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PORE FORMATION IN THE STRETCHED LIPID VESICLES

DUMITRU POPESCU[1] , ALIN GABRIEL POPESCU[2]
[1]Institute of Mathematical Statistics and Applied Mathematics, Romanian Academy of Science, Bucharest, ROMANIA,
popescu1947@yahoo.com
[2] Department of Computer Sciences, IT CORE SRL, Bucharest, ROMANIA

Abstract. *In this paper I have studied the transbilayer pore formation in a stretched lipid vesicle. The pore appearance in spherical bilayers is a process more intricate then in plane lipid bilayers. Here we have studied the influence of the elastic stretching of the membrane on the pore appearance. A minimum value of the spherical surface extension corresponding to a membrane stretching is required in order to open transbilayer pore. Also, we have calculated the energetic barrier for a pore opening and closing. The stability of the liposome with a pore on it was analysed.*
Keywords: *Stretched vesicle, energetic barriers, pore opening, pore closing*

1. INTRODUCTION

The passage of molecules, or genes, through a cellular membrane is a clue problem of drug delivery. The transbilayer pore formation is a way to increase the permeability of lipid bilayers. Some pores can appear due to structural and dynamic properties of lipid bilayers. Usually these pores are named stochastic pores [3]. The thickness fluctuations caused by thermal motion of lipid molecules superpose on local variations of the bilayer thickness which already exist due to selective association of phospholipids [1, 2] creating favourable conditions for pores appearance [3]. The fluctuation of the polar head groups density on the bilayer surface following the thermal motion produces local superficial defects. The neighboring lipid molecules rotate their polar heads such as to cover the to avoid the contact between hydrophobic chains and water. There are a lot of theoretical papers regarding the pores formation especially in plane lipid bilayers [4, 5].
The theoretical approach of transbilayer stochastic pores formation in lipid vesicles is more difficult because of their shape fluctuations [6]. Pores may be formed as a result of membrane expansion. In this case membrane tension change with vesicle expansion, what isn't the case at the black lipid membrane where the membrane tension is constant due to the Langmuir-Blodgett plateau, which is a lipid molecules reservoir.
In this paper, we have made a theoretical study of the pore formation across a vesicle membrane which is tensed by the osmotic pressure. We had in mind that the open of a pore is an important event for pulsatory liposome running.

2. THE CRITICAL EXPANDED SURFACE

At high pressure, the thermal undulations of lipid membrane can be ignored, and the membrane energy is due to elastic stretching.
Let us consider a vesicle into unstretched state. This is an equilibrium state and is characterized by $\sigma = 0$.
According to Hooke law, the membrane elastic energy is:

$$W = K\frac{(\Delta S)^2}{2} \tag{1}$$

where K is the two-dimensional stretching modulus and $\Delta S = 4\pi\left(R^2 - R_0^2\right)$, R_0 being the radius of the vesicle in the equilibrium state.
If the vesicle bilayer is stretched beyond the elastic limit, a pore is nucleated.
The pore appearance will lower the expansion surface, ΔS and consequently will decrese the elastic membrane energy, but contributes an additional energy due to the monolayers bending so that the lipid molescules head groups to cover the panza?? of the pore.

We consider a pore of area A is formed in a membrane initially expanded by ΔS. The elastic energy of such a membrane with a pore is:

$$W(R,r) = K\frac{(\Delta S - A)^2}{2} + 2\sqrt{\pi A}\,\gamma = K\frac{\left[4\pi\left(R^2 - R_0^2\right) - \pi r^2\right]^2}{2} + 2\pi r\gamma \tag{2}$$

where r is the pore radius and γ is the pore line tension.
The membrane surface tension is:

$$\sigma = \frac{\partial W}{\partial(\Delta S)} \tag{3}$$

According to formula (3) having in mind formula (1) the membrane surface tension is:

$$\sigma(R) = 4\pi K\left(R^2 - R_0^2\right) \tag{4}$$

for a stretched membrane without pore, and

$$\sigma(R,r) = K\left[4\pi\left(R^2 - R_0^2\right) - \pi r^2\right] \tag{5}$$

for a stretched membrane with pore on it.
The result of competition between the two terms from the equation (2) determines two different behaviours of the vesicle which depend on the initial value of the membrane expansion, ΔS.
The dependence of the membrane energy for different values of ΔS are presented in fig. 1.

