Biochimica et Biophysica Acta, 389 (1975) 13–19

BBA 76925

A MODEL FOR THE PACKING OF LIPIDS IN BILAYER MEMBRANES

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(Received November 4th, 1974)

SUMMARY

A number of known structural properties of mixed lipid bilayer membranes and monolayers are accounted for by a model in which lipids pack into bilayers and monolayers like building blocks, each characterized by a surface head group area and characteristic solid angle. In phospholipids above the melting transition the head group area (at a given temperature and degree of hydration) is fairly invariant while the hydrocarbon region may be liquid-like so long as the molecule is not compressed beyond its characteristic solid angle.

Phosphatidylcholine and phosphatidylserine are tapered lipids, i.e. their surface head group areas are greater than their non-polar end areas; cholesterol is frayed, i.e. its polar end area is less than its non-polar end area; while phosphatidylethanolamine is almost cylindrical. The "condensing" effect of cholesterol in mixed phospholipid-cholesterol films is seen as a taper-fray accommodation. The lipid distribution in erythrocyte membranes is shown to be conducive to a stable strain-free membrane.

THEORY

The radii of mixed lipid vesicles

Gent and Prestegard [1] found that cosonication of egg yolk phosphatidylcholine with cholesterol, and soybean phosphatidylcholine with phosphatidylethanolamine, in water at pH 7.5 resulted in single-shelled spherical vesicles whose radii increased as the mol fraction of cholesterol or phosphatidylethanolamine increased. Saunders [2] suggested that the type of micelle formed by phospholipids is governed by the solid angle into which these molecules can be fitted, and showed that in lysolecithin micelles the solid angle subtended by each molecule is close to that expected from molecular models.

Likewise, we find that the variable radii of the mixed lipid vesicles of ref. 1 are consistent with a model in which each lipid has a characteristic solid angle and surface head group area. For a lipid molecule i of head group area a_i and characteristic solid angle Ω_i the characteristic radius R_i of such a molecule is, by definition, given by

$$R_i^2 = a_i/\Omega_i \tag{1}$$

If the lipid is of length d, the non-polar end area a_i' is therefore given by

$$(R_i \pm d)^2 = a_i'/\Omega_i \tag{2}$$

The $-$ sign refers to tapered lipids, i.e. those whose non-polar end areas a_i' are less than their polar end areas a_i, while the $+$ sign refers to frayed lipids, i.e. those whose non-polar end areas are greater than their polar end areas. Consider a mixed vesicle composed of mol fractions f_1 and f_2 of lipids 1 (phosphatidylcholine) and 2 (cholesterol or phosphatidylethanolamine) of surface head group areas a_1 and a_2, and characteristic solid angles Ω_1 and Ω_2. The vesicle radius R will be determined by the way the lipids pack in the outer layer of the vesicle bilayer (as in a Roman arch). If this outer layer has thickness d, then the ratio of the areas occupied by the outer lipid layer at R and at $(R-d)$ is given by

$$\frac{R^2}{(R-d)^2} = \frac{f_1 a_1 + f_2 a_2}{f_1 a'_1 + f_2 a'_2} \tag{3}$$

Substituting for a_1' and a_2' using Eqns 1 and 2 we find that the vesicle radius R satisfies the quadratic equation

$$R^2 \frac{[2(f_1\sqrt{a_1\Omega_1} \pm f_2\sqrt{a_2\Omega_2}) - d(f_1\Omega_1 + f_2\Omega_2)]}{(f_1 a_1 + f_2 a_2)} - 2R + d = 0 \tag{4}$$

where $f_1 + f_2 = 1$. We may assume that lipid 1 (phosphatidylcholine) is tapered, whereas lipid 2 may be tapered or frayed (the $+$ sign refers to tapered lipids 2, and the $-$ sign to frayed lipids 2). For negligibly small d Eqn 4 may be written in the approximate form

$$R \approx \frac{f_1 a_1 + f_2 a_2}{(f_1\sqrt{a_1\Omega_1} \pm f_2\sqrt{a_2\Omega_2})} \tag{5}$$

