Microtubule-Associated Proteins and the Flexibility of Microtubules†

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ABSTRACT: Experiments were conducted to learn whether the binding of microtubule-associated proteins (MAPs) to microtubules alters the flexibility of the microtubules. Flexibility was measured *in vitro* by two established techniques. The first employed measurement of the bending of the microtubule in a flow of buffer; the second involved repeated measurement of random thermal fluctuations in the microtubule's shape. Similar values were obtained from microtubules prepared from purified tubulin and those prepared from microtubule protein containing saturating concentrations of MAPs isolated from bovine brain. When measured by the flow technique at 37 °C and pH 6.9, the persistence length of pure tubulin microtubules was found to be 8.4 ± 2.2 mm and that of MAP-containing microtubules was 9.4 ± 2.7 mm, not significantly different from each other. When measured by the thermal fluctuation technique under identical conditions, values of 6.2 ± 0.8 and 6.5 ± 0.8 mm were obtained, again not significantly different from each other. The results show that the binding of MAPs to native microtubules *in vitro* has little or no effect on their flexibility. MAP-induced effects on the cytoskeleton observed *in vivo* are likely to be due to other causes, such as formation of microtubule bundles.

Measurements of the flexibility of microtubules by different techniques have shown them to be remarkably rigid structures. A quantitative measure of their flexibility is their persistence length, essentially the distance at which the local direction of the microtubule (or other flexible rodlike structure) becomes uncorrelated. One recent study (Gittes et al., 1993), in which unanchored taxol-stabilized microtubules in random thermal motion were observed in dark-field optical microscopy and statistically analyzed, yielded a mean value of the persistence length of 5.2 ± 0.2 mm. A second study (Venier et al., 1994) of microtubules nucleated and anchored by sperm-tail axonemes, which were measured both by thermal fluctuations and by their deformation in hydrodynamic flow, obtained mean persistence lengths of 2.0 ± 0.2 mm. A third (Kurachi et al., 1995) made use of optical trapping to bend microtubules directly and obtained apparently length-dependent values in the range of 0.3−4.7 mm for microtubules stabilized by the drug taxol. These values, while not in complete agreement with each other, show microtubules to be about 2 orders of magnitude more rigid than the other well-characterized cytoskeletal filament, actin filaments (Gittes et al., 1993; Burlacu et al., 1992).

The measurements cited above were obtained *in vitro* with microtubules composed of highly purified tubulin. Cellular microtubules, though, contain both tubulin and microtubule-associated proteins (MAPs).[1] MAPs could play a role in modulating flexibility. Some results have suggested that the binding of MAPs to microtubules might make them more rigid. Kurachi et al. (1995), in addition to measuring taxol-stabilized microtubules, measured microtubules made from unchromatographed microtubule protein which contained significant quantities of MAPs, obtaining length-dependent persistence lengths in the range of 8−47 mm. Although this study showed clearly that MAP-containing microtubules are more rigid than MAP-free microtubules stabilized with taxol, it did not include measurements which would allow a direct comparison of the flexibilities of taxol-free microtubules assembled in the presence and absence of MAPs. Dye et al. (1993), studying microtubules to which the drug taxol had been bound, observed that such taxol-treated microtubules are both more wavy in appearance and more flexible than untreated microtubules. When MAPs (either MAP2 or tau) were added to the taxol-treated microtubules, a dramatic straightening and apparent increase in rigidity was observed. Although this qualitative study showed that MAPs were capable of reversing the flexibility induced by taxol, it did not address the question of whether they might have the same effect on untreated microtubules.

MAP-induced rigidity, if it proved to be a large effect in ordinary (i.e., non-taxol-treated) microtubules, could be important to their function within cells, where MAPs are always present. Attenuation of MAP1B expression by growth of PC12 cells in the presence of antisense oligo-DNA effectively blocks neurite outgrowth in response to nerve growth factor (Brugg et al., 1993). Expression of MAP2C in cultured cells that ordinarily lack this protein leads to the formation of bundles of microtubules which are straight relative to those seen in normal cells and which tend to bend where they encounter the cellular cortex (Weisshaar et al., 1992). Addition of cytochalasin B disrupts the cortical actin network of these MAP2C-expressing cells and allows them to form long neurite-like processes (Edson et al., 1993). One interpretation of these findings (Matus, 1994) is that some MAPs can modulate the flexibility of microtubules *in vivo*, leading to them becoming more rigid and thus able to deform

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[1] Abbreviations: MAPs, microtubule-associated proteins; 3X-MTP, microtubule protein purified by three cycles of assembly and disassembly; PC-MAPs, microtubule-associated proteins isolated by phosphocellulose chromatography; PM, 0.1 M 1,4-piperazinediethanesulfonic acid, 1 mM $MgSO_4$, 2 mM EGTA, 2 mM dithioerythritol, 1 mM GTP, pH 6.9; SDS, sodium dodecyl sulfate.

