Tubulin GTP Hydrolysis Influences the Structure, Mechanical Properties, and Kinesin-driven Transport of Microtubules*

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Microtubules, cylindrical polymers of α,β tubulin subunits, are involved in various forms of cell motility including ciliary beating, movement of chromosomes during mitosis, and organellar transport (1). Microtubules grow and shrink by adding subunits to their ends, and they exhibit considerable fluctuations in length even under steady state conditions. The growing and shrinking phases correspond to the net addition or loss of thousands of tubulin subunits, and transitions between these two states are stochastic and generally abrupt. This unusual behavior, termed dynamic instability (2), has been visualized for microtubules in vitro (3, 4) and in vivo (5–7). Dynamic instability enables the cell’s microtubule network to turn over rapidly and thereby change its configuration.

Dynamic instability, which does not occur with polymers that are in a simple binding equilibrium with a pool of monomers (8), is an energy-requiring phenomenon that is thought to be linked to GTP hydrolysis by tubulin. According to the most widely accepted model (the GTP cap hypothesis; reviewed in Ref. 9), microtubules grow when tubulin containing GTP (bound at the exchangeable site on the β subunit) adds to tubulin-GTP subunits at the end of the polymer; hydrolysis of the nucleotide occurs relatively rapidly after incorporation into the polymer. The microtubule continues to grow as long as it has a cap of tubulin-GTP subunits; however, if nucleotide hydrolysis exposes GDP-containing subunits at the end of the polymer, then tubulin-GDP subunits rapidly dissociate and the microtubule enters a shortening phase. Current estimates for the size of the GTP cap suggest that it may be smaller than 200 subunits (10).

Since microtubules contain a paucity of tubulin-GTP subunits, nonhydrolyzable analogues of GTP serve as useful tools for examining the structure and dynamics of microtubules composed entirely of tubulin with unhydrolyzed nucleotide. One such analogue is GMPCPP, which is hydrolyzed extremely slowly (4 × 10−7 s−1) after being incorporated into the microtubule (11). Tubulin-GMPCPP has an on-rate similar to that of tubulin-GDP, and the analogue binds to tubulin with an affinity comparable to that of GTP (11). These results indicate that GMPCPP is indeed a good analogue for studying the conformation and properties of tubulin with unhydrolyzed nucleotide. In accordance with the GTP cap hypothesis, microtubules composed of tubulin-GMPCPP are extremely stable (12, 13) and subunits dissociate at 5000-fold slower rates than those measured for tubulin-GDP (11).

In this study, we have examined the structure, mechanical properties, and kinesin-induced motility of microtubules composed of either tubulin-GMPCPP or tubulin-GDP. Kinesin is a microtubule-based motor protein, composed of two 120-kDa heavy chains and two 60–70-kDa light chains, that is thought to transport membranous organelles along microtubules (14, 15). We show here that the microtubules composed of tubulin-GMPCPP are stiffer and have a shallower protofilament twist angle than tubulin-GDP microtubules. We also find that kinesin transports tubulin-GMPCPP microtubules at rates ~30% faster than those of tubulin-GDP microtubules, even though both types of microtubules stimulate kinesin’s ATPase activity to the same extent. Together, these results suggest that GTP hydrolysis by tubulin significantly affects the structural and mechanical properties of microtubules.

** MATERIALS AND METHODS **

Preparation of Microtubules and Motor Proteins—Tubulin from bovine brain was prepared and modified with N-hydroxysuccinimide rhodamine, as described by Hyman et al. (11). Microtubules (~4 mg/ml) were polymerized in BRB80 (80 mM PIPES, pH 6.8, 1 mM MgCl2, 1 mM EGTA) supplemented with 4 mM MgCl2 in the presence of either 1 mM GTP (Boehringer Mannheim) or 1 mM GMPCPP (a gift from Dr. T. Mitchison) for 15 min at 37°C. Unless indicated, microtubules were subsequently stabilized by addition of 20 μM taxol (a gift from the National Cancer Institute). For the ATPase experiments, microtubules

