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Prion protein inhibits microtubule assembly by inducing tubulin oligomerization ☆

Krzysztof Nieznanski [a,*], Zoya A. Podlubnaya [b,c], Hanna Nieznanska [a]

[a] *Nencki Institute of Experimental Biology, Department of Muscle Biochemistry, Warsaw, Poland*
[b] *Institute of Theoretical and Experimental Biophysics, Laboratory of Structure and Function of Muscle Proteins, Pushchino, Russia*
[c] *Pushchino State University, Pushchino, Russia*

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Abstract

A growing body of evidence points to an association of prion protein (PrP) with microtubular cytoskeleton. Recently, direct binding of PrP to tubulin has also been found. In this work, using standard light scattering measurements, sedimentation experiments, and electron microscopy, we show for the first time the effect of a direct interaction between these proteins on tubulin polymerization. We demonstrate that full-length recombinant PrP induces a rapid increase in the turbidity of tubulin diluted below the critical concentration for microtubule assembly. This effect requires magnesium ions and is weakened by NaCl. Moreover, the PrP-induced light scattering structures of tubulin are cold-stable. In preparations of diluted tubulin incubated with PrP, electron microscopy revealed the presence of ~50 nm disc-shaped structures not reported so far. These unique tubulin oligomers may form large aggregates. The effect of PrP is more pronounced under the conditions promoting microtubule formation. In these tubulin samples, PrP induces formation of the above oligomers associated with short protofilaments and sheets of protofilaments into aggregates. Noticeably, this is accompanied by a significant reduction of the number and length of microtubules. Hence, we postulate that prion protein may act as an inhibitor of microtubule assembly by inducing formation of stable tubulin oligomers.

Keywords: Prion protein; Tubulin; Microtubules; Oligomerization; Electron microscopy

It is believed that the conformational conversion of the cellular form of prion protein (PrP^{C}) to an aberrantly folded isoform denoted PrP^{Sc} is a key event in the development of TSEs, known also as prion diseases [1]. Both the molecular mechanism of this pathogenic transition and the biological function of PrP^{C} remain enigmatic. Prion protein is a highly conserved sialoglycoprotein expressed mainly in the nervous system and, to a lesser extent, in many extraneural tissues [2]. The precursor of human PrP is composed of 253 aa residues, of which 22 N-terminal and 23 C-terminal ones constitute signal sequences. Posttranslational processing of PrP includes removal of the signal peptides, attachment of a GPI anchor, and *N*-glycosylation [3,4]. Moreover, PrP may be proteolytically truncated at the N-terminal part of the molecule [5,6]. Usually, PrP^{C} is anchored on the cell surface via a GPI moiety [3,7], however, this protein has also been found associated with many intracellular compartments ([8], reviewed in [9]). There are two described transmembrane forms of prion protein, called ^{Ctm}PrP and ^{Ntm}PrP, that cross the membrane of the endoplasmic reticulum [10–13]. Interestingly, a significant increase in the concentration of transmembrane PrP

☆ *Abbreviations:* CLIP, cytoplasmic linker protein; EB1, end-binding protein 1; GPI, glycosylphosphatidylinositol; GSS, Gerstmann–Sträussler–Scheinker disease; MAPs, microtubule-associated proteins; MCAK, mitotic centromere-associated kinesin; PrP, prion protein; PrP^{C}, cellular form of prion protein; ^{Ctm}PrP, transmembrane form of prion protein with the C-terminus residing in the lumen of endoplasmic reticulum; ^{Ntm}PrP, transmembrane form of prion protein with the N-terminus residing in the lumen of endoplasmic reticulum; PrP^{Sc}, scrapie form of prion protein; PrP106–126, peptide corresponding to PrP sequence 106–126; TSE, transmissible spongiform encephalopathy.

* Corresponding author. Fax: +48 22 8225342.
E-mail address: k.nieznanski@nencki.gov.pl (K. Nieznanski).

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is observed in Gerstmann–Sträussler–Scheinker disease (GSS), a form of inherited TSE [10,12,14,15]. Additionally, in a small population of brain neurons PrP^C is located predominantly in the cytosol [8].

Numerous attempts to identify the physiological function of prion protein have been carried out by searching for PrP-interacting proteins. The partners identified so far include: neural cell adhesion molecules, casein kinase 2, dystroglycan, aldolase C, heterogeneous nuclear ribonucleoprotein A2/B1, synapsin Ib, adaptor protein Grb2, Bcl-2, stress-inducible protein 1, laminin, and laminin receptor/precursor ([16–19], reviewed in [20]). Interaction with some of these molecules has led to the proposals that PrP^C may be implicated in cell adhesion, signal transduction, neurite outgrowth or act as an antiapoptotic protein. It has also been demonstrated that PrP may associate with microtubular cytoskeleton and its major component, tubulin [18,21,22]. Presumably this association is related to the active transport of prion protein in the cell [23]. On the other hand, a synthetic peptide corresponding to PrP sequence 106–126 (PrP106–126), with a point mutation linked to GSS, has been shown to inhibit tubulin polymerization [24].

