

# Identification of a 34-kD Polypeptide as a Light Chain of Microtubule-associated Protein-1 (MAP-1) and Its Association with a MAP-1 Peptide that Binds to Microtubules

Sergei A. Kuznetsov, Vladimir I. Rodionov, Elena S. Nadezhkina, Douglas B. Murphy,\* and Vladimir I. Gelfand

A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR; \*Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland 21205

**Abstract.** We examined the association of a 34-kD light chain component to the heavy chains of MAP-1 using a monoclonal antibody that specifically binds the 34-kD component and labels neuronal microtubules in a specific and saturable manner. Immunoprecipitation of MAP-1 heavy chains together with the 34-kD component by the antibody indicates that the 34-kD polypeptide forms a complex with MAP-1 heavy chains. Both major isoforms of MAP-1 heavy chains (MAP-1A and MAP-1B) were found in the immunoprecipitate. Digestion of MAP-1 with alpha-

chymotrypsin and analysis of the chymotryptic peptides reveals a 120-kD fragment of the MAP-1 heavy chain that binds to microtubules and is precipitable with the 34-kD light chain antibody, suggesting that the 34-kD light chain also binds to this domain of the molecule. Since microtubules that contain the 120-kD fragment lack the long lateral projections characteristic of microtubules with intact MAP-1, the 34-kD light chains may be localized at or near the microtubule surface.

**M**ICROTUBULES isolated from brain tissue extracts are composed of tubulin plus several microtubule-associated proteins (MAPs).<sup>1</sup> Of these, two classes of high molecular weight components termed MAP-1 and MAP-2 have been demonstrated to co-purify with tubulin during cycles of microtubule assembly and disassembly and to stimulate microtubule assembly *in vitro* (for a review see reference 22). There is considerable evidence that MAPs may mediate the binding of membranous organelles, actin filaments, and intermediate filaments to microtubules, leading to the speculation that they may therefore be important for cellular processes such as mitosis and organelle transport, and for determining the dynamic properties of the cytoskeleton. Immunofluorescence microscopic studies on the distribution of the brain high molecular weight MAPs have shown that MAP-2 is localized primarily in the dendrites of neurons in brain (2, 12) and possibly in small amounts in other cells (6, 17, 23), most notably chromatophores (18). In contrast, MAP-1 is more generally distributed, being found in both dendrites and axons of neurons and in glial cells in brain, in chromatophores, and on both interphase and mitotic microtubules in various tissue culture cells, suggesting that MAP-1 may have a more general function (1, 18).

Recently MAP-1 has been isolated and demonstrated to stimulate tubulin assembly *in vitro* (7, 8, 21) and to exhibit structural characteristics similar to those of MAP-2 as 20-nm

long projections on microtubule surfaces (9, 21). Purified preparations of MAP-1 from bovine brain have also been demonstrated to contain at least two low molecular weight components that remain tightly associated with MAP-1 heavy chains during ion-exchange chromatography and during gel filtration chromatography under nondenaturing conditions. The stoichiometric ratio of both light chains to one MAP-1 heavy chain is approximately 1:1 (21).

In this paper we examine further the relationship of the low molecular weight components to MAP-1 heavy chain using a monoclonal antibody to the larger of the two light chain components and describe the location of this polypeptide on a microtubule-binding, chymotryptic peptide of the MAP-1 heavy chain at or near the microtubule surface.

## Materials and Methods

### Biochemical Procedures

Microtubules, MAPs, MAP-1, and tubulin were purified from bovine brain in a buffer containing 50 mM imidazole-HCl (pH 6.7), 50 mM KCl, 0.5 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 1 mM GTP, 1 mM 2-mercaptoethanol (buffer A) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF). Unfractionated microtubule proteins were prepared by two cycles of assembly-disassembly (16) as modified by Rodionov et al. (14). Tubulin and MAPs were separated by chromatography on phosphocellulose in buffer A with 1 mM PMSF (25).

MAP-1 was purified as described in reference 7. Purified MAP-1 was often contaminated with polypeptides of lower molecular weight, including polypeptides that co-migrated with MAP-2 during SDS PAGE (see, for example, Fig. 1, lane C and Fig. 6A, 0 min). These polypeptides accumulated in preparations during storage, suggesting that they are products of MAP-1 proteolysis. Im-

<sup>1</sup> Abbreviations used in this paper: LC-1, LC-2, light chains 1 and 2 of MAP-1; MAP, microtubule-associated protein; PMSF, phenylmethylsulfonyl fluoride.

munoblotting with a monoclonal antibody to MAP-2 (see below) never showed this polypeptide or its degradation products in purified MAP-1 preparations.

Microtubule-binding activity of the MAPs and their proteolytic fragments was determined by a sedimentation assay in the presence of microtubules. Samples of total MAPs or purified MAP-1 were clarified by centrifugation at 150,000 *g* for 30 min. Microtubules were prepared by polymerizing phosphocellulose-purified tubulin (0.8–1.0 mg/ml) at 37°C for 30 min in buffer A supplemented with 10  $\mu$ M taxol, 1 mM GTP, and 1 mM EGTA. Microtubule assembly was monitored by light scattering at 330 nm (3). The fraction to be tested was added to the taxol-microtubules (1:10 MAP peptide/tubulin ratio), and after 30 min of incubation at 37°C, microtubules were pelleted for 30 min at 200,000 *g* (SW 50.1 rotor, Beckman Instruments Inc., Palo Alto, CA) through a cushion of 4 M glycerol in buffer A with 1 mM EGTA. The pellets were solubilized in 1% SDS and analyzed by SDS PAGE. A 33 kD-protein that inhibits tubulin polymerization was purified from bovine brain according to Kotani et al. (4). Another 35-kD microtubule-binding protein (glyceraldehyde-3-phosphate dehydrogenase) was prepared according to Kumagai and Sakai (5).

