Deformation and cleavage of microtubules studied with optical tweezers

Honglian Guo (郭红莲)[1], Chunxiang Liu (刘春香)[1], Chunhua Xu (徐春华)[2], E Qu (屈 娥)[1], Ming Yuan (袁 明)[2], Zhaolin Li (李兆霖)[1], Bingying Cheng (程丙英)[1], and Daozhong Zhang (张道中)[1]

[1] *Optical Physics Laboratory, Institute of Physics, Chinese Academy of Sciences, Beijing 100080*

[2] *State Key Laboratory of Plant Physiology and Biochemistry; College of Biological Sciences, China Agricultural University, Beijing 100094*

The mechanical properties of fluorescent microtubules (MTs) are probed with dual-optical tweezers system. The results indicate that the fluorescent MTs are much easier to be extended compared with those without fluorescence. Such MT can be extended by 30% and force for breaking up it is only several piconewtons. Furthermore, we find that the breakup of the protofilaments is not simultaneous but step-by-step. Finally, the mechanism of the breaking is discussed.

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Microtubule (MT) is a main component of cytoskeleton in eukaryotic cells. It consists of 13 parallel protofilaments joined laterally and the diameter is about 25 nm. The mechanical properties of MTs play a crucial role in some processes, such as intracellular transport and cell division. However, due to the small size, it cannot be seen with ordinary optical microscope. Therefore, labelling with dye is widely used to study the properties of MTs[1,2]. There have been some investigations on the dynamic characteristics of unlabelled MTs in resent years. Pablo *et al.*[3] probed the local mechanical properties of MTs in the nanometer scale by radial indentation in terms of a scanning force microscope tip. Kerssemakers *et al.*[4] measured assembly force of MT by multiple optical traps. However, in the most studies of biological functions MTs are labeled by different dyes. In this paper, the mechanical properties of fluorescent MTs are probed with dual-optical tweezers system. The results show that under illumination of excitation light the dye-labeled MTs become much easier to break up. The force needed to break up the MT is only several piconewtons, which is much smaller than that without fluorescence. Meanwhile, the MT can be extended by 30%, until it breaks up. At last a qualitative discussion is given to understand the extension and cleavage of MTs. Yet there have been no report about the dynamic characteristics of labelled MTs, so our results may offer reasonable explanation to related experimental results.

Porcine brain tubulin is purified according to the method in Ref. [5]. 5(and-6)carboxytetra-methylrhodamine succinimidyl ester (NHS-rhodamine) labelled tubulin is prepared by the method mentioned in Ref. [6]. Both NHS-rhodamine and NHS-biotin can be connected to the tubulin. Labelled tubulin is diluted with polymerization buffer (0.1 mol/L 1,4-piperazinediethanesulfonic acid (PIPES), 1 mmol/L GTP, 1 mmol/L $MgSO_4$, 1 mmol/L ethylene glycol-bis(2-amino-ethylether)-N,N,N',N',-tetra-acetic acid (EGTA), 10% dimethyl sulfoxide (DMSO), pH 6.9) to a final concentration of 10 μmol/L and incubated at 35 °C for 30–60 min. Then add 5× volume of PEMT (0.1 mol/L PIPES, 1 mmol/L EGTA, 1 mmol/L $MgSO_4$, 10 μmol/L taxol, 10% DMSO, pH 6.9) to the solution and centrifuged at 25000 g at 35 °C for 30 min. The pellets were re-suspended gently with 5× volume of PEMT.

In our experiment, the dual-beam optical tweezers system consists of inverted light microscope (Leica DMIRB, Germany, with 50mW mercury lamp) and Nd:YVO_4 laser (1064 nm, Coherent, USA). Two parallel laser beams enter the back aperture of the microscope objective (HCXAPO 100×, 1.30 numerical aperture (NA), oil immersion) and are focused at the specimen, forming dual-beam optical traps. The left trap can move in x-y direction by rotating a mirror driven by ultrasonic motor while the right one is fixed. A quadrant photodiode detector (QD) is used for measuring the displacement of the bead in the fixed trap and a high-resolution cooled CCD camera (CoolSNAP-fx, USA) is used for acquiring whole image of the trapped beads and MT. The stiffness of both optical traps is 0.042 pN/nm when the power of each beam measured in the front of the objective back aperture is 160 mW.

To prevent the attachment of MTs and beads to the bottom of sample cell, bovine serum albumin (BSA) is added to the sample cell and kept for 2–3 hours before experiment. Then, the residual BSA is aspirated and both MTs and beads are put in the sample cell. The adhesion of two beads and one MT, shown in Fig. 1(a), is performed as follows. First, each of the traps holds one bead, and then we move the stage along the y axis to make two ends of a MT attach respectively to the surfaces of two trapped beads. Due to the specific adhesion between biotin and neutravidin, a stable combination between the MT and beads is formed. The process of adhesion takes about 15 s. As long as the adhesion is achieved, we move the left trap toward the left direction along the x axis with a velocity of 158 nm/s in terms of the rotating mirror. At the same time, the QD detects the displacements of the right bead from the trap center in both x and y directions. And the CCD acquires a series of images which show the variations of the two trapped beads and the attached MT. The tensile force acting on MTs can be extracted from the x direction displacement of right bead. While change of the MT's length can be determined from the serial images acquired by CCD. When the left trap moves toward the left, the MT begins to be extended and the tensile force increases with the elongation of MT. When the force exceeds a

maximum value that the MT can endure, the MT is broken up, as shown in Fig. 1(b).