Recently, by means of chemical cross-linking and cosedimentation experiments, we have reported that PrP may directly bind to tubulin [25], the building block of microtubules. It is known that in the presence of GTP, heterodimers of α- and β-tubulin polymerize head-to-tail into linear protofilaments (reviewed in [26]). About 13 protofilaments associate laterally to form a tubular polymer. Microtubules are very dynamic structures that can switch stochastically between assembly and disassembly [27]. This unusual property, observed for purified tubulin as well as in vivo, is termed dynamic instability and is crucial to microtubule functions in the cell. The dynamic behavior of microtubules is connected with the intrinsic GTPase activity of tubulin and is controlled by the binding, hydrolysis, and exchange of the nucleotide (reviewed in [26]). Moreover, the stability of microtubules is regulated by interactions with a large number of proteins. Microtubule-associated proteins (MAPs), such as Tau, MAP2, MAP1A, MAP1B, doublecortin, EB1, and CLIP-170, belong to microtubule stabilizers (reviewed in [28]). These proteins may bridge several tubulin subunits in the polymer or bind to the microtubule ends. A tubulin-sequestering protein called stathmin, a protein disrupting contacts between tubulin heterodimers—katanin, and depolymerizing kinesins of the Kin-I subfamily, such as MCAK, represent microtubule-destabilizing factors ([29], reviewed in [28]).

In light of our recent findings [25], it was interesting to test whether a direct interaction with PrP may affect the ability of tubulin to polymerize into microtubules. Therefore, we examined the effect of full-length human recombinant PrP on tubulin polymerization by means of light scattering, sedimentation, and electron microscopy. Such a comprehensive experimental approach allowed us to demonstrate that prion protein induces rapid association of tubulin into oligomers thereby inhibiting microtubule formation. Our data strongly suggest that PrP may act as a tubulin-sequestering protein.

Materials and methods

Protein purification. Plasmid encoding human PrP (residues 23–231) with an N-terminal linker containing a His_6 tag and a thrombin cleavage site [30] was kindly provided by Prof. Witold K. Surewicz. Expression in *Escherichia coli*, refolding and purification of the recombinant protein were carried out as described by Zahn et al. [31]. Removal of the His tag was performed according to Morillas et al. [30] followed by dialysis against deionized water. PrP preparations were cleared before experiments by 30 min centrifugation at 200,000*g*, 25 °C.

Tubulin with associated MAPs was purified from fresh porcine brain according to the modified method of Mandelkow et al. [32], described in detail in [25]. Before experiments tubulin preparations were thawed from −70 °C and centrifuged for 20 min at 22,000*g*, 4 °C. The supernatants obtained were used in further studies.

Light scattering. Changes in the turbidity of tubulin were monitored spectrophotometrically at 350 nm. Two types of samples were examined: tubulin under conditions not allowing microtubule self-assembly and under conditions promoting polymerization into microtubules. In the first case tubulin at 0.1–0.2 mg/ml was incubated at 25 °C in the polymerization buffer containing: 10 mM sodium phosphate buffer, pH 7.0, 10% (w/v) glycerol, 1 mM GTP, and 16 mM $MgCl_2$. For the second type of conditions, tubulin at 2 mg/ml was incubated at 37 °C in the polymerization buffer supplemented with 20 μM taxol (Sigma, St. Louis, MO, USA). The desired temperature was maintained during the whole assay. In some experiments samples were cooled on ice for 15 min. The light scattering measurements were initiated immediately after addition of PrP (the first type of conditions) or tubulin (the second type of conditions) to the cuvette.

Sedimentation experiments. Tubulin at 0.2 or 2 mg/ml under the same conditions as those applied in the light scattering measurements was incubated for 30 min together with 0.024 mg/ml or 0.24 mg/ml PrP, respectively, and subsequently centrifuged for 1 min at 14,000*g*. The pellets were resuspended in a volume of deionized water equal to the volume of the supernatant and analyzed by SDS–PAGE with 12% separating gels according to Laemmli [33]. Electrophoresis was carried out under non-reducing conditions.

Transmission electron microscopy. The two types of samples assayed spectrophotometrically were subjected to electron microscopic examinations. In the first type, tubulin at 0.1 mg/ml was incubated in the polymerization buffer at 25 or 37 °C. The incubation was carried out for 5 or 30 min in the absence or presence of PrP at 0.048 mg/ml. In the second type of conditions, tubulin at 2 mg/ml was incubated for 1 or 30 min in the absence or presence of 0.32 mg/ml PrP at 37 °C in the polymerization buffer containing 20 μM taxol. The samples were subsequently diluted 10-fold in prewarmed buffer just before applying to electron microscopic grids. In some experiments, samples were incubated for 30 min on ice. For electron microscopic observations copper grids (400 mesh) covered with collodion (SPI Supplies, West Chester, PA, USA) and carbon were used. Ten microliters of samples at 0.1–0.2 mg/ml tubulin was applied to a grid for 40 s. Negative staining was performed with 1–2% (w/v) aqueous solution of uranyl acetate (SPI Supplies, West Chester, PA, USA) for 25 s. The grids were examined in a JEOL-1200EX electron microscope at an accelerating voltage of 80 kV with a 50-μm objective aperture.