SDS PAGE was performed according to Laemmli (10) in 7 or 10% polyacrylamide slab gels at an acrylamide to *N,N'*-methylene-bisacrylamide ratio of 100:1 (wt/wt). To resolve MAP-1 heavy chain isoforms by SDS gel electrophoresis we used tube gels containing a 4–10% linear gradient of polyacrylamide (acrylamide to *N,N'*-methylene-bisacrylamide ratio of 100:1) in the phosphate buffer system of Weber and Osborn (24). Coomassie Blue-stained gels, quantitated by densitometry at 500 nm, were found to be within the linear range of absorbance as a function of protein concentration.

Protein concentration was determined by the method of Lowry et al. (11) using bovine serum albumin as a standard.

### Immunological Procedures

The production of a monoclonal antibody (E12) to the 34-kD MAP was described previously by Rodionov et al. (15). This antibody was of the IgG class. Monoclonal antibodies to tubulin (Tu 0.1) and to MAP-2 (MA 1) were generously provided by Drs. P. Draber and V. Vicklicky (Institute of Molecular Genetics, Prague, Czechoslovakia).

Immunoblotting was performed according to Towbin et al. (19) as modified by Rodionov et al. (15). Antibody-reactive components were revealed by incubation in 4-chloro-1-naphthol (0.5 mg/ml) in 50 mM Tris-HCl (pH 7.5) containing 0.05% H<sub>2</sub>O<sub>2</sub>.

For immunoprecipitation, total MAPs (0.3–0.4 mg/ml) or alpha-chymotrypsin-treated MAP-1 (0.15–0.20 mg/ml) was incubated with E12 antibody (0.4–0.6 mg/ml) for 3 h in buffer A at room temperature. Monospecific rabbit antibody against mouse IgG was added to a final concentration of 0.5–0.7 mg/ml, and after 30 min of incubation at room temperature, immunoprecipitates were collected by pelleting at 150,000 *g* for 20 min through a layer of 4 M glycerol and 0.5 M KCl in buffer A. The precipitates were dissolved in 10% SDS and 5% 2-mercaptoethanol and analyzed by SDS gel electrophoresis. Small amounts of insoluble material usually remained in the immunoprecipitates, even after heating the samples in gel sample buffer containing 10% SDS and 10% beta-mercaptoethanol. However, the amounts of this insoluble material were small (<1%), did not contain LC-1, as determined by immunoblotting using a monoclonal antibody to LC-1 (Fig. 8B), and therefore did not interfere with our gel electrophoretic analyses of LC-1 in immunoprecipitates.

### Microscopy

For immunofluorescence staining, twice-cycled microtubules at 1.0–1.5 mg/ml in buffer A were adsorbed onto glass coverslips pretreated with poly-L-lysine (1 mg/ml). After adsorption microtubules were fixed with 4% formaldehyde in phosphate-buffered saline (PBS). Fixed microtubules were thoroughly washed with PBS, treated with 1% bovine serum albumin for 30 min, and then stained with the antibodies by the indirect method. E12 antibody (ascites) was used at a concentration of ~1 mg/ml.

For electron microscopy pelleted microtubules were fixed overnight with 1% glutaraldehyde in 10 mM sodium phosphate buffer (pH 7.0) containing 1% tannic acid and postfixed with 1% OsO<sub>4</sub>. Samples were stained with uranyl acetate in ethanol at 70°C and after ethanol-acetone dehydration were embedded in Epon. Ultrathin sections stained with lead citrate (13) were examined and photographed on an Hitachi HU-11B electron microscope (Hitachi Ltd, Tokyo) operated at 75 kV.

### Materials

Alpha-chymotrypsin (type VII TLCK treated) and molecular weight markers were purchased from Sigma Chemical Co. (St. Louis, MO); phosphocellulose

(P-11) was from Whatman Chemical Separation, Inc. (Clifton, NJ); taxol was from the National Cancer Research Institute (Bethesda, MD).

## Results

### Demonstration of Antibody Specificity

We prepared monoclonal antibodies to a total MAP fraction from bovine brain microtubules and obtained several antibodies, one of which binds specifically to a 34-kD component, which we demonstrate in this paper to be a light chain of MAP-1. The specificity of binding of this antibody (E-12) is demonstrated by immunoblotting in Fig. 1. The antibody binds to a single 34-kD polypeptide in preparations of cycled microtubules, total MAPs, and purified MAP-1.

The 34-kD component was determined to be distinct from the 35-kD microtubule-binding protein (glyceraldehyde-3-phosphate dehydrogenase) isolated by Kumagai and Sakai (5) and the 33-kD inhibitory factor of tubulin polymerization (4) (data not shown).

