Modification of microtubule steady-state dynamics by phosphorylation of the microtubule-associated proteins

(protein kinase/treadmilling/directionality/tubulin)

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ABSTRACT Phosphorylation of purified microtubule-associated proteins (MAPs) inhibits the rate and extent of MAP-stimulated microtubule assembly. The extent of microtubule assembly is reduced as a result of a decrease in the fraction of tubulin polymerized, without a significant change in the critical protein concentration. The decreased rate of microtubule assembly using phosphorylated MAPs reflects a reduction in microtubule nucleation resulting in fewer, but 2-fold longer, microtubules at steady state. Analysis of microtubule (MT) dynamics at steady state reveals that the rate of directional incorporation of subunits (flux) is 22 subunits·MT⁻¹·sec⁻¹ with phosphorylated MAPs, compared to 10 subunits·MT⁻¹·sec⁻¹ with unphosphorylated MAPs. The initial rate of disassembly determined by isothermal dilution is 232 subunits·MT⁻¹·sec⁻¹ for microtubules assembled with phosphorylated MAPs, compared to 102 subunits·MT⁻¹·sec⁻¹ for microtubules assembled with unphosphorylated MAPs. By using these results, the directionality (the number of successful subunit additions relative to the total number of association events per unit time) for subunit addition is found to be 0.1 for microtubules assembled with either phosphorylated or unphosphorylated MAPs. These observations are interpreted in terms of a mechanism in which phosphorylation of MAPs increases the rate of steady-state subunit flux by an equivalent enhancement of the rates of subunit association and dissociation, such that the critical protein concentration and directionality remain unchanged.

Microtubules assembled to steady state exhibit net assembly at one end and net disassembly at the opposite end, resulting in directional incorporation of subunits (flux) into the microtubule (1). This observation has suggested that factors which modify the rate of flux may be important in the regulation of microtubule function in dynamic cellular processes such as mitosis or intracellular transport (2).

Increased intracellular levels of 3',5'-cyclic AMP (cAMP) result in changes in microtubule arrangement and increased microtubule length (3, 4), suggesting that phosphorylation may play a regulatory role in microtubule-mediated functions. The microtubule-associated proteins (MAPs) have been found to be phosphorylated both *in vitro* and *in vivo* (5). MAPs enhance microtubule nucleation and increase the rate and extent of polymerization by stabilizing microtubules (6). Therefore, phosphorylation of MAPs could readily modulate microtubule dynamics. We recently demonstrated that phosphorylation of MAPs inhibited both the rate and extent of microtubule assembly (7). Margolis and Wilson have observed that ATP increased the rate of steady-state subunit flux, suggesting that phosphorylation may modulate this process as well (8).

In this paper, we have further characterized the effects of phosphorylation of MAPs on microtubule dynamics.

MATERIALS AND METHODS

Preparation of Microtubular Protein. Microtubular protein was purified by two cycles of assembly–disassembly, as described (7). Phosphocellulose chromatography was used for fractionation of MAPs and tubulin (9). The tubulin was concentrated by using an Amicon PM-30 membrane, and the MAP fraction was desalted by chromatography on Sephadex G-25. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (10) demonstrated that the MAPs fraction contained less than 10% contaminating tubulin and that the tubulin fraction was >95% pure.

Microtubule assembly and dilution-induced disassembly were measured by determining the change in absorbance at 350 nm (11). Protein phosphorylation was assayed as described (7).

Electron Microscopy. Microtubules were diluted 1:10 into 37°C 50% (wt/vol) sucrose in reassembly buffer [RB; 100 mM 4-morpholineethanesulfonic acid (Mes)/0.5 mM MgCl₂/1.0 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), pH 6.8] fixed with 1.5% (wt/vol) glutaraldehyde, applied to 50 × 200 mesh Formvar or collodion-coated grids, and stained with 1% uranyl acetate. As reported previously (1, 12), microtubules are stable under these conditions. Microtubule lengths were measured from electron micrographs taken at ×3000 magnification. The length distribution of microtubules at steady state allows one to determine the relative contribution of each microtubule length in a microtubule population. Therefore, for a given number of subunits polymerized, one can determine the relative number of microtubules formed.

