Mechanics of Microtubule-Based Membrane Extension

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We observe quasistatic deformation of lipid vesicles from within, due to the polymerization of confined microtubules. A pair of long, narrow membrane sleeves appears, sheathing the microtubule ends as they grow. Spontaneous buckling reveals that the force generated can be greater than 2 pN. The evolution of shape and magnitude of force are consistent with a simple theory for the membrane free energy. We consider a model of the force generating mechanism in which thermal fluctuations of the membrane are "rectified" by the binding of tubulin dimers to the microtubule end. [S0031-9007(97)04450-5]

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Since it was discovered that the boundaries of and within living cells are composed of lipid bilayer membranes [1] the material properties of such membranes and the morphology of closed bilayers, or vesicles, have captured the imagination of physicists. Early studies based on red blood cells [2], which have a two dimensional protein network anchored to the membrane, have been superseded by studies of cell-sized artificial vesicles of controlled lipid composition and no protein skeleton [3]. In such pure systems it has been possible to qualitatively reproduce biological phenomena such as budding and fission using distinctly nonbiological changes in temperature, pH, and membrane composition [4]. Technological advances in direct manipulation of single vesicles using micropipettes [5] and optical tweezers [6] has focused attention on surprising material and dynamic properties and away from the complications of biological relevance. In this Letter, we turn again toward the world of proteins and cells and study vesicles deformed from within by the polymerization of one of the ubiquitous cytoskeletal fibers, microtubules.

Microtubules by themselves have remarkable physical properties [7,8]. These extraordinarily stiff, crystalline, cylindrical aggregates of the protein tubulin are at the limit of detectability with the light microscope and display an intriguing nonequilibrium polymerization instability that is essential to cellular locomotion and division [9]. Encapsulation of microtubules inside vesicles was first demonstrated by Hotani and Miyamoto [10]. Similar experiments have been done using actin filaments, another important cytoskeletal fiber [11].

In this Letter, we observe and explain changes in vesicle shape due to the growth of confined microtubules. We place a lower bound on the mechanical force generated and propose a model of the force generating mechanism. The essential technique is an encapsulation of purified protein within vesicles of controlled composition, about 10 μm in diameter. Our best results were achieved with the freeze-thaw method [12] in which vesicles (1–5 mg/ml 60% DOPC/40% DOPS, Avanti) are mixed with 30 μM tubulin in solution, rapidly frozen by immersion in liquid nitrogen, and thawed on ice [13]. We use temperature (4 °C–37 °C) to control microtubule nucleation and assembly [8] and video-enhanced differential interference contrast (DIC) microscopy to observe microtubules inside a vesicle and its subsequent shape changes [14].

The characteristic evolution of shapes is shown in Fig. 1. One or few microtubules initially deform a

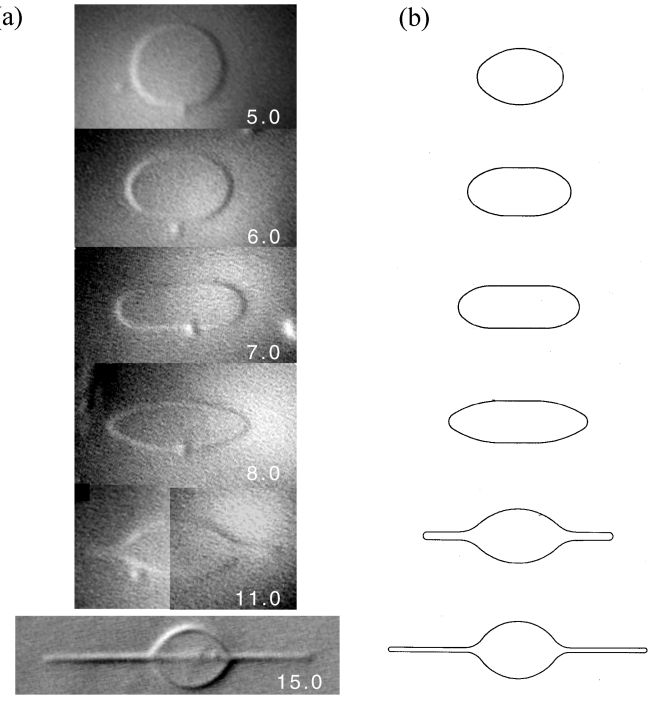

FIG. 1. (a) A phospholipid vesicle deformed by 1 to 3 microtubules (observed floating freely prior to vesicle deformation). The number at lower right is the length of the long axis in microns. This vesicle fortuitously stuck to the glass slide (spot below center), restricting its rotational diffusion. The final frame has a different vesicle since the other never lay in the focal plane as a ϕ shape. (b) Numerical minimization of the membrane free energy generates the observed vesicle shapes. See Table I.

