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Temperature dependence of the flexural rigidity of single microtubules

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Abstract

Although the flexural rigidity of a microtubule has previously been estimated by various methods, its temperature dependence has never been systematically examined. Here, we measured the flexural rigidity of a single taxol-stabilized microtubule from thermal fluctuation of the free end of a microtubule, the other end of which was fixed, at different temperatures; the results showed that the flexural rigidity is 2.54×10^{-24} N m^2 independent of temperature in the range of 20–35 °C. Next, we applied temperature pulse microscopy (TPM) [K. Kawaguchi, S. Ishiwata, Thermal activation of single kinesin molecules with temperature pulse microscopy. Cell Motil. Cytoskeleton 49 (2001) 41–47; H. Kato, T. Nishizaka, T. Iga, K. Kinosita Jr., S. Ishiwata, Imaging of thermal activation of actomyosin motors. Proc. Natl. Acad. Sci. USA 96 (1999) 9602–9606], which created the temperature gradient (1–2 °C/μm) along a microtubule gliding on kinesins in the presence of ATP. As a result, the gliding microtubule was buckled between two interacting kinesin molecules, when the microtubule had been propelled faster by the rear kinesin (higher temperature) and slower by the front one (lower temperature). By estimating the critical force to induce buckling of a microtubule, the flexural rigidity of a microtubule was estimated to be $(2.7–7.8) \times 10^{-24}$ N m^2, which was in good agreement with the value determined above. We discuss the buckling process based on the temperature dependence of the force–velocity relationship of kinesin motility.

Keywords: Flexural rigidity; Microtubule; Motor protein; Kinesin; Temperature pulse microscopy (TPM)

Microtubules are stiff tubular polymers composed of many tubulin heterodimers (a complex of α- and β-tubulins), which are one of the elements comprising the eukaryotic cytoskeleton. The mechanical properties of microtubules are therefore important for the basic cellular activities such as organelle transport, cell shape determination, and mitosis. Microtubules resist various forces to maintain the cell shape and also function as a track for motor proteins, such as kinesin and dynein, which generate the force required for cell movement and changes in shape. Kinesin-1, previously known as conventional kinesin, is a highly processive motor protein that keeps "walking" along a microtubule for >1 μm, using chemical energy released by ATP hydrolysis [1–5]. To resist the compressive and tensile forces exerted by molecular motors and maintain the cell shape, the microtubule must have an appropriate flexural rigidity. Several techniques have previously been used to measure the flexural rigidity of microtubules by correlating the buckling energy with positional deflections of the microtubule long axis. Buckling was induced by passive excitation through thermal energy [6–9] or by the controlled forces applied by optical tweezers or hydrodynamic flow [10–12]. These experiments were performed in the absence of the interaction with the molecular motors. In practice, however, the interaction with kinesin may change the structure of a tubulin heterodimer and allosterically cause a conformational change in the neighboring heterodimers, as suggested by Hoenger et al. [13]. Furthermore, it was recently reported that in the presence of ATP kinesin binds cooperatively to a microtubule [14], raising

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possibility that microtubules are not merely a passive track and that the binding of kinesin might change some physical properties of microtubules, such as the flexural rigidity.

We previously studied the effect of temperatures between 15 °C and 50 °C on the moving velocity, force production, and processivity (run length and duration) of single kinesin molecules by the bead assay and temperature pulse microscopy (TPM) [4,15–17]. The temperature can be spatially and temporarily elevated within 10 ms by illuminating with an infrared laser beam a thin metal layer evaporated on a glass surface [4] or an aggregate of metal particles [17]. The temperature of the solution adjacent to the metal can thus be elevated as high as up to a boiling temperature, creating a concentric temperature gradient (1–2 °C/μm) around the metal. When the laser beam is shut off, heat is dissipated into the surrounding medium within 10 ms, so that the protein function can be thermally activated beyond its physiological temperature determined at thermal equilibrium.

Applying the temperature gradient created by TPM to the gliding movement of a microtubule on kinesin molecules attached to the glass surface, we observed the buckling of a microtubule when it glided on two interacting kinesin molecules downward the temperature gradient. From the length of the buckled part of the microtubule in between two kinesin molecules, and by estimating the force exerted by the kinesin molecules, we were able to calculate the flexural rigidity of the taxol-stabilized microtubule. Thus, our TPM method introduces a new technique for studying the mechanical properties of microtubules.

Materials and methods

Materials and methods are described in the Supplementary material section.

