Microheterogeneity of Microtubule-associated Proteins, MAP-1 and MAP-2, and Differential Phosphorylation of Individual Subcomponents*

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High molecular weight microtubule-associated proteins 1 and 2 (MAP-1 and MAP-2), prepared by copolymerization with tubulin, were electrophoretically separated into three and two major subcomponents, respectively, using 5% sodium dodecyl sulfate-polyacrylamide gels. By two-dimensional gel electrophoresis, all five MAP components were shown to possess a pI of around 5. Four of these proteins, MAP-1A, MAP-1C, MAP-2A, and MAP-2B, present in comparable amounts, were iodinated after electrophoretic separation and analyzed by two-dimensional peptide mapping. With both trypsin and V8 protease, almost identical patterns were obtained from MAP-2A and MAP-2B. MAP-1A and MAP-1C, too, gave similar digestion patterns, although some differences were noted. Incubation with [γ-32P]ATP demonstrated that endogenous protein kinase activities phosphorylated individual subcomponents at different rates. MAP-2A, the highest labeled component, was phosphorylated 2.5-fold compared to MAP-2B both in the presence and the absence of cAMP. Labeling of MAP-1 subcomponents was 4 times less than that of MAP-2A in the absence and 16 times less in the presence of cAMP. 32P-labeled MAP-2A and MAP-2B bands were indistinguishable by one-dimensional peptide mapping, as were the three MAP-1 bands. For both MAP-1 and MAP-2 subcomponents, CAMP induced phosphorylation at new molecular sites. Incubation of radiolabeled microtubule proteins with 1 mM ATP affected, upon electrophoresis, a clear shift of MAP-2A and MAP-2B bands to positions of higher apparent molecular weights, while only slightly affecting MAP-1 bands.

High molecular weight MAPs1 from brain copolymerize with tubulin in high yield after isotonic homogenization of the tissue (1). Regular SDS-polyacrylamide gel electrophoresis distinguishes two major MAP components, MAP-1 (330 kDa) and MAP-2 (300 kDa) (2). In a previous study (3), we showed, by immunological and protein chemical methods, that MAP-1 and MAP-2 are partially homologous in structure. When analyzed by electrophoresis on low percentage gels, both MAP-1 and MAP-2 tend to split into a number of closely spaced bands. The major subcomponents of MAP-2, MAP-2A and MAP-2B, were recently compared by one-dimensional peptide mapping and found to have very similar structures (4, 5). MAP-1 can be resolved into three bands, MAP-1A, -1B, and -1C (6), which previously had not been compared by chemical methods. Work with monoclonal antibodies, however, suggests that individual MAP-1 bands share common molecular domains and have distinct ones as well (6-8).

MAP-1 and MAP-2 are developmentally regulated phosphoproteins (4, 9) and are substrates for various brain protein kinases (10-12). MAP-2 as isolated contained 8 to 13 mol of phosphate/mol of protein; with further in vitro phosphorylation values up to 24 were obtained (13-15). Furthermore, the phosphorylation of MAPs is of importance for the in vitro polymerization of microtubules (16), and it affects their binding affinity to microtubules (15-17). Therefore, phosphorylation is a likely mechanism for modulation of MAP functions in the cell.

In the present study, our primary goal was a detailed examination of the MAP-1 and MAP-2 subcomponents by comparative peptide mapping in order to clarify their structural relationship. In addition, we studied the phosphorylation of MAP subcomponents by endogenous kinases to examine whether this modification affects the microheterogeneity of MAPs.

