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Geometric packing constraints in egg phosphatidylcholine vesicles*

(inner and outer monolayers of vesicle bilayers/transmembrane asymmetry)

C. HUANG† AND J. T. MASON

Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22901

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ABSTRACT **This investigation presents a quantitative analysis of the asymmetric transmembrane lipid packing geometry in egg phosphatidylcholine vesicles. The analysis uses hydrodynamic and nuclear magnetic resonance data obtained for homogeneous egg phosphatidylcholine vesicles and demonstrates that the average area per lipid head group and effective length of the lipid are greater on the outer monolayer than on the inner monolayer of the egg phosphatidylcholine vesicle. Results also indicate that the bilayer of the egg phosphatidylcholine vesicle has an asymmetrical interface through its center.**

Spherically closed shell-like unilamellar phospholipid vesicles have gained wide acceptance as a membrane model system for the study of lipid bilayers, and to this end much work has been undertaken to illuminate their physical properties (1–3). One aspect of vesicle behavior that has drawn recent attention is the observation that the two monolayers comprising the vesicle bilayer are asymmetric with respect to many of their physical and compositional characteristics (4, 5). One such characteristic appears to be the geometric packing of the lipid molecules that make up the bilayer. This transmembrane asymmetric packing has been speculated to derive from the small radius of curvature of the vesicle bilayer (generally 100–110 Å) (6, 7).

Recently, Chrzeszczyk and her coworkers have derived an elegant approach for examining this asymmetric packing in dipalmitoylphosphatidylcholine vesicles, using a combination of hydrodynamic and nuclear magnetic resonance (NMR) techniques (8). Nonetheless, only a qualitative picture has emerged because these authors analyzed dipalmitoylphosphatidylcholine vesicles by using ^{31}P NMR data derived from unfractionated dipalmitoylphosphatidylcholine vesicles but hydrodynamic data derived from homogeneous egg phosphatidylcholine vesicles.

In this communication, we report our analysis of the transmembrane lipid geometric packing of egg phosphatidylcholine molecules using ^{31}P NMR and hydrodynamic data obtained in this laboratory for homogeneous egg phosphatidylcholine vesicles (9–12). This analysis yields quantitative information on the lipid geometry in the two halves of the vesicle bilayer. The results to be described indicate that the average area per lipid head group and effective length of the lipid are greater on the outer monolayer than on the inner monolayer of the egg phosphatidylcholine vesicle, suggesting thereby that similar transmembrane asymmetry of bilayer lipids may be present in highly folded regions of biological membranes.

METHODS

The lipid packing geometry of the egg phosphatidylcholine vesicle bilayer can be evaluated on the assumption that the partial specific volumes, and thus the average volume per lipid molecule, are the same for the phosphatidylcholine molecules in the two monolayers of the vesicle bilayer. Evidence for the validity of this assumption will be presented in the *Discussion*. With this assumption, the following two equations can be derived (8).

$$M = N(\tfrac{4}{3}\pi(R_C{}^3 - R_A{}^3))/\bar{v} \quad [1]$$

$$X = (R_C{}^3 - R_B{}^3)/(R_B{}^3 - R_A{}^3) \quad [2]$$

in which R_A, R_B, and R_C are three radial parameters of the vesicle defined in Fig. 1, N is Avogadro's number, M is the anhydrous vesicle weight, $\bar{v}$ is the vesicle partial specific volume, and X is the ratio of the number of the molecules in the outer monolayer (n_o) to that in the inner monolayer (n_i). The parameter X is derived from NMR data and the rest are from hydrodynamic measurements. The values for these parameters, as well as their sources, are shown in Table 1.

To begin our analysis, we assign a value for R_C based upon the hydrodynamic measurements for the hydrated vesicles radius 105 Å and the thickness for the outer hydration layer (6 Å). This gives a value for R_C of 99 Å as shown in Table 2. With this value for R_C, Eqs. 1 and 2 can be employed to calculate values for the radial parameters R_A and R_B.

Values for n_o and n_i as defined above can be obtained by using the following equations:

$$n_o/n_i = X \quad [3]$$

$$n_o + n_i = M/768 \text{ daltons} \quad [4]$$

In the latter equation, 768 daltons is taken as the average molecular mass of an egg phosphatidylcholine molecule.

