CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 6, (2001) pp 169 – 171

Received 5 February 2001
Accepted 14 May 2001

Short Communication

ON STABILITY OF CIRCULAR HOLE IN MEMBRANE BILAYER

MIHA FOŠNARIC[1], VERONIKA KRALJ-IGLIC[2],
HENRY HÄGERSTRAND[3] and ALEŠ IGLIC[1]
[1]Laboratory of Applied Physics, Faculty of Electrical Engineering, University of Ljubljana, SI-1000, Ljubljana, Slovenia, [2]Institute of Biophysics, Faculty of Medicine, University of Ljubljana, SI-1000, Ljubljana, Slovenia, [3]Department of Biology, Åbo Akademi University, FIN-20520, Åbo/Turku, Finland

Abstract: It was observed recently that nonionic surfactant octaethyleneglycol dodecylether ($C_{12}E_8$) decreases threshold for irreversible electroporation in membrane bilayers. In accordance, it is shown theoretically in this work that anisotropic $C_{12}E_8$ membrane inclusions may stabilize circular hole in a flat membrane segment.

Key Words: Surfactant, Electroporation, Membrane Free Energy, Anisotropic Inclusion, Bilayer

INTRODUCTION

By applying an electric field across the lipid membrane bilayer, the formation of transient pores in the membrane can be achieved. This phenomenon is known as electroporation. Many experimental studies have been made with electroporation in various conditions [1,2,3]. Recently, it has been reported that in planar lipid bilayers [4] and in transformed Chinese hamster lung fibroblast cells [5] nonionic surfactant octaethyleneglycol dodecylether ($C_{12}E_8$) decreases threshold for irreversible electroporation. Within the standard bending elasticity models of bilayer membranes [6] the equilibrium state of a circular hole in membrane bilayer (Fig.1), corresponding to the minimal bending energy of the membrane segment, cannot be satisfactorily explained. Therefore it is of interest to understand which additional mechanism beside the minimization of the membrane bending energy might take place in the shape stabilization of a hole in membrane bilayer.

$C_{12}E_8$ molecules in membrane bilayers are thought to induce anisotropic membrane inclusions [7]. Membrane inclusions are dynamic co-operative units composed of the embedded $C_{12}E_8$ molecule and adjacent phospholipid molecules, which are significantly distorted due to the presence of the embedded $C_{12}E_8$ molecule [7]. Therefore additional contribution of the $C_{12}E_8$ membrane inclusions to the membrane free energy was proposed recently [7].

In this work we consider a circular segment of a planar lipid bilayer with a circular hole in the center (Fig.1). The minimum of the membrane free energy which is the sum of the membrane bending energy and the contribution of the $C_{12}E_8$ membrane inclusions is searched for, to obtain the equilibrium state of a circular hole in a planar membrane bilayer segment.

THEORETICAL MODEL

To obtain the equilibrium shape of the hole in the planar lipid bilayer at given area A of the segment, we minimize the membrane free energy $F = W_b + F_i$, consisting of the contribution of the membrane bending energy (W_b) and the contribution of the $C_{12}E_8$ induced membrane inclusions (F_i). Membrane bending energy (W_b) can be written as [6]

$$W_b = \frac{1}{2} k_c \int 4\overline{C}^2 \mathrm{d}A + \frac{1}{2} k_G \int C_1 C_2 \mathrm{d}A, \tag{1}$$

where k_c is the local bending modulus, k_G is the Gaussian bending modulus, $\overline{C} = (C_1 + C_2)/2$, C_1 and C_2 are the principal membrane curvatures and dA is the infinitesimal membrane area element. In this work it is taken that $k_G = -2k_c$. The contribution of the nonlocal bending [6] is not considered in Eq. (1). The contribution of the $C_{12}E_8$ inclusions to the membrane free energy is [7,8]:

$$F_i = -NkT \ln\left(\frac{1}{A} \int e^{-x(\overline{C}-\overline{C}_m)^2/2kT - (x+x^*)(\hat{C}^2+\hat{C}_m^2)/4kT} \, \mathrm{I}_0\left((x+x^*)\hat{C}\hat{C}_m/2kT\right) \mathrm{d}A \right), \tag{2}$$

where x and x^* are the constants representing the strength of the interaction between the $C_{12}E_8$ inclusion and the membrane continuum, $\hat{C} = (C_1 - C_2)/2$, $\overline{C}_m = (C_{1m} + C_{2m})/2$, $\hat{C}_m = (C_{1m} - C_{2m})/2$, C_{1m} and C_{2m} are the principal curvatures of the intrinsic shape of the $C_{12}E_8$ inclusion, N is the total number of the $C_{12}E_8$ inclusions in the lipid bilayer, I_0 is the modified Bessel function, k is the Boltzmann constant and T is the temperature. In this work we take that $\boldsymbol{x} = \boldsymbol{x}^*$.