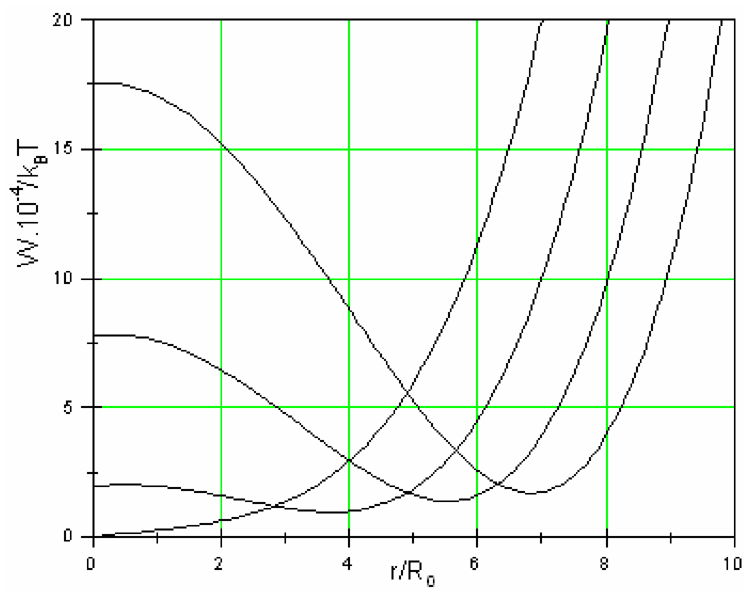

Figure 1. The elastic energy of the vesicle of initial radius $R_0 = 20$ µm as a function of pore radius, for different stretching degrees. The curves correspond rom bottom to top for: $R/R_0 = 1$; $R/R_0 = 1.002$; $R/R_0 = 1.004$; $R/R_0 = 1.006$. One observes that the greater the membrane expansion, the deeper the minimum on the energy curve.

Depending on the expansion size of ΔS, we can have two cases:

If ΔS is
- small, the membrane energy increases monotonously with the pore radius and the pore formation is unfavourable (the curve 1);
- greater than a critical value, on the energy curve appears. This means the formation of a pore in the membrane is possible (curves 2 - 4)

If a vesicle has an initial expansion ΔS, when a pore is nucleated, the vesicle bilayer chooses its final state so as to minimize its energy.

From the condition to minimize the energy function given by relation (2) with regard to ΔS, one find:

$$\Delta S = \pi r^2 + \frac{\gamma}{K r} \tag{6}$$

In fig.2 was represented the dependence of ΔS as a function of pore radius (r > 0) for which the vesicle energy has a minimum value.