No stable bilayer vesicles or lamellae should form when R is infinite or negative; this instability will occur with frayed lipids 2 at a critical mol fraction f_2 of approximately

$$f_2 \leqslant \frac{1}{1 + \sqrt{a_2\Omega_2/a_1\Omega_1}} \tag{6}$$

Pure phosphatidylcholine vesicles

The surface head group of egg phosphatidylcholine has been found to be the same in both lamellae [3, 4] and vesicles [5] and will be taken as $a_1 = 71$ Å^2. Thus for a pure phosphatidylcholine vesicle of radius 118 Å the solid angle per molecule is $\Omega_1 = 71/(118)^2 = 0.0051$ steradians. This is not to suggest that phosphatidylcholine is a rigidly tapered structure, but only that it opposes any compression that tends to compress or strain it beyond its characteristic solid angle as would occur in the outer vesicle layer at radii smaller than about 118 Å; inside the walls of the solid angle the hydrocarbon chains may be in a liquid-like state (at room temperature egg phosphatidylcholine is well above its melting transition temperature [6]). In

lamellar bilayers the hydrocarbon chain ends of the tapered phosphatidylcholine molecules could easily interdigitate in the central bilayer regions. In the inner layers of vesicle bilayers there are no steric or packing restrictions on the hydrocarbon chains which simply fill up the inner layer volume, the surface head group area [7] and specific volume per molecule [8] remaining unchanged.

Phosphatidylcholine-cholesterol vesicles

Monolayer studies have shown that cholesterol has a practically invariant mean cross-sectional area [9, 10] of about 38 Å^2. Fig. 1 shows a plot of the theoretical variation of vesicle radius R with cholesterol mol fraction f_2 based on Eqn 4 using $a_2 = 38$ Å^2 and frayed characteristic solid angle $\Omega_2 = 0.00015$ steradians (which gave the best fit to the experimental dotted curve [1]). A value of 20 Å was assumed for the outer layer thickness d (the theoretical radius R is very insensitive to variations in d for radii R appreciably greater than d as in the case of vesicles). The agreement with the experimental curve is fair.

The limiting mean area of 38 Å^2 of cholesterol in a pure cholesterol monolayer is not necessarily the same as that of the cholesterol surface head group in a mixed monolayer or bilayer. Indeed, the mean molecular area in mixed films of phosphatidylcholine with cholesterol [9–12] is well below that expected from the simple additivity rule, and similar "condensing" effects with cholesterol have been observed with other phospholipids [10]. In mixed egg phosphatidylcholine-cholesterol monolayers [9] and myelin phosphatidylcholine-cholesterol bilayers [11] cholesterol initially behaves as a lipid of area ≈ 19 Å^2 or less (depending on the pressure and degree of hydration) up to a mol fraction of about 0.5.

The "condensing" effect of cholesterol is often attributed to some sort of interaction [10, 12]; however, a number of observations are readily explained simply

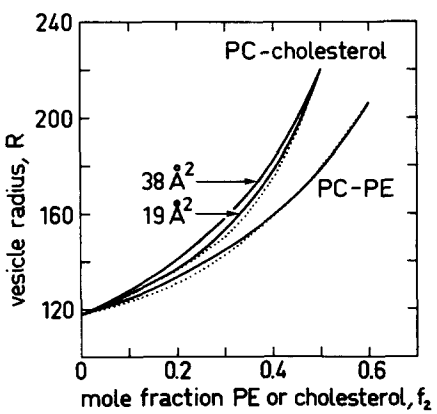

Fig. 1. Variation of vesicle radii of mixed phosphatidylcholine-cholesterol (PC-cholesterol) and phosphatidylcholine-phosphatidylethanolamine (PC-PE) vesicles with cholesterol or phosphatidylethanolamine mol fraction f_2. Dotted lines: experimental results [1]. Continuous lines: theoretical results based on Eqn 4 using phosphatidylcholine head group area $a_1 = 71$ Å^2, phosphatidylcholine solid angle Ω_1 (tapered) = 0.0051 steradians, and half bilayer thickness $d = 20$ Å. The values for cholesterol are $a_2 = 38$ Å^2, Ω_2 (frayed) = 0.00015 steradians, and $a_2 = 19$ Å^2, Ω_1 (frayed) = 0.00115 steradians. The values for phosphatidylethanolamine are $a_2 = 41.3$ Å^2, Ω_1 (tapered) = 0.000045 steradians.