Results

Prion protein induces formation of light scattering structures of tubulin

To test whether previously described direct interaction with PrP [25] may influence tubulin capability to assemble

into microtubules, we performed standard light scattering measurements at 350 nm. To assess whether PrP is able to induce polymerization of tubulin, the experiments were performed in a buffer containing GTP and magnesium ions that are known to be required for microtubule formation [34–36], but the concentration of tubulin was far below the critical concentration (~1 mg/ml) allowing self-assembly into microtubules [34]. Moreover, the turbidity measurements were carried out at 25 °C that is below the optimal temperature (37 °C) for microtubule formation [34,35]. Under these conditions tubulin alone did not form any detectable light scattering structures (data not shown). Interestingly, addition of PrP caused a raise in the turbidity of tubulin preparations (Fig. 1). The effect was observed immediately after addition of PrP. The rate and the level of tubulin oligomerization increased with the concentration of PrP. The reaction reached a plateau already after 10 min of incubation at a tubulin to PrP molar ratio equal to 1.5:1. The lowest PrP to tubulin molar ratio that allowed the detection of an increase in the turbidity was around 1:6 (Fig. 1). Control experiments confirmed that the described effect of PrP resulted from a specific interaction between the two proteins. A similar induction of tubulin turbidity was observed for prion protein lacking the His tag. PrP alone, at the concentrations applied in the experiments, did not display any absorption at 350 nm (data not shown). Furthermore, bovine serum albumin at a concentration corresponding to that of PrP did not induce any increase in the turbidity of tubulin preparations (data not shown).

Interestingly, the effect of PrP was dependent on the concentration of magnesium ions (Fig. 2A). In the absence of $MgCl_2$ prion protein induced only a slight transient raise in the turbidity followed by its decrease to a near-background level. Moreover, the effect of PrP was significantly reduced by 100 mM NaCl (Fig. 2B), as is also the case for the polymerization of tubulin containing MAPs into microtubules [35]. However, in contrast to microtubules which rapidly disassemble at low temperatures [34], the light scattering structures of tubulin formed in the presence of PrP were cold-stable (Fig. 1). This suggests that the above structures are different from microtubules.

We also checked whether PrP might influence tubulin polymerization under conditions promoting microtubule formation. In these experiments tubulin concentration was 2 mg/ml and measurements were carried out at 37 °C, in a polymerization buffer containing a microtubule-stabilizing agent, taxol [37]. As it is shown in Fig. 3, PrP induced a rapid increase in the turbidity of tubulin also under these conditions. At a fourfold molar excess of tubulin over PrP, a reaction plateau was

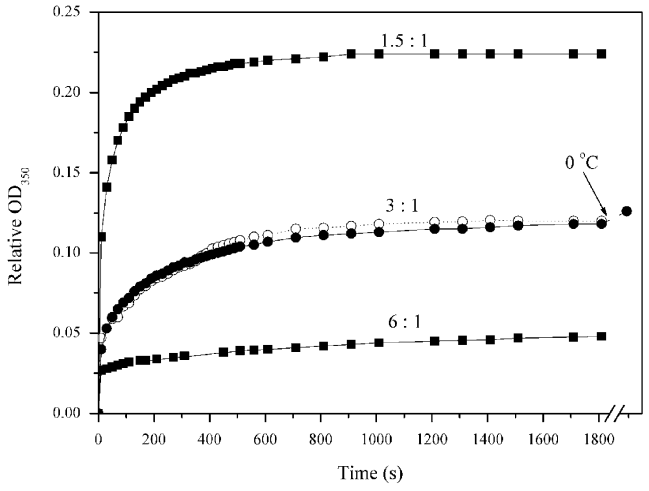

Fig. 1. Effect of PrP on the turbidity of diluted tubulin unable to self-associate into microtubules. Tubulin at 0.1 mg/ml was incubated in the polymerization buffer with increasing concentrations of PrP. The turbidity was measured against tubulin without PrP as a reference. The molar ratio of tubulin to PrP is indicated on each curve. The effect of PrP lacking the His tag is shown by a dotted line. At the time indicated (arrow), the samples were placed on ice for 15 min. Note that the turbidity level remained almost unchanged after cooling. The curves are representative for five independent experiments.

