Nanomechanics of Microtubules

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We have determined the mechanical anisotropy of a single microtubule by simultaneously measuring the Young's and the shear moduli *in vitro*. This was achieved by elastically deforming the microtubule deposited on a substrate tailored by electron-beam lithography with a tip of an atomic force microscope. The shear modulus is 2 orders of magnitude lower than the Young's, giving rise to a length-dependent flexural rigidity of microtubules. The temperature dependence of the microtubule's bending stiffness in the (5–40) °C range shows a strong variation upon cooling coming from the increasing interaction between the protofilaments.

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Microtubules are a filamentous assembly of protein subunits, α- and β-tubulin. They are hollow cylinders with external and internal diameters of ~25 nm and ~15 nm, respectively [1]. Along with actin and intermediate filaments, they form the eukaryotic cytoskeleton and participate in defining cell morphology. They also perform various vital functions unique to them: they act as building blocks for cilia and flagella and as tracks along which molecular motors move. These roles are determined by their structure and mechanical properties.

The complex dynamics of microtubules (MTs) and other cytoskeletal elements play a key role in cell division, motility, and determination of cell shape. Their elastic properties and interaction with the cell membrane are crucial in understanding cell morphology. Quantifying the mechanical properties of microtubules is also necessary for explaining the elasticity of, for example, sensory hair cells and sperm tails. Influence of various physical conditions such as temperature or chemical agents, for example, drugs, could also be better understood by precisely measuring the mechanical response of an MT, governed by Young's (tensile stiffness) and shear modulus. Conventional studies involving atomic force microscopy (AFM) demand a specific biochemical functionalization of the supporting surface, the sample, and the force-exerting tool, the AFM tip, in order to transmit a stretching load to biomolecules [2]. Although these methods work remarkably well for studying the stretchiness of single molecules (DNA, proteins, etc.), they do not give a complete description of complex systems like MTs, which are a loosely connected assembly of protofilaments. In such biomaterials, not only the properties of the individual components, but also the interactions between them — lateral as well as longitudinal — reflected in the elastic properties, play an important role. Here we report for the first time the elastic moduli of MTs stabilized *in vitro,* using a new approach for measuring mechanical properties of complex biological systems. This method gives both shear and Young's moduli, and demonstrates the high mechanical anisotropy of MT structure.

Previously performed experiments on mechanical properties of microtubules reported in the literature (with the exception of the only result obtained using AFM [3]) involved bending or buckling microtubules using optical tweezers [4], hydrodynamic flow [5], thermally induced vibrations or shape fluctuations [6], and buckling in vesicles [7]. The physical quantity determining the behavior of MTs under this kind of deformation is the flexural rigidity (bending stiffness), the resistance of a beam to bending. If microtubules were homogeneous and isotropic, the bending stiffness would correspond to Young's modulus E and could be written as EI, with I being the second moment of the tube's area. On that basis, the literature measurements would yield values of Young's modulus ranging between 1 MPa [3] (similar to soft rubber) and 7 GPa [4] (similar to Plexiglas). On the other hand, structural studies involving electron microscopy [8] have shown that tubulin molecules are practically indistinguishable inside single protofilaments. Individual protofilaments are separated with grooves and linked to each other between β-tubulin molecules from adjacent protofilaments. Cryo-TEM studies of disassembling microtubules [9] show that disassembly occurs by peeling apart the curved protofilament fragments at the ends of MTs. These are strong arguments in favor of MTs being inhomogeneous and anisotropic, as well as composed of subunits that are more strongly bound in the longitudinal direction (within protofilaments) than laterally (between protofilaments). Bending deformation in

this case occurs not only by stretching of filaments (governed by Young's modulus) but also by sliding between filaments (governed by shear modulus). To facilitate comparison with earlier work, we define a bending modulus E_{bending} that corresponds to the Young modulus that would be deduced if shear deformations were neglected. The flexural rigidity can then be written as E_{bending}. The bending modulus is dependent of the deformation length: for large lengths, there is little sliding between protofilaments, and the bending modulus corresponds more closely with the Young's modulus. For short deformation lengths, MTs act more like a bundle of loose protofilaments, and the mechanical response depends on the resistance to sliding between the protofilaments — the shear modulus. For an accurate mechanical description of microtubules the value of the shear modulus has to be extracted from the bending modulus and quantified.

In order to determine the shear modulus of an individual MT, we applied a method which has been used recently to measure the bending stiffness of carbon nanotube ropes [10]. There is a strong degree of structural analogy between ropes of single walled carbon nanotubes and MTs; the former are composed of weakly interacting single tubes [10] and the latter of protofilaments. The measurement method is based on the elastic deformation of MTs bound to a surface containing holes with a typical size of 200 nm, using the tip of an atomic force microscope. Data were collected in a liquid environment in the (5–42) °C temperature range. We additionally measured the elastic deformation of MTs deposited onto a surface with slits of different widths prepared by electron-beam lithography. These experiments allowed us to estimate simultaneously Young and shear moduli of a single MT.