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† † The abbreviations used are: GMPCPP, guanylyl α,β-methylene-diphosphate; PIPES, 1,4-piperazinediethanesulfonic acid.
Motility assays were performed as described under "Materials and Methods." The human kinesin expressed in Chinese hamster ovary cells contained amino acids 1-560 of the heavy chain. The velocities of 60 microtubules were measured, and the mean and standard deviation were determined. The ratio refers to the ratio of tubulin-GMPCPP microtubules divided by the velocity of tubulin-GDP microtubules. The p values were determined using a two-tailed t test.

<table>
<thead>
<tr>
<th>Motor</th>
<th>Velocity (μm/s)</th>
<th>Tubulin-GDP</th>
<th>Tubulin-GMPCPP</th>
<th>Ratio</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid kinesin</td>
<td>0.50 ± 0.04</td>
<td>0.67 ± 0.02</td>
<td>1.34</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Human kinesin</td>
<td>0.61 ± 0.03</td>
<td>0.67 ± 0.04</td>
<td>1.32</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>(E. coli)</td>
<td>(E. coli)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>14 S Tetratylaminae dynein</td>
<td>2.74 ± 0.07</td>
<td>2.68 ± 0.13</td>
<td>0.98</td>
<td>&gt;0.3</td>
<td></td>
</tr>
</tbody>
</table>

were sedimented in a TLA100 ultracentrifuge (280,000 × g; 5 min), the GTP- or GMPCPP-containing supernatant removed, and the microtubules resuspended in nucleotide-free buffer containing 10 μM taxol. The overall results described in this paper are the same whether the tubulin-GDP or tubulin-GMPCPP microtubules were assayed 10 min or 24 h after preparation. Nonfluorescent microtubules exhibited a behavior similar to that of microtubules composed of rhodamine-labeled tubulin subunits.

Chimeric microtubules composed of a tubulin-GMPCPP segment and a tubulin-GDP segment were prepared as described by Homan (16). Briefly, rhodamine-labeled tubulin was first polymerized with 1 mM GMPCPP at 37°C. These brightly fluorescent microtubules (200 μM/ml) were then used as seeds to nucleate the assembly of a mixture of unlabeled tubulin (1.7 μM/ml) and rhodamine-labeled tubulin (0.3 μM/ml) in the presence of 1 mM GTP at 37°C. After 15 min, the microtubules were diluted 5-fold into BRB80 containing 10 μM taxol. Because of their different fluorescent intensities, the tubulin-GDP and tubulin-GMPCPP segments of the microtubule could easily be distinguished by microscopy.

Kinesin was purified from squid optic lobes obtained at the Marine Biological Laboratory (Woods Hole, MA) by microtubule affinity followed by sucrose gradient density centrifugation, as described (17). Squid kinesin was stored for up to 6 months in liquid nitrogen. 14 S dynein was prepared from Tetratylaminae clia, as described by Johnson (18).

The purification and characterization of the bacterially expressed human kinesin proteins used in this study will be described in more detail elsewhere.2 In brief, constructs containing the first 379 or 560 amino acids of a human kinesin heavy chain (19) were cloned into the pET3A-based plasmid pH40P. This expression plasmid was transformed into the Escherichia coli strain BL21(DE3), and a saturated IPTG (isopropyl-1-thio-β-D-galactopyranoside) was used for induction, as the basal level of expression was sufficiently high. Cells were then centrifuged for 10 min at 8,000 × g. The liquid medium was discarded, and the cell pellets were quick-frozen in liquid nitrogen. For purification, the frozen cell pellets were resuspended in 10 volumes of KB buffer (25 mM PIPES, pH 6.8, 1 mM dithiothreitol, and protease inhibitors (50 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotonin, 100 μg/ml chymostatin, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 50 μg/ml 1-chloro-3-tosylamido-7-aminooctaheptanone). Resuspended cells were broken with a French press, and the insoluble material was removed by centrifugation at 27,000 × g for 15 min. The supernatant was then loaded onto a phosphocellulose P-1 column; the column was washed with 10 volumes of KB buffer and the kinesin protein eluted with a 0–1 M KC1 gradient in KB buffer. Fractions containing the kinesin protein were pooled, diluted 4-fold with KB, and then loaded onto a Pharmacia Mono-Q FPLC column. The kinesin protein was then eluted from column with a 0–0.5 M KC1 gradient in KB buffer. The purified kinesin-379 and kinesin-560 proteins (both >90% pure) were stored in KB20% sucrose in liquid nitrogen.

