Bending stiffness of lipid bilayers

I. Bilayer couple or single-layer bending?

Thomas M. Fischer

Institut für Physiologie, Medizinische Fakultät, Rheinisch-Westfälische Technische Hochschule, D-W-5100 Aachen, Germany

ABSTRACT To describe the resistance of a bilayer to changes in curvature two mechanisms are distinguished which are termed bilayer couple bending and single-layer bending. In bilayer couple bending, the resistance arises from the 2-D isotropic elasticity of the two layers and their fixed distance. Single-layer bending covers the intrinsic bending stiffness of each monolayer. The two mechanisms are not independent. Even so, the distinction is useful since bilayer couple bending can relax by a slip between the layers from the local to the global fashion. Therefore, the bending stiffness of a bilayer depends on the time scale and on the extent of the deformation imposed on the membrane. Based on experimental data, it is shown by order of magnitude estimates that (*a*) the bending stiffness determined from thermally induced shape fluctuations of almost spherical vesicles is dominated by single-layer bending; (*b*) in the tether experiment on lipid vesicles and on red cells, a contribution of local bilayer couple bending can not be excluded; and (*c*) at the sharp corners at the leading and the trailing edge of tanktreading red cells, local bilayer couple bending appears to be important.

INTRODUCTION

Two notions

In their classical work on membrane mechanics, Evans and Skalak (1979) start from the notion that a lipid monolayer offers no resistance to bending. The observed bending stiffness of lipid bilayers is attributed by these authors to the coupling, i.e., the fixed distance normal to the membrane plane, of the two monolayers and the resistance of the lipid molecules to a change in their mean lateral distance. In mechanical terms, this means that the membrane reacts like a sandwich in which two thin strata, responsible for most of the 2-D incompressibility, are separated by a soft core. In view of the molecular structure of a lipid bilayer, the thin strata had to be identified with the headgroups and the core with the hydrophobic part of the lipid molecules.

In his first work on membrane bending stiffness Evans (1974) assumed that the two monolayers are able to slide relative to each other. He termed the resulting kind of bending stiffness "global" because the bending energy is determined by the average curvature of the whole membrane envelope. In contrast, when slip of the two layers is not allowed the bending energy at each point on the membrane depends on the local curvature. Accordingly, the respective kind of bending stiffness was termed "local."

Helfrich (1973), on the other hand, by analogy to liquid crystals considered the resistance of the lipid molecules against a relative tilt. Here, the resistance to bending is distributed over the whole thickness of the monolayer. This resistance gives rise to an intrinsic bending stiffness of a single monolayer which is local in the sense defined above.

A combination of both notions was suggested by Svetina et al. (1982), Stokke et al. (1986), and Waugh and Hochmuth (1987).

Nomenclature

For the bending stiffness emanating from the coupling of layers, I will use the term bilayer couple bending, following Sheetz and Singer (1974). The respective stiffness parameter (B_c) depends, according to Evans and Skalak (1979), on the isotropic moduli (K_1, K_2) of the constituent layers and their distance (h);

$$B_c = h^2 \frac{K_1 K_2}{K_1 + K_2}. \quad (1)$$

For the intrinsic bending stiffness of a monolayer, I will use the term single-layer bending. Each monolayer has its own stiffness parameter in single-layer bending. These sum up to the total value of the bilayer (B_s). The distance does not enter.

Interlayer slip

Except for the very recent work of Waugh et al. (1992) the bending stiffness of vesicles or red cell membranes was determined by fitting to models based on local bending (Servuss et al., 1976; Evans, 1983; Schneider et al., 1984; Bo and Waugh, 1989; Faucon et al., 1989; Evans and Rawicz, 1990; Mutz and Helfrich, 1990; Duwe and Sackmann, 1990). According to the notion of Evans and Skalak (1979), this would correspond to the absence of interlayer slip. This, however, is hard to reconcile with the well known lateral diffusivity of lipid molecules in a bilayer as demonstrated in the following gedanken experiment. Fig. 1 shows schematically an unstressed plane piece of membrane in cross-section (Fig. 1 *A*) which is bent cylindrically into a positive and a negative half wave (Fig. 1 *B*). The edges of the piece are kept flush. We assume the bending was so fast that no lateral rearrangement of lipids could occur. The compressed lipids are shown in light shading (Fig. 1 *B*). At the line separating the two half waves, the isotropic tension in each monolayer changes the sign. The resulting step in 2-D pressure drives a lateral flow of lipids which is equivalent to a slip between the layers (Fig. 1 *C*).

