A Taxol-dependent Procedure for the Isolation of Microtubules and Microtubule-associated Proteins (MAPs)

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ABSTRACT The effect of the antimitotic drug taxol on the association of MAPs (microtubule-associated proteins) with microtubules was investigated. Extensive microtubule assembly occurred in the presence of Taxol at 37°C, at 0°C, and at 37°C in the presence of 0.35 M NaCl, overcoming the inhibition of assembly normally observed under the latter two conditions. At 37°C and at 0°C, complete assembly of both tubulin and the MAPs was observed in the presence of Taxol. However, at elevated ionic strength, only tubulin assembled, forming microtubules devoid of MAPs. The MAPs could also be released from the surface of preformed microtubules by exposure to elevated ionic strength. These properties provided the basis for a rapid new procedure for isolating microtubules and MAPs of high purity from small amounts of biological material. The MAPs could be recovered by exposure of the microtubules to elevated ionic strength and subjected to further analysis. Microtubules and MAPs were prepared from bovine cerebral cortex (gray matter) and from HeLa cells. MAP 1, MAP2, and the *tau* MAPs, as well as species of M_r = 28,000 and 30,000 (LMW, or low molecular weight, MAPs) and a species of M_r = 70,000 were isolated from gray matter. Species identified as the 210,000 and 125,000 mol wt HeLa MAPs were isolated from HeLa cells. Microtubules were also prepared for the first time from white matter. All of the MAPs identified in gray matter preparations were identified in white matter, but the amounts of individual MAP species differed. The most striking difference in the two preparations was a fivefold lower level of MAP 2 relative to tubulin in white matter than in gray. The high molecular weight MAP, MAP1, was present in equal ratio to tubulin in white and gray matter. These results indicate that MAP 1 and MAP2, as well as other MAP species, may have a different cellular or subcellular distribution.

Taxol is an antimitotic agent derived from the western yew plant (43). This compound has been shown to stimulate dramatically the polymerization of mammalian cytoplasmic microtubules in vitro (32) and in vivo (33). Taxol was found to be effective in promoting microtubule assembly at close to a 1:1 molar stoichiometry to tubulin, the principal subunit of the microtubule polymer. Under conditions normally favoring the assembly of cytoplasmic microtubules in vitro, taxol reduced the critical concentration of free tubulin subunits in equilibrium with the microtubule polymer. In addition, the drug promoted microtubule assembly in the presence of elevated concentrations of calcium ion and at low temperatures, conditions that are normally unfavorable for microtubule assembly.

Taxol in part mimics the effect of a group of naturally occurring proteins in eukaryotic cells, the MAPs (microtubule-associated proteins; 36). These proteins are components of cytoplasmic microtubules that promote the assembly of tubulin in vitro, and possibly in vivo (30). MAPs have been isolated and extensively characterized from two sources, brain tissue and HeLa cells. Under conditions where tubulin, the major subunit of the microtubule structure, fails to self-assemble, both brain (5, 16, 19, 26, 37, 46) and HeLa cell (6, 45) MAPs produce a marked stimulation of assembly. The MAPs bind uniformly along the length of the microtubule polymer with a defined stoichiometry (1, 19). Like Taxol, the MAPs appear to function in promoting microtubule assembly in a stoichiometric rather than a catalytic manner (5, 28, 47).

Thus, Taxol behaves like the MAPs, though the drug is effective in promoting microtubule assembly under a wider range of conditions than the MAPs. I initiated this study to further compare the interaction of the two agents with microtubules and to determine how Taxol would affect the association of MAPs with microtubules. I have found that under solution conditions that are favorable for microtubule assembly in vitro and which are used for most studies on MAP-containing microtubules, the association of MAPs with microtubules

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is not detectably affected by Taxol. In addition, I have found that at elevated ionic strength the association of the MAPs with microtubules is abolished, while the stabilization of microtubules by Taxol is unaffected. I have applied these findings to the isolation of MAP-containing microtubules as well as MAPs by a novel method which takes advantage of the dramatic microtubule assembly-promoting activity of Taxol.

MATERIALS AND METHODS

Chemicals

Taxol was obtained from the National Cancer Institute. It was dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 10 mM and stored at −80°C. DMSO was found to have no effect on microtubule assembly at the concentrations used.

The microtubule assembly buffer used in all experiments, unless noted otherwise, was 0.1 M piperazine-*N*,*N*-bis (2 ethanesulfonic acid), pH 6.6, containing 1.0 mM EGTA, 1.0 mM $MgSO_4$, and 1.0 mM GTP.

Protein Preparative Procedures

BRAIN MICROTUBULES, REVERSIBLE ASSEMBLY METHOD: Microtubule protein was purified from whole calf cerebrum by the reversible assembly method of Borisy et al. (4) with slight modification (42). Microtubule protein was stored at −80°C after two assembly/disassembly purification cycles and carried through a third cycle just before use. Tubulin was purified from microtubules prepared in this manner by DEAE-Sephadex chromatography (41).

BRAIN MICROTUBULES AND MAPS, TAXOL/SALT PROCEDURE: Preparation of extracts was performed at 0°–2°C. 1–3 g of calf cerebral cortex (gray matter) or corpus callosum (white matter) was homogenized in 1.5 vol of microtubule assembly buffer minus GTP in a Potter-Elvejhem Teflon pestle tissue grinder at 2,000 rpm. The homogenate was centrifuged at 30,000 *g* for 15 min and the pellet was discarded. The supernate was then centrifuged at 180,000 *g* for 90 min and the pellet again discarded. The supernate at this stage was referred to as the gray matter or white matter cytosolic extract. Taxol was added to 20 μM and GTP to 1 mM. The solution was warmed to 37°C for 10–15 min and the microtubules that were formed were centrifuged at the same temperature for 25 min through a cushion of 5% sucrose in microtubule assembly buffer containing 20 μM taxol in either a swinging bucket or a fixed angle rotor. At this stage (see Figs. 4 and 6) the pellet contained microtubules as pure as those obtainable by several cycles of assembly/disassembly purification without Taxol.