In Vitro Motility and ATPase Assays—Assays for kinesin (20, 21) and 14 S dynein (22) are explained in depth in previous studies. Essentially, squid kinesin (~10 μM/ml) was adsorbed onto the glass surfaces of a flow chamber consisting of glass slides and 18 × 18-mm coverslips separated by two strips of double-stick transparent tape that were pretreated with 2 J. Kull, R. Vale, and R. Fletterick, manuscript in preparation.

![FIG. 1. Transport velocities of tubulin-GDP microtubules, tubulin-GMPCPP microtubules, and chimeric microtubules by squid kinesin. Microtubules composed of tubulin-GDP, tubulin-GMPCPP, or chimeric microtubules containing a segment of each microtubule type were prepared as described under "Materials and Methods." Velocity measurements of 25 of each microtubule type were made on the same glass surface; the bars and the error bars represent the mean and standard deviation, respectively. Transport velocities of tubulin-GDP and tubulin-GMPCPP segments in the chimeric microtubules did not influence the velocity of transport; in many instances, the tubulin-GMPCPP segment was as long or longer than the tubulin-GDP portion of the microtubule.](https://www.jbc.org/content/2770/2/19876.full.html)
**Properties of Tubulin-GDP and Tubulin-GMPCPP Microtubules**

**RESULTS**

Kinesin-driven Transport of Microtubules Composed of Tubulin-GDP or Tubulin-GMPCPP—In this study, we examined whether the nucleotide state of tubulin influences kinesin-induced microtubule motility. Two types of microtubules were compared in this study. One set of microtubules was prepared by polymerizing tubulin in the presence of GTP; taxol was added subsequently to stabilize the polymer. Since tubulin hydrolyzes the nucleotide triphosphate rapidly upon polymerization (27), the vast majority of the tubulin subunits in such polymers contain bound GDP. A second set of microtubules was polymerized with GMPCPP, followed by subsequent addition of taxol. This nucleotide is not appreciably hydrolyzed within 24 h (<10%) and is thought to mimic a tubulin-GTP state (11).

To measure the moire repeat, images were printed at a final magnification of about ×100,000 and multiple repeats on single microtubules were measured from the prints. To determine the monomer spacing, suitable microtubule images were converted to optical density arrays with a Perkin-Elmer scanning microdensitometer. Spot and step sizes were equivalent to 7.14 Å.

Determination of Microtubule Stiffness: Measurement of Microtubule Curvature—Tubulin-GDP and tubulin-GMPCPP microtubules (42 each) were selected for analysis from about 10 randomly chosen electron micrographs (magnification, ×10,000). Images of microtubules in these micrographs were captured using the Perceps Pixel Buffer frame grabber on a Macintosh Quadra 950, and the shape of each microtubule was digitized manually with a mouse-driven cursor at an average interpoint distance of 0.4 µm along the microtubule using Grafeht Ultimage version 2.01. The curvature at each point was calculated as the angle, $d\theta$, between line segments passing through that point and through the fourth point in either direction along the microtubule, divided by half of the sum of the lengths of those two line segments.

To estimate the uncertainty in measuring curvature, $d\theta/d\alpha$, the same microtubule image was digitized with a mouse-controlled cursor 10 consecutive times. The curvature was calculated at each digitized point, and a mean curvature for the whole microtubule was calculated for each of the 10 trials. The standard deviation of the 10 trials was 10% of the mean, which represents the uncertainty in curvature measurements.