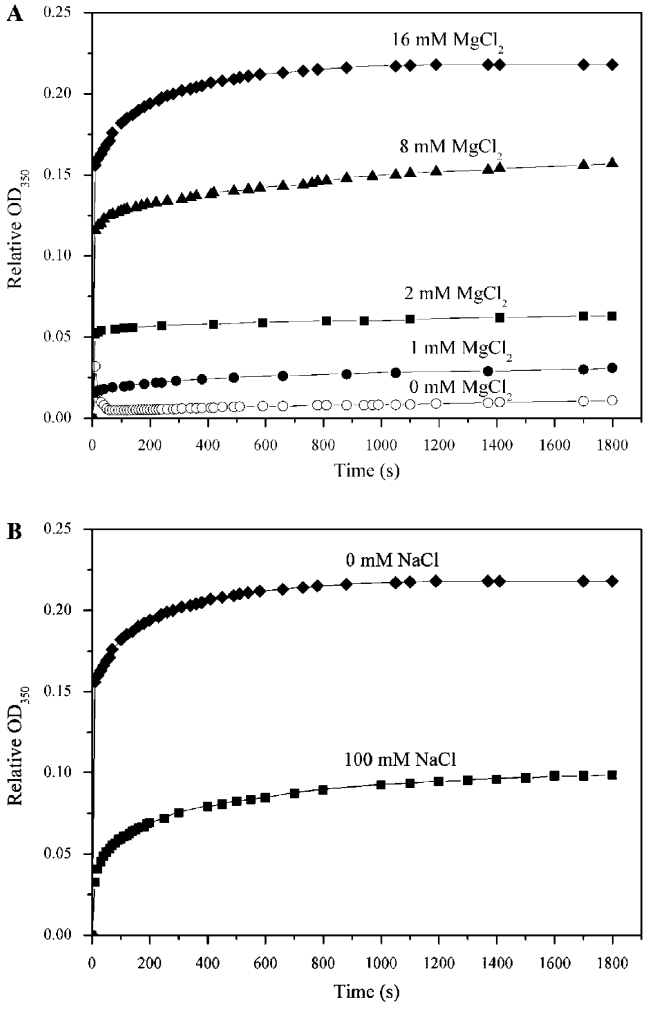

Fig. 2. Influence of $MgCl_2$ and NaCl on the effect of PrP on tubulin turbidity. Tubulin at 0.2 mg/ml was incubated with 0.024 mg/ml PrP in the polymerization buffer containing (A) 0, 1, 2, 8 or 16 mM $MgCl_2$; or (B) in the buffer containing additionally 100 mM NaCl. The turbidity was measured against tubulin alone as a reference. The results are representative for three independent experiments.

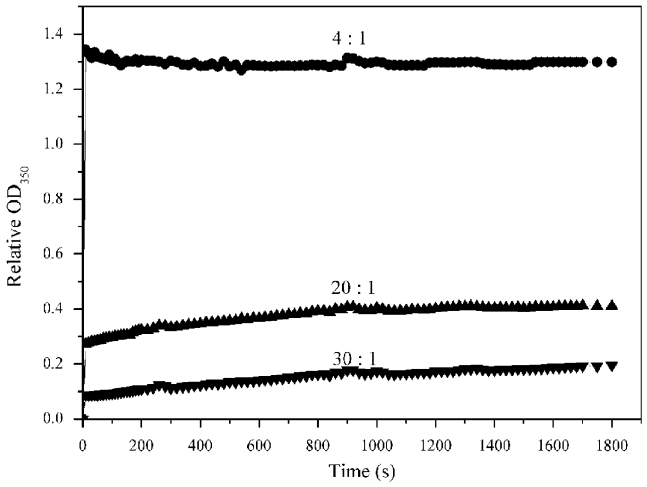

Fig. 3. Effect of PrP on the turbidity of tubulin under conditions promoting microtubule formation. Tubulin at 2 mg/ml was incubated in the presence of increasing concentrations of PrP in the polymerization buffer containing taxol. The turbidity was measured against tubulin without PrP as a reference. The molar ratio of tubulin to PrP is indicated on each curve. The results are representative for three independent experiments.

reached already within the first seconds of incubation. The effect was more intense than that described for diluted tubulin. An increase in the turbidity of the samples was observed already at a tubulin to PrP molar ratio of 30:1.

PrP induces sedimentation of tubulin

To further characterize the light scattering structures of tubulin formed in the presence of PrP, we performed sedimentation experiments for both types of samples that were also analyzed spectrophotometrically. To distinguish these structural species from microtubules, the samples were subjected to low-speed centrifugation not allowing sedimentation of microtubules. Tubulin at the concentration favoring microtubule formation (2 mg/ml) was incubated with PrP at a 4:1 molar ratio for 30 min at 37 °C, and subsequently centrifuged. As expected, tubulin polymerized without PrP remained in the supernatant (Fig. 4A), whereas in the presence of PrP almost all tubulin was pelleted. Similar low-speed sedimentation was performed for diluted tubulin (0.2 mg/ml) unable to form microtubules. Under these conditions PrP also induced the pelleting of tubulin (Fig. 4B), the effect was, however, less pronounced than that described for tubulin at 2 mg/ml. This observation is consistent with the results of the light-scattering experiments (compare Fig. 4 with Figs. 1 and 3). As it is seen in Fig. 4A and B, PrP undergoes co-sedimentation with tubulin, providing evidence that a direct interaction between the proteins occurs under both experimental conditions.

Electron microscopic analysis of tubulin oligomers induced by PrP

To characterize the morphology of the tubulin structures induced by PrP, samples after incubation under conditions similar to those described for the light scattering experiments were applied on grids and subjected to transmission electron microscopy, as described in Materials and methods.