Subsequent steps were used to isolate MAPs. Taxol was present in all steps at 20 μM. The microtubule pellet was washed by resuspension in ⅛ the starting volume of assembly buffer plus Taxol and the microtubules were recentrifuged at 30,000 *g* for 25 min. To dissociate the MAPs from the microtubules, the microtubule pellet was resuspended to volume in assembly buffer plus Taxol at 37°C, and NaCl was added to 0.35 M. The solution was centrifuged again at 30,000 *g* for 25 min, leaving the MAPs in the supernate.

HELA MICROTUBULES AND MAPS, TAXOL/SALT METHOD: HeLa cell extracts prepared as previously described (45) were generously supplied by Dr. James Weatherbee, or were prepared (45) from cells supplied by Dr. Thoru Pederson. Further steps were the same as for brain tissue as described in the previous section, with modifications as noted in Results. In a separate procedure the HeLa cells were swollen in H_2O containing 1 mM $MgSO_4$ and 1 mM EGTA. The cells were disrupted with a Dounce tissue grinder in two and a half times the original volume of packed cells and then ⅒ vol of × 10 assembly buffer minus GTP was added to the homogenate. Subsequent steps were as performed for brain tissue.

Isolated HeLa MAPs were further analyzed as follows. After recovery of the MAPs by exposure of Taxol-stabilized microtubules to 0.35 M NaCl followed by centrifugation, the MAP-containing supernate (0.3 ml) was passed over a 1-ml column of Sephadex G-25 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) pre-equilibrated with assembly buffer minus GTP. The MAPs were then combined with purified brain tubulin (1 vol of tubulin at 11 mg/ml combined with 6 vol of MAPs), GTP was added, and the mixture warmed to 37°C to allow polymerization to occur. Further microtubule purification steps by the reversible assembly method were as referred to above (4).

Analytical Methods

SDS gel electrophoresis was performed according to the method of Laemmli (21) using 9% acrylamide in the separating gel. Gels were stained with Coomassie Brilliant Blue R 250 (14). Gels were scanned with a Helena Quik-Scan, Junior scanning densitometer (Helena Laboratories, Beaumont, Texas). Quantitation of tubulin and the MAPs was carried out as described previously (42). Molecular weight standards were: calf brain MAP 2, rabbit skeletal muscle myosin, α-actinin, bovine serum albumin, actin, and α-chymotrypsinogen. Protein was determined using a modification (31) of the procedure of Lowry et al. (23). Electron microscopy was performed using a Philips 301 transmission electron microscope. Microtubules were pelleted through a sucrose cushion at 30,000 *g* at 37°C for 25 min. Pellets were fixed with 2% glutaraldehyde (Ladd Research Industries, Burlington, Vt.) and postfixed with 0.1% OsO_4 (Ladd Research Industries) for 30 min. Samples were stained en bloc with uranyl acetate and in sections with uranyl acetate and lead citrate.

RESULTS

Effect of Taxol on Microtubule Assembly and Composition

Both Taxol and the MAPs interact with tubulin and promote microtubule assembly. It seemed possible, therefore, that Taxol might interfere with the MAP-microtubule interaction. This would present a major problem in the use of Taxol for in vivo experiments and in the potential use of Taxol for the isolation of MAP-containing microtubules (this report). The following experiments were performed to determine the effect of Taxol on the association of MAPs with microtubules, and also to determine the effect of Taxol on microtubule assembly under conditions that were subsequently used in purifying microtubules and MAPs. It was found in the course of these experiments that the MAPs represented a significant fraction of the mass of the microtubule polymer. Therefore, the MAP content of microtubules could be determined using a mass assay for polymerization. This assay and an electrophoretic assay of microtubule composition were used for the experiments described in the first two figures.

Fig. 1 shows how microtubule polymerization was affected by Taxol under several sets of conditions. Extensive microtubule assembly occurred at 37°C in the absence of Taxol. In the presence of Taxol a slight increase in the amount of polymer was detected. No decrease in polymer was observed at the highest concentration of Taxol used (100 μM). As will be shown below, such a decrease would be expected if the MAPs were displaced by the drug.

Microtubule assembly was also assayed at 0°C. Polymerization was dramatically promoted by Taxol at this temperature (Fig. 1). The maximal extent of polymer formation was iden-

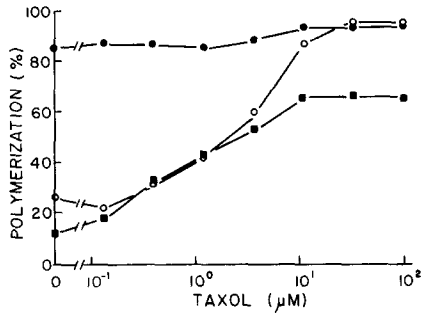

FIGURE 1 Promotion of microtubule assembly by Taxol. Taxol (10 mM in DMSO) was added to a series of concentrations to calf brain microtubule protein (2.0 mg/ml) that had been prepared by the reversible assembly procedure (4). The final concentration of DMSO was 1%. Polymerized microtubules were sedimented at 30,000 *g* for 25 min at 37°C. The percent of polymerization was obtained by measuring the concentration of protein in the supernates and subtracting these values from the total concentration before centrifugation. Some microtubule assembly or aggregate formation was evident at 0°C in the absence of Taxol. Microtubule assembly conditions were as follows: (●) 37°C for 15 min; (○) 0°C for 60 min; (■) 37°C for 15 min; 0.35 M NaCl added before polymerization.

