Tau Proteins: The Molecular Structure and Mode of Binding on Microtubules

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Abstract. Tau is a family of closely related proteins (55,000–62,000 mol wt) which are contained in the nerve cells and copolymerize with tubulin to induce the formation of microtubules in vitro. All information so far has indicated that tau is closely apposed to the microtubule lattice, and there was no indication of domains projecting from the microtubule polymer lattice. We have studied the molecular structure of the tau factor and its mode of binding on microtubules using the quick-freeze, deep-etch method (QF·DE) and low angle rotary shadowing technique. Phosphocellulose column–purified tubulin from porcine brain was polymerized with tau and the centrifuged pellets were processed by QF·DE. We observed periodic armlike elements (18.7 ± 4.8 nm long) projecting from the microtubule surface. Most of the projections appeared to cross-link adjacent microtubules. We measured the longitudinal periodicity of tau projections on the microtubules and found it to match the 6-dimer pattern better than the 12-dimer pattern. The stoichiometry of tau versus tubulin in preparations of tau saturated microtubules was 1:∼5.0 (molar ratio). Tau molecules adsorbed on mica took on rodlike forms (56.1 ± 14.1 nm long). Although both tau and MAP1 are contained in axons, competitive binding studies demonstrated that the binding sites of tau and MAP1A on the microtubule surfaces are mostly distinct, although they may partially overlap.

Microtubules are one of the main cytoskeletal elements in eukaryotic cells and are particularly abundant in nerve cells. It is well known that there are several microtubule-associated proteins (MAPs)[1] which copurify with brain tubulin during repetitive cycles of temperature-dependent assembly and disassembly. Among these proteins in neuronal tissues, high molecular weight proteins (MAP1 and MAP2), and tau factor are major species, and recently several minor proteins have also been identified (6, 7, 17, 27, 28, 33, 41).

The high molecular weight microtubule-associated proteins (MAP1, MAP2) are flexible, rodlike structures ∼100–200 nm long and form armlike projections when attached to microtubule surfaces (22, 31, 39, 40, 42). Recent structural studies demonstrated that microtubule domains in nerve cells are composed of microtubules, associated cross bridges, and granular materials (11, 14, 31). High molecular weight MAPs (MAP1, MAP2, 270,000-mol-wt MAP) were proven to be components of these cross bridges associated with microtubules in vivo (12, 14, 32).

Tau factor is composed of four to five polypeptides (55,000–62,000 mol wt) which were shown to be closely related by both peptide mapping and amino acid analysis (6), and were assumed to represent a highly asymmetric molecule by hydrodynamic data (7). This protein promotes polymerization of tubulin, is heat stable (41), and is able to bind to calmodulin in the presence of calcium (34). Recent immunocytochemical studies have shown that tau is mainly localized in axons (3). It has also been revealed recently that highly phosphorylated tau is a major element of paired helical filaments in Alzheimer's disease (8, 18, 23).

However, the molecular structure of tau and the mode of binding of tau to microtubules has remained unclear. This study was designed to disclose the molecular structure of tau by the quick-freeze, deep-etch method and low angle rotary shadowing technique, and has allowed us to reveal for the first time that tau is a rodlike structure (56.1 ± 14.1 nm long) and associates with microtubules with armlike projections (18.7 ± 4.8 nm long).

Materials and Methods

Isolation of Tau

Tau was prepared from porcine brain microtubules by the modification of a method described by Grundke-Iqbal et al. (8). Pellets of microtubule proteins obtained by cycles of temperature-dependent assembly and disassembly (30) were suspended in 3 vol of buffer containing 100 mM 2-(*N*-morpholino)ethane sulfonic acid Mes, 0.5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 0.75 M NaCl, 2 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), pH 2.7. The suspension was then heated at 95°C in boiling water for 5 min and centrifuged at 25,000 *g* at 4°C for 30 min. The pH of the resulting supernatant was adjusted to 6.8, and it was then subjected to Bio gel A 1.5 m (Bio-Rad Laboratories, Richmond, CA) column chromatography and concentrated in order to prepare purified tau fractions (1.89

1. *Abbreviations used in this paper*: MAP(s), microtubule-associated protein(s); PC, phosphocellulose column purified; PEM, 0.1 M Pipes, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.8.

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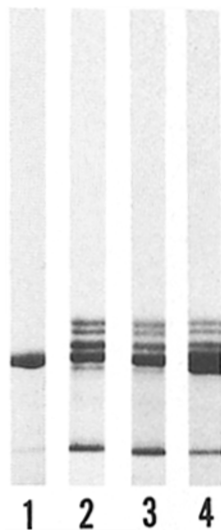

Figure 1. SDS-PAGE (7.5% running gel) of PC tubulin (lane *1*), purified tau factor (lane *2*), supernatant (lane *3*), and pellet (lane *4*) of suspensions of PC tubulin (1.0 mg/ml) and tau (0.4 mg/ml). The pellet was resuspended with PEM in a volume equal to the original suspension, and the same volume of the sample was applied (lanes *3* and *4*) on the gel.

mg/ml). Tau proteins were dialysed against PEM buffer (0.1 M Pipes, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.8).

Isolation of Tubulin

Tubulin was prepared from porcine brain by phosphocellulose chromatography (41).

Protein Determination

Protein concentrations were estimated by the assays of Bradford et al. (5) and Itzhaki and Gill (19) using BSA as a standard.

Quick-Freeze, Deep-Etch Electron Microscopy of Tubulins Polymerized with Tau

Phosphocellulose column-purified (PC) tubulin was mixed with tau fraction (final concentration: tau 0.4 mg/ml, tubulin 1.0 mg/ml) and incubated at 37°C in the presence of 1 mM GTP for 30 min. As a control, PC tubulin without tau was incubated at 37°C in the presence of 20 μM taxol and 1 mM GTP (38). The resulting polymers were centrifuged at 19,000 rpm at 30°C for 30 min, and pellets were quick frozen and deep etched as described previously (9-11, 14). In some cases pellets were resuspended with a small amount of PEM buffer (0.1 M Pipes, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.8) (four times the pellet volume), chilled on ice for 30 min, supplemented with taxol to 20 μM, and rewarmed at 37°C for 15 min. The suspension was quick frozen without centrifugation. The samples were dissolved in Purelox (Ohylox Corp., Tokyo, Japan), and replicas were washed with distilled water, and put on Formvar-carbon-coated grids.

Quick-Freeze, Deep-Etch Electron Microscopy of Axons

Sciatic nerves were dissected out of rats and incubated for 30 min at room temperature in 0.1% saponin, 70 mM KCl, 5 mM $MgCl_2$, 3 mM EGTA, 30 mM Hepes, pH 7.4, 10 μM taxol, and 0.1 mM phenylmethylsulfonyl fluoride as described previously (14). They were quick frozen as described previously (9-11, 14).

