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The elastic deformability of closed multilayered membranes is the same as that of a bilayer membrane

S. Svetina and B. Žekš

Institute of Biophysics, Medical Faculty and Institute "J. Stefan", University of Ljubljana, Lipičeva 2, SLO 61105 Ljubljana, Slovenia

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Abstract. The elastic behavior of closed multilayered membranes is analyzed with the assumption that the constituent layers are in close contact but are unconnected in the sense that they are free to slide by one another. The system exhibits three independent elastic deformation modes for any number of the constituent layers equal to or larger than two. These are the area expansivity of the membrane neutral surface, and the local and non-local membrane bending. The corresponding elastic moduli are expressed in terms of the elastic moduli of the constituent layers, their areas, and distances between their neutral surfaces. Closed multilayered membranes only differ from a closed bilayer membrane in that for any of their shapes some of the constituent layers are expanded and some compressed.

Key words: Multilayered membranes – Elasticity – Elastic constants

Introduction

A characteristic of several systems such as phospholipid vesicles, living cells or some cell organelles is that their interior is separated from the exterior by a closed membrane. The behavior of these systems in many aspects depends on the membrane elastic properties. For instance, membrane elastic properties affect the mechanical stability of phospholipid vesicles and play a role in active cellular processes such as exocytosis and endocytosis, or in the formation of different membrane protuberances. Therefore the mechanical properties of phospholipid and cellular membranes have been extensively studied (Evans and Skalak 1980; Evans and Needham 1987). With respect to their elasticity membranes were usually treated as single, thin viscoelastic shells. However, an essential feature of phospholipid and biological membranes is that they are composed of layers. Phospholipid membranes are formed by two opposing phospholipid monolayers which are in close contact at the ends of their hydrocarbon chains, and their polar heads face the two embedding aqueous solutions. Cellular membranes are even more complex in that there are typically cytoplasmic and external structures associated with the phospholipid bilayer. In many instances these structures can be considered as additional membrane layers. Other examples of multilayers include multilamellar phospholipid vesicles. The purpose of this communication is to show that closed multilayered membranes exhibit specific elastic behavior.

Elastic behavior of layered membranes is affected by the connectedness of the layers with respect to their relative lateral movement (Evans 1974). In the case of a membrane with connected layers its elastic constants such as area expansivity modulus and bending modulus can be readily derived from the elastic moduli of the constituent layers and interlayer distances (Evans and Skalak 1980) and the system behaves elastically in the same manner as a single layered membrane. That this situation is qualitatively different for closed membranes with unconnected constituent layers can be concluded from the case of a closed membrane composed of two unconnected layers where it is, in addition to the area expansivity and local bending contributions to the membrane elastic energy, also necessary to take into consideration the non-local bending contribution (Evans 1974; Svetina et al. 1985; Božič et al. 1992). The unconnectedness seems to be a characteristic property of phospholipid and biological membranes. In phospholipid bilayers the two composing monolayers are, because of the hydrophobic effect, in close contact but can be assumed to be free to slide one by the other and thus to be unconnected. Bilayers in multilamellar phospholipid vesicles are separated by layers of water and are therefore unconnected as well. In biological membranes a similar situation is encountered in cases of membrane cytoskeletons such as the one in the red blood cell (Bennett 1990). The red blood cell cytoskeleton is in contact with the phospholipid part of the membrane and is attached to it by bonds to the integral membrane proteins. These proteins can move laterally in the phospholipid milieu sufficiently freely so that the red blood cell cytoskeleton can be considered as an additional unconnected membrane layer on the time scales which are longer than the times characteristic for the lateral motion of membrane proteins. This condition is satisfied for static properties and also for slow enough dynamic properties. The examples presented indicate that there is a considerable biological interest in analyzing the elastic behavior of a general closed membrane composed of n unconnected layers.