Results and discussion

Temperature dependence of the flexural rigidity of single microtubules estimated by thermal fluctuation

First, we examined the effect of temperature on the flexural rigidity of single microtubules by analyzing positional fluctuations during thermal bending motion. We fixed a microtubule near one end and measured the deflection of the other, free, end (Fig. 1). Note that microtubules are not perfectly straight at equilibrium but are slightly curved as observed in Fig. 1A [6].

As shown in Fig. 2, we examined the flexural rigidity of microtubules of 5–18 μm in length (L) at 20–35 °C. The linear function fit of the length dependency of EI was expressed as $\mathrm{EI} = (0.007L + 2.35) \times 10^{-24}\ \mathrm{N\ m^2}$ (Fig. 2A). This small positive slope is not statistically significant, indicating that there is no length dependency of the flexural rigidity within the range of the examined lengths. The dependency of the flexural rigidity on temperature was not observed either (Fig. 2B). Thus, the obtained value of the flexural rigidity was $(2.54 \pm 0.52) \times 10^{-24}\ \mathrm{N\ m^2}$ (mean ± SD, $n = 58$), which is in good agreement with the previous reports [7,10–12].

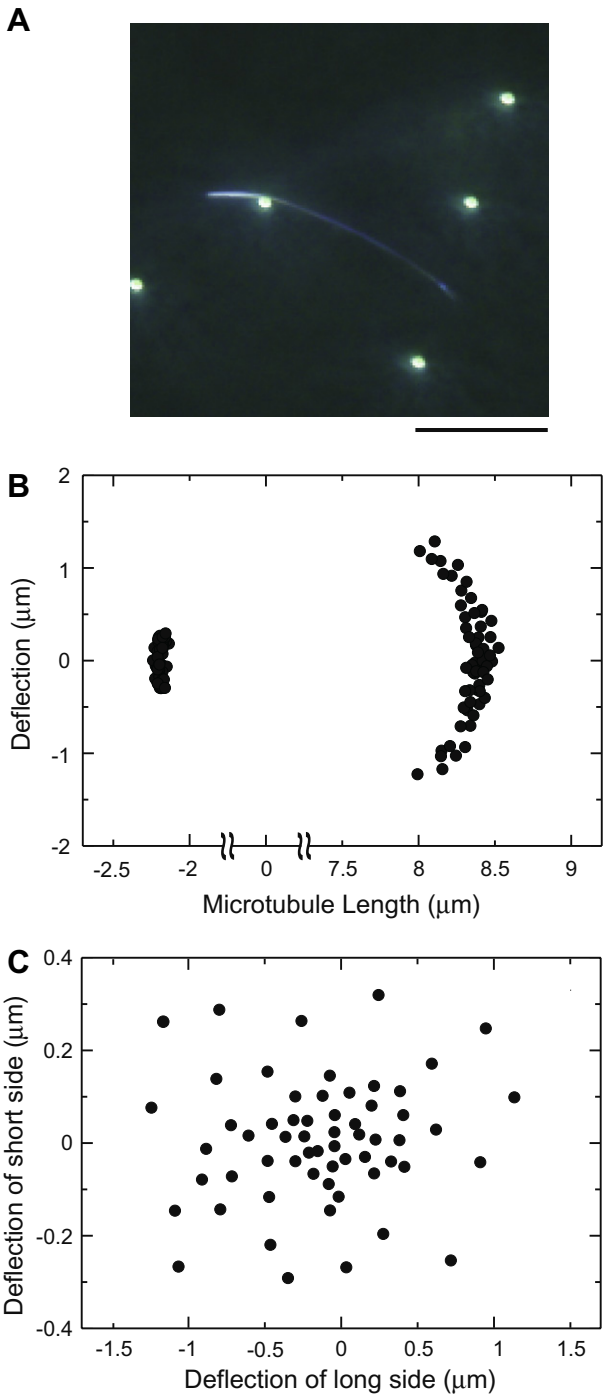

Fig. 1. Measurements of the flexural rigidity of single microtubules by their thermal bending motion. (A) A dark-field micrograph showing a microtubule, both ends of which are free to fluctuate in solution. The microtubule was attached near its one end to a casein-coated bead adsorbed onto a glass surface. Scale bar, 5 μm. (B) A distribution of 60 points indicating the positions of the fluctuating tips of the longer and the shorter ends of the microtubule at 25 °C. The estimated lengths of these ends and EI calculated from these distributions were 8.4 μm and $2.2 \times 10^{-24}\ \mathrm{N\ m^2}$, respectively, for the longer end and 2.2 μm and $2.0 \times 10^{-24}\ \mathrm{N\ m^2}$, respectively, for the shorter end. (C) A correlation between the positions of the tips of the two ends of the microtubule.