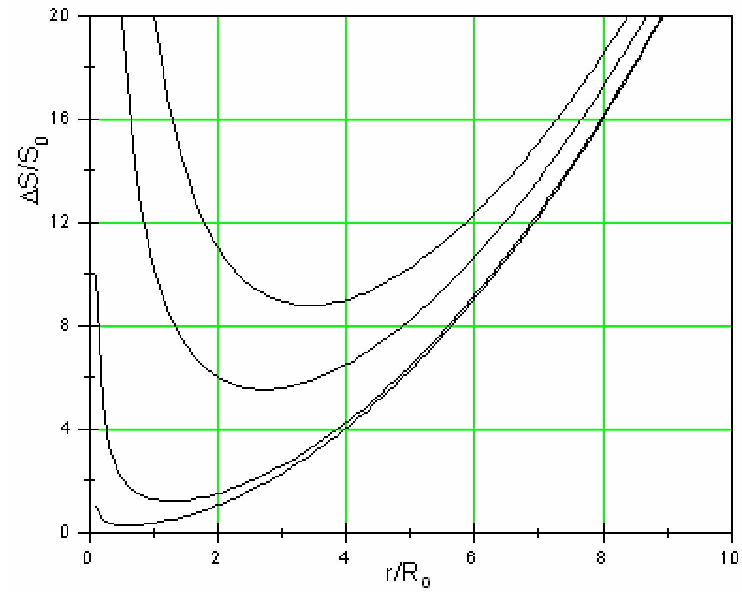

Figure 2. The vesicle expansion ΔS as a function of pore radius, for which the energy for the opening of a pore of radius r, is minimized. The curves have been drawn, from top to down, for vesicles with initial radius: R_0 = 0.1 µm; R_0 = 0.2 µm; R_0 = 2 µm; R_0 = 20 µm.

From this graphic we can see that there is a critical value of ΔS, marked with $\Delta\overline{S}$ for which the energy has the smallest value. This critical value of vesicle expansion, $\Delta\overline{S}$, corresponds to a transmembrane pore with the smallest radius, noted with $\bar{r}$.
Starting with the function ΔS(r) we can easily find the critical values of both vesicle expansion and pore radius:

$$\bar{r} = \sqrt[3]{\frac{\gamma}{2\pi K}} \tag{7}$$

$$\Delta\overline{S} = 3\pi\sqrt[3]{\left(\frac{\gamma}{2\pi K}\right)^2} \tag{8}$$

Between the smallest value of membrane expansion for which a transmembranar pore formation is possible, and the area of this pore there is a simple relation:

$$\Delta\overline{S} = 3\overline{A} \tag{9}$$

or an equivalent relation between their radius:

$$\bar{r}^2 = \frac{4}{3}\left(\overline{R}^2 - R_0^2\right) \tag{10}$$

We must retain from relation (10) that the critical pore radius depends on the initial size (R_0) of lipid vesicle.

Table.1.Critical values of the extended surface, $\Delta\overline{S}$, and of the pore radius, $\bar{r}$, for liposomes of four initial sizes

$R_0\times10^{-3}$ (nm)	$\Delta\overline{S}\times10^{-4}$ (nm^2)	$\bar{r}$ (nm)
20	63.3290762	317.480
2	2.939491	68.399
0.2	0.136440	14.736
0.1	0.054147	9.269

In order to forme a pore on a membrane extensed with ΔS, the membrane must overcome an enegetic barrier. We define the energy barrier for pore formation and note it with, $\mathbf{W_{bo}}$, as the difference between the maximum energy W of the membrane and the energy at point r = 0, when the pore is absent. It is equal with:

$$W_{bo}(r,R,R_0) = W(R,r) - W(R,0) = \frac{K\pi^2}{2}\left[r^4 - 8r^2\left(R^2 - R_0^2\right)\right] + 2\pi\gamma r \tag{11}$$

The initial energetic barrier of the pore formation corresponding to the membrane critical expansion, $\Delta\overline{S}$, is equal to:

$$\overline{W}_{bo} = W(\overline{R},\bar{r}) - W(\overline{R},0) = \frac{3}{4}\sqrt[3]{\frac{\pi^2\gamma^4}{2K}} \tag{12}$$

Also, we define the energy barrier for to close the pore as the difference between the maximum and minimum values of energy function. We note the energy barrier for close the pore with ΔW_{bc}.

