Buckling Microtubules in Vesicles

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We study microtubules in vesicles using micropipet aspiration. A single microtubule in a vesicle buckles as aspiration increases the tension in the membrane, yielding a measure of the persistence length, 6.3 mm. A linear bundle of microtubules deforms the membrane into the shape of the Greek letter ϕ. Under tension, this shape undergoes an abrupt and irreversible transition to a sphere and the microtubules are reorganized into a peripheral ring. [S0031-9007(96)00239-6]

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Microtubules are ordered polymers of the protein tubulin that are part of the flexible, plastic, and even self-effacing skeleton of eukaryotic cells [1,2]. They are hollow cylinders, 25 nm in diameter and tens of microns long [3]. Microtubules have large bending rigidity, with a persistence length of the order of millimeters [4]. The elastic properties of microtubules and their interaction with the cell membrane are essential in defining cell morphology.

This Letter is concerned with microtubules within artificial phospholipid vesicles, their shapes, and the shape transformations induced by membrane tension. A single microtubule deforms a vesicle into an oval. Under tension, the microtubule buckles and the vesicle recovers a spherical shape. This allows a measurement of the microtubule bending rigidity. A large number of microtubules organize inside a vesicle into a linear bundle which protrudes *without piercing the membrane.* The vesicle contains the bundle by extending a pair of narrow tubes about the microtubule extremities while maintaining a roughly spherical central portion. The resulting shape resembles the Greek letter ϕ in profile. Under tension, the bundle transforms into an equatorial ring and the vesicle recovers a spherical shape. Both constructs resemble organizations which occur naturally in some cells [5].

Under a high-magnification optical microscope, we use micropipet aspiration to vary the vesicle membrane tension [6,7]. The pressures required are small ($\sim$1 cm H_2O), so that the membrane elasticity comes mostly from entropic fluctuations [7]. The setup is sketched in Fig. 1. It supports the visualization of 25 nm diameter microtubules and 4 nm thick phospholipid membranes, as well as the manipulation of these minute objects.

We present two experiments [8]. The first focuses on a single microtubule. The microtubule deforms a vesicle from the shape of a floppy sphere to that of a football. Under gentle suction, the tension in the membrane forces the microtubule to buckle (Fig. 2). The result is a measurement of microtubule rigidity (Fig. 3) that falls within the broad range of published values obtained by very different methods [4]. The second experiment looks at a bundle of microtubules. The bundle is straight and long, about 3 times the diameter of the vesicle. Under tension, the membrane applies a compressive load on the ends of the microtubules. The bundle then buckles gradually [Fig. 4(a) and 4(b)] until, at a critical pressure [Fig. 4(c)], it suddenly collapses [Fig. 4(d)]. In the final state, the microtubules form a ring along the circumference of the vesicle, which is again nearly spherical. This exchange of stability (Fig. 5) can be compared to classical macroscopic observations [9,10].

Vesicles are prepared by a modification of standard methods [11]. Synthetic dioleoyl phosphatidyl choline (DOPC) and dioleoyl phosphatidyl serine (DOPS) are mixed in chloroform in a 70 to 30 ratio and dried on Teflon disks. Buffer (100 mM Na-pipes, 2 mM $MgSO_4$, 1 mM EGTA (ethylene glycol-tetraacetic acid), pH 6.9) is added to the lipid. The lipid solution is mixed with tubulin and GTP (guanosine tri-phosphate), to a final concentration of 30 μM and 1 mM, respectively. Finally, the mixture is flash frozen in liquid nitrogen and thawed on ice.

The observation cell contains 350 μl of 150 mM NaCl solution, which is 20% hypertonic to the microtubule buffer and less dense. The thawed vesicles are added, the micropipet is introduced, and, finally, a layer of mineral oil is spread across the surface to prevent evaporation.