Low Angle Rotary Shadowing of Tau Molecules

Tau fractions were suspended in glycerol (50% glycerol PEM) at 50 μg/ml protein concentration and sprayed on freshly cleaved mica flakes as described by Tylor and Branton (36) and dried by vacuum evaporation. The samples were rotary shadowed with platinum in a freeze-fracture machine (model 301; Balzers, Hudson, NH) at an angle of 6°. The replicas were detached from mica with hydrofluoric acid, washed with distilled water, and collected on Formvar-carbon-coated grids.

Electron Microscopy and Measurement

Replicas were examined with a JEOL 2000EX electron microscope at 100 kV and photographed. Micrographs were printed with their contrast reversed. The length of tau projections in the tau-saturated microtubule pellets and the length of tau molecules on mica were measured under a magnifying glass. The center to center distances between adjacent arms in replicas of tau-saturated microtubules were measured in the same way.

Stoichiometry of Tubulin and Tau

PC tubulin was mixed with an excess amount of tau fraction and incubated at 37°C in the presence of 1 mM GTP with or without 20 μM taxol for 30 min. As a control, PC tubulin without tau was incubated at 37°C in the presence of 20 μM taxol and 1 mM GTP. The suspensions were centrifuged at 19,000 rpm at 30°C for 30 min. The resulting pellets were resuspended in PEM, chilled thoroughly on ice, and homogenized thoroughly. Protein concentration of the pellets and supernatants was determined by the assays of Bradford et al. (5) and Itzhaki and Gill (19). SDS-PAGE of the pellets and supernatants was performed according to the method of Laemmli using 7.5% acrylamide (25). Gels were stained with Coomassie Brilliant Blue, scanned, and the areas of peaks were measured by densitometry (model CS 9000; Shimadzu Corp., Kyoto, Japan).

Tau-MAP1 and MAP1-MAP2 Displacement Experiments

MAP1A was purified by affinity chromatography on a cyanogen bromide-activated Sepharose 4B column containing our monoclonal antibody against MAP1A as described previously (31). After the application of crude extracts of rat brain, the column was washed with PEM containing 0.75 M NaCl and 0.5% NP-40. Bound polypeptides were eluted with 3 M $MgCl_2$ in 0.1 M PEM buffer, and peak fractions were dialyzed against 20 mM PEM buffer, and subjected to PAGE to check their purity.

PC tubulin was mixed with MAP1A at a weight ratio of 1:2.4 in the presence of 20 μM taxol and 1 mM GTP. Suspensions were incubated at 37°C for 30 min and were then layered onto 20% sucrose cushions containing tau at a weight ratio of 1 tubulin (in suspension) per 2 tau (in the cushion) or at a ratio of 1:0.2, 20 μM taxol and 1 mM GTP. Microtubules were centrifuged through the sucrose cushions at 30,000 *g* for 1 h at 30°C. Sucrose cushions lacking tau proteins were used as control.

In reciprocal experiments, PC tubulin was mixed with tau at a weight ratio of 1:0.4 in the presence of 20 μM taxol and 1 mM GTP. After incubation at 37°C for 30 min, the suspensions were centrifuged through 20% sucrose cushions containing MAP1A at a weight ratio of 1 tubulin (in suspension) per 2 MAP1A (in the cushion) or at a ratio of 1:1, 20 μM taxol, and 1 mM GTP. In these cases, the sucrose cushions of control samples contained no MAP1A.

In addition PC tubulin was mixed with tau and MAP1A at weight ratios of 1:1:1.2 in the presence of 20 μM taxol and 1 mM GTP. PC tubulin plus tau (1:1), PC tubulin plus MAP1A (1:1.2), and PC tubulin alone were also examined. After incubation at 37°C for 30 min, the suspensions were centrifuged through 20% sucrose cushions containing 20 μM taxol and 1 mM GTP. After their surfaces were washed with PEM, the pellets were resuspended in PEM. SDS-PAGE of the pellets and supernatants was performed according to the method of Laemmli using 7.5% acrylamide gels (25). Gels were stained with Coomassie Brilliant Blue, scanned with a densitometer (model CS 9000; Shimadzu Corp.), and the areas of peaks were measured.

MAP1 and MAP2 displacement experiments were performed similarly using MAP2 instead of tau proteins. MAP2 was purified from rat brains using a Superose 6 prepgrade gel filtration column (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (15). PC tubulin was mixed with MAP1A at a weight ratio of 1:2.4 in the presence of 20 μM taxol and 1 mM GTP. Suspensions were incubated at 37°C for 30 min and then layered onto 20% sucrose cushions containing MAP2 at a weight ratio of 1 tubulin (in suspension) per 2.4 MAP2 (in the cushion), 20 μM taxol and 1 mM GTP. Microtubules were centrifuged through the sucrose cushions at 30,000 *g* for 1 h at 30°C. Sucrose cushions without MAP2 were used as controls. Reciprocal experiments were also carried out. In additional experiments PC tubulin was mixed with MAP1A and MAP2 at a weight ratio of 1:2:2 in the presence of 20 μM taxol and 1 mM GTP. Tubulin plus MAP1A (1:2), tubulin plus MAP2 (1:2), and tubulin alone were also examined. After incubation at 37°C for 30 min the suspensions were centrifuged through 20% sucrose cushions containing 20 μM taxol and 1 mM GTP. Microtubules were cen-

