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Cytoplasmic Gamma-Tubulin Complex From Brain Contains Nonerythroid Spectrin

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ABSTRACT

The newer member of the tubulin superfamily, γ-tubulin, is known to mediate microtubule nucleation from the centrosome of eukaryotic cells with the aid of some other proteins. The major amount of γ-tubulin is believed to be located in the centrosome before the onset of mitotic division. However, a considerable amount has been found in the cytoplasm in the form of a complex whose function is not well known. Microtubules are most abundant in brain tissues and brain microtubules have been extensively used in many in vitro studies. Thus, it is relevant to use brain tissue to characterize cytoplasmic γ-tubulin complex. Here we show that cytoplasmic γ-tubulin in brain tissues exists as a ring complex as in other tissues. Interestingly, along with the common members of the γ-TuRC reported from several tissues and species, the purified brain cytoplasmic complex contains some high molecular weight proteins including α and β nonerythroid spectrin which are not found in other tissues. Immunohistochemical studies of brain tissue sections also show the co-localization of γ-tubulin and spectrin. The possible implications have been discussed. J. Cell. Biochem. 110: 1334–1341, 2010.

KEY WORDS: γ-TuRC; BRAIN; SPECTRIN; MICROTUBULES

Microtubules display different functions in eukaryotic cells. While the most noted function is their role in the cell division, transportation and movement are also highly important, and become the primary function in neurons, cilia, and flagella. Microtubules are organized from microtubule organizing centers (MTOC) which, in the case of animal cells, is the centrosome consisting of a pair of centrioles and the pericentriolar material. Nucleation of microtubules from its major component tubulin occurs at their minus end from the pericentriolar material. γ-Tubulin, a newer member of the tubulin super family, has been identified in many types of eukaryotic cells and is believed to play a key role in the nucleation of microtubules [for review, see Oakley, 2000]. γ-Tubulin also mediates the polarity of microtubules and dynamics at the minus end. Based on the immunofluorescence experiments, it is believed that γ-tubulin is primarily localized in the centrosome [Moritz et al., 1995]. However, a considerable amount is also present as complexes in the cytoplasm. In Xenopus oocytes, γ-tubulin exists as a large ring complex (γ-TuRC) whereas in drosophila, γ-tubulin is present both as a part of a large ring complex and a small complex (γ-TuSC) [Zheng et al., 1995; Oegema et al., 1999]. γ-TuRC has also been found in other types of mammalian cells including human cells in culture [Detraves et al., 1997; Murphy et al., 1998; Fava et al., 1999; Murphy et al., 2001]. The function of the cytoplasmic γ-tubulin complexes is not fully understood since in most mammalian cells, it does not nucleate microtubules in vivo. In contrast, γ-tubulin complex from Xenopus, drosophila, and cultured human cells can nucleate microtubules in vitro [Zheng et al., 1995; Oegema et al., 1999; Murphy et al., 2001]. However, it seems that cytoplasmic γ-tubulin complex is recruited to the centrosome and nucleation of microtubules from centrosome is dependent on the γ-tubulin complex. These are indicated by the facts that ring-shaped structures containing γ-tubulin have been observed in the pericentriolar material [Moritz et al., 1995; Vogel et al., 1997] and failure to recruit cytoplasmic γ-tubulin complex to the nascent sperm centrosome abolishes microtubule nucleation [Felix et al., 1994; Stearns and Kirschner, 1994]. It has been found

Abbreviations used: γ-TuRC, gamma tubulin ring complex; γ-TuSC, gamma tubulin small complex; HEK, human embryonic kidney; GTP, guanosine tri-phosphate; DAB, diaminobenzidine; ECL, enhanced chemiluminiscence; EM, electron microscope; OCT, optimum cutting temperature.The protein sequence data appears in the UniProt knowledge base under the accession numbers P85985 and P 85986.

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that γ-tubulin is recruited more in the centrosome at the onset of mitosis showing its importance in mitosis [Khodjakov and Rieder, 1999].

Brain microtubules have been extensively used to study the properties of microtubules and tubulin in vitro, since brain tissues contain the maximum amounts of microtubules. It has been shown earlier that microtubules in neurons are not attached to centrosome [Yu et al., 1993]. A later report showed that newly formed microtubules are attached to the centrosome for a short time [Ahmad and Baas, 1995]. Earlier studies described that in neurons, γ-tubulin was attached to the pericentriolar region and not to the microtubules of the axonal or dendritic region [Baas and Joshi, 1992]. Recent studies, however, showed the presence of γ-tubulin in microtubule preparations from mammalian brains [Detraves et al., 1997; Sulimenko et al., 2002]. Brain could be a convenient source for studying the function of cytoplasmic γ-tubulin complex. There is, however, one report that out of the two isoforms of γ-tubulin, one could have some brain-specific function [Yuba-Kubo et al., 2005]. These facts prompted us to study the brain γ-tubulin complex. We have found here that γ-tubulin complex in brain cytosolic fraction also exists as a ring. However, the most intriguing fact in brain γ-TuRC is the presence of a heterodimer α and β spectrin, which is not found in other tissues.