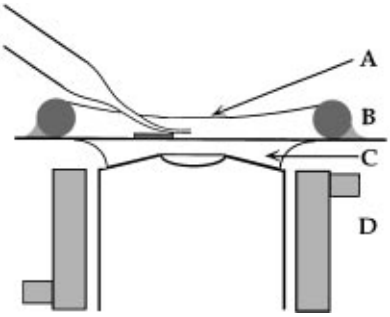

FIG. 1. The cell: (a) fluid medium covered by mineral oil, (b) Buna o ring sealed with paraffin to a glass cover slip, (c) immersion oil coupling to 63× Zeiss Plan Apochromat objective with DIC optics, and (d) water jacket for temperature control. Critical illumination by fiber optic coupled Xe lamp.

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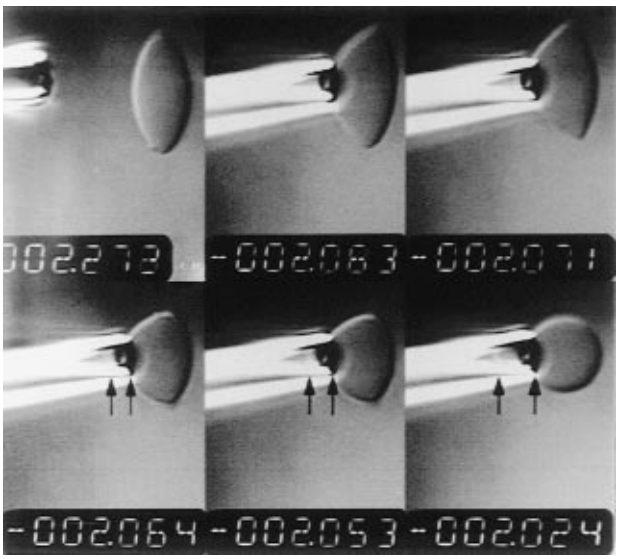

FIG. 2. From left to right, top to bottom, the selection, attachment, and aspiration of a vesicle deformed from within by a single microtubule spanning the long axis. Whereas in this series of pictures one cannot see the microtubule, it is clearly, though intermittently, visible in the live video. Aspiration increases the tension in the membrane and buckles the microtubule, leaving a spherical vesicle projected in the final frame. In three frames the membrane is visible inside the micropipet (arrows). Each frame is 16.0×21.5 μm. The numbers indicate the pressure in cm H_2O. Zero pressure is at -2.074 cm H_2O. The vesicle initially contained a bundle of microtubules (in a ϕ shape formed at 25 μM tubulin and 30 °C), most of which depolymerized after the temperature was lowered to 27 °C.

Tubulin polymerizes inside the vesicles as they sediment to the bottom of the cell. The cell is warmed by heating the microscope objective.

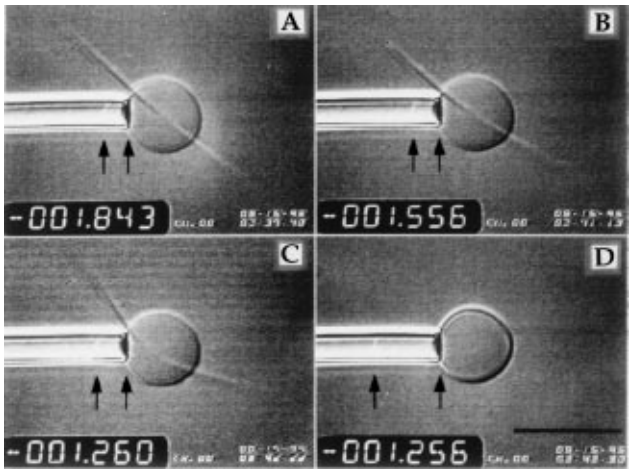

FIG. 4. Buckling and collapse of a microtubule bundle. A vesicle is distorted from within by a rigid bundle (seen as a linear object crossing the vesicle diagonally in the first three frames). Zero pressure is at -2.160 cm H_2O. The effective tubulin concentration is 37 μM and the temperature is 37 °C. Each frame is 39.1×31.3 μm. The membrane is visible inside the micropipet (arrows). At first the bundle is slightly buckled (a), and the bend increases slowly as the aspiration pressure rises (b,c). In the final image, the bundle has collapsed into a ring lining an inner diameter of the vesicle and the membrane has moved into the pipet (d).