Again, based on the assumption that the molecular volumes of the average lipids in the inner and outer monolayers of the vesicle bilayer are identical, the volume available to each lipid molecule within the outer monolayer (V_o) and the inner monolayer (V_i) can be calculated from Eqs. 5 and 6:

$$V_o = \tfrac{4}{3}\pi(R_C{}^3 - R_B{}^3)/n_o \quad [5]$$

$$V_i = \tfrac{4}{3}\pi(R_B{}^3 - R_A{}^3)/n_i \quad [6]$$

The values for the above calculated parameters can now be used to characterize the asymmetry of lipid geometric packing in the vesicle bilayer.

RESULTS

The values obtained in the above calculations and the parameters derived from them are summarized in Table 2. In no case

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Abbreviation: NMR, nuclear magnetic resonance.

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† To whom correspondence should be addressed.

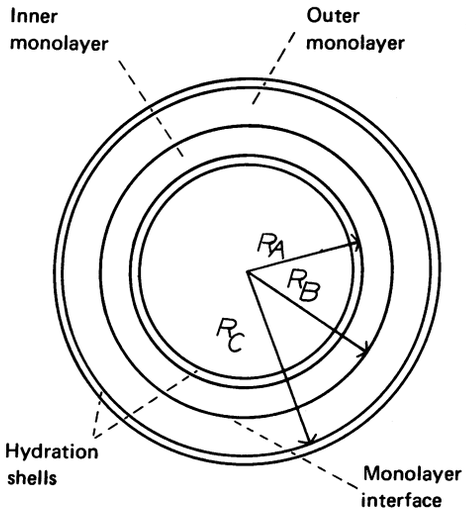

FIG. 1. Vesicle bilayer cross section showing the definitions of the radial parameters R_A, R_B, and R_C introduced in *Methods*.

were the errors in the parameters listed in Table 1, which were used to calculate the packing parameters listed in Table 2, greater than 5%. The radial parameters yield the thickness for the outer monolayer ($R_C - R_B$) and inner monolayer ($R_B - R_A$) which are 21 Å and 16 Å, respectively. The radial parameters and a knowledge of n_o and n_i will give the effective surface area per lipid head group for the two monolayers and also the effective cross sections available to the acyl chains per lipid molecule at the monolayer interface (R_B). For the outer and inner monolayers these values are 74 Å^2 and 61 Å^2, respectively, for the headgroup area, and 46 Å^2 and 97 Å^2, respectively, for the acyl chain cross sections. The volume available per lipid molecule in the vesicle bilayer can be calculated to be 1253 Å^3.

It should be emphasized that the above area and volumetric parameters are independent of any assumed geometry for the volume available to the individual lipid molecules in either of the monolayers. However, it is easier to visualize the difference in the molecular packing geometry imposed by the high surface curvature for lipids in the two faces of the vesicle bilayer if a defined geometry is assigned, and this is shown in Fig. 2. Here, we allow each lipid molecule to occupy a cell whose geometry is that of a cone intersecting the bilayer such that the apex of the cone is at the vesicle's center. The surface area and volumetric dimensions of these cells are made to conform to the parameters derived above.

DISCUSSION

The quantitative analysis of the lipid geometry in the two halves of the egg phosphatidylcholine vesicle bilayer has been achieved by a two-step procedure involving parameters taken from hydrodynamic and ^{31}P NMR analysis of egg phosphatidylcholine vesicles.

Table 1. Parameters for egg phosphatidylcholine vesicles

Parameter	Value	Measurement	Ref.
Outer hydration layer	6 Å	Hydrodynamic	9
Hydrated vesicle radius	105 Å	Hydrodynamic	10
Vesicle weight, M	1.88×10^6 daltons	Hydrodynamic	11
Partial specific volume, $\bar{v}$	0.9848 ml/g	Hydrodynamic	11
Bilayer lipid ratio, X	2.1	^{31}P NMR	12

Table 2. Geometric packing parameters for egg phosphatidylcholine vesicles

Parameter	Values
Radial parameters	R_A = 62 Å; R_B = 78 Å; R_C = 99 Å
Outer monolayer thickness	$(R_C - R_B)$ = 21 Å
Inner monolayer thickness	$(R_B - R_A)$ = 16 Å
Number of lipid molecules in each monolayer	n_o = 1658; n_i = 790
Volume per lipid molecule	$V_o = V_i$ = 1253 Å^3
Surface area per lipid head group	Outer, 74 Å^2; inner, 61 Å^2
Acyl chain cross section at R_B	Outer, 46 Å^2; inner, 97 Å^2
Anhydrous bilayer thickness	$(R_C - R_A)$ = 37 Å

The most prominent structural feature of the vesicle bilayer resulting from the present study is that the average phosphatidylcholine molecules of the inner and outer monolayers have different geometries, and the vesicle bilayer has an asymmetrical interface through its center. As shown in Fig. 2, the effective surface area available to the phosphatidylcholine's polar head group is greater on the outer monolayer surface than on the surface of the inner monolayer. In contrast, the effective cross-sectional area available to the phosphatidylcholine's acyl chains is greater within the inner monolayer than within the outer monolayer. These conformational differences are caused by the higher degree of surface curvature of the inner monolayer relative to the outer monolayer in the small vesicle with a radius of approximately 100 Å.

