BENDING RESISTANCE AND CHEMICALLY INDUCED MOMENTS IN MEMBRANE BILAYERS

E. A. EVANS

From the Departments of Biomedical Engineering and Surgery, Duke University, Durham, North Carolina 27706

ABSTRACT Pure bending of a membrane bilayer is developed including different properties for each membrane half. Both connected and unconnected bilayer surfaces are treated. The bilayer bending resistance is the resultant of parallel surface compression "resistances." The neutral surface is a function of the upper and lower surface compressibility moduli and does not necessarily coincide with the midsurface. Alterations in the interfacial chemical free energy density (surface tension) on either face can create induced bending moments and produce curvature; even small changes can have a pronounced curvature effect. Chemically induced moments are considered as a possible mechanism for crenation of red blood cells.

INTRODUCTION

The question of the relative contribution of bending moments and membrane tensions has arisen frequently in the consideration of plasma membrane deformations.[1] Rand and Burton (1964) theorized that the unusual behavior of micropipette aspiration of red blood cells resulted from "bending resistance" (resistance to producing curvature in the surface). Fung (1966) discussed the general relation between bending resistance and membrane tensions and pointed out the importance of bending in stability of the biconcave shape of red cells when stressed. Canham (1970) and Lew (1972) considered the biconcave shape to be the minimum bending configuration. Zarda (1974) has recently investigated the role of bending resistance in maintenance of the biconcave shape of the red cell during osmotic swelling. On the other hand, it is important to recognize that considerable evidence exists indicating that bending resistance is second order in comparison with surface shear resistance in large deformations (Evans, 1973; Evans and LaCelle, 1974; and Hochmuth, 1972, 1973). Therefore, the membrane bending resistance needs to be determined.

There have been extensive observations of induced curvature in red cell membranes resulting from alterations in chemical environment (Bessis, 1972). The red cell shape spectrum ranges from a normal biconcave disk to a tightly crenated sphere; the inter-

[1] In deformation of thin membrane shells, bending moments are most often negligible compared with membrane stresses or stress resultants (tensions) (Flügge, 1966; Fung, 1966). However, there are many situations that arise where bending moments not only cannot be neglected, but dominate and are essential for stability (Fung, 1966).

mediate states are spiculated shapes called echinocytes of different degrees. The reversible shape change from a biconcave disk into a crenated sphere was first described by Hamburger (1895) and still remains an unsolved puzzle for many a hematologist and cell physiologist. Ponder (1971) accumulated a vast amount of information concerning the multiplicity of agents and conditions that produce this transformation, but the underlying mechanism has not been established. Here, the possibility that the spiculation or curvature can be produced by chemically induced bending moments in the lipid bilayer will be investigated.

Pure bending of a membrane bilayer will be considered in this paper. However, it is reemphasized that additional structural rigidity exists for plasma membranes of biological cells (Evans, 1973; Evans and LaCelle, 1974); biological membranes have a non-zero shear modulus and resistance to in-plane shear. The shear modulus is significantly smaller than the modulus for resistance to area changes; therefore, for the bending resistance, isotropic tension created by small area changes in the layers will dominate. On the other hand, the deformation of the total surface (at essentially constant surface area) requires in-plane shear and will in general create anisotropic tension in biological membranes; the result of membrane tension will be discussed but not explicitly treated (see Evans [1973] and Skalak et al. [1973] for consideration of membrane shear resistance). This approximation to an actual composite membrane provides insight into a possible mechanochemical basis for equilibrium shape changes.

Lipid monolayers and bilayers behave as two-dimensional liquids: no resistance to in-plane shear, only isotropic tension (in the membrane plane) can be developed and is the result of surface dilation or compression. Bending of monomolecular layers can take place freely provided the radii of curvature of the surface are greater than intermolecular distances and no alteration in surface area occurs. The same is true for multilayers that are not connected and are free at the extremities (in other words, the freedom condition permits the layers to extend past one another at the edges to accommodate relative area changes). However, for connected layers or layers with constraints at the edges or boundaries, preventing the layers from slipping relative to each other, it is not possible to bend the composite without introducing area changes in the layers. For "pure bending," there will be a neutral surface that maintains constant area. Both connected and unconnected layers with fixed boundaries will be investigated to establish resistance to curvature production ("bending resistance") and induced bending moments.