In dynamic deformations of a lipid vesicle or of a red cell, one has to consider time. The velocity of lateral flow

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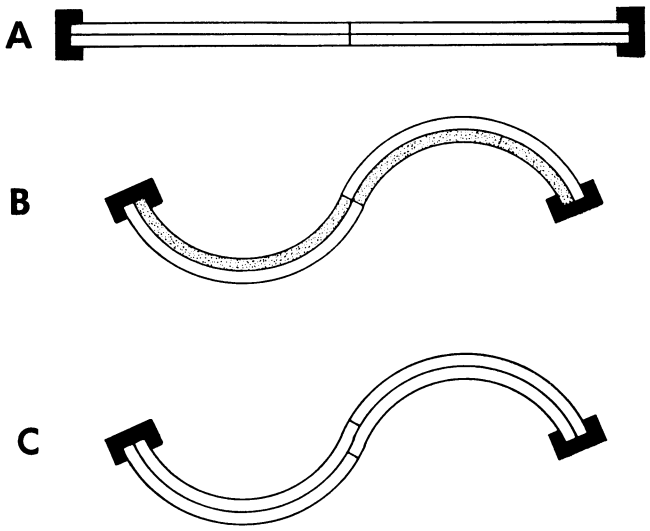

FIGURE 1 Local and global bilayer couple bending. Schematic drawing of an unstressed piece of a bilayer in cross section (*A*); after a cylindrical deformation in a positive and negative halfwave (*B, C*); no slip in local bending, the compressed portions of the two leaflets are shaded (*B*); slip in global bending (*C*).

of lipids depends on the gradient in 2-D pressure and on friction. In lipid bilayers without embedded intrinsic proteins exist two contributions to friction: (1) shear between the layers (the vorticity vector lies in the plane of the membrane); and (2) shear within each layer (the vorticity vector is normal to the plane of the membrane). In the red cell membrane we have another contribution: flow past the intrinsic proteins, which are to some extent connected to the membrane skeleton (the vorticity vector is normal to the plane of the membrane).

Energy stored in bending

Following Helfrich (1973), the expression for the elastic energy stored in (local) single-layer bending (E_s) can be written:

$$E_s = 2B_s \int (c - \xi_s)^2 \, dA, \tag{2}$$

where c is the local mean of the actual curvature of the membrane and ξ_s is the local mean of the spontaneous curvature in single-layer bending. The definition of ξ_s differs by a factor of 2 from the definition of a spontaneous curvature introduced by Helfrich (1973). In the Appendix it is shown that ξ_s is in accordance with what one would call, intuitively, the spontaneous curvature. dA denotes a surface element of the vesicle.

An analogous formula describes the energy (E_{lc}) stored in local bilayer couple bending:

$$E_{lc} = 2B_c \int (c - \xi_c)^2 \, dA, \tag{3}$$

where ξ_c is the local mean of the spontaneous curvature in bilayer couple bending (for a definition see Appendix). In contrast to ξ_s, ξ_c is, in general, not uniform on the membrane (see Appendix).

In global bilayer couple bending, the isotropic tension in each monolayer is uniform on the surface (Fig. 1). This means the lateral distribution of ξ_c is such that $(c - \xi_c)$ in Eq. 3 is uniform on the surface. The energy stored in global bilayer couple bending (E_{gc}) can therefore be written as:

$$E_{gc} = \frac{2B_c}{A}\left[\int c - \xi_c \, dA\right]^2. \tag{4}$$

Aims of the paper

In this work, order of magnitude estimates are presented to decide whether the published elastic constants are due to bilayer couple or single-layer bending. I will estimate whether slip is fast enough to follow the changes in curvature imposed by the external forces in two typical experiments set up for the measurement of membrane bending stiffness. Based on these estimates, I will exclude local bilayer couple bending as the kind of bending stiffness resisting the thermal shape fluctuations of freely suspended vesicles (Schneider et al., 1984; Faucon et al., 1989; Duwe and Sackmann, 1990). Based on experimental observations it is then inferred that global bilayer couple bending does not contribute either and that consequently single-layer bending dominates the elastic response. This in turn indicates that the measured bending stiffness corresponds to B_s. In case of the tether experiment on lipid vesicles and on red cells (Hochmuth et al., 1982; Bo and Waugh, 1989) local bilayer couple cannot be excluded. This would mean that partially B_c is measured in these experiments.

The considerations are then extended to the continuous bending deformation of the red cell membrane during the tanktread motion (Fischer, 1980).

LIPID VESICLES

Analysis of thermally induced shape fluctuations

First, we consider experiments in which thermally induced shape fluctuations of almost spherical, freely suspended vesicles (Duwe and Sackmann, 1990) were observed. For the estimate we assume that a typical fluctuation occurred so fast that a lateral flow of lipid could not take place. From vesicle geometry, local changes in 2-D pressure are calculated. Relaxation times (t) of lateral pressure differences are then estimated for the two frictional contributions. These times are compared to the experimentally observed decay times (τ) of the shape fluctuations.

Observed shape fluctuations have been decomposed in a combination of modes. Accordingly, the decay times t were determined for each mode separately. To estimate local changes in surface area and in linear di-

mensions of the monolayers the time-average contour of a vesicle is assumed as flat. Deviations from the plane are considered as spherical segments (sphere radius ρ and sector angle 2β). From the vesicle diameter ($D = 20\ \mu m$), the mode numbers (l), and the respective amplitudes (α) (Duwe, 1989) ρ and β are calculated according to:

$$\rho = \frac{[D\pi/(2l)]^2}{8\alpha} + \frac{\alpha}{2} \tag{5}$$

and

$$\cos\beta = 1 - \alpha/\rho. \tag{6}$$

It will turn out below that the neutral fiber of a monolayer is approximately in its middle. The relative change in surface area of a monolayer is then

$$\Delta A/A = (d/2)/\rho, \tag{7}$$

where d denotes the total thickness of the bilayer, taken as 4 nm. The change in length (Δs) of the neutral fiber of a monolayer along a meridian of the spherical segment is:

$$\Delta s = \beta d/4. \tag{8}$$

Both $\Delta A/A$ and Δs are given as absolute values. If we consider a fluctuation to the outside of the vesicle both changes are positive for the outer and negative for the inner monolayer and vice versa for a fluctuation to the inside.

We now assume that these changes prevail on the curved surface of the vesicle. We neglect the difference in curvature between outside and inside fluctuations and consider a spherical segment of the time-average vesicle surface. The altitude (H) and radius of base surface (R_b) of the segment can be calculated from mode number and vesicle diameter (D):

$$H = (D/2)(1 - \cos\varphi) \tag{9}$$

and

$$R_b = (D/2)\sin\varphi, \tag{10}$$

where $\varphi = \pi/(2l)$.

We assume the pressure difference between adjacent segments to decay by a time-independent flow of lipid molecules and take the time for complete pressure equilibration as a measure for t. The velocity in meridional direction of a segment is called v. At the border of the segment its value is $\Delta s/t$. To describe the distribution of v on the surface we use cylindrical coordinates: r for the radial position and h for the position along the axis of symmetry of the spherical segment. The distribution of v on the segment is assumed proportional to r. This keeps the surface density of the molecules on the surface approximately uniform. The velocity is then

$$v(r) = \frac{\Delta s}{t}\frac{r}{R_b}. \tag{11}$$

The power dissipated per volume by the first frictional contribution (vorticity tangent to the membrane plane) is approximated by $\eta_1\gamma^2$, where η_1 is the 3-D viscosity and γ a constant 3-D shear rate within the hydrophobic part of the bilayer. It is assumed that the shear flow is distributed over one third of the membrane thickness, according to the strong decrease of the order parameter of the hydrocarbon chains (Seelig and Seelig, 1974) in this region. The shear rate is then