Measurement of Subunit Flux at Steady State. Microtubules were assembled to steady state with 10 mM acetyl phosphate and acetate kinase at 0.1 unit/ml as described (8). A control mixture (200 μl) was assembled in the presence of [³H]GTP (1 μCi in 10 μl; 1 Ci = 3.7 × 10¹⁰ becquerels) in order to uniformly label the microtubules and define the maximal incorporation. Aliquots (200 μl) of unlabeled microtubules at steady state were mixed with 1 μCi of [³H]GTP and incubated at 37°C for various times. The amount of steady-state incorporation of [³H]GTP-tubulin into microtubules was determined after isolation of the microtubules by centrifugation through a glycerol cushion (11).

RESULTS

Phosphorylation of Purified MAPs. In a recent paper (7), we demonstrated that phosphorylated MAPs would inhibit MAP-stimulated microtubule assembly, but only when the MAP-tubulin oligomers were dissociated prior to polymerization. We have subsequently found that endogenous protein kinase activity copurifies with MAPs isolated on phosphocellulose and that

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Abbreviations: MAPs, microtubule-associated proteins; cAMP, 3',5'-cyclic AMP; MT, microtubules.

phosphorylation of isolated MAPs using 250 μM [$\gamma\text{-}^{32}\text{P}$]ATP and 25 μM cAMP results in a maximal incorporation of 20 nmol of phosphate per mg of protein. This is comparable to the maximal incorporation into MAPs when phosphorylation takes place prior to protein fractionation (7). Furthermore, when the proteins phosphorylated in the isolated MAPs fraction were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, the profile of ^{32}P -labeled proteins was indistinguishable from that obtained when the MAPs were isolated after phosphorylation of the unfractionated protein (7). Therefore, the current experiments were performed by phosphorylating MAPs after isolation from phosphocellulose and studying the effects of MAP-stimulated assembly of purified tubulin. This facilitates further characterization of the effects of phosphorylation on microtubules by allowing reconstitution directly after phosphorylation without an intermediate step to dissociate oligomers.

Inhibition of Microtubule Assembly by Phosphorylation of MAPs. Phosphorylation of MAPs after isolation from phosphocellulose results in inhibition of microtubule assembly upon reconstitution with purified tubulin (Fig. 1). Phosphorylation of MAPs prolongs the lag phase and inhibits both the rate and extent of microtubule assembly (Fig. 1). The maximal rate of polymerization is inhibited by approximately 87%, while the final extent of polymerization is reduced by approximately 25%. In control experiments, it was demonstrated that ATP and cAMP had no effect on microtubule assembly without a previous incubation with MAPs to allow phosphorylation to occur.

Critical Protein Concentration for Microtubule Assembly Using Unphosphorylated and Phosphorylated MAPs. The final extent of polymerization can be reduced either by raising the critical protein concentration in equilibrium with microtubules or by decreasing the fraction of tubulin polymerized at any concentration above the critical concentration. These may be distinguished by plotting the amount of microtubules formed as a function of tubulin concentration. As shown in Fig. 2, the critical protein concentration (x -axis intercept) is unchanged by phosphorylating MAPs. However, the slope of the line for microtubule formation as a function of tubulin concentration is decreased, indicating that phosphorylation of MAPs decreases the final extent of polymerization by reducing the fraction of tubulin polymerized.

FIG. 1. Inhibition of microtubule assembly by phosphorylation of MAPs. Microtubules were assembled by using tubulin at 2.9 mg/ml, 10 mM acetyl phosphate, acetate kinase at 0.1 unit/ml, 0.2 mM GTP, and MAPs at 0.6 mg/ml that had been preincubated at 37°C for 30 min either with no additions (●) or with 0.5 mM ATP and 0.05 mM cAMP (○).