MECHANICAL EQUILIBRIUM IN BILAYERS

The tension in two-dimensional, monomolecular lipid layers is isotropic (independent of direction in the plane of the membrane) but not necessarily homogeneous.

$$T_{ij} = T\delta_{ij}, \tag{1}$$

where tension is the force per unit length on the side of a material element; the subscripts (i, j) refer to the two coordinates in the plane of the surface; δ_{ij} is the Kro-

necker delta matrix. The isotropic tension is the balance of the interfacial, free energy density (surface tension between the monolayer and environmental solutions) and the two-dimensional compressibility of the surface.

$$T = \gamma + K\alpha, \tag{2}$$

where γ is interfacial, free energy density; K is the two-dimensional compressibility modulus; α is the areal strain (fractional change in area resulting from compression of the surface: $[A/A_0] - 1$).

For a bilayer, the surface properties will, in general, be different for each layer:

$$\begin{aligned} &\text{upper:} && T^u = (\gamma^u + K^u\alpha^u), \\ &\text{lower:} && T^l = (\gamma^l + K^l\alpha^l). \end{aligned} \tag{3}$$

If the bilayer is initially in equilibrium without any external forces, then the tensions are zero (where the subscript zero specifies initial equilibrium conditions):

$$T_0^u = T_0^l = 0.$$

The initial properties are related by,

$$\begin{aligned} \gamma_0^u + K^u\alpha_0^u &= 0, \\ \gamma_0^l + K^l\alpha_0^l &= 0. \end{aligned} \tag{4}$$

BENDING RESISTANCE AND RIGIDITY CONSTANT IN CONNECTED BILAYERS

If the connected bilayer is initially flat and then bent, a bending moment is produced by expansion of the upper surface and compression in the lower surface (Fig. 1).

The bending moment is given by,

$$M = h^u T^u - h^l T^l, \tag{5}$$

where h^u, h^l are the distances of the upper and lower surface from the neutral surface (which has not changed area). The total intersurface distance, h, is the sum of h^u plus h^l,

$$h = h^u + h^l. \tag{6}$$

In pure bending, the expansion of the upper surface relative to the compression in the lower surface is inversely proportional to the respective surface compressibilities:

$$-\delta\alpha^u/\delta\alpha^l = K^l/K^u. \tag{7}$$

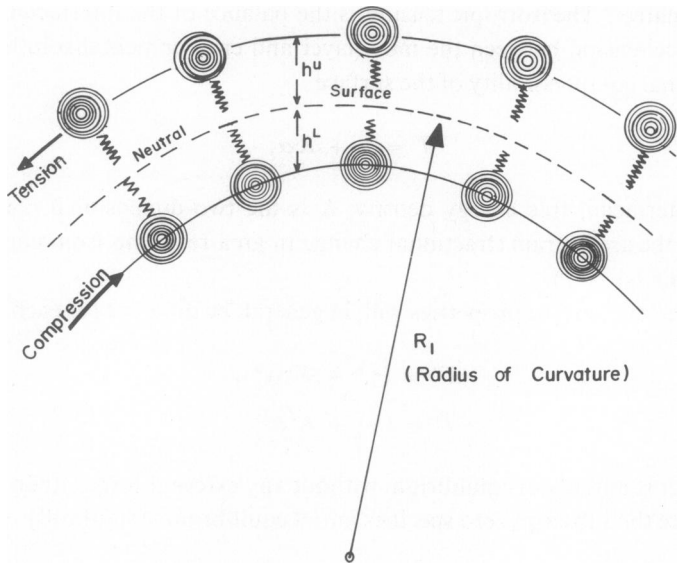

FIGURE 1 Schematic of lower surface compression and upper surface expansion resulting from bending of a membrane bilayer.

