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Low molecular weight microtubule-associated proteins are light chains of microtubule-associated protein 1 (MAP 1)

(protein complex/electron microscopy/microtubule projection/cytoskeletal crosslinker)

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ABSTRACT **Microtubule-associated protein 1 (MAP 1; M_r = 350,000) was analyzed by column chromatography of microtubule protein obtained from calf brain gray and white matter. Two low molecular weight proteins (LMW MAPs; M_r 28,000 and 30,000) were found to cochromatograph with MAP 1 under all conditions examined. MAP 1 and the LMW MAPs were purified from calf brain white matter as a complex containing approximately equimolar amounts of the three species. Urea (6 M) was used to remove the LMW MAPs from MAP 1. Binding of MAP 1 to microtubules was unaffected by urea and occurred with or without the LMW species. Electron microscopy of microtubules composed of purified tubulin and either MAP 1 preparation revealed that, like MAP 2, MAP 1 has the appearance of a filamentous arm on the microtubule surface.**

Various microtubule-associated proteins (MAPs) have been identified in preparations of cytoplasmic microtubules. The MAPs from brain tissue have been most extensively characterized. MAP 1 (M_r = 350,000)* and MAP 2 (M_r = 270,000)* are the most prominent nontubulin proteins in brain microtubule preparations. Both proteins copurify at constant stoichiometric relationship to tubulin through multiple cycles of assembly/disassembly purification (1, 4). Purified MAP 2 has the appearance of a regularly spaced filamentous arm on the microtubule surface (5, 6). It is not known whether the two high molecular weight MAPs are structurally related because, in contrast to MAP 2, virtually nothing is known regarding the structure of MAP 1. The τ MAPs (7) appear to represent a distinct structural class of MAPs, having a considerably lower molecular weight than MAP 1 and MAP 2 [M_r = 55,000–62,000 (8)] and a distinct morphology (9). A third class of low molecular weight MAPs (LMW MAPs, $M_r \approx$ 30,000) was identified by Berkowitz *et al.* (10). Although these proteins have received little attention, they fulfilled one significant criterion for being MAPs—i.e., they copurified with tubulin through as many as five cycles of assembly/disassembly purification at constant stoichiometric relationship to tubulin.

Of the two high molecular weight MAPs, MAP 2 has proven to be more amenable to purification. It may be readily purified by taking advantage of its unusual stability at higher temperatures (5, 6, 11). In addition, the protein has been purified in our laboratory under nondenaturing conditions (12). MAP 2 purified in this manner contained three accessory proteins (M_r = 39,000, 54,000, and 70,000), two of which (M_r = 39,000 and 54,000) represent the subunits of a cyclic AMP-dependent protein kinase (13).

We have now purified MAP 1. We have taken advantage of the recent finding (14) that MAP 1 is the most prominent MAP species in calf white matter and have used white matter as our source for MAP 1. We report here that, as previously found for MAP 2, proteins of lower molecular weight are associated with MAP 1. We have identified these components as the LMW MAPs that have been previously described (10). We have succeeded in removing these components from MAP 1 and describe here the morphology both of MAP 1 alone and of the MAP 1–LMW MAP complex.

MATERIALS AND METHODS

Preparation of Microtubules, MAPs, and Tubulin from Calf Brain Cerebral Cortex. Microtubule protein was prepared from calf cerebral cortex by using a modification (12) of the reversible assembly method of Borisy *et al.* (4). The buffer used for microtubule assembly was 0.1 M Pipes, pH 6.6/1.0 mM EGTA/1.0 mM $MgSO_4$ (PEM buffer) containing 1.0 mM GTP. MAPs and tubulin were separated by DEAE-Sephadex chromatography as described (15) but with PEM as the chromatography buffer. In addition, MAPs were prepared by adding taxol (20 μM) and NaCl (0.35 M) to microtubules purified by two cycles of assembly/disassembly purification. The microtubules were centrifuged at 30,000 × *g* for 30 min at 37°C; the MAPs were left in the supernatant (see ref. 14).

