Blocked Lipid Exchange in Bilayers and its Possible Influence on the Shape of Vesicles

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(Z. Naturforsch. **29 c**, 510–515 [1974]; received May 9, 1974)

Lipid Exchange, Vesicle Shape

The role of lipid exchange in the curvature elasticity of bilayers is studied theoretically. Blocking of exchange between the monolayers may give rise to a nonequilibrium lipid distribution going hand in hand with a spontaneous curvature. Some possible consequences for vesicular deformations are discussed. Lipid nonequilibrium is tentatively suggest as one possible cause for certain shape transformations of red blood cells.

Introduction

Recently we poposed [1, 2] a continuum theory of the presumable elasticity of lipid bilayers, regarding them as two-dimensional fluids. The theory may be useful to calculate the shape of bilayer vesicles, *i. e.* of closed membranes with water inside and outside the enclosure, which are the simplest models of biological cells. It was argued that the only elasticity controlling the shape of nonspherical vesicles is that of curvature, while the membrane area should be practically constant and the energy of stretching negligible. Formulas for some simple deformations of initially spherical versicles by magnetic [1, 2] and electric fields [3] and by excess outside pressure [2] were derived.

The curvature-elastic energy per unit area, w_c, was written as

$$w_c = \tfrac{1}{2}\, k_c (c_1 + c_2 - c_0)^2 + \bar{k}_c\, c_1\, c_2 \,. \qquad (1)$$

The two principal curvatures c_1 and c_2, the largest and the smallest, occur along orthogonal directions and are measured in radians per unit length. The elastic moduli k_c and $\bar{k}_c$ have the dimension of elastic moduls k_c and k_c have the dimension of energy. The integral of $\bar{k}_c\, c_1\, c_2$ over the entire vesicle surface was seen to be independent of the vesicle shape. Accordingly, the second term in (1) does not enter the differential equations for the shape and can be omitted. The theory allows for a spontaneous curvature c_0 of the bilayer. Such a curvature in the relaxed state may be expected if the two sides of the bilayer are unequal or facing different aqueous solutions. Therefore, the shape of vesicles is likely to be susceptible to chemical agents added to the outer aqueous phase and not instantly permeating to the inner one. Chemical deformations have not yet been observed with lipid vesicles, but they may underly certain shapes of red blood cells (see below).

Eq (1) for the elastic energy density can in general be used without reservation if the exchange of lipids between the four phases, *i. e.* the two monolayers and the two aqueous solutions, is faster than the process of deformation. Measurements by Kornberg and McConnel [4] with artificial phospholipid vesicles indicate, however, that the flip-flop of lipid molecules from one side of a bilayer to the other may take several hours at 30 °C. It seems, therefore, necessary to consider the possibility of virtually blocked or negligible flip-flop or *internal* lipid exchange.

For the *external* lipid exchange between a monolayer and the adjacent aqueous phase one may distinguish three "pure" cases: First, no external exchange, either because an energy barrier impedes the transition of lipids or because lipid diffusion in water is very slow. Second, bilayer-controlled external exchange, in which the lipid concentration in water (*i. e.* the lipid chemical potential) depends solely on the state of the vesicles. This condition should prevail whenever virtually all lipid molecules are tied up in the vesicle membranes. Third, solution-controlled external exchange, where only an insignificant fraction of the lipids is bound by the membranes. In cases 2 and 3 the exchange is, of course, assumed to be faster than the process to be measured. Cases 1 and 2 leave the number of tied-up lipid molecules exactly or practically con-

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stant, thus being indistinguishable in elastic measurements. In the following they will both be referred to as negligible or blocked external exchange.

Phenomenological relations

Let us imagine a symmetric bilayer, *i.e.* one whose two sides are of equal composition. A small piece is thought to be uniformly curved under the condition of blocked internal and external exchange. We may then replace in (1) the modulus for free exchange, k_c, by one for blocked exchange, k_c^{bl}. Alternatively, we can retain k_c and take account of blocking by inserting in (1) for c_0 a self-induced spontaneous curvature Δc_0 which is a linear function of the actual curvature. The second modulus in (1), k_c is the same with and without exchange, as may be inferred from simple symmetry considerations.