tical to that observed at 37°C. No decrease in polymer was observed at the highest Taxol concentrations, again indicating that the MAPs remained associated with microtubules in the presence of the drug. Maximal polymer formation occurred above 10 μM of Taxol, which was approximately the same concentration as that of tubulin in this experiment (calculated at 11 μM). This suggests that Taxol interacts with tubulin at close to a 1:1 molar stoichiometry (cf. reference 32). It may be noted that maximal polymerization occurred at the same concentration of Taxol under all conditions examined (Fig. 1).

From these results it was concluded that Taxol did not affect the association of MAPs with microtubules. This was directly confirmed by gel electrophoresis which also showed no detectable decrease in the MAP content of microtubules at elevated concentrations of Taxol. These results, therefore, suggested that it might be possible to isolate MAP-containing microtubules from cells and tissues using Taxol to promote assembly (see below). In an attempt to obtain conditions for the solubilization of the MAPs obtained in such a procedure, the effect of ionic strength on the composition of microtubules assembled in the presence of Taxol was also investigated. The selection of these conditions was motivated by the observation that the assembly-promoting activity of the MAPs can be mimicked by polycations (13) and inhibited by polyanions (5). This suggested that the interaction of the MAPs with microtubules might have an ionic basis. Since it was also reported that microtubule assembly is strongly inhibited by increased ionic strength (29), it was of interest to determine whether Taxol would stabilize microtubules under these conditions.

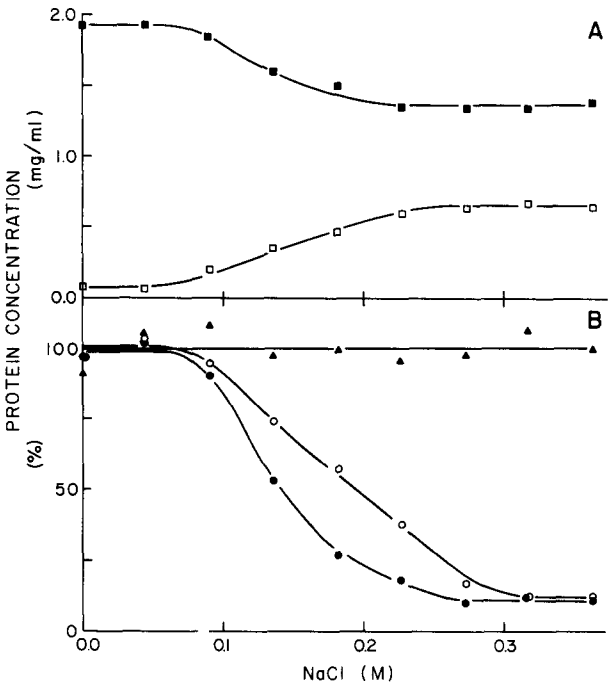

FIGURE 2 Polymerization and composition of microtubules as a function of ionic strength. Calf brain microtubules prepared by the reversible assembly procedure (4) were assembled at 37°C for 10 min in assembly buffer. Taxol was then added to 20 μM (0.2% DMSO), followed by NaCl added to the indicated concentrations. The microtubule protein (2.0 mg/ml) was centrifuged at 30,000 *g* at 37°C for 25 min through a cushion of 15% sucrose in assembly buffer plus 20 μM Taxol. The pellets were resuspended in assembly buffer, and the protein concentration and electrophoretic composition were determined for supernates and pellets. The composition data for the pellets only are shown. *A*: protein concentration of (■) microtubule pellets; and (□) supernates. *B*: amount of (▲) tubulin; (●) MAP 1; and (○) MAP 2 in microtubule pellets, in arbitrary units.

Fig. 1 shows that, in the absence of Taxol, microtubule assembly was abolished by the presence of 0.35 M NaCl. Addition of Taxol resulted in a dramatic stimulation of assembly. However, the maximal extent of assembly was considerably lower than that obtained at 37°C or at 0°C in the absence of added NaCl (Fig. 1). To learn the basis for this effect, the extent of microtubule assembly in the presence of Taxol was determined as a function of ionic strength, and the polymer that was formed was examined by electrophoresis (Fig. 2). Maximum assembly was observed in the absence of added NaCl. The amount of protein pelletable as polymer decreased as the concentration of NaCl was increased above ~0.1 M but then reached a constant lower level at higher NaCl concentrations (Fig. 2*A*). The range of ionic strength over which the decrease in polymer was observed was similar to that reported to block microtubule assembly in the absence of Taxol (29; see also this paper, Fig. 1). However, in the experiments reported here, polymerization was not totally blocked but, rather, only partially reduced. When the tubulin content of the polymer that was formed was assayed, it was found to be constant over the entire range of ionic strength examined (Fig. 2*B*). On the other hand, the MAP content of the microtubule polymer was greatly reduced as the ionic strength was increased. Both MAP 2, the major MAP species found in microtubules purified from brain tissue (see Fig. 4 and 6), and MAP 1, the second most prominent MAP species in brain microtubule preparations, were progressively displaced from microtubules as the NaCl was increased above ~0.1 M.[1] The dependence on NaCl concentration of the microtubule polymer mass and of the MAP content of the microtubules was quite similar. This suggested that the change in mass that was observed (30%) represented solely the loss of MAPs. It may be noted that all MAPs examined in this report in brain and HeLa cell microtubule preparations were removed from microtubules by exposure to NaCl (see below, Figs. 4, 5 and 6). Thus, the MAPs could be removed from microtubules under conditions that did not interfere with the stabilization of the microtubules by Taxol.