Preparation of MAP 1 from Calf White Matter. Microtubules were prepared from calf white matter by using taxol as described (14); starting with 60–80 g of tissue (wet weight) obtained from five calves; 10 mM 2-mercaptoethanol was included throughout the procedure. MAPs were recovered by salt dissociation from the taxol-stabilized microtubules as described (14). The predominant MAP species was MAP 1. The solution containing MAPs (4 ml) was applied to a 1.5 × 55 cm column packed with Bio-Gel A-15m preequilibrated with PEM buffer. The MAP 1 peak (Kd = 0.15–0.30) was recovered, pooled, and applied to a 4-ml column of CM-Sephadex preequilibrated with PEM buffer. The column was washed with 2 column volumes of buffer. The MAP 1–LMW MAP complex was eluted with a step of 0.25 M NaCl in PEM. The peak fractions were dialyzed for 2 hr against PEM. Approximately 1.5 mg of protein was recovered.

Alternatively, MAP 1 was eluted from the CM-Sephadex column with a step of 6.0 M urea in PEM buffer containing 10 mM 2-mercaptoethanol, leaving the LMW MAPs bound to the column.

Analytical Procedures. Gel electrophoresis was conducted according to the method of Laemmli (16) on 1.5-mm-thick slab gels with 9% polyacrylamide in the separating gel. Gels were

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Abbreviations: MAP, microtubule-associated protein; LMW, low molecular weight.

* Two bands referred to as MAP 1 and MAP 2 are observed in brain microtubule protein preparations on high percentage polyacrylamide gels (1). On lower percentage acrylamide gels, these bands are observed to be complex (2). The molecular weights used here are for the most prominent bands in the MAP 1 and MAP 2 complexes in calf microtubule protein (see ref. 3).

stained with Coomassie brilliant blue R250 (17). To determine the ratio of the LMW MAPs to MAP 1, protein was applied to the gel at low and high concentrations to ensure that both high and low molecular weight species, respectively, would be in the linear range of the dye absorbance–protein concentration relationship. Molecular weights of the LMW MAPs were estimated by using myosin, β-galactosidase, α-actinin, bovine serum albumin, tubulin, actin, α-chymotrypsinogen, and clathrin light chains as standards (18, 19); the last material was a generous gift of T. Kirschhausen and S. Harrison. Protein concentration was determined by the Bradford method (20) with the Bio-Rad protein determination reagent (Bio-Rad). Microtubule assembly was determined by light scattering measured as optical density at 320 nm (21).

Electron Microscopy. Microtubules were assembled from pure tubulin by using taxol to promote assembly. MAP 1 was either added to the preformed microtubules or was present during assembly. The microtubules were sedimented at 30,000 × g for 25 min at 37°C. All chemicals for electron microscopy were obtained from Ladd Research Industries (Burlington, VT). The pellets were fixed for 1 hr in 1% tannic acid plus 2% glutaraldehyde in PEM buffer, postfixed with 0.1% OsO_4/0.1 M sodium phosphate, pH 7.2, and embedded in Spurr's epoxy resin. Sections were stained with 20% uranyl acetate in methanol and 0.5% lead citrate in H_2O and examined in a Philips 301 transmission electron microscope at ×45,000 or ×57,000 magnification. Arm dimensions and spacing were determined by using a carbon replica of a 29,000-line-per-inch grating as standard.

RESULTS

Purification of the MAP 1–LMW MAP Complex. In attempts to purify MAP 2 (12) and MAP 1 (this paper) we have subjected microtubules and MAPs to various chromatographic procedures. An example of the chromatographic behavior of the MAPs is presented in Fig. 1. Most of the MAP 2 (peak at fraction 19) eluted early in the salt gradient along with a number of other protein species (which did not cochromatograph with MAP 2 in other chromatographic procedures). MAP 1 (peak at fractions 23–25) eluted after MAP 2 and was enriched relative to MAP 2 in the later fractions. Coeluting with MAP 1 were a pair of proteins of M_r 28,000 and 30,000 (denoted LMW 1 and LMW 2). These proteins coeluted quantitatively with MAP 1 under a wide variety of chromatographic conditions. These included Bio-Gel A-15m, DEAE-Sephadex, and CM-Sephadex chromatography and elution buffers ranging from low ionic strength (10 mM Pipes) to high ionic strength (100 mM Pipes/0.25 M NaCl) and in the presence or absence of 10 mM 2-mercaptoethanol, 5 mM EDTA, or 1% Triton X-100. The M_r 28,000 and 30,000 proteins appeared to be identical to the pair of proteins termed LMW MAPs by Berkowitz *et al.* (10), being of similar size, electrophoretic composition, and concentration in microtubule preparations (see below). Thus, our data suggested an association between the LMW MAPs and MAP 1. Under all chromatographic conditions examined with MAPs from calf cerebral cortex as starting material, we also observed significant, variable contamination of MAP 1 with MAP 2.