FIG. 2. Determination of the critical protein concentration for polymerization with unphosphorylated and phosphorylated MAPs. Microtubules were assembled by using tubulin at 3.2 mg/ml, 2 mM GTP, and MAPs at 0.46 mg/ml that had been preincubated at 37°C for 30 min either with no additions (●) or with 0.5 mM ATP and 0.05 mM cAMP (○). The assembled microtubules were diluted into reassembly buffer containing 2 mM GTP at 37°C and the final absorbance at 350 nm was determined after a new steady state was attained. The change in absorbance at 350 nm was determined at each protein concentration by subtracting the absorbance of blanks, which consisted of identical reaction mixtures diluted without previous polymerization and kept at 0°C.

Length Distribution of Microtubules Assembled by Using Unphosphorylated and Phosphorylated MAPs. As shown in Fig. 1, phosphorylation of MAPs increases the lag phase preceding polymerization and decreases the maximal rate of assembly. This suggests that phosphorylation of MAPs reduces their ability to promote microtubule nucleation. Because nucleation determines the number of microtubules formed, inhibition of this process would result in fewer but longer microtubules. We previously found this to be the case for phosphorylated microtubules assembled after dissociation of oligomers at low ionic strength (7). Under the present conditions, the mean length of microtubules formed by using phosphorylated MAPs ($\approx 25 \mu\text{m}$) is 2.3 times the mean length of microtubules reconstituted by using unphosphorylated MAPs ($\approx 11 \mu\text{m}$) (Fig. 3). Similar results have been obtained in six separate experiments.

From the length distribution shown in Fig. 3, it was found that there are 2.1 times the number of microtubule ends when polymerization is complete in the unphosphorylated reaction relative to the phosphorylated reaction. With the exception of changes in length, the microtubules assembled with or without phosphorylation of MAPs were indistinguishable by electron microscopy.

Determination of Relative Microtubule Number Concentration by the Initial Rate of Elongation. The rate of microtubule polymerization is proportional to the product of microtubule number concentration and unpolymerized tubulin concentration (13). Therefore, at a fixed concentration of unpolymerized tubulin, the initial rate of microtubule assembly after the addition of microtubule seeds is directly proportional to the number of microtubules in the added seeds (13). These observations provide an alternative means to determine the relative number of microtubules formed by using unphosphorylated and phosphorylated MAPs. Microtubules were assembled by using unphosphorylated or phosphorylated MAPs as described in Fig. 1. After steady state was attained, one-half of each reaction mixture (100 μl) was removed and replaced with an equivalent amount of unpolymerized microtubular protein containing unphosphorylated MAPs. The initial rates of micro-

FIG. 3. Length distribution of microtubules assembled by using unphosphorylated and phosphorylated MAPs. The microtubules assembled in the reactions shown in Fig. 1 were analyzed by electron microscopy; approximately 300 microtubules were measured in each reaction. The length of microtubules which had only one end on the grid space was estimated as described elsewhere (13).

tubule assembly after addition of the unpolymerized protein to microtubules formed with unphosphorylated and phosphorylated MAPs were $0.026 A_{350} \cdot \text{min}^{-1}$ and $0.013 A_{350} \cdot \text{min}^{-1}$, respectively. There was no lag preceding polymerization, indicating that the previously assembled microtubules were serving as nucleating sites for elongation. In unseeded polymerization reactions, there was no increase in absorbance over the time course used for measuring rates (90 sec), so the contribution of

FIG. 4. Dilution-induced disassembly of microtubules assembled by using unphosphorylated and phosphorylated MAPs. Microtubules were assembled by using either unphosphorylated (●) or phosphorylated (○) MAPs as described in the legend to Fig. 1. The change in absorbance at 350 nm was monitored in cuvettes with a 5-cm path length after dilution of 100 μl of microtubule solution into 2 ml of reassembly buffer at 37°C. (Inset) First-order plot of the rates for dilution-induced disassembly of microtubules formed with unphosphorylated MAPs (●) and phosphorylated MAPs (○). $A_{350}(t)$ and $A_{350}(\infty)$ are absorbance at a particular time and at completion of the disassembly.

spontaneous nucleation was negligible. These results indicate that twice as many microtubules were assembled with unphosphorylated MAPs as with the phosphorylated MAPs, confirming the observations made by using electron microscopy.

