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(Citation)
Biosystems,93(1-2):115-119

(Issue Date)
2008-08

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90000895

Formation and Maintenance of Tubular Membrane Projections: Experiments and Numerical Calculations

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March 18, 2008

abstract

To study the mechanical properties of lipid membranes, we manipulated liposomes by using a system comprising polystyrene beads and laser tweezers, and measured the force required to transform their shapes. When two beads pushed the membrane from the inside, spherical liposomes transformed into a lemon-shape. Then a discontinuous shape transformation occurred to form a membrane tube from either end of the liposomes, and the force dropped drastically. We analyzed these processes using a mathematical model based on the bending elasticity of the membranes. Numerical calculations showed that when the bead size was taken into account, the model reproduced both the liposomal shape transformation and the force-extension relation. This result suggests that the size of the beads is responsible for the existence of a force barrier for the tube formation.

1 Introduction

Living cells have their own characteristic shapes depending on their functions. For example, neuronal cells have many dendrites and long axons, and intestinal epithelial cells have numerous microvilli. Such cell morphologies are considered to be determined by the mechanical properties of cell membranes and of cytoskeletal networks, but the detailed mechanism has remained unclear.

To study the mechanism of morphogenesis of lipid membranes, we have used artificial lipid membrane vesicles (liposomes) in various experiments as a model of biological membranes (Hotani et al, 2003). One of the notable results was the formation of tubular membrane projections from liposomes by the action of cytoskeletal proteins. When tubulins were encapsulated in giant liposomes and polymerized into microtubules, spherical liposomes were transformed into lemon-shaped liposomes. Then tubular protrusions of membrane grew from either end or both ends of the lemon-shaped liposome (Hotani & Miyamoto, 1990; Kaneko et al, 1998). F-actin with actin-crossliked proteins had similar effects on the liposomal shape (Honda et al, 1999). It is well known that thin membrane tubes (tethers) are easily developed when an axial load is applied on liposomes or cells by using an aspiration pipette or laser tweezers (Hochmuth et al, 1973; Waugh, 1982; Evans et al, 1996). It is certain that the shape changes of liposomes with cytoskeletal proteins are also due to mechanical force, since polymerization of filamentous proteins generates a protrusive force.

Recently, new techniques have been developed to precisely measure the force-extension relation for the formation of tubes from giant liposomes (Inaba et al, 2005; Koster et al, 2005). In these studies, polystyrene beads, encapsulated in a liposome (Inaba et al, 2005) or bonded on the membrane surface (Koster et al, 2005), were

manipulated using laser tweezers. The results showed that a first-order shape transition occurred and the force drastically dropped when a tube was formed.

To investigate the shape transformation of liposomes caused by cytoskeletal proteins, several theoretical studies have been made based on the idea of bending energy of the membranes (Fygenson et al, 1997; Božič et al, 1997; Umeda et al, 1998; Heinrich et al, 1999). These studies assumed outward point forces on the membrane to successfully explain both the lemon-shapes and the spherical shapes with one or two tubes. However, the force-extension relations predicted by these models are not necessarily consistent with the experimental results mentioned above. Though there are some theoretical studies that focus on the overshoot of the force and first-order shape transitions (Derényi et al, 2002; Koster et al, 2005), tube formation from a planner membrane were considered in these studies, and the overall shape changes of liposomes have not been addressed.

In this paper, we study the formation of tubes when liposomes are manipulated by using laser tweezers. We first summarize the results of our experimental study (Inaba et al, 2005), and then analyze the overall shape changes of liposomes using a mathematical model based on the bending elasticity of the membranes. Unlike the bundle of microtubules, the beads that push the membrane are considerably larger compared with the diameter of the membrane tube. Therefore, we take account of the bead size in the calculation. The results show that both the shape changes and the force-extension relation can be explained by the bending elasticity model.

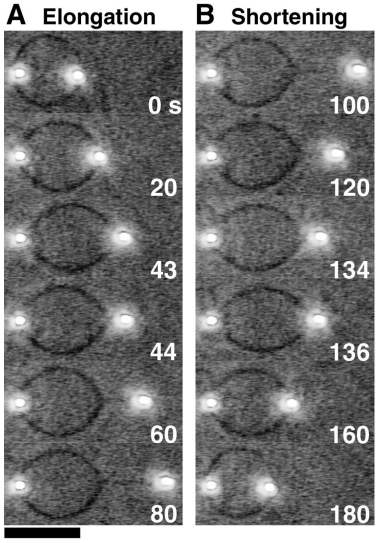

Figure 1: Process of liposome transformation induced by manipulation of beads. Time-lapse image sequences show liposome elongation (a) and shortening (b). Two bright spots show polystyrene beads. The left bead was trapped by a fixed position laser and the right one was manipulated by moving laser (0.15 μm/sec). The lapsed time (sec) after the start of the laser movement is shown on the bottom right of each image. Lipid composition was EggPC:EggPG = 4:1. The bar represents 10 μm.

