Effect of Tau on the Vinblastine-induced Aggregation of Tubulin

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ABSTRACT Two microtubule-associated proteins, tau and the high molecular weight microtubule-associated protein 2 (MAP 2), were purified from rat brain microtubules. Addition of either protein to pure tubulin caused microtubule assembly. In the presence of tau and 10 μM vinblastine, tubulin aggregated into spiral structures. If tau was absent, or replaced by MAP 2, little aggregation occurred in the presence of vinblastine. Thus, vinblastine may be a useful probe in elucidating the individual roles of tau and MAP 2 in microtubule assembly.

RESULTS

When 9.09 μM tubulin and 0.70 μM MAP 2 were incubated together, normal microtubule assembly occurred, as indicated by a gradual increase in turbidity (Fig. 2A) and by the appearance of microtubules as seen by electron microscopy. If 10 μM vinblastine was then added to these microtubules, the turbidity declined within 5 min to a plateau value ~25% lower than the turbidity of the untreated microtubule sample. The plateau value remained unchanged during 1 h of observation. When vinblastine was added to a mixture of tubulin and MAP 2 before incubation, a small increase in turbidity was sometimes observed upon heating to 37°C; this increase was about one-third as great as that generated under the same conditions in

MATERIALS AND METHODS

Microtubule protein was isolated from the brains of adult male Sprague-Dawley rats by the method of Fellous et al. (7). Tubulin was purified from microtubule protein by chromatography on phosphocellulose (Whatman P11; Whatman, Inc., Clifton, N. J.) (13). Thermostable microtubule-associated proteins, consisting of tau and MAP 2, were prepared from microtubule protein, as described previously (7), and were resolved into tau and MAP 2 by gel filtration on Ultrogel ACA 34 (LKB Instruments, Inc., Rockville, Md.) (13). The purity of each protein was determined by acrylamide gel electrophoresis (10). The MAP 2 fraction was found to be free of tau, and the tau preparation contained all four of the reported bands (3) and a 1–2% contamination with MAP 2 (Fig. 1). For light-scattering experiments, tau, MAP 2, tubulin, and vinblastine were mixed in various combinations in cuvettes, and incubated at 37°C in a Carl Zeiss PM 6KS spectrophotometer (Carl Zeiss, Inc., New York) with an automatic thermostated four-sample changer. The turbidity was determined at 345 nm. All proteins were dissolved in a buffer consisting of 100 mM 2(N-morpholino)ethanesulfonic acid, pH 6.4, 1 mM ethylene glycol-bis(β-aminoethyl ether) N,N'-tetraacetic acid, 0.1 mM ethylenediamine tetraacetic acid, 0.5 mM MgCl₂, 1 mM guanosine-5'-triphosphate, and 1 mM β-mercaptoethanol. Protein concentrations were determined by the method of Lowry et al. (11). Samples for electron microscopy were placed on Formvar-coated, carbon-stabilized 400-mesh copper grids and negatively stained with 1% aqueous uranyl acetate before viewing with a Philips 301 electron microscope at an operating voltage of 60 kV.
Electrophoretic analysis of tubulin and microtubule-associated proteins from rat brain. Samples of tubulin and microtubule-associated proteins from rat brain were reduced and carboxymethylated with sodium iodoacetate (4) and subjected to electrophoresis on discontinuous 6% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate (10). The gels were stained with fast green and photographed. The direction of electrophoresis is from top to bottom. The gel samples were as follows: a, tubulin purified on phosphocellulose; b, MAP 2; c, tau; d, tubulin (1.00 mg/ml) incubated for 30 min at 37°C with MAP 2 (0.20 mg/ml); e, tubulin (0.96 mg/ml) incubated for 30 min at 37°C with tau (0.11 mg/ml). The microtubules polymerized in the samples shown in gels d and e, as observed by turbidimetry and electron microscopy, whereas a sample identical to that shown in gel e generated spiral structures when 10 µM vinblastine was added.

DISCUSSION

Although both tau and MAP 2 could catalyze microtubule assembly, the properties of these two proteins, in the presence of vinblastine, differed from one another in two respects (Fig. 2). First, the turbidity generated by tubulin, MAP 2, and vinblastine was much less than that generated when tubulin and MAP 2 formed microtubules. In contrast, tubulin and tau generated a turbidity in the presence of vinblastine that was much greater than that seen when they formed microtubules. Second, addition of vinblastine to MAP 2-catalyzed microtubules caused only disassembly, whereas addition of the drug to tau-catalyzed microtubules caused substantial aggregation. These observations indicate a much greater difference between the absence of vinblastine (Fig. 2A). In many experiments, no increase in turbidity was observed when samples of tubulin containing MAP 2 were incubated at 37°C in the presence of 10 µM vinblastine. These results demonstrate that vinblastine inhibits MAP 2-catalyzed microtubule assembly and, in addition, induces partial disassembly of these microtubules.

