

Lipid Bilayer Thickness Varies Linearly with Acyl Chain Length in Fluid Phosphatidylcholine Vesicles

The thickness of the lipid bilayer in vesicles made of pure phosphatidylcholines, with acyl chain lengths ranging from 10 to 24 carbons, has been determined by analysis of continuous X-ray scattering data from vesicle pellets at temperatures above the lipid phase transition temperature. Bilayer thickness was found to vary linearly with the number of carbons per acyl chain. The lines for saturated and monounsaturated acyl chains were slightly displaced but had similar slopes. For the saturated species di-12:0, di-14:0, di-16:0, and di-18:0 phosphatidylcholine the surface areas per molecule were all 65.7 to 66.5 Å², while the monounsaturated species and di-10:0 phosphatidylcholine all occupied 67.7 to 70.1 Å² per molecule.

It is generally agreed that a lipid bilayer is the basic structural motif of most biological membranes (Stoeckenius & Engelman, 1969) and that interactions with lipids modulate the function of at least some membrane proteins in important ways. Since a bilayer arrangement of phospholipids is characterized by a distinct central hydrophobic region bounded by two polar interfacial regions, we expect the thickness of the hydrophobic region to influence such properties as ion permeability, capacitance, and structure and function of transmembrane proteins. In particular, optimal lipid bilayer thickness is required for maximal activity of at least two integral membrane proteins, the Ca²⁺-ATPase from sarcoplasmic reticulum (Caffrey & Feigenson, 1981; Johannsson *et al.*, 1981; Moore *et al.*, 1981) and the ion channel gramicidin (Haydon & Hladky, 1972). We have also explored the effect of lipid bilayer thickness on the planar organization of the transmembrane protein bacteriorhodopsin (Lewis & Engleman, 1983).

In order to interpret the results of such experiments, it is necessary to characterize the equilibrium thickness of pure lipid bilayers under similar experimental conditions. The simplest approach to measurement of the thickness of the hydrophobic region is to determine the distance between the phosphate groups across the bilayer, d , and then to subtract the thickness of the appropriate portion of the polar region. Therefore, we have used X-ray scattering to determine d in vesicles made with pure phosphatidylcholines with acyl chain lengths from 10 to 24 carbon atoms, each at a temperature above the phase transition temperature, t_m , of the phosphatidylcholine species. By using vesicles consisting of one or a few bilayers, strong sampling of the bilayer transform is avoided, and the resulting continuous X-ray scattering permits direct determination of d (Engelman, 1970, 1971; Wilkins *et al.*, 1971). This method avoids possible errors in the measurement of water content or in the assumptions of partial specific volumes, which are necessary to derive the bilayer thickness from the interlamellar distance in multilamellar samples.

A convenient method of analysis of such continuous scattering data is Patterson inversion (Blundell & Johnson, 1976), which requires no phase information and for a phospholipid bilayer yields a positive peak at the distance, d , between phosphate groups across the bilayer and a negative trough at the spacing between the bilayer center and the phosphate groups.

Saturated phosphatidylcholines were from Calbiochem, as was di-18:1 phosphatidylcholine (*cis*-9). Di-erucic (di-22:1, *cis*-13) and di-nervonic (di-24:1, *cis*-15) phosphatidylcholines were generously given by Drs Martin Caffrey and Gerald Feigenson (Caffrey & Feigenson, 1981). Unsaturated lipids were kept under inert gas until after the sonication step.

The lipids were lyophilized, hydrated in 0.1 M-sodium acetate (pH 5) at a temperature above the lipid phase transition temperature (t_m), and then sonicated and/or freeze-thawed to give vesicles larger than a few tens of nanometres. Vesicle pellets were placed into 1 mm glass capillaries, and a small amount of buffer was placed in the top of the capillary to prevent evaporation.

X-ray data were collected on a small-angle X-ray scattering system previously described (McDonald *et al.*, 1979; Newcomer *et al.*, 1981). For these experiments, a relatively short geometry was used; each distance (source to mirror, mirror to sample, and sample to detector) was 12 to 13 cm. The beam height was limited to 0.5 cm by an aperture between the mirror and guard slits. Sample temperature was controlled by a circulating water bath. For each set of samples, buffer data were collected, and the scale factors for buffer subtraction were individually chosen to bring the corrected data exactly to zero at the end of the interval used for calculating the Patterson function. This scaling was necessary to avoid ripples due to termination effects in the Fourier transform.

