THERMAL GROUP MOTION CREATES STOCHASTIC PORES IN PLANE PHOSPHATIDYLCHOLINE BILAYERS

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Abstract. Within the present work we determined the conditions for transbilayer pore formation due to thermal group motion of phospholipid bilayer molecules. The radius of the area containing molecules that move almost simultaneously perpendicular to the bilayer surface is within the range [R_{min}, R_{max}]. Bilayer deformation is characterized by its wavelength, which is also confined to a limited and continuous range. The limits of both R and the wavelength depend on the thickness of the bilayer's hydrophobic core, on temperature, polar group size, surface tension, and on the bilayer's elastic properties (elastic compression and splay). The conditions for transbilayer pore formation depend quantitatively on the radius of the perturbation area and on the bilayer deformation wavelength.

Key words: plane lipid bilayer, elasticity theory, transbilayer pore.

INTRODUCTION

It is well known that lipid molecules in biomembranes perform three types of motions: translations parallel to the bilayer, oscillations perpendicular to the bilayer, and rotations around their own axis. Also, transport systems, although studied for a long time, represent a main research subject in many laboratories worldwide, because of the structural and functional diversity of ion channels in living organisms. There are genetically determined pores, like the pore sieves within the walls of sinusoid vessels from mammalian liver [30], and pores formed by certain proteins, called porins, in lipid bilayers [19]. Pore formation in membrane bilayers has been experimentally confirmed [1, 2]. Also, a mechanism has been proposed, based on thermal movement of lipid components. Lateral displacement of lipidsin opposite senses can result in density fluctuations of polar groups at the bilayer surface [14, 23]. On the other hand, the lipid component of natural membranes consists in a mixture of lipids [15, 26, 27, 28]. Lipid molecules undergo

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selective associations depending on the length of the hydrophobic chains, resulting in lipid clusters [21, 22, 16, 29]. Both density fluctuations of polar groups at the bilayer surface and lipid clusters formation determine membrane defects, i.e. areas where hydrophobic surfaces come in direct contact with the external aqueous milieu [17, 18]. These defects can evolve into cylindrical hydrophobic pores and, after polar heads reorientation, into cylindrical hydrophilic pores [23, 24, 25]. Besides lateral displacement and rotation around their own axis, lipid molecules oscillate perpendicular to the bilayer surface. These oscillations induce local fluctuations in bilayer thickness [4, 6, 8, 13].

A legitimate question is whether the oscillations perpendicular to the bilayer can generate membrane pores. This phenomenon would represent a novel mechanism of transmembrane pore formation [20, 21, 23]. Because of intermolecular interaction forces, a lipid molecule can engage into its movement other neighboring molecules. The result is a group movement perpendicular to the bilayer surface, produced by thermal motion. If the amplitude of this group movement is equal to half the bilayer thickness, and the displacements of the two monolayers coincide, the bilayer can perforate, generating a pore [31].

A calculus of the variation in the bilayer free energy consequent to a variation in thickness due to the pore can confirm this mechanism of pore formation [8, 9, 10].

VARIATION IN THE BILAYER FREE ENERGY

Within the present work we consider a lipid bilayer with hydrophobic chains perpendicular to the bilayer surface. In this particular case, the lipid bilayer is similar to a type A smectic liquid crystal. The elasticity theory developed by de Gennes in 1972 can be applied to the plane lipid bilayer [7, 34].

In the initial state the bilayer surfaces are flat and the free energy is considered equal to zero. The monolayer perturbation generated by group thermal movement consists in a thickness perturbation, representing the displacement of the monolayer surface over the distance *u(x,y)* compared to the initial position. According to Huang's theory (1990), free energy variation per unit surface is equal to [10, 11, 12]:

$$\Delta F = hB\left(\frac{u}{h}\right)^2 + hK\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right)^2 + \gamma\left[\left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial u}{\partial y}\right)^2\right] \quad (1)$$

In smectic liquid crystals there are three ways of free energy variation, due to variations in thickness and changes in surface area. Thickness decrease produces a

compression of the lipid bilayer and a change in axis of the molecules in the perturbed area. The latter change is known as splay distortion.

In the formula of free energy variation due to a perturbation of amplitude *u(x,y)*, the first term represents the elastic compression energy, characterized by the elastic constant *B*; the second term represents the splay distortion, characterized by the elastic constant *K*; the third term represents the free energy variation due to superficial tension, characterized by the superficial tension coefficient γ.