Consider the circular segment of the double-layered membrane with the circular hole of radius R (Fig. 1). The area A of the membrane segment is taken to be constant, so when the size of the hole is changed, the radius l of the segment must change accordingly. In our model both membrane layers are flat in the region $l > x > R$ and bend toward each other in the region $R > x > R - r$, forming an inner half of the torus with the larger radius R and the smaller radius r (Fig. 1). The principal membrane curvatures C_1 and C_2 are zero in the region $l > x > R$, where the membrane is flat. In the region $R > x > R - r$ one of the principal membrane curvatures is constant, $C_1 = 1/r$, and the other is $C_2 = (x - R)/rx$.

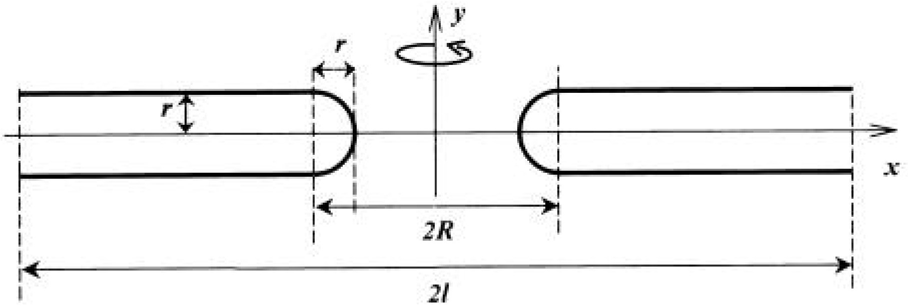

Fig. 1. Cross section of the geometrical model of the circular membrane segment with a hole in the center. The whole segment and the hole are assumed to have rotational symmetry around the *y*-axis. Here *R* is radius of the hole and *2r* is the distance between the surfaces of both layers at the flat part of the segment.

In the following analysis, dimensionless quantities are introduced. The unit of length is chosen to be the radius of the membrane segment without the hole: $l_0 = (A/2\boldsymbol{p})^{1/2}$. The membrane free energy F is normalized relative to the sphere: $f = F/8\boldsymbol{p}k_c = w_b + f_i$, where $w_b = W_b/8\boldsymbol{p}k_c$ and $f_i = F_i/8\boldsymbol{p}k_c$. Two new parameters (*e* and *k*) are introduced: $\boldsymbol{e} = NkT/8\boldsymbol{p}k_c$ and $\boldsymbol{k} = \boldsymbol{x}/kTl_0{}^2$. For fixed values of parameters *r, e, k* and for fixed C_{1m} and C_{2m}, the relative membrane free energy is only a function of the radius of the hole, $f = f(R)$, and we can search for such $R = R_{\min}$, that gives the minimal relative membrane free energy, $f_{\min} = f(R_{\min})$.