Electron micrographs of negatively stained samples of tubulin at the concentration not allowing microtubule formation (0.1 mg/ml) incubated in the polymerization buffer at 37 °C showed only small oligomers of different shapes and sizes ranging from 20 to 25 nm (Fig. 5A–C). Similar oligomers we observed at 25 °C and 0 °C (data not shown). These structures did not form large aggregates. Interestingly, in the samples of tubulin incubated in the presence of PrP we found numerous disc-shaped structures larger than those formed by tubulin alone (Fig. 5D–I). The diameter of these discs varied from 30 to 50 nm. Similar oligomers were formed at 25 °C (data not shown) and 37 °C (Fig. 5D–F, I), as well as at 0 °C (Fig. 5G and H). In addition to the disc-shaped structures also numerous ring-like structures of an outer diameter of ~45 nm were present (Fig. 5D). The disc-shaped oligomers associated into large aggregates in which they were bound together in various orientations. Some of these oligomers were associated with the rings

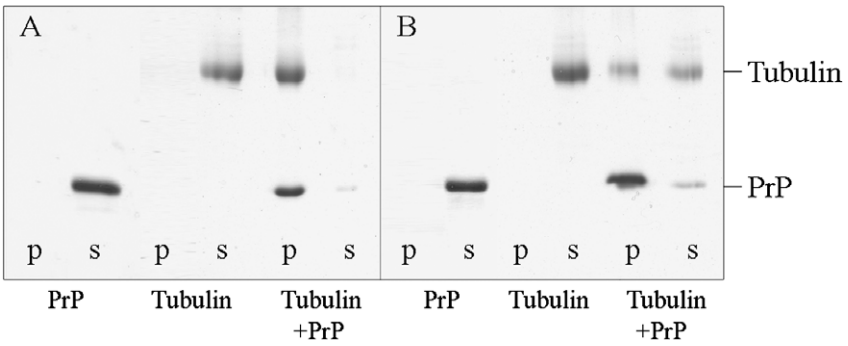

Fig. 4. Sedimentation of tubulin by PrP. (A) Tubulin under conditions favoring microtubule formation and (B) under conditions not allowing microtubule assembly was incubated in the presence or absence of PrP at a 4:1 (tubulin:PrP) molar ratio and subsequently centrifuged. The obtained pellets (p) and supernatants (s) were analyzed by electrophoresis. Note that neither PrP alone nor tubulin alone did not undergo pelleting. The image is representative for three independent experiments.

