Phospholipid-bilayer Vesicle Shapes and Shape Transformations: Theory vs. Experiment

Michael Wortis

Abstract

Laboratory preparations of micron-scale fluid-phase phospholipid vesicles exhibit a rich fauna of vesicle shapes and a complex systematics of shape transformations induced by changing accessible control parameters. At the same time, there is a well-developed theory of vesicle shapes based on the concept of minimizing the elastic bending energy. This contribution describes progress that has recently been made in comparing theory and experiment in a quantitative manner.

Michael Wortis
Physics Department
Simon Fraser University,
Burnaby, BC, Canada V5A 1S6
e-mail: wortis@sfu.ca

Biol. Skr. Dan. Vid. Selsk. 1998, **49**:59–63

Theory of Vesicle Shapes

Canham (Canham, 1970) and Helfrich (Helfrich, 1973) in the early 1970's were the first to propose that the shapes of fluid-phase phospholipid vesicles in aqueous solution are determined by simple minimization of an elastic bending energy. Deuling and Helfrich (1976) then produced a catalogue of minimum-energy shapes in good correspondence with the simple shape classes observed in the laboratory for both red blood cells (rbc's) and artificially prepared pure-lipid vesicles. Returning to this problem in the early 1990's with the aid of better computational facilities, we (Miao et al., 1991) and other groups (Seifert et al., 1991) began to map out the phase diagram of Helfrich's (Helfrich, 1973) model, showing the energy-minimizing shapes as a function of the control parameters. Two things became clear. First, we found that, for appropriate parameters, budded or vesiculated shapes, with one or more small (infinitesimal) necks, were minimizing shapes (Miao et al., 1991; Fourcade et al., 1994). Second, in comparing the calculated phase diagram with experiments in which shape transitions were induced by smoothly varying the control parameters (e.g., by varying the temperature), it became clear that the original Helfrich model was systematically inconsistent with experiment.

Several groups (Seifert et al., 1991; Miao et al., 1994; Svetina and Zeks, 1989) worked in the early 1990's to understand these discrepancies based

on ideas originally put forward earlier by Evans (1974; 1980), Helfrich (1974), and others. The physics behind these studies was the observation that the rate of flip-flop—the exchange of phospholipid molecules between the two leaves of the bilayer— is slow on mechanical time scales. Thus, the number of molecules on the inside and outside leaves of the bilayer—and, therefore, the relaxed area difference ΔA_0 between the two bilayer leaves—are conserved quantities. The upshot of this work (Miao et al., 1994) was the so-called area-difference elasticity (ADE) model, defined by the Hamiltonian,

$$H_{ADE}[S] = \frac{1}{2}\kappa_b\left[\oint dA\big(C_1(\mathbf{r}) + C_2(\mathbf{r}) - C_0\big)^2 + \frac{\alpha\pi}{AD^2}\big(\Delta A[S] - \Delta A_0\big)^2\right]. \tag{1}$$

In this expression, the first term is the original Helfrich bending energy (Helfrich, 1973), where $C_1(\mathbf{r})$ and $C_2(\mathbf{r})$ are the two local principal curvatures at the point $\mathbf{r}$ on the vesicle surface and C_0 is the so-called spontaneous curvature, which reflects any intrinsic asymmetry between the inner and outer bilayer leaves. The second term is the area-difference contribution, which measures the elastic energy cost of forcing the actual area difference ΔA (between inner and outer leaves) to differ from the relaxed area difference ΔA_0. The actual area difference ΔA depends on the shape $[S]$ of the vesicle via the relation,

$$\Delta A[S] = D\oint dA\big(C_1(\mathbf{r}) + C_2(\mathbf{r})\big), \tag{2}$$

where D is the interleaf separation (assumed fixed), A is the vesicle area, α is a dimensionless material constant (generically of order unity), and κ_b is the Helfrich bending rigidity. The fact that κ_b is 10—20 times the thermal energy k_BT for typical phospholipids near room temperature means that the shape problem is a low-temperature, purely mechanical problem to first approximation. The stable mechanical shapes are predicted to be the local minima of Eq. (1) subject to constraints of fixed volume V and area A. Because of the particular form of Eq. (2), it turns out that these shapes are precisely those of the Deuling-Helfrich catalogue (Deuling and Helfrich, 1976), only with a self-consistently determined effective value of the spontaneous curvature C_0.