Dilution-Induced Disassembly Rates for Unphosphorylated and Phosphorylated Microtubules. Rapid dilution of microtubules into isothermal buffer lowers the unpolymerized tubulin concentration, allowing the kinetics for microtubule depolymerization to be studied without interference from microtubule assembly (14). The initial rate of depolymerization upon dilution will depend upon the number of microtubule ends and the sum of the rate constants for subunit loss at the two ends of the microtubule, while the shape of the observed disassembly curve will depend upon the microtubule length distribution and microtubule number changes during disassembly (12).

It has previously been noted (12) that microtubules have a length distribution that is nearly exponential. As a result, the disassembly curve follows an exponential decay. Microtubules formed with either unphosphorylated or phosphorylated MAPs display dilution-induced disassembly kinetics that conform to an exponential decay process, reflecting the nearly exponential length distributions shown in Fig. 3 (Fig. 4 Inset). However, the observed rates for dilution-induced disassembly of unphosphorylated and phosphorylated microtubules are very similar despite a 2.1-fold difference in the number concentration of microtubule ends. This requires that the rate constant* for microtubule disassembly be proportionately larger for phosphorylated microtubules ($232 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}$) than for unphosphorylated microtubules ($102 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}$) in order to result in similar disassembly curves.

Rate of Steady-State Subunit Flux Through Microtubules Assembled by Using Unphosphorylated and Phosphorylated MAPs. Guanine nucleotides rapidly exchange at the E site of unpolymerized tubulin, but become nonexchangeable upon incorporation of the tubulin-nucleotide complex into microtubules (16). Therefore, the rate of uptake of labeled guanine nucleotides into microtubules directly reflects the rate of incorporation of tubulin subunits (1). Because at steady state net subunit addition equals net subunit loss, the rate of subunit flux through microtubules may be determined experimentally by measuring the rate of incorporation of labeled GTP into unlabeled microtubules.

The rate of [^3H]GTP incorporation into microtubules formed by using unphosphorylated and phosphorylated MAPs is shown in Fig. 5. The observed rate is expected to deviate from linearity for two reasons. First, because there is a distribution of microtubule lengths at steady state, the shorter microtubules will be completely labeled before the longer microtubules, and the

* Microtubules (MT) contain approximately 1600 dimers per μm (15). Therefore, the mean length of microtubules assembled with unphosphorylated MAPs is $17,600 \text{ dimers} \cdot \text{MT}^{-1}$, and the mean length with phosphorylated MAPs is $40,000 \text{ dimers} \cdot \text{MT}^{-1}$. The steady-state rate constant for disassembly, k_{-} , can be determined from the initial rate of disassembly after rapid dilution (11). For microtubules assembled by using either unphosphorylated MAPs or phosphorylated MAPs, the initial rate of dilution-induced disassembly is 0.58%/sec (Fig. 4). Therefore, the rate constant for disassembly of microtubules formed with unphosphorylated MAPs is:

$$\frac{\left(\frac{0.58 \text{ dimer released}}{100 \text{ dimers in the MT}} \right)}{\text{sec}} \times \frac{17,600 \text{ dimers in the MT}}{\text{MT}} = 102 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}.$$

A rate constant equal to $232 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}$ is calculated for the longer [$(40,000 \text{ dimers in the MT})/\text{MT}$] microtubules assembled with phosphorylated MAPs.

