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Tubulin, Actin, and Tau Protein Interactions and the Study of Their Macromolecular Assemblies

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Abstract The intracellular polymerization of cytoskeletal proteins into their supramolecular assemblies raises many questions regarding the regulatory patterns that control this process. Binding experiments using the ELISA solid phase system, together with protein assembly assays and electron microscopical studies provided clues on the protein–protein associations in the polymerization of tubulin and actin networks. In vitro reconstitution experiments of these cytoskeletal filaments using purified tau, tubulin, and actin proteins were carried out. Tau protein association with tubulin immobilized in a solid phase support system was inhibited by actin monomer, and a higher inhibition was attained in the presence of preassembled actin filaments. Conversely, tubulin and assembled microtubules strongly inhibited tau interaction with actin in the solid phase system. Actin filaments decreased the extent of in vitro tau-induced tubulin assembly. Studies on the morphological aspects of microtubules and actin filaments coexisting in vitro, revealed the association between both cytoskeletal filaments, and in some cases, the presence of fine filamentous structures bridging these polymers. Immunogold studies showed the association of tau along polymerized microtubules and actin filaments, even though a preferential localization of labeled tau with microtubules was revealed. The studies provide further evidence for the involvement of tau protein in modulating the interactions of microtubules and actin polymers in the organization of the cytsokeletal network. J. Cell. Biochem. 85: 315–324, 2002.

Key words: microtubules; actin polymers; tau; macromolecular interactions; cytoskeleton

One of the major paradigms of modern biology has been the capacity of certain macromolecular systems to assemble into polymers that constitute fundamental structural elements involved in the morphogenesis, shape determination, and motility of cells and intracellular traffic of organelles and vesicles. The structure and integrity of the cytoplasm in eukaryotic cells relies on the assembly and organization of the cytoskeletal network [Maccioni and Arechaga, 1987].

Important advances have been achieved in understanding how cytoskeletal proteins form the assembled filaments, e.g, actin filaments, microtubules, and intermediate filaments. These polymers are interlinked through a complex and coordinated set of protein interactions, whose dynamics is determining for the organization of this network [Maccioni and Cambiazo, 1995]. Homologous protein–protein interactions include those of actin polymerization into microfilaments, or tubulin assembly into microtubules, while heterologous interactions include those of actin binding protein with the actin filament network, microtubule-associated proteins with microtubules, and with other components of cytoskeletal network [Mandelkow and Mandelkow, 1995; Welch et al., 1997; Mandato et al., 2001]. In this context, since the Porter model on the organization of the cytoplasmic matrix [Ellisman and Porter, 1980], evidence has accumulated on the roles of MAPs in bridging microtubules and actin filaments in several cell types [Cross et al., 1993; Moraga et al., 1993; Henriquez et al., 1995; Maccioni and Cambiazo,

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1995], and also in the interaction of microtubular polymers with intermediate filaments [Capote and Maccioni, 1998]. The association between microtubules and actin filaments is critical for cellular morphogenesis. Models on tensegrity networks that maintain a stabilizing prestress through the incorporation of structural elements [Wang et al., 2001], have been proposed to explain the interplay of forces in the cytostructure. Living cells seem to behave like discrete structures of interconnected networks of polymers that modulate cell shape when mechanical stress is applied to cell surface.

Besides MAPs, other microtubule-interacting proteins, including Mip-90 [Gonzalez et al., 1998], appear to be critical in modulating cytoskeletal interaction of microtubules and microfilaments at the leading edge of fibroblasts, as well as in many other cellular processes involving changes in cell shape. The growth of neuronal extensions, heralded by the presence of growth cones at the distal end of neurons, represents an example of morphological changes during the course of neuronal development [Cunningham et al., 1997]. Tau proteins play a major role in maintaining axonal polarity, as well as in the stabilization of the differentiated neuronal architecture. Tau modulates the nucleation and growth of microtubular polymers, in a coordinated fashion during axonal development [Brandt and Lee, 1993]. The activity of tau has been shown in the regulation of the shape changes associated with the actin filament-microtubule networks in the growth cones of developing neurons [Pigino et al., 1997]. Tau association with tubulin/microtubules as well as its functional organization is controlled by posttranslational modifications, namely phosphorylations by several protein kinase systems. Particularly, the cyclin-dependent protein kinase Cdk5 modulates phosphorylation of tau and MAP1b as related with their interactions with the microtubular cytoskeleton and the organization of distal tips in the neuronal growth cones [Paglini et al., 1998]. Regulation of the main activator of Cdk5, p35 by laminin in developing neurons seems to play a major role in its activity. Therefore, regulatory aspects on tau phosphorylations are indeed a landmark in the normal axonal growth process [reviewed in Maccioni et al., 2001], and deregulation of this system appears to be involved in triggering neurodegeneration as occurs in Alzheimer's disease [Alvarez et al., 1999, 2001].