To obtain MAP 1 free of MAP 2 and to examine the association of the LMW MAPs with MAP 1 more directly, we prepared microtubules from bovine white matter. As shown previously (14), MAP 1 is the predominant high molecular weight MAP species in this preparation; MAP 2 is greatly decreased. Fig. 2 shows the results of chromatography of whole white matter MAPs on Bio-Gel A-15m. MAP 1 was the most prominent MAP species, and it eluted near but not at the void volume (Kd for MAP 1 = 0.10–0.20). MAP 2 also was present in the white matter MAPs, and most of it eluted from the column after MAP 1. The LMW MAPs coeluted with MAP 1, and no LMW MAP was observed elsewhere in the column profile. The LMW MAPs eluted in advance of MAP 2 despite the considerably higher subunit molecular weight of the latter protein.

To purify MAP 1 further, the MAP 2-containing fractions from a Bio-Gel A-15m column similar to that shown in Fig. 2 were passed over CM-Sephadex and were eluted with a 0.25 M NaCl step. Residual tubulin and MAP 2 were removed in the breakthrough fractions; both MAP 1 and the LMW MAPs eluted in the 0.25 M NaCl step. The purified LMW MAP–MAP 1 fraction is shown in Fig. 3. Both of the LMW MAPs were consistently

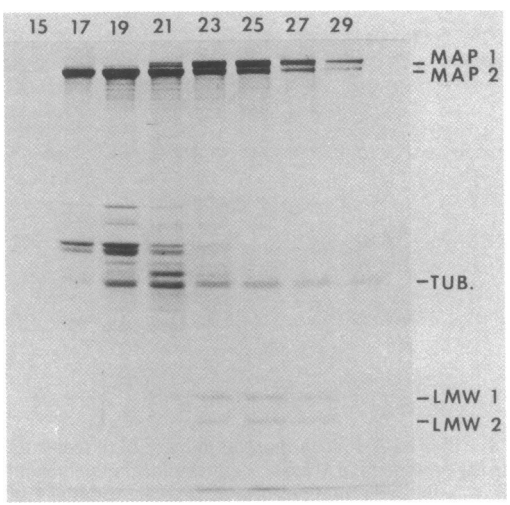

FIG. 1. Phosphocellulose chromatography of whole MAPs from calf cerebral cortex. MAPs were dissociated at increased ionic strength from taxol-stabilized microtubules and dialyzed against PEM buffer. The MAPs (2.5 ml at 1.1 mg/ml) were applied to a 4-ml bed of phosphocellulose preequilibrated with PEM buffer and eluted with a linear gradient of NaCl in PEM (50 1-ml fractions, 0.0–1.0 M NaCl). Most of the residual tubulin (TUB.) eluted in the breakthrough fractions, although some eluted with the MAPs. The MAPs eluted in the fractions shown, which corresponded to 0.0–0.39 M NaCl. The MAP 2 peak fraction (no. 19) corresponded to 0.12 M NaCl. The MAP 1 and LMW MAP peak fraction (no. 24) corresponded to 0.26 M NaCl.

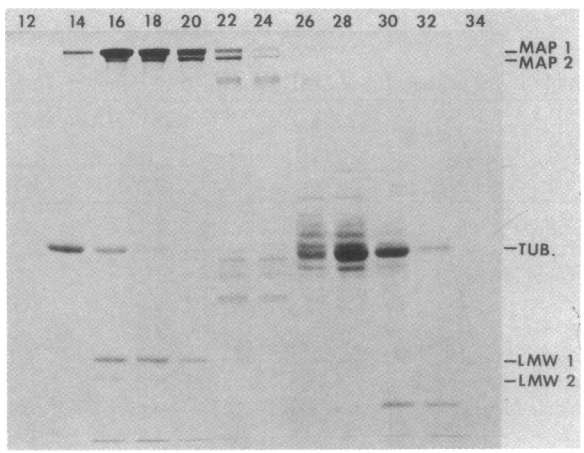

FIG. 2. Bio-Gel A-15m chromatography of MAPs obtained from white matter. MAPs (1.9 ml) were applied to a 0.9 × 55 cm column of Bio-Gel A-15m preequilibrated with 0.25 M NaCl in PEM buffer; 1-ml fractions were collected. The void volume was at fraction 14, and the included volume was at fraction 34. The bulk of MAP 1 and the LMW MAPs eluted in fractions 16–18 (Kd = 0.10–0.20). The MAP 2 peak was at fraction 20 (Kd = 0.30). This preparation of MAPs contained significant tubulin contamination.

