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Rapid report

Components of the lateral pressure in lipid bilayers deduced from H_{II} phase dimensions

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Abstract

The components of the effective internal lateral pressure arising from the lipid headgroups and the lipid chains in bilayer membranes are deduced from the conditions for interfacial equilibrium and the spontaneous bending moments within the component monolayers. The latter are obtained from the intrinsic curvature that is deduced from X-ray diffraction measurements on the corresponding inverted hexagonal (H_{II}) lipid phases.

Keywords: Lateral pressure; Intrinsic curvature; Elastic energy; Inverted hexagonal (H_{II}) phase; Phosphatidylethanolamine

The influence of lateral pressure on the activity of membrane enzymes, most notably phospholipases, in lipid monolayers is well known [1–3]. Also, the effects of an applied lateral membrane tension on the conducting properties of alamethicin ion channels in lipid bilayers [4] and on the hydrolytic activity of phospholipase A_2 with lipid vesicles [5] have been demonstrated recently. A lipid bilayer membrane in its natural state, however, is tension-free because at equilibrium the components of the internal lateral pressure are exactly balanced by the interfacial tension arising from the exposure of hydrophobic groups at the polar/apolar interface [6–8]. The internal lateral pressure in lipid membranes is therefore not a quantity that is amenable to direct measurement (except for the case of lipid bilayer vesicles in equilibrium with lipid monolayers, Ref. [9]) and even less so are the constituent components of the internal lateral pressure.

An interesting situation arises, however, for bilayers composed of phospholipids such as phosphatidylethanolamines that have a natural tendency towards spontaneous curvature. This spontaneous curvature is frustrated by the reflection symmetry of the component monolayers in a phospholipid bilayer but expresses itself in transitions (e.g., with increasing temperature) to non-lamellar phases such as the inverted hexagonal (H_{II}) phase that are composed of curved monolayers [10–12]. The radius of curvature of the resulting H_{II} phase is found to be close to that of the spontaneous intrinsic curvature of the lipid assembly, $c_o = 1/R_o$, where R_o is the intrinsic radius of curvature [12,13]. In a bilayer with frustrated spontaneous curvature, the monolayers experience equal and opposite spontaneous bending moments given by $M_o = k_c c_o$, where k_c is the curvature elastic constant of the monolayer. These intrinsic bending moments are a direct reflection of the components of the internal lateral pressure in the constituent lipid monolayers and of the offset in their centre of action from that of the hydrophobic tension at the polar/apolar interface. The functional consequences of this spontaneous bending moment have been demonstrated recently by the correlation of the ion-conducting activity of alamethicin channels with the intrinsic curvature of the phospholipids composing the host bilayer membrane [14].

The purpose of the present communication is to provide estimates for the size of the components of the lateral pressure in phospholipid membranes from the dimensions of the corresponding spontaneously formed H_{II} phases of these phospholipids. Strictly, this yields values that are appropriate to the bilayer at a temperature immediately below the transition to the H_{II} phase. The principles used in the calculation are those introduced in the preceding paragraphs. The motivation for such a study lies in the interpretation of experiments on the dependence of the

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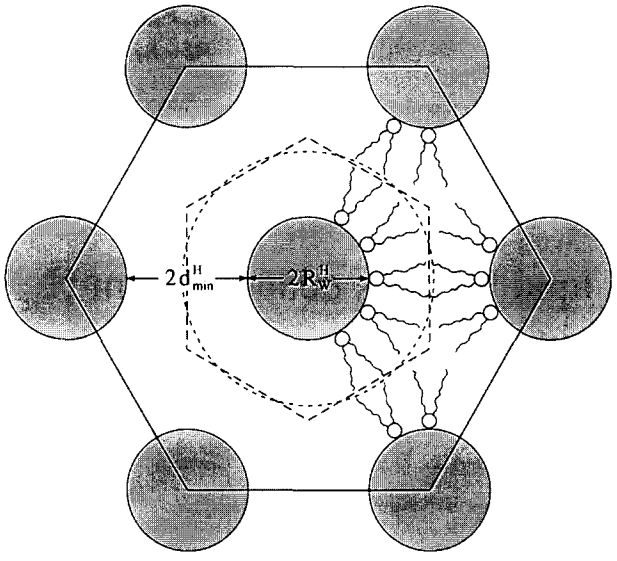