FIG. 5. Steady-state incorporation of tubulin subunits into microtubules assembled by using unphosphorylated and phosphorylated MAPs. Microtubules were assembled by using either unphosphorylated (●) or phosphorylated (○) MAPs as described in the legend to Fig. 1. After steady state was attained, aliquots were removed and incubated at 37°C with a trace amount of [³H]GTP for the times indicated. Incorporation of [³H]GTP was determined after isolation of the microtubules by centrifugation. Maximal incorporation was determined in control reactions in which polymerization took place in the presence of [³H]GTP. Each data point represents the mean determination from three experiments.

observed rate of incorporation will decrease with time. Second, the specific activity of the labeled GTP added to unpolymerized tubulin will slowly decrease as unlabeled guanine nucleotide is released from previously assembled microtubules. For these reasons, the initial rate of incorporation most accurately reflects the rate of steady-state flux. As shown by Fig. 5, the observed flux rates for microtubules assembled with unphosphorylated and phosphorylated MAPs are indistinguishable. However, because the microtubules formed with phosphorylated MAPs are much longer than those formed with unphosphorylated MAPs, it is apparent that for a given microtubule mass, there will be fewer microtubule ends available for subunit incorporation. Therefore, the flux rate per microtubule[†] in microtubules formed with phosphorylated MAPs (22 dimers·MT⁻¹·sec⁻¹) must be greater than that in microtubules formed with unphosphorylated MAPs (10 dimers·MT·sec⁻¹) in order for the same rate of subunit incorporation to be observed.

Effect of Phosphorylation on the Directionality of Steady-State Flux. The directionality of subunit flux refers to the number of successful subunit additions relative to the total number of association events per unit time (17). It therefore reflects the efficiency with which flux occurs. Steady-state directionality is given by flux/molecular rate constant for disassembly, k_- (Fig. 6). Therefore, by using the previously determined values for flux and k_- (Table 1), the directionality for microtubules assembled with unphosphorylated and phosphorylated MAPs is found to equal 0.1 in both cases.

Phosphorylation of MAPs Increases the Rate of Steady-State Subunit Flux by Proportionately Enhancing the Rate Constants for Subunit Addition and Loss. As illustrated in Fig. 6 and its legend, the dynamics of microtubules at steady state are largely defined by the critical protein concentration, rate of subunit flux, and rate of steady-state disassembly. The experimen-

FIG. 6. Microtubule dynamics at steady state. According to the condensation mechanism for assembly (18), microtubules are in equilibrium with a critical concentration, C_c , of tubulin subunits. The C_c is defined by the ratio of the off-rate constant, $k_- = k_{-1} + k_{-2}$, and the on-rate constant, $k_+ = k_1 + k_2$, such that $C_c = k_-/k_+ = (k_{-1} + k_{-2})/(k_1 + k_2)$. At steady state, the number of association events, defined by $C_c k_+ = C_c(k_1 + k_2)$, must equal the number of dissociation events defined by $k_- = k_{-1} + k_{-2}$. Subunit flux at steady state reflects the fact that there is a net gain of subunits at one end that is equivalent to the net loss of subunits at the opposite end. Thus, there is a net assembly end and a net disassembly end, which have different equilibrium constants for elongation. The rate of flux is defined by the difference in the number of association events and dissociation events at a given end such that flux = $C_c k_1 - k_{-1} = -C_c k_{+2} + k_{-2}$. Directionality refers to the efficiency of subunit flux and is the number of subunits gained at the assembly end relative to the total number of association events. At steady state, the number of association events equals the number of dissociation events. Therefore, steady-state directionality is equal to flux/ $k_- = (C_c k_1 - k_{-1})/(k_{-1} + k_{-2})$ (11). Experimentally, directionality for microtubules at steady state may be determined by measuring the rate of incorporation of [³H]GTP-tubulin subunits (flux) and the rate of dilution-induced disassembly, k_- (sum of the dissociation rate constants).