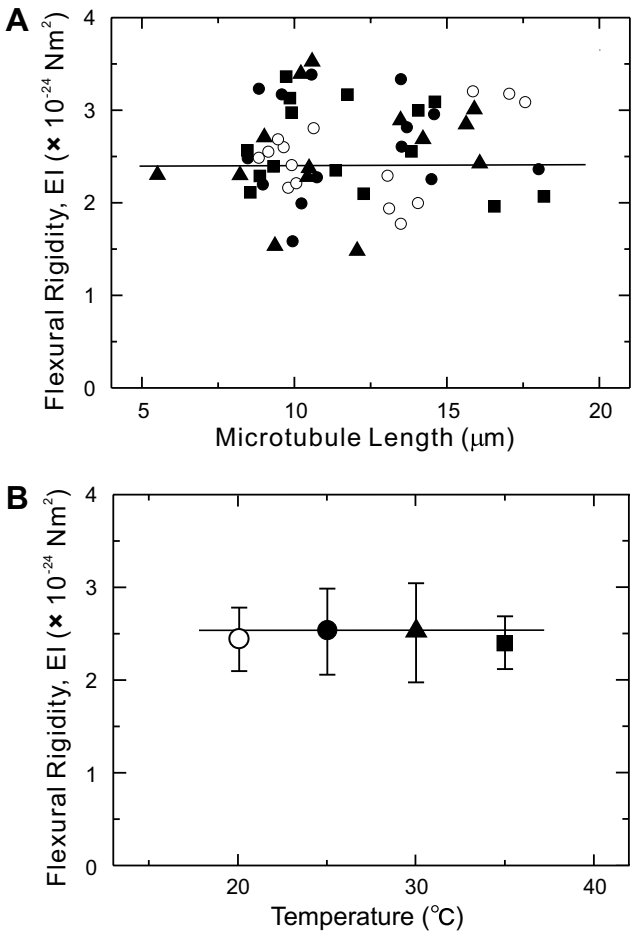

Fig. 2. The flexural rigidity of single microtubules determined from the thermal bending motion. (A) The dependence of the flexural rigidity of single microtubules on the length of microtubules at various temperatures. The linear fit shown by a thin line yielded $EI = (0.007L + 2.35) \times 10^{-24}$ N m^2 (L is the length of microtubules in micrometers). (B) Temperature dependence of the flexural rigidity of microtubules obtained from the data in (A). The error bars represent SD. The values of the flexural rigidity of microtubules (average ± SD) were 2.46 ± 0.36 ($n = 15$), 2.54 ± 0.46 ($n = 14$), 2.57 ± 0.58 ($n = 14$), and 2.36 ± 0.30 ($n = 15$) $\times 10^{-24}$ N m^2 at 20 °C (open circles), 25 °C (closed circles), 30 °C (triangles), and 35 °C (squares), respectively.

There were several reports previously on the temperature dependence of the flexural rigidity of microtubules. Howard and his associates [6,8] reported that with an increase in temperature the flexural rigidity increases from 22×10^{-24} N m^2 at 25 °C to 32×10^{-24} N m^2 at 37 °C. They also reported that the taxol-stabilized microtubule is more rigid than the taxol-free microtubule, which contradicts the results obtained by other groups [7,10–12]. On the other hand, the atomic force microscopy (AFM) measurements by Kis et al. [18] showed that the stiffness of glutaraldehyde-stabilized microtubules decreases as temperature increases. The apparent discrepancy on the effect of temperature may be attributable to the differences in the effect of stabilizing reagents, i.e., glutaraldehyde and taxol, and also to different methods of estimation based on simple theoretical analyses. On the other hand, it was reported that microtubules prepared from tubulin molecules with bound nonhydrolyzable GTP analog (GMPCPP) have higher rigidity than those prepared from GTP-bound tubulin [7]. Thus, it appears that the flexural rigidity of microtubules is sensitive to the local structure of the sites to which not only the stabilizing reagents but also the nucleotides bind. Therefore, to make clear the relationship between these previous reports and the present measurement of the flexural rigidity, it is indispensable to examine the nucleotide state and how much taxol is bound, at various temperatures. Such experiments must be performed in future. Furthermore, a recent model simulation study reported that microtubule has an anisotropic property [18,19]. Until now, all experiments and analyses dealing with the flexural rigidity have been done assuming that the microtubule is homogeneous and isotropic slender elastic rod. However, this assumption seems to be too simplistic, because, in fact, the structure of microtubules is complex. The examination under the well-controlled conditions is important for precise determination of the mechanical properties of microtubules.