$$\gamma = 6v/d. \tag{12}$$

For η_1 a value of 1 P was assumed according to spectroscopic measurements (Best et al., 1987). The choice of γ and η_1 results in a coefficient for viscous friction which is in the upper part of the range determined experimentally by Merkel et al. (1989). The power dissipated per monolayer by the first frictional contribution (PD_1) is obtained by integration over the surface of the segment:

$$PD_1 = \frac{d}{6}\eta_1\int_0^H \gamma^2 D\pi\, dh. \tag{13}$$

The limits of integration are constant. This is equivalent to a flow of material at the border of the segment from one monolayer into the other. In reality material flows across the border to an adjacent segment.

The power dissipated per surface area by the second frictional contribution (vorticity perpendicular to the membrane plane) is given by $2\eta_2(v_r/r)^2$ (Evans and Skalak, 1979), where η_2 is the 2-D viscosity of a lipid monolayer, and v_r the radial component of v. For a conservative estimate we used a value of 2.5×10^{-6} dyn s/cm (Waugh, 1982) for η_2. The power dissipated per monolayer by the second frictional contribution (PD_2) is obtained by integration:

$$PD_2 = 2\eta_2\int_0^H \left(\frac{v}{r}\frac{D/2 - h}{D/2}\right)^2 \pi D\, dh. \tag{14}$$

The dissipated power is provided by the areal relaxation of the layers. The energy stored per surface area is $(K/2)(\Delta A/A)^2$, where K is the isotropic modulus of a lipid monolayer. A value of 70 dyn/cm was used (Evans and Rawicz, 1990). Multiplication with the surface area of the segment and division by the time gives the available power (PA):

$$PA = K(\Delta A/A)^2 D\pi H/(2t). \tag{15}$$

Equating the dissipated power with the available power results in equations for the characteristic times t_1 (Eqs. 13 and 15) and t_2 (Eqs. 14 and 15) for the two frictional contributions. Their values for $l = 2, 3, 4$ are shown in Table 1 together with the experimentally observed values (τ). Higher order fluctuations are not shown because

TABLE 1 **Decay times in thermally induced shape fluctuations of lipid vesicles**

l	Mode number;
τ	Experimentally observed (Duwe and Sackmann, 1990) decay times for shape changes;
t_1, t_2	Theoretically estimated decay times for curvature-induced lateral pressure gradients;
t_1	Taking into account friction between layers;
t_2	Taking into account friction within each layer;

l	τ (s)	t_1 (ms)	t_2 (ns)
2	2.6	35	32
3	0.7	15	34
4	0.4	8	35

no corresponding values for τ have been reported (Duwe and Sackmann, 1990).

Addition of cholesterol to phospholipids has been shown to increase the isotropic modulus (K) of bilayers by a factor of 5 (Needham and Nunn, 1990). This would increase PA by the same factor. From spectroscopic measurements (Shinitzky and Inbar, 1976) we can expect η_1 and therefore PD_1 to be increased by about a factor of 5 as well. It is, therefore, likely that the values for t_1 (Table 1) apply for this case also.

Exclusion of local bilayer couple bending

It is obvious from Table 1 that the first frictional contribution dominates the second. But even t_1 is orders of magnitude smaller than τ, indicating that there is plenty of time for slip. It is concluded that bilayer couple bending acts in these measurements only globally. It is likely that this applies to the other methods as well which use thermally excited shape fluctuations (Evans and Rawicz, 1990; Mutz and Helfrich, 1990; Kummrow and Helfrich, 1991).

Exclusion of global bilayer couple bending

Having excluded one mechanism (local bilayer couple bending), the question arises how much each of the remaining mechanisms (global bilayer couple or single-layer bending) contributes or whether one of them dominates. A hint comes from the observation of shape fluctuations on oligolamellar vesicles. The thickness of the water layer between the lamellae depends on preparation history. Usually vesicles are selected that show a single contour under the light microscope. This means in large undulations (as are used to determine the bending stiffness) the lamellae are deformed in concert. In the small undulations which cannot be resolved by the light microscope, the lamellae may oscillate independently. This would hold the interlamellar distance constant due to the steric interaction introduced by Helfrich (1978).