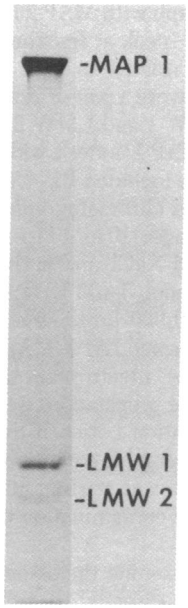

FIG. 3. Purified MAP 1–LMW MAP complex. MAP 1 was prepared from calf white matter MAPs by Bio-Gel A-15m and CM-Sephadex chromatography.

and quantitatively found in this fraction. Examination of this material with 4% polyacrylamide gels revealed that MAP 2 was either undetectable or was present at <1–2% of MAP 1, depending on the individual preparation. MAP 1 was itself complex, containing three prominent bands; similar heterogeneity has been reported (2). On 5–15% gradient gels, an additional band of $M_r \approx 17,000–19,000$ was also detected. The identity of this band is not known.

Stoichiometry of MAP 1–LMW MAP Complex. The ratio of LMW MAPs to MAP 1 in unfractionated microtubule preparations and at various stages of the purification of MAP 1 is shown in Table 1. The ratio of each of the LMW species to MAP 1 was approximately 1 mol per mol. The data suggest a possible difference in the ratios of LMW 1 to LMW 2 in gray matter and white matter.

Removal of the LMW MAPs from MAP 1 with Urea. Because we had observed no evidence of dissociation of the LMW MAPs from MAP 1 under a wide range of nondenaturing solution conditions, we examined the effect of chaotropic agents on the complex.

Table 1. Stoichiometry of MAP 1–LMW MAP complex

Sample	LMW MAPs/MAP 1, mol/mol	
	LMW 1	LMW 2
Gray matter		
Whole microtubules	1.0	1.1
Whole MAPs	1.1	1.1
	0.6	1.0
	1.3	1.5
Phosphocellulose MAP 1 peak (Fig. 1)	1.1	1.1
White matter		
Whole MAPs	1.0	0.4
	1.3	0.8
	1.1	0.5
Bio-Gel A-15m MAP 1 peak (Fig. 2)	1.5	0.8
Bio-Gel A-15m and CM-Sephadex purified MAP 1 (Fig. 3)	2.7	0.9
Mean ± SD	1.3 ± 0.6	0.9 ± 0.3

Mass ratios were determined by densitometry of Coomassie blue-stained electrophoretic gels. Molar ratios were calculated by assuming M_r = 350,000 for MAP 1, 30,000 for LMW 1, and 28,000 for LMW 2.

Fig. 4 shows the effect of 6 M urea on the behavior of MAPs chromatographed on Bio-Gel A-15m. MAP 1 eluted near the void volume, followed by MAP 2, as in Fig. 2. The elution position of the LMW MAPs, however, was markedly altered. In the presence of 6 M urea, these proteins eluted near the LMW end of the column profile (Kd = 0.74), indicating dissociation from MAP 1.

To determine whether urea destroyed the native properties of the MAPs, MAPs from calf cerebral cortex were incubated in 8 M urea/100 mM 2-mercaptoethanol for 1.5 hr at 0°C and then dialyzed overnight against two changes of PEM buffer containing 100 mM 2-mercaptoethanol. The MAPs were then centrifuged in the presence or absence of microtubules assembled from pure tubulin plus taxol (Fig. 5). In the absence of the microtubules (lanes 3 and 4), virtually all of MAP 1 and MAP 2 remained in the supernatant (lane 3) whereas in the presence of the microtubules (lanes 1 and 2), the MAPs were found in the microtubule pellet (lane 2). (Identical results were obtained when 6 M guanidine·HCl was used.) The LMW MAPs were detectable at higher gel loadings and showed only partial reconstitution with microtubules. Thus, it appeared that urea might be useful for isolating MAP 1 devoid of the LMW MAPs, although recovery of the LMW MAPs in active form was not ensured.