In this study, we analyze the interaction patterns of tau protein in systems in which microtubules and actin filaments coexist in vitro, and evaluate comparatively these patterns with the interactions of tau with free actin and tubulin and with their respective polymers. In vitro coassembly of tubulin and actin polymers was achieved in the presence of tau, and immunogold experiments at the electron microscopical level indicated its association with both types of cytoskeletal filaments. The solid phase immunoassay using microtiter plates proved to be an appropriate approach for studying these protein–protein interactions.

MATERIALS AND METHODS

Materials

Biochemical reagents were from Sigma Chemical Co., and the chemical compounds used in the study were of the highest analytical quality. The buffers used in this study were: (i) Buffer A: 0.1 M (2-*N*-morpholino)ethanesulfonic acid (Mes) containing 2.5 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, pH 6.4; (ii) Buffer B: 20 mM Tris, pH 8.0 containing 0.5 mM dithiothreitol, 0.2 mM ATP and 0.2 mM $CaCl_2$; (iii) PBS buffer, pH 7.3; and (iv) PBS-Tween-20 buffer, pH 7.3. The monoclonal antibodies against tau used in the solid phase system and electron microcopy studies as well as the anti-mouse IgG labeled with horseradish peroxidase were from Amersham. The site directed polyclonal antibody TRS1.2 [González et al., 1998] was also used in immunogold studies.

Purification of Proteins

Microtubular protein was purified from bovine brains after two temperature-dependent cycles of assembly and disassembly [Shelanski et al., 1973] with modifications as described by Capote and Maccioni [1998]. Protein was stored as microtubular pellets at $-80^{\circ}C$, and a third assembly cycle was carried out prior to tubulin purification. The microtubular pellets were resuspended in buffer A, and tubulin was purified from the three-cycled microtubular pellets by phosphocellulose chromatography. Tau protein was isolated from brain extracts as described in Farias et al. [1992]. MAP-2 was purified as indicated in Vera [1988]. Actin was prepared from chicken muscular tissue [Moraga et al., 1993]. Purified actin preparations were dialyzed in 3 × Tris buffer pH 8.0 for

2 days at 4°C. After dialysis, the aggregated material was removed by centrifugation at 100,000*g* for 60 min at 4°C. G-actin in Tris buffer containing 100 mM KCl, and 2 mM $MgCl_2$ was allowed to polymerize into microfilaments by incubation at 37°C for 30 min. The gels were stained with Coomassie Blue R-250 and destained in methanol:acetic acid.

Protein Determinations

Protein concentration was measured by the method of Vera [1988], using bovine serum albumin (BSA) as protein standard. Tau, tubulin, and actin purity was analyzed by 10% PAGE gels in the presence of SDS.

Electron Microscopy

Microscopical studies were carried out with a JeolX100 electron microscope, and the procedures for fixation and staining of in vitro assembled polymers were as described previously [Gonzalez et al., 1998]. The different samples containing microtubules, microfilaments, or networks of both types of polymers resulting from in vitro assembly experiments in the presence and absence of tau were fixed, placed on carbon/parlodion-coated grids for 1.5 min, stained with 1% aqueous solution of uranyl acetate and observed under the electron microscope. For immunogold experiments, copper grids with a Formvar film were used. Grids with polymer samples were washed 2 min in buffer TBS (30 mM Tris, 150 mM NaCl, pH 8.2) and preincubated for 15 min in buffer TBG (TBS plus 0.1% BSA, 1% gelatin, pH 8.2). Samples were incubated with first antibody (anti-tau monoclonal or anti-tau polyclonal TRS1.2) diluted in TBS during 30 min at room temperature. Grids were washed three times during 10 min in buffer TBG and incubated with second antibody anti-rabbit IgG conjugated with colloidal gold during 30 min at room temperature. Two gold particle sizes, 5 and 10 nm were used. The treated samples were then washed three times during 5 min in TBG buffer and another three-times in TBS. The negative staining was developed after embedding the grids in PBS, fixation for 2 min in 2% glutaraldehyde and staining with 1% uranyl.