3. ENERGETIC BARRIER FOR THE PORE CLOSING

So, in order to determine the energetic barrier for the pore close, we must calculate minimum and maximum values of the energy function W(R,r) as a function of the pore radius. With other words we must solve the equation:

$$r^3 - a\,r + b = 0 \qquad \textbf{(13)}$$

The leftt member of the equation (13) is the first derivate of W(R,r) as function of pore radius, r.
The coefficients, a and b, have the following meanings:

$$a = 4\left(f^2 - 1\right)R_0^2 = \frac{\left(f^2 - 1\right)S_0}{\pi} = \frac{\Delta S}{\pi}\,; \qquad b = \frac{\gamma}{\pi K} \qquad \textbf{(14)}$$

We can easily see that if:

$$a > \frac{3}{2}\sqrt[3]{2b^2} \qquad \textbf{(15)}$$

the equation (13) has three real solutions. We note with r_1 and r_2 the equation solutions for which the energy function has their maximum value and minimum value, respectively.
The two solutions accomplish the conditions:

$$0 < r_1 < \sqrt[3]{\frac{b}{2}}\,; \qquad r_2 > \sqrt[3]{\frac{b}{2}} \qquad \textbf{(16)}$$

With these notations, the energetic barrier for the pore close is :

$$\Delta W_{bc} = W(r_1) - W(r_2) \qquad \textbf{(17)}$$

After long and difficult calculations one obtains:

$$\Delta W_{bc} = \frac{2\sqrt{3}\pi^2}{3} a^2 K \cos\left(\frac{\pi}{6} + \frac{\alpha}{3}\right)\left[\frac{3}{2}\frac{b}{a}\sqrt{\frac{3}{a}} - \cos\left(\frac{\pi}{3} - \frac{\alpha}{3}\right)\right] \qquad \textbf{(18)}$$

where

$$\cos\alpha = -\frac{3}{2}\frac{b}{a}\sqrt{\frac{3}{a}} \qquad \textbf{(19)}$$

Table.2. The energetic barriers for opening and closing of a transbilayer pore formed on vesicle of radius R_0 = 2μm for different degree of surface stretching measured in k_BT units.

R/R_0	1.000	1.0001	1.0002	1.0004	1.0006	1.0008	1.0010
ΔE_{max}	-	-	-	-	2.1528	1.6146	1.2934
ΔE_{op}	-		-	-	2.1528	1.6146	1.2934
$\Delta\overline{E}_{min} \times 10^{-2}$	-	-	-	-	-1.6493	-12.3090	-27.2859
$\Delta E_{cls} \times 10^{-2}$	-	-	-	-	3.8071	13.9236	28.5793