in terms of the tapered and frayed shapes of phosphatidylcholine and cholesterol: The theoretical curve (Fig. 1) for cholesterol of area 38 $Å^2$ gives a fair fit for a frayed cholesterol solid angle of 0.00015 steradians. However, for cholesterol of area 19 $Å^2$ and larger frayed solid angle 0.00115 steradians the theoretical and experimental points practically overlap. The intrinsically frayed structure of cholesterol is consistent with its not being able to form into stable bilayers. The tapered shape of phosphatidylcholine allows it to form stable bilayers since the hydrocarbon chains can interdigitate in the bilayer. This would not be possible with cholesterol; the only way the frayed cholesterol molecule could be accommodated in a bilayer is by either having a large hydrophobic area in contact with water or by sustaining large compressive strains, both situations being energetically unfavourable. In mixed phosphatidylcholine-cholesterol monolayers and lamellar bilayers the cholesterol fray is taken up by the phosphatidylcholine taper so that the apparent mean molecular mean area is less than that expected from simple additivity of the mean area of each molecule. As the mol fraction of cholesterol increases the initial apparent area occupied by each lipid remains unchanged (approx. 71 $Å^2$ for phosphatidylcholine and approx. 19 $Å^2$ for cholesterol), while the phosphatidylcholine becomes more tapered (it is effectively cylindrical in pure phosphatidylcholine lamellae) so that the bilayer becomes thicker [5, 11] and more ordered or stiff [13–15]. Once the mol fraction of cholesterol has increased to the point where the characteristic solid angle of phosphatidylcholine is reached no more taper-fray accommodation is possible and lamellar bilayers should no longer be stable. The onset of this packing instability is given by Eqn 4 when $R = \infty$. For phosphatidylcholine-cholesterol films, using $a_1 = 71$ $Å^2$, $\Omega_1 = 0.0051$ steradians, $a_2 = 19$ $Å^2$, $\Omega_2 = 0.00115$ steradians (frayed), and $d = 20$ Å, we obtain a value of $f_2 \approx 0.77$ for the critical mol fraction of cholesterol above which the packing of the mixed lipids will be highly strained. In monolayers above this critical value a transition or discontinuity should be observed in the variation of the mean molecular area with further increase of cholesterol. Such a discontinuity has been observed by de Bernard [9] at a cholesterol mol fraction of about 0.75 in excellent agreement with the calculated value of 0.77. We may also expect cholesterol to have a smaller or no "condensing" effect on (a) polyunsaturated phospholipids that are likely to be less tapered than less saturated phospholipids [10], and on (b) phospholipids that are in the condensed state [16]. However, as pointed out by Demel et al. [10, 12] condensation effects in general cannot all be explained by steric factors alone; specific polar group interactions may well modify the surface head group areas and solid angles of lipids in some mixed lipid films.

Phosphatidylcholine-phosphatidylethanolamine vesicles

Less work has been done on phosphatidylethanolamine than on phosphatidylcholine or cholesterol, but it appears that egg phosphatidylethanolamine does not form stable vesicles [17], that it forms lamellar bilayers of high "porosity" [18], and that unlike phosphatidylcholine and many other phospholipids it forms inverted hexagonal micelles [4]. Thus phosphatidylethanolamine appears to behave as a frayed or slightly tapered phospholipid. The surface head group area of egg phosphatidylethanolamine in fully hydrated inverted hexagonal micelles [4] is 41.3 $Å^2$ at 55 °C; this value may well be less at lower temperatures. Fig. 1 shows the theoretical variation of vesicle radius with increasing phosphatidylethanolamine mol fraction f_2

based on Eqn 4 plotted for a head group area of $a_2 = 41.3$ Å^2 and a tapered solid angle of $\Omega_2 = 0.000045$ steradians which gave the best fit to the experimental dotted curve [1]. Thus phosphatidylethanolamine appears to be very slightly tapered, and by itself would form vesicles of about 2000 Å diameter. Such large vesicles would be highly unstable and quickly aggregate into lamellae through long range van der Waals forces. From the above it appears that phosphatidylethanolamine may be in the condensed state, since its almost cylindrical shape of mean area ≈ 40 Å^2 is close to that of condensed lipids [19]. For larger phosphatidylethanolamine head group areas the best fit theoretical curve is at progressively larger tapered solid angles; but even for a surface area as large as 59 Å [20], the best fit phosphatidylethanolamine solid angle remains much smaller ($\Omega_2 < 0.00025$ steradians) than that of phosphatidylcholine ($\Omega_1 \approx 0.005$ steradians).