Fig. 6 shows the removal of the LMW MAPs from purified MAP 1 by chromatography on CM-Sephadex in the presence of urea. MAPs were isolated from calf white matter and MAP 1 was further purified by chromatography on Bio-Gel A-15m. The pooled MAP 1-containing fractions (lane 1) were then applied to a column of CM-Sephadex. Both MAP 1 and the LMW MAPs bound to the column. Application of a 6 M urea step resulted in the elution of MAP 1 (lanes 4 and 5). The LMWs remained bound to the column but could be eluted with 0.5 M NaCl in 6 M urea (lane 6).

Structure of MAP 1. To examine the structure of MAP 1, the MAP 1–LMW MAP complex and MAP 1 purified by using urea each were combined with microtubules composed of pure tu-

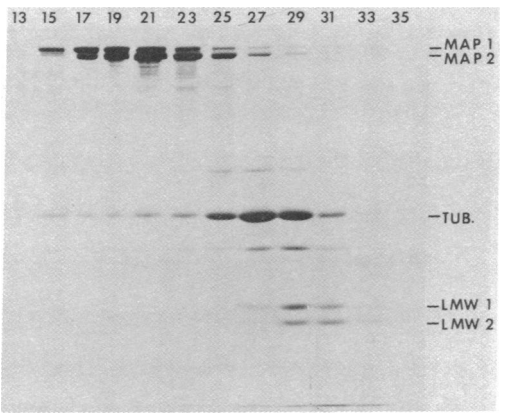

FIG. 4. Bio-Gel A-15m chromatography of high molecular weight MAPs in the presence of 6 M urea. A high molecular weight subfraction of MAPs was obtained from cerebral cortex microtubules (100 mg in 4 ml) prepared by two cycles of assembly/disassembly purification by chromatography on a 1.5 × 55 cm column of Bio-Gel A-15m in the presence of 0.25 M NaCl in PEM buffer. The early fractions (23 ml, Kd = 0.0–0.3) were pooled, precipitated with 50% $(NH_4)_2SO_4$, resuspended in PEM buffer, dialyzed overnight against 6 M urea/10 mM 2-mercaptoethanol, and centrifuged at 30,000 × g for 30 min at 4°C. The resulting MAPs preparation was applied to a 0.9 × 55 cm column of Bio-Gel A-15m preequilibrated with 6.0 M urea/10 mM 2-mercaptoethanol in PEM buffer; 1-ml fractions were collected. The void volume was at fraction 15, and the included volume was at fraction 34. The peaks were at fraction 18 (Kd = 0.16) for MAP 1 and fraction 29 (Kd = 0.74) for the LMW MAPs.

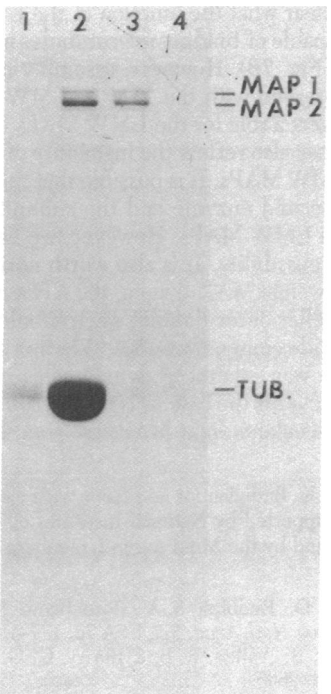

FIG. 5. Recovery of microtubule binding activity by MAPs after exposure to 8 M urea. MAPs (1.3 mg/ml) prepared from calf cerebral cortex microtubules by DEAE-Sephadex chromatography were incubated in 8 M urea/100 mM 2-mercaptoethanol in PEM buffer for 1.5 hr and then dialyzed overnight against two changes of PEM buffer containing 100 mM 2-mercaptoethanol. The MAPs (final concentration, 0.21 mg/ml) were combined with tubulin (final concentration, 1.4 mg/ml) or PEM buffer. Taxol was added to 25 μM and GTP to 0.80 mM. These solutions were warmed to 37°C for 5 min and centrifuged for 30 min at 30,000 × g. Lanes: 1, MAPs plus tubulin, supernatant; 2, MAPs plus tubulin, pellet; 3, MAPs alone, supernatant; 4, MAPs alone, pellet.

bulin. The microtubules were sedimented and thin sections of the pellets were examined by electron microscopy (Fig. 7). The calculated molar ratio of MAP 1 to tubulin was 1:6.7 for the