It should be pointed out that the validity of Eq. 2 is based on the assumption that the volumes occupied by average egg phosphatidylcholine molecules in the two halves of the vesicle bilayer are virtually identical. Nagle reported that the largest change in the bilayer density occurred during the gel → liquid crystalline phase transition and that the overall relative change in the density associated with the whole range of phase transition temperature for dipalmitoylphosphatidylcholine was merely 3.5% (13). The ^{31}P NMR experiments described in this paper were performed with egg phosphatidylcholine bilayer

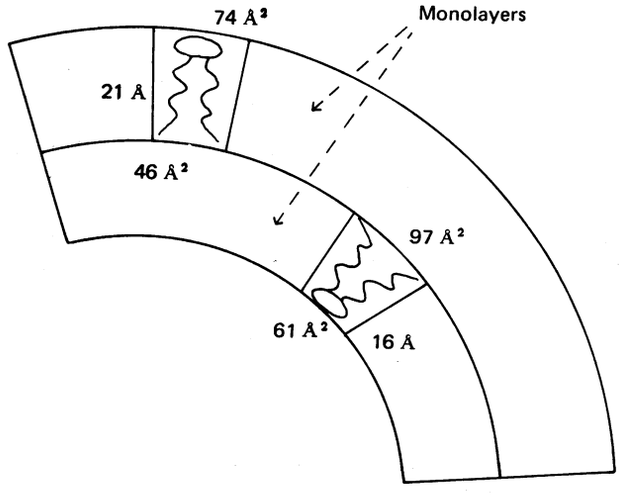

FIG. 2. Vesicle bilayer cross section showing the packing geometry of lipids within the inner and outer vesicle bilayer.

vesicles at 23 ± 2°, which is far above the broad phase transition of egg phosphatidylcholine bilayers centered at −5°; hence the difference in the lipid density between average egg phosphatidylcholine molecules in the two halves of the vesicle bilayer at the temperature of ^{31}P NMR measurements, if it exists, must be exceedingly small. Now, if we make the reasonable assumption that the density difference between lipids in the two monolayers is negligible, for mass to be conserved the volumes occupied by the average egg phosphatidylcholine molecules in the two halves of the vesicle bilayer must, therefore, be essentially identical.

The anhydrous vesicle bilayer thickness derived from this study is given by $(R_C - R_A)$ and yields a value of 37 Å (Table 2). This compares extremely well with the value of 36 Å of the vesicle bilayer thickness as derived from electron density profiles of x-ray diffraction patterns on egg phosphatidylcholine vesicles (2). These electron density profiles measure the distance between phosphate groups in opposing monolayers of the vesicle bilayer. Recent $^{31}P\{^1H\}$ nuclear Overhauser effect studies examining the phosphatidylcholine head group orientation in egg phosphatidylcholine vesicles would suggest that the electron density profiles give an adequate representation of the true bilayer thickness (14–16). The excellent agreement of the calculated bilayer thickness derived from the present study with x-ray diffraction data from egg phosphatidylcholine vesicles gives strong support of our analysis and conclusions about transmembrane molecular asymmetries in the bilayer structure.

Additional evidence supporting the validity of our calculations can be derived from monolayer studies. Nagel theorized that lipid molecules in a monolayer at the air–water interface should behave as those in the lipid bilayer if the surface pressure of the monolayer is made to be 50 dynes/cm (17). The monolayer work of Shimojo and Ohnishi (18) on egg phosphatidylcholine molecules at the air–water interface showed that the effective lipid head group area under an extrapolated external surface pressure of 50 dynes/cm is 66 Å^2. From our results the weight-average lipid head group area for the two monolayers comprising the egg phosphatidylcholine bilayer can be calculated to be (2.1/3.1) (74 Å^2) + (1/3.1) (61 Å^2) = 69 Å. This value is in good agreement with the extrapolated value for the monolayer data. Moreover, x-ray diffraction studies on egg phosphatidylcholine at 25° yield a value of 68 Å^2 as the limiting surface area occupied per lipid molecule for the uncurved or nonoriented preparations (19), again in excellent agreement with our calculated weight-average lipid head group area for the vesicle bilayer.

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