We have assumed that there is no asymmetric distribution of lipids on the inner and outer surfaces of the vesicles. Any asymmetry would affect the values obtained for the solid angles Ω_2. In other studies with mixed phosphatidylcholine-phosphatidylethanolamine vesicles Michaelson et al. [21] measured the mol fractions of phosphatidylcholine and phosphatidylethanolamine on the outer surface of vesicles (at pD 11) and found no asymmetry. Litman [17] measured the mol fraction of phosphatidylethanolamine on the outer surface to the total phosphatidylethanolamine and found that it decreased as the mol fraction of phosphatidylethanolamine relative to phosphatidylcholine increased, and thereby concluded that there was an asymmetry which increased as the amount of phosphatidylethanolamine increased. However, if the increase in vesicle size is taken into account we expect a decrease in the ratio of outer phosphatidylethanolamine to total phosphatidylethanolamine even for a symmetric distribution of phosphatidylethanolamine per unit surface areas on both sides of the vesicles. For example, at 0.1 and 0.6 mol fractions phosphatidylethanolamine Fig. 1 shows that the vesicle radii are about 123 and 207 Å, respectively. For a vesicle bilayer thickness of 40 Å, assuming no asymmetry, the outer phosphatidylethanolamine to total phosphatidylethanolamine mol fractions would be 0.69 and 0.60, respectively, to be compared with Litman's [17] measured values of 0.70 and 0.56 at these phosphatidylethanolamine/phosphatidylcholine mol fractions. Thus any asymmetry, if its exists, is small and when taken into account the analysis shows that the tapered solid angle of phosphatidylethanolamine is slightly less (i.e. phosphatidylethanolamine is even less tapered) than predicted on the assumption of a symmetrical distribution.

DISCUSSION

The general conclusion that may be drawn from the above analysis is that some lipids appear to pack into bilayer membranes like building block units. However, unlike the packing of molecules or submolecular groups in crystals and proteins these units do not have hard walls (cf. van der Waals radii of atoms), but neither are they entirely liquid like. Thus a compromise is made which sees lipids as being neither completely "hard" nor completely "soft". Indeed, any model purporting to account for the overall structure of bilayer membranes must treat the membrane-water interface as fairly rigid and ordered while allowing the inner bilayer hydrophobic regions to remain liquid-like. In the present model a lipid such as phosphatidyl-

choline is considered to have a fairly constant head group area (at a given temperature and degree of hydration) and a hydrocarbon region which behaves as a liquid so long as it is not compressed beyond a certain point.

The present model is clearly naive, yet it manages to explain a number of experimental results in terms of a simple picture in which each lipid has a characteristic surface area and solid angle. It is too difficult at present to calculate these areas and solid angles from basic principles or to calculate how lipids would pack in bilayers at different temperatures, degree of hydration, pH, ionic strength, degree of unsaturation, etc. All these factors will determine the packing characteristics of lipids. However, the concept of a solid angle may offer some guidelines. For example, pure phosphatidylcholine vesicles have the same radius in pure water as in 0.1 M NaCl solution [22], strongly suggesting that the solid angle per lipid, and hence the intermolecular forces between adjacent lipids, is independent of the ionic strength. This also implies that the electrostatic interaction between adjacent phosphatidylcholine zwitterions is unaffected by the presence of NaCl. However, in the case of the acidic phospholipid phosphatidylserine which carries a net negative charge at physiological pH the vesicle radius falls from approx. 125 Å in 0.1 M NaCl to approx. 82 Å in salt-free water [22]. Double-layer theory predicts an increased electrostatic repulsive force between acidic surface groups as the ionic strength decreases; but there is no reason to expect any change in the forces between the hydrocarbon chains as the ionic strength changes. Hence the expected overall effect on phosphatidylserine of decreasing ionic strength is to increase its characteristic solid angle (i.e. increase its taper) and thus to decrease the vesicle size, as observed.

Finally, the above conclusions regarding the packing structures of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and cholesterol may have a bearing on the distribution of lipids in biological membranes [23–26]. If these lamellar membranes are not to experience large strains then frayed lipids should always be found together with tapered liquids. Phosphatidylcholine, phosphatidylserine and probably sphingomyelin may be considered as tapered lipids, whereas cholesterol and possibly phosphatidylethanolamine may be considered as frayed lipids. This may partly explain why cholesterol is found together with phosphatidylcholine and sphingomyelin, and why phosphatidylethanolamine and probably cholesterol are found together with phosphatidylserine on the outer and inner layers of erythrocyte membranes, thus ensuring that both halves of the bilayer are separately stable and not subjected to unfavourable packing strains.

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