In our case, the lipid monolayer deformation shows a cylindrical symmetry, therefore equation (1) becomes:

$$\Delta F = B\frac{u^2}{h} + hK\left(\frac{\partial u}{r\partial r} + \frac{\partial^2 u}{\partial r^2}\right)^2 + \gamma\left(\frac{\partial u}{\partial r}\right)^2 \tag{2}$$

The total free energy variation of the deformation of radius *R* at the level of the plane surface is:

$$\overline{F} = 2\pi\int_0^R \left[B\frac{u^2}{h} + hK\left(\frac{\partial u}{r\partial r} + \frac{\partial^2 u}{\partial r^2}\right)^2 + \gamma\left(\frac{\partial u}{\partial r}\right)^2\right] r\mathrm{d}r \tag{3}$$

We consider only a local perturbation of the membrane surface of wavelength λ and amplitude equal to half the lipid bilayer thickness *h*:

$$u(r) = -h\cos\frac{2\pi r}{\lambda} \tag{4}$$

Elasticity theory applied to continuous media has an important advantage, because it takes into account the intrinsic properties of the bilayer, via the elasticity constant B^1, the elastic splay constant *K* and the surface tension coefficient γ. This represents the main reason for using elasticity theory in the calculus of the bilayer free energy variations due to thickness variations produced by group thermal motion.

Actually, we wanted to prove that group thermal motion of molecules, perpendicular to the bilayer surface, could produce a reduction in thickness able to perforate the bilayer, leading to the formation of a pore. We considered as the initial state of the bilayer that state where composing molecules do not move (0 K), resulting in a plane surface of the bilayer. By heating the bilayer up to the temperature *T*, lipid molecules gain kinetic energy, and their movement perpendicular to the bilayer causes its deformation and fluctuations in thickness.

[1] Within the present paper, *B* includes lateral compression; generally it is written as $\overline{B}$.

The variation of the mean free energy of deformation corresponding to a molecule has to be less than or equal to the thermal energy of a phospholipid molecule:

$$\frac{a_0}{\pi R^2}\overline{F} \le (3N - N_b)\frac{kT}{2} \tag{5}$$

where N represents the number of atoms in the lipid molecule and N_b the number of bonds within the molecule. For simplicity we have chosen $3N - N_b = 6$.

We were interested in finding the parameters λ and R that satisfy the equation:

$$\frac{a_0}{\pi R^2}\int_0^{2\pi}\int_0^{R} rF(r,\lambda)\mathrm{d}r\,\mathrm{d}\varphi = (3N - N_b)\frac{kT}{2} \tag{6}$$

With some arrangements, this equation can be written as:

$$a(y)x^4 + b(y)x^2 + c(y) = 0 \tag{7}$$

where the functions a, b, c and the variables x, y are given by:

$$\begin{aligned} x &= h\frac{2\pi}{\lambda}, \quad y = R\frac{2\pi}{\lambda} \\ a(y) &= \frac{K_1}{Bh^2}\left(1 + \frac{\sin 2y}{y} - 3\frac{\cos 2y - 1}{2y^2} + \frac{4}{y^2}\int_0^1 \frac{\sin^2 ty}{t}\mathrm{d}t\right) \\ b(y) &= \frac{\gamma}{Bh}\left(1 - \frac{\sin 2y}{y} - \frac{\cos 2y - 1}{2y^2}\right) \\ c(y) &= 1 + \frac{\sin 2y}{y} + \frac{\cos 2y - 1}{2y^2} - (3N - N_b)\frac{kT}{Bha} \end{aligned} \tag{8}$$

Unfortunately, equation (7) is too complicated to obtain an explicit formula for λ and R, but we can obtain a parametric representation. In order to do this, let us consider x as dependent variable and y as parameter. Thus, the equation becomes an algebraic equation in x that can be easily solved. Because the functions a and b are always positive, the equation has solutions only if c is negative. If this condition is satisfied one can obtain only positive solutions, and the parametric formulae are given by the expressions:

$$\begin{aligned} \lambda(y) &= 2\pi h\left(\sqrt{\frac{-b(y) + \sqrt{b^2(y) - 4a(y)c(y)}}{2a(y)}}\right)^{-1} \\ R(y) &= \frac{y\lambda(y)}{2\pi} \end{aligned} \tag{9}$$

Equation (6) has been solved for phosphatidylcholine bilayers, where the constants K, B and the surface tension coefficient γ are known.

HYDROPHOBIC CORE THICKNESS FLUCTUATION ANALYSIS – CONDITIONS FOR TRANSMEMBRANE PORE FORMATION

Within the present subchapter we intend to analyze the conditions required for transbilayer pore formation, starting from fluctuations in thickness of the hydrophobic core of lipid bilayers composed of single chain lipid molecules. In this respect it is important to know the values of parameters in equation (6).

