

Technical note

Cylindrical shapes of closed lipid bilayer structures correspond to an extreme area difference between the two monolayers of the bilayer

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Abstract

The shapes of extreme area difference between the outer and the inner layer (ΔA) of the closed lipid bilayer structures at fixed membrane area (A) and fixed volume (V) are determined by stating and analytically solving a variational problem for axisymmetric shapes. It is shown that the spheres with at most two different radii and the cylinder are the solutions of this variational problem. The cylinder ended by a hemisphere on each end is the shape combined from these solutions and is therefore, itself the shape of the extreme ΔA at fixed V and A . The related cylindrical shapes of stearyl-oleoyl-phosphocholine vesicles are shown. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The occurrence of red blood cell shape (RBC) changes and the vesiculation of RBC membrane have been observed in some human blood disorders (Palek, 1987; Wagner, 1986). Therefore better understanding of physical mechanisms determining RBC shape and RBC membrane vesiculation may help to develop a new medical treatment to reduce some complications of blood disorders (Wagner, 1986). The human RBC has no internal structure, therefore its shape at given cell volume is determined solely by the physical properties of the cell membrane (Evans, 1974; Deuling and Helfrich, 1976). The red blood cell membrane is essentially composed of two parts, the bilayer and the continuous network of proteins — the membrane skeleton (Evans and Skalak, 1980). Although the RBC membrane is structurally and mechanically more complex than that of the closed bilayer lipid vesicles (Evans and Skalak, 1980; Sackmann, 1994; Iglič, 1997), the study of the shape changes of lipid vesicles can be directed to many aspects of the RBC behaviour (Svetina and Žekš, 1996).

The transformations between the shapes of lipid vesicle or cell with no internal structure can be qualitatively well

explained within the bilayer couple model (Sheetz and Singer, 1974; Evans, 1974). In the view of this model the difference between the outer and the inner membrane lipid layer area of the membrane (ΔA) is taken to be an important parameter which influences the cell shape (Svetina and Žekš, 1996; Seifert, 1997; Iglič et al., 1998b).

Within the bilayer couple model the class of cell or vesicle shapes of a fixed area A contains all the stationary shapes of the same symmetry that can be continuously transformed one into other by continuously varying the two model parameters, i.e. the cell volume V and the difference between the areas of the two membrane lipid layers ΔA . The shapes of the extreme V at given A and ΔA (Svetina and Žekš, 1996) or the shapes of the extreme ΔA at given V and A (Iglič et al., 1998a) were defined as the shapes corresponding to the boundaries of certain classes of the phase diagram of possible shapes. We call these shapes the limit shapes (Seifert et al., 1991; Svetina and Žekš, 1996). It was shown analytically by means of solving a variational problem that the limit shapes can be composed of spheres or sections of spheres, where at most two different radii are possible (Svetina and Žekš, 1996; Iglič et al., 1998a). It was recently indicated that in addition to these, the limit shapes can be composed also from cylindrical and toroidal parts (Iglič et al., 1998a; Hägerstrand et al., 1998).

In this paper we theoretically describe the limit shape of closed bilayer structures composed of the cylinder ended with two hemispheres. Numerical calculations

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indicate the existence of such shapes at the boundary of the dumbbell class (Seifert et al., 1991; Svetina and Žekš, 1991). However, no analytical proof for the existence of the cylindrical limit shape has yet been offered. In this work it is shown analytically as well as calculated numerically that the cylinders ended with hemispheres are the limit shapes of prolate class of shapes. Moreover, experimental evidence of the existence of such limit shapes in the case of stearyl-oleoyl-phosphocholine (SOPC) vesicles is given.