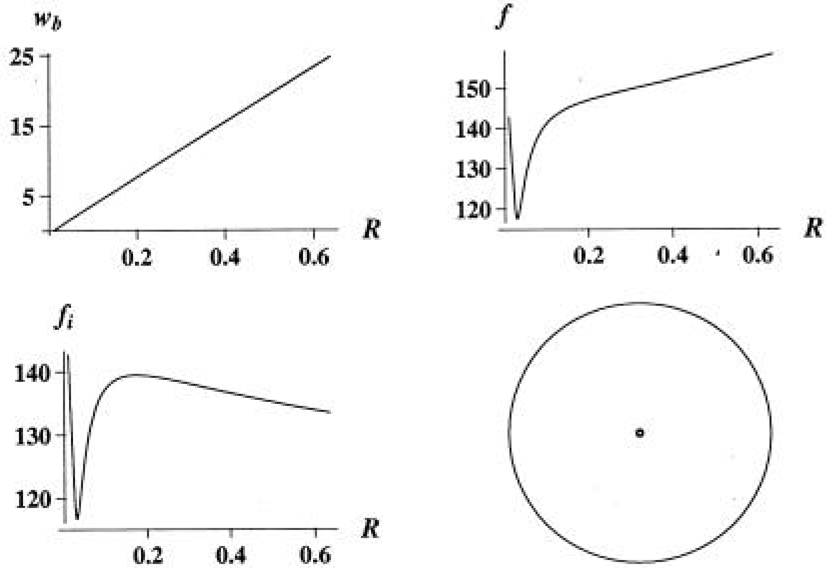

Fig. 2. The membrane bending energy w_b, the relative free energy of the inclusions f_i and the relative membrane free energy $f = w_b + f_i$ as functions of the relative radius of the hole. The membrane free energy reaches minimum at $R_{min} = 0.029$. The corresponding membrane segment with a hole with radius R_{min} is also shown. The values of parameters are: $e = 10$, $k = 0.01$, $r = 0.01$, $C_{1m} = -50$ and $C_{2m} = 100$. The values of model parameters are within the estimated range [6,8].

RESULTS AND DISCUSSION

The relative membrane free energy *f* as a function of the relative radius of the hole *R* was calculated (Fig.2). It can be seen in Fig.2 that the relative membrane bending energy w_b as a function of *R* does not have a minimum for a hole with radius *R* larger than *r* (the smallest possible hole in our model). This was of course expected, because membrane bending energy is minimal when the membrane surface is completely flat and without holes. Any anomaly in the flat membrane surface just increases its bending energy. On the other hand, the contribution of $C_{12}E_8$ inclusions to the relative membrane free energy f_i has a minimum for a specific size of the hole (Fig.2). This also results in a minimum of a total relative membrane free energy *f* of the segment.

On the basis of the results presented in this work it can be concluded that anisotropic $C_{12}E_8$ inclusions can stabilize the circular hole in a flat membrane segment. This is in accordance with the previous experimental results [4,5] which show that in planar lipid bilayers $C_{12}E_8$ molecules decrease threshold for irreversible electroporation. The assumption that $C_{12}E_8$ membrane inclusions are anisotropic (i.e. that they have nonzero intrinsic curvature deviator $\hat{C}_m$), is in accordance with the results of our previous studies [7]. Further experimental studies on membrane bilayers with inclusions of clearly anisotropic shape (i.e. dimeric amphiphiles [8]) would give a more detailed view on the influence of the intrinsic shape of the membrane inclusion on the efficiency of electroporation. In addition, the effect of $C_{12}E_8$ on electroporation should be compared to the effect of $C_{12}E_4$ which presumably does not form anisotropic membrane inclusions (Hägerstrand et al., unpublished results).

REFERENCES

1. Neuman E., Sowers A. E. and Jordan C. A. **Electroporation and electrofusion in Cell Biology**, Plenum Press, New York and London, 1989.
2. Janas T., Walinska K., Janas T. Electroporation of polyprenol-phosphatidylcholine bilayer lipid membranes. **Bioelectrochem. Bioenerg.** 45 (1998) 567-582.
3. Koronkiewicz S., Bryl K. Cholesterol-induced variations in fluctuations of the pores in bilayer lipid membrane. **Cell. Mole. Biol. Lett.** 4 (1999) 567-582.
4. Troiano G., Stebe K., Sharma V., Tung L. The electroporation of artificial planar POPC bilayers and the effects of $C_{12}E_8$ on its properties. **Biophys. J.** 74 (1998) 880-888.
5. Kandušer M., Miklavèiè D. Effect of polyoxyethylene surfactant $C_{12}E_8$ on electro-poration off cell line DC3F. 3rd International Conference on Bioelectromagnetism, Bled, 2000, 21-22.
6. Evans E. A., Skalak R. **Mechanics and Thermodynamics of Biomembranes**, CRC Press, Boca Raton, 1980.
7. Igliè A., Kralj-Igliè V., Bož iè B., Bobrowska-Hägerstrand M., Isomaa B., Hägerstrand H. Torocyte shapes of red blood cell daughter vesicles. **Bioelectrochemistry** 52 (2000) 203-211.
8. Kralj-Igliè V., Igliè A., Hägerstrand H., Peterlin P. Stable tubular microexovesicles of the erythrocyte membrane induced by dimeric amphiphiles. **Phys. Rev. E** 61 (2000) 4230-4234.