Equation (1) is at present qualitatively consistent with experimental observations (in a way that the Helfrich Hamiltonian (Helfrich, 1973) was not); however, until recently, it had never been directly tested. This may seem surprising; but, the reasons are rather simple. A direct test would be to measure the control parameters of a particular experimental vesicle, A, V, C_0, and ΔA_0 (κ_b, α, and D may be assumed known), and then to compare the observed and predicted shapes. There are two difficulties with this scenario. First, the control parameters cannot be simply measured: A and V must somehow be inferred from 2D phase-contrast microscope images, while C_0 and ΔA_0, not being geometrical, cannot be inferred from the microscope images. Second, thermal shape fluctuations are not really small (indeed, near shape instabilities they can be quite large!), so what one measures in the laboratory for a given vesicle is a time-sequence of shapes, constituting a thermal shape ensemble. One must find some way to infer from this shape ensemble the corresponding zero-temperature, mechanical shape, which is the object most directly predicted by the theory. We have developed (Döbereiner et al., 1997) a procedure, combining theory with observation of micron-scale vesicles by phase-contrast microscopy, which allows these difficulties to be circumvented and provides what we believe to be the first direct quantitative confrontation of theory with experiment. It is not, so far, a very extensive test, nor is it at all precise. On the other hand, it remains, in our view, the only one available. Theory appears to pass the test (Döbereiner et al., 1997).

As a consequence, we have at this point what appears to be a viable theory of single-component fluid-bilayer vesicle shapes and, thereby, an understanding of the influence on these shapes of a (small) set of control parameters—at the level of a fairly extensive but still incomplete shape/phase diagram. By systematically manipulating these control parameters, we know how to move vesi-

cles across instability boundaries (spinodals, in the language of phase transitions) so as to produce systematically in the laboratory shape transitions like budding and vesiculation, the discocyte-to-stomatocyte transition, etc.

What does this have to do with biological membranes? To first approximation, the honest answer may be "not much"; but, let me make a few remarks, anyway. The main issue here is that biological membranes, even those as simple as the rbc membrane, are much more complicated than one-component lipid bilayers.

Effect of the Cytoskeleton on the Red Blood Cell Shape

The rbc cytoskeleton is a tethered network of protein polymers anchored to the interior side of the plasma membrane (but not, as in many cells, extending into the cytosol). Unlike the (fluid) plasma membrane, the cytoskeleton has a well defined shape and its shape mechanics cannot be described by an energy functional of the form of Eq. (1) but must include local dilation and shear elasticities (in addition to bending). In situations where these contributions are important (as they certainly are at high deformation), an approach to rbc shapes based on Eq. (1) alone (and on the control parameters we have identified above) is bound to fail. However, there is some evidence that the cytoskeletal contribution to the energy is rather small for weak deformations of the normal discocyte, so that the discocyte and shapes not too far from it (including those accessed by thermal flickering) can probably be effectively described by Eq. (1). In this spirit, it may be argued that echinocyte shapes (which do not show up in the Deuling-Helfrich catalogue (Deuling and Helfrich, 1976) occur when the plasma membrane by itself would like to form multiple outward buds (i.e., when it has extra material in the outer leaf of the bilayer). Such buds require large shear deformations near the narrow necks and are thus suppressed by the cytoskeleton, resulting in the smooth crenellations characteristic of echinocytes. This hypothesis remains untested, as far as I know.

Effect of Lipid Mixtures

Typical biological membranes contain a mixture of many different lipids plus important proteins, etc. As long as this mixture remains spatially homogeneous, it can be characterized at long wavelength by appropriate (average) values of the parameters, κ_b, α, C_0, and D. On the other hand, as soon as inhomogeneities occur, in the form of either spatial fluctuations or full phase separation, then modification of Eq. (1) is required. (And, indeed, an additional term involving the Gaussian curvature cannot be ignored, as we have done above.) These modifications are not hard to incorporate and a few calculations have been carried out (Lipowsky, 1995). The issue is interesting when the compositional degrees of freedom are coupled to the geometric ones (e.g., different components having different κ_b's and/or different C_0's) and it is further complicated by the fact that compositional fluctuations at the same point on the bilayer but in different leaves may be coupled. When full phase separation takes place, the phase boundary acts as a line under tension, which under appropriate conditions can promote bud formation (just as an elastic band might pull closed the open neck of a bag)—a process called domain-induced budding (Lipowsky, 1992; Jülicher and Lipowsky, 1996).

Another possible consequence is the aggregation of species favoring large Gaussian curvature in a neck region, thus lowering the energy of neck formation. Such effects may also be associated with the experimental observation that buds, once formed, tend to fission spontaneously in some systems but not in others (Döbereiner et al., 1993).