2. Materials and Methods

2.1. Experiment

SOPC was purchased from Avanti Polar Lipids (Al, USA) and used without further purification. We dissolved the lipid powder in a mixture of chloroform and methanol (1 : 1 v/v) to 1 mg/ml and stored it at 258 K. Giant vesicles were prepared by modified method of Reeves and Dowben (Reeves and Dowben, 1969). Approximately 50 μ l of lipid solution was spread on the teflon disk and left for at least 2 h under low vacuum for the solvent to evaporate. The teflon disk with lipid film was placed at the bottom of 50 ml beaker and exposed to a gentle stream of water saturated nitrogen for 30 min. Then, 100 mM glucose solution was added and left to stand for some days at room temperature. The suspension with SOPC vesicles, kept in a glass chamber at room temperature, was observed by a phase contrast microscope (Obj.Ph 3, NA 1.3, 100 \times , Oil).

2.2. Possible limit shapes of vesicles corresponding to extreme values of ΔA

In order to obtain the limit vesicle shape of an extreme ΔA (Eq. (9)) at a given A and V , a variational problem is stated by constructing a functional (Iglič et al., 1998a)

$$Q = \Delta A - \lambda_A \left(A - \int dA \right) - \lambda_V \left(V - \int dV \right), \quad (1)$$

where λ_A and λ_V are the Lagrange multipliers which can be determined from the constraints for the membrane area and the enclosed vesicle volume. The analysis is restricted to axisymmetric vesicle shapes where the symmetry axis of the vesicle coincides with the x -axis, so that the shape is given by the rotation of the function $y(x)$ around the x -axis. The variation $\delta Q = 0$ is performed by solving the corresponding Euler–Poisson equation (Iglič et al., 1998a)

$$2y''(1 + y'^2)^{-2} + \lambda_A((1 + y'^2)^{-1/2} - yy''(1 + y'^2)^{-3/2}) - \lambda_V y = 0, \quad (2)$$

where λ_A and λ_V are renormalized. It has been shown (Iglič et al., 1998a) that the above Eq. (2) can be solved by the ansatz for the sphere with at most two different radii,

$$R_{1,2} = 2/(\lambda_A \pm (\lambda_A^2 - 2\lambda_V)^{1/2}), \quad (3)$$

as well by the the ansatz $y = \lambda_A/\lambda_V$ representing the cylinder of the radius

$$r = \lambda_A/\lambda_V. \quad (4)$$

Different combination of the above solutions are possible, provided that the combined shape fulfills the constraints and possible additional conditions. One of the possible combined shapes of the extreme ΔA at fixed V and A is the cylinder ended by a hemisphere on each end of the cylinder. For such combined shape the Lagrange multipliers are interdependent, $\lambda_A^2 = 2\lambda_V$. The shape is characterized by two parameters: the radius of the cylinder which is equal to the radius of the hemisphere (r) and the length of the cylinder (l) which can be determined from the constraints for the vesicle volume and area. As the number of the parameters in this case equals the number of the constraints, the cylinder ended with hemispheres fulfills the requirement for the limit shape corresponding to the extreme ΔA (Elsogolc, 1961).

To calculate the parameters of the cylinder ended with hemispheres, dimensionless quantities are introduced. For the unit length, the radius of a sphere R_S with the membrane area A is chosen. In accordance with the definition of the radius R_S , the relative area $a = A/4\pi R_S^2$ is equal to one, the relative volume is $v = 3V/4\pi R_S^3$ and the relative area difference is $\Delta a = \Delta A/8\pi h R_S$. At a given relative volume v the radius r and the length of the cylinder l can be determined from the constraints for the relative area and the relative volume of the vesicle:

$$1 = r^2 + rl/2, \quad (5)$$

$$v = r^3 + 3r^2l/4, \quad (6)$$

where r and l are measured in the units of R_S .

In experimental procedures the relative area difference Δa and the relative volume v of the artificial lipid vesicles can be varied continuously by changing the temperature or osmotic conditions (Helfrich, 1973; Berndl et al., 1990; Käs and Sackmann, 1991; Seifert et al., 1991). Also, the relative area difference Δa can be modified by redistribution of lipids from one monolayer to another by applying a transmembrane pH gradient (Farge and Devaux, 1992).

