Cluster Buckling as a Result of Compression-bending Balance in Lipid Bilayer

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Abstract. A new approach supporting the possibility of cluster buckling during the main phase transition in the lipid bilayer is presented. The elastic energy is calculated via harmonic approximation which yields the potential minimum in the case of buckling cluster, if critical cluster size is achieved. The significance of this event for interbilayer interaction in multilamellar vesicles is discussed.

Key words: Lipid bilayer — Phase transition — Elastic properties — Cluster buckling

Introduction

The elastic properties of lipid bilayers may account for many interesting shape transformations of the vesicles, e.g. budding (Julicher and Lipowsky 1993), topological genus (Michalet and Bensimon 1995) and others, some of which are of physiological significance. They also play an important role at microscopic length scale in the lipid bilayers which exhibit a variety of phase transitions. The best known, main phase transition, has significantly different behaviour dependent on the vesicle configuration. Observed transition characteristics show much more cooperativity in the case of multilamellar vesicles compared to unilamellar vesicles (van Osdol et al. 1991). In our previous work (Marek 1995) it was demonstrated that this difference might be explained by cluster mediated interaction of neighbouring bilayers. The presented paper proposes details of the mechanism likely driving this interaction. It is well known that lipid bilayer surface expands during melting but how this affects melting of the individual cluster has not been studied so far in spite of its significance with respect to the bilayer phase transition.

The model

The average molecular area increases during phase transition between the gel and

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the fluid bilayer which is accompanied by mechanical stress at the cluster boundary. Essentially, this strain can be compensated for in three ways:

I. in-plane stress redistribution between gel and fluid regions of the bilayer at a constant total vesicle surface,

II. out-of-plane cluster buckling at a constant cluster-gel interface length,

III. liposome growth (both surface and interface expansion).

The following section deals with the first two possibilities in a quantitative manner through calculation of the bilayer mechanical energies. The last case will be analysed in more detail in the discussion section. The bilayer is assumed to be a continuous flat layer. The appropriateness of such a macro-approach has already been discussed in the literature (Hianik and Passechnik 1995) with the conclusion that continuum approximation is acceptable at the length scale of several molecules. Another problem is that bilayer thickness at smaller cluster sizes is comparable with the cluster linear dimension. Such objects can also bend as it was observed at the lamellar-hexagonal phase transition in the lipid bilayer. Mean-step at this transition is formation of fluid bilayer with periodically undulated surface (Bradshaw et al. 1989). The size and the curvature of this ripple are similar to those proposed from plane deformation of fluid cluster in the gel matrix. In consequence of the continuum approach, to simplify calculations, we used spherical shape approximation of the cluster surface.

I. *In-plane relaxation*

At a constant total vesicle surface area A, there is a mechanical equilibrium state given by fluid-gel compression balance. An excess area produced by the fluid-gel molecular areas mismatch will be diminished by redistribution of the mechanical stress through vesicle surface. Each region, fluid and gel, is partly compressed according to their elastic area compression modulus (Fig. 1a). The total compression energy of vesicle E_{I} in harmonic approximation (Cevc and Marsh 1987) is then given by

$$\begin{aligned} E_{\mathrm{I}} &= \frac{1}{2}\left(k_1\frac{(\Delta A_1)^2}{A_{01}} + k_2\frac{(\Delta A_2)^2}{A_{02}}\right) \\ \Delta A_1 &= A_1 - A_{01} \\ \Delta A_2 &= A_2 - A_{02} \\ A &= A_1 + A_2 \end{aligned} \tag{1}$$

where A_1, A_2 are the actual gel and fluid areas, respectively, and A_{01}, A_{02} are their equilibrium non-stressed values. The area compression moduli in the gel and fluid state are k_1 and k_2, respectively. Let assign $x \in (0,1)$ as the fluid region compression coefficient ($x = 1$ if only fluid region is compressed, $x = 0$ if only gel