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the cell membrane to make projections. Although individual MAPs (e.g., τ) are dispensable for axonal elongation *in vivo* (Harada et al., 1994), it is possible that modulated flexibility could be important in cellular function. In a potentially analogous way, actin filaments are known to be modulated in their flexibility by the binding of myosin (Yanagida et al., 1978) and by Ca^{2+} and Mg^{2+} (Orlova & Egelman, 1993).

The present study makes use of methods described by Venier et al. (1994) and by Gittes et al. (1993) to address directly the question of modulation of flexibility by MAPs. We measured, *in vitro*, the flexibility of microtubules formed from pure tubulin and from microtubule protein containing saturating amounts of brain MAPs. MAPs proved to make little or no difference to the flexibility of microtubules. The speculation (Dye et al., 1993; Matus, 1994) that they modulate cellular events through modulation of rigidity of individual microtubules is therefore unlikely to be correct.

MATERIALS AND METHODS

Proteins and Axonemal Pieces. Microtubule protein (called 3X-MTP) was isolated from bovine brain by three cycles of assembly and disassembly, and tubulin was purified from 3X-MTP by chromatography on phosphocellulose, as described by Williams and Lee (1985) and modified by Correia et al. (1987). MAPs were eluted from a phosphocellulose column, gel-filtered into PM buffer (0.1 M Pipes, pH 6.9, 1 mM $MgSO_4$, 2 mM EGTA, 2 mM DTE, 1 mM GTP), and stored at −70 °C as described by Aamodt and Williams (1984). This mixture of MAPs is referred to as PC-MAPs. Analysis of 3X-MTP and of PC-MAPs by SDS−polyacrylamide gel electrophoresis is shown in Figure 1. Densitometric scanning was conducted with a Hewlett-Packard ScanJet 3P instrument and analyzed with the *NIH Image* software package. Subject to the limitations imposed by the possible nonlinearity of staining and differences in staining intensity between one protein and another, 3X-MTP had a ratio of high-molecular weight MAPs (primarily MAP1 and MAP2) to tubulin of 0.32 and a ratio of τ MAPs to tubulin of 0.14. PC-MAPs had a ratio of high-molecular weight MAPs to residual tubulin of 2.1 and a ratio of τ MAPs to residual tubulin of 0.54. Also visible in Figure 1 are the three bands, at apparent molecular weights of 200 000, 160 000, and 68 000, characteristic of the neuronal intermediate filaments present in small amounts in preparations of crude microtubules and MAPs (Berkowitz et al., 1977). Phosphocellulose-purified tubulin contained traces (less than 2%) of MAPs, which were removed from the tubulin by chromatography on DEAE-Sephadex, essentially as described by Detrich (1986). This extensively purified tubulin is called DEAE-PC-tubulin. The proteins were stored at −70 °C and gel-filtered into PM buffer just before use. Pieces of sperm-tail axonemes, used as nucleating structures, were prepared from sea urchin sperm (the kind gift of Dr. Leslie Wilson and Herb Miller) according to the method of Bell et al. (1982) as modified by Walker et al. (1988).

Microscopy. Microtubules, nucleated from pieces of sperm-tail axoneme which adhered to the glass slide, were observed by video-enhanced DIC microscopy and recorded on S-VHS videotape as described by Gildersleeve et al. (1992) and by Williams (1992). Magnification of the system was calibrated by measurement of a stage micrometer. Observations of microtubules bending in response to a flow