Many authors have studied membranes composed of phospholipids, therefore there are data for elastic constants. The hydrophobic core thickness for membranes composed of two chain molecules is $2h$ = 48 Å [3, 35]. The compressibility coefficient, obtained by Hladky and Gruen from White's experimental values [35], is equal to $5.36 \cdot 10^7$ N/m^2 [8]. The area per molecule is equal to 38.6 Å^2, while the surface tension coefficient γ is $8 \cdot 10^{-4}$ N/m [8]. The splay coefficient K has been obtained from measurements of the curvature elastic modulus $K_c = [2.8 – 6.5] \cdot 10^{-6}$ J [5]. The coefficient K_c has been measured for lecithin vesicles. We can obtain the elastic constant K from the relationship $K = K_c/2h$ [11, 32], and use it in equation (6). Because the thickness of vesicles where K_c has been measured was 30 Å, the splay elastic constant $K \in [0.933 – 2.167] \cdot 10^{-11}$ N.

The results presented in this subchapter pertain only to situations where the distortion energy is equal to the thermal energy of all the molecules involved in group movement resulting in local distortion of the lipid bilayer. Precisely, these results have been obtained by solving equation (6) for each case.

We have chosen as reference bilayer the solvent-free phosphatidylcholine bilayer, with a hydrophobic core thickness $2h$ = 48 Å, characterized by the above-mentioned parameters and by a splay elastic constant $K = 0.933 \cdot 10^{-11}$ N.

There is no one to one correspondence between the solutions of equation (6), therefore Table 1 and other similar tables in the text use a special designation. Except for the minimum and maximum values of λ, R, N, there are certain values within each interval, named using the index 2:1: $\lambda_{2:1}$, $R_{2:1}$, and $N_{2:1}$, respectively. These values divide the intervals of the corresponding variables into two subintervals, which are different regarding the nature of the correspondence (unilateral or not) between the solutions of equation (6).

Each value of the perturbation area radius in the interval $[R_{min,}\ R_{2:1}]$ corresponds to 2 solutions of the wavelength. If we represent by λ_{om} the value

corresponding to R_{min}, the 2 values of the wavelength corresponding to one value of R will satisfy the relationship $\lambda_1 < \lambda_{om} < \lambda_2$. The elongations corresponding to λ_1 and λ_2 will satisfy the relationship $u_1 < u_2$. Each value of the perturbation area radius within the interval ($R_{2:1}$, R_{max}) corresponds to a single value of λ. The reversal of these two statements is not true.

The interval of solutions of equation (6) for the wavelength is also divided in two subintervals following the same criterion. For each value of λ within the first subinterval [λ_{min}, $\lambda_{2:1}$], equation (6) presents 2 solutions for R, each of them corresponding to a certain value of the distortion energy and of the number of molecules involved in the pore generating group motion. If we represent by R_{om} the value of R corresponding to λ_{min}, the two values, R_1 and R_2, corresponding to a value of λ within the interval (λ_{min}, λ_{max}) will satisfy the relationship $R_1 < R_{om} < R_2$, and the corresponding relationship between the values of elongation ($u_1 < u_2$) will be also valid (Fig. 1). For each value of λ within the interval [$\lambda_{1:2}$, λ_{max}], equation (6) will present a single solution for R^2.

THE EFFECT OF THICKNESS

In order to study the effect of bilayer thickness on the radius of the area involved in thermal group motion and on the lipid bilayer distortion wavelength spectrum, we have solved equation (6) for lipid bilayers of hydrophobic core thickness $2h$ = 40.2 Å, $2h$ = 48 Å and $2h$ = 52 Å. Lipid bilayer thickness changes according to the nature of fatty acids composing the hydrophobic chains. We have studied lipid bilayers composed of DMPC ($2h$ = 40.2 Å), DPPC ($2h$ = 48 Å) and DSPC ($2h$ = 52 Å). Results are shown in Fig. 1 and presented in Table 1, where one can notice that the greater the hydrophobic core thickness, the higher the values of the perturbation area radius.

Obviously, the number of molecules involved in thermal group motion increases with the hydrophobic core thickness. We can infer by simple intuition that this increase is accounted mainly by compression energy. Considering that the greater the radius, the lower the probability of distortion, we can conclude that pore formation is less likely in thick lipid bilayers. The spectrum of wavelengths compatible with pore formation also shifts to lower values with increasing lipid bilayer thickness.

[2] In the last column of each table we have displayed the number of lipid molecules comprising the circular areas of radii R_{min}, $R_{2:1}$, R_{max}, located on the bilayer surface: $N = \pi R^2/a$. In every case the values of R and λ are in Å.