When microtubules prepared in the presence of Taxol and exposed to 0.35 M NaCl were examined by electron microscopy (Fig. 3) it was observed that the filamentous projections normally detected on the microtubule surface were absent. These projections have been shown to correspond to MAP 2 and, possibly, MAP 1 (11, 16, 19, 26, 40).

Use of Taxol to Purify Microtubules and MAPs

The results reported above suggested that Taxol might serve as a useful tool in the preparation of microtubules and the analysis of MAPs. While microtubules have been isolated from brain and from HeLa cells (4, 6, 35, 44), preparation of MAP-containing cytoplasmic microtubules has been difficult with many tissue and cell systems. This is probably partly due to

[1] MAP 2 (36) is defined as a heat-stable protein (16, 19) which is observed as a closely spaced pair of bands (M_r = 255,000 and 270,000) on electrophoretic gels. These bands have also been referred to as HMW 1 and 2 (4). MAP 1 (36) refers to a single major electrophoretic band (M_r = 350,000) and possibly several minor bands of molecular weight greater than MAP 2. Preliminary evidence suggests that this group of proteins may be heterogeneous with regard to some biochemical properties (W. Theurkauf and R. Vallee, unpublished observations).

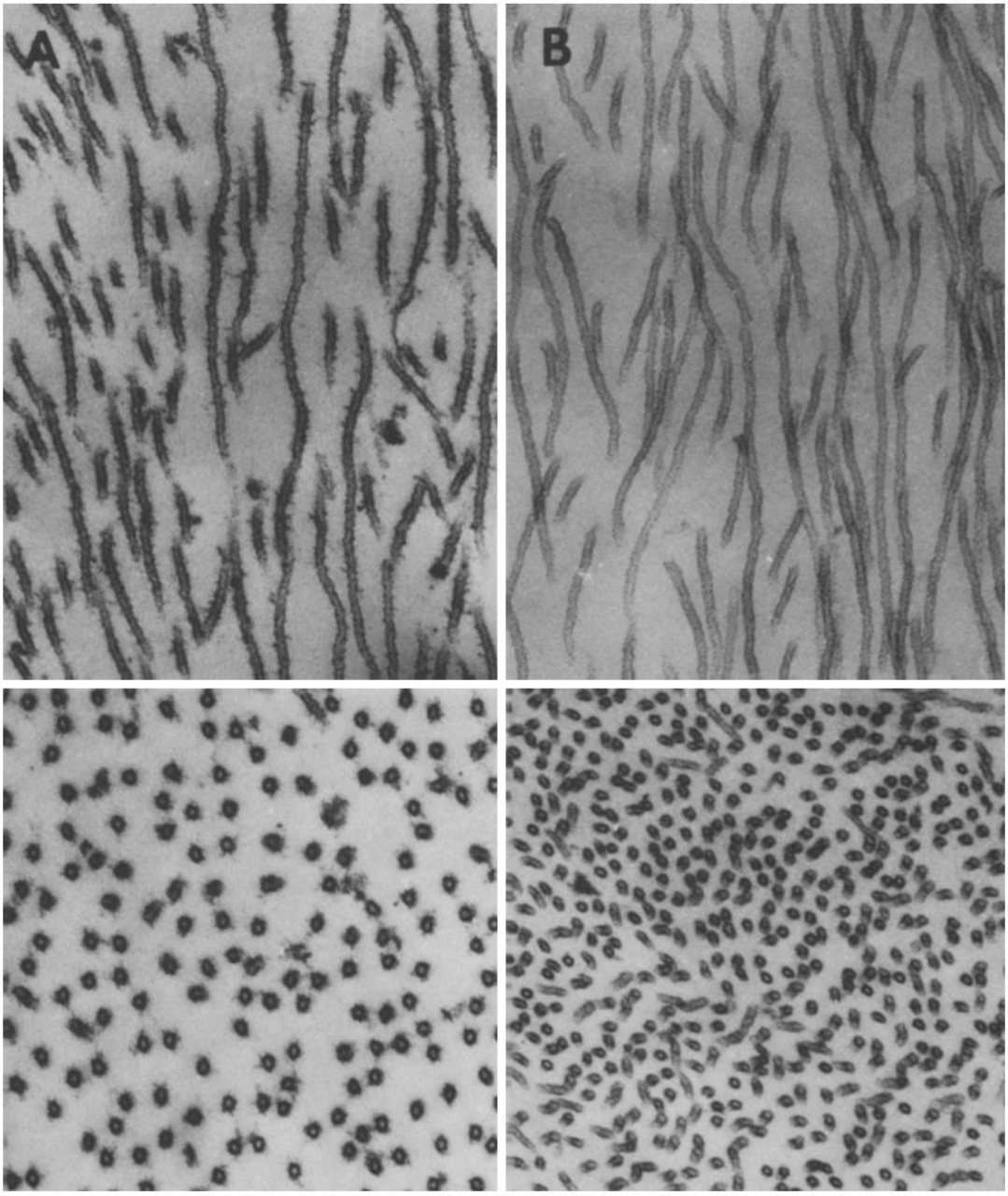

FIGURE 3 Electron microscopy of microtubules with and without MAP projections. Calf brain microtubules (2.0 mg/ml) prepared by the reversible assembly method (4) were polymerized for 10 min at 37°C in assembly buffer. Taxol was added to 20 μM (0.2% DMSO). To one-half of the solution NaCl was added to 0.35 M, while no additions were made to the other half. The two samples were layered over 15% sucrose in assembly buffer containing 20 μM Taxol and centrifuged at 30,000 *g* at 37°C for 25 min. *A*: no additions. *B*: 0.35 M NaCl added to remove MAPs. × 74,000.