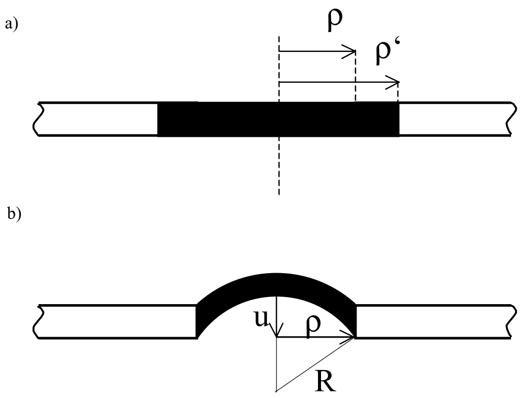

Figure 1. Schematic illustration of the cross-section of a lipid bilayer (dark region, fluid cluster; white, gel background; ρ, cluster radius; u, buckling distance; $1/R$, cluster surface curvature): a) cluster expansion; b) cluster buckling.

region is compressed) and ΔA as the total excess area, then

$$\begin{aligned} \Delta A_1 &= (1-x)\,\Delta A \\ \Delta A_2 &= x\,\Delta A \\ E_{\mathrm{I}}(x) &= \frac{(\Delta A)^2}{2}\left(\frac{k_1}{A_{01}}(1-x)^2+\frac{k_2}{A_{02}}x^2\right) \end{aligned} \tag{2}$$

The mechanical equilibrium state is given by the conditions

$$\left(\frac{\partial E_{\mathrm{I}}(x)}{\partial x}\right)_{A=\mathrm{const}} = 0, \qquad \frac{\partial A_1}{\partial x} = -\frac{\partial A_2}{\partial x} \tag{3}$$

which yields

$$\begin{aligned} x_{\mathrm{opt}} &= \frac{1}{1+\left(\dfrac{k_2}{k_1}\right)\left(\dfrac{A_{01}}{A_{02}}\right)} \\ E_{\mathrm{I}}(x_{\mathrm{opt}}) &= \frac{1}{2}\,k_2\,\frac{(\Delta A)^2}{A_{02}}\,x_{\mathrm{opt}} \end{aligned} \tag{4}$$

an optimal fluid region compression x_{opt} and the corresponding deformation energy $E_{\mathrm{I}}(x_{\mathrm{opt}})$. The foregoing statements can be expressed in terms of fluid and gel molecular areas a_{01} and a_{02}, respectively, using the following relations

$$\begin{aligned} A_{01} &= N_1\,a_{01} \\ A_{02} &= N_2\,a_{02} \\ \Delta A &= N_2\,(a_{02}-a_{01}) \\ N &= N_1+N_2 \end{aligned} \tag{5}$$

where N is the total and N_1, N_2 the gel and the fluid number of the molecules in the monolayer. Eq. (4) can be rewritten as a function of the fluid fraction n, using Eq. (5)

$$\begin{aligned} x_{\mathrm{opt}}(n) &= \frac{1}{1+\left(\frac{k_2}{k_1}\right)\left(\frac{a_{01}}{a_{02}}\right)\left(\frac{1}{n}-1\right)} \\ \varepsilon_{\mathrm{I}}(n) &= \frac{E_{\mathrm{I}}(x_{\mathrm{opt}})}{2N_2} = \varepsilon_{21}\, x_{\mathrm{opt}}(n) \\ n &= \frac{N_2}{N} \qquad \varepsilon_{21} = \frac{1}{4}\, k_2\, \frac{(a_{02}-a_{01})^2}{a_{02}} \end{aligned} \tag{6}$$

where $\varepsilon_{\mathrm{I}}(n)$, ε_{21} are the equilibrium deformation energy and the fluid-gel compression energy, respectively (per fluid molecule). Otherwise, the former is the case for optimal redistribution of compression between fluid and gel regions, and the latter when only the fluid phase is compressed.