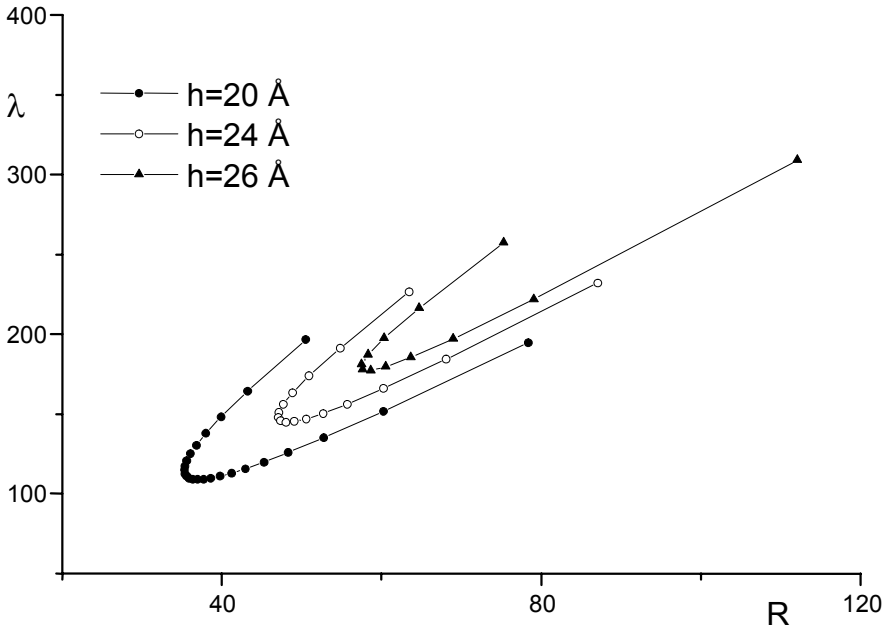

Fig. 1. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different values of the hydrophobic core thickness (2h).

Table 1

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on hydrophobic core thickness

$2h$ (Å)	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
40.2	[35.29 – 50.48 – 78.40]	114.77	[108.92 – 194.55 – 196.80]	36.96	[101 – 207 – 500]
48	[47.05 – 63.48 – 87.09]	147.81	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]
52	[57.52 – 75.31 – 112.1]	180.72	[177.14 – 257.16 – 308.85]	58.64	[268 – 461 – 1022]

Lipid bilayers have the characteristics $a_0 = 38.6$ Å^2, $T = 300$ K, $K = 0.933 \cdot 10^{-11}$ N, $B = 5.36 \cdot 10^7$ N/m^2, $\gamma = 8 \cdot 10^{-4}$ N/m.

THE EFFECT OF POLAR GROUP SIZE

The size of the polar head depends on the nature of the polar group, its angle with the bilayer surface and its degree of hydration. In case of complete hydration, the number of waters associated to each molecule is equal to 23 for DLPC, 25 for DMPC, 27 for DPPC, and 29 for DSPC [33].

For this reason we have studied the effect of polar group size on the conditions of transmembrane pore formation. For the reference bilayer, the polar group size is equal to 38.6 Å^2 and corresponds to a dehydrated polar head. Other

values were $a_0 = 41.3$ Å^2, corresponding to a 5% hydration, and $a_0 = 44$ Å^2, corresponding to a 10% hydration of a DMPC bilayer.

The results are shown in Figure 2 and Table 2. The increase in transversal area of polar heads leads directly to an increase in the radius of perturbation area, because of geometric reasons. Starting from the fact that the number of molecules increases with the transversal area of polar heads, we conclude that the effect of polar head size is reflected also in energy changes for splay, compression or surface tension.

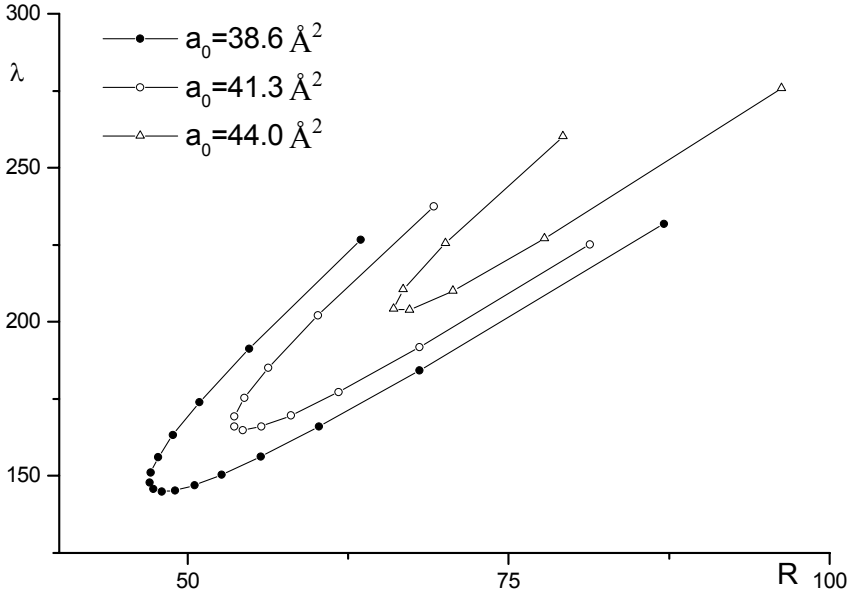

Fig. 2. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different values of the polar group transversal section (a_0), in a bilayer of hydrophobic core thickness $2h = 48$ Å.