Buckling of microtubule by two kinesin molecules

Next, the flexural rigidity of microtubules was estimated from the buckling that occurred upon interaction with kinesin in the gliding assay under the TPM. Taking advantage of the temperature gradient created by the TPM, we estimated the flexural rigidity by analyzing how the buckling of microtubule occurred during gliding between two interacting kinesin molecules located in the regions with different temperatures. For the analysis we selected single microtubules, with which only two kinesin molecules appeared to be interacting. Besides, we selected the microtubules that were gliding almost in parallel to the temperature gradient downward, such that the kinesin molecule interacting at the rear portion of the microtubule was located at higher temperature. The density of kinesin molecules adsorbed on the glass surface was reduced to as low as 50 ng/ml to ensure that only one or two kinesin molecules could interact with each microtubule (length <10 μm), simplifying the observation of its pivoting movement [1,15].

As Fig. 3 shows, under the temperature gradient created on the glass surface, the kinesin molecule interacting at the rear end of the microtubule pushes it faster than that at the front end. As a result, the middle portion of the microtubule placed between two kinesin molecule buckles. After a while, one of the kinesin molecules detaches from the microtubule, which leads to an abrupt change in the curvature of the buckled part. The average period of time during which the buckling was maintained was approximately 0.1 s ((0.11 ± 0.03) s, $n = 17$). Such short duration of the buckling was characteristic for these experiments, which is discussed in detail below.

For estimating the flexural rigidity of a microtubule from the analysis of the buckling, we took into account