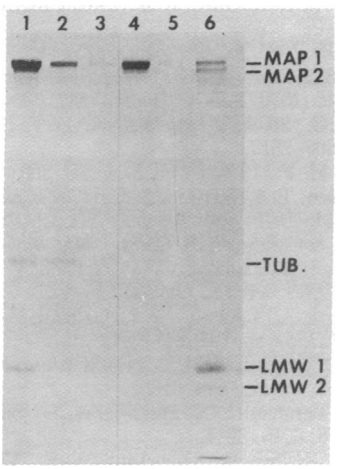

FIG. 6. Removal of LMW MAPs from MAP 1 by using urea. MAPs (4.3 ml) from white matter were passed over a 1.5 × 55 cm column of Bio-Gel A-15m. The MAP-1-containing fractions were pooled (lane 1) and applied to a 1-ml column of CM-Sephadex preequilibrated in PEM buffer. The breakthrough fractions (2 and 3) contained residual tubulin and some MAP 1. MAP 1 was eluted with a step of 6 M urea in PEM buffer containing 10 mM 2-mercaptoethanol (lanes 4 and 5). The LMW MAPs plus some residual high molecular weight protein species were eluted with a subsequent step of 0.5 M NaCl in PEM buffer (lane 6).

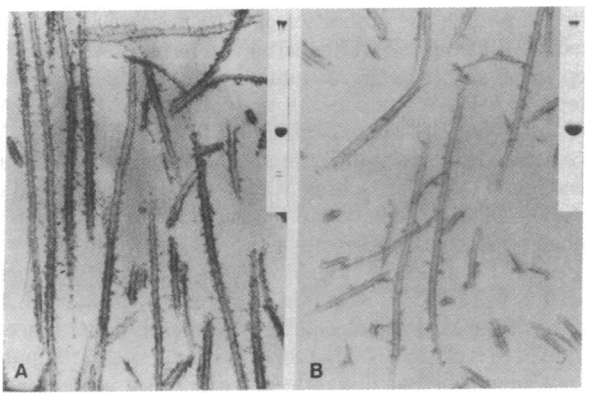

FIG. 7. Electron microscopy of MAP 1. Microtubules were assembled from pure tubulin, with taxol used to promote assembly, and then combined with MAP 1 and centrifuged at 30,000 × g for 25 min. Sections of the microtubule pellets are shown ×52,000. (*Insets*) Electrophoretic gels of the pelleted microtubules. Values for MAP 1/tubulin were corrected for MAP 1 sedimented in the absence of microtubules. (*A*) Microtubules composed of the MAP 1–LMW MAP complex prepared as described in Fig. 3 plus purified tubulin. The MAP 1/tubulin ratio in the sedimented microtubules was 0.5, corresponding to 1 mol of MAP 1 per 6.7 mol of tubulin dimer. (*B*) Microtubules composed of MAP 1 prepared as described in Fig. 6 plus purified tubulin. The MAP 1/tubulin ratio in the sedimented microtubules was 0.15, corresponding to 1 mol of MAP 1 per 20 mol of tubulin dimer.

complex and 1:20 for the purified MAP 1. The MAP 1–LMW MAP complex had the appearance of an arm on the microtubule surface (Fig. 7*A*). The arms were similar in morphology to MAP 2 arms (cf. refs. 5, 6, and 9) and often appeared slightly curved. The arms ranged up to 23 nm in length and had a mean (± SD) width of 6 ± 1 nm (n = 32). Arms crossbridging microtubules were seen frequently, and showed a mean length of 45 ± 10 nm (n = 16), close to twice the length of the longest free arms. The arms showed a regular spacing along the microtubule of 21 ± 5 nm obtained from 107 spacing measurements and representing 22 individual groups of clearly identifiable arms. The close spacing observed for the MAP 1–LMW MAP complex was not observed in the purified MAP 1 sample. This was probably due to a smaller number of molecules bound to the microtubules in the latter case as well as clumping of the purified MAP 1 arms. Nonetheless, many arms appeared identical in morphology to those observed in the complex.