Solid Phase Assays

Experimental conditions as described by Pedrotti et al. [1994] with some modifications were used. Solutions of microtubules, tubulin, and tau were prepared in Mes, pH 6.4 buffer, while actin and microfilaments were resuspended in Tris pH 8.0 buffer. The protein used as a substrate (tubulin, actin, or microtubular proteins) was coated in all the wells in the 96-well microtiter plates, and incubated for 3 h to allow adhesion to the plastic surface. After removal of the remaining solution with the unbound substrate by aspiration, the sites were saturated by incubation for 3 h with 100 μl of the blocking agent (Tween-20). The blocking solution was removed, and the microtiter wells were washed with the binding buffer and incubated with 50 μl of tau solution at different concentrations, or incubated with tau followed by actin or by a mixture of preassembled actin filaments and tau. After incubation for 3 h at 24°C, the plates were washed and incubated with the first antibody for 2 h (anti-tau monoclonal antibody). The first antibody was removed and the plates washed before the addition of 50 μl of the second antibody (mouse IgG labeled with horseradish peroxidase) (1:1,000 dilution) and incubated for 1 h at 37°C. After washing, the protein–protein interaction was detected using 100 μl of a solution that contained OPD (1 mg/ml *o*-phenylenediamine and 0.03% H_2O_2 in 0.1 M citric acid, pH 5.0) as substrates for the enzyme peroxidase, and the reaction terminated with 50 μl of 4 M sulfuric acid. The product formation was recorded by measuring the net change in absorbance at 490 nm. In order to test for the specificity of the interactions in the ELISA system, negative controls using BSA instead of actin were used in the competition assays for tau binding to tubulin. Incubation with purified MAP-2 instead of tau was used as a specific positive control to analyze the binding to tubulin in the presence or the absence of actin. As another positive control for the assay, the immunoreactivity of variable concentrations of tau immobilized in the microtiter plates was analyzed. As negative control tubulin was adsorbed in plates, actin added in the absence of tau and solid phase assays developed using the anti-tau monoclonal antibody. The experimental conditions were established in order to maintain a good signal in the solid phase assay with minimal background noise. Experiments were carried out in quadruplicate ($n = 4$) or with $n = 5$ in some experiments. The standard errors were determined in each case, and data analyzed for tests of significance with an ANOVA statistical program.

RESULTS

The association of tau with networks of actin filaments and microtubules was investigated in an attempt to elucidate the roles of this microtubule-associated protein in modulating the interactions of these cytoskeletal components. In a first approach, competition solid phase immunoassays were used to evaluate comparatively the interaction patterns of tau with free tubulin and actin, as well as with their respective polymeric species. Tubulin was immobilized in the plates and variable concentrations of tau alone, or tau followed by a fixed actin concentration or mixtures of both proteins were added, and the levels of reactive tau monitored by using an anti-tau monoclonal antibody. Purified tau protein bound to the microtiter plates coated with tubulin molecules in a concentration dependent fashion (Fig. 1). Tau concentrations used in these studies were chosen so to comply with linearity criteria after analyzing ΔA_{490} as a function of free tau concentration added to wells in the microtiter plates in the absence of other proteins (titration curve for tau). A competition with free actin for the tau–tubulin interaction was revealed by a significant decrease in tau binding upon addition of actin to the system. Maximal binding in this assay was attained at tau concentrations around 0.1 μM. A 23% inhibition of tau binding was observed upon actin addition, a difference which proved to be statistically significant ($P \leq 0.01$). Calculations of inhibition percentage were based on differences in maximal binding at saturation (B_{max}) obtained from the double reciprocal plots.

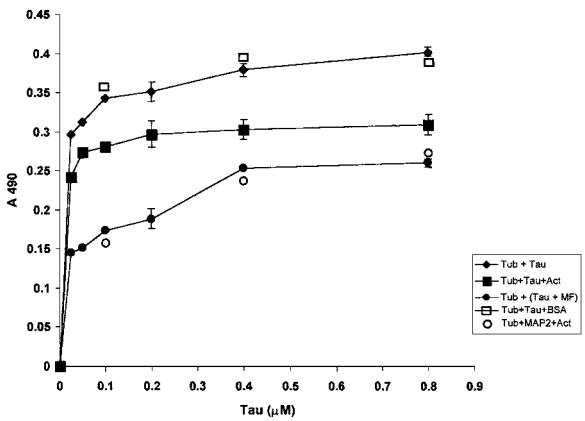

Fig. 1. ELISA experiment showing the competition of actin for tau association with tubulin. Hundred microliter tubulin samples (0.2 mg/ml) were coated on 96-well ELISA plates. After blocking, increasing concentrations of purified tau (◆), or tau protein followed by 2×10^{-4} μmoles monomeric actin (■) were added and immunoabsorbed in the microtiter plates. Binding of tau on the basis of its immunological reactivity was measured using an anti-tau monoclonal antibody (dil. 1:5,000), as indicated by reading the absorbance of the complex at 490 nm. In addition, tau was preincubated with polymeric actin, the mixture added to the solid phase system and analyzed as indicated (●). Immunoreactivity of pure tau immobilized in the plates and developed with anti-tau antibody was analyzed as a control. As a negative control, increasing concentrations of tubulin were added in the absence of tau and analyzed by solid phase assay using anti-tau monoclonal antibody. As a negative control for the protein inhibitor, the effects of 2×10^{-4} μmoles BSA on tubulin/tau interactions were analyzed (□). As a positive control for MAPs, the inhibitory effects of actin on MAP-2 interaction with tubulin was analyzed (○). Each point represents the mean of four or five determinations. Bars representing standard errors are shown for those measurements based on five determinations (n = 5).