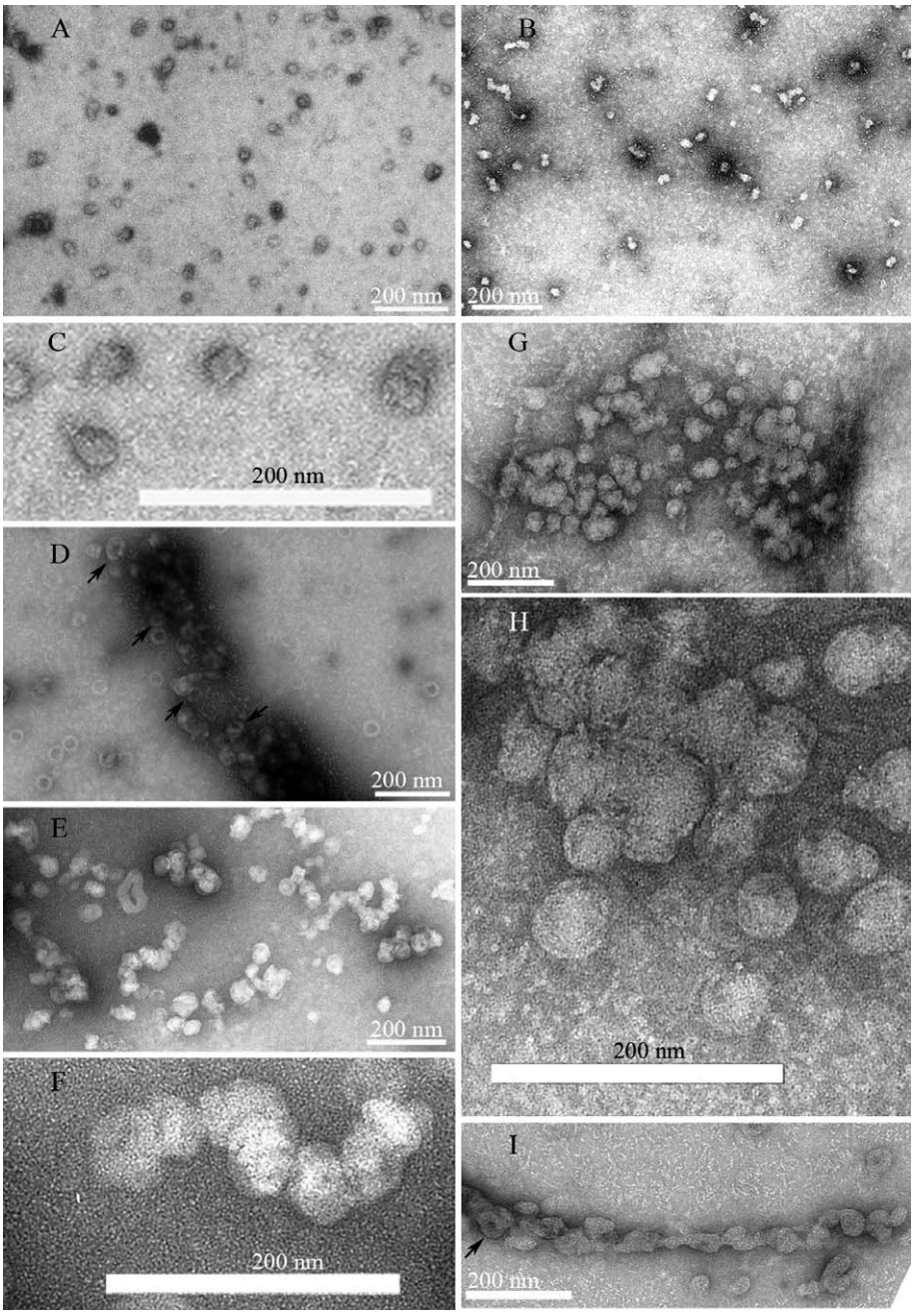

Fig. 5. Electron micrographs of tubulin at a concentration not allowing formation of microtubules. Tubulin alone incubated (A) for 5 min and (B) for 30 min. Fragment of micrograph (A) at a higher magnification is shown in (C). Tubulin incubated in the presence of PrP (D) for 5 min and (E,F,I) for 30 min at 37 °C. (D,I) Tubulin rings (arrows) associated with disc-shaped oligomers. (F) Magnified fragment of micrograph (E). Oligomers arranged in various orientations in amorphous and fibrous assemblages are shown in (E,F) and (I), respectively. (G) Tubulin incubated in the presence of PrP for 30 min on ice. (H) Fragment of micrograph (G) at a higher magnification.

(Fig. 5D and I). Samples incubated for 30 min, corresponding to the plateau phase of the light scattering curve, contained additionally long linear aggregates of disc-shaped oligomers (Fig. 5I). Whereas the ring structures of tubulin have been known for decades and are characteristic of self-assembled tubulin heterodimers containing GDP or of preparations of depolymerizing microtubules ([38], see [39] for a review), the disc-like oligomers formed in the presence of PrP have not been reported before. Under the conditions applied here, both in the presence and absence of PrP, we did not observe any structures similar to those of microtubules, protofilaments or sheets of protofilaments. PrP incubated alone in the polymerization buffer did not form any detectable structures (data not shown).

Micrographs of tubulin preparations at the concentration promoting formation of microtubules (2 mg/ml), incubated for 30 min at 37 °C, showed numerous microtubules of normal appearance (Fig. 6A). These microtubules were