II. *Out-of-plane relaxation*

The driving force of the cluster buckling is mechanical stress produced by the compression of the melted cluster in the gel matrix considering the area difference between the gel and fluid states. We will consider a circular cluster shape with radius ρ and spherical surface deformation with a radius of curvature R and buckling parameter u (Fig. 1b). The elastic energy per area unit of the cluster buckling E_{II} consists of two parts: area compression E_{comp} and 2D-bending E_{bend}, and is given by the following harmonic approximation (Cevc and Marsh 1987):

$$\begin{aligned} E_{\mathrm{II}} &= E_{\mathrm{comp}} + E_{\mathrm{bend}} \\ E_{\mathrm{comp}} &= \frac{1}{2}k_2\left(\frac{\Delta A(\rho,u)}{A_0}\right)^2 \\ E_{\mathrm{bend}} &= \frac{1}{2}k_{\mathrm{C}}\left[\frac{1}{R}+\frac{1}{R}\right]^2 = 2\,k_{\mathrm{C}}\left(\frac{1}{R}\right)^2 \end{aligned} \tag{7}$$

where $\Delta A(\rho,u) = A(\rho,u) - A_0$ and $A(\rho,u)$, A_0 are the actual cluster area and the fluid cluster area without mechanical stress, respectively; and k_{C} stands for the curvature elastic modulus. From the simple geometric relations (Fig. 1b),

$$\begin{aligned} R^2 &= (R-u)^2 + \rho^2 \\ \frac{1}{R} &= \frac{2\,u}{(\rho^2+u^2)} \\ A(\rho,u) &= 2\pi R u = \pi\left(\rho^2+u^2\right) \end{aligned} \tag{8}$$

Let n_m be the number of molecules in the cluster monolayer, then

$$\begin{aligned} A\left(\rho,0\right) &= n_\mathrm{m}\, a_{01} = \pi\rho^2 \\ A_0 &= n_\mathrm{m}\, a_{02} = \pi\rho^2 \frac{a_{02}}{a_{01}} \end{aligned} \tag{9}$$

Using Eq. (8) and Eq. (9),

$$\Delta A\left(\rho,u\right) = A\left(\rho,u\right) - A_0 = \pi\left[\left(1 - \frac{a_{02}}{a_{01}}\right)\rho^2 + u^2\right] \tag{10}$$

Hence, from Eqs. (7–10) the elastic energy per unit area is given by

$$E_\mathrm{II}\left(\rho,u\right) = \frac{1}{2}k_2\left[\left(\frac{\rho^2+u^2}{\rho^2}\right)\frac{a_{01}}{a_{02}} - 1\right]^2 + 8k_\mathrm{C}\left(\frac{u}{\rho^2+u^2}\right)^2 \tag{11}$$

and per fluid molecule

$$\varepsilon_\mathrm{II}\left(\rho,u\right) = a_{02}\, E_\mathrm{II}\left(\rho,u\right)/2 \tag{12}$$

The equilibrium buckling parameter u_opt follows from the condition

$$\left(\frac{\partial E_\mathrm{II}\left(\rho,u\right)}{\partial u}\right)_{\rho=\mathrm{const}} = 0 \tag{13}$$

which yields the minimum energy of cluster buckling per fluid molecule

$$\varepsilon_\mathrm{II}\left(\rho\right) = a_{02}\, E_\mathrm{II}\left(\rho,u_\mathrm{opt}\right)/2. \tag{14}$$

Results

The elastic energy profiles (models I, II) were calculated for the lecithin bilayer as functions of the cluster size (which reflects the number of molecules corresponding to the cluster radius ρ) and the buckling distance u, using the experimental values of elastic modulus $k_1 \approx 1 \cdot 10^{-20}$ JÅ^{-2} (DMPC ∼ 20 ℃, Hianik and Passechnik 1995), $k_2 = 0.14 \cdot 10^{-20}$ JÅ^{-2} (fluid lecithin bilayer, Cevc and Marsh 1987) and $k_\mathrm{C} = 4 \cdot 10^{-20}$ J (DMPC ∼ 26 ℃, Engelhardt et. al 1985) and average molecular areas $a_{02} = 68$ Å^2, $a_{01} = 40.8$ Å^2 for the fluid and gel phases, respectively. (DMPC = dimyristoyl phosphatidylcholine) Fig. 2a shows the dependence of the elastic energy versus buckling distance u at the cluster sizes 80, 120, 180, 240 and 300 molecules calculated from Eqs. (9), (11), (12). A series of local potential wells are observed. However, not all correspond to the equilibrium state. The optimal buckling parameter u_opt was calculated numerically from Eqs. (11), (13) in function of the cluster size (Fig. 3a). The size effect is well visible via a typical first order behaviour of the buckling parameter. The global energy minimum at a nonzero u exists above