EXPERIMENTAL PROCEDURES

Materials—Sodium [3H]borohydride (10 Ci/mmol), Na2[H] (13.5-16.2 Ci/mg), I-32P-Protein A (80-100 mCi/mg), and carrier-free 32P, were from The Radiochemical Centre, Amersham, United Kingdom; [γ-32P]ATP (specific activity, 132 to 1200 Ci/mol) was prepared according to the method of Walsh and Johnson (18). Nucleotides and enzymes were purchased from Boehringer Mannheim. Acrylamide, urea (pro analysi), carrier ampholytes, and aprotinin were from Serva (Heidelberg); V8 protease was from Miles Laboratories, Inc. (Elkhart, IN); chloramine T, theophylline, bovine serum albumin (fraction V), phenylmethylsulfonyl fluoride, and all detergents and buffers were purchased from Sigma.

Preparation of Microtubule Proteins—Homogenization of grey matter from pig brain and subsequent rounds of in vitro polymerization/depolymerization of microtubule proteins (cycling) were essentially performed by the method of Karr et al. (1). Two times cycles material was stored at ~70°C. Before use thawed material was taken up in 100 mM Mes (pH 6.8), 1 mM EGTA, 1 mM MgCl2 (buffer A), or 100 mM Mes (pH 6.8), 5 mM MgCl2, 4 mM 2-mercaptoethanol (buffer B), and the insoluble material was removed by centrifugation in a Beckman 65 rotor at 4°C and 40,000 rpm for 20 min. The soluble material was desalted into the desired buffer by chromatography on Bio-Gel P-10 columns. For two-dimensional gel electrophoresis, tubulin-free MAPs were prepared by chromatography of microtubule proteins on phosphocellulose columns (19).

Preparation of Boiled Brain Extracts—Small pieces of gray matter (~1 g) were dissected on ice with a razor blade, transferred to a cooled homogenizer, and mixed with 1 ml of ice-cold 50 mM Mes (pH 6.8),

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1 The abbreviations used are: MAP, microtubule-associated protein; SDS, sodium dodecyl sulfate; Mes, 4-morpholinoethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
10 mM EGTA, 10 mM 6-aminohexanoic acid, 10 mM MgCl₂, 4 mM phenylmethanesulfonyl fluoride, 50 μg/ml benzamidine, 100 μg/ml aprotinin, and 2% Nonidet P-40. After one stroke with a Teflon pestle, aliquots were supplemented with equal volumes of boiling two times concentrated electrophoresis sample buffer (20) and heated for 3 min in a boiling water bath. DNA was removed by centrifugation at 10,000 × g for 1 h.

Radio labeling of Proteins—Tritium labeling of proteins was carried out by reductive methylation (21) using [3H]borohydride (10 Ci/mmol) as previously described (3). Radiolabeling of proteins in polyacrylamide gel slices was performed by the method of Elder et al. (22). After electrophoresis, proteins were visualized by staining with 0.2% Coomassie Brilliant Blue in 50 mM Tris/HCl, pH 7.2, desired bands cut out, and the stain removed by washing with 50 mM Tris/HCl, pH 7.2, and water. After drying, gel bands were allowed to absorb a mixture of 170 μl of 100 mM sodium phosphate buffer (pH 7.4), 1.5 μl (150 μCi) of [32P]-sodium, and 10 μl of 0.2% chloramine T in water. After 30 min at room temperature reactions were stopped by addition of 10 μl of sodium bisulfite (10 mg/ml) and gel slices were 6 times washed with 10 ml of 10% methanol followed by drying.

MAPs were phosphorylated using aliquots of microtubule protein that had been supplemented with 1% aprotinin and desalted into buffer A or B. For cyclic AMP-dependent phosphorylation, samples were preincubated with 10 μM cyclic AMP and 2 μM theophylline for 3 min. Reactions were started by the addition of [γ-32P]ATP to 2.5 μM and carried out at 30 °C with occasional vortexing. Reactions were stopped by addition of three times concentrated electrophoresis sample buffer (20) and heating to 95 °C for 3 min.