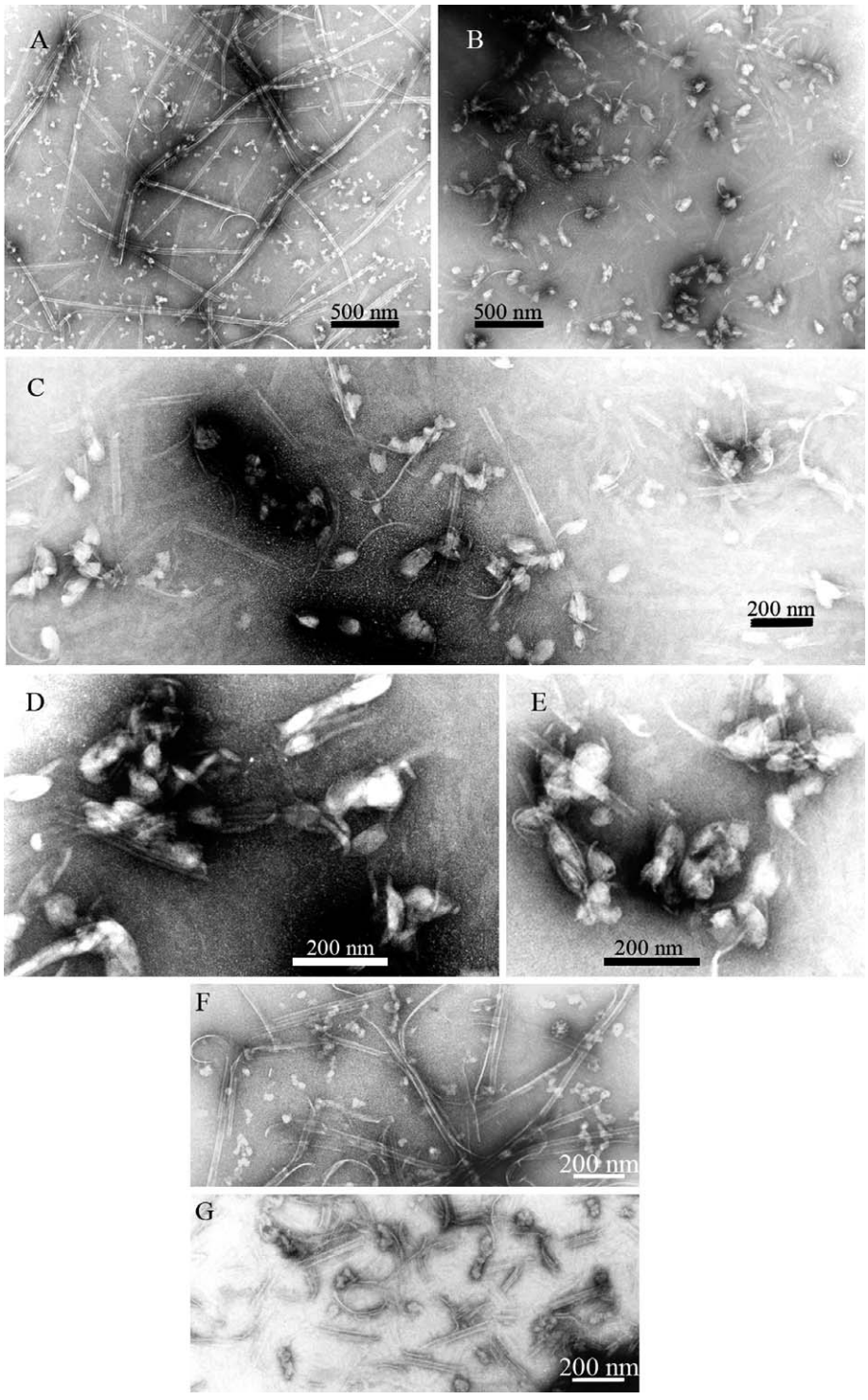

Fig. 6. Electron micrographs of tubulin under conditions promoting microtubule assembly. Tubulin incubated (A) for 30 min and (F) for 1 min in the absence of PrP. Tubulin incubated in the presence of PrP (B–E) for 30 min and (G) for 1 min. (B–E) Oligomers resembling disc-shaped structures seen in Fig. 5 seem to be wrapped by very short curved protofilaments. Some of the protofilaments appear to emerge directly from the aggregates of oligomers. (B,C) Numerous sheets of protofilaments are seen in the background. Note that the length and number of microtubules are reduced in the presence of PrP.

~25 nm in diameter and heterogeneous in length (0.5–2 μm). There were also multiple oligomers of a diameter up to 25 nm dispersed in the background and resembling structures seen for diluted tubulin (compare Figs. 5A–C and 6A). Some of these structures associated into small aggregates composed of several oligomers. Separated protofilaments and sheets of protofilaments were infrequent. Noticeably, samples of tubulin polymerized for 30 min in the presence of PrP were of a completely different appearance. As it is seen in the micrographs (Fig. 6B–E), PrP reduced significantly the number and length of microtubules. The microtubules found in these samples usually

did not exceed 250 nm. At the same time PrP did not affect the diameter of microtubules. The prevailing structures were sheets of protofilaments and aggregates of oligomers (Fig. 6B and C). These oligomers were larger than those observed in samples of tubulin alone and associated predominantly with short protofilaments. Some of them were also bound to short sheets of protofilaments. We did not find similar structures in tubulin preparations incubated in the absence of PrP. The shape of the observed oligomers was determined by their tight association with curved protofilaments (Fig. 6C–E). Since those oligomers seemed to be wrapped by very short protofilaments, it was impossible to calculate their diameter. As it is seen in Fig. 6G, the effect of prion protein was already visible after 1 min incubation of tubulin with PrP under the conditions favoring microtubule assembly. This is consistent with the results of turbidity measurements (Fig. 3). In the presence of PrP, tubulin was found in the form of short microtubules ranging from 60 to 250 nm, short protofilaments and sheets of protofilaments. Numerous oligomers of roughly globular shape associated into aggregates together with short protofilaments and sheets of protofilaments were already formed at this stage. From a comparison of Fig. 6G and Fig. 6B and C, it becomes apparent that prolongation of the incubation time with PrP led to an increase in the concentration of oligomers. In contrast, Fig. 6F shows that after a one-minute incubation, tubulin alone formed much longer microtubules measuring up to 650 nm, long circled protofilaments, and small globular oligomers. The data presented above demonstrate that prion protein inhibits the assembly of microtubules.

We have also characterized the cold-stable structures induced by PrP in samples of concentrated tubulin. Electron microscopic studies were performed for tubulin at 2 mg/ml incubated for 30 min on ice in the polymerization buffer. In the samples of tubulin incubated in the absence of PrP, we observed numerous oligomers of 20–25 nm similar to the structures found in the preparations of diluted tubulin (compare Figs. 7A and 5A and B). These oligomers assemble into small, composed of only several units, or large aggregates. We also observed numerous tubulin rings of the usual diameter. The cold-stable structures formed in the presence of PrP strongly resembled the disc-shaped oligomers described above (compare Figs. 7B and 5E–H). Their diameter ranged from ~30 to 50 nm, and they could associate into aggregates. Tubulin rings were also found in these samples. As expected, microtubules, sheets, and protofilaments were not detected either in the absence or presence of prion protein.

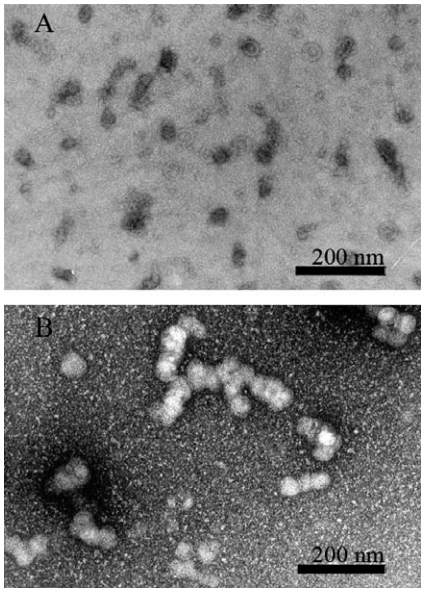

Fig. 7. Electron micrographs of cold-stable tubulin oligomers. Samples of concentrated tubulin incubated on ice (A) in the absence and (B) in the presence of PrP. Note the disc-shaped oligomers associated in different orientations in amorphous aggregates (B).