TABLE I. Observed vesicle shapes. Z is the length of the horizontal axis (i.e., microtubule), α is the proportion of area stored in membrane undulations, r is the radius of the end caps, and $f = \partial F/\partial Z$ is the force on the microtubule ends. Details of the calculation are described in the caption of Fig. 3.

	Z (μm)	α	r (μm)	f (pN)
ellipsoid	5.00	0.16	1.05	0.22
sphericylinder	5.99	0.11	0.92	0.25
sphericylinder	7.00	0.048	0.84	0.27
pointed prolate	7.99	0.022	0.49	0.72
ϕ	10.99	0.018	0.21	1.80
ϕ	14.96	0.015	0.13	2.94

near-spherical vesicle into an ellipsoid. Their rate of growth ~ 2 μm/min is slow compared to the slowest relaxation velocities of the vesicle ~ 4 μm/sec, so the deformation is quasistatic and Brownian motion aligns the microtubules along a common axis [15]. In flaccid vesicles, the ellipsoid gives way to a sphericylindrical shape. As visible thermal fluctuations diminish, the vesicle assumes a taut, pointed, prolate shape with the microtubules aligned along the axis of rotational symmetry. Despite apparent end-on contact with the membrane, microtubule growth does not slow. The vesicle develops regions of negative curvature ("necks") and collapses into a pair of narrow membrane tubes around the microtubule extremities. The resulting shape has a profile resembling the Greek letter ϕ (Fig. 1). The ϕ shape accommodates a wide range of microtubule lengths, from 2 to at least 10 times the initial vesicle diameter. As the arms extend, the central portion becomes increasingly spherical, but the overall morphology is preserved. Occasionally the characteristic microtubule dynamic instability [9] is observed, but surprisingly often, particularly at long lengths, growth slows until the microtubules appear constant in length [16].

The membrane does not rupture. The microtubules do not break. The vesicle regains its initial shape when the microtubules disassemble completely. Variations in the initial shape and size of the vesicle have slight effects: Initially prolate vesicles bypass the ellipsoid shape; initially taut vesicles bypass the sphericylindrical shape; large vesicles (>20 μm diameter) are distorted differently because microtubules that span their diameter are individually unstable to buckling [see Eq. (2)]; small vesicles (<0.5 μm diameter) are not distorted because the microtubule that spans the diameter depletes the tubulin supply so much ($>67\%$) that further growth and nucleation are suppressed.

Pulling on a flaccid vesicle with optical tweezers results in a similar extension and relaxation of membrane shape (Fig. 2), indicating that long, narrow membrane extensions do not require an attractive interaction between membrane and microtubule [17]. We therefore look to understand the vesicle shapes from membrane elasticity alone.

The free energy of a vesicle has two components, one due to curvature and the other due to surface area expansion [5,18]. At finite temperatures it is convenient to consider the contribution of large scale curvature separately from that of microscopic membrane undulations excited by thermal energy. Equipartition yields an effective surface tension [18] which reflects the cost of increasing projected area at the expense of flattening thermally excited undulations. A general expression for the free energy is

$$F = \tfrac{1}{2}k_c \int (2H)^2 d^2r + \begin{cases} A_0(\tau_0/\gamma)e^{-\gamma\alpha}, & \alpha \geq 0, \\ A_0(\tau_0/\gamma - \tau_0\alpha + \tfrac{1}{2}k_s\alpha^2), & \alpha \leq 0, \end{cases} \quad (1)$$

where H is the mean curvature, k_c ($\sim 15k_BT = 6 \times 10^{-13}$ ergs) is the membrane bending modulus [5], A_0 is the true membrane area [given by the (fixed) number of lipid molecules at their preferred density], and $\alpha \equiv (A_0 - A)/A_0$ is a measure of the extent to which the true area is greater or less than the apparent (projected) membrane area A. When $A_0 > A$, α is the fraction of membrane area "stored" in undulations. When $A_0 < A$, α is the relative area dilation. Equation (1) uses