Moreover, when increasing concentrations of tau were preincubated with polymeric actin, and then added to the system, a stronger inhibition of tubulin-tau interaction was observed (ΔA_{490} decreased by 35%, Fig. 1). BSA used instead of actin did not modify tubulin-tau interaction, thus indicating the specificity of the competitive effect of actin on this interaction (Fig. 1). Besides the analysis of Figure 1 in the tau concentrations range 0–1 μM, double reciprocal plots analyses based on additional data in the range 0–0.1 μM tau allowed estimations of apparent association constants (Kapp) of 4×10^7 M^{-1} for the tau binding to tubulin in the absence of actin in close agreement with binding constants determined for tau/tubulin interactions [Ackmann et al., 2000], while Kapp $= 1.6 \times 10^7$ M^{-1} was estimated from data in the presence of polymeric actin. As a positive control for specificity, the presence of actin lowered tubulin-tau association when MAP-2 was used a positive control (Fig. 1), in agreement with previously reported data [Ozer and Halpain, 2000]. In another set of experiments, free actin was immobilized on the microtiter plates and the binding of tau was analyzed. A statistically significant inhibition ($P \leq 0.005$) of tau/actin association by 26% was observed upon the addition of tubulin molecule to the system (Fig. 2). However, when tau was preincubated with preassembled tubulin polymers, the inhibitory effects on actin–tau interaction were higher than those exerted by free tubulin (around 44% inhibition). In another study, increasing amounts of microtubular polymers were coated on the solid phase support and the binding of tau analyzed (Fig. 3). A

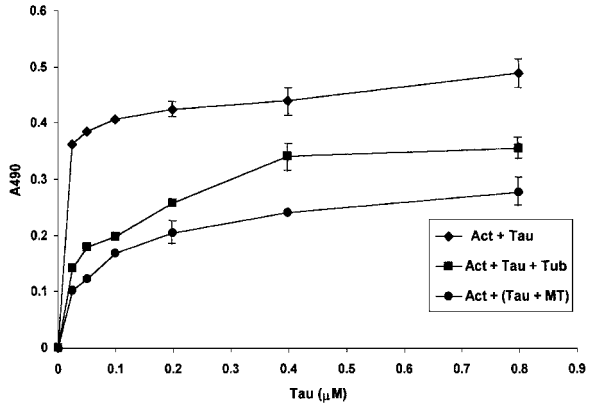

Fig. 2. ELISA study of the competitive effect of tubulin on actin–tau interaction. The ELISA plates were incubated with 100 μl of an actin solution (0.2 mg/ml). Aliquots of increasing concentrations of tau protein (◆), or aliquots of tau followed by tubulin at a concentration of 0.2 mg/ml were added and the immunoreativity monitored by the solid phase assay (■) using anti-tau antibody (dil. 1:5,000). In another experiment, tau was preincubated with polymerized tubulin, then added to the system and the immunoreactivity analyzed by the immunoassay (●). Each point represents the mean of four or five determinations. Bars representing standard errors are shown for those measurements based on five determinations (n = 5).

weaker competitive effect of actin filaments on tau/microtubule interaction was also observed after addition of a mixture of tau with preassembled actin filaments (around 16% inhibition). Estimates of the extent of inhibition were made on the basis that the plateau levels observed in the solid phase assays represent the maximal binding of tau (B_{max}). This was corroborated by sedimentation experiments of assembled microtubules and determinations of the tau stoichiometries. For these studies, microtubules were assembled with taxol, incubated with increasing tau concentrations and the assembled polymers sedimented by centrifugation. Determinations of molar ratios of tau and tubulin in the assembled polymers allowed tau:tubulin stoichiometries of $1:(6.4 \pm 0.4)$ moles per mole to be estimated. The electrophoretic gels showing the protein components of purified proteins and microtubular and actin filament preparations are shown in Figure 4. Electrophoretic quantitative analysis showed that tubulin as well as actin and tau used in this study were between 96 and 99% pure.

It was of interest to analyze the in vitro assembly of tubulin into microtubules in the presence of actin, the coassembly of tubulin and actin polymers and the effects of tau in the assembly processes. Tubulin was induced to assemble into microtubules with 10% DMSO in the presence or absence of preassembled actin filaments, and the assembly kinetics monitored