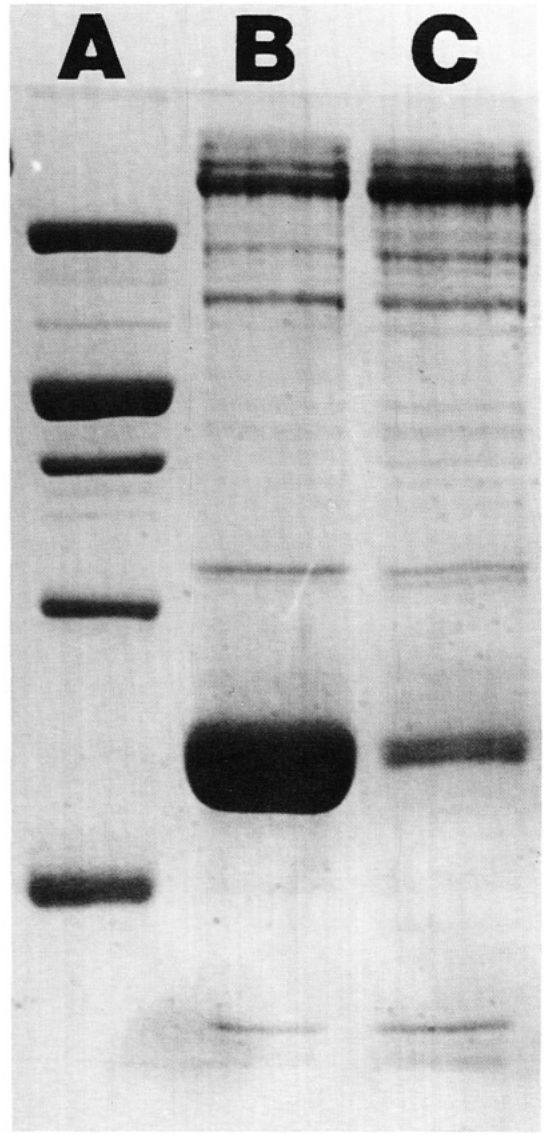

FIGURE 1: SDS gel electrophoresis of MAP-containing preparations: lane A, standards; lane B, 10 μg of 3X-MTP (i.e., microtubule protein prepared by three cycles of assembly and disassembly); and lane C, 5 μg of PC-MAPs (i.e., microtubule-associated proteins separated from 3X-MTP by phosphocellulose chromatography). Electrophoresis was performed on a 7.5% polyacrylamide gel, stained with B/T Blu (BT Scientific Technologies, Carlsbad, CA). Molecular weights of standards were as follows: 200 000, 116 250, 97 400, 66 200, and 45 000.

of solution were carried out in a flow cell built according to a modification (Williams, 1992) of the design of Berg and Block (1984). Flow was induced and maintained by a syringe pump (Harvard Apparatus, Model 950) arranged to withdraw liquid from the cell. The relevant velocity of flow was measured by tracking particles in the same focal plane as the microtubules as the particles were swept through by the flow (Venier et al., 1994). Those microtubules which grew (in stationary solution) in a direction perpendicular to the direction of flow, which were also substantially in focus along their entire length, and which appeared to be free of collision with other microtubules, other objects, or the glass substrate were selected for observation. The solution passing through the flow cell was either tubulin or 3X-MTP at a concentration approximating the appropriate critical concentration (in experiments with DEAE-PC-tubulin, microtubule assembly was initiated at a tubulin concentration of 1.3 mg/mL, and during flow, the concentration was maintained at

1.0 mg/mL; in experiments with 3X-MTP, growth was initiated with a protein concentration of 0.25−0.4 mg/mL and flow was maintained with 0.25−0.3 mg/mL). The large difference between the tubulin concentration required for assembly of the pure protein and the lower concentration required for 3X-MTP reflects the assembly-stimulating property of the MAPs. The range of concentrations of 3X-MTP employed reflects differences between preparations of 3X-MTP. The solution used to generate flow was kept on ice and warmed by its passage through tubing on the way to the flow cell, which was maintained at 37 °C. Constant flow was maintained for 1−3 min to obtain a representative sample of videotaped images. Occasionally, a particular microtubule was subjected to flow at more than one rate.

Observations not involving a flow of solution during measurement were carried out in a chamber, similar to that described by Hitt et al. (1990), made with spacers of Parafilm and sealed with VaLaP (a 1:1:1 mixture of vaseline, lanolin, and paraffin). Axonemes in suspension were introduced and allowed to settle for 10 min. A volume of protein-containing solution (tubulin or tubulin + MAPs) sufficient to rinse the cell three times was then forced to flow slowly through the cell in order to equilibrate the glass surfaces with tubulin. The cell was then sealed at both ends with VaLaP to prevent further flow. The volume of solution was 15−20 μL, the thickness of the chamber was 50−80 μm, and the protein concentration was 1.5−1.8 mg/mL pure tubulin or 0.8 mg/mL tubulin plus 0.1− 0.3 mg/mL MAPs (the MAP concentration was adjusted to produce good microtubule assembly with different preparations of protein).

Data Analysis. Measurements of flexibility of microtubules bent by a flow of solution were carried out, essentially as described by Venier et al. (1994), by observation of single video frames. Several (8−24) frames were chosen, at intervals of 0.3−1.0 s, in which the microtubule was in focus over its entire length. The contour of the microtubule in each frame was measured at 10−25 points (depending on its length) along its contour by the use of an image digitizer which superimposes a cursor directly on the video image. The velocity of the flow was measured by tracking small particles in the flow as described by Venier et al. (1994). Sufficient particles were adventitiously present in the 3X-MTP, but polystyrene beads (0.1 μm diameter, Polysciences, Inc.) had to be introduced into the solutions of DEAE-PC-tubulin, which were otherwise particle-free. A 10 000-fold dilution of the stock concentration gave good results. Profiles of microtubules bending in flow were measured and fitted to obtain the value of the variable K by a simple least-squares technique to eq 5 of Venier et al. (1994):

$$y = \{\pi\eta VL^4/[6K \ln(L/2d)]\}[(x/L)^4 - 4(x/L)^3 + 6(x/L)^2] \quad (1)$$

where x and y are the coordinates of a position on the contour of the microtubule, η is the viscosity of the solution (taken to be equal to that of water at 37 °C, 0.69 × 10^{-3} kg m^{-1} s^{-1}), V is the velocity of flow, L is the length of the microtubule, and d is its diameter (taken to be 28.4 nm). K is the flexural rigidity, and

$$K = EI \quad (2)$$

where E is Young's modulus of the rod and I is the moment of inertia of its cross section. K and the persistence length, L_p, are related by

$$L_p = K/kT \quad (3)$$

where k is Boltzmann's constant and T is the temperature. The values of L_p obtained from several measurements of each microtubule were averaged, and their standard deviation was calculated.