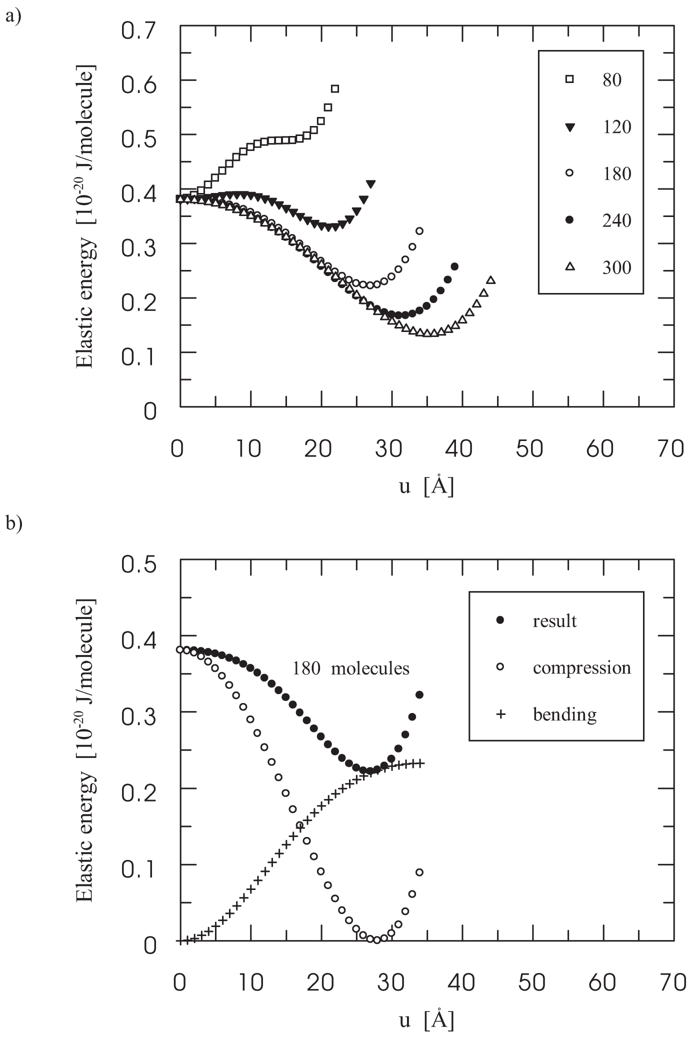

Figure 2. a) Dependence of the elastic cluster energy on buckling distance u at various cluster sizes (inset: the total number of molecules in the cluster). b) Demonstration of compression-bending balance at the cluster size of 180 molecules (individual components and the total elastic cluster energy are drawn).

a certain critical cluster size only. This limit is about 105 molecules for the used values of elastic parameters. The individual contributions to the total elastic energy of the cluster, compression and bending parts, are illustrated in Fig. 2b. The equilibrium energy of the cluster buckling was determined by the obtained values of u_{opt} using Eqs. (11), (14). The equilibrium in the case of the in-plane model