Table 2

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on polar head transversal area, in Å^2

a_0 (Å^2)	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
38.6	[47.05 – 63.48 – 87.09]	147.81	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]
41.3	[53.62 – 69.16 – 81.35]	169.30	[164.11 – 225.16 – 237.46]	54.32	[219 – 364 – 503]
44.0	[66.06 – 79.22 – 96.27]	204.17	[177.14 – 257.16 – 308.85]	67.28	[311 – 448 – 661]

Lipid bilayers have the characteristics $2h = 48$ Å, $T = 300$ K, $K = 0.933 \cdot 10^{-11}$ N, $B = 5.36 \cdot 10^7$ N/m^2, $\gamma = 8 \cdot 10^{-4}$ N/m.

THE EFFECT OF TEMPERATURE

Temperature is the only external factor acting directly on transmembrane pore formation, because it represents the energy source for bilayer distortion. As we have previously mentioned, the aim of our work was to find out if thermal energy is enough to insure lipid bilayer perforation. Therefore, we have chosen three values of temperature below the transition temperature of the reference bilayer: $T = 290$ K, $T = 300$ K, $T = 310$ K.

The results are presented as graphs in Fig. 3 and as data in Table 3. Taking into account that an increase in temperature means an increase in thermal energy of each molecule, it becomes clear that the number of molecules in motion necessary to produce the pore generating distortion, and consequently the radius of the perturbation area, decreases at higher temperatures. This fact is confirmed by the solutions of equation (6) at the three above-mentioned temperatures. An increase in temperature produces a decrease in the radius R and in the wavelength, because the decrease in the perturbation area also decreases the total free energy of distortion required for lipid bilayer perforation. Although the minimal value of the perturbation area radius decreases with increasing temperature, the maximal radius increases up to a temperature (between 300 K and 310 K) and then decreases (Table 3). Before attempting a phenomenological explanation, we have to study the behavior of the solutions of equation (6) in the vicinities of the integration limits.

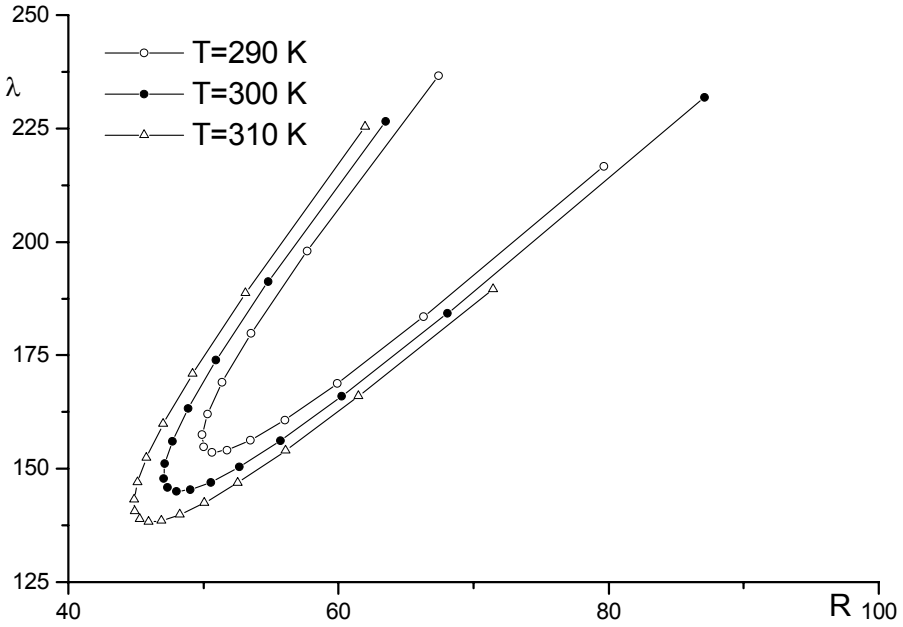

Fig. 3. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different absolute temperatures (T), in a bilayer of hydrophobic core thickness $2h = 48$ Å.

Table 3

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on absolute temperature in *K*

T (K)	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
290	[49.87 – 67.40 – 79.63]	157.47	[153.47 – 216.59 – 236.57]	50.62	[202 – 370 – 516]
300	[47.05 – 63.48 – 87.09]	147.81	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]
310	[44.84 – 61.96 – 71.43]	143.17	[138.21 – 189.53 – 225.30]	45.93	[164 – 312 – 414]

Lipid bilayers have the characteristics $2h = 48$ Å, $a_0 = 38.6$ Å^2, $K = 0.933 \cdot 10^{-11}$ N, $B = 5.36 \cdot 10^7$ N/m^2, $\gamma = 8 \cdot 10^{-4}$ N/m.