2 Experiments

To quantify the mechanical properties of lipid membranes, we constructed a simple model system that can manipulate giant liposomes and measure the force required to transform their shapes simultaneously (Inaba et al, 2005). Giant liposomes in which polystyrene beads (1 μm in diameter) were encapsulated were prepared by adding bead-containing solution to the lipid films. When liposomes swelled, they spontaneously captured beads by chance. We chose liposomes that had a spherical shape and encapsulated just two beads. By using double-beam laser tweezers, the two beads in a liposome were trapped and manipulated to push the membrane from the inside. In more detail, one of the beads was trapped by a fixed position laser, while the other was manipulated to move apart at a constant speed (0.15 μm/sec) for 80 seconds, rest for 20 seconds, and then move back for 80 seconds. The position of the beads and the shape change of the liposome were observed using a phase contrast microscopy and recorded. The force applied to the beads was determined from the displacement of the bead trapped in the fixed position laser.

A typical example of the microscopic image sequences is shown in Fig.1. When the two beads were pulled apart, an initially spherical liposome was continuously transformed into a lemon-shape (Fig.1a, 0 – 43 sec). When the liposome had elongated to a critical length, however, a discontinuous transition occurred and a membrane tube was abruptly protruded from either end of the lemon-shaped liposome (Fig.1a, 44

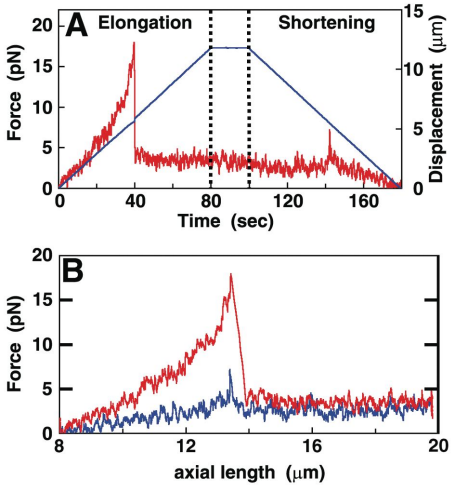

Figure 2: The force required to transform liposome. (a) The red line shows the time-course of the force. The blue line shows displacement of the manipulated bead. This bead was moved to elongate the liposome from 0 to 80 sec, rest for 20 sec between the broken lines and then moved back to shorten the liposome, from 100 to 180 sec. (b) The force as a function of the axial length of liposome during the liposome elongation (red line) and shortening (blue line).

sec). As a result, the liposome differentiated into two distinct portions, a spheroidal portion and a membrane tube. The spheroidal portion was shorter in length and larger in diameter compared with the lemon-shaped liposome just before the membrane tube formation, which suggests that the membrane tension was lowered when the tube was developed. The shape of the membrane at the close vicinity of the bead lying at the opposite side of the tube also became rounder. The membrane tube was very thin compared with the bead size, though the exact diameter could not be determined from the microscopic images. The length of the tube was elongated with further bead movement (Fig.1a, 44 – 80 sec).

During the reverse movement of the bead, the membrane tube gradually shortened (Fig.1b, 100 – 134 sec). After decreasing to about 2 μm in length, the membrane tube suddenly retracted into the spheroidal portion that transformed back into a lemon-shape (Fig.1b, 136 sec). The lemon-shaped liposome transformed back to a spherical-shape after further bead movement (Fig.1b, 136 – 180 sec).

Figure 2 shows the force required to transform the liposome. Depending on the expansion of the lemon-shaped liposome, the force increased monotonically and reached its maximum ($\sim$18 pN) at the critical length. Once a membrane tube was formed, the force dropped down to less than 5 pN. Then the force remained almost constant independent of the tube length during its elongation, rest, and shortening, though the value during the shortening was slightly lower. At the reverse transition of the liposomal shape, the force rose to 7 pN. After that, the force gradually decreased with the shortening of the liposome. The shortening process was similar to the elongation process in shape change, but required weaker force compared with the elongation process at the same end-to-end length of the liposome.

