bars have been added where possible. In many instances, however, insufficient information was available to allow for an assessment of errors.

the respective lipids. Janiak et al. [5] have demonstrated that most of the changes in dimensions associated with the main phase transition are complete within 10–15°C above the transition temperature.

The densities employed in calculating the molecular areas of the saturated phospholipids studied here: DLPC (1.0387 mg/ml), DMPC (1.0005 mg/ml) and DSPC (0.8981 mg/ml), were deduced from a combination of the dilatometric results of Nagle and Wilkinson [15], and those of Knoll [16]. We have assumed in all cases that the starting material was the monohydrate. The area estimates in Fig. 1 derived from the published literature were recalculated from the originally published long spacing data together with the more recent measurements of the lipid density. In addition to the densities quoted above the density of egg yolk phosphatidylcholine (EYPC) [6] was taken as 1.017 mg/ml and of dipalmitoylphosphatidylcholine (DPPC) [15,16] as 0.9829 mg/ml.

Using the volume of the hydrocarbon segment of the lipid [15,16] it is also possible to calculate the thickness of the glycerohydrocarbon portion of the bilayer; i.e.

$$\text{The thickness of the glycerohydrocarbon region} = \frac{\text{Volume of the glycerohydrocarbon region}}{\text{Molecular area per lipid}}$$

$$= \frac{(\mathrm{MW}_{\mathrm{lipid}}/(N_A \cdot \rho_l)) - V_{pc}}{S}$$

where V_{pc} is the volume of the phosphocholine group which has been taken from Small [6] as 0.204 nm^3 and is assumed to be independent of temperature. Estimates of the glycerohydrocarbon thickness are shown in Fig. 2 based upon the same raw data as used in Fig. 1.

Reference to Fig. 1 shows that there is a trend for the area per phospholipid molecule to increase with an increase in the number of acyl chain groups within the phospholipid. Using a least-squares linear fit to the data in Figs. 1 and 2 we obtain:

(a) the area per phospholipid in a fully hydrated multilamellar dispersion

$$S = (0.31 + 0.024\,N)\,\mathrm{nm}^2 \qquad (1)$$

and

(b) the thickness of the glycerohydrocarbon region of a fully hydrated multilamellar dispersion

$$t = (1.87 + 0.066\,N)\,\mathrm{nm} \qquad (2)$$

where N is the number of acyl chain carbons in the

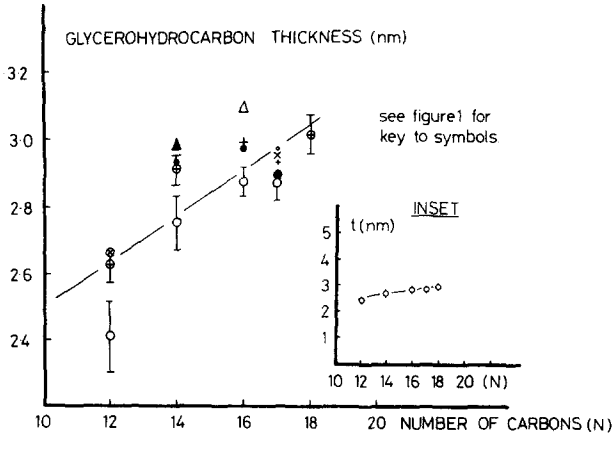

Fig. 2. The hydrocarbon core thickness (t) in nm for dispersions of different chain length (N) diacylphosphatidylcholines. Inset. Same data plotted on an ordinate scale with its origin at zero.

series of saturated diacyl phosphatidylcholines from $N = 12$ to 18.

Clearly with fully hydrated fluid phase phospholipids there is a compensation between the acyl chain length and the area per molecule which causes the bilayer thickness to be less sensitive to the lipid chain length than might have been expected. Indeed as can be calculated from expression 2 for the dispersions of the saturated phosphatidylcholines studied here the thickness of the hydrocarbon core ranges from 2.65 to 3.05 nm.

These results may be compared with the differences in length of the fully extended hydrocarbon chain of lipids with different numbers of acyl carbons, N. This has been determined by Tanford [17] as $(0.3 + 0.253\ N)$nm for a bilayer. Thus is appears that by adding an additional six carbon atoms to the length of the lipid chain of the membrane phospholipid, the fully extended length of the lipid chain would increase by 1.52 nm. The observed increase in bilayer thickness is only 0.4 nm.

Relatively little information is available on unsaturated lipids. A number of studies have been reported for egg yolk phosphatidylcholine which predominantly comprises chains of 16 : 0 and 18 : 1 and one study of dioleoyl phosphatidylcholine [14] for which the chains are both 18 : 1. The area and thickness of bilayers prepared from these lipids are consistent with the disaturated lipids if they are related to the disaturates according to their hydrocarbon volume. For the purpose of illustration the properties of EYPC are plotted on Figs. 1 and 2 at $N = 17$ as an approximate average of the 16 and 18 carbons in each chain. A very comprehensive study of lipid chain lengths and saturation has been reported by Caffrey and Feigensen [18]. Although insufficient information has been provided to derive the lipid area or the membrane thickness, little effect is seen in the overall long spacing due to a *cis* or *trans* unsaturation site along the lipid hydrocarbon chain.