For thin membranes, the changes in the area strains ($\delta\alpha^u$, $\delta\alpha^l$) are given by,

$$\delta\alpha^u = h^u\left(\frac{1}{R_1} + \frac{1}{R_2}\right),$$
$$\delta\alpha^l = -h^l\left(\frac{1}{R_1} + \frac{1}{R_2}\right). \tag{8}$$

R_1, R_2 are the principal radii of curvature for the surface.

Using Eqs. 6–8, the neutral surface distances are determined,

$$h^u/h^l = K^l/K^u,$$
$$h^u = h/(1 + [K^u/K^l]),$$
$$h^l = h/(1 + [K^l/K^u]). \tag{9}$$

The moment, Eq. 5, can be expressed in terms of the compressibilities and fractional area changes,

$$M = K^u\delta\alpha^u h^u - K^l\delta\alpha^l h^l,$$

which is given by,

$$M = h^2\left(\frac{K^lK^u}{K^u + K^l}\right)\left(\frac{1}{R_1} + \frac{1}{R_2}\right). \tag{10}$$

The bending rigidity constant, D, is

$$D = h^2\left(\frac{K^l K^u}{K^u + K^l}\right). \tag{11}$$

The area compressibility moduli, K, are in units of dynes per centimeter and would be divided by a single molecular layer thickness in order to obtain the "equivalent" stress units (dynes per square centimeter); however, such a conversion can not be made because the membrane is not a continuum in the thickness dimension. In Eq. 11, the result is that the bending rigidity constant is proportional to total bilayer thickness squared. For a three-dimensional, isotropic material, the bending rigidity constant is $Eh^3/12(1 - \sigma^2)$ where E is Young's modulus in dynes per square centimeter and σ is Poisson's ratio. The numerical coefficient results from the integration of shell stresses that linearly vary through the shell thickness (Landau and Lifshitz, 1970). Also, Young's modulus, E, is proportional to the shear modulus of the material which is zero in this case; therefore, bending theory for a three-dimensional isotropic material is clearly inappropriate to lipid bilayers. An important aspect of Eq. 11 is the effect of different surface compressibilities. The surface compression "resistances" act in parallel; therefore, the total bending resistance is limited by the smallest modulus. The neutral surface is not necessarily the "mid-surface".

CHEMICALLY INDUCED MOMENTS IN CONNECTED BILAYERS

Changing the surface chemical equilibrium by altering the interfacial free energy densities (γ^u, γ^l) would result in adjustments in upper and lower surface areas, thereby inducing a bending moment. In order to establish the new equilibrium, the surface free energy change must be minimized. The surface free energy per unit area is given by,

$$F = \gamma^u(\alpha^u + 1) + \gamma^l(\alpha^l + 1) + 1/2K^u(\alpha^u)^2 + 1/2K^l(\alpha^l)^2. \tag{12}$$

Using the initial equilibrium conditions (Eq. 4) and examining the change in free energy,

$$\Delta F = \delta\gamma^u\delta\alpha^u + \delta\gamma^l\delta\alpha^l + 1/2K^u(\delta\alpha^u)^2 + 1/2K^l(\delta\alpha^l)^2, \tag{13}$$

where,

$$\Delta F \equiv F - F_0,$$

$$F_0 = \gamma_0^u(\alpha_0^u + 1) + \gamma_0^l(\alpha_0^l + 1) + 1/2K(\alpha_0^u)^2 + 1/2K^l(\alpha_0^l)^2,$$

$$\delta\gamma = \gamma - \gamma_0,$$

$$\delta\alpha = \alpha - \alpha_0.$$

For pure bending of a connected bilayer with the neutral surface area unchanged, the variation in free energy can be expressed in terms of local curvature.