To obtain the thickness of the lipid hydrocarbon region from d , the measured distance between phosphate groups across the lipid bilayer, one subtracts twice the distance between the phosphate group and the beginning of the hydrocarbon region on the phosphatidylcholine molecule in its fluid bilayer configuration. The hydrocarbon region of each acyl chain is defined here as beginning at carbon 2 (C-2), the first carbon after the carbonyl group. Using the results of neutron diffraction studies of specifically deuterated di-16:0 phosphatidylcholine in

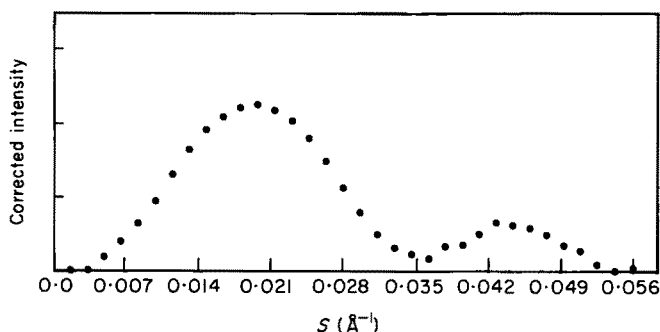


FIG. 1. X-ray scattering data from di-22:1 phosphatidylcholine at 24°C after buffer subtraction and Lorentz polarization corrections. $S = 2 \sin \theta/\lambda$.

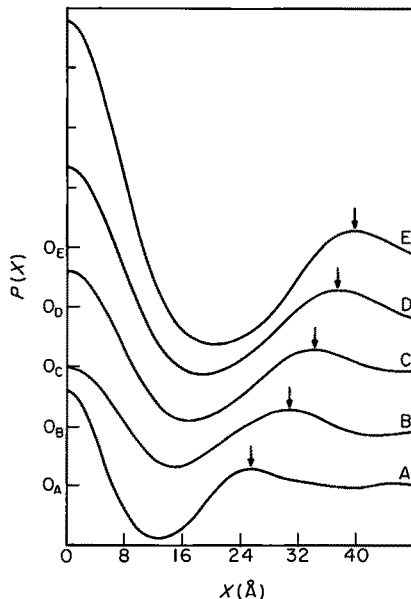


FIG. 2. Patterson functions for vesicles made with the saturated, even-chain phosphatidylcholines, in 0.1 M-sodium acetate (pH 5). The plots are vertically displaced for clarity, and O indicates the origin of each. Arrows indicate the first non-origin peaks. PC, phosphatidylcholine: A, di-10 : 0 PC, 20°C; B, di-12 : 0 PC, 20°C; C, di-14 : 0 PC, 36°C; D, di-16 : 0 PC, 44°C; E, di-18 : 0 PC, 60°C.

multilayer samples (Buldt *et al.*, 1979; Zaccai *et al.*, 1979), the phosphate-HC distance in fluid bilayers was estimated to be 5.5 Å per phosphatidylcholine molecule (Lewis, 1981). Thus the thickness of the hydrocarbon region of a bilayer can be obtained by subtracting 11 Å from the measured value of d .

Figure 1 shows an example of the scattering data after background and Lorentz polarization corrections. Figure 2 shows the Patterson inversions of the data for the saturated even chain series di-10 : 0 through di-18 : 0 phosphatidylcholine, each above its respective t_m value; Patterson inversions (not shown) for the monounsaturated di-18 : 1, di-22 : 1, and di-24 : 1 species are similar. Identical Pattersons were obtained from samples in 0.1 M-Tris · HCl (pH 8). The positions of the first positive non-origin peaks in the Patterson functions and the calculated bilayer thicknesses are listed in Table 1. In some samples, a second peak at higher spacing was observed in the Patterson plot; these peaks can be attributed to correlations between adjacent bilayers in samples containing a small number of multilamellar vesicles.

Plots of the hydrocarbon region thicknesses *versus* the number of carbons across the bilayer (starting with C-2) are shown in Figure 3. We assume that phospholipids are essentially incompressible, so the molecular volume at a given temperature is a function only of the chemical composition of the bilayer lipids. Thus the bilayer thickness and the surface area per head group for a given phosphatidylcholine species are inversely proportional.

The surface area per phospholipid molecule was calculated by dividing the

TABLE I
Bilayer thicknesses of pure phosphatidylcholines

Lipid	Reported t_m (°C)	Measurement temperature (°C)	Phosphate peak spacing (+ or - 1 Å)	Thickness of hydrocarbon region (+ or - 1 Å)	Molecular surface area (Å ²)
10:0	—	20	26.5	15.5	69.7 4.8
12:0	-1.8	20	30.5	19.5	66.5 3.6
14:0	23†	36	34	23	65.7 3.0
16:0	41.5†	44	37	26	66.5 2.6
18:0	54.9‡	60	40.5	29.5	65.9 2.3
18:1	-14†	20	38	27	70.1 2.7
22:1	11§	24	45	34	68.4 2.0
24:1	24§	36	48.5	37.5	67.7 1.9

† From Van Dijk *et al.* (1976).

‡ From Mabrey & Sturtevant (1976).