**In the motility assay, kinesin was adsorbed onto the surface of a glass slide, and the movement of fluorescently labeled microtubules across the motor-coated surface was assayed by video microscopy. Only those microtubules traveling in a straight path were scored for velocity. Table I shows that kinesin isolated from squid optic lobe transported tubulin-GMPCPP microtubules at 34% faster rates than tubulin-GDP microtubules at saturating ATP concentrations. Although this rate difference is small, it is statistically significant ($p < 0.001$) and reproducible. As reported previously for tubulin-GDP microtubules (20), the velocity with which kinesin transported tubulin-GMPCPP microtubules was the same over a wide range of kinesin densities on the surface. At kinesin densities of ~0.5 and 200 molecules/µm², the velocities of movement were 0.65 ± 0.07 and 0.63 ± 0.03 µm/s, respectively. At the lower density, microtubules pivoted about nodal points of attachment to the surface, suggesting that such movement was produced by single kinesin molecules (20, 28).

We also expressed a portion (amino acids 1–560) of a human kinesin heavy chain gene (19) in E. coli that contained the motor domain and approximately half of the α-helical coiled coil stalk, but did not include the C-terminal domain. The purified, bacterially expressed protein translocated microtubules in vitro; again, tubulin-GMPCPP microtubules were transported at 32% faster rates than tubulin-GDP microtubules ($p < 0.001$) (Table I). This result indicates that the different rates of transport of tubulin-GMPCPP and tubulin-GDP microtubules reflect distinct interactions with the kinesin motor domain, and not with a second microtubule binding site located in the C-termi-
Fig. 4. Bending of microtubule segments composed of tubulin-GDP or tubulin-GMPCPP. The brightly fluorescent portion of this microtubule is composed of tubulin-GMPCPP, while the more dimly fluorescent portion is composed of tubulin-GDP. Although the tubulin-GDP segment was usually at the plus-end of the tubulin-GMPCPP seeds, the reverse was occasionally observed, probably because of annealing between a tubulin-GMPCPP microtubule and a tubulin-GDP microtubule. The tubulin-GDP segment, which is leading the direction of movement toward the upper right corner, travels along a sinuous path on the squid kinesin-coated surface (panel A). When the tubulin-GMPCPP segment moves over the curved track of kinesins on the glass, it initially bends (panel B) (point of maximal curvature indicated by the arrow), but then dissociates from the kinesins and recoils to a straight configuration (panel C). Such dissociations of tubulin-GMPCPP segments occurred repeatedly as the chimeric microtubules moved across the surface, presumably because the elastic restoring force of the bending microtubule exceeded the binding energy of kinesins for the microtubules. This observation demonstrates that the tubulin-GMPCPP segment is more resistant to deformation by kinesin motors. Bar, 5 µm.

In contrast to the above results for kinesin, 14 S dynein from Tetrahymena axonemes transported tubulin-GMPCPP and tubulin-GDP microtubules at similar rates (Table I). This indicates that not all microtubule motor proteins transport these two types of microtubules with different velocities.

The velocity of chimeric microtubules composed of a tubulin-GDP segment and a tubulin-GMPCPP segment (see “Materials and Methods” for preparation) on squid kinesin-coated surfaces was also measured. As shown in Fig. 1, the chimeric microtubules translocated at the same average speed as tubulin-GDP microtubules, which was slower than the transport rate of tubulin-GMPCPP microtubules. A drag imposed by motors engaged with the tubulin-GDP segment of the microtubule may limit the velocity of transport and hence explain the comparable velocities of chimeric and tubulin-GDP microtubules. Similar results have been observed in a smooth muscle myosin motility assay, where unphosphorylated myosins impose a load that slows down the velocity of transport of actin filaments by phosphorylated myosins (30).