Fig. 1 demonstrates that the procedure used to purify MAP-1 results in the purification of both MAP-1 heavy chains and two low molecular weight components (31 and 34 kD) as described by Vallee and Davis (21). The binding of antibody to native cycled microtubules was also demonstrated to be saturable in a microtubule pelleting assay (Fig. 2), which revealed that saturation was near that expected for equimolar binding of antibody to the 34-kD component as determined

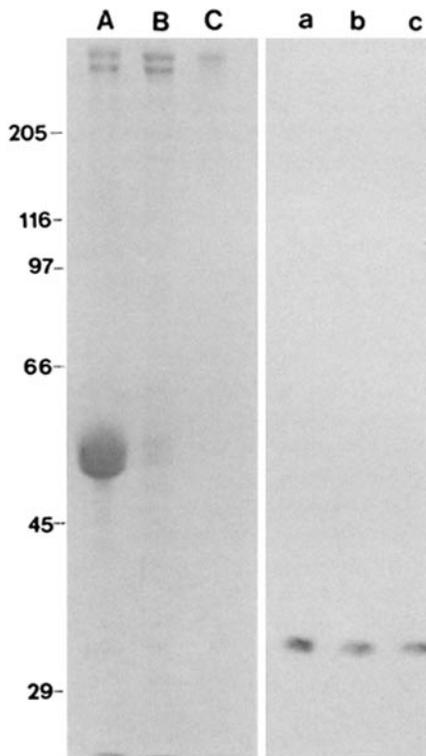
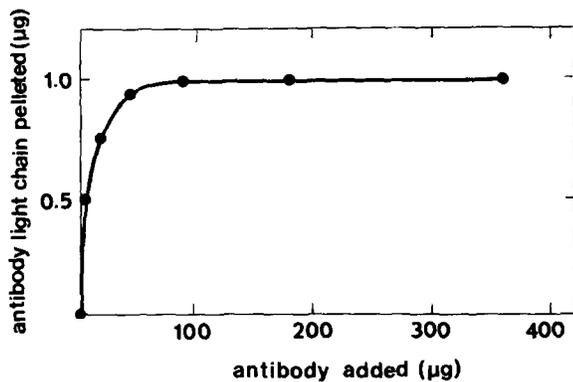


Figure 1. Demonstration of antibody specificity by immunoblotting. The figure shows Coomassie Blue-stained gels (lanes A–C) and corresponding immunoblots (lanes a–c) for E-12 antibody to the 34 kD-light chain component of MAP-1. Lanes A and a, microtubules prepared by two cycles of assembly and disassembly; lanes B and b, total MAP fraction; lanes C and c, purified MAP-1. The antibody is specific for the 34-kD polypeptide associated with MAP-1. Numbers at left indicate molecular weight markers ( $\times 10^3$ ).

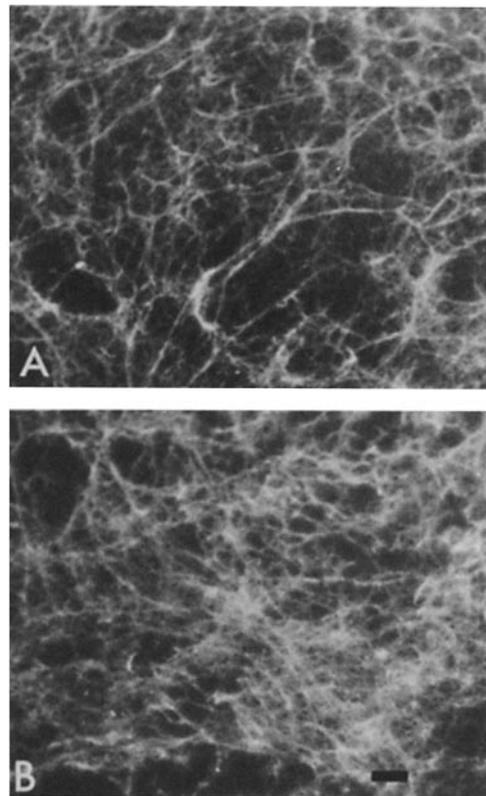


**Figure 2.** Demonstration of the saturability of antibody binding to microtubules. Increasing amounts of E-12 antibody (0–360  $\mu\text{g}$ ) were added to aliquots of a total MAP fraction (10  $\mu\text{g}$ ) and incubated for 3 h at 4°C. The mixture was added to 100  $\mu\text{g}$  of microtubules assembled from phosphocellulose-purified tubulin in the presence of 10  $\mu\text{M}$  taxol, incubated for 30 min at 37°C, and pelleted as described in Materials and Methods. The amount of antibody light chains present in the resulting supernates and pellets was estimated by densitometry of Coomassie Blue-stained SDS polyacrylamide gels. Only the antibody light chains were quantitated because IgG heavy chains migrate on SDS gels with an electrophoretic mobility close to that of tubulin. Examples of the resolution of LC-1 and antibody light chains after fractionation on 10% polyacrylamide gels are shown in Figs. 4, lane C and 5, lane A. Saturation of antibody binding occurred rapidly and at a ratio consistent with that expected for specific binding.

by densitometry of Coomassie Blue-stained polyacrylamide gels. The twofold difference in the molar ratio (one antibody/two low molecular weight components) may be due to differences in Coomassie Blue staining, heterogeneity of the antigen, or other factors. As expected, the antibody also stained cycled brain microtubules that were fixed before examination by immunofluorescence microscopy (Fig. 3). These observations establish that the binding of antibody to the 34-kD component of cycled brain microtubules is specific and saturable.