THE EFFECT OF VARIATIONS IN ELASTIC SPLAY PROPERTIES

The energy of molecule tilt compared to the direction perpendicular to the lipid bilayer is named splay energy and is characterized by the elastic splay constant *K*. This energy depends most probably on interactions between polar groups. The measured values of splay constant are confined within an interval $K \in [0.933 – 2.167] \cdot 10^{-11}$ N.

Because for lipid bilayers consisting of single chain molecules there is a critical splay constant value that separates two dependency modes between *R* and λ, we have chosen 4 values of the splay constant in arithmetic progression, as shown in column I of Table 4.

As depicted in Table 4 and Fig. 4, there is no critical value of *K* for lipid bilayers composed of double chain molecules. The increase in elastic constant shifts the interval of *R* towards higher values. This shift is proportional to the increase in elastic constant. Interestingly, if the constant increases from $0.933 \cdot 10^{-11}$ N up to $2.167 \cdot 10^{-11}$ N, the effect on the perturbation area radius is the same as in the case of an increase in bilayer thickness from $2h = 48$ Å to 52 Å.

THE EFFECT OF ELASTIC COMPRESSION CONSTANT

For double chain lipid bilayers that do not contain solvent, the experimental value of the compression constant is equal to $B = 5.36 \cdot 10^7 \cdot$N/m^2, while for lipid bilayers that contain solvent $B = 5.75 \cdot 10^4$ N/m^2. Generally, the increase in elastic compression constant decreases both the perturbation area radius and the distortion wavelength (Table 5). For values of B greater than $14.5 \cdot 10^7$ N/m^2 the dependence of wavelength on the perturbation area radius is not unilateral.

In Figure 5 one can notice that only the branch of large wavelength values is influenced by an increase in elastic compression constant. The width of the non-unilateral interval decreases with increases in *B*, thus for $B = B_c = 14.5 \cdot 10^7$ N/m^2 the upper branch of the curve representing the dependence of the wavelength on the perturbation area radius becomes parallel to the axis Oλ, which is equivalent to an independence of the wavelength on the radius *R*.

For values of B smaller than the critical value the dependence of the wavelength on the radius R becomes unilateral and the wavelength interval narrows with the decrease of B. There is also a case where the minimal radius becomes equal to zero. Thus, for B values smaller than the critical value there is a chance that a single molecule is brought with its polar head in the middle of the bilayer. In this case the flip-flop phenomenon is possible, because the molecule that has arrived in the middle of the lipid bilayer can pass in the opposite monolayer. For B values of about $12.5 \cdot 10^6$ N/m^2, the sensitivity of the wavelength dependence on the radius R is very low. When B is less than 10^5 N/m^2 the effect of a decrease in the compression constant is very weak.

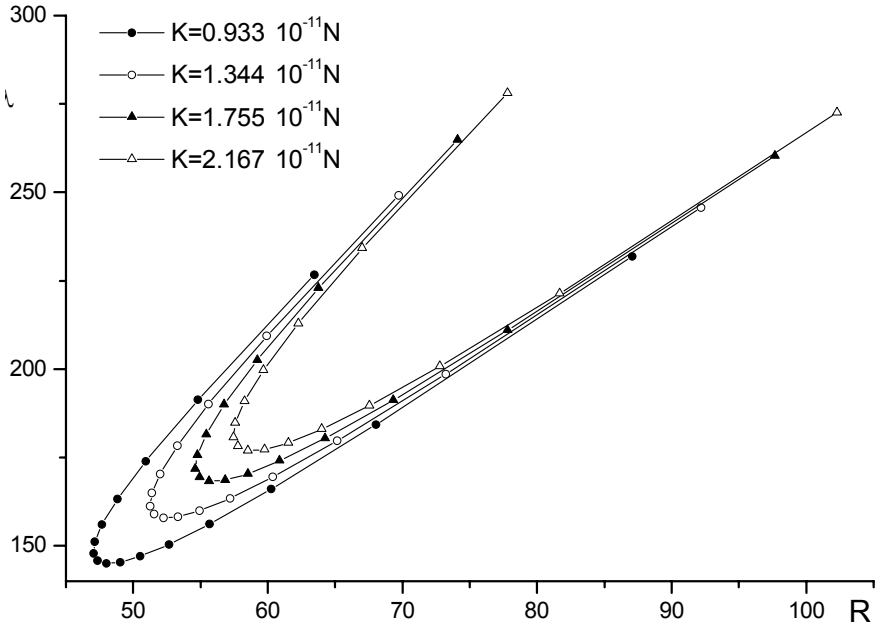

Fig. 4. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different values of the splay elastic constant

(K), in a bilayer of hydrophobic core thickness $2h$ = 48 Å.