Polymerization of 32P-labeled Microtubule Protein—Microtubule protein was labeled in the presence of cyclic AMP for 20 min in buffer A. The incubation was stopped by loading the sample (equivalent to 120 μg of sedimented microtubule protein in 0.5 ml of buffer A) on a 2.5-ml column of Bio-Gel P-10 and transfer into half-concentrated stock solutions and incubated at 37 °C. After 15 min GTP was supplemented to 2 mM and after 30 min 400 μl of samples were layered onto a cushion of 500 μl of 5% sucrose in buffer A. Centrifugation was for 60 min in a Beckman 65 rotor at 40,000 rpm at 37 °C. The supernatant was carefully collected and supplemented with three times concentrated electrophoresis sample buffer (20). The pellet was resuspended in 0.6 ml of electrophoresis buffer and heated for 3 min at 95 °C.

Gel Electrophoresis, Immunoblotting, and One-dimensional Peptide Mapping—Sodium dodecyl sulfate-polyacrylamide gels (5 or 6.25% acrylamide) were used for analytical and preparative purposes (23). The inclusion of 25% glycerol (24) helped to improve the separation of individual MAP subcomponents and was done whenever necessary. The electrophoresis buffer at the cathode contained 0.1% 2-mercaptoethanol (5 or 6.25% acrylamide) were used for analytical and preparative purposes (23). The inclusion of 25% glycerol (24) helped to improve the separation of individual MAP subcomponents and was done whenever necessary.

RESULTS

Microtubule protein, prepared from hog brain by two cycles of in vitro polymerization/denpolymerization under isotonic conditions, was primarily contained, aside from tubulin, high molecular weight MAPs, each consisting of two major bands, were revealed by staining with Coomassie Blue (Fig. 1). Third and fourth MAP-1 group bands (marked MAP-1A and MAP-1B in Fig. 1C) became clearly visible only after silver staining. In addition, this treatment revealed a set of bands migrating ahead of the MAP-2 doublet. The designation of the Coomassie-stained bands in Fig. 1 as MAP-1A, 1B, and 1C and MAP-2A and 2B follows a suggestion of Bloom et al. who found a similar splitting of bands with high M, MAPs from calf brain (6).

Quantitation of individual bands from microtubule preparations labeled by reductive methylation revealed that MAP-1 and MAP-2 group bands accounted for 57 and 43% of total high M, components, respectively; MAP-1A, 1C, 2A, and 2B were present in approximately equal amounts (20–26%), while MAP-1B constituted much less (11%) (Fig. 1D).

In order to test whether proteolysis occurs during the lengthy preparation of the microtubule proteins and contributes to the multiplicity of MAP bands, brain extracts were boiled in SDS electrophoresis sample buffer and analyzed on two-dimensional gel electrophoresis.

FIG. 1. Resolution of high M, MAPs on low percentage gels. Two times concentrated microtubule proteins from hog brain (A–D) or directly boiled brain extracts (E and F) were separated on 7.5% or 5% acrylamide gels. A and B, Coomassie Blue staining; C, silver staining; D, quantitation (in per cent of total label) of MAP bands marked in C after labeling by reductive methylation (see Materials and Methods); E and F, immunoblotting using antiserum to MAP-1 and MAP-2, respectively, followed by [125I]protein A. In B to F only relevant parts of the gels were processed. Tub, tubulin.
Western blots. A group of four bands in the MAP-1 region was found to be immunoreactive to antiserum raised against the entire MAP-1 group (30) (Fig. 1E). Several bands of lower molecular weight were also stained with this antiserum. Similarly, an antiserum to MAP-2 components (30) preferentially stained a double band corresponding to MAP-2A and MAP-2B and, to a lesser degree, material of lower molecular weight (Fig. 1F). Therefore, we conclude that multiple MAP-1 and MAP-2 components are indeed present in brain, but that some proteolysis apparently occurs even when brain tissue is prepared directly for SDS-gel electrophoresis.