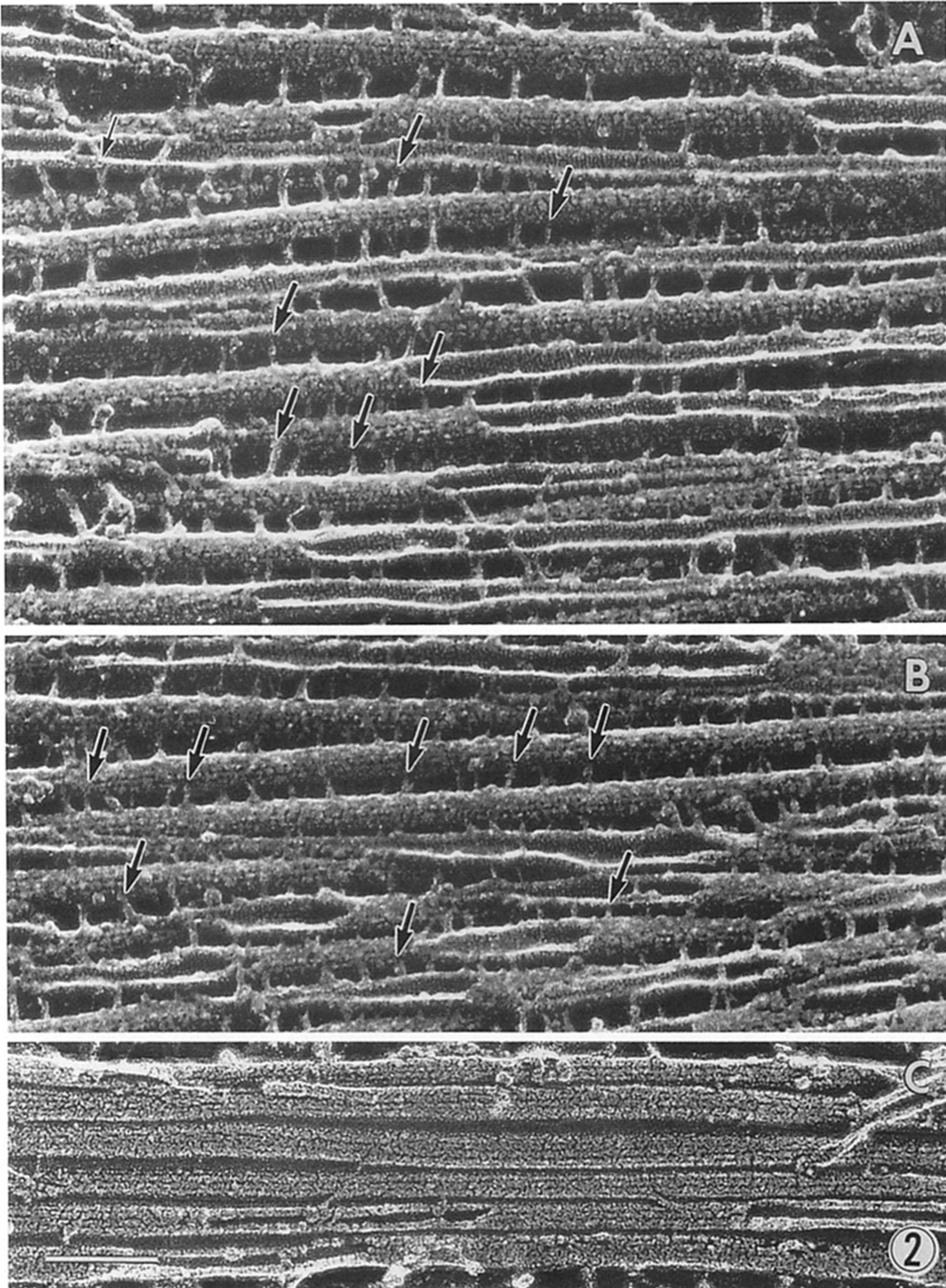

Figure 2. Higher magnification views of quick-frozen, deep-etched microtubule pellets polymerized with (*A* and *B*) and without tau (*C*). Although only tightly packed microtubules are observed in *C*, numerous projections (*arrows*) exist on the microtubules polymerized with tau (*A* and *B*). Bar, 100 nm.

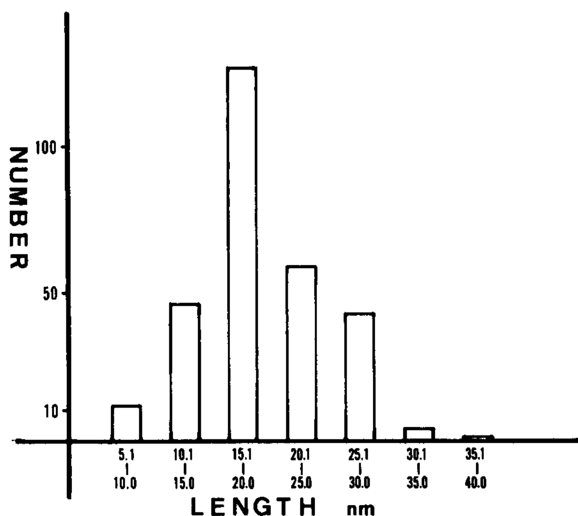

Figure 3. A histogram showing the length–frequency distribution of tau projections on microtubules. $n = 292$.

trifuged at 30,000 g for 1 h at 30°C. SDS-PAGE of the pellets and supernatants was performed.

Results

Fig. 1 shows SDS-PAGE analysis of PC tubulin (lane *1*), purified tau factor (lane *2*), the supernatant (lane *3*), and pellet (lane *4*) of a suspension of PC tubulin plus tau incubated at 37°C for 30 min in the presence of 1 mM GTP. The tau factor from porcine brain prepared by heat treatment at a low pH was composed of five bands (molecular weight of 50,000–65,000) (lane *2*). Boiling at a low pH removed the high molecular weight MAPs while tau remained soluble (8). The presence of tau promoted polymerization of PC tubulin, and thus, in the pellet we found tubulin plus tau.

Tau Forms Armlike Projections on Microtubule Surfaces

Fig. 2 displays high magnification views of the tau–microtubule pellet processed by the quick-freeze, deep-etch method. As can be seen, the pellet contained numerous parallel microtubules, each with numerous armlike projections (<20 nm long) attached to the microtubule surfaces. The projections were short and straight and appeared to cross-link adjacent microtubules. When PC tubulin was polymerized with tau in the presence of 10 μM taxol and 1 mM GTP, we observed similar projections on the microtubules. Because samples which contained only PC tubulin exhibited bare surfaces, (Fig. 2 *C*), we could conclude that tau forms straight, short, armlike projections on the surfaces of microtubules.

Fig. 3 shows a histogram of the length–frequency distribution of tau projections on microtubules. The average length was 18.7 ± 4.8 (SD) nm.

The projections appeared to cross-link adjacent microtubules. To check the possibility that centrifugation induced the patterns we observed, we resuspended the tau-saturated microtubules in a small volume of PEM, incubated them on ice for 30 min, and after homogenization rewarmed the suspension in the presence of 20 μM taxol and 1 mM GTP. The suspension was then quick frozen without centrifugation. In such preparations most of the microtubules tended to run

