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# THE MOLECULAR PACKING AND STABILITY WITHIN HIGHLY CURVED PHOSPHOLIPID BILAYERS

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## Summary

It is shown that the area occupied per phospholipid molecule and the thickness of the bilayer are the same in vesicles as in a planar bilayer. From this it is concluded that the lower limit to the size of a vesicle depends on the packing of the head groups of the inner monolayer.

Unilamellar, spherical phospholipid vesicles have been extensively used as a model system with which to study the properties of biological membranes. A physical basis for the formation of phospholipid vesicles has been suggested recently by Israelachvili et al. [1]. Using the approach of Tanford [2], these authors concluded that phospholipid vesicles possess an energetically favoured diameter due to a balance between an entropy term which tends to minimize the number of lipid molecules in a vesicle and a geometric term which prevents the radius of curvature falling below a critical minimum value. The critical value that they obtain is dependent upon the area occupied per molecule at the hydrocarbon/water interface, the volume of the hydrocarbon region and the fully extended length of the hydrocarbon chain in the outer monolayer.

A re-examination of physical data obtained from sonicated, fractionated egg yolk lecithin vesicles has been reported recently by Huang and Mason [3]. Using the approach of Chrzeszczyk et al. [4], these authors calculated the vesicle dimensions shown in Table I. Table I also shows the dimensions of a multilamellar egg yolk lecithin dispersion taken from the X-ray data of Small [5]. In his calculations, based on the X-ray data, Small has chosen to distribute the 'bound' water, associated with the bilayer, throughout the polar groups of the phospholipid. The approach which has been followed by Huang

TABLE I

COMPARISON OF THE BILAYER DIMENSIONS MEASURED IN SONICATED VESICLES AND PLANAR LAMELLAR DISPERSIONS

| | Huang and Mason [3] (vesicle bilayer) | Small [5] (planar bilayer) |
|---|---|---|
| Anhydrous bilayer thickness | 16 + 21 = 37 Å | — |
| Hydrated planar bilayer thickness | — | 45.6 Å |
| Surface area per molecule in a planar bilayer | — | 71.7 Å$^2$ |
| Surface area per lipid headgroup at the inner and outer surface of a vesicle | | |
| inner surface | 61 Å$^2$ | — |
| outer surface | 74 Å$^2$ | — |

and Mason assumes, however, that the bound water associated with the vesicle is distributed as hydration shells covering its inner and outer surfaces.

The purpose of the present communication is to modify the approach followed by Huang and Mason to enable a direct comparison to be made between the vesicle and planar bilayer structure.

We shall use the hydrated vesicle molecular weight and the hydrated phospholipid partial specific volume in our calculation for the vesicle inner radius. By doing this and by taking Small's value for the volume of the phosphorylcholine group it is possible to calculate the penetration of the water into the phospholipid bilayer and to locate the water/hydrocarbon chain interface. It is also possible to calculate the area occupied per molecule at this interface.

1. From the results of Small, 12.4 water molecules are known to be associated with each phosphorylcholine group in an egg yolk lecithin bilayer. Thus, from the value of Huang and Mason for the anhydrous vesicle molecular weight we can calculate that there are $1.88 \cdot 10^6 / 768 = 2448$ molecules in each vesicle of radius 105 Å and thus, $12.4 \cdot 2448 = 30\,355$ water molecules to be included in the molecular weight. This results in a hydrated vesicle molecular weight of $2.43 \cdot 10^6$.

2. Likewise, the hydrated partial specific volume of a lipid molecule can be calculated knowing that there are $18 \cdot 12.4/768$ g of water per g of lipid. Taking $\bar{v}$ for the lipid [3] to be 0.9848 ml/g we may calculate the hydrated partial specific volume, $\bar{v}$, as 0.9882 ml/g.

In the absence of any experimental evidence to the contrary, it is assumed that the partial specific volume of the water associated with the fully hydrated phosphorylcholine group of the lipid is unity.

3. Using the expression:

$$M = 4\pi N/3\bar{v}(R_o^3 - R_i^3)$$

and a value [3] of $R_o$ of 105 Å, substituting the hydrated value for $M$ and $\bar{v}$ results in an inner radius ($R_i$) of 59.2 Å.

4. From the experimental value of 2.1, obtained by Huang and Mason,

for the bilayer lipid ratio, there are 1658 and 790 lipid molecules in the outer and inner monolayers of the vesicle, respectively.

5. Thus, the inter-monolayer radius may be calculated from:

$$\frac{1658}{790} = \frac{R_o^3 - R_{int}^3}{R_{int}^3 - R_i^3}$$

whence $R_{int}$ = 80.1 Å.