Fig. 1. Structure of the inverted hexagonal lipid phase. The minimum lipid length, d^{H}_{min}, and the radius of the water cylinders, R^{H}_{W}, are indicated. The area per lipid molecule, A^{H}_{o}, is that defined at the lipid/water interface. The dashed circle has an area equal to that of the hexagonal unit cell (defined by the dashed lines) and has a radius $R_{eq} = (R^{H}_{W} + d^{H}_{min})\sqrt{2\sqrt{3}/\pi}$.

activity of membrane proteins on membrane lipid composition, in particular on intrinsic curvature of the lipids as in the example cited above.

The structure of the inverted hexagonal lipid phase is indicated schematically in Fig. 1. The thicknesses of the lipid headgroup and lipid chain regions are d_{HG} and l_{ch}, respectively, and the radius of the water cylinders in the H_{II} phase is R^{H}_{W}. The spontaneous radius of curvature is then:

$$R_o = R^{H}_{W} + d_{HG} \quad (1)$$

where it is assumed that the neutral surface lies close to the polar/apolar interface. The headgroup thickness for

Table 1
Dimensions (R^{H}_{W}, A^{H}_{o}, d^{H}_{min}) of the H_{II}-phases of phosphatidylethanolamines from X-ray diffraction measurements, and molecular dimensions for the constituent lipids (d_{HG}, l_{ch}) deduced from Eqs. 2 and 3

Lipid	T (°C)	d^{H}_{min} (nm)	A^{H}_{o} (nm^2)	R^{H}_{W} (nm)	d_{HG} (nm)	l_{ch} (nm)	Ref.
diC(12:0)PE	135	1.015	0.631	1.60	0.483	0.663	[11]
diC(20:0)PE	99	1.765	0.490	2.175	0.622	1.430	[11]
diC(18:1)PE	10	1.62	0.48	2.29	0.635	1.181	[12]
diC(18:1)PE	90	1.51	0.51	1.62	0.598	1.069	[12]

phosphatidylethanolamines is estimated from that in the crystal structure of dilauroylphosphatidylethanolamine ($d_{DLPE} = 0.79$ nm), together with the measured surface area per lipid, A^{H}_{o}, in the H_{II} phase:

$$d_{HG} = d_{DLPE}(A_{DLPE}/A^{H}_{o}) \quad (2)$$

where $A_{DLPE} = 0.386$ nm^2 is the area per lipid molecule in the dilauroylphosphatidylethanolamine crystal [15]. Eq. 2 allows for the effective thinning of the headgroup region depending on the molecular packing at the lipid/water interface. Because the apparent lipid chainlength varies in the H_{II} phase, the thickness of the lipid chain region is taken to be the equivalent thickness for a uniform cylinder of cross-sectional area equal to that of the hexagonal H_{II} cylinders (cf., Ref. [11]):

$$l_{ch} = (d^{H}_{min} + R^{H}_{W})\sqrt{2\sqrt{3}/\pi} - (R^{H}_{W} + d_{HG}) \quad (3)$$

where d^{H}_{min} is the minimum lipid length (that along the line joining the water cylinders) in the H_{II} phase (see Fig. 1). Values for the dimensions of the H_{II} phases of various phosphatidylethanolamines obtained from X-ray diffraction measurements, and the corresponding lipid molecular dimensions deduced from Eqs. 2 and 3 are given in Table 1. Data are presented for a short-chain dialkyl lipid didodecylphosphatidylethanolamine, diC(12:0)PE, for a longer