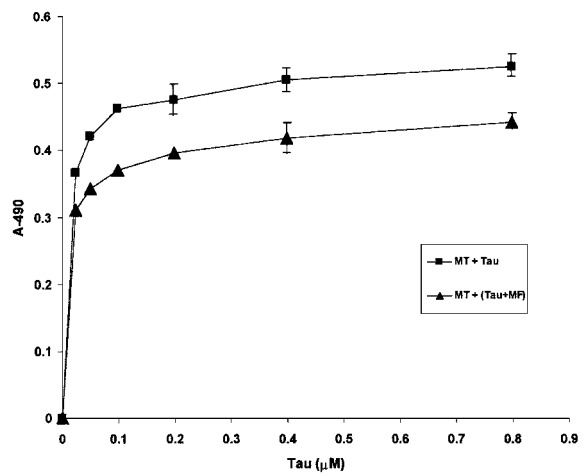

Fig. 3. ELISA study of the competition of actin for tau association with polymerized microtubules. The ELISA plates were incubated with 100 μl of preassembled microtubules (final concentration of 0.2 mg/ml tubulin). Increasing concentrations of tau (■), or mixtures of increasing tau concentrations preincubated with actin filaments (▲) were added to the plates, and ELISA immunoreactivity analyzed using an anti-tau polyclonal antibody. Each point represents the mean of four or five determinations. Bars representing standard errors are shown for those measurements based on five determinations (n = 5).

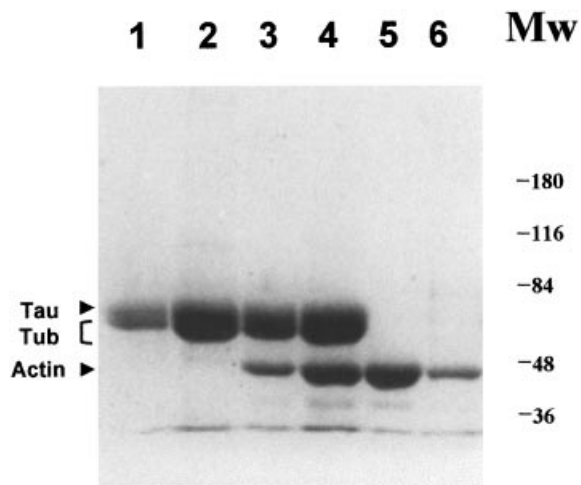

Fig. 4. Electrophoretic analysis of the protein components of actin and microtubules used in the study. Tubulin from microtubules assembled with 10% DMSO (**lane 1**). Microtubules derived from three cycles of assembly-disassembly containing high molecular weight MAPs and tau (**lane 2**). Macromolecular assemblies of tubulin and actin (**lane 3**). Macromolecular assemblies of preassembled actin and microtubules induced to polymerize with tau (**lane 4**). Actin from actin polymers (**lane 5**), and free unpolymerized actin (**lane 6**). Molecular weight markers are indicated on the right.

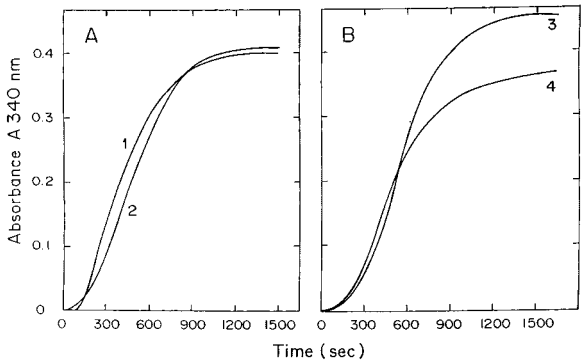

Fig. 5. Microtubule assembly kinetics in the presence of actin filaments. **A**: Tubulin (1.2 mg/ml) was induced to polymerize in the presence of 10% DMSO, either in the presence (curve 1) or the absence (curve 2) of preassembled actin filaments (0.9 mg/ml actin), and the assembly kinetics monitored at 340 nm. **B**: In another experiment, tubulin samples were induced to assemble with purified tau (0.6 mg/ml) in the presence (curve 3) or the absence (curve 4) of preassembled actin filaments, and the assembly kinetics monitored by the turbidity change at 340 nm. Experiments were repeated four times (n = 4) with different preparations of purified tubulin, actin and tau, and identical results were obtained after quantification of the assembly kinetics.

by the time-dependent change in absorbance at 340 nm. Even though no major differences were observed in the extent of polymerization of microtubular polymer assembly with and without actin filaments (Fig. 5A), a 20% inhibition in the extent of polymerization was observed as a result of four different assembly experiments (n = 4) in the presence of actin filaments in tau-promoted tubulin assembly into microtubules (Fig. 5B). On the basis of our previous studies indicating that tau peptides containing the microtubule-binding motifs corresponding to first tau repeat induce tubulin assembly into normal microtubules [Maccioni et al., 1989], experiments were carried out that showed a 22–26% inhibition in the assembly upon addition of actin filaments to the tau-peptide promoted microtubule assembly (data not shown).