The stability of tubulin-GMPCPP microtubules provides a means of examining whether taxol affects the velocity of motor-driven movement. In the experiments described above, taxol was added to both tubulin-GDP and tubulin-GMPCPP microtubules. However, since the critical concentration for assembly of tubulin-GMPCPP is very low (20 nM; Ref. 11), tubulin-GMPCPP microtubules could be diluted to the low concentrations used in the motility assay in the absence of taxol. We found that the velocity of transport of tubulin-GMPCPP microtubules by squid kinesin was the same in the absence (0.68 ± 0.04 µm/s) or the presence (0.69 ± 0.04 µm/s) of 20 µM taxol (p > 0.4).

To ensure that taxol does indeed interact with tubulin-GMPCPP microtubules, we assessed whether taxol could stabilize these microtubules against cold-induced depolymerization (11). A 5-min incubation at 0 °C was sufficient to depolymerize all tubulin-GMPCPP microtubules, as determined by fluorescence microscopy. However, when tubulin-GMPCPP microtubules (0.15 µM tubulin) were incubated with 0.05 µM taxol for 5 min prior to the incubation at 0 °C, then 83% of the microtubules remained. Hence, even substoichiometric amounts of taxol are sufficient to stabilize tubulin-GMPCPP microtubules. Thus, taxol does indeed bind to tubulin-GMPCPP microtubules but does not noticeably affect kinesin-driven motility.

Microtubule Stimulation of Kinesin ATPase Activity—The higher velocity of transport of tubulin-GMPCPP microtubules compared with tubulin-GDP microtubules could be due either to a faster rate of ATP turnover by kinesin or to a larger distance moved by kinesin per ATP hydrolyzed. To distinguish between these possibilities, stimulation of ATP hydrolysis rate of a bacterially expressed human kinesin motor domain (amino acids 1–379) by tubulin-GDP or tubulin-GMPCPP microtubules was examined. In the absence of microtubules, the kinesin motor domain exhibited a low level of ATPase activity (0.04 ATP/s/head). This basal ATPase rate was stimulated approximately 500-fold by microtubules. As shown in Fig. 2, the stimulation of kinesin ATPase activity as a function of tubulin concentration was very similar for both tubulin-GMPCPP and tubulin-GDP microtubules. The curves could be best fit using a Michaelis-Menten equation with \( k_{\text{cat}} \) values of 21.1 and 21.0 s\(^{-1}\) and apparent \( K_m \) values of 0.87 and 0.98 µM for tubulin-GDP and tubulin-GMPCPP microtubules, respectively. These results indicate that the faster rates with which tubulin-GMPCPP microtubules are transported compared with tubulin-GDP microtubules cannot be explained by faster rates of ATP turnover by kinesin.

Mechanical Properties of Tubulin-GDP and Tubulin-GMPCPP Microtubules—Amos and Amos (31) previously reported that taxol-stabilized, tubulin-GDP microtubules form tight loops and bends when traveling over bovine brain kinesin-coated surfaces. We also noted pronounced bending of tubulin-GDP microtubules moving on surfaces coated with a high density of squid kinesin (particularly obvious examples are shown in Fig. 3). In some cases, the microtubules would move in a circular manner with a radius of curvature as small as 0.5–0.7 µm, although such extreme curvatures represented a small proportion (<5%) of the moving microtubules. Amos and Amos (31) suggested that extreme curvatures may be due to kinesin-induced deformation of the microtubule. In contrast to tubulin-GD microtubules, comparable length microtubules composed of tubulin-GMPCPP traveled as relatively straight rods and did not form the tight bends and loops observed for tubulin-GDP polymers (Fig. 3).