Theory

The elastic properties of a closed membrane composed of n layers in close contact but unconnected are governed by the elastic properties of individual layers and the geometrical constraints which these n layers impose on each other. We assume simple elastic properties for the individual layers, i.e. that they behave elastically in the same manner as a phospholipid monolayer in the liquid crystalline phase. Then the elastic energy of a closed single layer (denoted by the index i) is given as a sum of the area expansivity term and the bending term, respectively:

$$W_i = \frac{1}{2}\frac{K_i}{A_{0,i}}(A_i - A_{0,i})^2 + \frac{1}{2}k_{c,i}\int(c_1 + c_2 - c_{0,i})^2\,\mathrm{d}A_{0,i} \qquad (1)$$

where in the area expansivity term A_i is the area of the neutral surface of the i-th layer, $A_{0,i}$ the equilibrium value of this area, and K_i the area expansivity modulus. In the bending term c_1 and c_2 are the two principal curvatures (reciprocals of the principal radii of curvature) defined so that they are positive for a sphere, $c_{0,i}$ is the spontaneous curvature (Helfrich 1973) of this layer, and $k_{c,i}$ its local bending modulus. Integration is over the whole area of the neutral surface of the layer.

The geometrical constraints that the n layers exert on each other arise as a consequence of the contact between the neighbouring layers. This contact causes the distances between the neutral surfaces of all the layers to be fixed. For instance, because the interlayer distances are much smaller than the dimensions of vesicles and cells, and the curvatures are varying slowly over the membrane, the area of the j-th layer is related to the area of the k-th layer by the relation

$$A_j = A_k + h_{jk}\,C \qquad (2)$$

where h_{jk} is the distance between the neutral surfaces of these two layers (indices run from the most inner to the most outer layer) and C is the integral of the mean curvature over the whole membrane area:

$$C = \int(c_1 + c_2)\,\mathrm{d}A_0 \qquad (3)$$

This integration can be performed over any surface A_0 within the membrane because membrane width is much smaller than the principal radii appearing in the integrand in (3).

(i) Elastic energy of a bilayer

It has been previously shown for a closed membrane composed of two layers (Svetina et al. 1985) that in view of the constraint (2) it is appropriate to express its elastic properties in terms of three independent elastic deformation modes, i.e. the area expansivity of the neutral surface of the membrane, the relative expansivity of membrane layers, and the membrane bending:

$$W^{(2)} = \frac{1}{2}\frac{K^{(2)}}{A_0^{(2)}}(A - A_0^{(2)})^2 + \frac{1}{2}\frac{K_r^{(2)}}{A_0^{(2)}}(\Delta A - \Delta A_0^{(2)})^2 + \frac{1}{2}k_c^{(2)}\int(c_1 + c_2 - c_0^{(2)})^2\,\mathrm{d}A_0^{(2)} \qquad (4)$$

where in the area expansivity term A is the area of the neutral surface of the bilayer, $A_0^{(2)}$ is its equilibrium value, and $K^{(2)}$ is the area expansivity modulus. In the relative expansivity term ΔA is the difference between the areas of the outer and the inner layers $(A_2 - A_1)$, $\Delta A_0^{(2)}$ is the corresponding equilibrium value $(A_{0,2} - A_{0,1})$, and $K_r^{(2)}$ is the relative expansivity elastic modulus. In the bending term $k_c^{(2)}$ is the membrane local bending modulus and $c_0^{(2)}$ is the spontaneous curvature.

Equation (4) was obtained (Svetina et al. 1985) by summing elastic energies (1) for the layers 1 and 2 and by taking into consideration (2). The neutral surface of the membrane is defined in such a way that the area expansivity and relative expansivity terms become independent elastic deformation modes. Then we have

$$A = \frac{K_1/A_{0,1}}{K_1/A_{0,1} + K_2/A_{0,2}}A_1 + \frac{K_2/A_{0,2}}{K_1/A_{0,1} + K_2/A_{0,2}}A_2\,, \qquad (5)$$

