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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Measurement of the Force Developed by Disassembling Microtubule during Calcium-induced Depolymerization

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Microtubules (MT) are hollow linear polymers which are formed as a result of polymerization of αβ-heterodimers of tubulin. The longitudinal interactions between the tubulin molecules form linear protofilaments, which, in turn, generate the MT wall due to lateral interactions.

The GTP molecule bound to β-monomer is rapidly hydrolyzed after binding of the dimer; the main part of MTs consists of GDP-tubulin [1]. The hydrolysis of GTP molecule influences the conformation of the tubulin molecule: GDP-tubulin is more curved. The GDP-tubulin in the MT wall cannot return to natural conformation and stays in the wall under the tension. Such MT is unstable. Its stability is determined by the presence of a small region of molecules which did not hydrolyze GTP at the end of MT. When such "GTP-cap" is lost, microtubule is depolymerized. The tubulin dimers during depolymerization return to their natural "curved" form and break connections between the protofilaments. The energy of GTP hydrolysis reserved in the MT wall as mechanical tension is released and may perform work [2]. It seems that this energy mainly determines movement of chromosomes during mitosis. Study of mechanisms of conversion of this energy into work is of great interest. Recently we showed that the depolymerizing MTs may develop considerable forces and move glass beads [4, 5]. The mechanism of the force development during depolymerization and properties of this force such as magnitude and duration are not studied well. It is known that MT may be polymerized in the presence of nonhydrolyzable GTP analogue–GMPCPP. Such microtubules are stable but their depolymerization may be induced by calcium ions [3]. We believe that comparison of characteristics of the forces developed during such depolymerization with the forces produced by depolymerization of usual MTs may help to understand the mechanisms of force development under normal conditions. The purpose of this work was to measure the magnitude of the force developed by bending protofilaments during depolymerization of GMPCPP-stabilized MTs.

MATERIALS AND METHODS

Experimental installation. The force was measured using a "laser tweezers" installation [6]. The main components of the installation were as follows: an AxioPlan 2 fluorescent microscope (Carl Zeiss, Germany), equipped for DIC microscopy, and an intense laser which provided continuous gauss beam of infrared light (model BL-106C, Spectra Physics, United States). The laser beam focused by the microscope objective generates three-dimensional light gradient which works as a trap which entraps objects of micron size whose dielectric permittivity is larger than the permittivity of the medium. A deviation of bead from the center of the beam induces force directed to the center and proportional to the deviation. A deviation of the bead from the beam center may be measured with an accuracy to several nanometers by the quadrant photodetector (specially elaborated at the Department of Physics, University of Colorado at Boulder, CO) using the changes in the interference pattern of light transmitted and refracted by the bead.

Reagents and proteins. The majority of reagents were purchased from Sigma (United States) and Molecular Probes (United States). GMPCPP was purchased from Jena Bioscience (Germany). Tubulin was extracted from cow brain [7]. The labeling of tubulin with rhodamine and biotin was performed according to the standard protocol [8]. Tetrahymena pellicles lysed in the standard buffer (0.1 M PIPES, 1 mM EGTA,

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1 mM $MgCl_2$, pH 6.9 (adjusted with KOH)) in the presence of 0.5% Nonidet P-40 (Sigma, United States) were used as MT nucleation centers. Glass beads (1 μM) were purchased from Bangs Laboratories, Inc. (United States)

Experimental procedures. The experiment was performed as described in [6] with some modifications. Tetrahymena pellicles were placed on a cover slip, whereupon the chamber was filled with a buffer, assembled, and sealed with sealant. Tubulin (0.5 mg/ml), 1/10 of which was labeled with biotin, was added to the chamber in the presence of GMPCPP (1 mM). After washing, the glass beads of micron size covered with streptavidin [9], which randomly adhere to biotin-labeled microtubules, were added to the chamber. The bead selected for measurements was captured by the trap. Depolymerization of the microtubule was induced by infusion of a buffer containing 5 mM $CaCl_2$ to the chamber (Fig. 1). After initiation of the microtubule depolymerization (1 to 10 min), the bead adhered to the microtubules became free. This was accompanied by different movements of the bead, which were analyzed with the use of the laser trap.