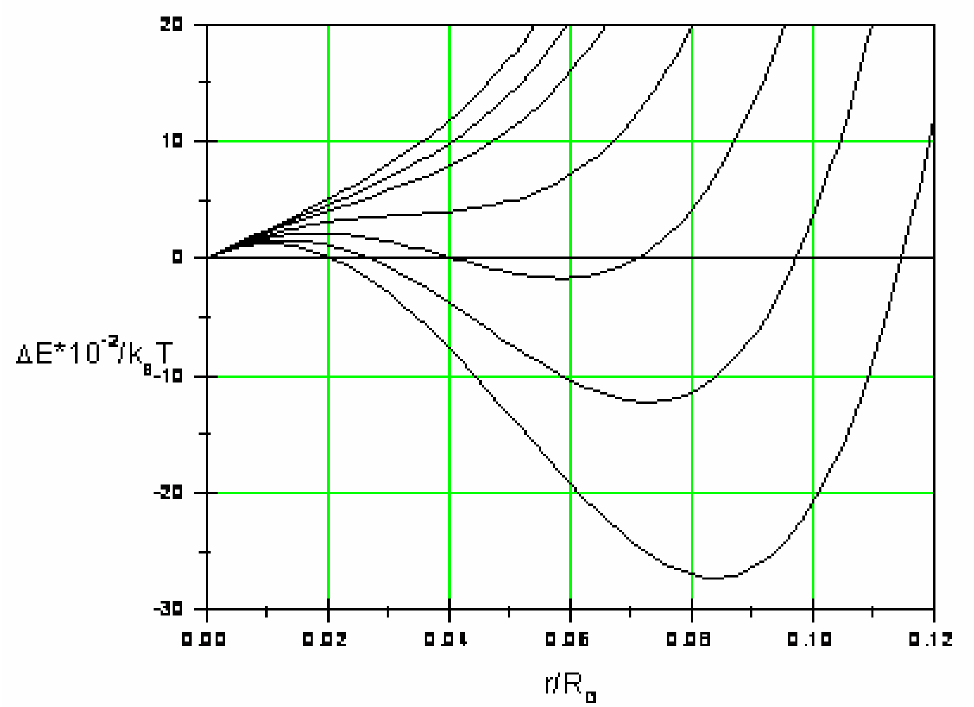

Figure 3. The energy $\Delta E_{bo}(R,r)$ necessary to open a pore of radius r in lipidic vesicle of radius R_0 =2 μm, for different degree of membrane stretching. The vesicle membrane stretching is described by the ratio R/R_0. The graphs, from top to down, were sketched for: $R/R_0 = 1$; $R/R_0 = 1.0001$; $R/R_0 = 1.0002$; $R/R_0 = 1.0004$; $R/R_0 = 1.0006$; $R/R_0 = 1.0008$; $R/R_0 = 1.001$.

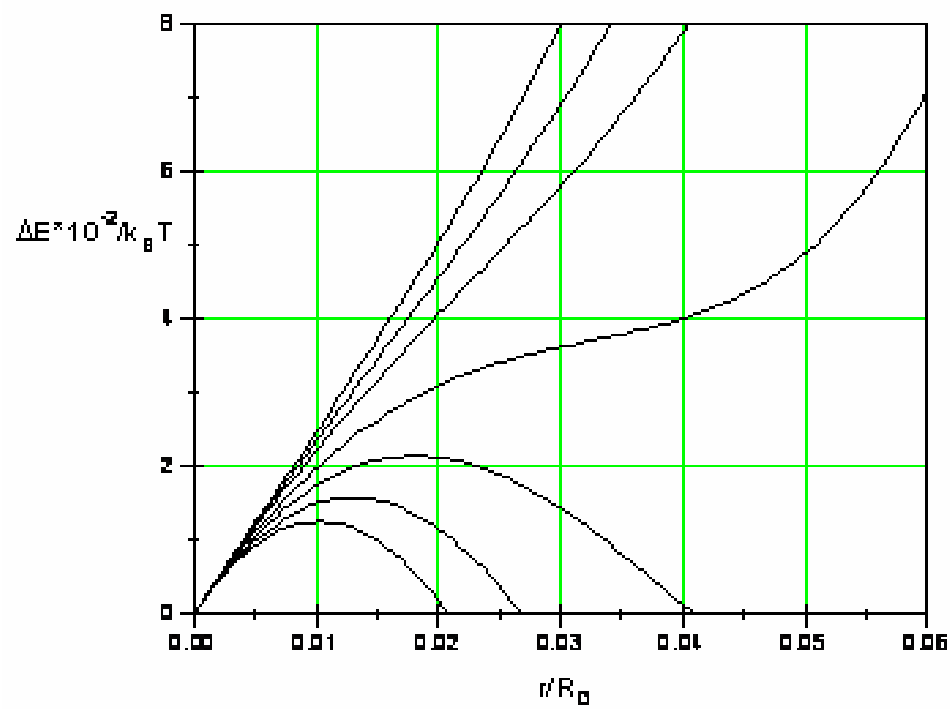

Figure 4. A detailed sketch o the first part of the curves drawn in fig. 3. In order to see the existence of a maximum value of the membrane energy change due to the pore appearance.