Individual MAP bands revealed very similar isoelectric points in two-dimensional gel electrophoresis (Fig. 2). The MAP-1 group (marked by brackets) did not focus well relative to the MAP-2 group, as indicated by its pronounced streaking between the start (arrow) and its isoelectric points. Consequently, the amount of MAP-1 components at their isoelectric points was low relative to that of MAP-2. Furthermore, the MAP-2 group appeared to consist of several isoelectric variants, which possibly represented MAP-2 species at different degrees of phosphorylation. In addition, degradation products were evident within two major zones, one descending to the acidic, the other to the basic side. Components corresponding in size to MAP-1A, 1B, and 1C and focusing in the pH region of MAP-2 were also clearly identified by antibody overlay of two-dimensional gels using antiserum to MAP-1 (Fig. 2, inset). In this experiment the previously demonstrated (3, 29, 30) cross-reactivity of the anti-MAP-1 antiserum to MAP-2 components became extraordinarily prominent, probably due to the very small proportion of MAP-1 to MAP-2 components accessible for the antibodies.

To compare individual MAP bands by tryptic “fingerprint” analysis, proteins were first separated on gels and then iodinated individually. After extensive tryptic digestion the peptide patterns generated from MAP-1A and MAP-1C were identical in nine major spots and similar in an additional ring-like cluster of spots (Fig. 3, A and B). Slight structural differences between MAP-1A and MAP-1C must exist, however, since several spots were either specific for MAP-1A or MAP-1C or were present in different proportions (Fig. 3, large arrowheads). A similar situation was encountered with V8 protease, where 5 identical major spots were revealed (Fig. 3, C and D). Fig. 4 shows a corresponding analysis of MAP-2A and MAP-2B. With both trypsin and V8 protease, virtually identical peptide maps were obtained which indicates a high degree of structural homology between the two MAP-2 components.

To examine whether the individual subcomponents of the MAP-1 and the MAP-2 groups were phosphorylated to a similar extent and at identical sites the endogenous kinase activities cocycling with microtubule proteins were utilized to label MAPs with radioactive phosphate. Fig. 5, lane I, shows the pattern of radiolabel in MAPs after standard incubation of microtubule protein with [γ-32P]ATP. Most prominently labeled was MAP-2A, followed by MAP-2B, MAP-1A, MAP-1C, and MAP-1B. When the reaction time was prolonged from 10 to 25 min the label in all bands increased proportionally (Fig. 5, lane 4). Preincubations with cAMP led to a marked increase in radioactivity of the MAP-2 bands (Fig. 5, lane 2). The nature of the cAMP-independent protein kinase copurifying with microtubules is unclear. We tested whether this activity was inhibited by 1 mM Ca2+ like the casein kinases I and II (reviewed in Ref. 34) or other kinases (35). However, the phosphorylation of MAPs was unchanged (Fig. 5, lane 3).

Quantitation of the 32P radioactivity after cutting out individual stained MAP bands from gels followed by Cerenkov counting revealed that, under standard conditions of labeling (Fig. 5, lane 1), MAP-2 accounted for 70% of the radioactivity.

**Fig. 2. Two-dimensional gel electrophoresis of high M₅ MAPs.** Microtubule proteins were separated on phosphocellulose columns (19) and MAP fractions subjected to isoelectric focusing (IEF). The gel was stained with Coomassie Blue after removal of ampholytes. The inset shows an autoradiography of a gel run in parallel that was subjected to antibody gel overlay employing antibodies to MAP-1 (see “Materials and Methods”). The arrow indicates the start of the isoelectric focusing gel; brackets indicate the position of MAP-1 subcomponents. Note three MAP-1 bands recognized by the antiserum (arrowheads) and two very prominent tracks of immunoreactive degradation products.

**Fig. 3. Two-dimensional peptide mapping of MAP-1 subcomponents.** Protein bands were excised from 5% acrylamide gels and radioiodinated with chloramine T. A and C, MAP-1A; B and D, MAP-1C (see Fig. 1). A and B, trypsin; C and D, V8 protease. Electrophoresis was from left to right and chromatography from bottom to top. Numbers indicate major peptides common to MAP-1A and -1C. In A and B, small arrowheads point to peptides arranged in a ring-like cluster of spots not completely resolved; in C and D, small arrowheads indicate common minor peptides. Large arrowheads mark distinct fragments.
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To Fig. 3. A and C, MAP-SA; and -2B.