#### *The 34-kD Component Is a Light Chain of MAP-1*

We demonstrated previously that purified fractions of MAP-1 contain two low molecular weight components with molecular weights of <40 kD (7). Vallee and Davis (21) showed that the low molecular weight components co-purify with the MAP-1 heavy chain when MAP preparations are subjected to various chromatographic procedures. To confirm that MAP-1 preparations contain low molecular weight components and to show that the light chains were not binding to other MAPs such as MAP-2, we incubated antibody with a preparation of total high molecular weight MAPs and examined the composition of supernates and pellets after immunoprecipitation with a rabbit anti-mouse IgG antibody. The immunoprecipitates were observed to contain both the 34-kD component and the heavy chain of MAP-1 (Fig. 4) but did not contain MAP-2, which remained in the supernate. However, the pellets did contain a component with an electrophoretic mobility near that of MAP-2, but by immunoblotting using a MAP-2 specific antibody, it could be shown that this component was not MAP-2 itself, suggesting that it may be a proteolytic fragment of the MAP-1 heavy chain. Electrophoresis of MAP-1 on gradient gels in 0.1 M phosphate revealed



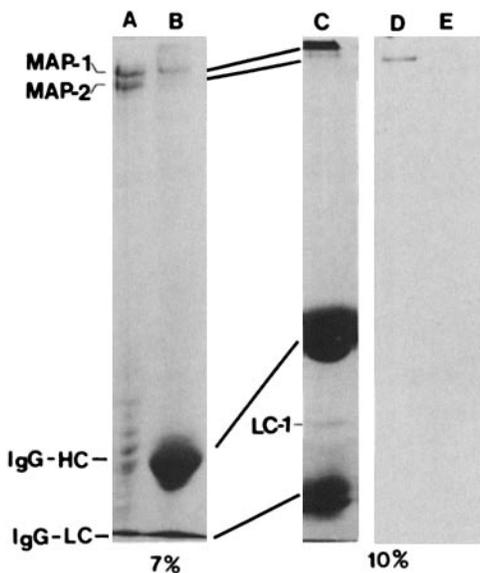
**Figure 3.** Immunofluorescence microscopy of cycled microtubules stained with tubulin antibody (A) and with E-12 antibody (B). Microtubules were adsorbed onto poly-L-lysine-treated glass coverslips, fixed with formaldehyde, and stained with antibodies as described in Materials and Methods. No staining was observed using nonspecific ascites and secondary fluorescent antibody or with the secondary antibody alone (not shown). Bar, 5  $\mu\text{m}$ .

that MAP-1 contains three principal heavy chains, as was demonstrated previously by Bloom et al. (2). Electrophoretic analysis of immunoprecipitates prepared from a purified MAP-1 fraction revealed that the 34-kD component bound to at least two major classes (A and B) of the MAP-1 heavy chains (Fig. 5). These observations demonstrate the specificity of binding of the 34-kD component to MAP-1 heavy chains and justify its designation as a light chain of MAP-1 (LC-1).

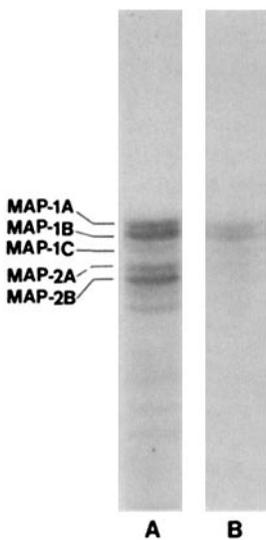
Antibody binding experiments showed that E-12 antibody interacts with MAP-1 both in solution and on microtubule surfaces. Therefore, the site of antibody binding and the site of tubulin binding on the MAP-1 molecule may not overlap. The observation that preincubation of MAP-1 with the antibody does not inhibit the ability of MAP-1 to promote tubulin polymerization *in vitro* is consistent with this interpretation (data not shown).

#### *Localization of LC-1 on the Heavy Chain of MAP-1*

We and others have shown previously that MAP-1 binds to the lateral surfaces of microtubules and contains a distinctive projection portion that can be distinguished by electron microscopy after tannic acid staining and thin sectioning (9, 21). If partial proteolysis removed the projection portion of MAP-1 from microtubules as it does for MAP-2, we could use this method to determine which domain of the MAP-1 heavy chain binds LC-1. When preparations of purified MAP-1 free