6. Also, the area per hydrated lipid molecule at the inner surface is:

$$\frac{4\pi R_i^2}{790} = 55.7 \text{ Å}^2$$

and at the outer surface:

$$\frac{4\pi R_o^2}{1658} = 83.6 \text{ Å}^2$$

7. The penetration of the water into the phospholipid bilayer may be calculated from Small's value for the volume of the hydrated phosphorylcholine group (573.8 Å$^3$), the number of molecules on each surface, and the internal and external vesicle radii. Thus, the radius of the inner hydrocarbon/water boundary, $R_{hc_i}$, can be found from:

$$790 \cdot 573.8 = \frac{4\pi}{3}(R_{hc_i}^3 - 59.3)$$

which results in $R_{hc_i}$ = 68.1 Å.

Similarly, the radius of the outer hydrocarbon/water boundary, $R_{hc_o}$, is given by:

$$1658 \cdot 573.8 = \frac{4\pi}{3}(105^3 - R_{hc_o}^3)$$

which results in $R_{hc_o}$ = 97.6 Å.

8. This immediately gives the thickness of the inner ($l_{pc_i}$) and outer ($l_{pc_o}$) hydrated phosphorylcholine layers as 8.9 and 7.4 Å, respectively.

9. Finally, the area per hydrated lipid molecule at the interface is:

$$\frac{4\pi R_{hc_i}^2}{790} = 73.7 \text{ Å}^2 \text{ for the inner monolayer, and}$$

$$\frac{4\pi R_{hc_o}^2}{1658} = 72.2 \text{ Å}^2 \text{ for the outer monolayer.}$$

The assumption throughout all of the preceding calculations has been that the partial specific volume and hydration of the lipid molecules on the inner and outer monolayers are the same. This assumption is in part justified in Ref. 3. The volume of a hydrated lipid molecule in the inner and outer monolayers is, by simple geometry, 1626 Å$^3$. Allowing for a hydrated phosphorylcholine group volume of 573.8 Å$^3$ gives the volume of the hydrocarbon region of each molecule as approx. 1052 Å$^3$.

The vesicle dimensions have been summarized in Fig. 1. A comparison of $A_{hc_o}$, $A_{hc_i}$ and the area occupied per lipid molecule at the lipid/water interface of a planar bilayer quoted by Small reveals that all three lie within the range 72.5 ± 1.5 Å$^2$. Likewise, the overall bilayer thicknesses in both cases are in excellent agreement and lie within the range 45.7 ± 0.1 Å. A direct result of the equality of these areas is that the contribution of the hydrocarbon

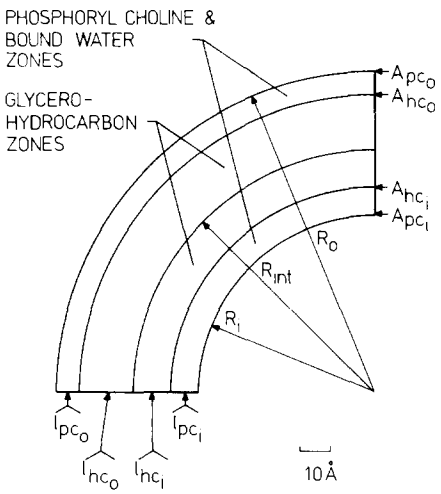


**Fig. 1. The dimensions of a hydrated egg yolk lecithin vesicle derived from the data of Huang and Mason (1978) [3]. $R_o$ = 105 Å, radius to the outer monolayer surface. $R_{int}$ = 80.1 Å, radius to the interface between the inner and outer monolayers. $R_i$ = 59.2 Å, radius to the inner monolayer surface. $A_{pc_o}$ = 83.6 Å, area per lipid molecule of the hydrated phosphorylcholine group at the outer monolayer surface. $A_{hc_o}$ = 72.2 Å$^2$, area per lipid molecule at the interface of the polar and glycerohydrocarbon region of the outer monolayer surface. $A_{hc_i}$ = 73.7 Å$^2$, area per lipid molecule of the interface of the polar and glycerohydrocarbon region of the inner monolayer surface. $A_{pc_i}$ = 55.7 Å$^2$, area per lipid molecule of the hydrated phosphorylcholine group at the inner monolayer surface. $l_{pc_o}$ = 7.4 Å, length of the hydrated phosphorylcholine group at the outer monolayer. $l_{hc_o}$ = 17.5 Å, length of the glycerohydrocarbon chain group of the outer monolayer. $l_{hc_i}$ = 12 Å, length of the glycerohydrocarbon chain group of the inner monolayer. $l_{pc_i}$ = 8.9 Å, length of the hydrated phosphorylcholine group of the inner monolayer.**

chain region to the overall bilayer thickness also remains constant, independent of the membrane curvature if the density of the hydrocarbon chains within each monolayer is the same. The effect of the bilayer curvature is absorbed in two ways. The first is a shift, towards the centre of curvature, of the interfacial region between the hydrocarbon chains of the two monolayers. A consequence of this shift is an increase in the area occupied by the ends of the hydrocarbon chains of the inner monolayer and a decrease in the area of the equivalent groups at the outer monolayer.