Measurements of the random thermal fluctuations in the shapes of microtubules grown from anchored axonemes in sealed chambers were made by repeated (60−70 frames) measurement of their contours (15−25 points) in frozen images at time intervals of 2−3 s. The variation of their shapes was analyzed by Fourier decomposition as described by Gittes et al. (1993), whose notation is employed here. The amplitudes of the Fourier modes (denoted a_n for the nth mode) of the microtubule's contour at each time were determined from the data as described. The variance of each mode (denoted $\mathrm{var}(a_n)^{\mathrm{measured}}$ for the nth mode) was then calculated. Two major phenomena contribute to the variance: the actual thermal fluctuation of the microtubule's shape and the random error of measurement. Their separate contributions are reflected in the two terms on the right-hand side of eq 17 of Gittes et al. (1993):

$$\mathrm{var}(a_n)^{\mathrm{measured}} = (L/n\pi)^2(1/L_p) + (4/L)\langle\epsilon_k^2\rangle[1 + (N-1)\sin^2(n\pi/2N)] \quad (4)$$

Here, L is the length of the microtubule, N is the number of points less one, n is the mode number, L_p is the persistence length, and $\langle\epsilon_k^2\rangle$ is the mean square measurement error. The variables L_p and $\langle\epsilon_k^2\rangle$ were determined by a least-squares fit of the data to this equation.

As a means of checking the effects of random measurement error, a single still video frame of a microtubule was measured 20 times. The measurements were then analyzed as if they were a time series.

Other Methods. Protein concentrations were determined by the method of Bradford (1976), with tubulin as a standard. The concentration of the standard tubulin was determined from its extinction coefficient: ϵ_{280} = 1.20 mL/(mg cm) (Detrich & Williams, 1978).

RESULTS

Microtubules Bending in Flow. Figure 2 shows observed and fitted profiles of microtubules, grown from DEAE-PC-tubulin, in a flow directed perpendicular to the original direction of the microtubule's axis. Figure 2A shows a video-DIC image of a microtubule bending in flow. Figure 2B shows the measured contour of this microtubule at two velocities of flow. One can see that it bends more sharply as the velocity of flow increases and that the quality of fit of the theoretical line to the actual shape of the bent microtubule is quite good in each case. Figure 2C represents several successive measurements, from different video frames, of the same microtubule in constant flow. The shape varies over times of a few seconds. Likely sources of this variation are minor instabilities of the pattern of flow and the ordinary random flexing of the microtubule due to Brownian motion. To accommodate the variation, measurements of many video frames were averaged in arriving at a value for the persistence length of each microtubule.