Discussion

In a previous work we have reported that prion protein may directly interact with tubulin [25]. Here, we examined the effect of this interaction on the intrinsic ability of tubulin to polymerize. Experiments performed both under conditions not allowing and those promoting microtubule formation showed that PrP may induce rapid oligomerization of tubulin. Under the second type of conditions this oligomerization was accompanied by a significant reduction of the length and number of microtubules formed, which could be observed by electron microscopy already after one minute of incubation. PrP seems to limit the number of tubulin heterodimers available for polymerization into microtubules, most probably by engaging them in the formation of oligomers. Thus PrP may be implicated in the modulation of microtubule dynamics as a tubulin-sequestering protein. In this PrP slightly resembles the effect of stathmin, which protein, however, sequesters tubulin in a complex composed of only two αβ–tubulin heterodimers [40].

To our knowledge, oligomers of tubulin similar to those formed in the presence of PrP have never been described before. During the assembly and disassembly of microtubules, numerous transient structures such as tubulin double rings, protofilaments, and sheets of protofilaments, as well as small tubulin oligomers have been detected by means of electron microscopy (reviewed in [26]). Stabilization of these structural intermediates by cellular factors may play an important role in the microtubule dynamics. It is accepted that tubulin sheets formed of protofilaments may close into microtubules, whereas rings correspond to oligomers of GDP-containing tubulin unable to assemble into microtubules. Formation of tubulin rings is favored at high concentrations of Mg^{2+}, low temperature or in the absence of GTP [41]. Similarly, the light scattering structures of tubulin induced by PrP were dependent on magnesium ions and were cold-stable. Moreover, the diameter of the disc-shaped oligomers formed in the presence of PrP corresponds to that of the rings [36,42,43]. Interestingly, under conditions not allowing microtubule formation, in the pres-

ence of PrP we observed an increased number of tubulin rings. The various small oligomers of tubulin reported to date include more-or-less globular-shaped agglomerates and elongated particles of a controversial role in the microtubule dynamics. Globular structures not exceeding 30 nm were found at the early stages of tubulin polymerization and in preparations of microtubules oscillating between the growing and shrinking phases [43]. Mandelkow and colleagues have proposed that these oligomers are assembly-competent species implicated in microtubule nucleation. On the other hand, it has been assumed that formation of some oligomers of erythrocyte tubulin may reduce the rate of microtubule assembly [44].

Interestingly, the oligomerization of tubulin by PrP bears also some common features with tubulin polymerization into microtubules. The effect of PrP is significantly reduced by NaCl, a phenomenon described previously for polymerization of tubulin and especially of tubulin containing MAPs [35]. Moreover, magnesium ions known to stimulate tubulin polymerization [36] are also required for the tubulin oligomerization induced by PrP. In contrast to microtubule assembly, formation of the disc-shaped structures induced by PrP may occur at a low tubulin concentration and at a low temperature. Furthermore, in contrast to microtubules, these oligomers readily sediment upon low-speed centrifugation, which may result from their association into large aggregates.

It has previously been demonstrated that the neurotoxic peptide corresponding to PrP sequence 106–126 binds to tubulin [22]. To resolve the mechanism of the toxicity, the effect of this synthetic peptide on tubulin polymerization was examined [24]. It was shown by standard turbidity measurements that PrP106–126 slightly inhibits microtubule assembly. The peptide carrying the point mutation A117V, linked to GSS, decreased the rate of microtubule formation more efficiently than the wild type peptide. The effect was observed only in the presence of tau protein. Interestingly, in contrast to our results obtained for the full-length prion protein, Brown [24] has reported a decrease of tubulin turbidity by the PrP106–126 peptide. Since PrP106–126 was unable to induce formation of light scattering oligomers of tubulin, the mechanisms of inhibition of microtubule assembly by the synthetic peptide and full-length protein are apparently different. This discrepancy may be explained by the hydrophobic character of the 106–126 sequence of PrP that may be buried in the structure of full-length molecule and hence cannot interact with tubulin. Our results suggest that there is another region in the prion protein, located outside the 106–126 sequence, responsible for the effect described here.

The strong effect of PrP reported in this study may be weaker in vivo since the overall concentration of PrP in the cytoplasm is much lower than that of tubulin. However, it is still possible that the interaction with prion protein may modulate polymerization of tubulin in the cell. The influence on tubulin assembly, under the conditions allowing microtubule assembly, observed already at a 30-fold molar excess of tubulin over PrP, suggests that it might be of physiological relevance. It is likely that PrP may act in particular regions of the cell where it is concentrated and/or may affect only a restricted pool of tubulin heterodimers. Presumably, tubulin properties could be affected by the transmembrane form of prion protein, termed ^{Ctm}PrP, whose N-terminus resides in the cytoplasm. At least in neurons of the hippocampus, neocortex, and thalamus, where PrP^{C} is present predominantly in the cytosol [8], prion protein could exert an effect on tubulin polymerization. Notably, the level of intracellular PrP increases significantly in TSE [10,12,14,15]. We hypothesize that the neurotoxicity of cytoplasmic prion protein [45,46] may be related to its effect on the oligomerization of tubulin described here.

Acknowledgments

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