In order to derive a few phenomenological relations we now consider a bounded piece of bilayer which we curve, like a cylinder, in only one direction, the curvature being c. For free exchange the curvature-elastic energy per unit area is

$$w_c = \tfrac{1}{2} k_c c^2. \tag{2}$$

For blocked exchange it may be expressed by

$$w_c^{bl} = \tfrac{1}{2} k_c^{bl} c^2. \tag{3}$$

The flat piece and deformations with and without a redistribution of lipids between the monolayers are sketched in Fig. 1 for a bilayer consisting of a

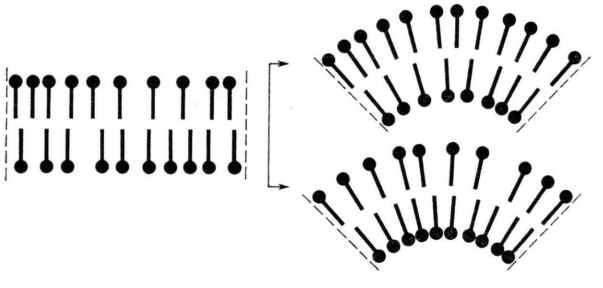

Fig. 1. Curving of a bilayer with redistribution of lipid molecules (top) and without (bottom).

single speciel of molecules. Because lifting the block can only diminish the free elastic energy, we have

$$k_c^{bl} \geqq k_c. \tag{4}$$

With a view to the later discussion of vesicles we call the upper and lower monolayers in Fig. 1 outer and inner and, correspondingly, define the shown curvature as positive. In addition, we denote the densities of lipid molecules per unit area in the outer and inner halves by ν_{out} and ν_{in}, restricting ourselves to one-component systems. If the bilayer is curved the densities are meant to refer to the center surface between the monolayers. With blocked flip-flop the concentrations may differ even in the absence of curvature, as indicated in Fig. 2.

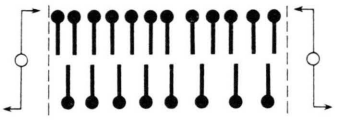

Fig. 2. Lipid unbalance between the monolayers and the torque preventing the membrane from curving spontaneously.

In order to maintain flatness a torque per unit length of boundary, τ, has in general to be applied. The stress is related to a spontaneous curvature by

$$\tau = -k_c c_0. \tag{5}$$

A torque stress shall be positive if it tends to produce a positive spontaneous curvature. The spontaneous curvature in (5) will be proportional to the density difference,

$$c_0 = \alpha \frac{\nu_{out} - \nu_{in}}{\nu_0}, \tag{6}$$

where ν_0 is the mean density. The parameter α should satisfy

$$\alpha \geqq 0 \tag{7}$$

for any bilayer which in complete equilibrium is flat and has $\nu_{out} = \nu_{in}$.

Returning to the curved piece of membrane, we adopt a parameter β linking the density difference which arises with free exchange to the actual curvature

$$(\nu_{out} - \nu_{in})/\nu_0 = \beta c. \tag{8}$$

By definition, no spontaneous curvature is generated. This implies that for blocked exchange the self-induced spontaneous curvature is

$$\Delta c_0 = -\alpha \beta c. \tag{9}$$

Again for blocked exchange, a deforming torque stress τ can now be expressed in two ways:

$$\tau = \begin{cases} k_c^{bl}\, c \\ k_c (c - \Delta c_0) \end{cases} . \tag{10}$$

Equating the two forms yields

$$k_c^{bl} = (1 + \alpha\beta) k_c . \tag{11}$$

The inequalities (4) and (7) entail

$$\beta \geqq 0 . \tag{12}$$

The relationship between the lipid densities and the respective chemical potentials μ_{in} and μ_{out} can be expressed by a third coefficient, γ,

$$\nu_{out} - \nu_{in} = \gamma (\mu_{out} - \mu_{in}) . \tag{13}$$

If the lipid concentration in the enclosed volume is negligible, *i. e.* bilayer controlled, one deduces from simple elastic considerations

$$\nu_{out} - \nu_{in} = 2\gamma (\mu_{out} - \mu_0) . \tag{14}$$

The equilibrium value of the chemical potential, μ_0, should be largely independent of the radius of the vesicles. We disregard lipid ionization which would require a more complex description.