the low concentrations of tubulin and MAPs in many cells and to the relatively high critical concentrations (15, 18) of microtubule subunits that must be exceeded to allow assembly to occur (6). In addition, microtubule assembly is highly sensitive to the lysis (6, 44) and centrifugation (3) conditions employed in preparing cytoplasmic extracts. Taxol offers the promise of promoting microtubule assembly under normally unfavorable conditions and, in light of the experiments reported here, of yielding MAP-containing microtubules. The limitation posed by Taxol is the potential difficulty in resolubilizing microtubules after polymerization, which has traditionally been an essential element in microtubule purification procedures (4, 35). This problem has been circumvented with the use of elevated ionic strength (see below) for directly solubilizing MAPs, which may then be further analyzed and purified by traditional procedures.

Fig. 4 shows the stages in the preparation of MAPs from brain tissue using Taxol to promote microtubule assembly. In the procedure used for this experiment the brain homogenate was subjected to successive low-speed (30,000 *g*) and high-speed (180,000 *g*) centrifugation, the former to remove large cellular debris and the latter to remove filaments and small vesicles which represent one major source of contamination in microtubule preparations (2). This procedure can only be performed with the aid of Taxol, since microtubule assembly has been found to be totally inhibited by centrifugation at high

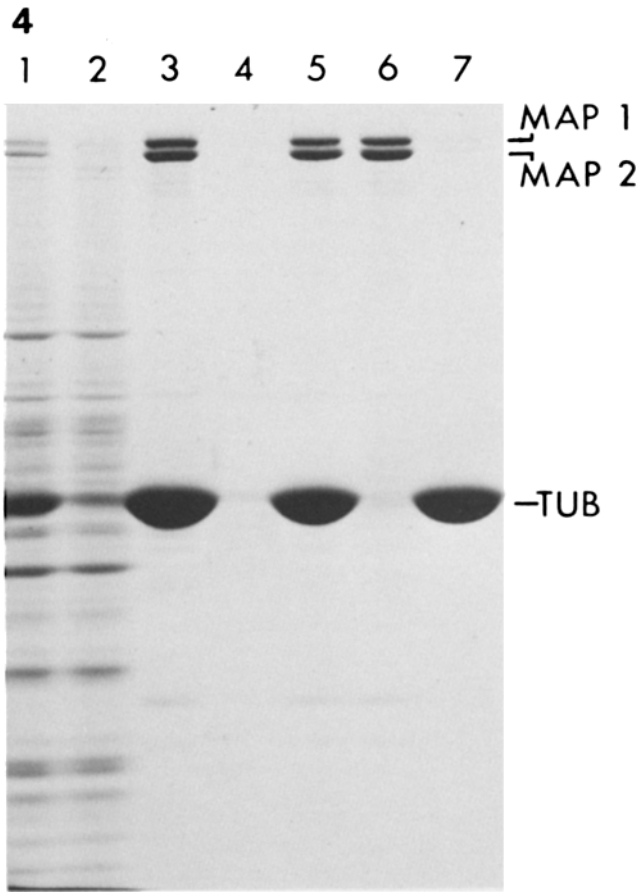

FIGURE 4 Microtubules and MAPs prepared from calf cerebral cortex by Taxol/salt method. A cytosolic extract was prepared from 2.7 g of calf cerebral cortex (gray matter) as described in Materials and Methods. Subsequent steps to isolate microtubules and MAPs were performed in complete assembly buffer containing 20 μM Taxol as described in Materials and Methods. Samples from each stage in the procedure were examined by gel electrophoresis and are labeled as follows: *1*, extract; *2*, first supernate, and *3*, first microtubule pellet; *4*, supernate, and *5*, microtubule pellet after wash of microtubules in assembly buffer plus Taxol; *6*, MAP-containing supernate, and *7*, tubulin-containing pellet after wash in assembly buffer containing Taxol and 0.35 M NaCl. Lanes *3–7* were loaded at five times the volume of sample used for lanes *1* and *2*. The yield of microtubules was 2.35 mg.