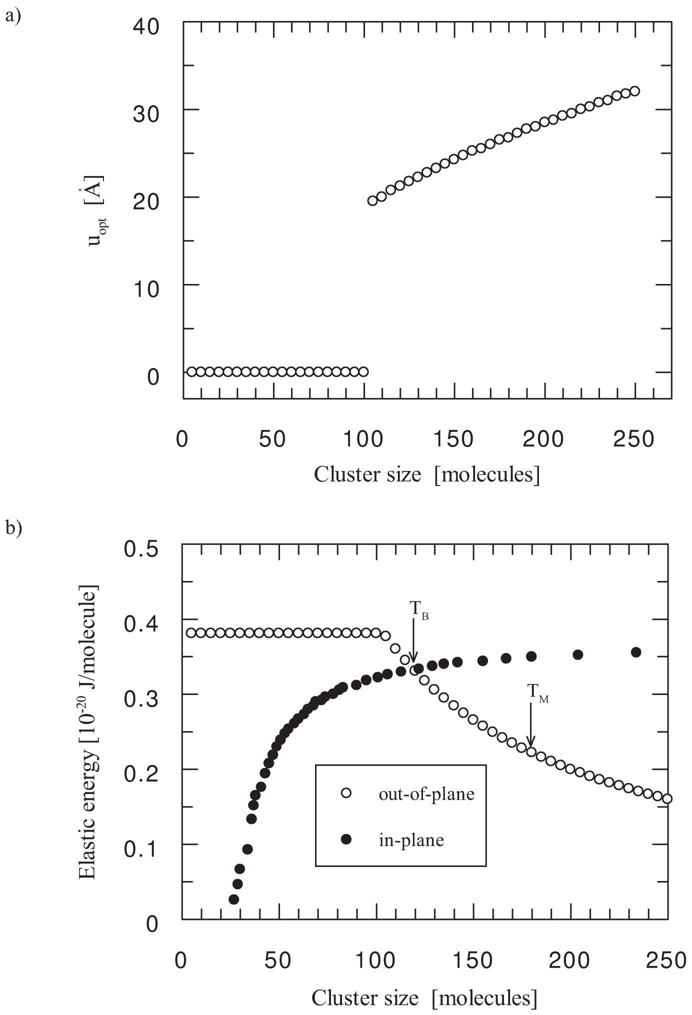

Figure 3. a) Dependence of the optimal buckling parameter u_{opt} on cluster size. b) Dependence of the optimal elastic energies per fluid molecule for the case of in-plane (I) and out-of-plane (II) relaxation on cluster size (T_{B} is the crossing point between compression and buckling regions, and T_{M} represents the main phase transition point).

(I, Eq. (6)) depends on the fraction melted $n(T)$. To obtain this, the experimental heat capacity data of high purity unilamellar DPPC vesicles (DPPC = dipalmitoyl phosphatidylcholine) measured at very low scan rates were used (van Osdol et al. 1991). Application of the deconvolution method (Freire and Biltonen 1978) on the excess heat yields the temperature dependence of the average cluster size $\sigma(T)$. In order to compare model I and II of the mechanical stress relaxation it is necessary to express Eq. (6) as a function of the cluster size using both foregoing functions. The corresponding curves are illustrated in Fig. 3b. Two significant points are shown, the chain melting transition temperature T_M and intersection point T_B of in- and out-of-plane curves. The saturation values of both curves are the same: $\varepsilon_\mathrm{I}(x_\mathrm{opt} = 1) = \varepsilon_\mathrm{II}(u_\mathrm{opt} = 0)$ corresponding to the same starting mechanical state after cluster melting.

Discussion

In order to analyse the possible evolution of the mechanical state of a vesicle, it is necessary to consider not only the energy of bilayer deformation but also the process of molecular rearrangement in the lattice and therefore the time scale. A big difference between the behaviours of the fluid and the gel phases, as for their mechanical properties, should be taken into account. According to experimental observations (Hianik and Passechnik 1995), the gel bilayer is much stiffer compared to the fluid one. The lipid flow invoked by the strain at the phase boundary is significantly slowlier in the gel state as follows from a comparison of the corresponding diffusion rates (Ivkov and Berestovsky 1981). Hence, from the time-scale point of view, local lipid rearrangement near the interface is preferred (Cevc and Marsh 1987).