Spontaneous curvature in vesicles

The spontaneous curvature of the bilayer around a vesicle is zero if the two halves including the adjacent aqueous phases are in thermodynamic equilibrium [6]. A vesicle with zero spontaneous curvature and no forces acting on it will be spherical (apart from fluctuations) because this shape has the lowest elastic energy. (The only possible exception, not to be treated here, may occur in connection with elastic effects of higher order than the quadratic approximation (1). They could be of importance for very small vesicles whose radius is not much larger than the thickness of the bilayer.) How fast chemical equilibrium is reached from a given initial state depends on the rate (or rates) of lipid exchange and on the permeabilities of the bilayer for the various other molecules involved.

Only spherical vesicles have a uniform curvature. For all other shapes it is essential to note that the two monolayers of a two-dimensionally fluid bilayer can probably slide upon each other without elastic restraint. If this is true, the only sensible way to express the elastic energy is by means of k_c. Even in the case of blocked lipid exchange the modulus k_c^{bl} should not be used. The reason is that any self-induced spontaneous curvature will be uniform all over the vesicle: It does not depend on the local curvature, but is a functional of the vesicle shape as a whole. Henceforth, we will assume the bilayers to possess the additional freedom of internal sliding, thus being, in a sense, three-dimensional fluids.

Assuming now that the deformation of, for instance, a spherical vesicle starts from a condition of chemical equilibrium we have for the self-induced spontaneous curvature

$$\Delta c_0 = -\frac{\alpha\beta}{A} \left[\oint_{\text{after}} (c_1 + c_2)\, dA - \oint_{\text{before}} (c_1 + c_2)\, dA \right] , \tag{15}$$

where A is the (constant) area of the center surface and the two integrals over it are taken before and after the deformation. It seems safe to expect the equalization of Δc_0 during the deformation to be quite fast except, perhaps, for very large vesicles [7].

An interesting class of vesicle deformations are those starting from the spherical shape. So far only ellipsoids of revolution of weak ellipticity have been considered. They may be expressed in polar coordinates by

$$s = r - r_0 = \tfrac{3}{2} s_2 (\cos^2\theta - \tfrac{1}{3}) , \tag{16}$$

where r_0 is the radius of the initial sphere. The second Legendre polynomial on the right-hand side keeps the membrane area linearly independent of s_2. The integral of curvature over a sphere is

$$\oint_{\text{sphere}} (c_1 + c_2)\, dA = 8\pi r_0 . \tag{17}$$

With an ellipsoid of revolution one obtains in the lowest order of s_2

$$\oint_{s_2} (c_1 + c_2)\, dA = 8\pi r_0 + \frac{8\pi}{5} \frac{s_2^2}{r_0} \tag{18}$$

The integration is outlined in the appendix. Consequently, the self-induced spontaneous curvature of the transition sphere-ellipsoid is

$$\Delta c_0 = -\frac{2\alpha\beta}{5 r_0} \left(\frac{s_2}{r_0} \right)^2 . \tag{19}$$

The importance of self-induced curvature will be assessed after an estimate of $\alpha\beta$ to be given in the next section. Generally speaking, the occurrence of self-induced curvature renders it more difficult to calculate vesicular deformations. Δc_0 being a function of the final shape, one must compute shapes

for various Δc_0's and then select that (or those) for which Eq (15) gives the same value. However, it follows from Eq (18) and the formulas for the deformation by magnetic [1, 2] and electric [3] fields that Δc_0 is negligible for small enough ellipticities. Also, the instability threshold of spherical vesicles under excess outside pressure [2] is not affected.

A change of the outer aqueous phase making it different, at least temporarily, from the inner one may result in a chemically induced spontaneous curvature. It may also give rise to osmosis (and possibly affect k_c). If water leaves the vesicle the sphere must in general be deformed with the possible exception of very small vesicles [2]. The new shape will be influenced by c_0, including the self-induced part. A purely chemical deformation of spheres, though possible in principle [2] for large enough positive c_0, may be strongly hindered by slow water permeation. This is because in most cases the internal excess pressure produced by the spontaneous curvature is probably quite small [2]. However, a shape which is already nonspherical should be sensitive to a decrease or increase of c_0 (see below).