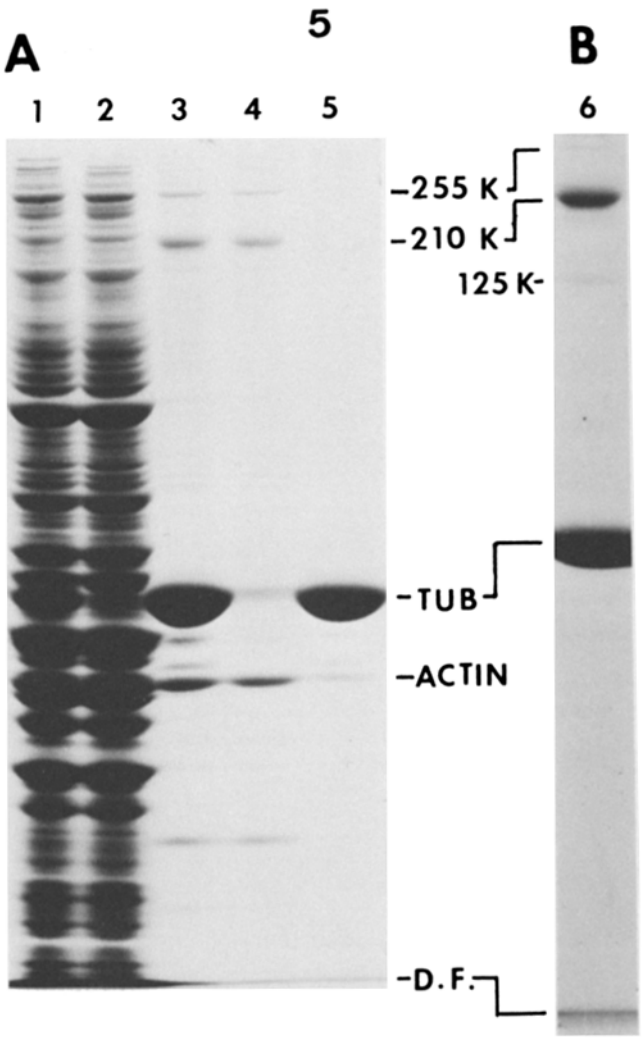

FIGURE 5 Microtubules and MAPs prepared from HeLa cells by Taxol/salt method. *A*: a sample of extract prepared from sonically disrupted HeLa cells (45) was kindly provided by Dr. James Weatherbee. The extract was further centrifuged at 180,000 *g* for 90 min at 2°C. Other steps were as for brain microtubules, with the following differences. 10 μM Taxol was used throughout. The first microtubule pellet was resuspended to ⅓ vol and was washed directly in 0.35 M NaCl in assembly buffer containing Taxol and 0.35 NaCl, without an intermediate low-ionic-strength washing step. Samples were examined by gel electrophoresis and are labeled as follows. *1*, high-speed extract; *2*, first supernate, and *3*, first microtubule pellet; *4*, MAP-containing supernate, and *5*, tubulin-containing pellet after wash in elevated ionic strength solution. Lanes *3–5* were loaded at five times the volume of sample used for lanes *1* and *2*. *B*: 3.0 g of packed HeLa cells were sonically disrupted in 1.5 vol of assembly buffer minus GTP, containing 1 mM DTT and 100 KIU/ml trasylol (cf. reference 45) and centrifuged at low speed (60,000 *g* for 30 min at 2°C). Microtubule assembly was performed as described above in the presence of 20 μM Taxol, and the first microtubule pellet was taken up in ⅒ vol of assembly buffer containing Taxol plus 0.35 M NaCl. Other details were as described in *A*. The MAP-containing supernate was passed over a column of Sephadex G-25 pre-equilibrated with assembly buffer, combined with purified calf brain tubulin, and carried through two cycles of assembly/disassembly purification (4). Gel lane *6* shows the resulting microtubule pellet.

speed (3). In addition, the microtubules were pelleted through a 5% sucrose cushion to eliminate a second major source of contamination, soluble cytoplasmic proteins entrapped in the microtubule pellet. The particular procedure used for Fig. 4 led to the rapid isolation of microtubules of a high degree of purity. Other extraction, centrifugation and polymerization conditions were also used successfully and may be appropriate for other types of experiment (see Fig. 5 *b*, for example).

Polymerization of brain microtubules in the presence of Taxol led to the extensive recovery of MAPs. MAP 1 and MAP 2 as well as tubulin were depleted (Fig. 4, lane *2*) from the cytoplasmic extract (lane *1*) and recovered in the microtubule pellet (lane *3*). Densitometric scanning of lanes *1* and *2* indicated that 90% of MAP 1 and MAP 2 co-sedimented with microtubules. Washing of the microtubules failed to remove appreciable quantities of any of the pelleted proteins (lanes *4* and *5*). Subsequent washing of the microtubules with assembly buffer plus 0.35 M NaCl, however, led to virtually complete removal of the MAPs (lanes *6* and *7*). All of the MAP species previously identified in brain microtubule preparations were found in the MAP fraction (lane *6*, and see Fig. 6 *c*), including MAP 1, MAP 2, *tau* (9), and a pair of LMW (low molecular weight) MAPs (2). The cyclic AMP-dependent protein kinase

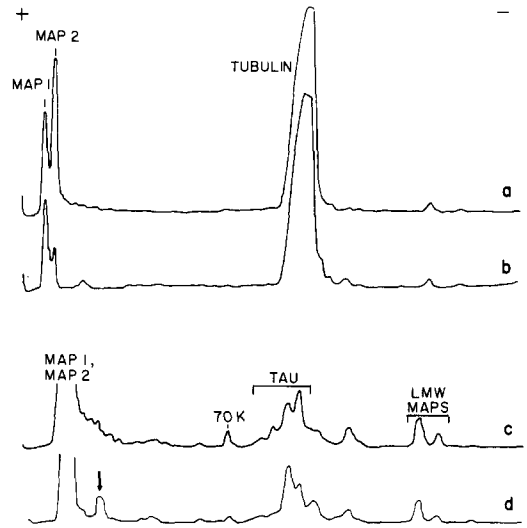

FIGURE 6 Microtubules and MAPs from gray and white matter. Microtubules were prepared using Taxol as described in Materials and Methods (see the legend to Fig. 4), and MAPs were released from the microtubules by exposure to 0.35 M NaCl. The gray matter preparation was the same as that shown in Fig. 4. The figure shows densitometric scans of gel lanes loaded with (*a*) gray matter microtubules; (*b*) white matter microtubules; (*c*) gray matter MAPs; and (*d*) white matter MAPs. The amount of tubulin loaded in *a* vs. *b* was the same (ratio of tubulin in *a*:*b* was 1.06). The MAPs are shown in *c* and *d* at about five times the scale in *a* and *b*. The arrow indicates a putative MAP species of ~200,000 mol wt (see text).

recently shown to be associated with MAP 2 (42) also cosedimented with microtubules in the presence of Taxol and was released from the microtubules as a complex with MAP 2 in the presence of 0.35 M NaCl (W. Theurkauf and R. Vallee, *J. Biol. Chem.*, in press).