Microtubule Assembly-Promoting Activity of MAP 1. The MAP 1–LMW MAP complex showed considerable assembly-promoting activity as determined by the increase in light scattering upon addition of the MAP fraction to purified tubulin. Combination of MAP 1 at 0.20 mg/ml with tubulin at 1.7 mg/ml resulted in a cold-reversible change in OD_{320} at 37°C of 0.24 with $t_{1/2}$ = 50 sec. Numerous microtubules were observed in the sample by negative-stain electron microscopy. These results are consistent with one recent study indicating that MAP 1 promotes microtubule assembly (22, 23) and contradict an earlier negative report which was based on less direct evidence (24). We have also examined the effect, on microtubule assembly, of our preparation of MAP 1 from which the LMW MAPs were removed. No significant promotion of assembly was detected. We do not know whether this indicates that the LMW MAPs are required for assembly or that assembly-promoting activity has been destroyed during purification.

DISCUSSION

Extensive characterization of MAP 2, the major MAP isolated from brain tissue, has been carried out but little has been re-

ported regarding the properties of MAP 1. We have now purified MAP 1 from calf white matter and have shown that MAP 1 represents an arm on the microtubule surface (Fig. 7). We have detected two LMW components, previously identified as MAPs (10), that copurified as a complex with MAP 1 (Figs. 1–3). We have succeeded in disrupting the complex with urea (Figs. 4 and 6) and found that the resulting MAP 1 preparation still had microtubule-binding activity and appeared as an arm when examined by electron microscopy (Fig. 7).

The MAP 1 molecule (Fig. 7) appears to be morphologically similar to MAP 2 (5, 6, 9) in thin sections of microtubules. The spacing of the MAP 1 molecules on the microtubule surface reported here is closer than that reported for MAP 2 (6, 9), but we do not know whether this reflects a difference in the binding sites for the two MAPs on the microtubule surface. Our results do indicate that the two proteins are related structurally to some extent, despite a recent report that they show significant differences in their peptide maps (22). In view of the preferential localization of MAP 2 in the dendritic processes of neurons (14, 25, 26), our results suggest that MAP 1 could represent the arms observed on axonal microtubules (27–29) and perhaps on other cytoplasmic microtubules as well (30). It is interesting in this context that the spacing reported here for MAP 1 is identical to that reported for crossbridges in the HeLa mitotic spindle (30).

Whereas the high molecular weight MAPs and the τ MAPs have received considerable attention since their discovery nearly a decade ago, the LMW MAPs have been little investigated. This is probably due to their low content in purified microtubule preparations when evaluated on a mass basis. When their content is evaluated on a molar basis, it is evident that they are present in purified microtubules at a level approximately equal to that of the high molecular weight MAPs (this paper, Table 1; see also ref. 10).

The existence of three polypeptides bound to the MAP 2 polypeptide chain has been demonstrated (12). Two of these polypeptides represent the subunits of a type II cyclic AMP-dependent protein kinase (13). The function of the third polypeptide (M_r = 70,000) remains unknown. In contrast to the present results, the molar ratios of all three polypeptides to MAP 2 were low. Thus, whereas only 1 MAP 2 molecule in 40 was found to contain an associated kinase holoenzyme, it appears likely that many or all of the MAP 1 molecules in brain microtubule preparations must contain an associated LMW MAP. One important question that remains to be resolved is whether the several closely spaced electrophoretic species identified as MAP 1* actually represent related proteins and whether all of these species bind the LMW MAPs.

Two of the MAPs, MAP 2 and τ, are notable for their unusual resistance to denaturation at increased temperature (5, 6, 11). A recent report has indicated that another MAP, the M_r 210,000 species isolated from HeLa cells, is also heat stable (31). In contrast to this behavior, MAP 1 has been found to coprecipitate with tubulin when microtubules are exposed to heat (5, 6, 11). The present results indicate that, despite the different response of MAP 1 to heat, this protein also has unusual stability characteristics, particularly for a large protein. In the presence of urea, no change was observed in the elution behavior of MAP 1 on Bio-Gel A-15m (Fig. 4 vs. Fig. 2) or in the ability of the protein to bind to microtubules (Figs. 5 and 7). We have noted that, after separation of MAP 1 from the LMW MAPs in the presence of urea, MAP 1 has an increased tendency to precipitate. This may be due to exposure of sites on the MAP 1 molecule that are normally masked by the light chains. In contrast to MAP 1, the LMW MAPs showed considerable variability in their ability to bind to microtubules and to rebind to MAP 1 after exposure to urea (data not shown).