The morphological aspects of the macromolecular assemblies of tubulin and actin in the presence and absence of tau, as well as the in vitro assembled polymeric networks of both cytoskeletal filaments were analyzed by electron microscopical studies (Fig. 6). Actin filaments assembled in the presence of tau (Fig. 6b) showed greater numbers of filament bundles as compared with normal actin filaments (Fig. 6a). Tau-promoted microtubular polymers (Fig. 6c), when assembled in the presence of actin filaments, exhibited structural associations with microfilaments at the electron microscopic levels (Fig. 6e, the insert 6e′). In some experiments, crossbridges between these filamentous polymers were also identified. These interactions were not revealed in microtubules assembled with actin filaments in the absence of tau (Fig. 6d). Tau association into these structures was also visualized at the electron microscopic level by immunogold. Studies at low magnification revealed immunolabeled tau colocalized mainly with microtubular polymers (Fig. 7a) as compared with negative controls in which a lower level of colocalization was revealed (Fig. 7b). At higher magnification, tau colocalization with microtubules (Fig. 7c) and with coexisting microtubules and actin filaments (Fig. 7d) were observed. In the later experiment, higher levels of tau binding to microtubules as compared with actin polymers were revealed. In the study of tau/microtubules colocalization (Fig. 7c), a significantly higher fraction (91.5%) of immunogold-detected tau with larger-size gold particles bound to microtubules, while only a small fraction (8.5%) remained unbound. On the contrary, in the negative control (Fig. 7b), 86% of the immunogold-detected tau corresponded to the unbound fraction.

DISCUSSION

We carried out an integrative study to analyze at the in vitro level the interactions of brain tau protein with actin filaments and microtubules, and the tau association with coexisting assembled networks of these polymers. This study is an approach for an analysis of the in vitro reconstitution of these cytoskeletal networks and the role of tau in these interaction patterns. In a first set of experiments, we used a solid phase immunoassay for studying protein–protein associations of tau with the cytoskeletal proteins tubulin and actin as well as with their polymers. Solid phase systems proved to be reliable assays for quantifying interactions of cytoskeletal proteins [Pedrotti et al., 1994]. The studies revealed that monomeric actin and actin filaments inhibited tau–tubulin interactions, and that complexes formed by tau and actin filaments exerted a stronger competition for tau–tubulin association. These studies were reinforced by evidence that tubulin molecules and preassembled microtubules strongly inhibited tau–actin interactions. Interestingly, these binding experiments indicate