The micropipet tip has an inner diameter of 3 to 4 μm. It is partially filled with 150 mM NaCl and connected to an adjustable closed volume of air. Pressure in the closed volume is measured by a Baratron (in cm H_2O units; 1 cm $H_2O = 980$ dyne/cm^2), with a resolution of ± 1 dyne/cm^2 over a range of 10^4 dyne/cm^2. Zero pressure is calibrated to correct for the meniscus inside the pipet.

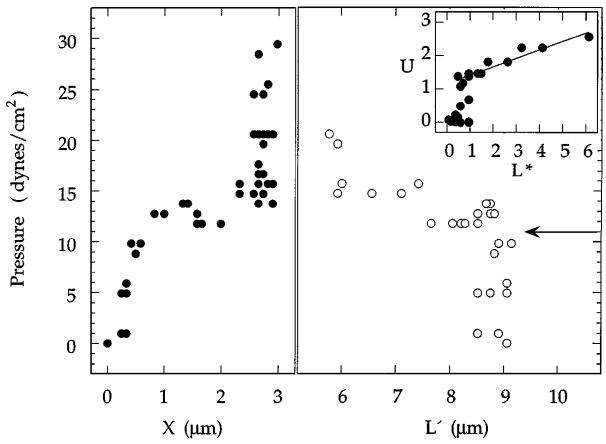

FIG. 3. Aspiration pressure vs intrusion of the membrane into the micropipet X, and vs end to end distance of the microtubule L'. The arrow indicates critical buckling. The insert shows the work done on the vesicle, $U = \int P\,dV = A \int P\,dX$ (pipet cross section $A = 9.1$ μm^2) in units of 10^{-10} erg, vs the bending of the microtubule, $L^* = (L - L')/L'^2$ in units of 10^2 cm^{-1}. The slope yields a measure of the bending modulus, $EI = 2.6 \times 10^{-14}$ dyne $\cdot$ cm^2.

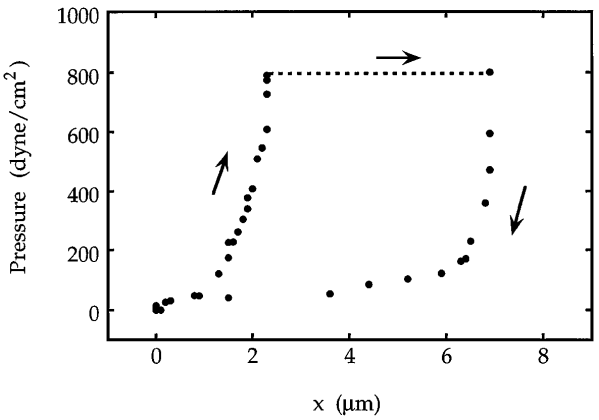

FIG. 5. Aspiration pressure P vs displacement of the membrane intrusion inside the micropipet X. Arrows indicate the time course. A small pressure produces a large displacement as membrane fluctuations are damped. At higher pressures the microtubule bundle supports the membrane until it collapses and the membrane leaps into the pipet (dotted line). Releasing the pressure, the slope is reduced as the elasticity of the bent microtubules is felt.

Measurement of the position of the membrane inside the micropipet is crucial and best done when the micropipet lies in the focal plane. This is achieved, after grabbing a vesicle, by translating the stage to bring the pipet to the edge of a 25 μm thick metal foil, and lowering it onto the foil so that the tip bends to horizontal (Fig. 1).

Figure 2 shows the aspiration of a vesicle deformed by a single microtubule. Figure 3 is a plot of aspiration pressure versus intrusion of the membrane into the pipet X, and versus microtubule endpoint separation, L'.

The ends of the microtubule are free to rotate, so the critical force for buckling is $F_{\rm cr} = \pi^2 EI/L^2$, where L is the unbent length and EI, the bending rigidity, is the product of Young's modulus and the moment of inertia [12]. Assuming that the membrane area is constant, the pressure-area product in the pipet gives the force on the microtubule ends. At critical buckling, the pressure is 11 dyne/cm^2 (Fig. 3). Combined with the cross-sectional area of the pipet, 9.1 μm^2, this corresponds to a force $F = 1.0 \times 10^{-6}$ dyne $= 10$ pN. Given $L = 9.2$ μm, we find $EI = 8.6 \times 10^{-14}$ dyne $\cdot$ cm^2.