The proportion of radioactivity residing in MAP-2. The increase in buffer B and separated on 5% acrylamide gels as described with [γ-32P]GTP.

The labeling of MAP-1s was elevated only slightly, raising the individual MAP-1 bands were labeled according to their mass ratio (see Fig. 1D), while, based on mass, MAP-2A incorporated radiolabel 2.5-fold more efficiently than MAP-2B (data not shown).

ATP was followed by a “chase” with 1 mM unlabeled GTP for 25 min; autoradiography was for 1 h (lanes 1–4) and 20 h (lane 5).

When the incubation of microtubule protein with [γ-32P]ATP was followed by a “chase” with 1 mM unlabeled GTP for 20 min in order to assess the influence of GTP on the above described labeling patterns, neither the amount of incorporated radioactivity nor the labeling of MAP bands showed significant change. Moreover, when [γ-32P]GTP was employed instead of labeled ATP (Fig. 5, lane 5), the incorporation of 32P activity accounted for only 5% of that observed with ATP and, notably, the labeling pattern was altered significantly. With [γ-32P]GTP, MAP-2A received 69% of the label compared to 19% for MAP-2B, resulting in a ratio of 3.7 instead of the ratio of 2.5 observed with labeled ATP. The MAP-1 group was labeled by 12% and thus significantly lower than in controls employing labeled ATP (data not shown). These results indicated that the microtubule preparations from hog brain contained, though in low levels, a kinase activity which exhibited a different substrate specificity than the one utilizing ATP.

In contrast to GTP, a “chase” with 1 mM unlabeled ATP caused a striking alteration in the position of MAP-2 bands but not in that of MAP-1 bands. As determined by Coomassie staining (Fig. 6, lanes 1–3) as well as autoradiography (lanes 4–6) the two major MAP-2 bands showed a decrease in electrophoretic mobility. The major labeled MAP-2 band was observed intermittent between MAP-1C and the original position of MAP-2A (Fig. 6, lane 5). Its staining intensity with Coomassie Blue (lane 2) was comparable to that of MAP-2A from the control sample (lane 1). Both bands at the original position of MAP-2A and MAP-2B were stained about half as intensely as the MAP-2B band from the control. Mixing equal amounts of “chased” and “not chased” material resulted in the appearance of a new MAP-2 band (Fig. 6, lane 4) was about equal to that in the new uppermost MAP-2 band after ATP “chase” (Fig. 6, lane 5). Similarly, the label associated with MAP-2B of controls appeared to migrate at the position of the original MAP-2A. The radioactivity now located at the position of control MAP-2B resulted, we assume, from an upward shift of the phosphorylated bands below control MAP-2B. One-dimensional peptide maps indicated that these bands were degradation products of MAP-2 (data not shown). Quantitation by Cerenkov counting of bands cut from gels confirmed that MAP-2A and MAP-2B were shifted in unison. This suggested that MAP-2A and MAP-2B are not interconvertible by phosphorylation and that MAP-2A is, therefore, not a higher phosphorylated variant of MAP-2B.

Cyclic AMP increased the labeling of MAP-2s 4-fold, while the labeling of MAP-1s was elevated only slightly, raising the proportion of radioactivity residing in MAP-2. The increase of incubation time from 10 to 25 min enhanced the labeling of both MAP-1 and MAP-2 by a factor of 2.5. On the whole, individual MAP-1 bands were labeled according to their mass ratio (see Fig. 1D), while, based on mass, MAP-2A incorporated radiolabel 2.5-fold more efficiently than MAP-2B (data not shown).