It should be noted that clusters are dynamical objects which change their size and position (Mouritsen 2000) with an average lifetime τ in the region of 10^{-3}–10^{-4}s (Groll et al. 1996). During this time, according to the gel diffusion rate $D \sim 10^{-10}\ \mathrm{cm^2s^{-1}}$, the lipid rearrangement attains the length-scale $\sqrt{D\tau} \sim 10^1$ nm near the interface (Cevc and Marsh 1987), which is two orders less than the vesicle perimeter ($\sim 10^3$ nm). In this sense, the global molecular rearrangement necessary for the liposome growth in the gel phase (as a way of extra area compensation) is too slow regarding the cluster lifetime which makes this possibility less probable. In addition, according to the cluster fluctuations, this process should be periodic, i.e. a permanent lipid flow should occur along the vesicle surface accompanied with energy dissipation.

Hence, from the time-scale point of view, the first two types of the excess area compensation are favourable – stress redistribution between the gel and the fluid bilayer regions and out-of-plane cluster buckling. Because of the sound velocity in the lipid bilayer the former is fast enough to follow the changes in the cluster distribution. However, this is not possible without some local rearrangement near the closed cluster-gel interface, due to its length-change at cluster extension-compression. This requires some lipid flow in the cluster neighbourhood which decelerates this process but does not eliminate it in the time-scale of the cluster lifetime. Thus, the equilibrium energy calculated in the previous section (I) should be understood as the lower limit. The real value is always larger since the strain fails to spread over the whole vesicle surface, and so the relative gel area compression and consequently the elastic energy are increasing.

In the latter case (II), the length of the interface is assumed to be constant, the cluster area increases and surface curvature appears. The harmonic approximation of the elastic energy was used (Cevc and Marsh 1987) with a constant elastic modulus. A contingent non-linear behaviour occurring at the larger curvatures or compressions might become manifest at the beginning of compression and the final part of bending-curves, because a larger curvature occurs at a smaller compression and vice-versa (Fig. 2b). It will result in an increase of the absolute value of the total elastic energy and could shift, but should not suppress, the local potential wells in the elastic energy and thereby the existence of buckling. The area mismatch between the fluid and the gel phases is compensated for by the cluster buckling connected with getting the cluster surface out of the bilayer plane. This effect is hindered by a repulsion force from the bilayer bending. The final cluster state is a result of the balance between bilayer compression and bending (Fig. 2b).

Fig. 3b shows both dependencies of the equilibrium mechanical state of the bilayer regarding the average cluster size. While the buckling curve is constant up to some critical point after which it breaks and falls down, which corresponds to the first order behaviour of buckling parameter u_{opt} (Fig. 3a), the in-plane curve continuously increases and, at some temperature T_{B}, crosses the previous curve. This point separates two regions: $T < T_{\mathrm{B}}$ preferring the in-plane stress redistribution between the gel and the fluid phases (model I), and the second one, $T > T_{\mathrm{B}}$ where out-of-plane cluster buckling occurs as the preferred state (model II). In such a way, the real vesicle state evolution could be a combination of in-plane and out-of-plane relaxation depending on the average cluster size.

There is indirect experimental support to the proposed cluster buckling (model II) as a mechanism of mechanical stress relaxation during melting from the gel to the fluid phases. Analysing the differences in the heat capacity between uni- and multi-lamellar vesicles it was found that the interaction between the bilayers is very strong (van Osdol et al. 1991; Marek 1995). The models of liposome growth (III) and stress redistribution (I) provide only weak interaction because the clusters remain in the bilayer plane. On the other hand, cluster buckling and out-of-plane moving (II) causes a steric contact with the adjacent bilayer and might more likely explain the founded decay of the cluster displacement freedom on the vesicle surface (Marek 1995). This means that the neighbouring bilayer is a strong barrier for the melted cluster and relevant repulsion forces product strain in a portion of the opposite bilayer which increases the local probability of a new cluster forming immediately above and below the cluster buckling. In such a way, groups of out-of-plane clusters occur in directions upright to the vesicle surface which is connected with a large increase of the transition cooperativity as has been observed (van Osdol et al. 1991).

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