It is not yet clear what the function of the LMW MAPs may be. MAP 1 is capable of binding microtubules in the absence of these proteins (Fig. 7*B*). However, assembly-promoting activity was only observed with the MAP 1–LMW MAP complex. This might suggest a role for the LMW MAPs in assembly regulation, but it may also reflect the instability of MAP 1 after removal of the LMW MAPs. It is possible that, like MAP 2, MAP 1 bears an associated enzyme and the subunits of it are represented by the LMW MAPs. However, we have no evidence to support this possibility. It is also worth noting that another high molecular weight MAP, dynein, the ATPase associated with ciliary and flagellar outer doublet microtubules, also contains LMW polypeptide components (32). Whether the MAP 1 complex is in some way related to dynein remains an interesting question. Whatever is the case, it is now becoming clear that all of the high molecular weight MAPs are complex molecules.

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1. Sloboda, R. D., Rudolph, S. A., Rosenbaum, J. L. & Greengard, P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 177–181.
2. Murphy, D. B., Vallee, R. B. & Borisy, G. G. (1977) *Biochemistry* **16**, 2598–2605.
3. Vallee, R. B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3206–3210.
4. Borisy, G. G., Marcum, J. M., Olmsted, J. B., Murphy, D. B. & Johnson, K. A. (1975) *Ann. N. Y. Acad. Sci.* **253**, 107–132.
5. Herzog, W. & Weber, K. (1978) *Eur. J. Biochem.* **92**, 1–8.
6. Kim, H., Binder, L. & Rosenbaum, J. L. (1979) *J. Cell Biol.* **80**, 266–276.
7. Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y. & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1858–1862.
8. Cleveland, D. W., Hwo, S.-Y. & Kirschner, M. W. (1977) *J. Mol. Biol.* **116**, 207–225.
9. Zingsheim, H.-P., Herzog, W. & Weber, K. (1979) *Eur. J. Cell Biol.* **19**, 175–183.
10. Berkowitz, S. A., Katagiri, J., Binder, H.-K. & Williams, R. C., Jr. (1977) *Biochemistry* **16**, 5610–5617.
11. Fellous, A., Francon, J., Lennon, A. M. & Nunez, J. (1977) *Eur. J. Biochem.* **78**, 167–174.
12. Vallee, R. B., DiBartolomeis, M. J. & Theurkauf, W. E. (1981) *J. Cell Biol.* **90**, 568–576.
13. Theurkauf, W. E. & Vallee, R. B. (1982) *J. Biol. Chem.* **257**, 3284–3290.
14. Vallee, R. B. (1982) *J. Cell Biol.* **92**, 435–442.
15. Vallee, R. B. & Borisy, G. G. (1978) *J. Biol. Chem.* **253**, 2834–2845.
16. Laemmli, U. (1970) *Nature (London)* **227**, 680–685.
17. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617.
18. Pearse, B. M. F. (1978) *J. Mol. Biol.* **126**, 803–812.
19. Kirschhausen, T. & Harrison, S. C. (1981) *Cell* **23**, 755–761.
20. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
21. Gaskin, F. & Cantor, C. R. (1974) *J. Mol. Biol.* **89**, 737–758.
22. Kuznetsov, S. A., Rodionov, V. I., Gelfand, V. I. & Rosenblat, V. A. (1981) *FEBS Lett.* **135**, 237–240.
23. Kuznetsov, S. A., Rodionov, V. I., Gelfand, V. I. & Rosenblat, V. A. (1981) *FEBS Lett.* **135**, 241–244.
24. Villasante, A., de la Torre, J., Manso-Martinez, R. & Avila, J. (1980) *Eur. J. Biochem.* **112**, 611–616.
25. Matus, A., Bernhardt, R. & Hugh-Jones, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3010–3014.
26. Miller, P., Walter, U., Theurkauf, W. E., Vallee, R. B. & De Camilli, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5562–5566.
27. Smith, D. S., Jarlfors, U. & Cameron, B. G. (1975) *Ann. N. Y. Acad. Sci.* **253**, 472–506.
28. Ellisman, M. H. & Porter, K. R. (1980) *J. Cell Biol.* **87**, 464–479.
29. Hirokawa, N. (1982) *J. Cell Biol.* **94**, 129–142.
30. McIntosh, J. R. (1974) *J. Cell Biol.* **61**, 166–187.
31. Weatherbee, J. A., Sherline, P., Mascardo, R. N., Izant, J. G., Luftig, R. B. & Weihing, R. R. (1982) *J. Cell Biol.* **92**, 155–163.
32. Bell, C. W., Fronk, E. & Gibbons, I. R. (1979) *J. Supramol. Struct.* **11**, 311–317.