3. Results

Fig. 1 shows the values of Δa of the cylinder ended with hemispheres in dependence of its relative volume v . It can be seen in Fig. 1 that the length of the cylindrical part of the vesicle l increases while the radius r decreases with

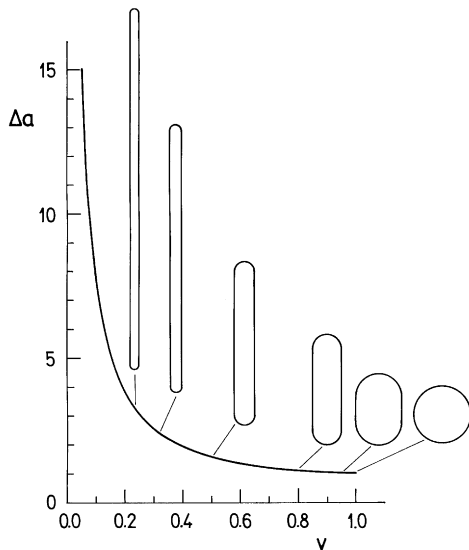


Fig. 1. Relative area difference Δa of the cylindrical vesicle ended with hemispheres in dependence of the relative volume (v). Figure also shows some characteristic vesicle shapes.

decreasing v . It can be also seen in Fig. 1 that the relative area difference Δa increases with decreasing relative vesicle volume v . Consequently, the enclosed relative volume of the cylindrical vesicle ended with hemispheres should be as small as possible in order to render the value of Δa the largest.

The above theory gives the shapes at the boundary of the phase diagram of the possible shapes. Due to confirmation it is of convenience to find a sequence of shapes within a given class of vesicle shapes that leads to the boundary of the phase diagram upon a continuous change of the relative area difference Δa . Fig. 2 shows that the cylindrical vesicle shape ended with hemispheres represents the lower limit of a sequence of the prolate vesicle shapes with equatorial mirror symmetry. The shapes B–E in Fig. 2 are obtained numerically by solving the variational problem of varying the local bending energy for the axisymmetric vesicle shape at fixed A , V and ΔA (Deuling and Helfrich, 1976; Svetina and Žekš, 1996; Seifert, 1997). The corresponding differential equation for the axisymmetric vesicle shape is given in the appendix. The shape F was calculated numerically from Eqs. (5) and (6) using the tangential method. The limit shape A was calculated analytically from the constraints for the relative area and the relative volume of the limit vesicle shape composed of three spherical vesicles with the same radius connected by the ideal necks.

The theoretically predicted cylindrical (prolate) shapes are compared with the observed ones. Fig. 3 shows the phase contrast micrograph of a SOPC cylindrical vesicle. The vesicle shape remained stable for at least half an hour. In Fig. 4 shape transformation from initially undulated shape (Fig. 4A) to a more cylindrical shape

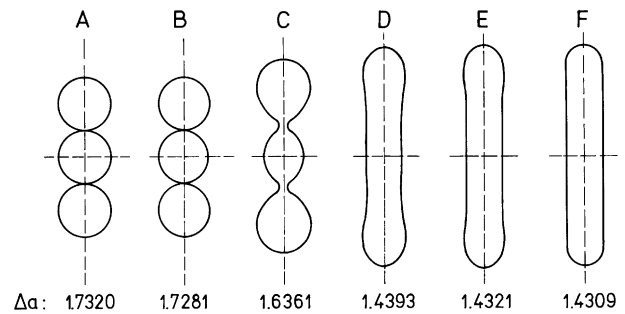


Fig. 2. The sequence of the prolate vesicle shapes with equatorial mirror symmetry for $v = 3^{-1/2}$. The shape A represents the limit shape composed of spheres of equal radii while the the shape F is the limit shape composed of the cylinder ended with hemispheres on both ends.