When the incubation of microtubule protein with [γ-32P]ATP was followed by a “chase” with 1 mM unlabeled GTP for 20 min in order to assess the influence of GTP on the
To examine whether the differential labeling of individual MAP-bands demonstrated in Figs. 5 and 6 correlated with the labeling at different molecular sites, we performed one-dimensional peptide mapping of various MAP bands. Fig. 7 shows the V8 protease degradation patterns of MAP-2A and MAP-2B phosphorylated under the conditions shown in Figs. 5 and 6. Two groups of peptides generated, one in the M, range from underdigested MAP-2 to around 100 kDa and another consisting of 5 major fragments from 76 to 42 kDa (Fig. 7A, lanes 3 and 4; small arrowheads), were characteristic for MAP-2A as well as MAP-2B, independent of the conditions of labeling. The fragmentation patterns of MAP-2s labeled in the presence of 1 mM Ca²⁺ ions (data not shown) were indistinguishable from those of MAPs labeled in the absence of Ca²⁺ ions (Fig. 7A, lanes 1 and 2) nor were significant differences observed between MAPs labeled for 10 min (Fig. 7A, lanes 1 and 2) and those labeled for 25 min (data not shown). However, strongly labeled fragments at 33, 18, and 12.5 kDa were generated only from those MAP-2A and -2B bands that had been labeled in the presence of cAMP (large arrowheads in Fig. 7A, lanes 3 and 4). These fragments were observed in comparable proportions irrespectively of whether labeled MAP-2s were "chased" with GTP (data not shown), ATP (Fig. 7A, lanes 5 and 6), or neither of these (Fig. 7A, lanes 3 and 4). When MAP-2A and MAP-2B, labeled in the presence and absence of cAMP, were compared after enhanced digestion in high percentage gels (Fig. 7B), again, identical patterns were observed for MAP-2A and -2B. In this case cAMP-dependent MAP fragments were of molecular weight 18,000, 12,500, 11,500, and 9,000. Since in all cases the degradation pattern of MAP-2A was virtually identical to that of MAP-2B, the following conclusions can be drawn: (i) the same sites were phosphorylated in MAP-2A and MAP-2B; (ii) neither prolonged labeling nor the presence of Ca²⁺ ions resulted in the phosphorylation of new sites; (iii) cAMP induced the phosphorylation of both MAP-2A and MAP-2B at new, identical sites; and (iv) phosphatase activity in the microtubule preparations must have been low, because "chasing" with excess unlabeled ATP did not result in the disappearance of any of the phosphorylated fragments and the amount of radioactivity in MAPs was not reduced by the "chase."

Peptide mapping of the individual MAP-1 bands shown in Fig. 5 produced major fragments of 76, 71, 64, 54, and 42 kDa under all conditions of labeling (Fig. 8A). These fragments were of identical M, as those generated from MAP-2s. Like with MAP-2s the fragmentation patterns of MAP-1s were not influenced by Ca²⁺ or longer incubation time (data not shown). In contrast to MAP-2, however, under these conditions of digestion no additional fragments were observed after

![Fig. 7. Autoradiography of one-dimensional peptide maps of MAP-2A and MAP-2B phosphorylated in vitro.](image1)