For measurements of the bending modulus, MTs were assembled from pure (> 99% tubulin) in a standard assembly buffer (80 mM Na-1,4-piperazine-diethane-sulfonic acid, 1 mM $MgCl_2$, 1 mM guanozine 5'-triphosphate, 10% glycerol) at 37 °C. The assembly was monitored by measuring the UV absorbance at 340 nm. Microtubules were then stabilized during 1 min using 0.5% glutaraldehyde, centrifuged, and resuspended. Microtubules prepared in this way support kinesin movement and attachment [11] while containing a minimal number of structural defects, e.g., kinks [12]. This treatment also stabilizes microtubules against temperature changes and enables one to study the temperature dependence of the interaction which holds together the protofilaments in a wide temperature range. Furthermore, glutaraldehyde promotes adhesion of MTs to the substrate. Attempts to stabilize MTs using taxol or microtubule associated proteins did not result in tubes that would adhere strongly enough to the substrate to resist imaging in the buffer with AFM operating in contact mode.

A drop of solution containing MTs was deposited on the surface of an alumina ultrafiltration membrane, which was then placed in an AFM (Thermomicroscopes M5) equipped with a liquid cell. MTs occasionally lie over a pore of ~200 nm in diameter. A series of AFM images was taken in contact mode, each with different normal force using a Si_3N_4 lever with a nominal spring constant of $k = 0.01$ N/m. The spring constant was calibrated by measuring the cantilever's resonant frequency [13]. Analysis of the images gives a relation of deflection [Fig. 1(a) and 1(b)] versus applied force. The elastic deformation of the suspended part of the tube can be regarded as the sum of deflections due to bending and shearing (which becomes significant if the anisotropic component of shear modulus is small compared with the longitudinal Young's modulus or if the diameter is not a lot smaller than the suspended length) [8,11]. Neglecting end effects (which might be enhanced for a highly anisotropic stiffness tensor) in accordance with Saint-Venant's [14] principle, as well as using the unit-load method for a concentrated load F and modeling the MT as a clamped beam, the deflection in the middle [15] becomes

$$\begin{aligned}\delta &= \delta_B + \delta_S \\ &= FL^3/192EI + f_s FL/GA \\ &= FL^3/192E_{\text{bending}}I,\end{aligned}$$

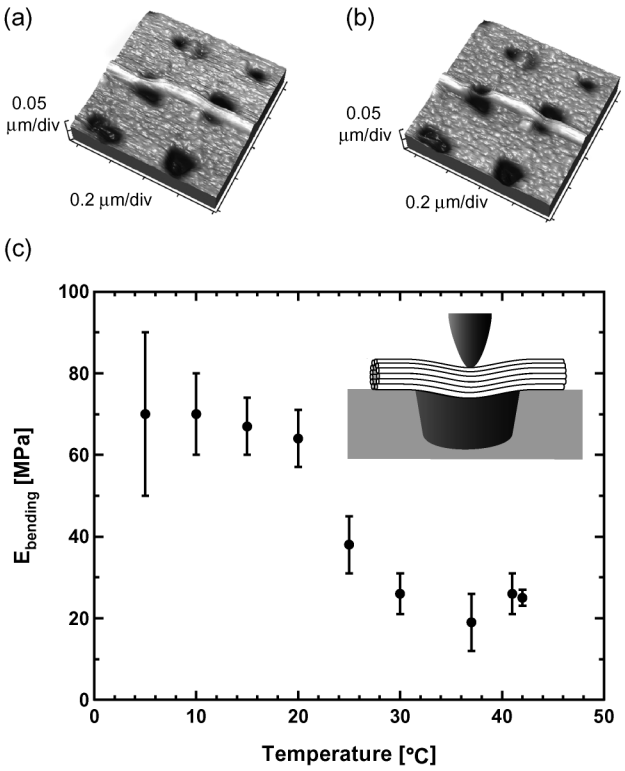

FIG. 1. Pseudo-3D rendering of a microtubule lying on a porous substrate under two nominal loading forces: (a) 100 pN; (b) 150 pN. The deformation of suspended parts is reversible and increases linearly with increasing force. (c) Measured dependence of the bending modulus on the temperature and a sketch of the experimental configuration.

where L is the suspended length, E is the Young's modulus, f_S is a constant numerical factor determined by the geometry, G is the shear modulus, and I is the second moment of the tube's area A. This describes a linear elastic compliance, which can be written as

$$1/E_{\text{bending}} = 1/E_{\text{Young}} + (10/3G)[(D_{\text{ext}}^2 + D_{\text{int}}^2)/L^2],$$

where D_{ext} and D_{int} represent the external and internal MT diameter. Since the MT is firmly attached to the substrate outside the pore, due to stretching of the suspended length there will also be a contribution to the force, which is cubic with the deflection. There may also be contributions to the compliance due to buckling at the hinge points, as well as squashing of the circular cross section. Within the accuracy of the data the force distance curves are linear, and measurements where the MTs are not over the pores show that the contribution to compliance from squashing is small.