Figure 2 shows the dependence of tensile force on the extension. It can be seen that the force versus extension curve is highly nonlinear and can be described by an exponential function shown in solid line. The initial length of MT is 3.36 μm and the extension is about 30%. When the tensile force reaches 6.59 pN, the MT breaks up.

In order to know how the MT is broken and if the cleavage of the thirteen protofilaments, which constitute MT by joining laterally, are simultaneous or not, we detect the process of breakup under an external force acting on it. Firstly, we exert several piconewton force on the MT as shown in Fig. 3, then observe the change

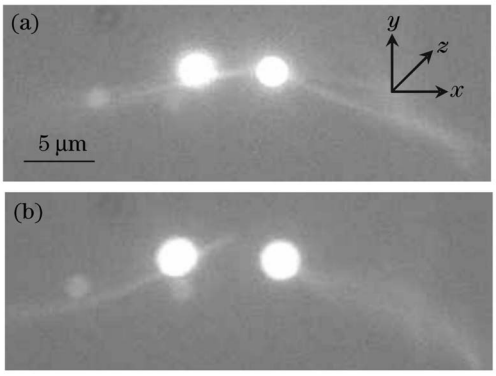

Fig. 1. The fluorescence image of the adhesion between two beads and a MT (a), and the breakup of MT (b), in which x-y plane and z axis denote horizontal plane and vertical direction, respectively.

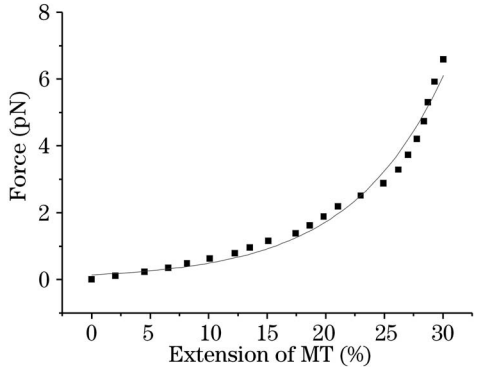

Fig. 2. The force versus extension of the MT, solid line is the exponential fit.

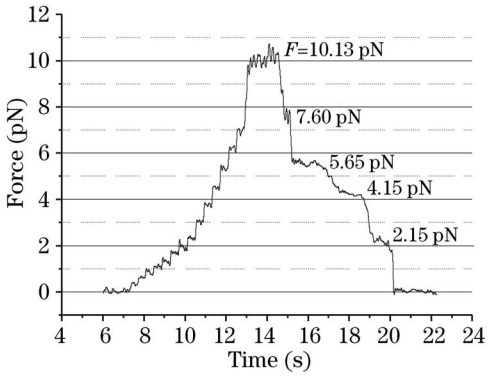

Fig. 3. The process of breakup of the fluorescent MT.

of the force, from which the process of breakup can be revealed. Figure 3 is one of the representative results. In this experiment, the initial force is 10.13 pN, under which the MT is extended, then the force decreases step by step and each step is about 2 pN. This means that the breakup of the thirteen protofilaments are not simultaneous, but one-by-one. If protofilaments' cleavages happen at the same moment, the force should immediately drop to zero, rather than gradually decreases. Furthermore, the nearly even step ((2.03 ± 0.41) pN) indicates that each protofilament can stand about 2-pN force.

For non-fluorescence MTs, the extended force is very large and MTs are hardly elongated. However, in our experiment for fluorescent MTs exposed to excitation light, the force that the MT can stand is about two orders of magnitude smaller than that without fluorescence. The mechanism for elongation and cleavage of fluorescent MTs may be qualitatively understood as follows. There are some kinds of photo-chemical reactions between fluorescent dyes and excitation light. Reactive oxygen species (ROS) produced by these reactions[7,8] may cause some conformational changes of MTs, which will result in the decrease of interactions between tubulin-tubulin, dimer-dimer and protofilament-protofilament. Previous investigations have proved that the interaction between protofilaments is much smaller than that between the others[9]. In addition, the taxol, which is added into sample cell to keep MT stable, can reduce the interactions between protofilaments[10], so each protofilament of MT can be seen as an individual component. Because not every protofilament is tagged with a dye molecule, the damage position caused by ROS may be random. When the MT is pulled, some protofilament will break up firstly at the damaged site, so that the other unbroken protofilaments will be extended in order to balance the external force, such process is repeated until all the protofilaments break up. Besides, our results further prove that under the present experimental conditions the interaction between protofilaments is very weak.

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