![Fig. 8. Autoradiography of one-dimensional peptide maps of MAP-1 subcomponents phosphorylated in vitro.](image2)
labeling in the presence of cAMP (compare lanes 1–3 with 4–6 in Fig. 8A). MAP-1A, -1B, and -1C gave similar fragmentation patterns in the M, region of 39,000 to 19,000 with major fragments of 32, 28, and 26 kDa (Fig. 8A, small arrowheads). With MAP-1A and MAP-1B, the intensity of bands in the 39-kDa region was enhanced relative to MAP-1C, indicating slight differences in the components’ accessibility to the protease. This finding is supported by data from electrophoresis on high percentage gels following increased proteolysis (Fig. 8B). A group of 10 bands of M, 31,000 to 12,000 was generated from each MAP-1A, -1B, and -1C (Fig. 8B, lanes 2–4, respectively) and also from MAP-1A’, the minor band migrating above MAP-1A (Fig. 8B, lane 1). The two upper MAP-1 bands, however, gave rise to a more pronounced fragment at 27 kDa while MAP-1B and 1C yielded relatively prominent fragments at 24 and 12 kDa (Fig. 8B, arrowheads). This indicated that the four resolvable bands in the MAP-1 region were phosphorylated at identical sites but that their susceptibility toward proteolysis was slightly altered probably due to minor differences in primary structure. However, when MAP-1s labeled in the presence of cAMP were analyzed in parallel, two additional fragments, not resolved on lower percentage gels (see Fig. 8A), were observed (data not shown).

The results obtained by one-dimensional peptide mapping of phosphorylated MAP species were confirmed by tryptic “fingerprinting.” For each MAP-1A and -1C, and MAP-2A and -2B, 8 to 9 phosphorylated fragments with identical two-dimensional patterns were observed (data not shown).

To analyze the fate of phosphorylated MAP subcomponents during polymerization of microtubules, in particular to assess whether individual phosphorylated MAP-bands “cycled out,” microtubule proteins were labeled with [γ-32P]ATP, desalted into polymerization buffer, incubated with GTP at 37 °C, and polymers formed were centrifuged. In total, more than 90% of the radioactivity associated with the MAPs subjected to polymerization was recovered in the microtubule pellet. The analysis of MAPs in various fractions showed that the degree of phosphorylation was fairly constant for all five subcomponents tested. During the whole experiment, which lasted 2 h, the percentage of label in the MAP-1 group rose by 2.4% corresponding to a decrease of label in MAP-2A of 1.7% and in MAP-2B of 0.7%. The soluble MAP fraction contained less than 0.7% of the radioactivity of the insoluble microtubule-associated MAP fraction, and soluble MAP-1 and MAP-2 were labeled in a ratio of 1:2. Thus it appeared that MAP-2 did not “cycle out” preferentially but was degraded slightly more than MAP-1.

**DISCUSSION**

In this study, our previous finding of partial homology between the high molecular weight microtubule-associated proteins MAP-1 and MAP-2 was extended to the level of individual subcomponents of these polypeptides. In two-dimensional peptide maps, the two major components of each group exhibited only minor differences indicating that MAP-1 as well as MAP-2 consists of a number of microheterogeneous polypeptides. Phosphorylation of individual subcomponents by endogenous copurifying protein kinases occurred at different rates. However, the phosphorylated domains of individual components of each group were indistinguishable by one-dimensional peptide mapping. This indicated that the molecular phosphorylation sites are highly conserved and that the heterogeneous regions are located in other parts of the molecules.

Comparing the major two bands of MAP-1, MAP-1A and -1C, by two-dimensional tryptic peptide mapping, a much higher degree of similarity was observed than when the whole MAP-1 group was compared to the MAP-2 group (3). This confirmed that the MAP-1s are molecular variants distinct from MAP-2s. The slight differences observed for the two MAP-1 species indicated that they differed in some parts of their sequence, whereas the indistinguishable fingerprints of MAP-2A and -2B established their identity at this level of analysis. A high degree of similarity of MAP-2A and -2B was recently suggested also on the basis of one-dimensional peptide maps (4, 5).

The splitting of polypeptide bands on SDS gels has been shown to be a result of differential phosphorylation of single proteins. This kind of microheterogeneity occurs, for instance, with the regulatory subunit RII of the cAMP-dependent protein kinase (36) and the calmodulin-dependent protein kinase from brain (37). Regarding MAPs we noted that the apparent M, of both MAP-2A and -2B shifts up upon extensive phosphorylation. A similar shift of MAP-2A and MAP-2B was observed when taxol-stabilized microtubules from rat brain were phosphorylated under stimulation of cAMP, and the mobility of MAP-2s was compared with those of unstimulated incubations (9). In our system, however, the critical factor for the shift clearly was not the presence or absence of cyclic nucleotide but the concentration of ATP.