3 Theoretical model

To elucidate the mechanism of liposomal shape transformation, we calculate the equilibrium shape of liposomes when axial loads are applied. The free energy of a liposome has two components, bending energy and the energy due to surface area expansion. For simplicity, we assume the following form of the bending energy:

$$W_{\rm bend} = \int \frac{k_{\rm c}}{2}(2H)^2 dA, \qquad (1)$$

where H is the mean curvature of the membrane and $k_{\rm c}$ is the bending modulus (Helfrich, 1973). The effects of spontaneous curvature, non-local bending elasticity, and Gaussian bending elasticity are neglected. As to the surface area expansion, there are two types of elasticity (Evans & Rawicz, 1990). In the low-tension regime, microscopic undulations are excited in the membrane so that strain energy is stored thermally. In this case, projected area of the membrane A becomes smaller than the true area A_0 which is determined by the relaxed area per lipid molecule and the number of molecules. In the high-tension regime, on the other hand, the projected area becomes larger than A_0 and the elastic energy is stored due to direct expansion of area per molecule. We here adopt the following form for

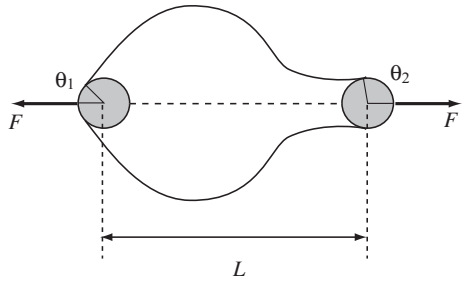

Figure 3: Schematic diagram of a liposome pushed by two beads from the inside.

the area expansion energy:

$$W_{\rm area} = \begin{cases} A_0(\tau_0/\gamma)e^{-\gamma\alpha} & \alpha \geq 0, \\ A_0(\tau_0/\gamma - \tau_0\alpha + \frac{1}{2}k_{\rm s}\alpha^2) & \alpha < 0, \end{cases} \quad (2)$$

where

$$\alpha \equiv (A_0 - A)/A_0 \quad (3)$$

is a measure of the shrinkage of the membrane area, $\gamma = 8\pi k_{\rm c}/k_{\rm B}T$ is a dimensionless constant, τ_0 is the membrane tension at $\alpha = 0$, and $k_{\rm s}$ is the membrane stretching modulus (Fygenson et al, 1997).

Since the permeability of water through membrane is low, and the experimental process is within a few tens of seconds, the volume change of liposomes may be very small. We here assume that the volume is invariant during the experiment. Then the equilibrium shape of the membrane is obtained by minimizing $W = W_{\rm bend} + W_{\rm area}$ under the constraint of constant volume. The variation method applied to this model leads to the following Euler-Lagrange equation:

$$2k_{\rm c}\triangle H + 4k_{\rm c}H(H^2 - K) - 2\tau H - p = 0, \quad (4)$$

where $\triangle$ represents the two-dimensional Laplace operator on the membrane, K is the Gaussian curvature of the membrane surface, and $p \equiv p_{\rm OUT} - p_{\rm IN}$ is the pressure difference across the membrane (Ou-Yang & Helfrich , 1989). The mean tension τ acting in the membrane is given by

$$\tau = \frac{\partial W_{\rm area}}{\partial A} = \begin{cases} \tau_0 e^{-\gamma\alpha} & \alpha \geq 0, \\ \tau_0 - k_{\rm s}\alpha & \alpha < 0. \end{cases} \quad (5)$$

We now consider the case that the two beads encapsulated in a liposome are pulled apart from each other (Fig.3). The shape of the membrane can be considered as rotationally symmetric with respect to the line connecting the centers of the two beads. At the two ends of the liposome, a part of the membrane will be in contact with the surface of the beads. We denote by θ_1 (θ_2) the angle up to which the membrane is in contact with the bead from the direction of the force at the left (right) end of the liposome. If we assume that there is no adhesion or friction between the membrane and the bead surface, the membrane shape will be smooth at the points where the membrane leaves the bead surface. Moreover, the curvature of the membrane will be continuous at those points. Under these boundary conditions, we can calculate the membrane shape from a set of integro-differential equations derived from equations (3)-(5) and the volume constraint.

4 Numerical results

To obtain the equilibrium shape of liposomes, we numerically solved the above equations. Parameters used in the calculation are shown in Table 1. The volume of the liposome was set to be $V = vV_0$ with $v = 0.94$ and V_0 is the volume of a sphere whose area is A_0. This means that the initial liposomal shape was a shrunken sphere with $\alpha = 1 - 0.94^{2/3} = 0.0404$. The value of α did not go below 0 in all the calculations described below.