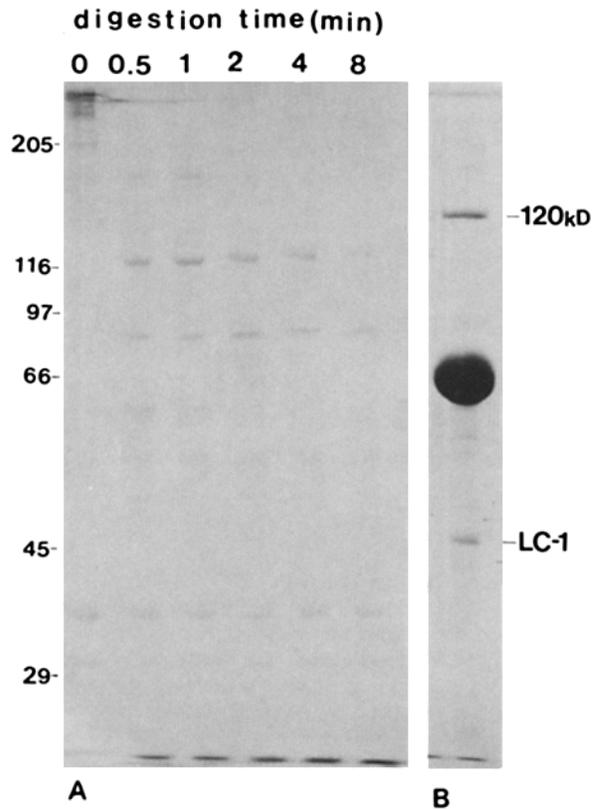


**Figure 4.** Immunoprecipitation of MAP-1 from total MAPs fraction with E-12 antibody. For immunoprecipitation, antibody (45  $\mu\text{g}$ ) was incubated for 3 h at room temperature with the total MAP fraction (30  $\mu\text{g}$ ). Rabbit monospecific antibody against mouse IgG (65  $\mu\text{g}$ ) was added, and after a 30-min incubation immunoprecipitates were collected and analyzed by Laemmli gel electrophoresis. Lane A, starting total MAP fraction, electrophoresis on 7% polyacrylamide gel; lanes B and C, immunoprecipitate, electrophoresis on 7% (lane B) and 10% (lane C) polyacrylamide gels. The prominent bands in the immunoprecipitates shown in lanes B and C are the heavy and light chains of immunoglobulins. Only the heavy chains are resolved on the 7% gel shown in lane B. Lanes D and E, immunoblots of the samples shown in lanes A and B respectively, stained with MAP-2 antibody after electrophoresis on a 10% gel. Results indicate that MAP-2 is stained in a control preparation of total MAPs (lane D) but is absent from the immunoprecipitate (lane E). Therefore, the faint component seen in both lanes B and C with an electrophoretic mobility similar to MAP-2 is not MAP-2 itself, but probably represents a proteolytic fragment of the MAP-1 heavy chain.



**Figure 5.** Precipitation of isoforms of MAP-1 heavy chains by LC-1 antibody. Immunoprecipitates prepared as described in the legend to Fig. 4 were fractionated on tube gels (acrylamide gradient 4–10%, acrylamide/bisacrylamide ratio, 100:1) in 0.1 M phosphate, pH 7.0, to resolve the heavy chain components of MAP-1. The figure shows the high molecular weight region of two identical gels containing total MAPs (lane A) and the corresponding immunoprecipitate (lane B). Both of the major MAP-1A and MAP-1B isoforms of heavy chains were contained in the immunoprecipitate.

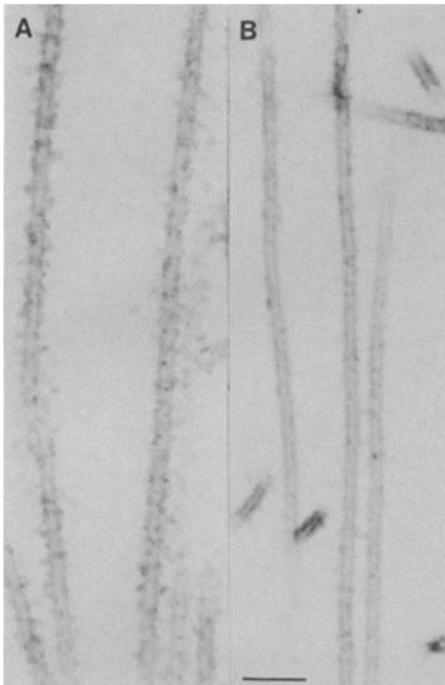
of microtubules were digested with alpha-chymotrypsin, several peptides were formed, including a stable 120-kD fragment of the MAP-1 heavy chain, LC-1, and other minor components (Fig. 6A). When the peptide mixture was combined



**Figure 6.** Generation of a stable 120-kD fragment of MAP-1 by digestion with alpha-chymotrypsin and demonstration of its binding to microtubules. (A) Time course of digestion of purified MAP-1 shows the generation of a stable 120-kD fragment (digestion conditions: 6  $\mu\text{g}$  MAP-1, 0.03  $\mu\text{g}$  alpha-chymotrypsin, at room temperature). The positions of molecular weight standards ( $\times 10^3$ ) are shown on the left. (B) Binding of proteolytic fragment of MAP-1 to microtubules. Proteolytic fragments were added to microtubules composed of phosphocellulose-purified tubulin assembled in the presence of 10  $\mu\text{M}$  taxol, pelleted, and examined by gel electrophoresis. Gel analysis of the microtubule pellets shows, in addition to tubulin, the specific binding of the 120-kD heavy chain fragment, LC-1, plus other trace components. A and B are separate but otherwise identical gels, hence the slight differences in electrophoretic mobilities.

with taxol-stabilized microtubules containing phosphocellulose-purified tubulin (taxol microtubules) and pelleted, all of the 120-kD fragment and LC-1 were observed to bind to microtubules, as determined by sedimentation and gel electrophoresis (Fig. 6B). Examination of thin sections of these pellets by electron microscopy revealed that a considerable portion of the MAP-1 projection was absent (Fig. 7), indicating that the fragments that bound to microtubules were part of the microtubule-binding domain of MAP-1.