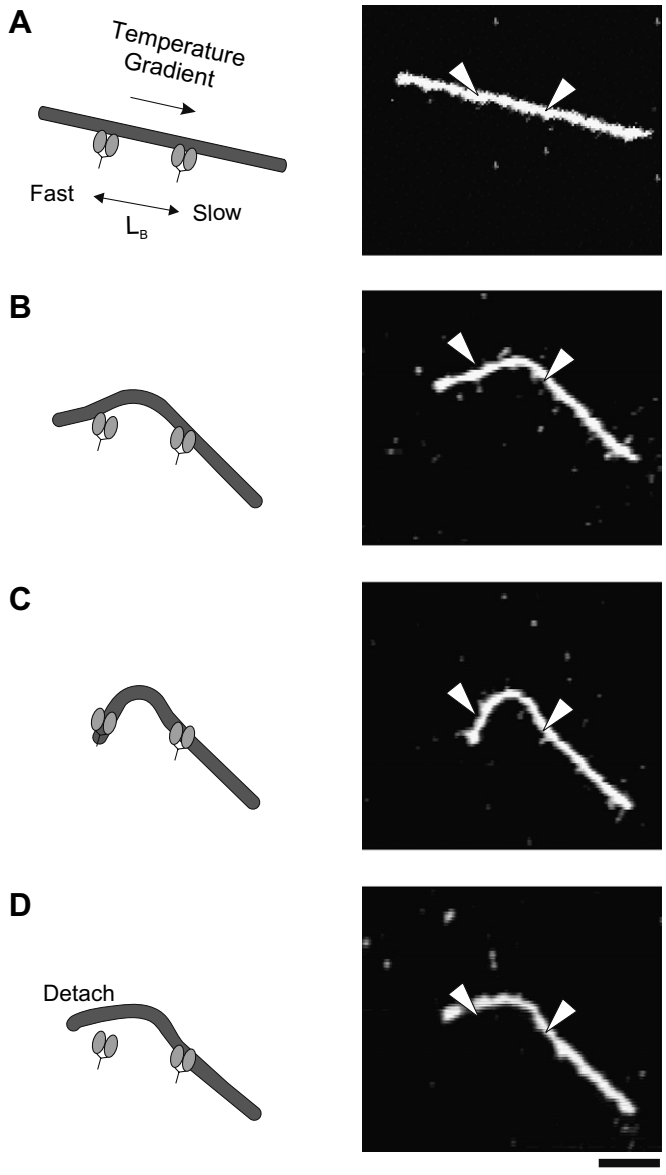

Fig. 3. Estimation of the flexural rigidity of single microtubules from the buckling observed under temperature gradient. (A–D) Schematic illustrations (left column) and corresponding fluorescence images (right column) of a gliding microtubule viewed under fluorescence microscopy. The time proceeds from 0 s (A), 0.07 s (B), 0.1 s (C) to 0.13 s (D) after the temperature pulse. The sizes of kinesin molecules and microtubules in the illustrations are greatly exaggerated. The fluorescence micrographs show the microtubule, with which two kinesin molecules separated by L_B are interacting, gliding under the temperature gradient created on the glass surface. Because the gliding velocity of the rear end of the microtubule is larger than that of the front end due to the temperature gradient, the middle portion of microtubule is buckled. We assume that the rear kinesin motor pushes the microtubule toward a minus end with the loading force F_B. After a while (approximately 0.1 s), the kinesin molecule at either the rear or the front end detaches (in this example, the rear kinesin detached). The arrowheads indicate the position of kinesins. Scale bar, 5 μm.

the following: (1) In the interval between the temperature pulse and the occurrence of the buckling, we assume that the gliding velocity of a microtubule must increase transiently. (2) When the compressive force, which quickly increases because of the higher ATPase activity of the rear kinesin (at higher temperature), exceeds some threshold value (Euler force; critical force for buckling), the buckling starts to occur. (3) To estimate the flexural rigidity from the buckling behavior, we need to deduce the force required for the buckling to occur. (4) From the gliding velocities at the front and the rear ends of the microtubule just prior to and during the buckling, we estimate the load imposed on kinesin molecules based on the nearly linear force–velocity relationship obtained at different temperatures (20 °C and 35 °C, see Ref. [4] and the following description).

Thus, the flexural rigidity was estimated according to the above reasoning as follows: First, the gliding velocity of microtubules at 20 °C was 0.51 ± 0.07 μm/s ($n = 17$). Just after the application of temperature pulse, the gliding velocity instantaneously increased to about 0.7 μm/s without buckling. After the buckling started to occur, the gliding velocity at the rear end (35 °C) increased to 1.18 ± 0.11 μm/s ($n = 17$), which was slightly lower than that obtained under no load (1.35 μm/s), whereas the velocity at the front end (20 °C) was maintained at 0.68 ± 0.09 μm/s ($n = 17$), which is denoted as α in Fig. 4. This situation is schematically shown in Fig. 4, where we assume that the forces borne by the front kinesin (at 20 °C) and produced by the rear kinesin (at 35 °C) are counterbalanced, i.e., have the same amplitude with opposite directions according to the action-reaction low. Following this consideration, we estimated that the force applied just before the buckling was about 3.5 pN, and the force borne during buckling was about 1.2 pN. Thus, we conclude that the critical force that induced the buckling was in the range between 1.2 pN and 3.5 pN.

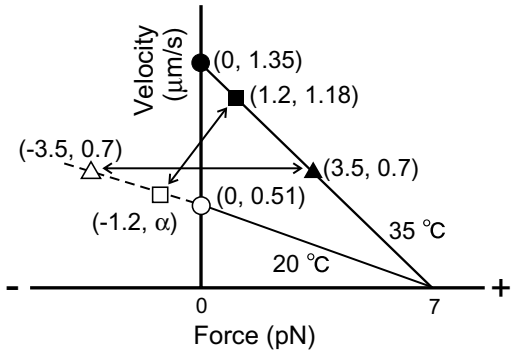

Fig. 4. Schematic illustration of the force–velocity relationship of kinesin motility at different temperatures at 20 °C (open symbols) and at 35 °C (closed symbols). This Figure was obtained according to the experimental data reported previously [4] and in this study. The relationship in the region of negative forces at 20 °C (dashed line) was drawn according to the assumption that the force developed at the front and the rear kinesin molecules are balanced. Before the temperature pulse is applied, the microtubule glides under no load at 0.51 μm/s at 20 °C (the open circle). Just after the application of temperature pulse, the gliding velocity instantaneously increased to about 0.7 μm/s without buckling (the triangles). After the buckling occurred, the gliding velocity of the rear end of a microtubule increased significantly to about 1.18 μm/s, whereas that of the front end remained nearly unchanged, α μm/s ($0.5 < \alpha < 0.7$) (the squares). The both-sided arrows indicate the data corresponding to the front and the rear ends of gliding microtubules.