§ From Caffrey & Feigenson (1981).

|| Error carried through from ± 1 Å in thickness.

volume of two pairs of acyl chains (Reiss-Husson & Luzzati, 1964; Nagle & Wilkinson, 1978), starting at C-2, by the thickness of the bilayer hydrocarbon region. The values all fall within the range of 65 to 70 Å², with the monounsaturated species being at the high and the saturated species at the low end of the range. The four saturated species di-12:0 through di-18:0 have essentially identical areas, but in the unsaturated series the molecular area decreases with increasing chain length. The vertical displacement of the two lines

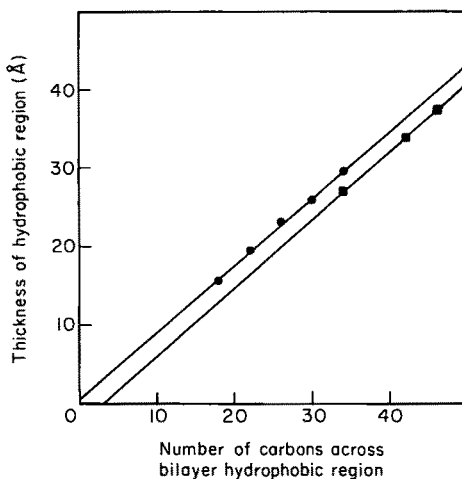


FIG. 3. Plot of the measured thickness of the bilayer hydrocarbon region *versus* the number of carbons across the hydrophobic region. The number of carbons is taken starting at C-2 of each acyl chain. Estimated errors in the thickness measurement correspond to the size of the plotted points. The lines are least-squares fits to the data. Circles, saturated acyl chains; squares, monounsaturated acyl chains.

in Figure 3 includes contributions both from the reduced volume of the two double-bonded carbons per chain and from the increased molecular surface area of the monounsaturated species.

The finding that the surface area remains almost constant as the lengths of the acyl chains are more than doubled suggests that the interactions of the phospholipid head groups at the bilayer surfaces predominate in determining the equilibrium configuration of the fluid phosphatidylcholine bilayer. This suggestion is consistent with the liquid-like nature of hydrocarbon chains and with the strength of the hydrogen bonding and hydrophobic interactions that govern the structure of the lipid-water interface. Nevertheless, it is also apparent that the chemical nature of the acyl chains, especially in the region close to the interface, also plays a role in determining the molecular configuration.

Two other groups, Caffrey & Feigenson (1981) and Lis *et al.* (1982), have measured thicknesses of fluid phosphatidylcholine bilayers for a series of acyl chain lengths. Both used X-ray diffraction to determine the lamellar repeat distance (d') in hydrated multilamellar samples, and then estimated the lipid bilayer thickness (dl) by subtracting the thickness of the water layer (dw). This thickness was either assumed (Caffrey & Feigenson, 1981) or inferred from the measured water content of the samples (Lis *et al.*, 1982). In both cases, the measured d' values closely paralleled the values obtained in the present work for d , the distance between phosphate groups across the bilayer; for the saturated phosphatidylcholines, the d' values were 28 to 30 Å larger than d , and for the unsaturated species, the d' values exceeded d by 26 to 27 Å. Thus, if dw was about 29 to 27 Å, respectively, the results of the multilamellar experiments agree exactly with the present results.

In the work of Lis *et al.* (1982), the water content and d' were measured for four of the lipids examined in the present work. In two cases, the di-12:0 and di-14:0 species, the dw values were calculated to be 29 and 28 Å, respectively, giving excellent agreement. In the other two cases, however, di-16:0 and di-18:1, the dw values were determined to be 33 and 32 Å, implying thinner bilayers and larger molecular areas (71 and 82 Å²) than indicated by our results.

The equilibrium thicknesses of phosphatidylcholine bilayers in predominantly single-bilayer vesicles may differ slightly from those in multilamellar samples due to the interbilayer forces in the latter case, but such differences might be expected to follow a systematic pattern as the acyl chain is varied. Any sources of error in the present determination of the phosphate peak spacing should also produce only systematic differences. Therefore, the discrepancies between the previously reported dl values and the d values obtained in the present work may best be explained by errors in measuring the water content of, or lack of homogeneity of, the multilamellar di-16:0 and di-18:1 phosphatidylcholine samples. This explanation is consistent with the agreement of both sets of results for the di-12:0 and di-14:0 species.

Thus the range of molecular areas of 65 to 70 Å² agrees well with some, but not all, of the areas calculated by Lis *et al.* (1982). This range also agrees with the range of 66 to 68 Å² proposed by Kwok & Evans (1981) to be most consistent with elasticity measurements on egg phosphatidylcholine vesicles and multilayers. The

present results indicate that di-18:1 (*cis*-9) phosphatidylcholine occupies a slightly larger surface area in fluid than do the saturated species of moderate chain length, but the surface areas of the longer-chain unsaturated species approach those of the saturated species examined here.

In summary, we have established that the thickness of fluid phosphatidylcholine bilayers varies linearly with the acyl chain length in vesicles containing one or a small number of bilayers. The area per molecule remains remarkably constant as the chain length is varied from 10 to 24 carbons. Introduction of a *cis* double bond results in a light increase in molecular surface area; however, the increase found here is smaller than that reported by other groups.

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