In a bilamellar vesicle, the distance between the two outer lipid monolayers is at least twice as large as the interlayer distance in a bilayer. It is reasonable to assume that the velocity of interlamellar slip is at least as large as that of the interlayer slip estimated above. Due to the quadratic dependence on h (Eq. 1), B_c as measured from global bilayer couple bending would, therefore, be at least four times as large compared to a unilamellar vesicle. In contrast, values for the bending stiffness were measured in the various kinds of experimental setups that were two, three, and four times as large as the minimal value (Duwe and Sackmann, 1990; Mutz and Helfrich, 1990; Schneider et al., 1984). This indicates that global bilayer couple bending does not appreciably resist the shape fluctuations. The only mechanism remaining is single-layer bending where the values for the bending stiffness of the lamellae simply add up, which is in keeping with the experimental observations.

Molecular basis of bending rigidity

With the dominance of single-layer bending the resistance against area changes cannot, as in the sandwich model, be ascribed exclusively to the headgroup region. On the contrary, the headgroups appear to contribute very little as indicated by the geometrical data on phospholipids in the various liquid cristalline phases. Upon transitions between these phases the area per headgroup changes about 10 times as much as the thickness of the layers and the thickness in turn changes 10 times as much as the volume of the layers (Kirk et al., 1984). These observations suggest that each layer behaves as an incompressible continuum of finite thickness. In such a layer both a change in surface area and in curvature involve a transverse shear deformation. It, therefore, appears that the resistance of the hydrocarbon chains against transverse shear is responsible for the resistance of a monolayer against area change as well as bending.

Here, we assume the properties of a monolayer to be uniform in thickness direction. A similar approximation was made by Waugh and Hochmuth (1987) for small radii of curvature. The neutral fiber of such a layer lies approximately in its middle.

It can easily be shown that in a membrane assembled by tight apposition of two such layers $B_c = 3B_s$. An experimental determination of both parameters in lipid vesicles gave approximately this relation (Waugh et al., 1992).

Interpretation of data

Because Eqs. 2 and 3 are formally identical, bending stiffness parameters obtained from the measurement of thermally induced shape fluctuations that were previously interpreted as B_c can now be interpreted as B_s.

With this reinterpretation, it is possible to explain a discrepancy noted by Evans and Rawicz (1990). The values determined experimentally by these authors for the

bending stiffness were an order of magnitude lower than the values expected from Eq. 1 when a value of 4 nm was taken for h. The isotropic moduli (K) to be inserted had been determined experimentally in the same experiment. Instead of using the whole bilayer thickness for h we now use half this value according to our assumption of the location of the neutral fiber of the individual monolayers. This reduces the calculated value for B_c by a factor of 4. The remaining discrepancy which averages to 2.8 for the five kinds of lipids and lipid mixtures used by Evans and Rawicz (1990) compares well with the expected ratio of B_c/B_s.

Considering just the data on pure lipids collected by Evans and Rawicz (1990), the values of B_c/B_s correlate with the degree of unsaturation of the hydrocarbon chains. The deviation of B_c/B_s from the mean value becomes even larger when instead of a common value for h individual values (Rand and Parsegian, 1989) are used for each kind of lipid. These deviations can be explained when the curvatures of the monolayers are accounted for individually (Fischer, 1992*a*).

Analysis of tether extension

The measurements are performed in mechanical equilibrium, i.e., at small rates of change in tether length (Bo and Waugh, 1989). It is, however, not guaranteed that the lateral position of the lipids in the membrane is in equilibrium as well. This is checked by the following estimate using the published experimental data.

For simplicity it is assumed that the rate of increase in tether length (called tether velocity in the following) is constant during the experiment. As can be seen from the time registration in Fig. 4 of Bo and Waugh (1989) the mean tether velocity (v_p) is ~ 5 μm/s. The relative difference in surface area between outer and inner layer of the tether membrane is $d/(2R_t)$, where R_t denotes the radius of the tether.