Table 4

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on the elastic splay constant K

K	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
0.933	[47.05 – 63.48 – 87.09]	147.8	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]
1.344	[51.27 – 69.71 – 92.20]	161.2	[157.95 – 245.68 – 249.13]	52.24	[214 – 395 – 692]
1.755	[54.62 – 74.09 – 97.66]	171.8	[168.27 – 260.24 – 264.80]	55.65	[243 – 447 – 776]
2.167	[57.44 – 77.79 – 102.3]	180.6	[176.95 – 272.58 – 278.01]	58.52	[268 – 492 – 851]

Lipid bilayers have the characteristics $2h$ = 48 Å, a_0 = 38.6 Å^2, T = 300 K, $B = 5.36 \cdot 10^7$ N/m^2, $\gamma = 8 \cdot 10^{-4}$ N/m. Values in column I are in 10^{-11} N.

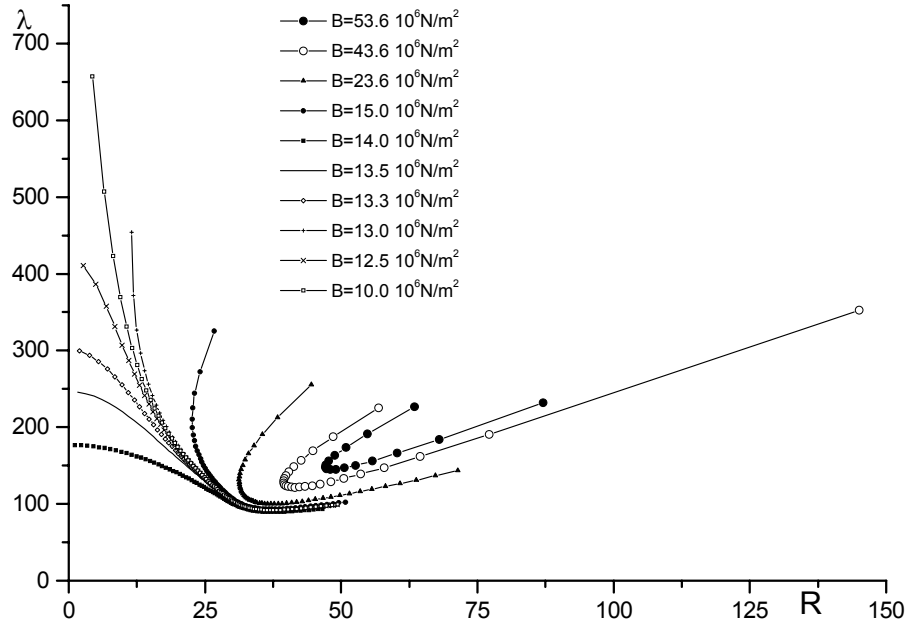

Fig. 5. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different values of the elastic compression constant (B), in a bilayer of hydrophobic core thickness $2h$ = 48Å.

Table 5

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on the elastic compression constant B

B	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
53.6	[47.05 – 63.48 – 87.09]	147.81	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]
43.6	[39.41 – 56.88 – 77.13]	129.97	[121.68 – 190.42 – 225.46]	41.54	[126 – 263 – 484]
23.6	[31.27 – 44.48 – 71.40]	131.58	[99.83 – 143.19 – 255.68]	37.06	[80 – 161 – 415]
15	[22.64 – 26.69 – 50.75]	210.77	[93.24 – 102.36 – 325.61]	37.32	[42 – 58 – 210]
14	[1.95 – 48.96]	299.24	[91.79 – 98.57 – 299.24]	36.83	[1 – 195]
13.5	[0.067 – 49.23]	420.91	[92.00 – 99.12 – 420.91]	36.91	[1 – 197]
13.3	[0.134 – 49.33]	839.59	[92.07 – 99.30 – 839.59]	36.94	[1 – 198]
13	[1.56 – 49.39]	454.15	[92.15 – 99.47 – 983.14]	36.96	[1 – 198]
12.5	[1.60 – 48.53]	245.81	[91.43 – 97.70 – 112.95]	37.26	[1 – 192]
10	[1.15 – 46.61]	176.96	[89.65 – 93.83 – 176.96]	37.11	[1 – 177]

Lipid bilayers have the characteristics $2h$ = 48 Å, a_0 = 38.6 Å^2, T = 300 K, $K = 0.933 \cdot 10^{-11}$ N, $\gamma = 8 \cdot 10^{-4}$ N/m. Values in column I are in 10^6 N/m^2.

The elastic compression constant can also change if the bilayer is composed of lysophospholipids instead of double chain lipids.

THE EFFECT OF SURFACE TENSION

The surface tension contant describes interactions at the separating surface between two different non-mixing media. It obviously depends on the composition of the two media in the vicinity of the interface. For lipid bilayers the surface tension coefficient depends on the pH of the medium adjacent to the bilayer. Experimental values are ranging between 10^{-8}·N/m and $5 \cdot 10^{-2}$ N/m. In the present study we have chosen three values of γ: $2.5 \cdot 10^{-4}$ N/m, $8 \cdot 10^{-4}$ N/m, $15 \cdot 10^{-4}$ N/m. As can be noticed in Table 6 and Figure 6, surface tension exerts a weak influence on the conditions for transmembrane pore formation.