From this procedure alone, however, it was not possible to determine if LC-1 and the 120-kD fragment of the MAP-1 heavy chain bound to microtubules independently or were associated together as a complex. To distinguish between these alternatives, we examined the composition of immunoprecipitates of a purified MAP-1 preparation after partial digestion with chymotrypsin, which produced the 120-kD fragment (Fig. 8). The immunoprecipitates contained both LC-1 and the 120-kD heavy chain fragment, indicating that the two components are associated together in a complex. Thus, there is no evidence to indicate that LC-1 and MAP-1 heavy chain

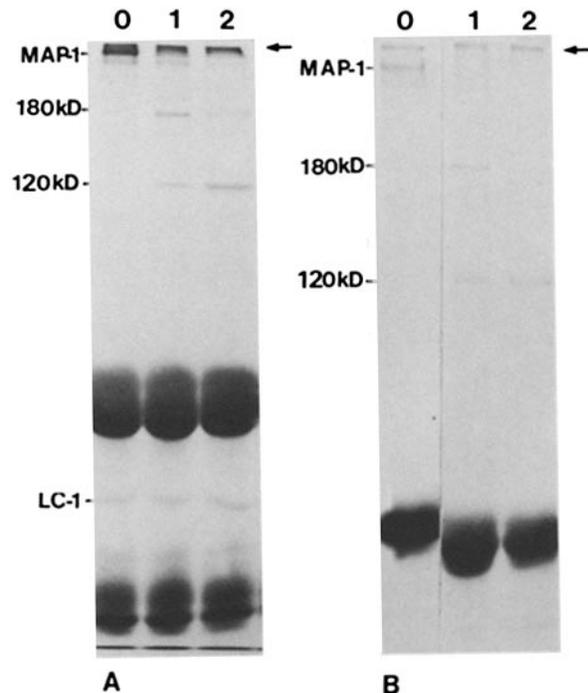


**Figure 7.** Removal of the projection portion of MAP-1 by digestion with alpha-chymotrypsin. Purified MAP-1 (0.2 mg/ml) was treated with alpha-chymotrypsin (1:200 enzyme/MAP1 ratio) for 3 min in buffer A at room temperature. The reaction was arrested with 2 mM PMSF, and the digest was added to taxol-microtubules assembled from phosphocellulose-purified tubulin. Microtubules were pelleted and prepared for thin sectioning after staining with tannic acid as described in Materials and Methods. (A) Control preparation of microtubules decorated with undigested MAP-1; (B) decoration of microtubules with chymotrypsin-digested MAP-1. Enzyme treatment removes most of the MAP-1 projections. Bar, 0.1  $\mu$ m.

bind independently to microtubules. We also observed that the pattern of cleavage products and peptide binding to microtubules was the same when taxol-stabilized microtubules containing both MAP-1 and MAP-2 were partially digested with chymotrypsin, pelleted, and examined by gel electrophoresis and immunoblotting (Fig. 9). No differences in the pattern of cleavage were observed, indicating that MAP-1 is cleaved similarly in both the bound and unbound states.

## Discussion

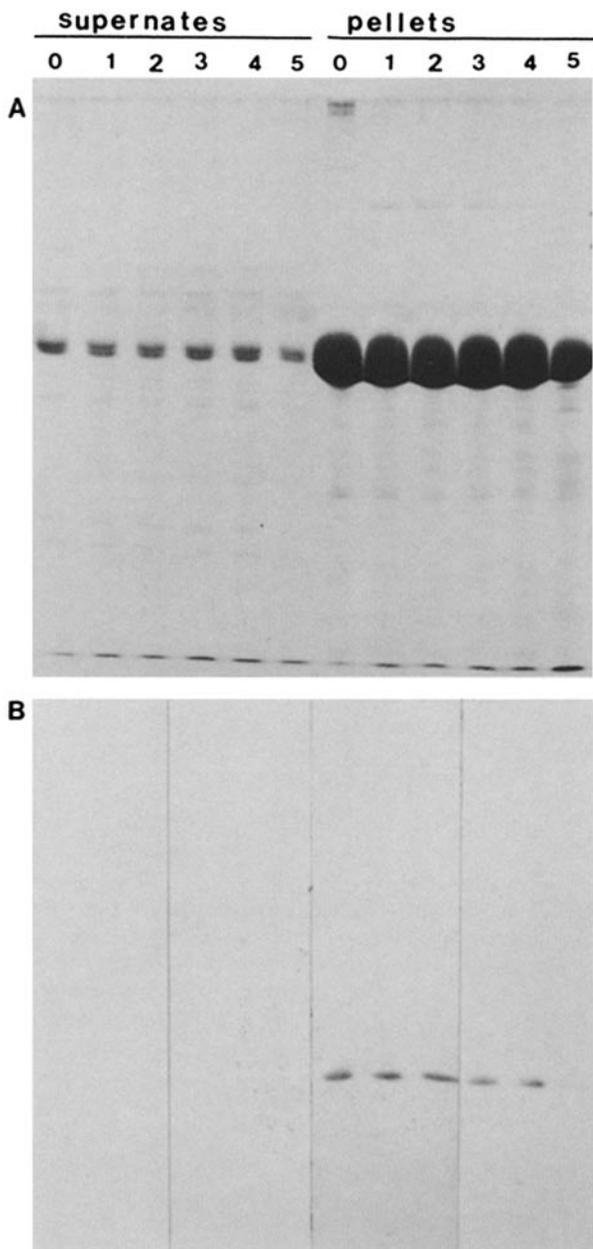
With a monoclonal antibody specific for the 34-kD component (LC-1) of MAP-1 and an immunoprecipitation assay, we have been able to demonstrate that LC-1 is tightly and specifically bound to MAP-1 heavy chain and that LC-1 shows no affinity for MAP-2. Saturating amounts of antibody to LC-1 were observed to precipitate most of the heavy chain components of MAP-1, including the two principal A and B isoforms of MAP-1. However, resolution of the immunoprecipitates was insufficient to determine if all of the MAP-1 heavy chain species were present. This indicates that at least the A and B variants of MAP-1 heavy chain contain light chain binding sites and are structurally related, which is in agreement with previous comparisons by peptide mapping (2). This observation also suggests that most of the MAP-1 heavy chains are complexed with LC-1. We also observed that LC-2 was removed from solution by immunoprecipitation,