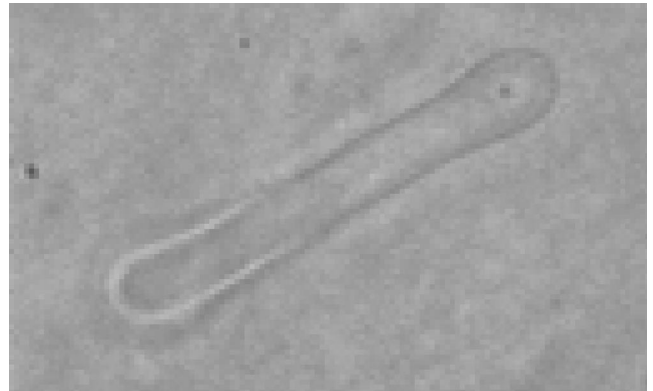


Fig. 3. Phase contrast micrograph of the SOPC cylindrical vesicle.

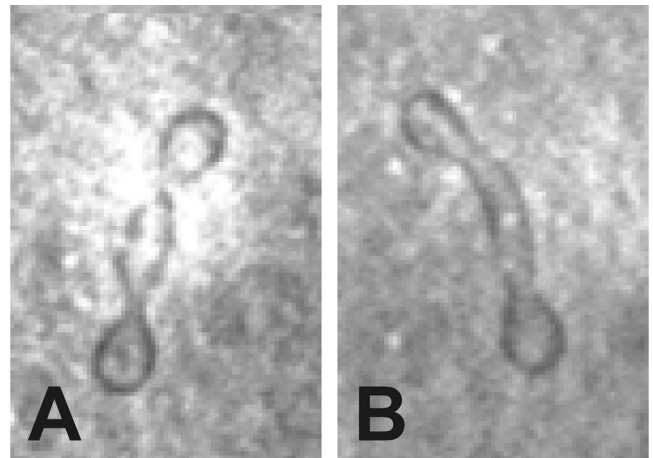


Fig. 4. Phase contrast micrographs of the SOPC prolate vesicle at two different times. The undulated shape of the vesicle (A) has larger ΔA than the cylindrical shape (B).

(Fig. 4B) took place within some minutes. We believe that the shape change occurred due to the decrease of ΔA caused by the drag of the lipids from the outer membrane layer by the glass of the chamber. After the vesicle had reached the cylindrical shape it remained unchanged for half an hour and was no longer observed. It can be seen

that good qualitative agreement between the observed shape transformations (Fig. 4) and the calculated shape transformations of the prolate undulated vesicle (Fig. 2) induced by decreasing of ΔA is obtained.

4. Conclusions and Discussion

The described limit cylindrical vesicle shape composed of a cylindrical part and two hemispheres renders a discontinuity of the principal curvature along the meridians at the junction between the cylindrical part and the spherical part (Fig. 2F). Therefore, in reality, the observed cylindrical vesicle shapes (Fig. 3) never reach the exact limit cylindrical shape ended with hemispheres. However, they are very close to the calculated shape E in Fig. 2 where the discontinuities at the cylindrical–spherical junction are smoothed out, i.e. the radius of the cylinder smoothly ends by two caps of a slightly enlarged extension perpendicular to the cylinder axis.

The prolate shapes of the vesicle at given A , ΔA and V (Fig. 2) was determined by minimization of the local bending energy. Including the nonlocal bending energy (Evans and Skalak, 1980) in the minimization procedure would not change the calculated shape at given ΔA , only the total energy of the vesicle membrane would be different (Svetina and Žekš, 1996; Iglič et al., 1998b). Including some other contributions to the membrane free energy such as the energy due to inhomogeneous distribution of the membrane components (Lipowsky, 1993; Kralj-Iglič et al., 1996) would influence also the calculated equilibrium vesicle shape at given ΔA . However, the limit shape corresponding to the extreme ΔA would remain the same.

The spherical and the cylindrical limit shapes and their possible combinations are not the only possible limit shapes of the extreme ΔA . We have recently shown that the torus and the torocyte shapes (Hägerstrand et al., 1998) are also the shapes of the extreme ΔA .