A direct measurement of the dependence of the lipid thickness on acyl chain length is seen in the electron density profiles derived from the diffraction data. These show that the separation of the phosphate groups in dimyristoyl ($N = 14$) [19,20], dipalmitoyl ($N = 16$) [13] and egg yolk [21–23] (predominantly $N = 16 : 0$ and $N = 18 : 1$) phosphatidylcholines all lie within a range of 0.2 nm. An earlier report by McIntosh [24] shows that if such a comparison is made at less than full hydration or below the main phase transition temperature the inter phosphate group spacing is more sensitive to increases in chain length. This effect of hydration is not unexpected since the area per molecule increases steadily as the water content is increased up to the maximum hydration accepted by the lamellar phase. Measurements at less than full hydration therefore underestimate the fully hydrated molecular area and force the chains to adopt a more extended configuration. Likewise the inter phosphate group spacings of the gel crystalline phase are dominated by the properties of the crystalline, and thus fully extended, lipid chains.

Nuclear magnetic resonance (NMR) techniques also provide information about the thickness of the hydrocarbon region of phospholipid membranes [25]. This is possible through a measurement of the molecular order parameter, S_{mol}, from the quadrupolar splittings, for example, of specifically deuterated methylene groups along the hydrocarbon chain. The S_{mol} is a measure of the disorder experienced at each site and is related to the length each carbon-carbon bond projects onto the average molecular long axis. By summing over the projected bond distances for each group along the chain it is possible to determine [26] the thickness of the hydrocarbon layer of the membrane. Browning [26] has recently reviewed the currently available data on the S_{mol} obtained in this manner. At a reduced temperature * of 0.03 the S_{mol} for DMPC is found to be systematically greater than that for DPPC over the initial portion of the chain from $N = 2$ to 10. This shows that, at a common reduced temperature in the fluid phase, DMPC is more ordered and thus occupies a smaller area per molecule than DPPC. Because the NMR data needs to be summed over each site along the chain to obtain an estimate of the membrane thickness the effect on the order parameter of each methylene group is relatively subtle.

Additional evidence for the compensation between lipid area and acyl chain length is seen with

* The reduced temperature is $(T - T_c)/T_c$ where T_c is the main phase transition temperature of the lipid and T is the temperature at which the measurement was actually made.

the incorporation of alkanes into the interior of the membrane. White et al. [27] have recently employed neutron diffraction difference techniques designed both to locate deuterated hexane within bilayers of dioleoylphosphatidylcholine and to determine its effect on the thickness of the bilayer lipid. Despite the partitioning of hexane into the bilayer centre, no detectable variation was observed in either the lamellar phase long spacing or in the glycerohydrocarbon thickness. The area per lipid, however, was found to increase by 5%. These results point to a general effect when adding hydrocarbons to the core of a bilayer membrane. Regardless of whether the additional hydrocarbon is covalently attached to the lipid acyl chain or simply partitioned into the central chain region as a solute, the additional alkane is distributed in a manner so as to minimally disturb the thickness of the lipid core.

The first systematic correlation of the molecular area with lipid acyl chain length reported in the literature was by Janiak et al. [5] in which the results of Tardieu [7] were collected and compared with their own data on DMPC and DPPC. These authors demonstrated that the molecular areas appear to a first approximation to be related to the phase transition temperature of the lipid although no further interpretation was placed on the reported chain length dependence.

These effects may in part be explained by the additional lipid causing the membrane to swell whilst maintaining the same overall proportions. Owing to the area increase being related to the volume increase, ΔV, by an expression of the form $\Delta V^{2/3}$, whereas the thickness changes according to $\Delta V^{1/3}$, the major effects are seen as a change of area and not of thickness. In fact, there is a further bias towards a smaller variation in membrane thickness. Refering to expressions 1 and 2 for the area (S) and the hydrocarbon thickness (t) of a lipid bilayer, we may define a molecular aspect ratio S/t (having dimensions of nm) which describes the distribution of volume within the membrane. Using dilauroylphosphatidylcholine ($N = 12$) as a reference, $S/t = 2.25$. Should the additional hydrocarbon volume be distributed so as to hold the area per lipid constant, S/t would fall to 1.5 for distearoyl phosphatidylcholine ($N = 18$) or alternatively rise to 3.2 if the thickness did not alter. The experimentally observed value of S/t for the eighteen carbon chain length lipid is 2.43 indicating a slight additional preference for a constant hydrocarbon thickness. It is interesting to note that as a result of this, the degree to which the lipid chains are compressed relative to their fully extended crystalline length is quite different for different acyl chain lengths. For example for $N = 12$ the chain is extended to 80% of its crystalline length whereas for $N = 18$ this ratio is approx. 62%. It is this increased compression of the longer chain which causes the membrane thickness to be less sensitive to the acyl chain length than might have been expected.

In summary we have shown that the hydrocarbon thickness of lamellar phase dispersions of phosphatidylcholines is less sensitive to the hydrocarbon volume or acyl chain length than might be expected based on the change in length of the fully extended acyl chain. The major change which occurs with variation of acyl chain length is associated with the molecular area.

This finding requires consideration when interpreting many aspects of membrane biophysics.

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