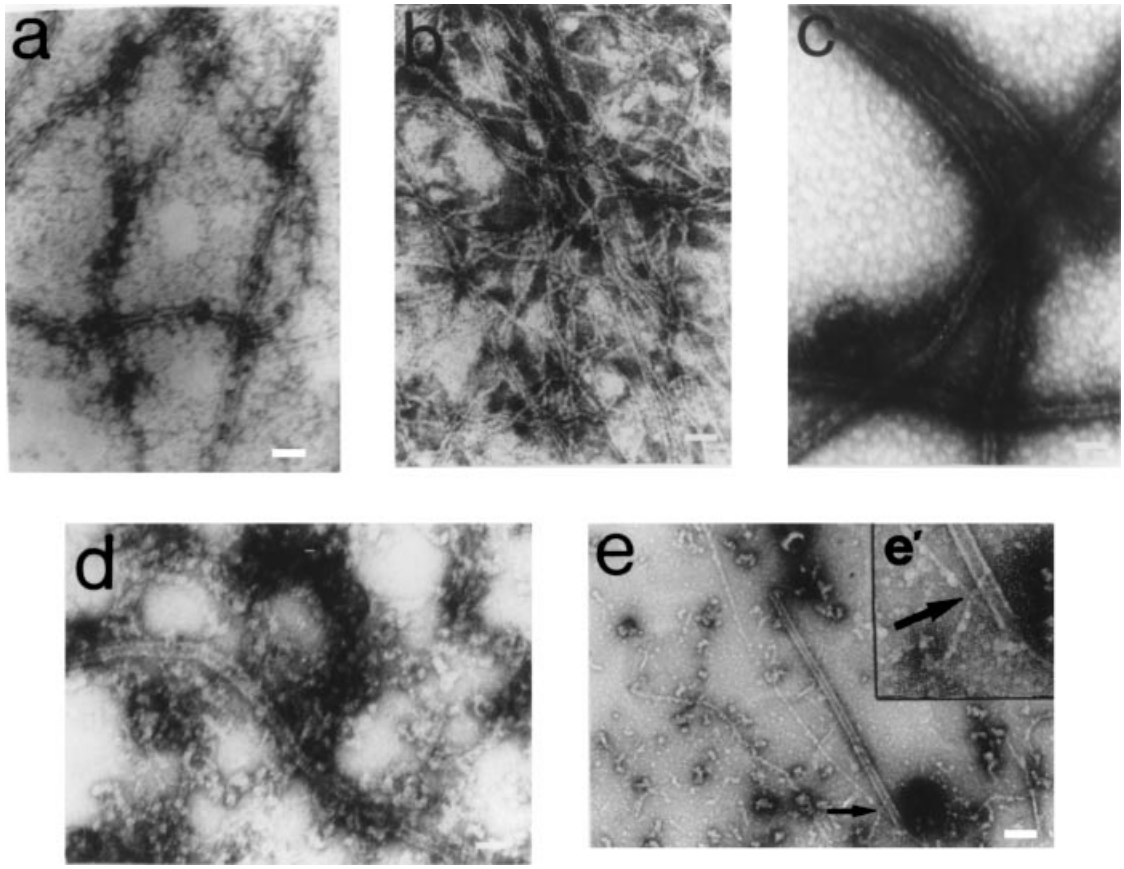

Fig. 6. Electron microscopy of tubulin polymers assembled with tau in the presence of actin filaments. **a**: Actin filaments assembled in the absence of tau. **b**: Bundles of actin filaments after assembly in the presence of 5 μM tau. **c**: Microtubular polymers in vitro assembled from purified tubulin in the presence of brain tau protein. **d**: Microtubules assembled with 10% DMSO in the presence of actin filaments and in the absence of tau. **e**: Microtubules assembled in the presence of tau and 2.5 μM preassembled actin filaments. **e′** (inset): Higher magnification of an area of panel shown in (e). Bars, 75 nm for a through e. Bars equals 20 nm for the inset e′.

that the extent of tubulin inhibition on tau–actin interactions was slightly higher than that of actin inhibition on tau–tubulin association. When microtubules were immobilized on the solid phase support, the tau–actin filament complex was less effective in inhibiting tau binding to the microtubular polymers. Considering the high level of purity of the protein preparations as shown in Figure 4, it is unlikely that possible impurities in actin or tau preparations may account for these competitive effects on tau binding to either tubulin or actin. In fact, identical binding results were obtained when tau with a purity higher than 99% was used, as compared with the experiment using 96% pure tau and highly purified tubulin. Furthermore, the estimates of tau stoichiometries (1:6.4 moles/ mole) are in good agreement with previous studies on these interactions based on other biochemical approaches [Cambiazo et al., 1995; Ackmann et al., 2000].

Our previous studies using ultracentrifugation analysis of the binding of tubulin peptides from the MAPs binding domain on tubulin to tau molecule have shown a cooperative association of tau to unpolymerized tubulin with four binding sites on tau [Maccioni et al., 1988]. Moreover, tau fragments corresponding to the repetitive microtubule binding motifs interacts with tubulin with an association constant $K_a = 2.5 \times 10^5\ M^{-1}$ [Maccioni et al., 1989]. In addition, tau binds to actin filaments as evidenced from previous studies in neuronal cells as well as in non-neuronal cell types [Cross et al., 1993; Henriquez et al., 1995]. The observations of a stronger competition of actin filament tau mixtures on tau association with microtubules as compared with that of monomeric actin