Tau’s effects were very different from those of MAP 2. Although 1.06 µM tau induced rapid polymerization of 9.09 µM tubulin (Fig. 2B) into normal looking microtubules (Fig. 3A), the addition of 10 µM vinblastine to these samples caused the turbidity to decline by 50% within 1 min and then to increase rapidly after 2 min, until, at 10 min after addition of the drug, the turbidity reached a value three times as great as that of the untreated microtubule sample. Similarly, when vinblastine was added to a sample of tau and tubulin before incubation, a very large increase in turbidity occurred upon heating to 37°C (Fig. 2B). The increase in turbidity due to vinblastine was much larger than that observed when microtubules were formed in the same system in the absence of vinblastine. The rate of increase in turbidity after the addition of vinblastine was approximately the same whether the drug was added before or after microtubule assembly. When the drug-treated samples were examined by electron microscopy, the only organized structures visible were spirals (Fig. 3B), circles, and paracrystalline assays, whose common structural element was a filament 40-50 Å in diameter. In samples in which tau and tubulin had formed microtubules as seen by turbidimetry and electron microscopy, addition of vinblastine to such microtubule preparations caused the almost complete disappearance of microtubules and the appearance of large numbers of spirals. Samples lacking tau, that is, containing only tubulin or tubulin with vinblastine, showed no increase in turbidity and contained no organized structures when viewed in the electron microscope. In other experiments, we found that the rate of increase in turbidity was directly proportional to the concentrations of tau, tubulin, and vinblastine and that, at concentrations <5 µM, vinblastine inhibited microtubule assembly without inducing tubulin aggregation.

![Figure 1](image1.png)

**FIGURE 1** Electrophoretic analysis of tubulin and microtubule-associated proteins from rat brain. Samples of tubulin and microtubule-associated proteins from rat brain were reduced and carboxymethylated with sodium iodoacetate (4) and subjected to electrophoresis on discontinuous 6% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate (10). The gels were stained with fast green and photographed. The direction of electrophoresis is from top to bottom. The gel samples were as follows: a, tubulin purified on phosphocellulose; b, MAP 2; c, tau; d, tubulin (1.00 mg/ml) incubated for 30 min at 37°C with MAP 2 (0.20 mg/ml); e, tubulin (0.96 mg/ml) incubated for 30 min at 37°C with tau (0.11 mg/ml). The microtubules polymerized in the samples shown in gels d and e, as observed by turbidimetry and electron microscopy, whereas a sample identical to that shown in gel e generated spiral structures when 10 µM vinblastine was added.

![Figure 2](image2.png)

**FIGURE 2** Effect of vinblastine on tubulin polymerization in the presence of MAP 2 (A) and tau (B). 500-µl aliquots of tubulin (1.0 mg/ml) were incubated at 37°C with MAP 2 (0.212 mg/ml) (A) or tau (0.063 mg/ml) (B) in the presence (●) or absence (○) of 10 µM vinblastine. In some experiments, microtubules were assembled in the absence of vinblastine and, at the time indicated by the arrow, vinblastine (VBL) was added, to a final concentration of 10 µM. Development of turbidity was monitored at 345 nm.

![Figure 3](image3.png)

**FIGURE 3** Electron micrographs of tubulin polymerization in the presence of MAP 2 (A) and tau (B). (A) Tubulin (1.0 mg/ml) was polymerized in the absence of vinblastine, and the microtubules were fixed, subjected to electron microscopy, and then stained. τ (3.3 mg/ml) and tubulin (1.0 mg/ml) were polymerized in the presence of 10 µM vinblastine, and the microtubules were fixed, subjected to electron microscopy, and then stained. (B) Tubulin (1.0 mg/ml) was polymerized in the absence of vinblastine, and the microtubules were fixed, subjected to electron microscopy, and then stained. τ (3.3 mg/ml) and tubulin (1.0 mg/ml) were polymerized in the presence of 10 µM vinblastine, and the microtubules were fixed, subjected to electron microscopy, and then stained.
vinblastine. In contrast, MAP2-microtubules partially disassembled induced substantial aggregation in the presence of 10 μM vinblastine. When MAP2 was present at a concentration much lower than tau, or whether they have the same effect unless vinblastine is present, or whether vinblastine prevents the binding of MAP 2 to tubulin, cannot be determined from these data and remains the subject of future investigation.

In summary, our results show that, in the presence of vinblastine, tau and MAP 2 differ greatly in their ability to polymerize tubulin and suggest that vinblastine may be a useful probe in future studies to distinguish between the roles of the individual microtubule-associated proteins in the regulation of microtubule assembly.

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