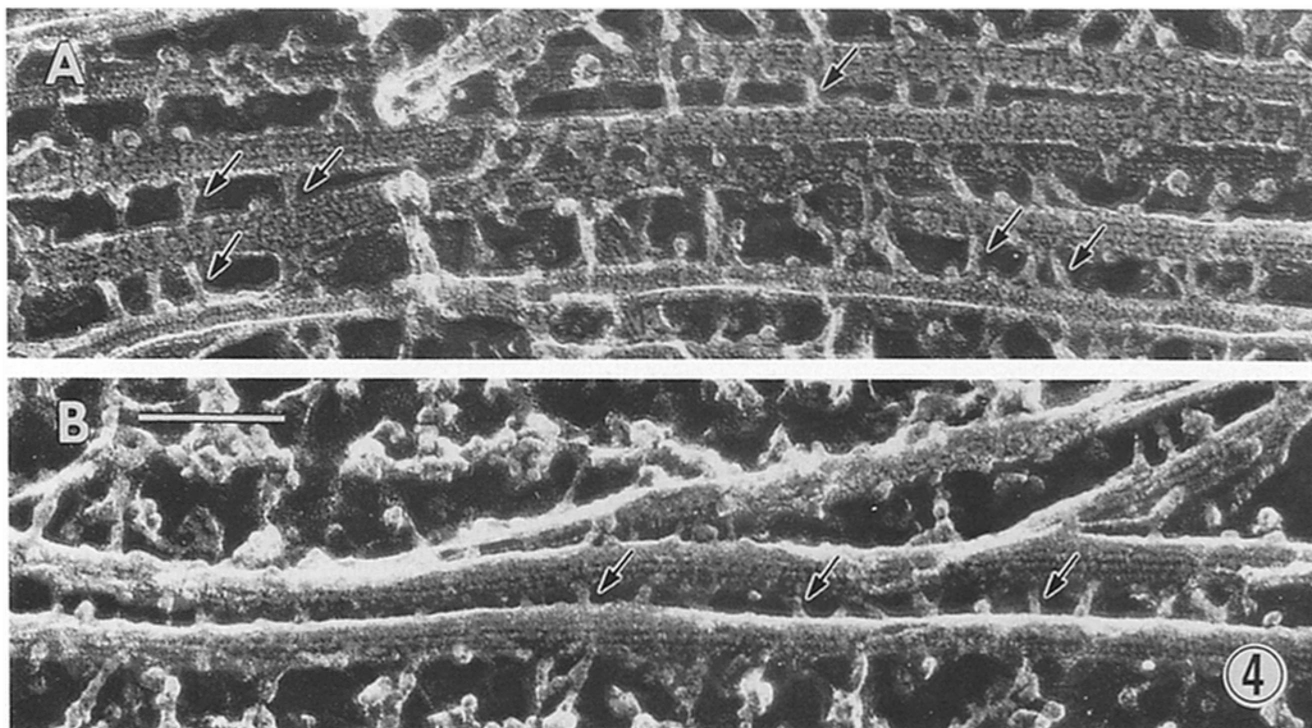

Figure 4. Quick-frozen, deep-etched suspension of microtubules saturated with tau proteins. Note the frequent cross bridges (*arrows*) between the microtubules. Bar, 100 nm.

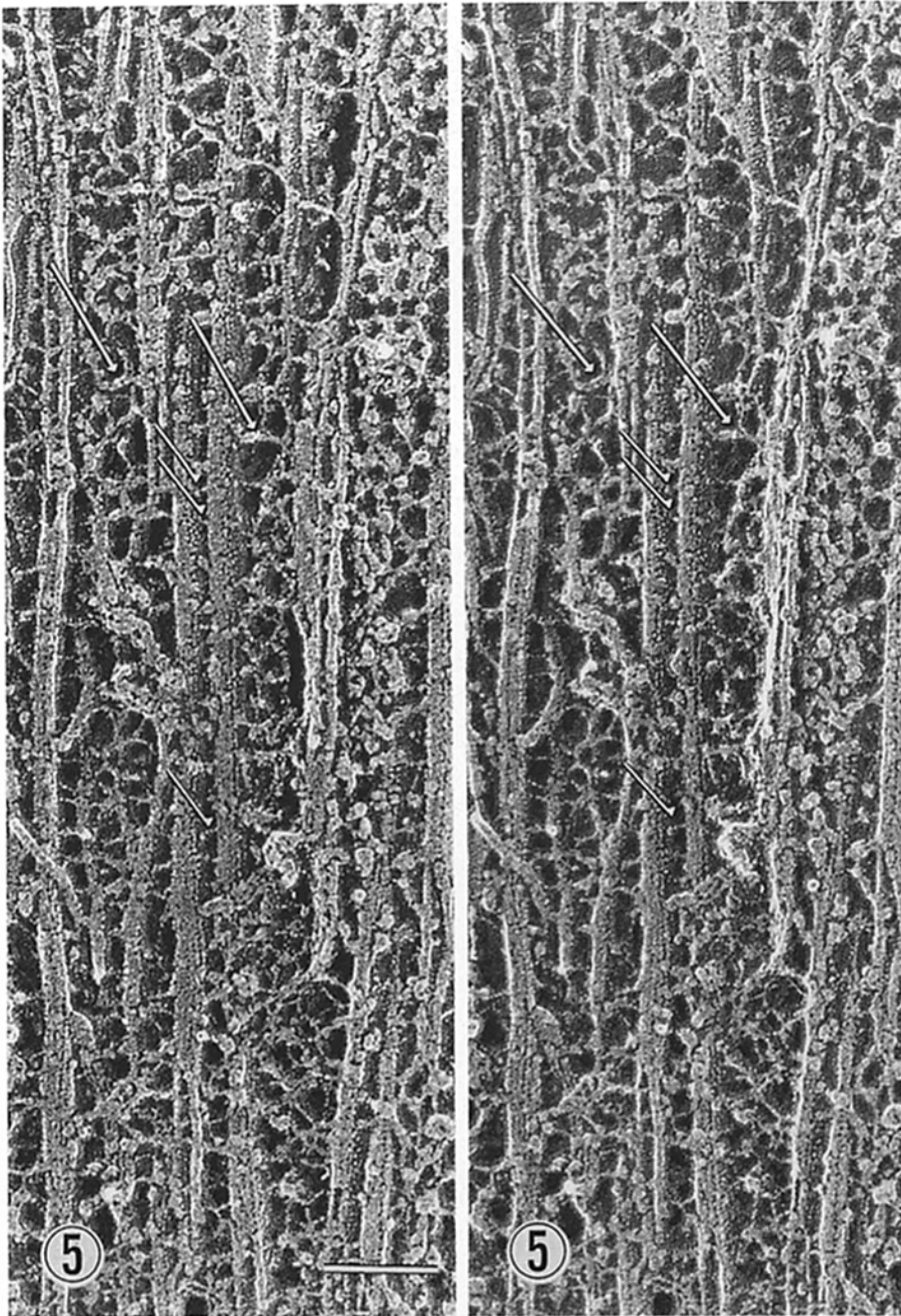

Figure 5. A stereopair of an axoplasm in a rat sciatic nerve quick-frozen and deep-etched after saponin extraction. Frequent short cross bridges (*short arrows*) are found between microtubules. Longer strands are also associated with microtubules (*long arrows*). Bar, 100 nm.

randomly and granular structures were attached on the microtubule surfaces; however, when microtubules ran parallel and close to each other we found frequent cross-links between adjacent microtubules as shown in Fig. 4. These results suggest that tau proteins cross-link microtubules when microtubules are in very close proximity to each other.