Measurements recorded at 20 °C gave a typical bending modulus of 20 ± 10 MPa. The main source of the experimental error is the determination of the diameter and the length of the suspended beam, the MT. For example, an uncertainty of ~15% in the measurement of the suspended length gives a final error of ~50%. Additional recordings of the bending modulus as a function of temperature were performed in the (5–42) °C range. These measurements were accomplished on the same MT, which reduces the systematic errors mentioned above. Results displayed in Fig. 1(c) show a significant decrease of the bending modulus with increasing temperature. The observed temperature dependence is reminiscent of glass transition in polymeric systems. We note, however, that the glass transition in polymers is a collective phenomenon that cannot be applied to microtubules.

To elucidate the dependence of the stiffness on the suspended length, microtubules were deposited on the surface of a silicon substrate coated with a layer of poly(methyl methacrylate) (PMMA). Slits with widths varying between 80–170 nm were cut into PMMA using electron-beam lithography. By measuring the bending modulus of the same MT suspended over slits of different sizes, shown in the inset of Fig. 2, the relative contributions of shearing and bending can be varied from one slit to the other. Values obtained at 25 °C are plotted as bending compliance in Fig. 2. In the limit of small deflection the slope gives the shear modulus of MTs (at this temperature) to be $G = 1.4 \pm 0.4$ MPa, and the intercept gives the Young's modulus at least 2 orders of magnitude higher, in the 100 MPa range, close to the bending modulus measured at 5 °C. Such a large difference between shear and Young's moduli can occur only in highly anisotropic materials. This measurement quantifies the concept that the tubulin subunits are relatively strongly bound in the longitudinal direction, along protofilaments, while the lateral interaction between protofilaments is much weaker. We note that this low shear

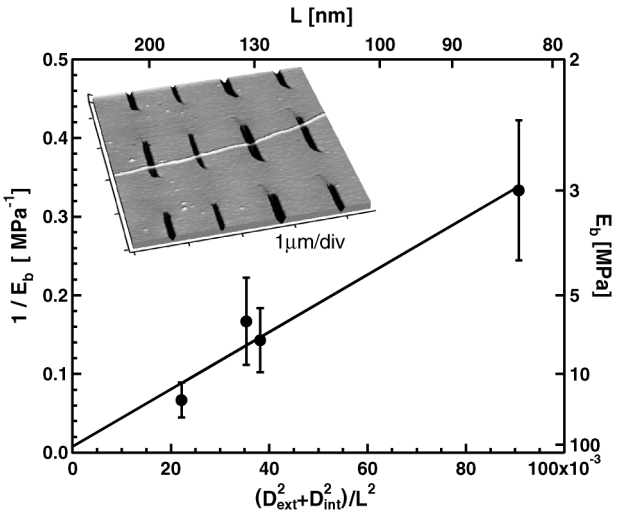

FIG. 2. Inverse bending modulus (E_b) as a function of suspended length (the D_{ext} and D_{int} which represent the external and internal MT diameter are constant). In this representation, shear modulus ($G = 1.4 \pm 0.4$ MPa) is proportional to the inverse slope. The Young's modulus (E) is the inverse of the intercept on the abscissa, with the lowest estimate of 100 MPa. The inset shows a pseudo-3D image of a single microtubule lying over slits with widths (from left to right) of 133, 83, 168, and 128 nm.

modulus of 1.4 MPa is an upper limit, since glutaraldehyde might somewhat increase the interfilament interaction. The two measurements, one performed on a constant temperature with various suspended lengths and the other on a fixed-sized pore but in a large temperature window, suggest that the interaction which holds together the protofilaments is strongly temperature dependent with the shear modulus increasing at lower temperatures. Protofilaments are therefore more stiffly bound at lower than higher temperatures, which could explain the decrease in shortening velocity of disassembling MTs [16] with decreasing temperature. Interactions between tubulin subunits are determined by hydrophobic, van der Waals, and polar interactions of amino acids at the interface between the molecules. Electron microscopy studies [8] have shown that within protofilaments α and β subunits dock well into each other and interact over a relatively large surface. On the other hand, the interaction of β-tubulins in neighboring filaments has a marked electrostatic character and is associated with a close contact of an M loop of one tubulin molecule with H3 and a part of the H1-S2 loop in the adjacent protofilament, with a much lower contact area than for intraprotofilament interaction. This is why MTs have a Young's modulus at least 2 orders of magnitude higher than the shear modulus. Changes in temperature induce small conformational changes in the tubulin molecule that are less constrained in the lateral direction due to a relatively large free space between the protofilaments.

Because of a low interaction surface between the protofilaments, even small conformational changes can lead to significant changes in the interaction surface and therefore the shear modulus. Other ways to influence the interprotofilament interaction are by changing the *p*H of the surrounding medium or adding Mg^{2+} or Zn^{2+} ions that could also act as screening charges.

This high mechanical anisotropy of MT illustrates the compromises that arise when nature gives multiple roles to a single biological structure and shows the importance of knowing mechanical properties for understanding the function of biological assemblies. The MT's high stiffness on length scales associated with the cell size aids in their role of maintaining mechanical stability of the cell, while the MT's end can still oscillate close to the cellular membrane enabling the attachment of tubulin molecules to the end that leads to membrane protrusion by the Brownian ratchet mechanism. This mechanism would not work for high shear modulus or for an isotropic structure.

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