In a separate experiment (data not shown), microtubules were induced to polymerize in a brain extract at 0°C by addition of Taxol. The extent of microtubule assembly and the composition of the microtubules were not detectably different from those of microtubules assembled at 37°C. Polymerization at 0°C may be useful for extracts of cells and tissues containing high levels of protease activity.

To determine whether the Taxol/salt procedure was applicable to biological material other than brain tissue, microtubules were also prepared from HeLa cells (Fig. 5). Several proteins were released from the microtubules by exposure to 0.35 M NaCl (lane *4*). The most prominent of these proteins had an electrophoretic mobility identical to that of the major MAP identified in HeLa cells (6, 45), kindly provided by Dr. James Weatherbee (Worcester Foundation for Experimental Biology) for comparison. This protein is labeled 210,000 in Fig. 5 (6), though it ran slightly below myosin in the present experiments. A minor species labeled 255,000 corresponds in molecular weight to another HeLa MAP (7). A band apparently representing actin was also observed in the microtubule and MAP fractions (lanes *3* and *4*), as well as a LMW protein (M_r = 29,000) which did not behave like a MAP according to other criteria (see below).

MAPs in the molecular weight range 100,000 to 125,000 (6, 45) were not detected. The absence of these species was apparently not an effect of Taxol. HeLa microtubules provided by Dr. Weatherbee, which contained MAPs in the molecular weight range 100,000–125,000, showed no change in composition through a complete cycle of assembly/disassembly purification in the presence of 10 μM Taxol. To determine whether the 100,000–125,000 MAPs might have been lost in the high-speed centrifugation employed in preparing the HeLa cell extract (Fig. 5*A*), the speed of centrifugation was reduced (Fig. 5*B*). Under these conditions the microtubule and MAP fractions obtained were less pure than after high-speed centrifugation. However, the MAPs were easily identified by co-assembly with pure tubulin (Fig. 5, lane *6*). The MAPs quantitatively cosedimented with tubulin and promoted its assembly. Under these conditions of preparation a MAP species of M_r = 125,000 was detected, corresponding to the second most prominent MAP in HeLa microtubule preparations (6). Thus, the MAPs identified were identical to those observed by Bulinski and Borisy (6, 7) and, in addition, to those identified in HeLa cells by Duerr et al. (12) by selective extraction of HeLa cells. The absence of the 125,000 mol wt band after high-speed centrifugation probably reflects the tendency of this species to form high molecular weight aggregates (7).

In addition to the experiment shown in Fig. 5 in which sonication was employed to disrupt the HeLa cells, cells were also disrupted in a Dounce tissue grinder after hypotonic swelling. The composition of the microtubules obtained by this procedure was the same as that obtained after sonication (data not shown).

Isolation of Microtubules from White and Gray Matter

The results reported here in preceding sections indicated that Taxol could be used to identify and isolate MAPs from sources for which traditional procedures have been successful. The following experiment was performed to assess the usefulness of the new technique with material not previously analyzed. Recent evidence has suggested that HMW MAPs in brain microtubule preparations may be preferentially localized in the dendritic processes of neurons (25). In that study the identity of the HMW MAPs was not established, nor were other MAP species investigated. Using the procedure outlined in the present report, I have prepared microtubules from bovine white matter and compared them with microtubules from gray matter as an independent method of determining the distribution of MAPs in nervous tissue. The results are shown in Fig. 6.

MAP 1 and MAP 2 were found in both gray and white matter (Fig. 6*a* and *b*). The yield of tubulin from white matter was 57% of that from gray matter on the basis of tissue wet weight. On the basis of recovered tubulin, MAP 2 was present at a much lower level in white matter than in gray. The ratio of MAP 2 to tubulin in gray matter was 0.13 and in white matter 0.025, a fivefold difference. On the other hand, the ratio of MAP 1 to tubulin in microtubules from both white and gray matter was 0.070. Thus, MAP 1 appeared to be evenly distributed on microtubules in different brain regions. In whole cerebellum (not shown), MAP 1 and MAP 2 were present in almost equal ratio to tubulin. The value for MAP 1 was 0.067, close to the value in cerebral gray and white matter, and for MAP 2 0.064.

Two other classes of MAP—*tau* (four bands of M_r = 55,000–62,000 [9]) and two LMW MAPs (2) M_r = 28,000 and 30,000—were also present in both white and gray matter at roughly equal ratio to tubulin (Fig. 6*c* and *d*). Differences in the intensity of the individual *tau* bands may be noted. In addition to these established species, a polypeptide (arrow) that comigrated on electrophoretic gels with the HeLa MAP of M_r = 210,000 was enriched in white matter (see Fig. 6). Whether this

species represents a new MAP and whether it is related to the major MAP of HeLa cells (Fig. 5) remain to be determined. A species of M_r = 70,000 was enriched in microtubules from gray matter. This species comigrated on electrophoretic gels with a polypeptide that appears to be associated with MAP 2 (42), which, as indicated above, is also enriched in microtubules from gray matter. The identity of other minor species lying between *tau* and the LMW MAPs is not known.

DISCUSSION

Interaction of MAPs and Taxol with Microtubules

I have developed a new procedure for the isolation of microtubules and MAPs using the drug Taxol to promote microtubule assembly. The mode of action of this drug is not well understood, and several new observations were made regarding its effect on microtubules and on the nature of the MAP/microtubule bond.