Furthermore, we examined microtubule domains in the axons of rat peripheral nerves after saponin extraction. As shown in a stereopair in Fig. 5, microtubules are linked with each other via fine short cross bridges exactly like those found in the microtubules saturated with tau in vitro (See Fig. 4 in reference 11). Longer strands tending to form networks were also associated with microtubules (Fig. 5).

Longitudinal Periodicity of Tau Projections

We measured distances between adjacent projections on individual microtubules. Fig. 6 is a histogram of longitudinal spacing of adjacent arms on the same microtubules. The dots and arrows indicate spacings predicted by a 12-dimer superlattice model and a 6-dimer superlattice model, respectively (1, 20). Fig. 7 shows a schematic diagram of the 6-dimer superlattice model. The numbers 11, 15, 22, 27, 33, 37, and 48 on Fig. 7 indicate predicted spacings by a 6-dimer superlattice model. As shown in Fig. 6, the longitudinal spacings observed in tau-saturated microtubule pellets match the 6-dimer pattern better than the 12-dimer pattern. The actual data fit with spacings of 11, 15, 22, 26, 33, and 48 nm, while there

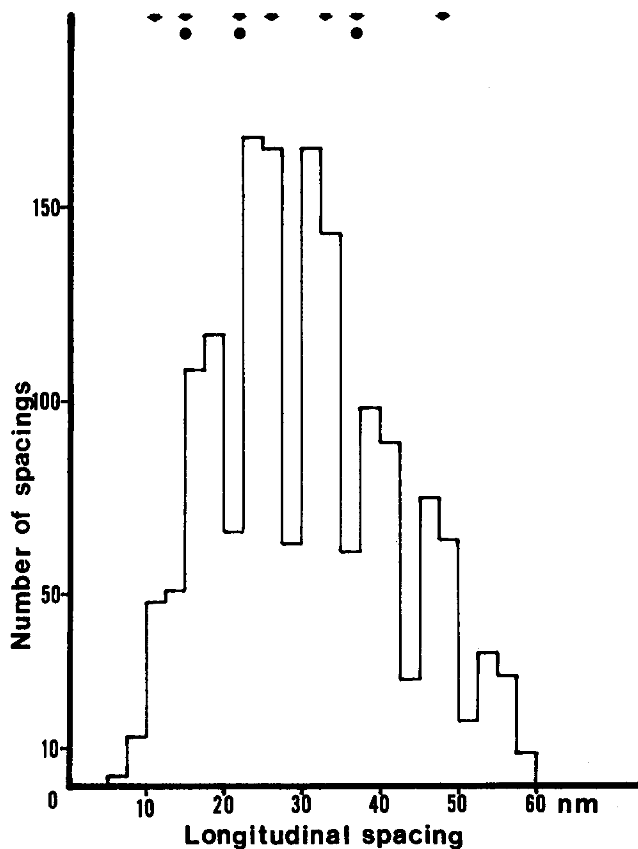

Figure 6. A histogram showing the frequency of longitudinal spacings between adjacent arms on the same microtubules saturated with tau. Arrows and dots indicate spacings predicted by the 6-dimer superlattice and 12-dimer supperlattice models, respectively. n = 1,605.

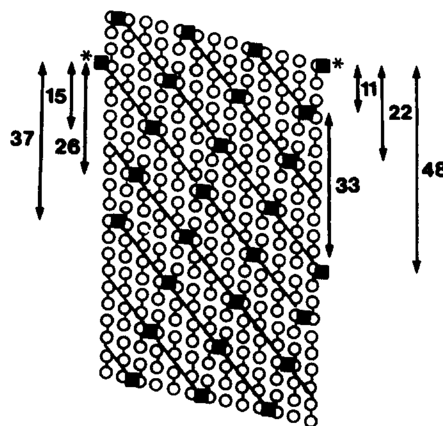

Figure 7. A schematic drawing of a 6-dimer model for the arrangement of tau projections. The opened-out lattice as viewed from the outside. The circles represent tubulin monomers. The squares indicate the binding sites of tau. The numbers indicate predicted spacings (in nanometers) between binding sites.

were additional peaks at 19, 30, 38, 42, and 46 nm. Therefore, the pattern of longitudinal spacing resembles, but is not identical to, the sites defined by the 6-dimer superlattice.

Stoichiometry of Tubulin and Tau

PC tubulin was mixed with a saturating amount of tau proteins. After incubation at 37°C for 1 h in the presence of 1 mM GTP with or without 20 μM taxol, the resulting pellet and supernatant were subjected to SDS-PAGE. We estimated the amount of proteins in the pellets and supernatants by the assay of Bradford (5). Peak areas corresponding to tubulin and tau proteins were measured by densitometry. As shown in Fig. 1 and 8 *A*, tau proteins from rat brains were composed of five distinct bands. Because in the microtubule pellets saturated by tau the third, fourth, and fifth bands overlap the tubulin bands, we estimated the area of the third plus fourth plus fifth peaks relative to the area of the first plus second peaks. As a result the ratio of the area of the first plus second peaks to the third plus fourth plus fifth peaks was 1:2.2. Since this ratio did not change in the supernatants of tau-saturated microtubules polymerized in the presence of taxol, we estimated the binding affinities of each five bands to tubulin to be equal.

Thus, we estimated the total amount of tau proteins in the tau-saturated microtubule pellets based on these assumptions; the total area of tau peaks are 3.2 times the area of the first plus second peaks. Accordingly, the area of tubulin peaks was estimated as the third peak plus a large tubulin peak −2.2 times (first plus second peaks of tau proteins). The molar ratio of tau/tubulin ranged from 1:3 to 1:6, using an average molecular weight of 60,000 for tau. The average was 1:∼5.

An another approach, we calculated the molar ratio of tau to tubulin from the amount of proteins in the pellets. The PC tubulin was polymerized with or without tau in the presence of taxol and GTP. The amount of tubulin in the tau-saturated microtubules was estimated from that in the pellet containing only tubulin. From this approach the molar ratio of tau to tubulin was also found to be 1:∼5.