MAP-1 subcomponents were phosphorylated with [γ-32P]ATP at a much lower rate than MAP-2s, and they shifted only slightly on SDS gels upon “chase” with 1 mM ATP. Furthermore, their phosphorylation was stimulated significantly by cAMP only at high concentrations of ATP (3, 38). This may indicate that MAP-1, in marked contrast to MAP-2, was nearly saturated with phosphate and that its dephosphorylation during cycling may be low. Accordingly, after in vivo incubation with 32P, MAP-1 from chick brain carried more radiolabel than MAP-2 (10).

Both cAMP-independent and -dependent protein kinase activities discriminated between MAP-2A and MAP-2B, in that MAP-2A was phosphorylated by both kinases 2.5 times more than MAP-2B. In the light of the identical peptide maps of MAP-2A and -2B, their differential phosphorylation could be explained in various ways: (i) MAP-2B may be a higher phosphorylated isoform of MAP-2A but possess a lower molecular weight and therefore migrate ahead of MAP-2A; (ii) MAP-2B might differ in secondary structure from MAP-2A and thus be a less accessible substrate for the protein kinases; (iii) MAP-2B may be comprised of two very similar but not identical polypeptides, only one of which is phosphorylated in vivo (this would explain why, following “chase” with 1 mM ATP, both MAP-2 bands labeled by [γ-32P]ATP in the “pulse” shift in unison, while the Coomassie Blue-stained band, remaining at the original position of MAP-2B, carried hardly any label). Each of these possibilities is consistent with the demonstrated identity of the two-dimensional peptide maps of MAP-2A and MAP-2B.

One- and two-dimensional peptide maps obtained from phosphorylated MAP-2A and MAP-2B were virtually identical under various conditions of labeling and digestion. Thus, the apparent microheterogeneity of the MAP-2 group does not seem to be confined to the major phosphorylated domain, which has been located on a 20-kDa peptide of the 35-kDa microtubule binding fragment (39). A similar situation might apply for all three MAP-1 components, which also yielded virtually identical phosphopeptide maps independent of the conditions of labeling and digestion.

When phosphopeptide maps of MAP-1 and MAP-2 were compared, differences became apparent only after complete digestion of polypeptides below 40 kDa. However, even under
these conditions, the fragmentation patterns were similar, showing differences only in terms of the relative proportion of labeled fragments. This indicated that MAP-1 and MAP-2 are phosphorylated within similar molecular domains but that individual phosphorylated sites may differ in primary sequence and thus be phosphorylated at different rates. Since the major phosphorylated domain of MAP-2 is located in the tubulin-binding domain (see above), this observation argues for a considerable conservation of the microtubule-binding region between both MAP-1 and MAP-2.

From the data presented, we conclude that high molecular weight MAPs like many other proteins, e.g. the tubulins (40), spectrins (41), and type II CAMP-dependent protein kinases (36), exist in isoforms. In the case of spectrins, for example, it has been shown that α- and β-spectrin are composed of rather identical building blocks, whereby the faster migrating β-unit is about 150 amino acid residues shorter than the α-subunit and, in contrast to the α-subunit, is phosphorylated at the carboxyl terminus (42). Similar criteria might apply to MAP-1 and MAP-2 subcomponents. Therefore, the microheterogeneity observed for MAP-1 and MAP-2 may be restricted to small domains that have been altered by post-translational modifications or differential gene expression. This would result in the synthesis of a number of molecules with diverse in addition to conserved functions. Their expression may depend not only on species and tissue heterogeneity observed for MAP-1 and MAP-2 but that in the carboxyl terminus (36), their expression may depend not only on species and tissue heterogeneity observed for MAP-1 and MAP-2 but that in the carboxyl terminus (36).

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