The bending properties of the tubulin-GMPCPP segment in chimeric microtubules were also examined. In some cases, the tubulin-GDP segment was at the front end of the chimeric microtubule traveling across the kinesin-coated surface, while the GMPCPP segment was trailing. The tubulin-GDP segment could move in a serpentine pattern, as noted in previous studies (21, 32), while the trailing tubulin-GMPCPP segment was generally unable to follow such circuitous path. In the example
Properties of Tubulin-GDP and Tubulin-GMPCPP Microtubules

To 120 GDP + TAXOL

Curvature (rad/μm)

Number of MT segments

GDP

GMPCPP

GMPCPP + TAXOL

Fig. 5. Distributions of curvatures for tubulin-GDP and tubulin-GMPCPP microtubules in solution in the presence and absence of taxol. Microtubules were prepared, frozen, and examined by cryo-electron microscopy and subsequently analyzed as described under "Materials and Methods." Distributions of curvatures for 1.6-μm segments along 42 randomly chosen microtubules are shown.

shown in Fig. 4, the tubulin-GMPCPP segment began to bend, but then abruptly dissociated and returned to its straight configuration, presumably because the restoring force of the bending microtubule exceeded the binding energy of the kinesins attached to the microtubules.

We also sought to compare the flexibility of tubulin-GDP and tubulin-GMPCPP microtubules in solution that were subjected to thermal agitation but not to kinesin-induced forces. To accomplish this, tubulin-GDP and tubulin-GMPCPP microtubules in the presence and absence of taxol, but without fixation, were rapidly frozen in liquid ethane and then examined by cryo-electron microscopy. From the electron micrographs, the change in the orientation of the tangent to the microtubule, dθ, along the length of 42 randomly chosen microtubules (described under “Materials and Methods”), and the distributions of curvatures, dθ/θ, are shown in Fig. 5. The mean curvatures of microtubulin-GDP microtubules were 0.127 ± 0.03 rad/μm and 0.120 ± 0.032 rad/μm, while those of tubulin-GMPCPP microtubules were 0.075 ± 0.07 rad/μm and 0.061 ± 0.06 rad/μm. These measurements demonstrate that the mean curvature of tubulin-GDP microtubules in solution is greater than that of tubulin-GMPCPP microtubules both in the presence or absence of taxol. The effect of taxol, the difference in the mean curvatures of tubulin-GTP microtubules in the presence or absence of taxol was not statistically significant (p > 0.15). For tubulin-GMPCPP microtubules, taxol decreased the mean microtubule curvature by a small (~20%) but significant (p < 0.005) amount. However, in contrast to the effect of bound nucleotide (GTP versus GMPCPP), taxol appears to have only a small effect on microtubule rigidity.

Structural Features of Tubulin-GDP and Tubulin-GMPCPP Microtubules—Tubulin-GDP and tubulin-GMPCPP microtubules were examined by cryo-electron microscopy to look for any changes in structure that might provide an explanation for the observed differences in motility and stiffness. The axial repeat was measured on computed power spectra of the two types of microtubules and found to be 41.93 Å (S.D. ± 0.15, n = 24) and 41.87 Å (S.D. ± 0.27, n = 14) for tubulin-GMPCPP microtubules in the absence and presence of taxol, respectively, and 40.74 Å (S.D. ± 0.27, n = 31) and 42.02 Å (S.D. ± 0.31, n = 21) for tubulin-GDP microtubules in the absence and presence of taxol, respectively. The axial repeat of tubulin-GDP microtubules in the absence of taxol was significantly different (p < 0.001) from that of tubulin-GMPCPP microtubules (presence or absence of taxol). Hence, both taxol and nucleotide have small effects on the tubulin lattice spacing. However, there is no substantial difference in the tubulin monomer spacing that could account for the 30% difference in kinesin-driven motility of tubulin-GDP and tubulin-GMPCPP microtubules.