If v_p were infinitely large the area per headgroup would increase in the outer layer of the tether and decrease in the inner one by $d/(4R_t)$. At finite v_p the lipid flow producing the tether will be faster in the outer than in the inner layer, leading to a relative change ($\Delta A/A$) in area per headgroup in each layer the absolute value of which is smaller than $d/(4R_t)$. The deviation (v_d) from the average flow velocity (v_p) at the border between tether and body of the vesicle is obtained from a flow balance. For an approximate calculation we use the linearized form:

$$v_d = v_p\left(\frac{d}{4R_t} - \frac{\Delta A}{A}\right). \tag{16}$$

The difference in lateral pressure between vesicle and tether that drives in each layer the deviation from the average flow is considered to be proportional to $\Delta A/A$. The difference in isotropic tension between inner and outer layer that accumulates in the body of the vesicle with increasing tether length is neglected in this estimate. This is equivalent to the assumption that the surface area of the vesicle is much larger than that of the tether.

At constant tether velocity, $\Delta A/A$ will be constant along the tether. Accordingly, the slip between the layers occurs in the body of the vesicle, which is approximated by a flat disk of radius D, D being the diameter of the vesicle. The distribution of the velocity (v) on this disk is assumed to be proportional to $1/r$. This choice makes the flow incompressible. With the boundary condition at the border between tether and vesicle we obtain:

$$v(r) = v_d R_t/r. \tag{17}$$

From $\Delta A/A$, the force (F) at the border between the tether and the body of the vesicle is calculated:

$$F = (\Delta A/A)K2\pi R_t. \tag{18}$$

F is balanced by the force emanating from friction. For the first contribution we obtain:

$$F_1 = \int_{R_t}^{D} \eta_1 \gamma 2\pi r \, dr. \tag{19}$$

Equating Eqs. 18 and 19 and using Eqs. 12, 17, and 16 results in an equation for $\Delta A/A$. It is interesting to note that R_t drops out. Inserting the numerical values gives a value of 0.18 for $\Delta A/A$ relative to $d/(4R_t)$. As above, it can be shown that the influence of the second frictional contribution is much smaller.

Interpretation of data

As such, this result indicates that 18% of bilayer couple bending is local. Because of the approximative nature of the calculations, no quantitative statement can be made. It is, however, instructive to look at the consequences if this number were true. First, we neglect global bilayer couple bending against single-layer bending in accordance with Waugh and Hochmuth (1987). We are left with two local contributions and assume that the stiffness in bilayer couple bending is $0.2B_c$. With the approximate relation $B_c = 3B_s$, it follows that the measured bending stiffness would be equal to $1.6B_s$. This could be an explanation for the relatively large value that was obtained by Song and Waugh (1990) and Waugh et al. (1992) compared to others (Faucon et al., 1989; Mutz and Helfrich, 1990; Evans and Rawicz, 1990).

RED CELLS

Before we apply the results obtained for vesicles to the red cell case we have to deal with the presence of (intrinsic) proteins embedded in the bilayer. The flow of lipid molecules past these proteins gives rise to the third frictional contribution in which the vorticity is again perpendicular to the membrane plane. The force (F) on a single protein is adopted from calculations of the flow past an infinite periodic array of cylinders (Drummond and Tahir, 1984):

$$F = \frac{8\pi\eta_2 v}{\ln(1/\epsilon) - 1.4975 + 2\epsilon - \epsilon^2/2}, \quad (20)$$

where η_2 is the 2-D viscosity of the lipids, v the average flow velocity past the proteins, and ϵ the surface fraction of the intrinsic proteins within the bilayer.

Only bands 3 and 4.5 were taken into account in this estimate. The glycophorins were neglected because of their smaller size and number. It is not clear whether band 3 and band 4.5 exist as dimers or tetramers in the red cell membrane. The calculation of their surface density (σ) was based on a trimeric state (number of copies = 7.1×10^5 and surface area of the red cell = 135 μm^2). For simplicity, an equidistant arrangement of the intrinsic proteins was assumed although the laterally mobile ones may be dragged towards the border of a "corral" (Sheetz, 1983). The cross-section of the membrane spanning portion of the proteins was estimated by assuming a close packing of alpha helices (diameter = 1 nm). This results in a value of 15% for ϵ.