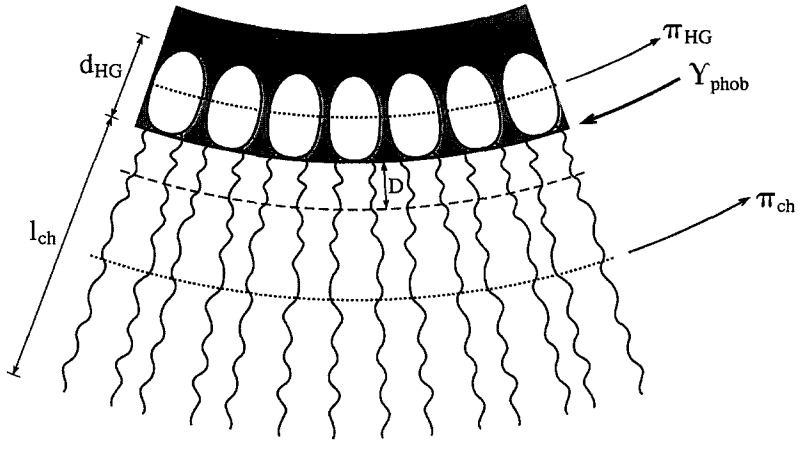

Fig. 2. Schematic indication of the balance of lateral forces in a lipid monolayer. The components of the internal lateral pressure from the lipid headgroups (π_{HG}) and from the lipid chains (π_{ch}) balance the cohesive hydrophobic tension (γ_{phob}) which acts at the polar/apolar interface. The resultant centre of action of the internal lateral pressure is offset by a distance D from the polar/apolar interface, creating a spontaneous bending moment.

chain diacyl lipid diarachinoylphosphatidylethanolamine, diC(20:0)PE, and for an unsaturated-chain lipid dioleoylphosphatidylethanolamine, diC(18:1)PE.

The model used for the calculation is that illustrated in Fig. 2. The effective components of the internal lateral pressure from the lipid headgroups (π_{HG}) and from the lipid chains (π_{ch}) are assumed to act at distances $d_{HG}/2$ and $l_{ch}/2$, respectively, from the polar/apolar interface at which the cohesive hydrophobic tension, γ_{phob}, acts. In general, π_{HG} and π_{ch} are determined by integration over the lateral pressure distribution in the headgroup and chain regions, respectively, and the points of action by the centres of gravity of these distributions. The balance of apposing forces required to maintain equilibrium of the lipid assembly gives the following relation between the components of the lateral pressure and the hydrophobic interfacial tension:

$$\gamma_{phob} = \pi_{HG} + \pi_{ch} \tag{4}$$

If the resultant of the lateral pressure components acts at a distance D from the polar/apolar interface (cf., Fig. 2 and Ref. [7]), then the balance of their moments about this point is given by the relation:

$$\pi_{HG}[(d_{HG}/2) + D] = \pi_{ch}[(l_{ch}/2) - D] \tag{5}$$

From Eqs. 4 and 5, the components of the lateral tension are given by:

$$\pi_{HG} = \gamma_{phob}(l_{ch} - 2D)/(l_{ch} + d_{HG}) \tag{6}$$

and

$$\pi_{ch} = \gamma_{phob}(d_{HG} + 2D)/(l_{ch} + d_{HG}) \tag{7}$$

where $l_{ch} + d_{HG}$ is the equivalent total lipid thickness in the H_{II} phase. A useful dimensionless quantity that can be extracted from Eqs. 6 and 7 is the fraction f_{ch} of the internal lateral pressure that is contributed by the lipid chains. This is given by:

$$f_{ch} = \pi_{ch}/\gamma_{phob} = 1 - \pi_{HG}/\gamma_{phob} \tag{8}$$

where use has been made of Eq. 4.

The value of the offset distance D may be obtained from the intrinsic bending moment, M_o, in the lipid monolayer which is given by:

$$M_o = \gamma_{phob} \cdot D \tag{9}$$

because the balanced forces, γ_{phob} and $(\pi_{HG} + \pi_{ch})$, in the resultant couple are separated by a distance D (cf., Fig. 2). As mentioned above, the spontaneous bending moment is also related to the intrinsic radius of curvature via the curvature elastic constant by the defining relation: $M_o = k_c/R_o$ (see, eg., Ref. [8]). From these two relations for the bending moment, the following expression is then obtained for D:

$$D = (k_c/\gamma_{phob})/R_o \tag{10}$$

The curvature elastic constant for the monolayer, k_c, may be obtained by integrating the elastic energy for extension throughout the thickness of the region over which the resultant pressures act, i.e., from $-l_{ch}/2$ to $+d_{HG}/2$.