In conclusion, we have shown analytically for the first time that the vesicle shape composed of the cylinder ended with two hemispheres is the limit vesicle shape corresponding to the extreme value of ΔA . The related structures in the case of the SOPC vesicles were observed (Figs. 3 and 4). On the basis of the presented results it can be suggested that while describing the boundaries of the phase diagram of the vesicle shapes the cylindrical limit shapes and their combinations with spherical shapes should also be considered in addition to the previously described spherical and toroidal limit shapes.

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Appendix A

The vesicle shape with minimal local bending energy (Deuling and Helfrich, 1976)

$$W_b = \frac{1}{2}k_c \int (C_1 + C_2)^2 dA. \quad (7)$$

at given A , V and ΔA is obtained by minimizing the functional (Deuling and Helfrich, 1976; Svetina and Žekš, 1996)

$$F = W_b - \lambda_A A - \lambda_V V - \lambda_\Delta \Delta A, \quad (8)$$

where the Lagrange multipliers λ_A, λ_V and λ_Δ can be determined from the constraints for the area, volume and area difference. Here k_c is the local bending modulus of the membrane, C_1 and C_2 are the principal membrane curvatures. Integration in Eq. (7) is performed over the bilayer neutral surface area of the segment. Since the distance between the neutral surfaces of the bilayer leaflets (h) is much smaller than the dimensions of the vesicle, the area difference ΔA can be written as

$$\Delta A = h \int (C_1 + C_2) dA. \quad (9)$$

where C_1 and C_2 are defined so that they are positive for a sphere.

In the following analysis dimensionless quantities are introduced. To describe the equilibrium shape, we introduce independent coordinates $\rho(s)$ and $z(s)$ (Jülicher and Seifert, 1994) where ρ is the distance between the symmetry axis and a certain point on the contour, z is the position of this point along the symmetry axis and s is the arclength along the contour. These coordinates are normalized with respect to R_s . The angle $\psi(s)$ made by surface normal and z axis is defined by the equation $\tan \psi = dz/d\rho$ (Deuling and Helfrich, 1976).

The bending energy W_b and the functional L are normalized relative to the bending energy of the sphere $8\pi k_c$:

$$f = F/8\pi k_c = w_b - \lambda_A a - \lambda_V v - \lambda_\Delta \Delta a, \quad (10)$$

where $w_b = W_b/8\pi k_c$ and the new Lagrange multipliers are $\lambda_A = A_A R_s^2/2k_c$, $\lambda_V = A_V R_s^3/6k_c$ and $\lambda_\Delta = A_\Delta h R_s/k_c$. The restriction for the geometrical relations between the angle ψ and the coordinate ρ is taken into account by introducing an additional Lagrange multiplier $\gamma(s)$ (Heinrich, 1991; Jülicher and Seifert, 1994). Using the

definition $f = \int L ds$, where

$$L = (\rho(d\psi/ds)^2 + \sin^2 \psi/\rho) + \lambda_A \rho/2 + 3\lambda_V \rho^2 \sin \psi/4 + \lambda_\Delta(\rho d\psi/ds + \sin \psi) + \gamma(d\rho/ds - \cos \psi), \quad (11)$$

the Lagrange–Euler equations for the described variational problem can be then written as

$$d\omega/ds = \sin \psi \cos \psi/\rho + 3\lambda_V \rho^2 \cos \psi + 4\gamma \sin \psi, \quad (12)$$

$$d\gamma/ds = (\omega^2 - \sin^2 \psi)/8\rho^2 + \lambda_A/2 + 3\lambda_V \rho \sin \psi/2 + \lambda_\Delta \omega/4\rho, \quad (13)$$

$$d\psi/ds = \omega/\rho, \quad (14)$$

$$d\rho/ds = \cos \psi. \quad (15)$$

In Eqs. (12)–(14) the function ω is defined as

$$\omega = \rho d\psi/ds. \quad (16)$$

Eqs. (12)–(16) are solved numerically as described in detail elsewhere (Heinrich, 1991).

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