Budding Mechanisms

It is well established that the mechanisms described above, based on Eq. (1) and its associated control parameters, can and do produce budding (and other shape instabilities) in artificial vesicles and in rbc's. It is also known that many instances of cellular and intracellular budding are driven by specific, energy-consuming processes involving proteins such as clathrins. I cannot point to any cases where it has been established that a particular biological budding processes proceeds via (biochemical) manipulation of the control parameters of Eq. (1); however, there are many budding processes the mechanisms of which remain to be understood. What is clear in any case is that, to initiate budding, nature must control the energy landscape set by Eq. (1) or its generalization in the case of lipid mixtures and/or cytoskeletal involvement. In this connection, it is interesting to note that estimates of the closure energy of clathrin cages come in just about an order of magnitude larger than the energy scale κ_b of typical membrane lipids. This is probably not an accident. One may speculate as to whether at early stages in cellular evolution, before the development of clathrin and clathrin-like mechanisms, nature made more general use of the "physical" control parameters whose action we have studied.

Summary

In summary, the significance of understanding the shape mechanics of one-component lipid bilayer membranes probably does not lie principally in any direct or immediate application to cellular processes. Such applications will usually have to incorporate elaborations of the simple theory discussed here to account for the richer biochemical environment of the working cell. What our work (and that of our many collaborators and competitors) has shown is that we are at least on our way to understanding at a quantitative, predictive level the mechanical properties of one of the simplest biological materials, the fluid-phase phospholipid bilayer. Such bilayers (in the many cellular contexts where they occur) form a substrate for much important cellular machinery. And, it is increasingly being appreciated that this material is far from passive but modulates in crucial ways the structure and behavior of the proteins and other biomolecules that use it as a substrate.

***

This research was supported by the Natural Sciences and Engineering Research Council of Canada.

References

Canham, P.B. 1970. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *J. Theor. Biol.*, 26, 61–81.

Deuling, H.J., and W. Helfrich. 1976. The curvature elasticity of fluid membranes: A catalogue of vesicle shapes. *J. Physique (Paris)*, 37, 1335–1345.

Döbereiner, H.-G., E.A. Evans, M. Kraus, U. Seifert, and M. Wortis. 1997. Mapping vesicle shapes into the phase diagram: A comparison of experiment and theory. *Phys. Rev. E* , 55, 4458–4474.

Döbereiner, H.-G., J. Käs, D. Noppl, I. Sprenger, and E. Sackmann. 1993. Budding and fission of vesicles. *Biophys. J.*, 65, 1396–1403.

Evans, E.A. 1974. Bending resistance and chemically induced moments in membrane bilayers. *Biophys. J.*, 14, 923–931.

Evans, E.A. 1980. Minimum energy analysis of membrane deformation applied to pipette aspiration and surface adhesion of red blood cells. *Biophys. J.*, 30, 265–284.

Fourcade, B., L. Maio, M. Rao, M. Wortis, and R.K.P. Zia. 1994. Scaling analysis of narrow necks in curvature models of fluid lipid-bilayer vesicles. *Phys. Rev. E*, 49, 5276–5286.

Helfrich, W. 1973. Elastic properties of lipid bilayers: Theory and possible experiments. *Z. Naturforsch.*, 28c, 693–703.

Helfrich, W. 1974. Blocked lipid exchanges in bilayers and its possible influence on the shape of vesicles. *Z. Naturforsch.*, 29c, 510–515.

Jülicher, F., and R. Lipowsky. 1996. Shape transformations of vesicles with intramembrane domains. *Phys. Rev. E*, 53, 2670–2683.

Lipowsky, R. 1992. Budding of membranes induced by intramembrane domains. *J. Phys. II France*, 2, 1825–1840.

Lipowsky, R. 1995. The morphology of lipid membranes. *Curr. Opin. Struct. Biol.*, 5, 531–540. See also references contained therein.

Miao, L., B. Fourcade, M. Rao, M. Wortis, and R.K.P. Zia. 1991. Equilibrium budding and vesiculation in the curvature model of fluid lipid vesicles. *Phys. Rev. A*, 43, 6843–6856.

Miao, L., U. Seifert, M. Wortis, and H.-G. Döbereiner. 1994. Budding transitions of fluid-bilayer vesicles: The effect of area-difference elasticity. *Phys. Rev.*, 49, 5389-5407.

Seifert, U., K. Berndl, and R. Lipowsky. 1991. Shape transformations of vesicles: Phase diagrams for spontaneous-curvature and bilayer-coupling models. *Phys. Rev. A*, 44, 1182–1202.

Svetina, S., and B. Zeks. 1989. Membrane bending energy and shape determination of phospholipid vesicles and red blood cells. *Eur. Biophys. J.*, 17, 101–111. This article contains earlier references.