The elastic energy at a given level within the bent monolayer is $K_A(A - A_o)^2/2A_o$ where the area extension at a distance x from the polar/apolar interface is $(A - A_o)/A_o \approx x/R_o$ and K_A is the elastic constant for area extension/dilation (see, e.g., Ref. [8]). Integrating over the effective thickness, the mean elastic energy then becomes: $(1/24) \cdot K_A A_o(d_{HG}^2 + l_{ch}^2 - d_{HG} l_{ch})/R_o^2$, where the average is taken over the range $x = -l_{ch}/2$ to $+d_{HG}/2$. This value can be identified with the elastic curvature energy, i.e., $(1/2) \cdot k_c A_o/R_o^2$, from which it can be seen that the curvature elastic constant of the monolayer is given by: $k_c = (1/12) \cdot K_A(d_{HG}^2 + l_{ch}^2 - d_{HG} l_{ch})$, which is of the order of magnitude 10^{-20} J.

Simplifications can be obtained by expressing the elastic constant for area dilation, K_A, in terms of the model given in Fig. 2. Because the hydrophobic tension (or free energy density) γ_{phob} is essentially independent of area, the area elastic constant is given by: $K_A = -A(d\pi_{tot}/dA)_{A_o}$, where $\pi_{tot} = \pi_{HG} + \pi_{ch}$ is the total internal lateral pressure [6,8]. If the dependence of the latter on molecular surface area is given by $\pi_{tot} \approx -1/A^2$, then $K_A = 2\pi_{tot}(A_o) = 2\gamma_{phob}$, which yields numerical values (≈ 150 mN/m) that are close to those measured experimentally [7,8]. Making this reasonable approximation in the expression for the curvature elastic constant, the expression for the offset distance D then becomes:

$$D = \tfrac{1}{6}(d_{HG}^2 + l_{ch}^2 - d_{HG} l_{ch})/R_o \tag{11}$$

which in combination with Eqs. 6 and 7 may then be used to estimate the values for the components of the internal lateral pressure.

The values of the spontaneous curvature, R_o, the offset distance, D, and the fractional contribution, f_{ch}, of the chains to the internal lateral pressure for various phosphatidylethanolamines are given in Table 2. These are obtained from Eq. 1, Eqs. 7 and 11 by using the dimensional data deduced from X-ray diffraction studies of H_{II} phases that are given in Table 1. The offset distances are of the order of 0.1 nm or less, consistent with the lipid

Table 2
Values deduced for the spontaneous radius of curvature, R_o, centre of action, D, of the internal lateral pressure and fractional contribution, f_{ch}, of the internal pressure from the lipid chains obtained from Eqs. 17 and 11, and the data of Table 1

Lipid	T (°C)	R_o (nm)	D (nm)	f_{ch}
diC(12:0)PE	135	2.083	0.028	0.47
diC(20:0)PE	99	2.797	0.080	0.40
diC(18:1)PE	10	2.925	0.060	0.42
diC(18:1)PE	90	2.218	0.065	0.44

assemblies forming stable bilayers at lower temperatures, for which π_{ch} will be reduced and π_{HG} correspondingly increased (because of reduced chain isomerism) and therefore D becomes smaller (cf. Eq. 5). The fractional contribution to the internal lateral pressure from the lipid chains lies in the range 0.40 to 0.47, indicating that the relative contributions from the chain and headgroup regions of the lipid molecules are comparable in magnitude. The contribution from the lipid chains is greater for diC(12:0)PE than for diC(20:0)PE, presumably because of the effect of the ether chain linkage in the former which outweighs the effects of the shorter chainlength and is reflected also in the higher curvature for this lipid. The effect of chain unsaturation in diC(18:1)PE cannot be deduced directly because comprehensive X-ray data is not available for the corresponding saturated-chain phosphatidylethanolamine and interpolation is not possible because of the dominant effect of the different chain linkages in diC(12:0)PE and diC(20:0)PE. However, the values of f_{ch} are greater than that for the longer chain diacyl lipid diC(20:0)PE, suggesting that a relatively greater pressure is exerted by the unsaturated chains. The temperature dependence for diC(18:1)PE is also interesting. The contribution from the chains appears to become somewhat more significant with increasing temperature, corresponding to the increased chain rotational isomerism.