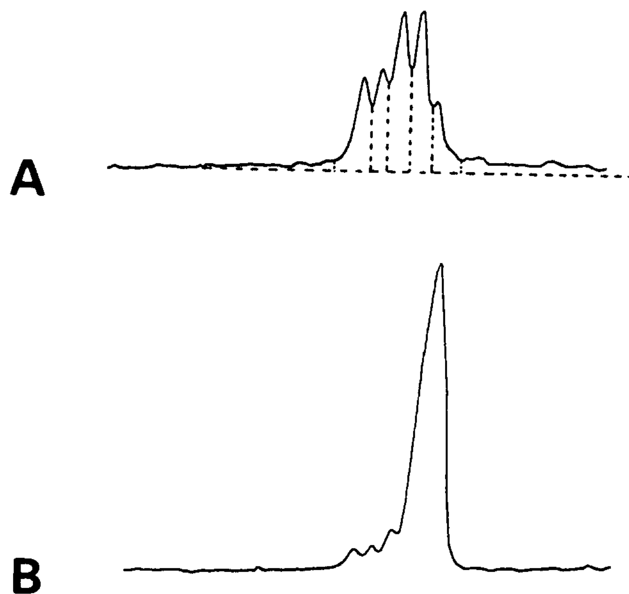

Figure 8. Densitometric scans of SDS gels of tau proteins (*A*) and microtubules saturated with tau proteins (*B*) equivalent to the gels shown in Fig. 1.

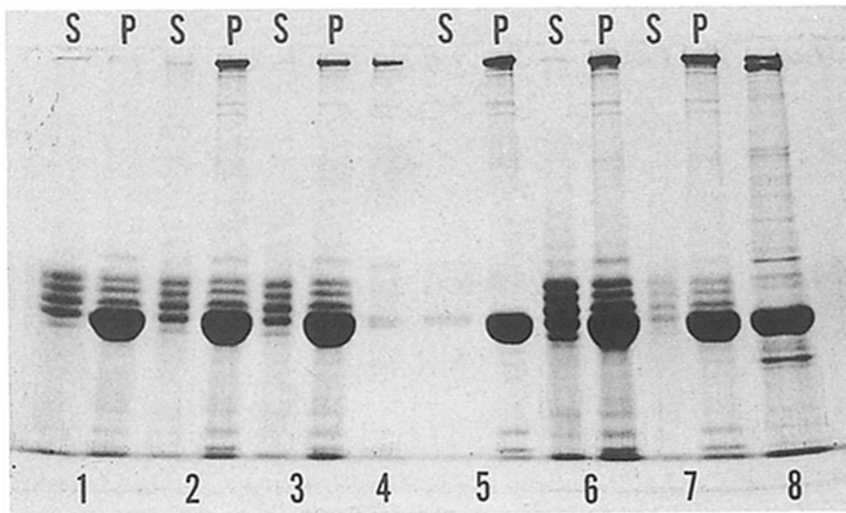

Figure 9. The SDS gel of a tau and MAP1A displacement experiment. Lane *1*, Supernatant (*S*) and pellet (*P*) of a suspension containing tau and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol and 1 mM GTP only. Lane *2*, supernatant (*S*) and pellet (*P*) of a suspension containing tau and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol, 1 mM GTP, and MAP1A. Lane *3*, supernatant (*S*) and pellet (*P*) of a suspension containing tau and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol, 1 mM GTP, and MAP1A (concentration of MAP1A is half of that in the 20% sucrose cushion of lane 2). Lane *4*, MAP1A purified by affinity chromatography on a cyanogen bromide-activated Sepharose 4B column using our monoclonal antibody against MAP1A. Lane *5*, supernatant (*S*) and pellet (*P*) of a suspension containing MAP1A and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol and 1 mM GTP only. Lane *6*, supernatant (*S*) and pellet (*P*) of a suspension containing MAP1A and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol, 1 mM GTP, and tau. Lane *7*, experiment similar to lane *6*, except that the concentration of tau in the 20% sucrose cushion was one-tenth of that in the cushion of lane *6*. Lane *8*, microtubule proteins from rat brain.

Binding Sites of Tau and MAP1A on Microtubules

Both tau and MAP1 have been found to be localized in the axons of neurons in brain (3, 4, 14, 17). Concerning the binding sites of tau proteins on microtubules, it has been shown that tau and MAP2 bind competitively to the same sites (21, 29). Therefore, we attempted to analyze whether tau and MAP1 bind to microtubules competitively or not. MAP1A was purified by an affinity column using a monoclonal antibody against rat brain MAP1A. We performed tau–MAP1 displacement experiments according to a procedure used by Kim et al. (21). First, tubulin was polymerized with an excess amount of the first MAP (tau or MAP1A). Then the suspension was centrifuged through 20% sucrose cushions containing a large amount of the second MAP (MAP1A or tau). In control experiments tubulin was polymerized with the first MAP and was centrifuged through 20% sucrose cushions containing no second MAP. As shown in Figs. 9 and 10, the amount of the first MAP (tau or MAP1A) relative to tubulin in the pellets was not significantly changed regardless of the presence or absence of the second MAP in the cushions. If the amount of the second MAP in the cushions was decreased, a smaller amount of the second MAP bound to tubulin, but the ratio of tubulin to the first MAP remained unchanged.

We carried out other experiments as well. Tubulin was polymerized with excess amounts of tau, MAP1A, or tau plus MAP1A. Then the suspensions were centrifuged through 20% sucrose cushions. Fig. 11 shows SDS-PAGE of supernatants and pellets of these experiments. After scanning with a densitometer, we found that the amounts of tau relative to tubulin in the pellets of tau plus tubulin and tau plus MAP1A plus tubulin had not significantly changed. However, the amount of MAP1A in the pellet of tubulin plus tau plus MAP1A was ~81% of that in the pellet of MAP1A plus tubulin. From these experiments, then, we could conclude that the binding sites of tau and MAP1A on the microtubule surfaces are mostly distinct, but may be partially overlapping.

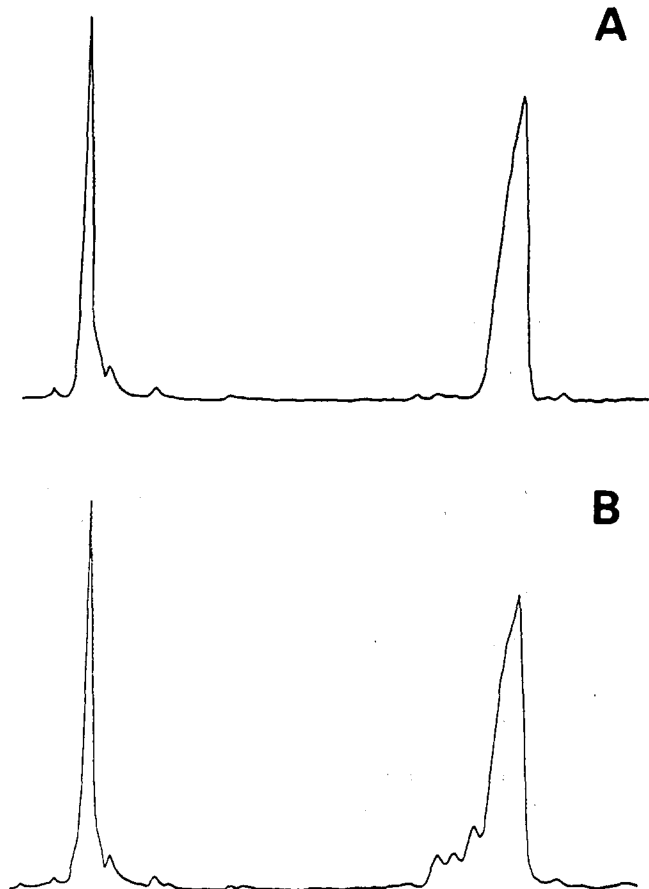

Figure 10. Densitometric scans of SDS gels of a tau and MAP1A displacement experiment. (*A*) Pellet of microtubules saturated with MAP1A equivalent to lane *5P* in Fig. 9. (*B*) Pellet of microtubules saturated with MAP1A after centrifugation through 20% sucrose containing 20 μM taxol, 1 mM GTP, and an excess amount of tau equivalent to lane *6P* in Fig. 9. The ratio of MAP1A to tubulin does not change after tau binds to microtubules.