The results are shown in Fig.4 in which the contact angle θ_1 at the left end of the liposome is plotted against the stretch length $\Delta L = L - L_0$. The curve that connects points O-B-B' is a branch of symmetric solutions. As ΔL increases, a spherical liposome changes its shape to become ellipsoid-like, and lemon-like. When $0 \leq \Delta L < 1.89$ μm, the contact angles θ_1 and θ_2 are almost zero, which means that the equilibrium shapes are almost the same as the shapes caused by point forces on the membrane. When $\Delta L > 1.89$ μm, however, a significant part of the membrane is in contact with the beads at the both ends of the liposomal body.

Two branches of mirror-asymmetric solutions bifurcate from the branch of symmetric solutions at $\Delta L = 4.37$ μm (point B), and are extended leftward to $\Delta L = 3.59$ μm (points C). The shape is roughly lemon-like but a mem-

Table 1: Parameters used in calculation

symbol	meaning	value
k_c	local bending modulus of membrane	5×10^{-20} [J]
k_s	stretching modulus of membrane	0.25 [J/m^2]
$\gamma = 8\pi k_c/k_B T$	constant	300
$\tau_0 \sim k_s/\gamma$	membrane tension at $\alpha = 0$	0.001 [N/m]
$R_0 = (A_0/4\pi)^{1/2}$	radius of spherical liposome at $\alpha = 0$	5 [μm]
a	radius of beads	0.5 [μm]

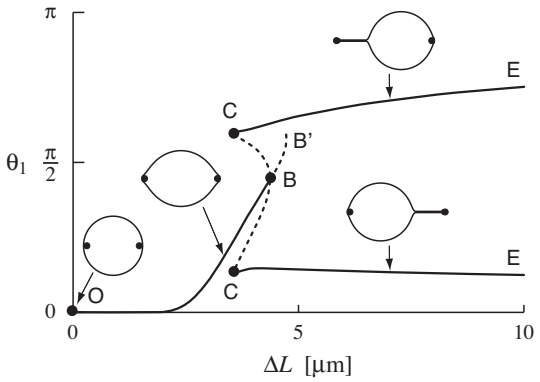

Figure 4: Equilibrium shapes of liposomes. The contact angle θ_1 at the left end is plotted against the stretch length ΔL. Typical overall liposomal shapes are also depicted. Shapes on the branch O-B-B' are mirror-symmetric. Shapes on the branches B-C and C-E are mirror-asymmetric. Unstable shapes are shown by dashed lines. Parameters used in the calculation are shown in Table 1. The relative volume was set to be $v = 0.94$.

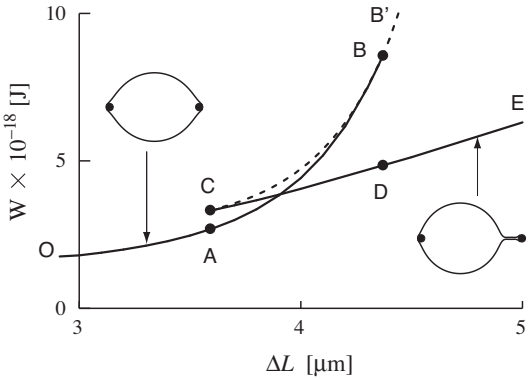

Figure 5: Total energy $W = W_{\rm bend} + W_{\rm area}$ of an equilibrium liposome as a function of stretch length ΔL. The neighborhood of the mirror symmetric-asymmetric bifurcation point B is magnified.

brane tube is protruded from one end of the body. From each of points C, another branch is extended rightward to E. On these branches, the body of the liposome becomes rounder and the membrane tube becomes thinner and longer as ΔL increases. Note that the shapes on the upper asymmetric branches are the same as the shapes on the lower asymmetric branches except for the direction of the protrusion.

Comparison of the total energy $W = W_{\rm bend} + W_{\rm area}$ between the shapes suggests that the asymmetric shapes on the branches B-C are unstable because they have higher energy (Fig.5). The shapes on the symmetric branch beyond the bifurcation point B are also considered to be unstable. Therefore, there are at least three types of locally stable solutions: symmetric shapes on the branch O-A-B, asymmetric shapes on the branch C-D-E with the membrane tube at the right end, and asymmetric shapes with the tube at the left end. Though other types of solutions exist, e.g. shapes with two membrane tubes at the both ends, we omitted them from Fig.4 and Fig.5 because they have considerably higher energy.