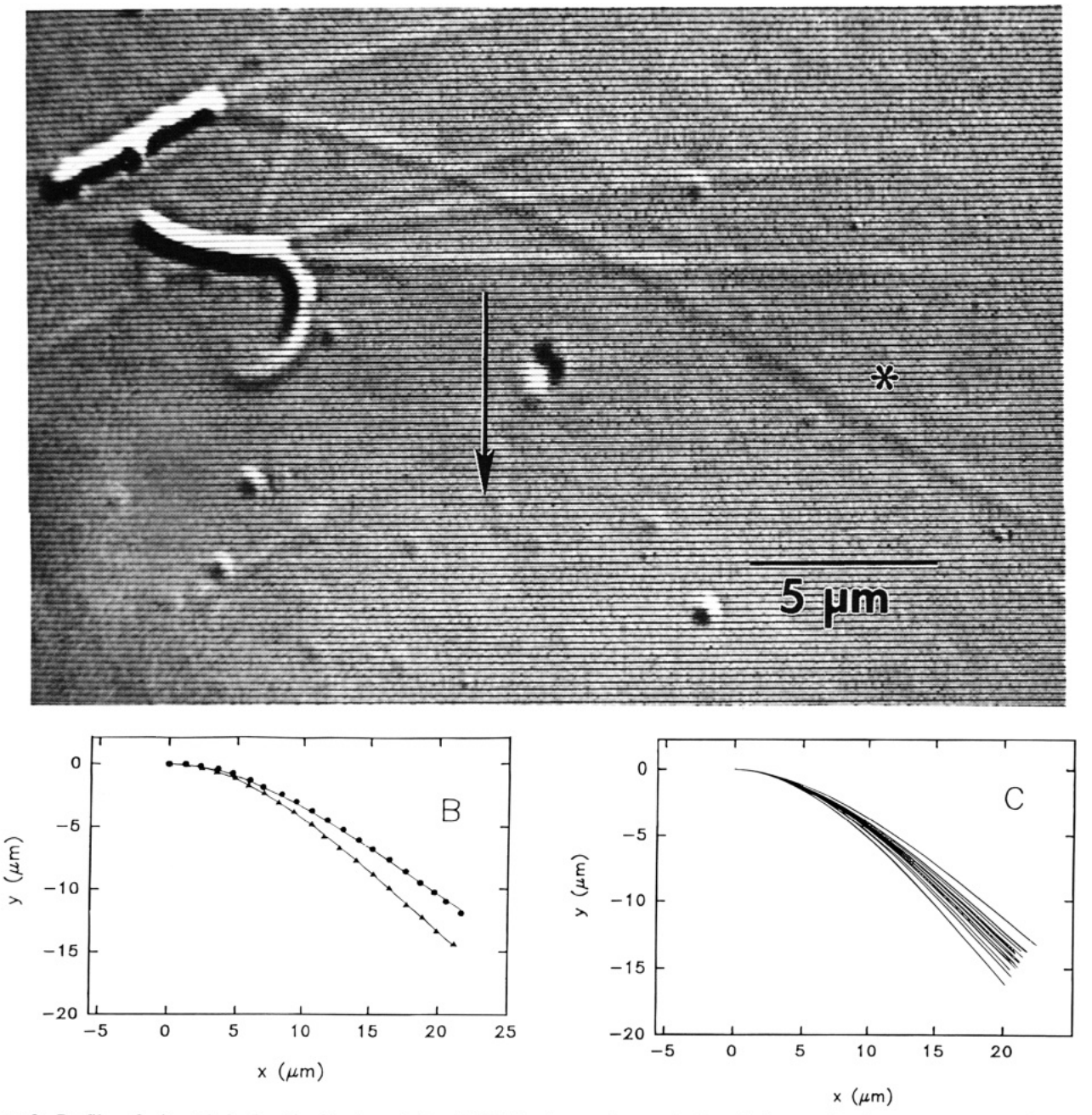

FIGURE 2: Profiles of microtubules bent by flowing solution. (A) Video image of several microtubules growing from two axonemal fragments. The arrow represents the direction of flow of solution. The two heavy-appearing structures are axonemal fragments, which adhere to the glass substrate. An asterisk marks a microtubule bent by the flow (several other microtubules visible in the same field are less deformed, partly because they are shorter and partly because they lie closer to the glass substrate than the one under observation). Scale bar = 5 μm. (B) Measured (points) and fitted (line) contour of the asterisked microtubule in panel A, recorded at two velocities of flow. The less sharply bent microtubule (●) corresponds to a flow velocity of 6.2 (±1.6) μm/s and the more sharply bent (▲) to a velocity of 8.8 (±2.5) μm/s. The coordinates of the junction between the axoneme and the microtubule are (0,0). (C) Successive profiles of a microtubule deformed by a constant flow. The range of variation in the profiles due to random movement of the microtubule is evident. The resulting range of fitted persistence lengths corresponding to the fitted lines shown was 8.8−11.7 mm [mean 9.66 ± 0.77 (standard deviation)].

Qualitatively similar behavior was observed whether the microtubules were grown from pure tubulin or from 3X-MTP.

Table 1 summarizes quantitative observations of microtubules bending in flow. The mean persistence length of microtubules made from pure tubulin (8.4 ± 2.2 mm) does not differ significantly from that of microtubules made from 3X-MTP (9.4 ± 2.7 mm). The presence of MAPs in 3X-MTP therefore does not measurably alter the flexibility of the microtubules. The observed absolute values of the persistence length are greater than those obtained in other studies (see Discussion), but they fall within the range of reliable measurement of the methods employed. The measured persistence lengths did not appear to depend on microtubule length or the flow velocity. Determinations of flow velocity were limited by the factors discussed by Venier et al. (1994). Specifically, the particles that were tracked to observe the flow were subject to Brownian motion and so moved rapidly in directions perpendicular to the main direction of flow as well as moving randomly in the direction of flow. These random movements contributed to the uncertainty of measurement of their velocity, as did the finite

Table 1: Persistence Lengths of Microtubules Bending in a Flowing Solution

microtubule	length (μm)	velocity of flow (μm s^{-1})	EI (N m^2)	persistence length (mm)	n[a]
		Without MAPs (DEAE-PC-tubulin)			
1	30.5 ± 0.9[b]	17.2 ± 4.0	3.40 (±0.85) × 10^{-23}	7.9 ± 2.0	8
2	14.9 ± 0.5	45.0 ± 13.6	3.31 (±0.37) × 10^{-23}	7.7 ± 0.9	14
3	23.7 ± 0.3	23.9 ± 4.4	5.09 (±0.61) × 10^{-23}	11.9 ± 1.4	14
4	22.6 ± 0.5	22.5 ± 4.0	3.65 (±0.57) × 10^{-23}	8.5 ± 1.3	20
5	19.6 ± 0.3	17.6 ± 5.0	2.46 (± 0.33) × 10^{-23}	5.8 ± 0.8	19
			3.58 (±0.95) × 10^{-23}[d]	**8.4 ± 2.2**[d]	
		With MAPs (3X-MTP)			
1A[c]	25.2 ± 0.3	6.2 ± 1.6	3.12 (±0.19) × 10^{-23}	7.3 ± 0.4	19
1B[c]	26.0 ± 0.3	8.8 ± 2.5	3.70 (±0.29) × 10^{-23}	8.7 ± 0.7	22
2	25.8 ± 0.4	5.4 ± 2.1	5.42 (±0.60) × 10^{-23}	12.7 ± 1.4	24
3	25.0 ± 0.3	7.6 ± 2.9	3.93 (±0.51) × 10^{-23}	9.2 ± 1.2	21
4	21.8 ± 0.3	9.0 ± 1.4	5.34 (±0.75) × 10^{-23}	12.5 ± 1.8	20
5	14.6 ± 0.3	13.3 ± 4.0	2.22 (±0.28) × 10^{-23}	5.9 ± 0.7	20
			3.95 (±1.25) × 10^{-23}[d]	**9.4 ± 2.7**[d]	