The second effect of curving a bilayer membrane is the variation in thickness of the polar group region on the inner and outer monolayers. From Fig. 1 it can be seen that the outer polar region is approx. 20% thinner than the inner region.

Israelachvili et al. [1] have produced an expression for the limiting radius ($R_{hc_c}$) of a vesicle at the outer monolayer hydrocarbon-water interface:

$$R_{hc_o} = l_c \left( \frac{3 + \sqrt{3\,(4v/l_c a_i - 1)}}{6\,(1 - v/l_c a_i)} \right)$$

The basic idea behind this expression is that the smallest radius which may be adopted by a phospholipid vesicle is determined by the critical length of the hydrocarbon chains in the outer monolayer of the vesicle bilayer. This critical length although related to, is less than, the fully extended length of the hydrocarbon chain. For example, from Ref. 1, p. 1553, for a range of $l_c$ from 16 to 19 Å the radius changes from 212 to 80 Å. A value of $l_c$ equal to 17.5 Å is chosen as the critical length of the hydrocarbon chains. It is then noted that the critical chain length, $l_c$, corresponds to 80% of the fully extended length of the hydrocarbon chain. The choice of this value of $l_c$ is based entirely on its use in the above expression giving the value of $R_{hc_o}$ observed experimentally. In fact, the volume used in this calculation by Israelachvili et al. is that appropriate to the total glycerohydrocarbon region and not simply that of the chain. This means that approx. 3 Å need to be subtracted from the above estimate of $l_c$, giving a value of 14.5 Å for the hydrocarbon chain contribution. This reduces $l_c$ from 80% to 66% of the fully extended chain length. Furthermore, this estimate is based on assuming the fully extended length of the chains in egg yolk lecithin is the same as that of a 16 carbon chain phospholipid. Given that egg yolk lecithin possesses a mixture of 16 and 18 carbon chain length it would seem more appropriate to assume an average of 17 carbons per chain giving an even greater reduction from the fully extended length. Now whether this value of 63% or the original value of Israelachvili et al. of 80% of the fully extended chain length is reasonable is quite unclear to us. The choice of 17.5 Å made by Israelachvili et al. is quite arbitrary.

A clue to another possible mechanism may be seen in both the area available per head group and the thickness of the hydrated head group region at the inner surface of the vesicle. Recent neutron diffraction and nuclear magnetic resonance data suggest that the phosphorylcholine group is normally lying roughly parallel to the membrane surface in a planar bilayer (Seelig [6]). In this configuration the total thickness of a hydrated phosphorylcholine group is approx. 8 Å. These groups in a curved vesicle appear, however, to occupy 7.4 and 8.9 Å on the outer and inner monolayers, respectively. The different head group configurations would be expected to result in detectable differences in the nuclear magnetic resonance spectra of these groups. Such an effect has indeed been reported for the $NMe_3^+$ protons of egg yolk lecithin vesicles (Kostelnik and Castellano [7]). By maintaining the phosphorylcholine O-C-C-N system in the approximately *gauche* conforma-

tion, in which it is thought to exist in a planar membrane system [6], a rotation of the entire group about the $O_1$-$C_3$ bond (using the notation of Seelig [6]) results in the phosphorylcholine group adopting a more extended configuration with the $NMe_3$ nitrogen directly above the phosphorus. This places an upper limit on the thickness of the hydrated polar region of approx. 9 Å in agreement with our calculated value of 8.9 Å. Furthermore, the area per molecule at the outer surface is 83.6 $Å^2$ and at the inner surface 55.7 $Å^2$ which is in good agreement with the lower limit quoted by Shah and Schulman [8] and by de Bernard [9] for the limiting surface area at which monolayers collapse. These dimensions suggest that the maximum curvature adopted by the bilayer is determined by the extent to which the phosphorylcholine group may realign to maximize its length and minimize its area at the inner surface of the vesicle. For aggregates formed from molecules having very small head groups (e.g., fatty acids) it is likely that the restraints effected by the hydrocarbon chains would be the limiting factor as postulated by Israelachvili et al [1].

In conclusion, we suggest that the curvature of the smallest vesicle bilayer is not a consequence of the natural packing geometry of the constituent molecules. Instead, we suggest that energy must be fed into the system before the lipid bilayers will form into these small highly curved structures, and further that these structures are not stable, and given time will combine with neighbouring vesicles in a manner to relieve the stress built into the altered packing of the head groups of the inner monolayer. This means that natural biological membranes will only have highly curved surfaces if sufficient energy is available to maintain their structure.

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