A model

The following simple model of bilayer elasticity may be helpful to get an idea of the strength of self-induced spontaneous curvature. The monolayers are viewed as isotropic membranes with vanishing shear modulus to ensure two-dimensional fluidity. The energy of curvature is ascribed to density changes, *i. e.* compression and dilation tangential to the membrane. If the edges of the monolayers are clamped to each other and if there is no spontaneous curvature the elastic energy density of the bilayer may be written as

$$w_c = \tfrac{1}{2}\varkappa \int_{-b/2}^{b/2} z^2 (c_1 + c_2)^2 \, dz = \frac{\varkappa b^3}{24} (c_1 + c_2)^2 . \quad (20)$$

The integration goes from one side of the membrane to the other, b being the thickness and z the distance from the middle surface. $\varkappa$ is a bulk elastic modulus [8]. Since material exchange between the two sides of the membrane is not allowed, this case represents blocked flip-flop, implying the identity

$$k_c^{bl} = \varkappa b^3/12 . \quad (21)$$

On the other hand, if internal lipid exchange takes place and results, *e. g.*, in equal material density in the two monolayers, as referred to their respective center planes, one may apply the same calculation separately to the monolayers. Replacing in (21) b^3 by $2(b/2)^3$ yields

$$k_c = \varkappa b^3/48 = \tfrac{1}{4} k_c^{bl} . \quad (22)$$

With respect to the bilayer's center surface the "molecular" densities are different:

$$(\nu_{in} - \nu_{out})/\nu_0 = (b/2)(c_1 + c_2) . \quad (23)$$

Therefore, the parameters introduced above are in the present model

$$\begin{aligned} \alpha\beta &= 3 , \\ \beta &= b/2 , \\ \alpha &= 6/b . \end{aligned} \quad (24)$$

Insertion of (24) in (19) gives

$$\Delta c_0 = -\frac{6}{5}\frac{1}{r_0}\left(\frac{s_2}{r_0}\right)^2 . \quad (25)$$

We may conclude that the self-induced curvature becomes comparable to the curvature of the sphere, $2/r_0$, only for fairly strong deviations from the spherical shape.

Finally we write down a formula for the coefficient γ,

$$\gamma = 1/(\varkappa b \nu_0^{-2}) \quad (26)$$

where ν_0^{-1} stands for the monolayer area occupied by a single molecule. It is obtained by equating $\mu - \mu_0$ and the elastic energy needed to add one molecule to a piece of monolayer of fixed unit area.

For an estimate of k_c we use $k = 1 \cdot 10^9$ dyn cm^{-2} and $b = 5 \cdot 10^{-7}$ cm and obtain

$$k_c \approx 2.6 \cdot 10^{-12} \text{ erg} .$$

This number is five times a previous estimate [2]. It should be noted that the new model is again a gross simplification. For instance, the inevitable asymmetry of the monolayers (which separately may have very strong spontaneous curvatures offsetting each other in the bilayer) is disregarded. We hope, however, that the estimates are helpful in designing experiments to measure the curvature-elastic moduli of bilayers.

Some shapes of red blood cells

This section is speculative and added mainly for lack of data on artificial lipid bilayer vesicles.

Some observations with red blood cells are interpreted in terms of spontaneous curvature due to lipid unbalance. The assumption is that at least in its elastic properties the red cell membrane behaves much like a lipid bilayer.

Living red blood cells usually possess a biconcave-discoid shape, probably caused by an osmotic restriction of water uptake which, in turn, is due to metabolic processes (active transport). Shapes of this type can be reproduced by computer calculations on the basis of curvature elasticity, as is shown elsewhere [9].

It is well known that the addition of certain chemical agents to the outer medium transforms the so-called discocytes into either echinocytes or stomatocytes, depending on the agent. The former display a fairly large number of finger-like spicules on top of a discoid or spherical contour. The latter are cup-like, having a hollow on one side. These two classes of mostly reversible deformations are clearly distinct and can be achieved under iso-osmotic conditions, *i. e.* at constant vesicle volume.

It appears attractive to explain the formation of echinocytes and stomatocytes as the response to strong chemically-induced spontaneous curvatures. The tips of the fingers are regions of large positive curvature, thus indicating $c_0 \gg 0$. At the feet of the spicules the two principal curvatures are of opposite sign which implies at least partial compensation in Eq (1). The hollow of stomatocytes suggests $c_0 \ll 0$ because in it the membrane is negatively curved. On the rim the two principal curvatures are of opposite sign so that the formation of a hollow is likely to lower, for $c_0 \ll 0$, the total curvature-elastic energy in comparison with the discocyte. Of course, these preliminary interpretations remain to be checked by rigorous calculations.