The value for η_2 was estimated from lateral diffusion measurements of lipid probes using a relation given by Saffman and Delbrück (1975). The diffusion constant was adopted from the measurements of Cribier et al. (1990) on artificial vesicles made of lipids extracted from red cells. Correction for the difference between the temperature at which the diffusion measurements were done (37°C) and room temperature (23°C) at which the mechanical experiments were performed was made using the data of Bloom and Webb (1983). For the viscosity of a monolayer (average between inner and outer one) we obtained 1.7×10^{-5} dyn s/cm. This is much larger than the value reported by Waugh (1982) for vesicles made from egg-phosphatidylcholine. But note that for this lipid, Cribier et al. (1990) obtained a smaller value than Waugh (1982) which is, in keeping with the assessment of this author, that his data represent an upper bound of the real value.

Comparison of the force per surface area resulting from the first frictional contribution ($\eta_1\gamma$) with that resulting from the third one ($F\sigma$) shows that the third one is larger by more than an order of magnitude.

Tether extension

In applying the vesicle analysis to the red cell we use the following experimental values: a tether velocity of 0.5 μm/s (Hochmuth et al., 1982), an equivalent red cell diameter of 6.6 μm, and an isotropic modulus of a monolayer of 225 dyn/cm (Evans and Skalak, 1979). From the third and dominating frictional contribution the force F_3 emanates:

$$F_3 = \int_{R_t}^{D} F\sigma 2r\pi \, dr. \quad (21)$$

From Eqs. 18, 20, and 21 we obtain 4.6% for $\Delta A/A$ relative to $d/(4R_t)$. For this estimate it was assumed that the whole membrane flows unchanged from the red cell body into the tether. Experimental evidence (Berk and Hochmuth, 1992) indicates, however, that the membrane skeleton is strongly disturbed within or may not even enter the tether. Concomitant with this disturbance a concentration of intrinsic proteins could occur at the location where the tether is pulled from the red cell body. To make a rough estimate of this effect we assume that no intrinsic proteins enter the tether but that they are collected in the red cell body at twice their normal density in a ring concentric to the tether. This would increase the above ratio from 4.6% to 5.9%. Again, these numbers are approximate. If indeed 6% of bilayer couple bending were local, the bending stiffness deduced for the red cell membrane would be $1.2B_s$.

Membrane tanktreading

In the tanktread motion of the red cell, the membrane flows with high velocity around a sharp corner at the leading and the trailing edge of an elongated red cell. It is therefore, interesting to ask whether bilayer couple bending is local or global at this location.

For an estimate, we consider the flow of a plane membrane around a half cylinder to make a 180° bend. The radius of the cylinder (R_c = 1 μm) and the velocity of the membrane (v_m = 50 μm/s) are adopted from an experiment (Fischer, 1980) where a cell is subjected to a shear rate of 42/s. In the cylindrical portion the lipids are compressed in the inner and expanded in the outer layer by $d/(4\ R_c)$. In dynamic equilibrium there is a flow v_l of lipids relative to the membrane skeleton between regions of different surface pressure. For simplicity we assume the lipids to move down a constant gradient within a strip that separates the flat and the curved region. v_l is obtained from a flow balance:

$$v_l = v_m d/(4R_c). \quad (22)$$

The force balance for the third frictional contribution reads:

$$Kd/(4R_c) = F\sigma\Delta y, \quad (23)$$

where Δy is the width of the strip. Inserting v_l for v into Eq. 20, and using the same numbers as before results in a value of 1.4 μm for Δy. As such this result means that in about half of the curved region bilayer couple bending is local. In the transition regions between the different surface pressures the elastic energy is between that for pure local and for pure global bilayer couple bending. We conclude that local coupling should be important in this case.

It is interesting to ask whether the power dissipated in the transition region would contribute appreciably to the total energy dissipation in the red cell membrane. From velocity times force the dissipated power (PD_3) is obtained as:

$$PD_3 = 2v_1F\sigma A_t, \quad (24)$$

where A_t denotes the surface area of the transition regions on the red cell membrane. It was taken as 60 μm^2 which is one third of the total surface area of this particular cell. The factor of 2 in Eq. 24 is to cover both monolayers. Inserting the numbers into Eq. 24 gives a dissipated power of 10^{-8} erg/s. For the power dissipated due to shear deformation of the membrane a value five times as large was calculated (Fischer, 1980). It therefore appears that the friction involved in the continuous membrane bending during tanktreading contributes appreciably to the overall energy dissipation.