**Figure 8.** Immunoprecipitation of chymotryptic fragments of MAP-1. Aliquots of purified MAP-1 (40  $\mu$ g) were digested for 0, 1, and 2 min at room temperature with alpha-chymotrypsin (0.1  $\mu$ g), arrested with 2 mM PMSF, and immunoprecipitated as described in the legend to Fig. 4. Antibody to LC-1 pellets LC-1 as well as the stable peptides of the MAP-1 heavy chains (molecular weights 180 and 120 kD), and intact MAP-1 heavy chains. Digestion time in minutes is indicated for each gel lane. Electrophoresis on 10% (A) and 7% (B) polyacrylamide gels. Comparison of lanes in A and B shows that 1–2 min of hydrolysis leaves no MAP-1 heavy chains intact (B, lanes 1 and 2). The material at the tops of the gel lanes (arrows) represents trace amounts of residual insoluble immunoprecipitates. No conditions were found that completely dispersed these aggregates.

but this observation was difficult to quantitate by gel electrophoresis of immunoprecipitates, since LC-2 was usually obscured by the presence of antibody light chains. It therefore appears likely that MAP-1 heavy chains, LC-1, and LC-2 are associated together in a complex.

Alpha-chymotrypsin cleaves MAP-1 into peptides, some of which retain the ability to bind to microtubules. However, the sizes and relative amounts of the microtubule-binding peptides are different from those obtained from MAP-2 using the same enzyme treatment. This agrees with the observations made previously that MAP-1 and MAP-2 are different proteins as determined by partial proteolysis with *Staphylococcus* protease V8 or alpha-chymotrypsin and peptide analysis on Cleveland gels (7). Under conditions of limited proteolysis with chymotrypsin, MAP-2 is cleaved into a major 240-kD fragment that no longer binds to microtubules (projection portion) and 32–39-kD fragments that retain microtubule-binding activity (microtubule-binding domain) (20). In contrast, we observed that MAP-1 is cleaved into a large 120-kD proteolysis-resistant fragment that associates with both LC-1 and LC-2 and contains microtubule-binding activity. A number of other peptides of intermediate to low molecular weight were also generated but did not contain microtubule-binding activity. Since LC-1 is not strongly affected by limited proteolysis and is associated with the 120-kD fragment, this molec-



**Figure 9.** Chymotryptic digestion of microtubules containing total MAPs. Microtubules were prepared by polymerization of phosphocellulose-purified tubulin in the presence of  $10 \mu\text{M}$  taxol, and total MAPs were added to the microtubule polymers. Polymers containing  $200 \mu\text{g}$  tubulin and  $20 \mu\text{g}$  MAPs were digested with  $0.05 \mu\text{g}$  alpha-chymotrypsin for 0–5 min at  $37^\circ\text{C}$ . Digestion was arrested with 2 mM PMSF, microtubules were pelleted, and pellets were analyzed by SDS gel electrophoresis (A) and immunoblotting (B). (A) Time course of chymotryptic digestion. A comparison of the supernates and pellets shows the specific binding of a 120-kD fragment and other minor components (30–50 kD) to microtubules. (B) Immunoblot of the same fractions shows that LC-1 binds to microtubules and is affected relatively little by chymotrypsin treatment. The sharp reduction in the amount of LC-1 seen in the immunoblot of the microtubule pellet after 5 min is due primarily to the prolonged digestion with chymotrypsin.

ular association may in part account for the relative stability of the 120-kD peptide from the MAP-1 heavy chain. Thus, in contrast to digestion of MAP-2, digestion of MAP-1 with

chymotrypsin generates a single large peptide that contains the microtubule-binding domain of the molecule as well as a site for the binding of LC-1.

So far it has not been possible to identify which components in the microtubule-binding domain of MAP-1 contain microtubule-binding activity. Our antibody does not block the binding of MAP-1 to microtubules, inhibit MAP-1 induced polymerization of tubulin, or displace MAP-1 from preformed microtubules containing MAP-1. Therefore it is possible that microtubule-binding activity is not contained in LC-1 but in other components such as the MAP-1 heavy chain or LC-2. Alternatively, LC-1 may bind to microtubules but at a site not blocked by the presence of bound antibody. Vallee and Davis (21) reported previously that MAP-1 heavy chain, isolated by ion-exchange chromatography in the presence of 6 M urea, binds weakly to microtubules, but it was not clear from this study if the low binding activity was due to the removal of MAP-1 light chains or to partial denaturation of the protein. To answer this question definitively, it would be desirable to examine the microtubule-binding activity of native MAP-1 heavy chains in the presence and absence of MAP-1 light chains.

Although we can demonstrate the selective binding of LC-1 to the heavy chains of MAP-1 and localize the light chain binding site to a 120-kD peptide of MAP-1, we have so far been unable to demonstrate that LC-1 is specifically localized on microtubules in situ. LC-1 was not detected by immunoblotting and microtubules were not labeled with the antibody in several different types of tissue culture cells (mouse embryo fibroblasts, bovine tracheal epithelial cells [FBT cell line] and CHO cells). It is possible that MAP-1 light chains in these cells are present in low amounts, or contain different antigenic sites, or are absent. However, it may be possible to culture neuronal cells that contain more MAP-1 to determine if LC-1 is localized on microtubules in situ. With cultures of neuronal cells it may also be possible to inject living cells with antibody, and by observing subsequent cellular behavior, ascertain if LC-1 is important for determining some of the functions of microtubules in vivo. Further work will be required to explore these possibilities.