Among the many echinocytogenic agents [10] are fatty acids (*e. g.* oleic acid) and phospholipids (*e. g.* lysolecithin). Both lipids should be readily incorporated into the membrane. Provided the membrane is largely a bilayer and the flip-flop of the amphiphilic molecules is not too fast, the addition of molecules to the outer monolayer should indeed generate a positive spontaneous curvature.

Interestingly, Lichtman and Marinetti [11] found most of the echinocytes caused by adding lysolecithin to become discocytes again after about 6 hours (at 37 °C). The authors suspected lipid exchange within the membrane to be the reason for the return to the disc shape. The relaxation time is of the same order as Kornberg and McConnel's [4] who studied small artificial lipid vesicles (at 30 °C), monitoring the lipid distribution by means of spin labels.

One of the agents producing stomatocytes is triton X, a well-known nonionic detergent. Micellar solutions of triton X are routinely used to extract lipids from biological membranes by solubilization. Therefore, they are likely to induce a negative spontaneous curvature, in agreement with the proposed explanation of the discocyte-echinocyte transition.

Concluding remarks

In this paper we intended to show that the deformation of bilayer vesicles may be influenced by self-induced spontaneous curvature of the membrane provided the deformation proceeds faster than the exchange of lipids between the two constituent monolayers. Furthermore, we are now in a position to justify the tacit neglect of lipid flip-flop in our previous calculations concerning only small deformations of bilayer spheres. The observations with red blood cells and the formulas derived earlier for the deformation of bilayer spheres in magnetic and electric fields point to possibilities of measuring the rate of lipid flip-flop in very direct ways.

Appendix:

To calculate

$$\oint (c_1 + c_2)\, \mathrm{d}A$$

for an ellipsoid of revolution up to second order in s_2 we may start from the representation

$$\mathrm{d}A = 2\pi a \cos t\,(a^2 \sin^2 t + b^2 \cos^2 t)^{1/2}\, \mathrm{d}t\,,$$
$$c_\mathrm{m} = a\,b\,(a^2 \sin^2 t + b^2 \cos^2 t)^{-3/2}\,,$$
$$\mathrm{d}A = 2\pi a \cos t\,(a^2 \sin^2 t + b^2 \cos^2 t)^{-1/2}\,.$$

Here $a = r_0 + s_2$ and $b = r_0 - s_2/2$ are the half axes of the ellipsoid and c_m and c_p are the principal curvatures which are along the meridians (c_m) and parallels (c_p). These expressions are expanded up to second order in s_2. Integrating over the angle t from 0 to π one finds a contribution of second order, with the first order terms cancelling each other. As the integral of the surface of the ellipsoid

also contains a term proportional to s_2^2 one has to renormalize r_0 so that the area is equal to that of the sphere. This results in another second-order contribution to the integral.

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[2] W. Helfrich, Z. Naturforsch. **28 c**, 693 [1973].
[3] W. Helfrich, Z. Naturforsch. **29 c**, 183 [1974].
[4] R. D. Kornberg and H. M. McConnell, Biochemistry **10**, 1111 [1971].
[5] R. Smith and C. Tanford, J. Mol. Biol. **67**, 75 [1972].
[6] Exactly speaking, the equilibrium is metastable because the number of visicles in solution in thought to be fixed. Otherwise, the vesicles would become fewer and larger until, ideally, only one is left.
[7] As a rough formula for the relaxation time τ of Δc_0 we may write

$$\tau \approx (\eta/k\, b^2) \cdot (1/q^2),$$

where q is the wave vector of a fluctuation of $(v_{\text{out}} - v_{\text{in}})$ and η a viscosity governing the sliding of the monolayers past each other. For an example, we take $\eta = 100$ poise, $q = 10^4\ \text{cm}^{-1}$ (corresponding to the size of red cells), $\varkappa = 10^9\ \text{dyn cm}^{-2}$ and $b = 5 \cdot 10^{-7}$ cm. The result is $\tau \approx 4 \cdot 10^{-3}$ sec.
[8] In this model the elastic modulus of stretching, as introduced previously [2], would be $k_S = b\,\varkappa$.
[9] W. Helfrich and H. J. Deuling, in preparation.
[10] See, *e. g.*, R. I. Weed and B. Chailley, Red Cell Shape (eds. M. Bessis, R. I. Weed, and P. L. Leblond), Springer-Verlag, New York 1973.
[11] M. A. Lichtman and G. V. Marinetti, ibid.