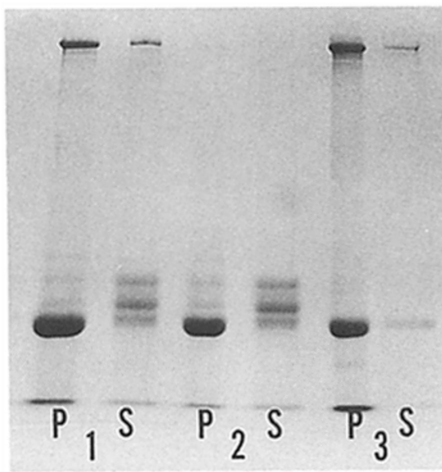

Figure 11. The SDS gel of a competition-experiment of tau and MAP1A. Lane *1*, pellet (*P*) and supernatant (*S*) of microtubules saturated with tau and MAP1A. Lane *2*, pellet (*P*) and supernatant (*S*) of microtubules saturated with tau. Lane *3*, pellet (*P*) and supernatant (*S*) of microtubules saturated with MAP1A.

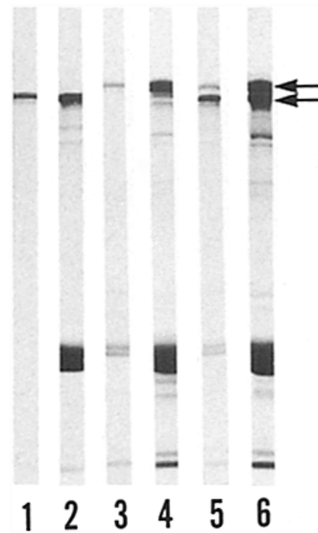

Figure 12. The SDS gel of a MAP1A and MAP2 displacement experiment. Lanes *1* and *2*, supernatant (*1*) and pellet (*2*) of microtubules saturated with MAP2. Lanes *3* and *4*, supernatant (*3*) and pellet (*4*) of a suspension containing MAP1A and PC tubulin after centrifugation through 20% containing 20 μM taxol and 1 mM GTP only. Lanes *5* and *6*, supernatant (*5*) and pellet (*6*) of a suspension containing MAP1A and PC tubulin after centrifugation through 20% sucrose containing 20 μM taxol, 1 mM GTP, and MAP2. The ratio between MAP1A and tubulin is not significantly changed after centrifugation through an excess amount of MAP2. Upper and lower arrows in lane *6* indicate MAP1A and MAP2, respectively.

In these experiments we also estimated the molar ratio of MAP1A to tubulin in the MAP1A saturated microtubules. The molar ratio was 1:12 (MAP1A/tubulin). In addition, we also performed MAP1A and MAP2 displacement experiments. As shown in Fig. 12, the ratio of tubulin to MAP1A was not changed regardless of the presence or absence of MAP2 in the cushion. When tubulin was polymerized with MAP1A alone, MAP2 alone or MAP1A plus MAP2, amounts of MAP1A and MAP2 relative to tubulin were not significantly changed. This indicates that MAP1A and MAP2 probably have distinct binding sites on microtubules.

Tau Is a Short Rodlike Molecule

Fig. 13 demonstrates a gallery of tau molecules adsorbed on mica. Low angle rotary shadowing revealed that tau (~50 nm long) is a rodlike structure much shorter than the high molecular weight MAPs (100–200 nm long). We measured the length of tau molecules adsorbed on mica (Fig. 14) and found the average length to be 56.1 + 14.1 (SD) nm.

Discussion

Molecular Structure of Tau

The present study for the first time demonstrates that tau proteins are rodlike molecules (~50 nm long) and bind to microtubules as periodic, short, armlike projections (<20 nm long). Previous examination by thin section, and negative staining methods and metal shadowing did not reveal their features (35, 42). This is probably because tau forms very short arms on the microtubules when microtubules are close to each other, making their visualization very difficult by conventional methods.

When high molecular weight MAPs such as MAP2 or 270,000-mol-wt MAP were incubated with PC tubulin and processed similarly, long projections (longer than 30 nm) were observed on the microtubule surface (12). They sometimes took on the appearance of anastomosing cross bridges between microtubules, while tau always appeared as a straight, short, rodlike component and never formed anastomosing networks.

Since tau arms on the microtubules were shorter than 20 nm, certain parts of the molecule could comprise the binding domain associated with the microtubule surface. It is well known that limited chymotryptic digestion of MAP2 produced a fragment with a molecular weight of 35,000 which promotes microtubule assembly and corresponds to the microtubule-binding domain of MAP2 (37). Recently we found that the 190,000-mol-wt MAP from adrenal medulla is also a long, rodlike protein, and forms armlike projections on microtubules (26). A 27,000-mol-wt fragment of the 190,000-mol-wt MAP produced by limited chymotryptic digestion was also observed to stimulate tubulin polymerization and to correspond to the binding domain that associates with microtubules (1). Because both tau and MAP2 promote tubulin polymerization, are rodlike molecules, and bind competitively to microtubules, their binding domains may have a common nature. This binding domain of tau may play an active role when tau promotes the polymerization of tubulin.