MATERIALS AND METHODS

Anti-β- and γ-tubulin antibodies and secondary antibodies used were from Sigma. PIPES, HEPES, GTP, and DAB were also from Sigma. Nonerythroid spectrin antibody was from Abcam or Chemicon, erythroid spectrin was from Chemicon. Alexa fluors were purchased from Molecular Probe, Inc. ECL reagent and protein A agarose were from Millipore and GIBCO BRL, respectively. γ-Tubulin C-terminal peptides were custom synthesized from Sigma Genosys. All other chemicals used were of reagent grade. OCT was from Electron Microscopy Sciences.

AMMONIUM SULFATE PRECIPITATION

Brain extract was made from homogenized goat brains by centrifugation at 105,000*g* at 4°C. The supernatant was subjected to different percentages of ammonium sulfate precipitations viz, 0–15% (S_{11}), 15–25% (S_{12}), and 25–50% (S_{13}). The pellets after centrifugation were dissolved in HEPES buffer (0.1 M HEPES, 1 mM EGTA, 1 mM $MgSO_4$, 100 mM NaCl, pH 6.9) containing 0.1 mM GTP and were cleared by centrifugation. The protein concentrations were determined by Bradford's method [1976].

ELECTROPHORESIS AND IMMUNOBLOT

Proteins were electrophoresed by SDS–polyacrylamide gels (8% and 5%) in a mini PROTEAN II gel system (Biorad) and stained with colloidal Coomassie blue. For immunoblot, proteins separated on SDS–PAGE gels were transferred to nitrocellulose membrane in a Biorad Trans blot apparatus. Blots were blocked with 5% nonfat dry milk in TBST (Tris-buffered saline containing 0.05% Tween-20) and incubated with primary antibody. Subsequently they were incubated with horse radish peroxidase-conjugated secondary IgG. The blots were developed either with DAB or ECL substrate.

AFFINITY CHROMATOGRAPHY

Antibodies were raised in rabbits against a 15mer peptide (AATRPDYISWGTQDK) corresponding to the C-terminal sequence of Xenopus γ-tubulin (custom made from Sigma Genosys), which is known to have about 97% sequence homology with mammalian γ-tubulin. The antibodies were purified from the serum using protein A agarose and could recognize γ-tubulin from goat brain homogenate in a similar way as a commercial antibody from Sigma (data not shown). An affinity column was prepared by coupling the purified antibodies to protein A agarose using the protocol of Harlow and Lane [1988]. Samples prepared by dissolving the required ammonium sulfate pellet were passed through the affinity column and was eluted using the 15mer peptide (0.4 mg/ml). The excess peptide was removed and the protein was concentrated by passing the eluent through a PD-10 column followed by spinning through an Amicon Ultra 5 kDa concentrator.

IMMUNOPRECIPITATION OF THE PROTEINS

The proteins obtained from 15% to 25% ammonium sulfate precipitation of brain extract were immunoprecipitated with 5 μg of anti-spectrin or anti-γ-tubulin antibody or 0.5 μl (1:100) of Bid antibody bound to 50 μl of protein A agarose (50% bead concentration). The protein A agarose beads were washed with PEM and blocked with 10% BSA or 0.6 M NaCl before binding to the antibodies. The immunoprecipitated beads were washed three times in PEM buffer with protease inhibitors (aprotinin 1 μg/ml, pepstatin 1 μg/ml, leupeptin 1 μg/ml, sodium orthovanadate 0.25 μg/ml, and PMSF 0.5 μg/ml). The bound proteins were resolved by 8% SDS–PAGE and transferred to PVDF membrane and the blots were sequentially developed with the required primary antibodies using chemiluminescence.

IN GEL TRYPSIN DIGESTION

For analysis of the new proteins, SDS–PAGE was performed and the bands were cut from the gel stained with colloidal Coomassie and subjected to trypsin in gel digestion. In gel digestion was carried out using a modified version of the protocol of Shevchenko et al. [1996]. The peptides were analyzed by LC tandem MS.

ANALYSIS BY NANOSPRAY LC–TANDEM MS

Peptides were separated on monolithic capillary HPLC columns. They were eluted directly into the mass spectrometer (Finnigan LCQ Deca XP Ion Trap) equipped with a nanospray ion source (ThermoFinnigan, San Jose, CA). All tandem MS spectra were manually examined and interpreted for sequence information and the derived sequences were searched for similarity profiles with related mammalian genomes using BLAST.

ELECTRON MICROSCOPY

The purified γ-tubulin complex were fixed with EM stabilization buffer (0.1 M PIPES, 2 mM EGTA, 2 mM $MgSO_4$, pH 6.95, containing 0.25% glutaraldehyde) and stained with 1% uranyl acetate on

carbon-coated grids. Subsequently they were viewed under a JEOL JEM-1011 electron microscope.