$$\Delta F = \left[\frac{\delta\gamma^u}{K^u} - \frac{\delta\gamma^l}{K^l}\right]\frac{D}{h}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \frac{D}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right)^2. \tag{14}$$

The first term in Eq. 14 is the chemically induced, moment free energy, and the second term is the bending resistance. In the absence of external forces and boundary constraints, the free energy is minimized by setting the variation with respect to total curvature equal to zero,

$$\delta(\Delta F)/\delta C = 0, \tag{15}$$

where

$$C \equiv (1/R_1) + (1/R_2),$$

the total curvature. Eqs. 14 and 15 give the relation between local curvature and chemical alterations for a connected bilayer:

$$-\left(\frac{1}{R_1} + \frac{1}{R_2}\right) = \left(\frac{\delta\gamma^u}{K^u} - \frac{\delta\gamma^l}{K^l}\right)\frac{1}{h}. \tag{16}$$

If the surface has fixed boundary conditions or is a closed surface, then the equilibrium condition involves transverse shear, membrane tensions, and moments in a complicated set of relations (see Flügge, 1966). However, as will be discussed in a later section, lipid bilayers are apparently unconnected; therefore, it will not be necessary to carry the development further here.

BENDING IN UNCONNECTED BILAYERS

As previously mentioned, an unconnected bilayer without edge restrictions would offer no resistance to bending because the lower layer would merely "slip" past the upper layer. If, on the other hand, the edges of the bilayer are constrained such that the layers are prevented from relative movement at the edge (or if the membrane is a closed surface, e.g. encapsulating a cell), then there will be net area differences between upper and lower layers when curvature is produced. But local adjustment and relative movement can take place between the layers. In this case, the difference in total upper and lower surface areas is determined by the integral of curvature over the whole membrane.

$$\delta\alpha^u - \delta\alpha^l = \frac{h}{A_m}\int\left(\frac{1}{R_1} + \frac{1}{R_2}\right)ds, \tag{17}$$

where $\int ds$ is the integral over the total membrane and A_m is the total membrane area. Again, the fractional area changes are related by Eq. 7 and the surface com-

pressibilities:

$$\delta\alpha^u = \frac{h}{A_m}\left(\frac{1}{1 + (K^u/K^l)}\right)\int\left(\frac{1}{R_1} + \frac{1}{R_2}\right)ds,$$

$$\delta\alpha^l = \frac{-h}{A_m}\left(\frac{1}{1 + (K^l/K^u)}\right)\int\left(\frac{1}{R_1} + \frac{1}{R_2}\right)ds. \tag{18}$$

Using Eq. 18, the variation in free energy density can be obtained from Eq. 13 for an unconnected, bounded bilayer.

$$\Delta F = [(\delta\gamma^u/K^u) - (\delta\gamma^l/K^l)](D/h)\langle C\rangle + (D/2)\langle C\rangle^2, \tag{19}$$

where $\langle C\rangle$ is the surface average total curvature.

$$\langle C\rangle = \frac{1}{A_m}\int\left(\frac{1}{R_1} + \frac{1}{R_2}\right)ds.$$

Here, it is observed that the induced moment and bending resistance are active in a collective sense over the whole surface rather than at a specific location.

The equilibrium condition is given by the variation of the free energy change with respect to average curvature:

$$\frac{\delta(\Delta F)}{\delta(\langle C\rangle)} = 0,$$

$$-\frac{1}{A_m}\int\left(\frac{1}{R_1} + \frac{1}{R_2}\right)ds = \left(\frac{\delta\gamma^u}{K^u} - \frac{\delta\gamma^l}{K^l}\right)\frac{1}{h}. \tag{20}$$