Now we consider how the shape transformation of liposomes occurs. If the distance between the two beads increases, a spherical liposome will change its shape along the stable branch O-A-B to become a lemon-like shape (see Fig.5). When it reaches point B, the symmetric shape becomes unstable and the shape will jump to one of the asymmetric shapes (point D) at which the liposome has a long membrane tube at either side. The tube elongates as ΔL is increased further (point E). Conversely, a liposome starting from point E changes its shape along the branch E-D-C as ΔL decreases. When it reaches point C, the solution vanishes and the shape jumps to point A at which the liposome has a lemon-

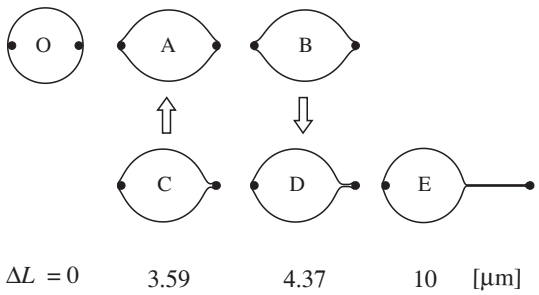

Figure 6: Pathways of liposomal shape changes in the case of $v = 0.94$. When ΔL increases, an initially spherical liposome changes its shape along the pathway O-A-B-D-E. When ΔL decreases, the liposome is transformed along E-D-C-A-O.

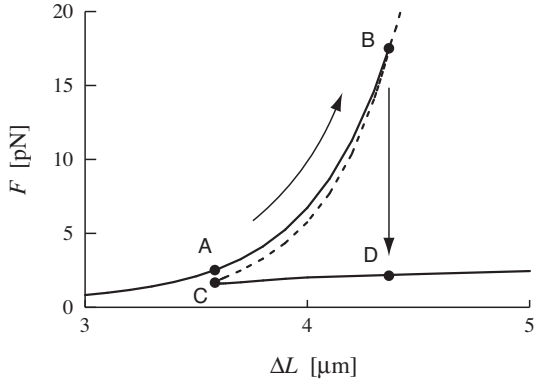

Figure 7: Force acting on the beads when $v = 0.94$.

like shape. The shape changes are summarized in Fig.6. The calculated shapes and the shape transformation pathway quite agree with the observations.

The force acting on the beads during the liposome transformation is shown in Fig.7. When the shape of the liposome is mirror-symmetric, the force increases almost exponentially with ΔL, and becomes very strong at state B. Once a tube is projected, however, the force drops considerably and increases gradually with ΔL. In the reverse process, the force is slightly lifted up when the liposome is transformed from state C to A. These features are consistent with the experimental measurement shown in Fig.2, though the details are slightly different.

Calculations using different parameters showed that the liposomal shape and the force depend greatly on the liposomal volume. Figure 8 shows the calculated force when the relative volume v was set to 0.93. Comparison of this

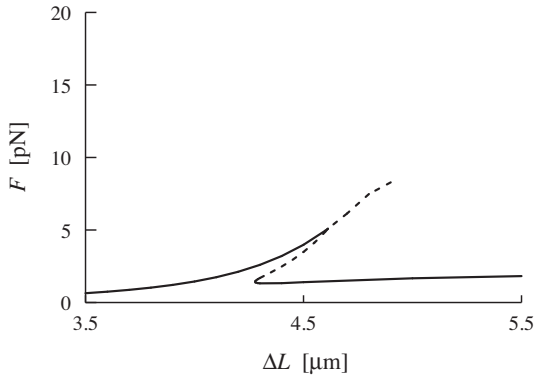

Figure 8: Force applied on the beads when $v = 0.93$.

figure with Fig.7 indicates that a decrease of only 1% in the volume causes a large reduction of the force. This fact may account for why the maximum force varied from one experiment to another. Moreover, the experimental result presented in Fig.2(b) showed that the shortening process required weaker force than the elongation process when the liposome was lemon-like. This may indicate that a very small amount of water leaked out from the liposome during the elongation-shortening process.

5 Conclusion

In the present study, we analyzed the formation of tubular membrane projections from liposomes when they were manipulated by using beads and laser tweezers. When the size of the beads was adequately taken into account, the mathematical model based on the bending elasticity of the membranes reproduced very well the observed liposomal shape transformation, i.e. continuous shape change from a sphere to a lemon-like shape, a discontinuous transition to a spheroidal shape with a thin tube, elongation of the tube, and a backward transition with hysteresis. The model also reproduced the basic features of the force-extension relation observed in the experiments. The force dropped largely at the forward transition and rose slightly at the backward transition. The difference of the forces observed during the elongation and the shortening of the lemon-shaped liposomes may be accounted for by a small amount of water leakage from the liposomes.

The discontinuous shape changes and jumps

of the force had not been explained by the previous models that assumed point forces on the membranes. This suggests that the size of the beads is responsible for the existence of a force barrier for the tube formation. Once a tube is completely projected, the tube becomes thinner than the diameter of the bead. This may make the membrane relaxed and the force to be reduced.

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