In addition to the monomer spacing, other structural differences between tubulin-GTP or tubulin-GMPCPP microtubules were found. First, a higher percentage of 14-protofilament microtubules was observed when microtubules were polymerized...
The difference was particularly striking in the in vitro motility assay; pronounced bends of tubulin-GDP microtubules were observed that were never seen with tubulin-GMPCPP microtubules. The most extreme curvatures, however, seem in excess of those resulting from Brownian motion of the microtubule end and may be due to kinesin-induced deformation of the microtubule (31). In contrast to the effect of nucleotide on microtubule flexibility, we observed relatively little effect of taxol on microtubule curvature. Previous studies have obtained different results concerning the effect of taxol on microtubule rigidity. In preliminary work described by Gittes et al. (33), taxol was reported to produce a small increase in microtubule rigidity, while Dye et al. (34) reported that taxol considerably decreases the stiffness of microtubules nucleated from axonemes. The variation in results may reflect differences in the assays used to perform the measurement, and further studies will be required to resolve this matter.

Electron microscopic studies indicate that changes in the curvature of microtubules appear to be accommodated primarily by increased twisting of protofilaments rather than by compression/extension in the spacing between subunits within a protofilament (31). Thus, the greater stiffness of tubulin-GMPCPP microtubules relative to that of tubulin-GDP polymers might be due to a stronger lateral interactions between protofilaments. In support of this idea, a considerable difference in the protofilament twist angle of tubulin-GMPCPP versus tubulin-GDP microtubules was observed, which is suggestive of different lateral protofilament interactions in these two nucleotide states. The notion of stronger lateral interactions is also consistent with the finding that disassembling microtubules (presumably tubulin-GDP) have frayed and coiled protofilaments at their ends (35), while growing microtubules (presumably tubulin-GTP) and microtubule composed of tubulin-GMPCPP have straight, nonfrayed protofilaments (11, 35).

Native or bacterially expressed kinesin also transported tubulin-GMPCPP microtubules faster than tubulin-GDP microtubules. The greater speed of movement was not associated with an increase in ATPase rate. Although solution ATPase may not reflect the ATPase rate of motors that are attached to the glass surface and engaged in motility, these finding raise the possibility that kinesin, on average, travels farther per ATP hydrolyzed with tubulin-GMPCPP microtubules. Since the elemental step in the kinesin chemomechanical cycle involves movement of kinesin to an adjacent tubulin dimer (36), an increase in the distance between dimers could yield a greater step size. This model, however, is ruled out by diffraction patterns of cryo-electron microscopic images of microtubules, which show very little, if any, change in the spacing between tubulin subunits in the microtubule lattice. The difference in flexural rigidity between tubulin-GMPCPP and tubulin-GDP microtubules also is unlikely to change the speed of transport, since Young's modulus is sufficiently great to preclude any significant change in effective step size through microtubule strain. Using $E_{\text{tub}} = 1162 \text{ pN/nm}^2$ (33), an average inter-kinesin spacing of 100 nm along the microtubule, power stroke force of 4 pN, inner radius of microtubule = 11.48 nm, and outer radius = 14.18 nm (37), one can calculate that the reduction in step size at most would only be $7.9 \times 10^{-4}$ nm. These values are negligible compared to the normal step size of ~8 nm (36). An alternative idea to a change in step size is that the coupling efficiency (hydrolysis of an ATP followed by completion of an 8-nm step) is higher with tubulin-GMPCPP microtubules. An understanding of the physical basis of this phenomenon, however, must await more detailed information regarding the kinesin-microtubule interaction and the power stroke.
Properties of Tubulin-GDP and Tubulin-GMPCPP Microtubules

Although microtubules in cells are composed almost entirely of tubulin-GDP, the results presented here may be relevant for understanding the growing ends of microtubules, which are thought to be composed of tubulin-GTP. Such ends, whose size is still in debate, might be more rigid and have a different protofilament organization and monomer spacing than the remainder of the microtubule. This prediction could be examined in subsequent studies. The finding that the nucleotide state of tubulin influences kinesin interactions also raises the possibility that other types of microtubule-associated proteins might interact differently with tubulin-GTP and tubulin-GDP. Proteins that preferentially interact with tubulin-GTP, for example, might cap the plus-ends of microtubules and stabilize them to disassembly. Tubulin-GMPCPP microtubules might serve as useful reagents for identifying such proteins.

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