$$A_0^{(2)} = \frac{K_1 + K_2}{K_1/A_{0,1} + K_2/A_{0,2}}\,, \qquad (6)$$

$$K^{(2)} = K_1 + K_2\,, \qquad (7)$$

$$K_r^{(2)} = (K_1 + K_2)\frac{K_1 K_2}{A_{0,1}A_{0,2}(K_1/A_{0,1} + K_2/A_{0,2})^2}\,, \qquad (8)$$

$$k_c^{(2)} = k_{c,1} + k_{c,2}\,, \qquad (9)$$

and

$$c_0^{(2)} = \frac{k_{c,1}c_{0,1} + k_{c,2}c_{0,2}}{k_{c,1} + k_{c,2}}\,. \qquad (10)$$

Integration is chosen to be over the neutral surface of the membrane.

It is evident from inspection of (4) that the behavior of the membrane composed of two unconnected layers is governed by three elastic moduli which are determined by the elastic moduli of the composing layers. The membrane area expansivity modulus (7) and the local bending modulus (9) are simply the sums of the corresponding moduli of the composing layers. The third elastic modulus which appears in (4), $K_r^{(2)}$ (8) is a measure of the relative expansivity of the areas of the two layers. It can be seen if we take in (8) $A_{0,1} \approx A_{0,2}$ that for a symmetrical bilayer (i.e. $K_1 = K_2$) the value of $K_r^{(2)}$ is one fourth of the value of the bilayer area expansivity modulus.

For the purpose of subsequent generalizations it is appropriate, by utilization of (2), to reexpress the relative expansivity term of (4) as

$$\frac{1}{2}\frac{k_r^{(2)}}{A_0^{(2)}}(C - C_0^{(2)})^2 \qquad (11)$$

where C, an integral of the sum of principal curvatures over the membrane neutral surface (3) is given by (2), and $C_0^{(2)}$ is the corresponding equilibrium value equal to

$$C_0^{(2)} = (A_{0,2} - A_{0,1})/h_{12}\ . \tag{12}$$

The effect of relative expansivity is expressed in this representation as a non-local bending which is measured by the non-local bending modulus $k_r^{(2)}$ related to the relative expansivity modulus $K_r^{(2)}$ by

$$k_r^{(2)} = h_{12}^2\, K_r^{(2)} \tag{13}$$

It has been shown only recently that for phospholipid membranes $k_r^{(2)}$ is a measurable quantity (Waugh et al. 1992).

(ii) Elastic energy of a multilayer

It is the purpose of this communication to generalize the treatment which leads to the description of the elastic properties of the bilayer in order to describe the elastic properties of the membrane composed of n layers where $n > 2$. It is shown that the elastic energy of the multilayer can be expressed by the same elastic deformation modes as appear in the expression (4) for the elastic energy of a membrane composed of two layers.

For the n-layered membrane the total membrane elastic energy is the sum of expressions (1) for all n layers. To obtain an expression for this energy ($W^{(n)}$) that takes into account the constraints on interlayer spacing (2), the area expansivity terms and the bending terms are summed separately. The sum of the area expansivity terms ($W_s^{(n)}$) can be expressed in terms of two variables only, the area of the neutral surface of the membrane (A) and the integrated mean curvature (C). This can be shown by writing each layer area analogously to (2) in terms of the area of the membrane neutral surface and the distance (h_i) between the neutral surface of the i-th layer and the neutral surface of the membrane:

$$A_i = A + h_i\, C \tag{14}$$

Then the sum of the area expansivity terms reads:

$$\begin{aligned} W_s^{(n)} &= \frac{1}{2}\sum_{i=1}^{n} \frac{K_i}{A_{0,i}}(A_i - A_{0,i})^2 = \frac{1}{2}\sum_{i=1}^{n} \frac{K_i}{A_{0,i}} A^2 - \sum_{i=1}^{n} K_i\, A \\ &\quad + \frac{1}{2}\sum_{i=1}^{n} \frac{K_i h_i^2}{A_{0,i}} C^2 - \sum_{i=1}^{n} K_i\, h_i\, C + \sum_{i=1}^{n} \frac{K_i\, h_i}{A_{0,i}} AC \\ &\quad + \frac{1}{2}\sum_{i=1}^{n} K_i\, A_{0,i} \end{aligned} \tag{15}$$