[a] Number of times the contour of this microtubule was measured. Each measurement was made on a separate video frame. [b] Error estimates represent one standard deviation. The standard deviation shown in each of the line-by-line entries is calculated directly from the values of the multiple measurements. The error estimates attached to the mean values of EI and persistence length represent the standard deviation of the five listed values about their respective means. [c] Entries 1A and 1B represent the same microtubule observed at two different flow rates. [d] Mean values.

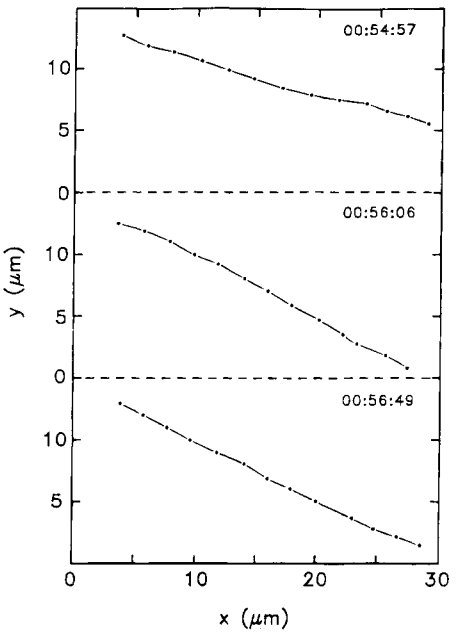

FIGURE 3: Representative measured profiles of a microtubule in random thermal motion in stationary solution. The nucleating axoneme was located near coordinates (0,15). Each panel represents a single still video frame. The points show measured values; the line has been added for convenience. The numbers in the upper right-hand corner of each panel represent the time of measurement in minutes:seconds:hundredths of a second.

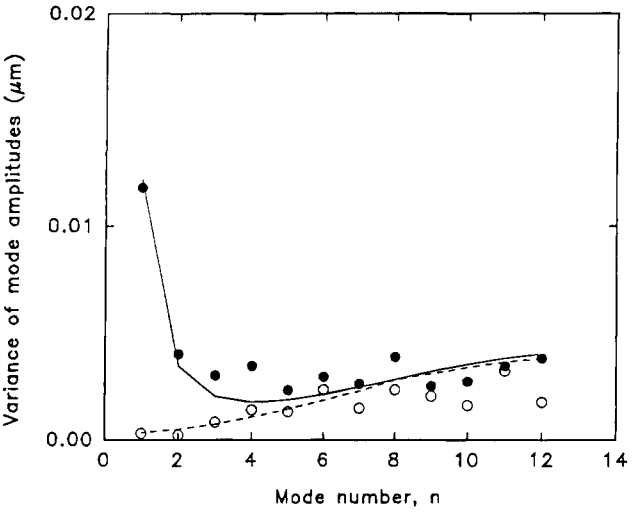

FIGURE 4: Variances of amplitudes of Fourier modes. Solid circles (●) show variances (denoted var$(a_n)^{measured}$ in eq 4) for a particular microtubule. The solid line represents the best least-squares fit of eq 4 to the data. The dashed line represents the contribution of random error of measurement (the second term on the right-hand side of eq 4) to the value of var$(a_n)^{measured}$. The open circles (○) show the variances estimated by repeated measurement of a single microtubule in a stationary video frame, as described in Materials and Methods.

focal depth of the objective lens (approximately 1 μm) which, when combined with the variation in the rate of flow with the distance from the glass, produced additional uncertainty. These causes contribute to the standard deviation of the flow velocity recorded in Table 1 and are reflected in the uncertainty of measurement of the persistence length.

Bending Due to Thermal Fluctuations. Figure 3 shows typical profiles of a microtubule in stationary solution measured at intervals of a few seconds. Fluctuations in curvature are seen to be slight but measurable, reflecting the rigid nature of the structure. For each of several microtubules, 60−70 measurements of the contours were obtained at successive times. Each was subjected to Fourier analysis into bending modes by the method of Gittes et al. (1993).

Figure 4 shows the variance of the amplitude of each of the first 12 modes for one particular microtubule (closed points and solid line) as well as the estimate of the contribution of random measurement error obtained from the second term of eq 4 (dashed line). It can be seen that the fractional contribution of measurement error to the variance of mode amplitudes is least for Fourier mode 1, as would be expected. Therefore, mode 1 yields the best precision of measurement. Also shown in Figure 4 is the variance of repeated measurements of a single microtubule, included as a control. One can see that the variance due to estimated measurement error (dashed line) closely approximates that obtained by repeated measurement (open points) of a stationary image of a microtubule.