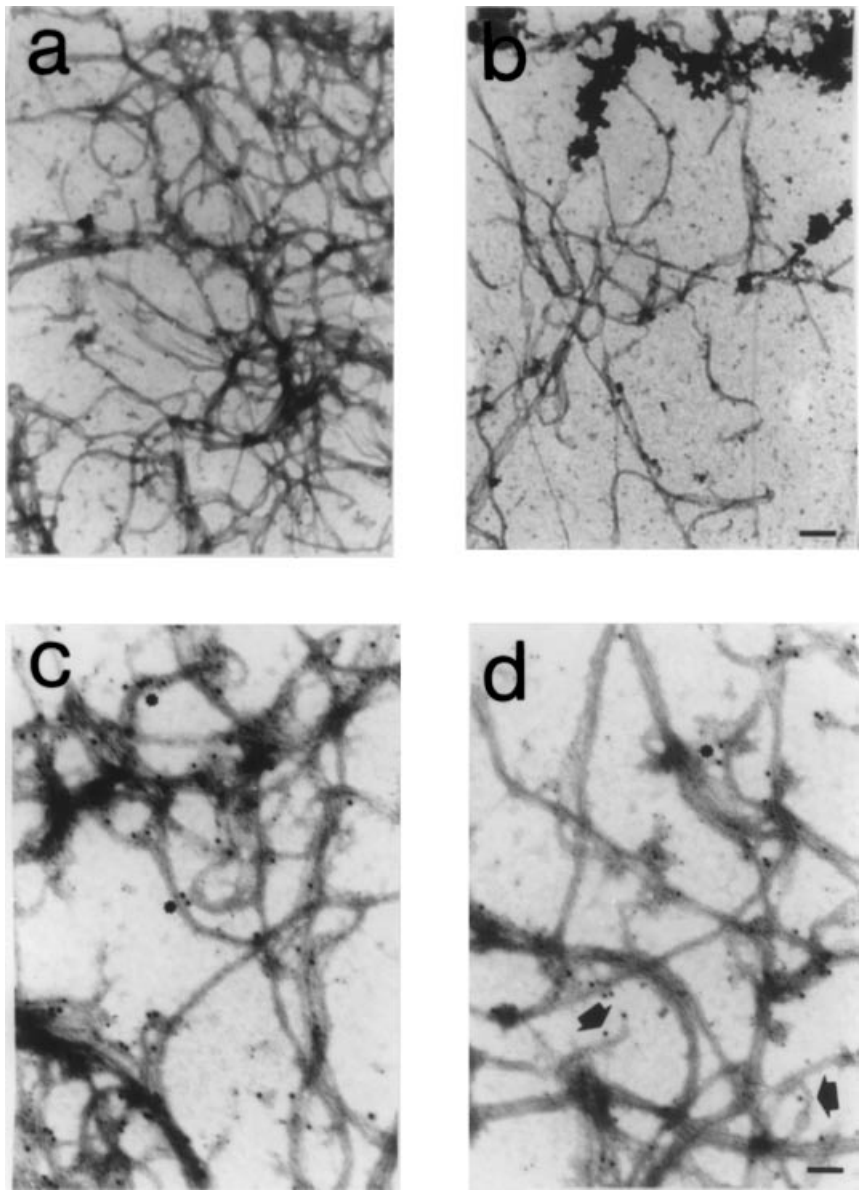

Fig. 7. Colocalization of immunolabeled tau protein along in vitro assembled microtubular polymers and actin filaments as analyzed by electron microscopy. **a**: Microtubules assembled in the presence of tau protein and immunogold localization of tau protein with the microtubular structures. **b**: Control for the secondary antibody in which no colocalization is revealed. **c**: Immunogold localization of tau with microtubular polymers. **d**: Immunogold localization of tau protein with in vitro assembled networks of microtubules and actin filaments. Arrows, tau localization with some in vitro assembled microfilaments. Asterisks, tau localization with microtubules. Bars, 200 nm on top and 75 nm on the bottom panels of the figure.

suggest a higher efficiency of tau/actin polymer binding as compared with tau interaction with the actin monomer. This could be responsible for the inhibitory effect on tau association with the solid phase-immobilized tubulin. In essence, solid phase studies provided valuable information to evaluate the roles of tau in mediating interactions between actin and tubulin polymers. Studies by other laboratories have also shown the interaction of MAP-2 with microtubular polymers [Pedrotti et al., 1994], and also with microtubules and actin networks [Sattilaro, 1986; Cunningham et al., 1997], and with intermediate filaments [Bloom and Vallee, 1983]. Interaction of MAP2c to the actin cytoskeleton was also reported [Ozer and Halpain, 2000].

Within the framework of this analysis, it was important to evaluate the effects of actin polymers on the in vitro assembly of microtubules.

Even though no major effects were observed in the extent of tubulin polymerization as a result of the presence of pressembled actin polymers in the assembly system, in which tubulin was polymerized with DMSO, a clear inhibition exerted by the actin polymers was revealed in the tau-promoted microtubule assembly (Fig. 5). These results indicate that association of free tau in the system with actin polymers may be responsible for its decreased activity in promoting microtubule assembly, thus corroborating the competition of actin polymers for tau association with microtubules. Similar inhibition was also observed in tubulin assembly induced by the tau peptide corresponding to first tau repeat. The different experiments raise the question of the morphological features of tau associations with actin and tubulin polymers, and how tau may be involved in mediating these interactions. These studies showed associations between in vitro assembled networks of filaments, and in some experiment, the presence of crossbridges between actin filaments and microtubules induced to assemble in the presence of tau. Studies on copolymerization (Fig. 6) revealed regular patterns for the links between actin filaments and microtubules, suggesting that the associations involve the tau motifs corresponding to the binding repeated sequences and that tau modulates actin filaments/microtubule interactions. Furthermore, immunogold experiments showed that tau associated with both in vitro assembled polymers. These findings are in agreement with studies by Hirokawa et al. [1988] of periodic arm-like projections of tau from the microtubule surface, which appear to play a role as crossbridging elements. It is possible that tau oligomers, instead of tau, may serve as linkers between these filamentous structures [Henriquez et al., 1995]. In this regard, studies have provided evidence of the capacity of tau to dimerize and oligomerize in solutions, thus supporting its potential structural role in crossbridging actin and tubulin polymers [Ackmann et al., 2000]. The regulatory roles of tau protein in modulating the integrated patterns of macromolecular assemblies of tubulin and actin polymers has been evidenced from studies in cultured cells [Cross et al., 1993], but also from in vitro evidence [Brandt and Lee, 1994; Gustke et al., 1994; von Bergen et al., 2000]. In summary, these studies shed new light onto our understanding of the molecular events underlying macromolecular assemblies of cytoskeletal filaments and the roles of tau proteins in modulating these associations.

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