tal determination of these properties allows insight into the mechanism by which phosphorylation of MAPs alters steady-state dynamics. By using the notation defined in the legend of Fig. 6 and the results compiled in Table 1, it may be seen that when microtubules are assembled with phosphorylated MAPs, the rate constant for steady-state disassembly, k_- , is increased from 102 to 232 dimers·MT⁻¹·sec⁻¹, indicating that phosphorylated MAPs facilitate the loss of subunits from the microtubule ends. Furthermore, for the steady-state condition to be met for microtubules assembled with phosphorylated MAPs, the number of association events, $C_c k_+$, must be increased in proportion to the increase in the number of disassembly events, k_- . The number of association events, $C_c k_+$, may be increased by raising C_c , by increasing the rate constant for association, k_+ , or both. As shown in Fig. 2, microtubules assembled with either unphosphorylated or phosphorylated MAPs have the same C_c , within experimental error. Therefore, it follows that the increase in the number of association events in microtubules assembled with phosphorylated MAPs results from an increase in the rate constant for association, k_+ , which is proportionate to the increase in the rate constant for dissociation, k_- . Although the steady-state rate constants for association and dissociation are both increased in microtubules assembled with phosphorylated MAPs, it is not possible to predict how this will affect the rate of subunit flux without knowing the values of the individual rate constants for association (k_1 and k_2) and dissociation (k_{-1} and

Table 1. Summary of the properties of microtubules assembled with unphosphorylated and phosphorylated MAPs

Experimental observation	Unphosphorylated	Phosphorylated
Maximal rate of polymerization, $A_{350} \cdot \text{min}^{-1}$	0.15	0.019
Critical protein concentration, $\text{mg} \cdot \text{ml}^{-1}$	0.40	0.46
Mean microtubule length, μm	11	25
Disassembly rate, k_- , dimers·MT ⁻¹ ·sec ⁻¹	102	232
Flux rate, dimers·MT ⁻¹ ·sec ⁻¹	10	22
Directionality	0.1	0.1

[†] Incorporation of [³H]GTP-tubulin at steady state is 50% complete in 900 sec for both types of microtubules (Fig. 5). Therefore, the flux rate is $(0.5/900) \times 17,600 = 10 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}$ with unphosphorylated MAPs and $(0.5/900) \times 40,000 = 22 \text{ dimers} \cdot \text{MT}^{-1} \cdot \text{sec}^{-1}$ with phosphorylated MAPs.

k_{-2}) at the two ends of the microtubule (Fig. 6). However, experimentally, the rate of subunit flux in microtubules assembled with phosphorylated MAPs was found to increase by the same factor as that by which the disassembly rate, k_{-} , had been increased (Table 1). The significance of having proportionate increases in the rate of flux and the rate of disassembly is that the directionality for subunit incorporation, flux/k_{-} , is unchanged.

The observations that the critical protein concentration and directionality are unchanged when microtubules are assembled with phosphorylated MAPs results in an important simplification that allows the mechanism by which phosphorylated MAPs modulate the rate of subunit flux to be elucidated. Namely, it may be seen from the definitions in Fig. 6 that any disproportionate alteration in the individual rate constants (k_1 , k_{-1} , k_2 , and k_{-2}) for association or dissociation will change either the directionality or the critical protein concentration. Therefore, the increased rate of flux observed in microtubules assembled with phosphorylated MAPs must result from a proportionate increase in each of the individual rate constants for association and dissociation. For example, a 2.2-fold increase in k_1 , k_{-1} , k_2 , and k_{-2} would result in the experimental observations (Table 1) of a 2.2-fold increase in the rate of subunit flux and dilution-induced disassembly, without a change in the critical protein concentration or directionality.