First, Taxol did not inhibit the association of MAPs with the microtubule surface (Figs. 1 and 3). Competition was not detected at levels of Taxol as high as 100 μM. This represented approximately a tenfold excess over tubulin and, therefore, approximately a 100-fold excess over the most prominent MAP in brain microtubules, MAP 2 (see 1, 16, 19). While failure to detect competition may simply indicate that the MAPs bind to microtubules more strongly than does Taxol, this seems unlikely since Taxol failed to displace the MAPs even under conditions where the drug was more effective than the MAPs at promoting microtubule assembly (see Fig. 1, 0°C samples). Therefore, another explanation may be correct, that Taxol and the MAPs occupy distinct, independent sites on the microtubule surface. Since Taxol interacts with microtubules at close to a 1:1 stoichiometry with tubulin (32; this paper, Fig. 1) it is unlikely that Taxol would bind solely to the microtubule ends as has been reported for other antimitotic drugs (24). Thus, the binding sites for Taxol and the MAPs may be spatially close but, nonetheless, independent. Since all of the known MAPs of brain and HeLa cells retained the ability to bind to microtubules in the presence of Taxol (Fig. 4–6), all of the MAP binding sites may be distinct from the Taxol binding site.

While Taxol did not affect the association of MAPs with microtubules, this association could be abolished at elevated ionic strength (Figs. 2 and 3). The binding of Taxol was apparently not influenced by ionic strength, as indicated by the persistence of its assembly-promoting activity over the range of ionic strength examined (Fig. 2). Thus, the mechanism for the association of Taxol and the MAPs appears to be different. MAP 1 and MAP 2 (Fig. 2) as well as all of the other known brain and HeLa MAPs (Fig. 5 and 6) lost the ability to bind to microtubules over the same range of ionic strength, suggesting a common basis for the interaction of all of the MAPs with microtubules. The present results strongly suggest a role for ionic bonds in these interactions. The earlier finding that polycationic macromolecules could substitute for MAPs in promoting microtubule assembly (13, 22, 27) is consistent with this conclusion and suggests that the portion of the MAP molecule involved in binding to microtubules (39) may be rich in basic amino acids.

These results, may, at least in part, explain the ionic strength dependence of microtubule assembly that has been observed in vitro (29) despite the predominant hydrophobic character of the assembly reaction (see reference 17). In this context it may be noted that the formation of the MAP-microtubule bond was not detectably temperature dependent (Fig. 1, cf. 0°C and 37°C samples at concentrations of Taxol ≥10 μM), in contrast to the overall assembly reaction (see, for example, Fig. 1, 0°C vs. 37°C samples in the absence of Taxol).

Isolation of Microtubules and MAPs

The binding of the MAPs to Taxol-stabilized microtubules has provided the basis for a new procedure for isolating MAP-containing microtubules (Figs. 4–6). Recovery of the MAPs for further analysis and purification was then accomplished by exposure of the microtubules to elevated ionic strength. The new procedure is an addition to the available procedures for microtubule purification (4, 30, 35) and for the identification of MAPS (8, 10, 12, 20, 38) but offers several advantages over existing procedures. (*a*) Taxol dramatically promotes complete microtubule assembly under a variety of inhibitory conditions. (*b*) Recovery of MAPs was unaffected by Taxol as it is by other assembly-promoting agents such as glycerol (34, 44). (*c*) The procedure can be performed on a microscale (pure microtubules were isolated from as little as 200 mg of tissue–data not shown). (*d*) The procedure can be performed in the cold, which inhibits proteolysis. (*e*) Rapid recovery of MAPs at high yield can be obtained.

The new procedure was used to isolate microtubules from brain and HeLa cells. All of the well-characterized MAPs from both sources were identified and isolated as MAPs with the new procedure (Figs. 4, 5, and 6). The procedure was also used to compare the composition of microtubules in gray and white matter from calf brain (Fig. 6). Differences as well as similarities in the MAPs from the two sources were observed. The most striking difference between the two preparations was a fivefold lower concentration of MAP 2 in white matter microtubules than in gray (Fig. 6). This result is consistent with a recent immunohistochemical study (25) indicating that HMW MAPs from brain were preferentially localized in the dendritic processes of neurons, which are present in gray but not in white matter. While MAP 2 was not specifically identified in the antigen preparation used in the earlier study, my results are consistent with the preferential localization of this particular protein in dendrites and, possibly, neuronal cell bodies.[2] My results do allow for the possibility that MAP 2 may also be present in axons, though at a lower concentration than in dendrites, since some MAP 2 was detected in white matter. Thus, a role for MAP 2 in axons cannot be ruled out, though a role in dendrites now seems certain.

In contrast to MAP 2, MAP 1 was present at equal concentration in both gray and white matter microtubules, in apparent disagreement with the results of Matus et al. (25). No real disagreement may exist, however, since it is not clear whether MAP 1 was present in the antigen preparation used in the earlier study. It is also possible that, if the antiserum prepared by Matus et al. contained antibodies to both MAP 1 and MAP 2, the uniform distribution of MAP 1 might have been obscured by the nonuniform distribution of MAP 2.

Why MAP 1 and MAP 2 should have different distributions is not at present clear. It seems reasonable to conclude that the two proteins, rather than operating in concert in the cell, must each be responsible for a separate cellular function. What the nature of these functions may be remains to be determined.

[2] In addition, a monospecific antibody against MAP 2 has been found to react preferentially with dendrites and neuronal cell bodies (P. DeCamilli, W. Theurkauf, and R. Vallee, manuscript in preparation).

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