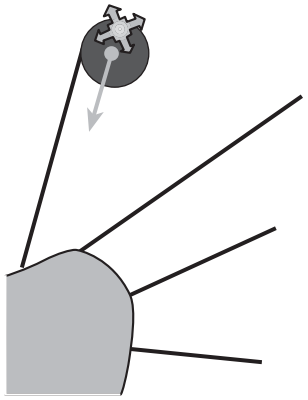

Fig. 1. Schematic representation of experiment. One microtubule is connected with a bead used for the experiment. When the depolymerization wave approaches the bead, it is affected by the force shown by the arrow. This results in the movement of the bead relative to the trap center marked by the cross.

RESULTS

The movements of the bead induced by the microtubule depolymerization strongly varied from experiment to experiment: some beads detached rapidly with a characteristic time of hundredths seconds, whereas others performed quite complex movements which lasted to minute. However, finally, the bead always detached from the microtubule and moved to the trap center.

We believe that the complex bead movements were determined by binding of the bead with several microtubules and its detachment from the different microtubules occurred at different time moments. Approach of the depolymerization wave of each microtubule to the bead induced the bead movements. We analyzed only the last event because we did not know how many microtubules were initially attached to the bead. This guaranteed that we analyzed detachment of the last microtubule. The fact that the bead was free was confirmed by tracking the bead position during movement of the platform: if the bead was free, it stayed all the time in the center of the trap.

The mean force measured in our experiments was 0.17 ± 0.03 pN. The magnitude of the forces developed depended neither on the microtubule length nor the initially applied tension. The distribution of forces decreased to zero at 0.33 pN (Fig. 3). We believe that

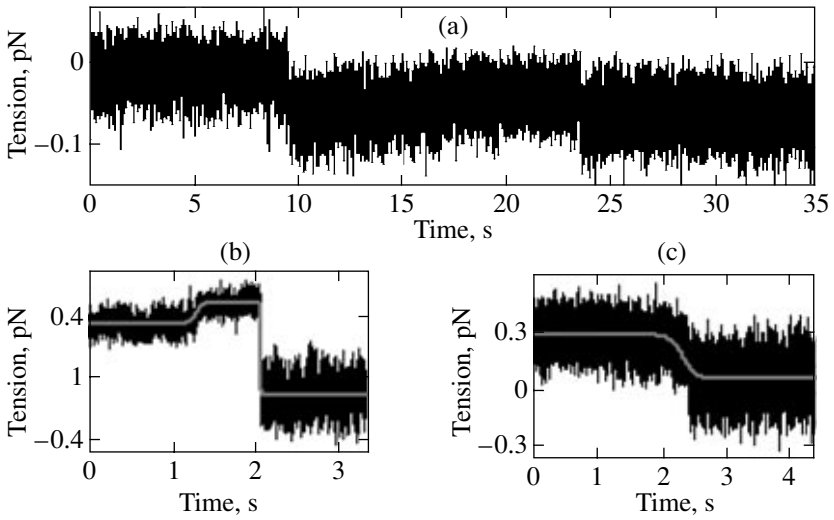

Fig. 2. Examples of signals obtained from the quadrant detector: (a) Both events occurred after the addition of calcium and before complete liberation of the bead; (b) a signal which demonstrates the force development; (c) an experiment without any force, with relaxation to the free state. The solid curve shows approximation by the smooth function.

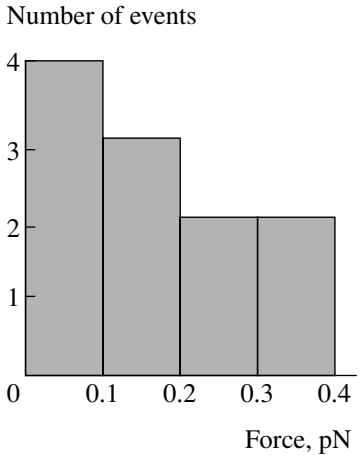

Fig. 3. Histogram of the force amplitudes obtained in the experiment.