The neutral surface of the membrane is again obtained from the requirement that the membrane area and the integrated mean curvature become independent elastic deformations which is attained if the coefficient of the term in (15) involving the product AC is zero:

$$\sum_{i=1}^{n} \frac{K_i\, h_i}{A_{0,i}} = 0 \tag{16}$$

From (16) it is possible for instance to determine the distance between the neutral surface and a chosen layer from the $n-1$ distances between the neighbouring layers.

The sum of the bending energies ($W_b^{(n)}$) of the n layers is

$$\begin{aligned} W_b^{(n)} &= \frac{1}{2}\sum_{i=1}^{n} k_{c,i} \int (c_1 + c_2 - c_{0,i})^2\, \mathrm{d}A_0^{(n)} \\ &= \frac{1}{2}\sum_{i=1}^{n} k_{c,i} \int (c_1 + c_2)^2\, \mathrm{d}A_0^{(n)} - C \sum_{i=1}^{n} k_{c,i}\, c_{0,i} \\ &\quad + \frac{1}{2}\sum_{i=1}^{n} k_{c,i}\, c_{0,1}^2\, A_0^{(n)} \end{aligned} \tag{17}$$

The elastic energy of the n-layered membrane can then be written as

$$\begin{aligned} W^{(n)} = W_s^{(n)} + W_b^{(n)} &= \frac{1}{2}\frac{K^{(n)}}{A_0^{(n)}}(A - A_0^{(n)})^2 + \frac{1}{2}\frac{k_r^{(n)}}{A_0^{(n)}}(C - C_0^{(n)})^2 \\ &\quad + \frac{1}{2} k_c^{(n)} \int (c_1 + c_2 - c_0^{(n)})^2\, \mathrm{d}A_0^{(n)} + W_0^{(n)} \end{aligned} \tag{18}$$

where

$$A_0^{(n)} = \frac{\sum_{i=1}^{n} K_i}{\sum_{i=1}^{n} \frac{K_i}{A_{0,i}}} \tag{19}$$

is the equilibrium area of the neutral surface of the n-layered closed membrane, and

$$K^{(n)} = \sum_{i=1}^{n} K_i \tag{20}$$

is its area expansivity modulus.

$$C_0^{(n)} = \frac{\sum_{i=1}^{n} K_i\, h_i}{\sum_{i=1}^{n} \frac{K_i\, h_i^2}{A_{0,i}}} \tag{21}$$

is the equilibrium value for the integral of the mean curvature over the area of the membrane neutral surface.

$$k_r^{(n)} = \frac{\sum_{i=1}^{n} \frac{K_i\, h_i^2}{A_{0,i}} \sum_{i=1}^{n} K_i}{\sum_{i=1}^{n} \frac{K_i}{A_{0,i}}} \tag{22}$$

is the non-local bending modulus,

$$c_0^{(n)} = \frac{\sum_{i=1}^{n} k_{c,i}\, c_{0,i}}{\sum_{i=1}^{n} k_{c,i}} \tag{23}$$

is the spontaneous curvature, and

$$k_c^{(n)} = \sum_{i=1}^{n} k_{c,i} \tag{24}$$

is the local bending modulus of the membrane. The constant term $W_0^{(n)}$ in (18) denotes the fact that in the closed multilayered membrane in general in the equilibrium the composing layers are either expanded or compressed.