Now, the Euler stability formula for a homogeneous rod under a compressive force with a clamp at one end and zero torque at the other (we assume this because of the large rotational compliance of the kinesin molecule [20]) provides the minimum buckling force, F_B, by the following equation [1,21]:

$$EI \cong \frac{L_B^2 \times F_B}{20.19} \quad (1)$$

According to Gittes et al. [21], we measured L_B, the contour length of the part of microtubule where the buckling started to occur between two kinesin molecules (Fig. 3A). In the present experiment, one end of the rod must be completely clamped in order to deduce the relative velocity. For 17 different microtubules examined above, the average length of the buckled part of a microtubule, L_B, was 6.64 ± 1.30 μm. Thus, using the values of 1.2 pN and 3.5 pN for F_B and 6.6 μm for L_B, we obtained the flexural rigidity as $(2.66 \pm 0.37) \times 10^{-24}$ and $(7.76 \pm 1.08) \times 10^{-24}$, respectively. The maximum temperature determined from the fluorescence intensity was 35 ± 1 °C. To obtain 35 °C at the rear portion of gliding microtubules, we controlled the laser pulse by using the appropriate ND filter. Note that the coverslip temperature was maintained at 20 ± 1 °C. Thus, the value of the flexural rigidity of a microtubule estimated by TPM was in good agreement with that measured from thermal fluctuation. This result also indicates that sparsely bound kinesin molecules do not substantially affect the flexural rigidity of a microtubule. For further investigation, the measurements of the flexural rigidity without using taxol are desirable. It has to be stressed that our method facilitates the estimation of the flexural rigidity in the situation where the microtubule interacts with motor proteins.

Table 1 summarizes the flexural rigidity of taxol-stabilized microtubules reported by several groups including the present results.

Interpretation of shorter interaction time and implications for the mechanism of kinesin motility

As described above, the average period during which the buckling was maintained (duration of the buckling) under TPM was about 0.1 s, which was much shorter than the duration under the external load (the period of time for each run) obtained by the conventional assay using optical tweezers [4]. Taking into account such short duration of kinesin-microtubule interaction, temperature pulse was applied immediately after confirming that the motors bound to the microtubule. The duration of each run of single kinesin molecules under no load became shorter with increasing temperature: the average duration at 20 °C was 1.5 s, which is ~2-fold longer than at 35 °C (0.8 s), obtained by a bead assay [16]. Even under the external load, the duration is not significantly shortened, being much longer than 0.1 s, namely, ~1 s at 20 °C and ~0.5 s at 35 °C, even at stall force [4].

What is the reason for the short duration of the processive run during buckling? One plausible interpretation is that the twisting and/or pulling force, which is imposed on the kinesin molecules during the buckling, shortens the lifetime of kinesin-microtubule interaction. As mentioned above, the duration of bound state is not shortened, but is rather extended, when the maximum (stall) force is developed. In this situation, the external load balancing the force developed by kinesin (the action-reaction force) must be applied nearly in parallel to the long axis of a microtubule, although it may be slightly tilted along a protofilament of a microtubule. Thus, the twisting and/or pulling force (especially the component perpendicular to the microtubule's long axis) may be responsible for the shortening of the duration of the buckling. Indeed, Gittes et al. reported [21] that pulling a walking kinesin molecule upwards increases the stepping rate. In addition, there is also a possibility that the contortion of tubulin molecules induced by the buckling may weaken the interaction with kinesin. Besides, the temperature gradient created on the microtubule's surface may modulate its conformation and the surface properties, similarly to the polyelectrolytes, which results in the reduction of the affinity of microtubules for kinesin. Such properties should be examined in future by the single-molecule measurements of the unbinding force applied in various directions with various loading rates and under different temperature gradients [4,5,22].

Table 1
Flexural rigidity of taxol-stabilized microtubule

Flexural rigidity (10^{-24} N m^2)	Microtubule length (μm)	Temperature (°C)	References
22.0	25–65	25	[6]
4.7	3–8	37	[7]
32.0	24–68	37	[8]
2.0–22.0	4.4–16.5	37	[10]
2.0	6–24	37	[11]
1.0 (RELAX)	6.1–14.0	22–25	[12]
1.9 (WIGGLE)	3.8–6.3	22–25	[12]
2.5 (Thermal fluctuation)	5.6–17.2	20–35	This study
2.7–7.8 (TPM)	5.3–7.8	20–35	This study

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.11.162.

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