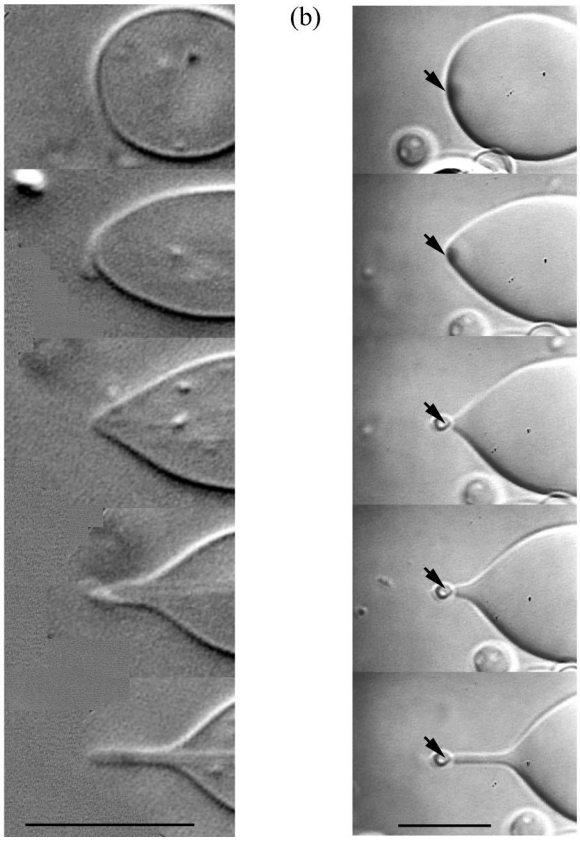

FIG. 2. (a) Closeup of a membrane extension generated by several growing microtubules encapsulated within a vesicle. Scale bar: 10 μm. (b) A membrane extension drawn from a fluctuating vesicle using optical tweezers (830 nm single-mode diode, 200 mW). Scale bar: 10 μm. Arrowhead indicates the location of the tweezer. The vesicle is in an osmotically matched NaCl solution which creates a large refractive index mismatch with the interior for better contrast and easier tweezing. The diameter of the extension is ~ 1 μm.

an empirical interpolation to preserve continuity of the function and its first derivative at $\alpha = 0$ [19]. Of the remaining parameters, $\gamma = 8\pi k_c/k_BT$ is a dimensionless constant, the prefactor τ_0/γ represents the membrane tension at $\alpha = 0$ [18], and k_s ($\sim 6 \times 10^7 k_BT/\mu\text{m}^2 = 250\ \text{ergs/cm}^2$) is the membrane stretching modulus [5]. The initial α of a vesicle depends on the ratio of surface to volume with which the vesicle formed.

The free energy [Eq. (1)] is an implicit function of the microtubule length through A and H. Its calculation is simplified, in keeping with the observations, by considering only figures of revolution about the microtubule axis that are reflection symmetric across the bisecting plane [20]. Working with a class of shapes parametrized by straight lines and circular arcs, sketched in Fig. 3, we computed the minimum free energy of a vesicle constrained to contain a microtubule of length Z and a fixed volume V [21]. It is plotted as a function of the length Z in Fig. 3. Shapes corresponding to points marked on the curve are drawn opposite the observed membrane deformations in Fig. 1 to emphasize the close resemblance. Calculated values of α based on the optimal shape parameters are listed in Table I. Note that the radius of the arms of the ϕ shape, r, is 10 times the radius of a single microtubule at longest axial length.

The changing free energy indicates that a force $f = \partial F/\partial Z$ is sustained at the microtubule ends. This becomes dramatically apparent when the force exceeds the critical force for buckling [22]:

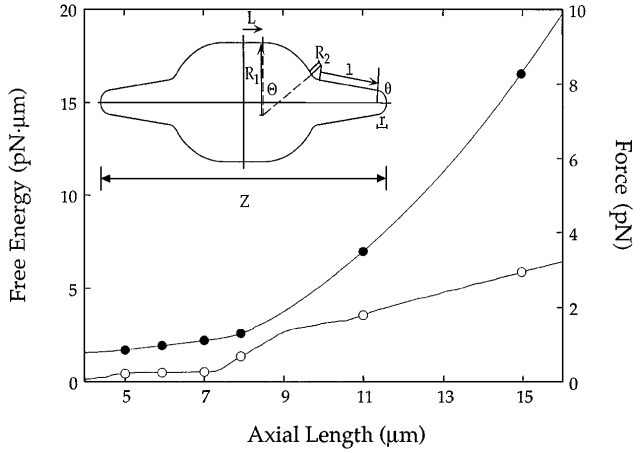

FIG. 3. Minimum free energy (F, filled circles) and associated mechanical force (f, open circles) vs axial length (Z) for a parametrized shape (inset). Volume was fixed at $V = 27\ \mu\text{m}^3$ and true area was fixed at $A_0 = 53.1\ \mu\text{m}^2$ (i.e., initial $\alpha = 0.18$) to correspond with the vesicle shown in Fig. 1(a). Note that, although our stretching free energy is strictly quantitative only when the fluctuation correlation length is small compared to vesicle size ($\alpha < 0.03$), the results are reasonable at large α, because the volume constraint and the bending energy dominate. Inset: parametrization of a vesicle shape using straight lines and circular arcs. Z is the overall length. Rotational symmetry about the Z axis and reflection symmetry across the bisecting plane are assumed.