CONCLUSIONS

The general conclusion reached in this paper is that the bending stiffness of a bilayer depends on the time scale and the extent of the deformation imposed on the membrane. The particular results are summarized as follows. (*a*) The bending stiffness determined from thermally induced shape fluctuations of quasispherical vesicles is dominated by single layer bending. The published values have to be interpreted as B_s. (*b*) In the tether experiment on lipid vesicles and on red cells, a contribution of local bilayer couple bending can not be excluded. If local bilayer couple bending would actually contribute B_s would be smaller than the published value for the bending stiffness. (*c*) At the sharp corners at the leading and trailing edge of tanktreading red cells, local bilayer couple bending appears to be important. The energy dissipation involved in bilayer couple bending can not be neglected against that from shear deformation.

APPENDIX

Definition of spontaneous curvatures

The spontaneous curvatures used above can be operationally defined as follows. We consider a circular piece of the membrane of a vesicle (Fig. 2). It should be large enough for continuum mechanics to apply and small enough to have uniform actual curvature.

We now imagine to cut the piece out of the closed membrane envelope and neglect edge effects. The piece will then assume a uniform curvature. There is good evidence that the deviatoric part of this curvature vanishes (Fischer, 1992*b*). The isotropic part (or mean value) I choose to call the spontaneous curvature.

If the two lipid layers are kept flush at the rim (no slip) one would observe the net spontaneous curvature (ξ). If a slip between the two layers but no separation of them is allowed one would observe the spontaneous curvature due to single-layer bending (ξ_s). The spontaneous curvature due to bilayer couple bending (ξ_c) could be obtained from the difference (Fig. 2) taking into account that ξ is the sum of the two contributions ξ_c and ξ_s weighted by their share in total bending stiffness.

It follows from this definition that ξ_c is a local value. Due to interlayer slip, its distribution on the membrane depends on shape history of the whole membrane envelope. Its value averaged over the membrane surface, on the other hand, is a constant that depends on the surplus in surface area of one of the two layers. As to single-layer bending, it can be shown by thermodynamical reasoning that in the absence of strong intermolecular attraction different molecular species remain essentially uniformly distributed on the surface, i.e., ξ_s is a constant. Each layer has an extra spontaneous curvature which depends on the packing properties of the molecules in this layer.

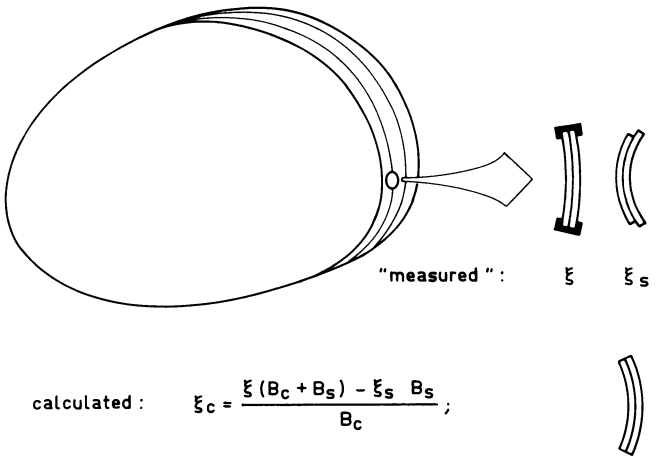

FIGURE 2 Spontaneous curvatures of a vesicle membrane. Schematic drawing of an operational definition of the spontaneous curvatures (ξ_c in bilayer couple bending and ξ_s in single layer bending). For details see text.

The sign convention is such that the spontaneous curvatures are positive in a spherical vesicle irrespective of the orientation of the monolayers within the bilayer. ξ_s is equal to the sum of the values of both monolayers weighted by their share in B_s. It is clear that ξ_s is zero for symmetrical membranes while this is not necessarily the case for the average value of ξ_c.

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