Table 2 shows the collected thermal fluctuation results for microtubules made from DEAE-PC-tubulin and from aliquots of the same tubulin to which PC-MAPs were added. These results are less extensive than those obtained by the flow method and yield mean values for the flexibility somewhat

Table 2: Persistence Lengths of Microtubules Measured from Thermal Fluctuations

microtubule	length (μm)	measured variance[b] (μm)	corrected variance[c] (μm)	persistence length (mm)	n[a]
		Without MAPs (DEAE-PC-tubulin)			
1	26.5 ± 0.9	0.0135 ± 0.0025	0.0129 ± 0.0026	5.5 ± 1.2	59
2	20.5 ± 1.0	0.0094 ± 0.0016	0.0087 ± 0.0018	4.8 ± 1.1	68
3	22.8 ± 0.5	0.0068 ± 0.0017	0.0064 ± 0.0012	8.2 ± 1.8	66
				6.2 ± 0.8[d]	
		With MAPs (DEAE-PC-tubulin + PC-MAPs)			
1	27.2 ± 0.5	0.0118 ± 0.0021	0.0115 ± 0.0021	6.5 ± 1.3	65
2	24.5 ± 0.6	0.0094 ± 0.0016	0.0089 ± 0.0017	6.8 ±1.4	66
3	21.5 ± 0.5	0.0081 ± 0.0014	0.0075 ± 0.0015	6.2 ± 1.4	69
				6.5 ± 0.8[d]	

[a] Number of measurements of the microtubule included in calculation of the variance. [b] Variance of the amplitude of Fourier mode 1, uncorrected for the effects of random error of measurement, denoted $\mathrm{var}(a_1)^{\mathrm{measured}}$ in eq 4. [c] Variance of the amplitude of Fourier mode 1 after subtraction of the estimated contribution of measurement error (shown by the dashed line in Figure 4 and calculated as $\mathrm{var}(a_n)^{\mathrm{measured}} - (4/L)\langle\epsilon_k^2\rangle[1 + (N-1)\sin^2(n\pi/2N)]$ in the notation of eq 4). [d] Mean value.

smaller than those measured by the flow technique. Nevertheless, they confirm that the presence of MAPs causes no detectable change in flexibility of microtubules.

DISCUSSION

The measurements, both those made by the flow technique and those made by observation of flexing due to Brownian motion, yield the main conclusion that the presence of MAPs does not greatly alter the flexibility of microtubules. This is true both of the MAPs present in 3X-MTP and of those MAPs isolated from microtubule protein and added back to purified tubulin.

Saturation of Sites by MAPs. It was not possible to measure directly the extent to which the microtubules were covered with bound MAPs. In the case of microtubules made from 3X-MTP, one would expect MAPs to occupy nearly all of the binding sites on the tubulin lattice because the MAP:tubulin ratio (approximately 0.32 g/g, corresponding to a rough molar ratio of MAPs to tubulin dimer[2] near 0.20, or one MAP per five tubulin dimers) and the concentration of protein (1.5−1.8 mg/mL) approximate those that maintain a constant MAP:tubulin ratio during cycles of assembly and disassembly (Berkowitz et al., 1977). In the case of added PC-MAPs, the amounts added were such as to reconstitute the 3X-MTP from which they were originally isolated. One can also infer from the data of Wallis et al. (1993) that the resulting concentration range of 0.1−0.3 mg/mL (about 0.6−1.8 μM) is more than sufficient to produce saturation of the available sites on the microtubules, provided that the MAPs behave similarly to MAP2. For these reasons, it appears that the available MAP-binding sites must have been largely occupied in these experiments.

Reliability of the Measurements. The measurements made use of well-characterized techniques. The good fit of the observed bending of microtubules in flow to the theoretical equation describing their shape (Figure 2B) and, in the case of the Brownian flexibility measurements, the appropriate dependence of the variance of the Fourier modes on the mode number (Figure 4) both indicate that the methods applied are appropriate and that the microtubules behave as uniform rods flexing freely in solution.

The standard deviations of each of the measurements of persistence length by the flow technique (Table 1) fall in the vicinity of 25% of the values measured. Inspection reveals that these statistical variations are smaller than the difference in persistence length between microtubules. This difference between measurements probably results from the fact that both the flow rate and the shape of the bent microtubules enter into the mean numbers. The differences between individual microtubules can thus be accounted for largely by the large uncertainty (approximately 23% of the values measured) in the measurement of the flow rates; they probably do not reflect heterogeneity of flexibility of the microtubules. The lengths of the microtubules chosen for measurement clustered about 20 μm. Insufficient variation in length was present to reveal the dependence of flexibility on microtubule length noted by Kurachi et al. (1995).