It should be noted that the precise values of f_{ch} depend on the choice of the model taken for estimating the curvature elastic constant, k_c. However, the relative values are unchanged by assuming some other value for k_c, within a reasonable range. Also, the values of the chain and headgroup pressure components will be correspondingly scaled if their centres of action do not coincide with the geometric centres, as is assumed. To obtain estimates of the absolute values of the components of the internal lateral pressure requires knowledge of the hydrophobic tension, γ_{phob} (cf., Eqs. 6 and 7). Taking values of $\gamma_{phob} \approx 35$ mN m^{-1} obtained from the transfer free energy of alkanes from oil to water [8], the contributions to the lateral pressure lie in the ranges 14–17 mN m^{-1} and 21–19 mN m^{-1}, for the lipid chains and headgroups, respectively. In principle, the experimentally measured shifts in the lamellar to inverted hexagonal transition temperature with lipid structure [10] may also be used to estimate the corresponding incremental changes in the components of the internal lateral pressure. From the dependence of the transition temperature on lipid chainlength, it can be estimated that $\delta\pi_{ch}/\pi_{ch} \leq 0.01$ per methylene group and, from the dependence on headgroup structure, that $\delta\pi_{HG}/\pi_{HG} \approx 0.02$ for addition of either an N-methyl group or a methylene group, for dialkyl phosphatidylethanolamines. These estimates depend rather sensitively on the value assumed for the free energy of the inverted hexagonal phase, and the relative values are likely to be more reliable than the absolute values.

As already noted, functional consequences of the lateral pressure components in the bilayer have been demonstrated by the dependence of the properties of alamethicin ion channels on the intrinsic curvature of the bilayer lipids [14]. It was found that the relative occupancies, p_{i+1}/p_i of adjacent conductance levels of the alamethicin ion channels increased approximately exponentially with the spontaneous curvature, c_0, of the membrane lipids. The measured gradient was found to be $d\ln(p_{i+1}/p_i)/dc_0 \approx 5.5$ nm. This behaviour has strong parallels with the dependence of the ratio p_{i+1}/p_i for the adjacent conductance levels of alamethicin in bilayers subjected to a uniform applied tension, $\bar{T}$ [4]. In the latter case, p_{i+1}/p_i was found to depend exponentially on $\bar{T}$, corresponding to the Boltzmann factor:

$$p_{i+1}/p_i = \exp\left(-\bar{T}\Delta A_{i+1,i}/kT\right) \qquad (12)$$

where the increase in cross-sectional area of the alamethicin pore on transition between adjacent conductance levels was found to be $\Delta A_{i+1,i} \approx 12 \pm 1$ nm^2. For membranes composed of lipids with non-zero intrinsic curvature, the component monolayers are under a non-uniform tension arising from the frustrated spontaneous curvature. From the expression for the intrinsic bending moment, $M_0 = k_c c_0 \approx \delta\bar{T}_{c_0}(d_{HG} + l_{ch})$, the effective tension will be maximally: $\delta\bar{T}_{c_0} \approx k_c c_0/(d_{HG} + l_{ch})$. Assuming that the exponential dependence on c_0 and hence on $\delta\bar{T}_{c_0}$ corresponds also to that given by Eq. 12, the effective area increase in response to the frustrated spontaneous curvature is $\Delta A_{i+1,i} \approx 4$ nm^2 (for $k_c \approx 10^{-20}$ J). This is smaller than that found for the response to a uniform external tension, possibly in part because of the inhomogeneous distribution of tension. In the latter case, the changes in population of the conductance states may arise solely from molecular rearrangements of the channel pore in response to the internal stress and not also from changes in the number of alamethicin molecules incorporated into the membrane channels as suggested for the response to uniform stress [4]. Whatever the mechanism, the correlation with the spontaneous curvature indicates that changes in the components of the internal lateral pressure via changes in bilayer lipid composition are capable of affecting the ion channel activity.

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