Concerning the molecular structure of MAPs, most of the high molecular weight MAPs, including MAP1A and B, MAP2, 270,000-mol-wt MAP, and 190,000-mol-wt MAP exhibit long rodlike forms (12, 26, 31, 40). However, recently we found that one of the main MAPs in the mitotic spindle of sea urchin eggs, which has a molecular weight (75,000) close to tau, is a globular molecule, and covers the microtubule surface as round buttonlike structures (13, 16). We initially thought that tau might be a similar molecule to this 75,000-mol-wt MAP (buttonin), but buttonin was not heat stable and did not bind to calmodulin in the presence of calcium (13). So buttonin was determined as expressing characteristics distinct from tau. In addition, the present study clearly shows that tau possesses quite a different molecular shape from buttonin (rodlike vs. spherical). So far, microtu-

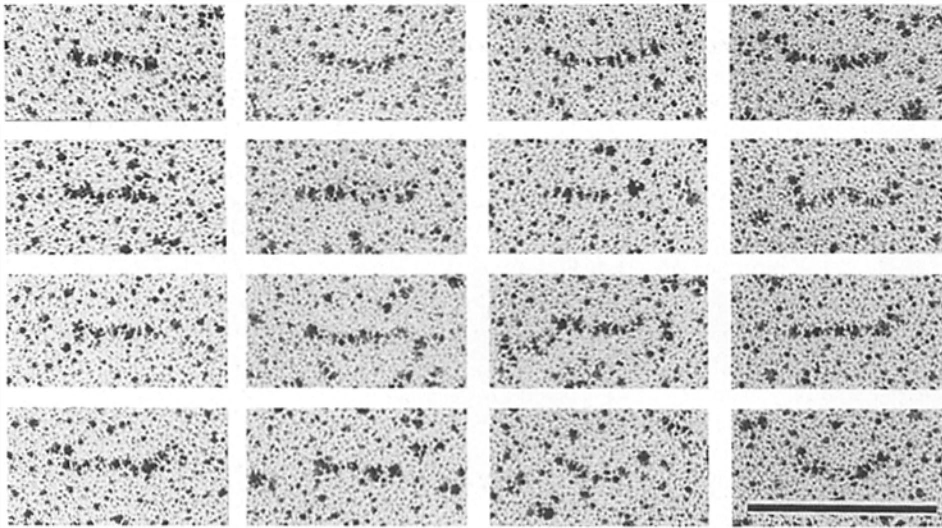

Figure 13. A gallery of tau molecules adsorbed on mica revealed by the low angle rotary shadowing technique. Bar, 100 nm.

bule-associated proteins of neuronal origin mostly tend to take on rodlike forms. This fact is in agreement with the cytoskeletal structure in the nerve cells in vivo. With the quick-freeze deep-etch technique we found various kinds of strands associated with microtubules in nerve cells (11, 14, 31, 32), and proved that MAP1A and B, and MAP2 are components of these strands (14, 15, 31, 32). When the microtubules run close together in the axon, the distance between them is 15–20 nm. This spacing is similar to the length of tau molecules bridging adjacent microtubules. In fact, we found short straight cross bridges between microtubules in the axons exactly like those found in the tau microtubule pellets. So it is very likely that tau forms short cross bridges between microtubules in vivo.

Periodicity of Tau Arms on Microtubules

From our data, spacings between adjacent arms on microtubules overlap with the sites defined by the 6-dimer superlattice, but are not identical. Jensen and Smaill studied the arrangement of MAP2 on microtubules by microdensitometer–computer correlation techniques and proposed that MAP2 projections are arranged in a "saturated 12-dimer, unsaturated 6-dimer" superlattice (20). Because MAP2 and tau compete for binding sites on microtubules in vitro (21), our results about tau binding sites coincide well with Jensen and Smaill's model about MAP2-binding sites. The binding sites of MAP2 and tau showed some tendency to overlap.

Our stoichiometry data indicated the molar ratio of tau versus tubulin to be 1:~5. This value is in agreement with a previous report by Kim et al. (21), who used a different approach. From their data the molar ratio of tau/tubulin in tau-saturated microtubules ranged from 1:3 to 1:5. The spacings between adjacent arms in the tau-saturated microtubules overlap with the 6-dimer supperlattice, while there are also additional spacings. From these data, if we accept the molar ratio of tau to tubulin as being 1:5, a simple possibility would be that one arm is composed of one tau. In the present study the tau arms appeared to cross-link with adjacent microtubules. Because the microtubules were cross-linked even in suspensions and because we found similar short cross bridges between microtubules in vivo, we suppose that tau proteins are able to cross-link microtubules only when the latter are in close proximity to one another.

Concerning the further question of how many molecules it would take to form a cross bridge between microtubules, there are two possibilities. One is that if one arm has two tubulin-binding sites, one arm could form a cross bridge. The other possibility is that one cross bridge is composed of two arms from adjacent microtubules. From our data it is also supposed that a tau molecule may possess a strong binding site to tubulin and other weak binding site to tubulin or tau. It may be possible to resolve this question in future studies using monoclonal antibodies.

Binding Sites of Tau Proteins on Microtubules

Previous immunocytochemical studies revealed that although MAP1 localizes in neuronal dendrites, cell bodies and axons, MAP2 is located mainly in dendrites and cell bodies and tau exists mainly in the axon (3, 4, 14, 15, 17, 24). The intracellular mechanisms for the sorting and transport of these proteins to their destinations is unknown. In this regard the binding sites of these MAPs on the microtubules are

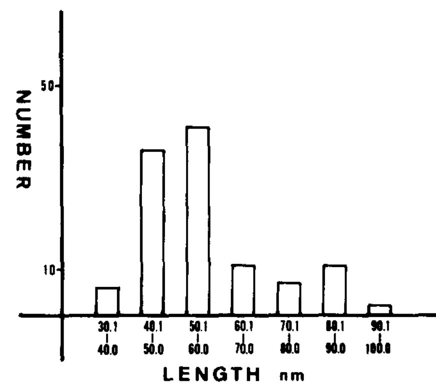

Figure 14. A histogram of the length of tau molecules adsorbed on mica. $n = 114$.

important. A previous study presented evidence suggesting that tau and MAP2 competitively bind to the microtubules (21). We showed that the binding sites of tau may be mostly different from those of MAP1A although some of them are overlapping. This is a particularly interesting point because both MAP1 and tau are localized in axons. We have also demonstrated in the present study that MAP1A and MAP2 may bind to microtubules noncompetitively. In a previous work we showed that both MAP1A and MAP2 colocalize on the same microtubules in dendrites in vivo (32).

These observations suggest that tau and MAP1A could colocalize on the same microtubules in the axon. However, as we mentioned above, microtubules in the axon tend to form small fascicles. These microtubules could play the role of rails for organelle transport. Because tau could form short cross bridges and because MAP1A is a longer rodlike molecule we assume that tau could work as short cross bridges to form small bundles of microtubules very close to each other, while MAP1A could play the role of a matrix, forming channels of microtubules for the translocation of membrane organelles or cross-link microtubules more distant from each other. Of course these structures would not be expected to be static and their binding to microtubules could be dynamically controlled by possible modification mechanisms such as Ca^{+}-calmodulin or phosphorylation.

The function of tau molecules in vivo and the mechanisms responsible for the intracellular sorting of this protein indeed pose as interesting subjects for future studies.

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