MECHANISM FOR RED BLOOD CELL CRENATION

As mentioned in the Introduction, the human red blood cell exhibits progressive crenation in response to increasing amounts of various chemical agents and to electrical fields (Rand et al., 1965). The action of induced bending moments is proposed here as the mechanism for crenation. In this case, crenation would be the result of expansion of the outer surface relative to the inner surface. Such conditions would arise either when the outer interfacial free energy density (γ^u) decreased (e.g. the addition of electrical charges to the outer layer) or when inner interfacial free energy (γ^l) increased (e.g., Ca^{++} binding between negatively charged amphiphilic molecules of the inner surface, see Bretscher [1973]). In order to evaluate the evolution of the spiculation, it is necessary to determine whether or not the bilayer is connected. Direct mechanical evidence would require measurement of relative shear resistance between the layers, but this has not been done. However, extensive X-ray diffraction data exists showing that the nonpolar tails are *not* connected (Mateu, et al. [1973] and Tardieu et al. [1973]). Therefore, it is reasonable to assume that the two layers can move relative to one another.

If the spicules are produced uniformly over the surface, Eq. 20 shows that the average curvature per spicule must increase as the interfacial free energy density of the outer surface decreases:

$$-\langle C\rangle_{\text{Spicule}} = ([\delta\gamma^u/K^u] - [\delta\gamma^l/K^l])\,1/h. \tag{21}$$

For a fixed surface like the red cell membrane, the average curvature per spicule increases with increasing number of spicules. Therefore, the number of spicules progressively increases with the interfacial free energy change. In addition, it is apparent from Eq. 21 that reducing the surface compressibility amplifies the effect (e.g. by incorporating material in the membrane like cholesterol).

Only the crenation of red cells has been discussed here; but, the concept is also compatible with the red cell disk to cup shape transformation (Bessis, 1972). In this case, the opposite situation exists: the inner surface is expanding relative to the outer surface (in other words, the cell is trying to turn "inside-out"). Here, either the inner surface interfacial free energy density (γ^l) decreases or the outer surface interfacial free energy density (γ^u) increases.

Implicit in the preceding discussion has been the assumption that the structural rigidity possessed by the red cell membrane can be neglected. In order to evaluate this assumption, the elastic free energy of the structural component must be compared with that of Eq. 13. The elastic free energy density is given by (Evans, 1973),

$$\Delta F_E = (\mu/2)(\epsilon_x + \epsilon_y),$$

where μ is the two-dimensional shear modulus (dynes per centimeter) of the membrane; ϵ_x, ϵ_y are the finite strains in the plane of the membrane. When the induced curvature of the surface is very small, the sum of the strains is the order of $\delta\alpha$; therefore, the interfacial free energy change must be greater than the membrane shear modulus in order to initiate bending,

$$\delta\gamma \sim \mu.$$

However, when the curvature is not small, the sum of strains greatly exceeds the fractional area change of a layer. In this case, the condition is approximately,

$$\delta\gamma \sim \mu(\epsilon_x + \epsilon_y)/h\langle C\rangle,$$

and could be the order of 100 times the shear modulus μ. The red cell membrane shear modulus has been measured to be about 10^{-2} dyn/cm; therefore, the interfacial free energy change would have to be between 10^{-2} and 1 dyn/cm for the progressive crenation of red cells.

CONCLUSION

The bending resistance and chemically induced moments in membrane bilayers have been developed with different properties for each layer; both connected and unconnected bilayers have been treated. It was shown that the location of the neutral surface is a function of the upper and lower surface compressibility moduli. Note, these moduli may change in value when going from compression to tension. Therefore, the neutral surface may move in response to curvature reversal. The bending resistance of a connected bilayer is a function of local curvature whereas bending resistance of unconnected bilayers is related to the surface average curvature. Alterations in the interfacial, chemical free energy densities can create induced moments and produce curvature. Small changes in environment can cause appreciable curvature production because large curvature must counter the small layer separation in order to give the fractional area change needed.

The chemically induced moment is proposed as a mechanism to produce crenated and cup-shaped red cells. The shear rigidity of the red cell membrane must also be overcome along with the bending resistance and may be appreciable for large spicules.

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