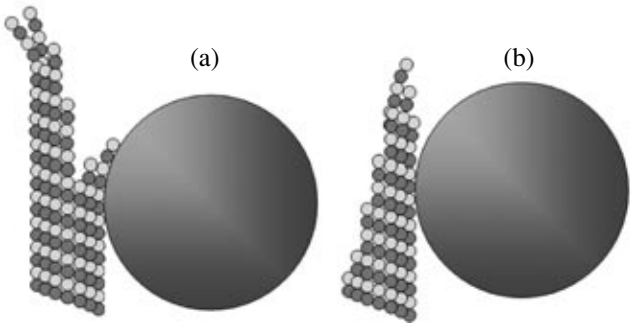

Fig. 4. Two principal scenarios of the microtubule depolymerization in our experiments. The tubulin dimer is shown by two small circles. The bead is shown ten times smaller than the real size: (a) Protofilaments attached to a bead detach earlier than others, they bend and develop the maximum force; (b) the bead is attached for a long time to a structure comprised of many protofilaments, which is less rigid than the microtubule. In the presence of initial tension, the microtubule is bent.

this value is close to the maximum force which may be developed in our experimental system.

Approximately in half of cases, we observed the development of the force, and in another half we found relaxations shown in Fig. 2c. We analyzed this result with the use of a mathematical model in which the depolymerization wave approaches the place of the bead attachment and induces bead movements. The maximum force always grows continuously (Fig. 2b) during depolymerization. The reason why the force amplitude was not measured in each experiment is the asymmetry of the ends of disassembling MTs. The maximum force is developed when the protofilaments attached to the bead detach first, because in this case the protofilaments is initially bent (Fig. 4a). In the case when the protofilaments unconnected to the bead detach first at the level of the bead, the bead remained anchored to MTs consisting of more than two protofilaments. Their mutual curvature is not sufficient for development of maximum force or, if the number of protofilaments is very large, the force is not developed (Fig. 4b). If the bead is initially under the tension, the protofilaments may bend in opposite directions and decrease the initial tension because the rigidity of a pack of several protofilaments is considerably lower than the rigidity of the entire microtubule. This underlies the slow relaxations.

Comparison of parameters of forces developed during depolymerization of GMPCPP and normal MTs

MT	Force amplitude, pN	Relaxation, s	Force duration, s	Reference
GMPCPP	0.17 ± 0.03 ($n = 9$)	0.07 ± 0.03	6 ± 2	
Normal	0.28 ± 0.04 ($n = 48$)	0.08 ± 0.02	1.3 ± 0.3	Nature, 2005

We compared the properties of forces described in this work with the properties determined in the experiments performed with normal GDP-microtubules, which were reported in [6]. The comparison is shown in the table. The force developed by GMPCPP MTs was smaller than the force developed by normal MTs because the curvature of the GMPCPP-protofilaments is two times smaller than the curvature of the GDP-protofilaments [6, 10].

The considerable fluctuations of force durations and relaxations are apparently associated with the considerable fluctuations in the depolymerization rates, which is typical of a homogeneous MT population [11]. GMPCPP and normal MTs significantly differ in the force duration, which is the sum of the plateau time and the time of approach to the plateau (Fig. 2b). This reflects the fact that the disassembling rate of GMPCPP MTs in the presence of 5 mM Ca^{2+} is lower than the depolymerization rate of normal MTs under these conditions.

The large relaxation time suggests that the protofilaments that lost connections with their neighbors exist under tension for quite a long time, whereas the protofilaments under usual conditions are rapidly disassembled to single dimers after the loss of connections between the protofilaments. As was mentioned in [6], this fact may indicate that the presence of tension increases the stability of connections in the protofilaments.

The mechanism of force development described in this article is "one-shot." The depolymerizing MT that pulled a bead becomes disassembled. It is necessary to have a certain coupling device to continuously exploit the force developed by the MT for movement along with the disassembling end of the protofilament. Such a device may be, for example, a ring attached to the MT end. The bending protofilaments may push the inner border of the ring and continuously move it along the

surface of MT. The recently extracted DASH complex [12] forms a ring around MT and is a perfect candidate for the role of such a coupling device.

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