The addition of MAPs must increase the hydrodynamic resistance of the microtubules, and no correction for this effect has been introduced into the data. Such increased resistance would cause the apparent persistence length of MAP-containing microtubules to be smaller than the true value. Such an underestimation would affect only the values obtained from the flow technique and not those obtained from the Brownian motion measurements. Because of the likely flexibility of the projecting portions of the bound MAPs and the substantial distance between them, this effect is expected to be small.

Absolute Values of the Persistence Lengths. The values of the persistence length obtained by the two techniques

[2] Largely because of the heterogeneity of the MAPs, only a rough approximation of their molar concentration can be given. A rough mean molecular weight for the MAPs can be estimated as the weight average of their molecular weights. These are taken to be 300 000 for MAP1A (Langkopf et al., 1992), 255 000 for MAP1B (Noble et al., 1989), 199 000 for MAP2A and -2B (Lewis et al., 1988), and 41 500 for the collection of τ MAPs [cf. Goedert and Jakes (1990)]. With the assumptions that both 3X-MTP and PC-MAPs contain 4 times as much MAP2 species as MAP1 species, that much more MAP1B is present than MAP1A, that the binding of MAP1C is negligible under the conditions of measurement, and that the ratio of high-molecular weight MAPs to τ MAPs corresponds to the measured value of 2.3:1, the rough mean molecular weight becomes (0.46 × 280 000 + 1.84 × 199 000 + 1 × 41 500/3.3 = 163 000. A solution of 1 g/L of MAPs in the observed ratio thus has a molar concentration of 1/163 000 M. Employing this number in the estimation of amounts of bound MAPs requires the further simplification of assumptions that no species other than those enumerated are present and that all MAP molecules bind to microtubules with the same affinity. When this is done, the 0.32 weight fraction of MAPs present in 3X-MTP can be converted to a rough mole fraction of 0.32 × (100 000/163 000), or approximately one MAP molecule per five tubulin dimers. This fraction approximates the accepted values for saturation of the tubulin lattice with MAP2 (Wallis et al., 1993) as well as earlier values (Kim et al., 1986; Burns et al., 1984) after they are corrected for the error in the molecular weight of MAP2.

differ moderately from each other ($P = 0.04$). Although the reason for the difference is not known, the comparison between microtubules with and without MAPs would not be expected to be affected by this difference. The persistence lengths observed here for MAP-free microtubules by the technique of Gittes et al. (1993) are marginally larger than the values (5.2 ± 0.2 mm) found by those workers. The values found by the technique of Venier et al. (1994) are substantially greater than the value (2.0 ± 0.43 mm) reported by them. We cannot account for the latter difference. The values found for MAP-saturated microtubules are smaller by about a factor of 2 than the mean value obtained for similar microtubules of 20 μm length by Kurachi et al. (1995) but are within the range of variation of their data. The agreement can thus be considered good. The comparison between MAP-free and MAP-containing microtubules within the present data is, of course, unaffected by the differences in the absolute values observed here and those reported elsewhere.

A previous study (Dye et al., 1993) showed that addition of MAP2 to microtubules which had initially been made wavy and apparently flexible by addition of the drug taxol caused a visually striking reduction in both properties, leading to a straightening of the microtubules against a flow of buffer. Subsequent investigation of taxol-treated microtubules by Venier et al. (1994) emphasized the waviness induced in microtubules by the drug and showed the increase in flexibility induced by taxol to be only approximately 2-fold, less than the 10-fold change initially estimated. The observed straightening of taxol-treated microtubules may have been partly the result of reduction in the taxol-induced waviness of these structures. The present results suggest that the flexibility-reducing effect of MAPs on microtubules, while it may be observed as a restoration of taxol-distorted microtubules to normal straightness, does not apply to normal microtubules. Putting all these studies together, one may infer the following relationships between the flexibility of microtubules of different kinds:

taxol-stabilized, pure tubulin > pure tubulin = MAP-decorated tubulin

taxol-stabilized, pure tubulin > taxol-stabilized, MAP-decorated tubulin

Cellular Consequences. These *in vitro* findings suggest that the rigidity of single microtubules is not greatly altered by the binding of MAPs, at least by the mixture of MAPs that is isolated from brain. Flexibility of microtubules is not altered by the binding of MAPs in a manner analogous to the myosin-induced alteration of the flexibility of actin filaments mentioned in the introduction section. It would seem, therefore, that if the effects of intracellular expression of MAPs on the formation of neurites and similar structures involve an induction of rigidity, that rigidity must result from such mechanisms as the formation of bundles of cytoskeletal elements [reviewed by MacRae (1992)], rather than from changes of the properties of individual microtubules.

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NOTE ADDED IN PROOF

While this study was in press, a paper appeared which also addresses the question of the effect of a MAP, recombinant τ protein, on microtubule flexibility [Mickey, B., & Howard, J. (1995) *J. Cell Biol. 130*, 909–917]. By microscopic observation of spontaneously nucleated and fluorescently labeled microtubules prepared with and without τ, that work found that binding of τ protein increases microtubule rigidity only slightly (about 30%). It thus appears that neither MAP-2 nor τ makes a large difference in microtubule flexibility.

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