Discussion

From an inspection of (4) and (18) it is evident that the number of independent elastic deformation modes in the closed bilayer and in the closed multilayer is the same. These modes are of the same nature. Two of them, the area expansivity mode and the bending mode, are the same as the modes for a membrane comprising only a single layer. However, if there are at least two layers, another mode must be included, taking into account the relative expansivity of the layers. In the case of the bilayer the corresponding energy term represents directly the elastic energy which appears if the difference between the areas of the two layers is different from the corresponding equilibrium difference. In the case of the multilayered membrane this energy term is more conveniently expressed in terms of the deviation of the integral of the mean membrane curvature (3) from its equilibrium value. For $n=2$ the expressions (21) to (23) reduce to (12), (13), and (10), respectively.

The elastic moduli of the membrane appearing in (18) depend on the elastic parameters of the constituent layers, their equilibrium areas and the distances between their neutral surfaces (19 to 24). The area expansivity modulus $K^{(n)}$ (20) is simply the sum of the area expansivity moduli of the constituent layers. The same is true for the bending modulus $k_c^{(n)}$ (24). The non-local bending modulus $k_r^{(n)}$ exhibits a more complex dependence on the number of layers. This is clearly seen if (22) is simplified by taking the layer areas to be equal:

$$k_r^{(n)} = \sum_{i=1}^{n} K_i\, h_i^2 \tag{25}$$

To see in what manner the relative expansivity modulus depends on the number of layers, the summation in (25) is worked out for a simple membrane with n unconnected layers where it is assumed that the layers are equidistant and that their area expansivity moduli are equal ($h_{i,i+1}=h$, $K_i=K_1$). In this case we get

$$k_r^{(n)} = K_1\, h^2\, n\,(n^2-1)/12 \tag{26}$$

The strong dependence of the non-local bending modulus on the number of layers as predicted by (26) is shown in Table 1. It is of interest to note that in the case of the connected layers the same dependence on n was obtained for the local bending modulus (Evans and Skalak 1980).

In this report we have shown that multilayered membranes with unconnected layers exhibit the same general characteristics in elastic deformation regardless of the number of layers. Previously it was shown (Svetina et al. 1988) that in the case of the trilayer the number of independent elastic modes is the same as in the case of the bilayer. This result is now generalized for any number of layers. Our findings can be understood on the basis of the notion that the number of geometrical constraints for the areas of n layers is $n-1$. Each additional layer provides a new variable (its area) but also a new constraint so that the number of independent variables does not change as the number n increases.

Table 1. Non-local bending modulus of the n-layered membrane as obtained from (26) relative to the non-local bending modulus of a bilayer as a functon of the number of layers n

n	2	3	4	5	6	7	8
$\frac{k_r^{(n)}}{k_r^{(2)}}$	1	4	10	20	35	56	84

It can be concluded that the elastic behavior of closed membranes composed of unconnected layers differs fundamentally from the elastic behavior of a single-layered membrane. Already in the case of a bilayer there is an additional energy term which arises owing to the possibility that the two layers can be expanded or compressed relative to each other at a constant value of the area of the neutral surface of the membrane. We have shown that in closed multilayers there is an analogous energy term arising owing to relative expansion and compression of all the layers. There is, however, a notable difference between the elastic behavior of the bilayer and $n>2$ multilayers. In the case of a bilayer with the equilibrium area of the outer layer larger than the equilibrium area of the inner layer it is always possible to find the vesicle shape for which the two layers are neither expanded nor compressed (Svetina and Žekš 1989). In multilayers even in the case that the area expansivity and the relative expansivity energy terms of (18) are equal to zero, in general some layers are expanded and some compressed, the corresponding energy being contained in the constant term of this equation.

There are different indications that the elastic behavior of phospholipid and biological membranes is governed by their layered structure. Recently this notion gave rise to an interpretation of the temperature dependence of the shapes of phospholipid vesicles (Berndl et al. 1990). We have earlier suggested that the elastic behavior of closed layered membranes may form the basis of different biological phenomena such as those related to red blood cell shape transformations (Svetina and Žekš 1989) and hemolysis (Svetina et al. 1989), and such as cellular polarity, cytokinesis or gastrulation (Svetina and Žekš 1990; Svetina and Žekš 1991).

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