Fig. 6. – Dependence of the bilayer distortion wavelength on the radius of the perturbation area caused by thermal group motion, at different values of the surface tension coefficient (γ), in a bilayer of hydrophobic core thickness $2h$ = 48 Å.

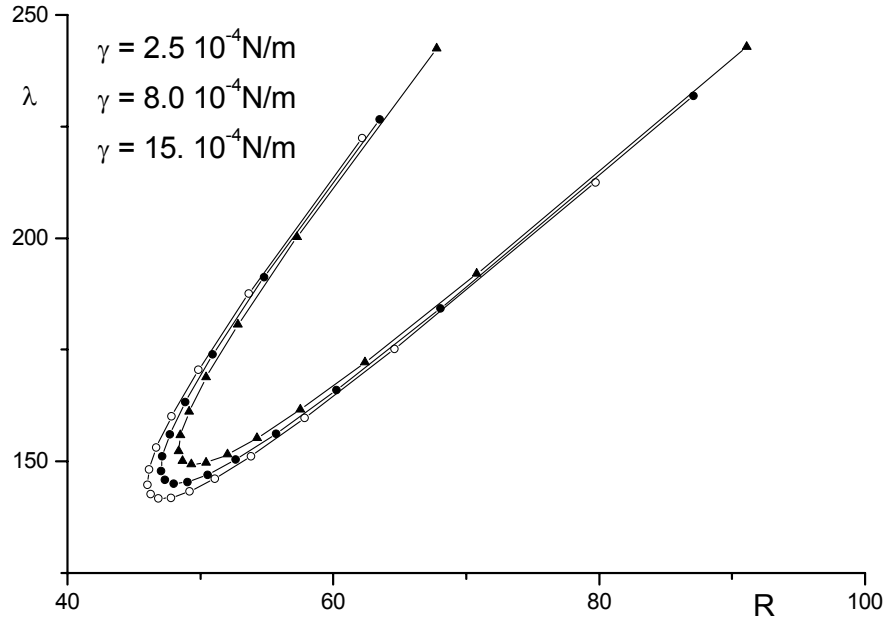

Table 6

Significant values of perturbation area radius, distorted surface wavelength, and number of molecules in the area, depending on the surface tension

γ	$[R_{min} - R_{2:1} - R_{max}]$ (Å)	λ_{ox} (Å)	$[\lambda_{min} - \lambda_{2:1} - \lambda_{max}]$ (Å)	R_{ox} (Å)	$N_{min} - N_{2:1} - N_{max}$
2.5	[46.02 – 62.18 – 79.72]	144.79	[141.72 – 212.51 – 222.34]	46.85	[172 – 314 – 517]
8	[47.05 – 63.48 – 87.09]	147.81	[144.96 – 226.62 – 231.87]	47.99	[180 – 328 – 617]

15	[48.37 – 67.79 – 91.08]	152.12	[149.23 – 242.42 – 242.81]	49.33	[190 – 374 – 675]

Lipid bilayers have the characteristics $2h = 48$ Å, $a_0 = 38.6$ Å^2, $T = 300$ K, $K = 0.933 \cdot 10^{-11}$ N, $B = 5.36 \cdot 10^7$ N/m^2, $\gamma = 8 \cdot 10^{-4}$ N/m. Values in column I are in 10^{-4} N/m.

CONCLUSIONS

In this work we have proven that thermal energy of molecules involved in thermal group motion can produce lipid bilayer distortions large enough to generate transmembrane pores. Meanwhile, we have studied the effects of structural properties (bilayer thickness, transversal area of the polar group), of temperature and of properties depending on inter- and intramolecular interactions (molecular axis tilt, compression and interface interactions) on the size of perturbation and the shape of the bilayer. The results presented herein pertain only the case where the entire thermal movement energy of lipid molecules involved in group motion is used to distort the lipid bilayer. As shown in Tables 1 – 6 and in Figures 1 – 6, there is a minimal and a maximal value of the group motion area that can generate pores. The existence of the minimal value is understandable, because the energy required for bilayer distortion can be provided by a minimal number of lipid molecules that, obviously, occupy a certain area. The maximal value has only a phenomenological explanation: the molecules within a very large area cannot be perfectly synchronous in their movement perpendicular to the bilayer.

The flip-flop phenomenon, i.e. the passage of a single molecule from one monolayer to the other, is possible only for very small values of the elastic compression coefficient. In other terms, the flip-flop phenomenon is possible for double chain bilayers containing a solvent, or in any other situation where the compression constant is small (it is highly probable in lipid bilayers composed of lysophospholipids).

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