DISCUSSION

We recently found that when MAP-tubulin oligomers were dissociated prior to polymerization, phosphorylation of MAPs inhibited both the rate and extent of microtubule assembly (7). In studies on microtubule steady-state dynamics, Margolis and Wilson found that ATP increased the rate of subunit flux (8). They proposed that this resulted from phosphorylation of MAPs, because the effect of ATP persisted through a cycle of assembly-disassembly and was not seen after the addition of a nonhydrolyzable analog of ATP.

In this paper, we have extended these earlier studies to further analyze the effects of phosphorylation of MAPs on microtubule assembly and dynamics. Utilizing the observation that the effects of phosphorylation of MAPs on microtubule assembly require dissociation of MAP-tubulin oligomers, we have studied microtubules assembled with purified tubulin and unphosphorylated or phosphorylated MAPs.

Phosphorylation of purified MAPs was found to inhibit both the rate and extent of microtubule assembly (Fig. 1), as previously described for MAPs phosphorylated prior to fractionation of microtubular protein (7). The prolonged lag phase and marked inhibition in the rate of microtubule assembly with phosphorylated MAPs (Fig. 1) provide kinetic evidence that nucleation has been inhibited. Thus, one would expect to find that phosphorylation of MAPs results in fewer but longer microtubules. This was found to be the case, as microtubules assembled with phosphorylated MAPs were 2.3 times as long as those assembled with unphosphorylated MAPs (Fig. 3). Furthermore, the initial rate of polymerization after addition of microtubules formed with unphosphorylated MAPs was twice that of microtubules formed with phosphorylated MAPs, providing additional evidence that phosphorylation inhibits nucleation and results in fewer microtubules.

The small reduction in the extent of polymerization with phosphorylated MAPs (Fig. 1) was found to result from a change in the fraction of tubulin polymerized rather than an increase in the critical protein concentration. Because the critical protein concentration is defined by the inverse of the equilibrium constant for elongation (18), this indicates that phosphorylation of MAPs does not alter the ratio of the disassembly rate constant, k_{-} , and the assembly rate constant, k_{+} .

Analysis of microtubule dynamics at steady state revealed that microtubules assembled with phosphorylated MAPs had a 2.2-fold increase in the rate of subunit flux and the rate of dilution-induced disassembly relative to microtubules assembled with unphosphorylated MAPs (Table 1). The proportionate increase in these properties reflects the fact that phosphorylation of MAPs does not alter the directionality for subunit incorporation. Therefore, phosphorylation of MAPs increases the rate of subunit flux but does not accomplish this by increasing the efficiency of subunit incorporation (directionality). Rather, it was shown that a proportionate increase in each of the individual rate constants (k_1 , k_{-1} , k_2 , and k_{-2}) for subunit association and dissociation at the microtubule ends would result in the experimental observation of a proportionate increase in the rate of subunit flux and dilution-induced disassembly, without a change in the critical protein concentration or directionality of subunit flux. Proportionate increases in the rates of association and dissociation do not change the equilibrium constants for subunit addition at the two ends of the microtubule and are therefore accomplished by lowering the activation energy barrier for subunit addition and loss.

Murphy *et al.* have concluded that MAPs stabilize microtubules by decreasing the rate of subunit dissociation without affecting the rate of subunit addition (6). Our results indicate that phosphorylation of MAPs alters the rate of association as well as dissociation. This would suggest that phosphorylated MAPs undergo different interactions with tubulin in the microtubule than do unphosphorylated MAPs. This view is consistent with the observation that the effects of phosphorylation on microtubule assembly (7) and dynamics (11) are not manifest unless MAPs and tubulin are dissociated prior to polymerization. Therefore, we suggest that phosphorylation of MAPs results in the formation of microtubules in which the MAP-tubulin interactions are altered in such a way as to facilitate the rates of subunit addition and loss at the microtubule ends and thereby modulate the rate of subunit flux. Modulation of microtubule dynamics by phosphorylation of MAPs may be an important mechanism for regulating the physiological functions of microtubules.

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