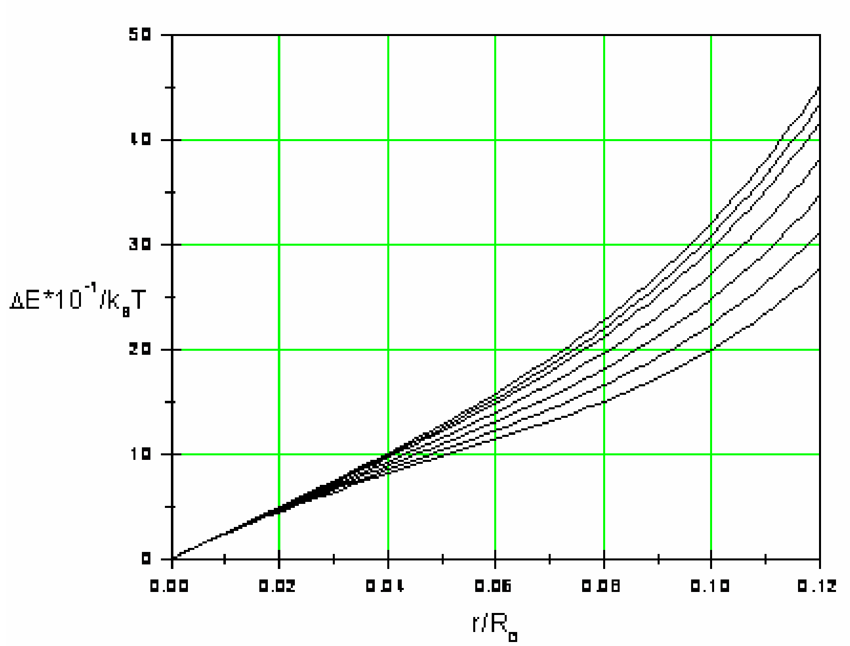

Figure 5. The energy $\Delta E(R.r)$ necessary to open a pore of radius r in lipidic vesicle of radius R_0 =0.1 μm, for different degree of membrane stretching. The vesicle membrane stretching is described by the ratio R/R_0. The graphs, from top to down, were sketched for: $R/R_0 = 1$; $R/R_0 = 1.0001$; $R/R_0 = 1.0002$; $R/R_0 = 1.0004$; $R/R_0 = 1.0006$; $R/R_0 = 1.0008$; $R/R_0 = 1.0012$.

4. DISSCUTIONS AND CONCLUSIONS

The pore appearance in lipid unilamelar vesicle is has two characterisctics. Firstly, there is a minmum value of membrane extension (which corresponds to membrane stretching) for which a pore can appear such as the membrane bearing this pore to be stable. This value is given and the radius of corresponding pore are given by equation (8) and (7) respectively. The second characteristic is the existence of the energetic barrier for pore closing. The existence of closing energetic barrier depends by the vesicle size in unstretching state.

The pore opening is an essential clue for the working of a pulsatory liposome, which may be regarded as a two stroke bioengine.

REFERENCES

[1] POPESCU, D., VICTOR, G., *Association Probabilities Between the Single–Chain Amphiphiles Into A Binary Mixture in Plan Monolayers ,* Biochim. Biophys. Acta, **1030(2)**, pp. 238–250, 1990.

[2] POPESCU, D., *Association Probabilities Between the Single–Chain Amphiphiles Into A Binary Mixture in Plan Monolayers (II),* Biochim. Biophys. Acta, **1152**, pp. 35–43,1993.

[3] POPESCU, D., RUCAREANU, C., VICTOR, G., *A Model for the Appearance of Statistical Pores in Membranes Due to selfoscillations*, Bioelectrochem. Bioenerg., **25**, pp. 91–103, 1991

[4] POPESCU, D., VICTOR, G., *The Transversal Diffusion Coefficient of Phospholipid Molecules Through Black Lipid Membranes,* Bioelectrochem. Bioenerg., **25**, pp. 105–108, 1991.

[5] POPESCU, D., RUCAREANU, C., *Membrane selfoscillations model for the transbilayer statistical pores and flip–flop diffision*, Mol. Cryst. Liquid Cryst., **25**, pp. 339–348, 1992.

[6] POPESCU, D., ION, S., POPESCU, A. I., MOVILEANU, L., *Elastic properties of bilayer lipid membranes and pore formation.* In Planar Lipid Bilayers (BLMs) and Their Applications, H. Ti Tien and A.Ottova, editors. Elsevier Science Publishers, Amsterdam, **3**, pp. 173–204, 2003.