We thank N. V. Minina for excellent technical assistance and Drs. P. Draber and V. Vicklicky for tubulin and MAP antibodies.

Received for publication 8 August 1985, and in revised form 12 November 1985

#### References

1. Bloom, G. S., F. C. Luca, and R. B. Vallee. 1984. Widespread cellular distribution of MAP 1A in the mitotic spindle and on interphase microtubules. *J. Cell Biol.* 98:331–340.
2. Bloom, G. S., T. A. Schoenfeld, and R. B. Vallee. 1984. Widespread distribution of the major polypeptide component of MAP-1 (microtubule associated protein 1) in the nervous system. *J. Cell Biol.* 98:320–330.
3. Gaskin, F., C. R. Cantor, and M. L. Shelanski. 1974. Turbidometric studies of the in vitro assembly and disassembly of porcine microtubules. *J. Mol. Biol.* 89:737–758.
4. Kotani, S., M. Murufushi, E. Nishida, and H. Sakai. 1984. 33 K protein—an inhibitory factor of tubulin polymerization in porcine brain. *J. Biochem.* 96:959–969.
5. Kumagai, H., and H. Sakai. 1983. A porcine brain protein (35 K protein) which bundles microtubules and its identification as glyceraldehyde-3-phosphate dehydrogenase. *J. Biochem.* 93:1259–1269.
6. Kuznetsov, S. A., V. I. Rodionov, A. D. Bershadsky, V. I. Gelfand, and V. A. Rosenblat. 1980. High molecular weight protein MAP-2 promoting microtubule assembly in vitro is associated with microtubules in cells. *Cell Biol. Int. Rep.* 4:1017–1024.

7. Kuznetsov, S. A., V. I. Rodionov, V. I. Gelfand, and V. A. Rosenblat. 1981. Purification of high  $M_r$  microtubule proteins MAP-1 and MAP-2. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 135:237-240.
8. Kuznetsov, S. A., V. I. Rodionov, V. I. Gelfand, and V. A. Rosenblat. 1981. Microtubule-associated protein MAP-1 promotes microtubule assembly in vitro. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 135:241-244.
9. Kuznetsov, S. A., V. I. Rodionov, V. I. Gelfand, and V. A. Rosenblat. 1984. MAP-2 competes with MAP-1 for binding to microtubules. *Biochem. Biophys. Res. Commun.* 119:173-178.
10. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
12. Matus, A., R. Bernhardt, and T. Hugh-Jones. 1981. High molecular weight microtubule-associated proteins are preferentially associated with dendritic microtubules in brain. *Proc. Natl. Acad. Sci. USA.* 78:3010-3014.
13. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208-212.
14. Rodionov, V. I., V. I. Gelfand, and V. A. Rosenblat. 1978. Polymerization of purified tubulin in the presence of glycerol. *Dok. Akad. Nauk. SSSR.* 239:231-233.
15. Rodionov, V. I., E. S. Nadezhdina, E. V. Leonova, E. A. Vaisberg, S. A. Kuznetsov, and V. I. Gelfand. 1985. Identification of 100-kDa protein associated with microtubules, intermediate filaments, and coated vesicles in cultured cells. *Exp. Cell Res.* 159:377-387.
16. Shelanski, M. L., F. Gaskin, and C. R. Cantor. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. USA.* 70:765-768.
17. Sloboda, R. D., and K. Dickerson. 1980. Structure and composition of the cytoskeletons of nucleated erythrocytes. I. The presence of microtubule-associated protein 2 in the marginal band. *J. Cell Biol.* 87:170-179.
18. Stearns, M. E. 1984. Cytomatrix in chromatophores. *J. Cell Biol.* 99(1, Pt. 2):144s-151s. (Suppl.)
19. Towbin, H., T. Stachelin, and J. Gordon. 1979. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
20. Vallee, R. B. 1980. Structure and phosphorylation of microtubule-associated protein 2 (MAP-2). *Proc. Natl. Acad. Sci. USA.* 77:3206-3210.
21. Vallee, R. B., and S. E. Davis. 1983. Low molecular weight microtubule-associated proteins are light chains of microtubule-associated protein 1 (MAP-1). *Proc. Natl. Acad. Sci. USA.* 80:1342-1346.
22. Vallee, R. B., G. S. Bloom, and W. E. Theurkauf. 1984. Microtubule-associated proteins: subunits of the cytomatrix. *J. Cell Biol.* 99(1, Pt. 2):38s-44s. (Suppl.)
23. Weatherbee, J. A., P. Sherline, R. N. Mascardo, J. G. Izant, R. B. Luftig, and R. R. Wehing. 1982. Microtubule-associated proteins of HeLa cells: heat stability of the 200,000 molecular weight HeLa MAPs and detection of MAP-2 in HeLa cell extracts and cycled microtubules. *J. Cell Biol.* 92:155-163.
24. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.
25. Weingarten, M. D., A. H. Lockwood, S.-Y. Hwo, and M. W. Kirschner. 1975. A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. USA.* 72:1858-1862.