$$f_c = N\,\frac{\pi^2 B}{Z^2}\,, \tag{2}$$

where N is the number of microtubules, B is the microtubule bending rigidity [23] ($B \sim 10^{-14}\ \text{dyn cm}^2$), and Z is the microtubule length at the onset of buckling. We have observed microtubules in ϕ shaped vesicles buckle under the force of their own growth. As illustrated in Fig. 4, they gradually bend over completely and form a one-armed, paddlelike shape. Assuming the vesicle in Fig. 4 contains a single microtubule and measuring the length at which it began buckling, we deduce a lower bound on the force $f \geq 2$ pN. This is of the same order as forces generated by specialized motor proteins ($\sim$5 pN) [24], and agrees with the scale of force expected from the free energy calculation (Table I).

The results of this calculation suggest a mechanism for the force generation. That $\alpha > 0$ even at the long extensions (Table I) means entropic elasticity of the membrane balances the mechanical force generated by microtubule growth. Although the membrane appears taut, it continues to fluctuate. This suggests that thermally excited undulations of the membrane allow tubulin dimers to access the microtubule ends. Once tubulin binds, the longer microtubule prevents the membrane from retracting.

For this mechanism to be plausible, membrane undulations must (1) be large enough and (2) have long enough lifetimes to permit tubulin to access the microtubule ends. Membrane undulations near the tip of the microtubule exist on top of a mean radius of curvature r (Fig. 3, inset). Thermal undulations of wavelength $\lambda < r$ have amplitude $\approx (k_BT/k_c)^{1/2}\lambda$ while undulations with $\lambda > r$ are quenched by the membrane tension [25]. For tubulin access, a minimum undulation amplitude of a dimer length Δ is needed, or $r > \lambda_{\text{min}} \approx (k_c/k_BT)^{1/2}\Delta$. Given $k_c/k_BT \sim 15$ and $\Delta \sim 10$ nm, we find membrane fluctuations are large enough to accommodate tubulin provided $r > 40$ nm. In the example given [Fig. 1(b)], this condition is satisfied even in the ϕ shape.

During the lifetime [25] of a thermal undulation $\eta r^2\lambda/k_c$ a tubulin dimer can diffuse a distance $(r^2\lambda k_BT/k_c\Delta)^{1/2}$. For tubulin to access the microtubule end, this distance should exceed the typical distance between free dimers. For our tubulin concentration

FIG. 4. Spontaneous buckling of microtubules inside a ϕ shaped vesicle. In the final image, the microtubules are bent completely and continue to grow with both ends sheathed in a single membrane sleeve. Immediately prior to buckling, $Z \sim 7.5\ \mu$m. Scale bar: 5 μm.

of 30 μM, the constraint is $r > 80$ nm. Again, this condition is satisfied in our example [Fig. 1(b)].

We conclude that a force-generating mechanism whereby thermal undulations of the membrane are rectified by microtubule growth is likely to be responsible for force generation in this system. Specifically, we propose that the binding energy of tubulin to the end of the microtubule drives the system out of equilibrium. If true, there should be a well-defined length, determined by the initial ratio of surface area to volume of the vesicle, at which microtubule growth will stop. Microtubules in ϕ shaped vesicles that appear "stalled" indicate that such predictions can be tested experimentally [26].

The relevance of this work to processes in living cells, where microtubule assembly rates and membrane elasticity are quantitatively distinct from our model system, remains an open question. Observations of microtubule-based membrane extensions in cells designed to generate excess microtubules [27], and in cell extracts [28], as well as observations of microtubules buckling in cells [29], invite speculation that a similar mechanism may pertain *in vivo.*

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[14] Details of the apparatus are the same as in [8].

[15] The relaxation velocity for membrane curvature on a vesicle of radius R in a medium of viscosity η is $v = k_c/\eta R^2$. For our vesicles, $k_c \sim 10^{-12}$ erg, $\eta \sim 10^{-2}$ P, and $R \sim 5$ μm. Transient shapes are observed when microtubule lengths change at a rate 10 μm/min, as commonly occurs during shortening.

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