A more rigorous deduction of EI comes from equating the work done on the membrane to the energy stored in the buckled microtubule [13]

$$U = \int P\, dV = (EI/2) \int \kappa^2\, dL\,,$$

where κ is the local curvature. For small displacements κ is equal to the second derivative of the shape,

$$(EI/2) \int \kappa^2\, dL \approx (EI/2) \int y''^2\, dx\,.$$

At the same level of approximation, a buckled rod takes a half-wave sinusoid shape, so

$$(EI/2) \int y''^2\, dx = \pi^2 EI(L - L')/L'^2,$$

where L' is the end to end distance of the buckled microtubule. Thus, the expression for the potential energy becomes

$$U = \int P\, dV = A \int P\, dX \approx \pi^2 EI(L - L')/L'^2,$$

where A is the pipet cross section. The inset of Fig. 3 is a plot of U vs $L^* = (L - L')/L^2$, giving $EI = 2.6 \times 10^{-14}$ dyne $\cdot$ cm^2 and persistence length [14] $L_p = EI/k_BT = 6.3$ mm. Uncertainties in the measured parameters bound the measurement of EI to the range $[3.0{-}1.6] \times 10^{-14}$ dyne $\cdot$ cm^2.

The experiment is different when a large number of microtubules is involved. Figure 4 shows the aspiration of a vesicle deformed by a microtubule bundle. The bundle buckles under aspiration. However, after the onset it bends only slightly even though the load on it increases greatly. This is in sharp contrast to simple buckling [15].

At high suction pressure, 800 dyne/cm^2, the configuration becomes unstable. The bundle folds suddenly into the spherical portion of the vesicle (within 0.20 sec). As a result the membrane jumps further into the pipet. Figure 5 traces the intrusion of the membrane inside the pipet through the collapse documented in Fig. 4. This collapse occurs at a pressure almost 4 times the one corresponding to the onset of buckling.

To understand this abrupt transition, recall that the membrane is a two-dimensional fluid which can apply a force only normal to its surface. It exerts a radial compression, confining the microtubules to a bundle, as well as a force on the ends directed along the length of the bundle, even when it is bent. As soon as buckling begins, the force on the ends develops a component perpendicular to the axis joining the microtubule ends. This component is balanced by the asymmetric distortion of the vesicle at the necks, where the spherical portion joins the cylindrical extensions of the membrane. As a result, the extremities of the bundle are held straight and the curvature is concentrated in the spherical portion of the vesicle. A qualitative picture emerges; beyond critical buckling there is a separation into two domains, straight and bent, and a barrier to transform the entire system to the bent state.

The force applied to the end points of the bundle can be calculated from the membrane tension [16]. Since the main part of the membrane is roughly spherical, the tension τ is obtained directly from the aspiration pressure [7] $P : \tau = PR_p/2(1 - R_p/R_0)$, with $R_p = 1.6$ μm, the pipet radius, and $R_0 = 4.8$ μm, the radius of the spherical portion of the vesicle. The force on the microtubule ends is related to the tension by geometry: $F = 2\pi r\tau$ where $r = 0.17$ μm is the radius of the cylindrical membrane sleeve that surrounds the extremities of the bundle and is calculated supposing a close packing of the $\sim$170 microtubules in the bundle [17]. Onset of buckling occurred at a pressure $P = 240$ dyne/cm^2, corresponding to a tension $\tau = 0.029$ dyne/cm, and a force $F_{\rm cr} = 3.1 \times 10^{-6}$ dyne. In this instance, the unbent length L is 38 μm. As $F_{\rm cr} = \pi^2 EI/L^2$, we find $EI = 4.5 \times 10^{-12}$ dyne/cm^2 for the bundle. Dividing by the microtubule bending rigidity measured above, we can confirm the estimated number of microtubules in the bundle $N = 174$.

Spontaneous shape transformations of lipid vesicles by polymerization of entrapped microtubules and actin filament bundles have been observed previously [18]. This work introduces micropipet aspiration as a controllable coupling to the membrane tension, applying piconewton forces to measure elastic properties of these micron-scale objects.

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