IMMUNOHISTOCHEMISTRY

Small pieces (0.5 cm × 0.5 cm) from the cortical region of fresh goat brains were cut and fixed overnight in 4% paraformaldehyde. They were dehydrated sequentially in 5% and 30% sucrose in PBS and embedded in OCT medium and stored at −80°C till microsectioning. Subsequently 13 μm tissue sections were prepared using cryomicrotome at −20°C and placed on gelatin-coated slides. The sections were washed to remove OCT and dipped in chilled methanol for 5 min and blocked for 30 min at 37°C in blocking solution (5% BSA, 5% normal donkey serum, and 0.2% triton X). They were then sequentially stained with rabbit anti-α nonerythroid spectrin and mouse anti-γ-tubulin (1:200 in blocking buffer) overnight at 4°C followed by alexa 568-conjugated anti-rabbit and alexa 488-conjugated antimouse IgGs for 1 h 30 min at room temperature. Subsequently they were stained with DAPI (0.1 μg/ml) and mounted in 70% glycerol in PBS and imaged in an Olympus BX 61 microscope fitted with a CCD camera.

RESULTS

PURIFICATION OF γ-TUBULIN COMPLEX

The cytosolic fraction and portions from different stages of purification of microtubules from goat brains, were tested for γ-tubulin content by SDS–PAGE and Western blot analysis. It was found that microtubule protein contained γ-tubulin after first or second cycle of polymerization, but no enrichment from the cytosolic fraction was achieved in these different stages of microtubule purification. The cytosolic fraction was subjected to different percentages of ammonium sulfate precipitations. Western blot analysis followed by densitometric scanning showed that 15–25% ammonium sulfate precipitate (designated as S_{12}) contained the maximum amount of γ-tubulin (data not shown).

The dissolved S_{12} precipitate was subjected to immunoaffinity chromatography using a column conjugated with an antibody raised against a 15mer peptide from the C-terminal sequence of γ-tubulin. The bound γ-tubulin was eluted using the same peptide. The SDS–PAGE gel profile of the eluted protein fractions showed a band at 48 kDa, two closely spaced bands at 55 kDa, four other proteins of molecular weights 75, 109, 135, 195 kDa and two major protein bands above 212 kDa (Fig. 1A). The bands at 48 and 55 kDa were confirmed as γ-tubulin and αβ-tubulin, respectively, by Western blots using γ-tubulin and β tubulin antibodies (data not shown). Excluding the high molecular weight proteins, the protein profile was consistent with the earlier reports, where γ-tubulin was shown to exist as a large complex [Zheng et al., 1995; Detraves et al., 1997; Murphy et al., 2001]. The relative amount of each protein in the purified γ-tubulin complex was determined by densitometry analysis using tubulin as standard. For 1 mol of γ-tubulin, 0.56 mol of αβ tubulin and approximately 0.47 mol of 195 kDa, 0.5 mol of 75 kDa, and 0.4 mol of each of the 109 kDa and 135 kDa proteins were observed. It is possible that the affinity purified fractions contained a heterogeneous population of large and small complexes as reported by Detraves et al. [1997]. This was not checked further. The concentration of γ-tubulin was found to be 3–7 μg/ml in the complex which was about 0.00035% of the total cytosolic protein.

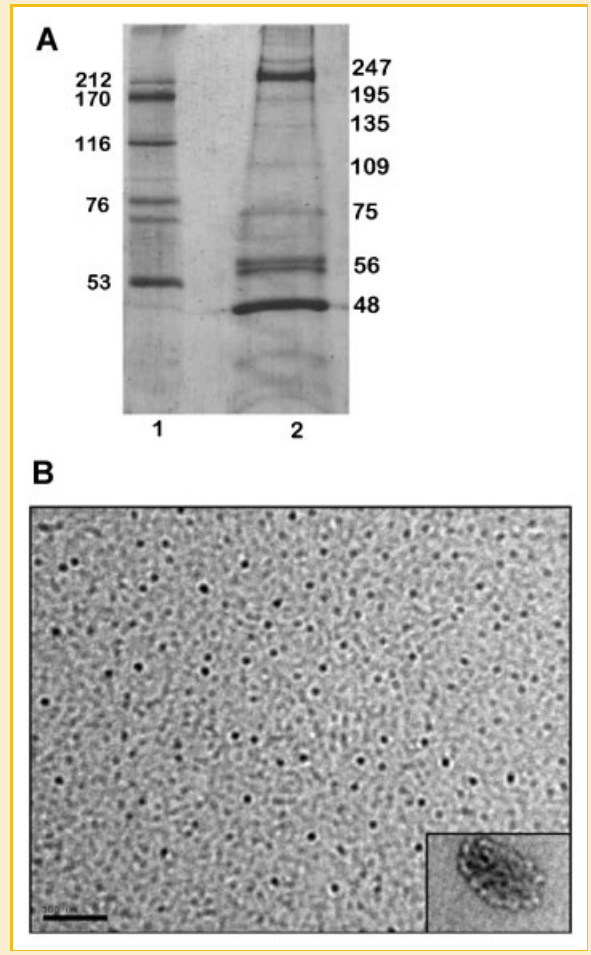

Fig. 1. Affinity purification of the γ-tubulin complex. A: The 15–25% ammonium sulfate precipitate of goat brain homogenate was dissolved in HEPES buffer and passed through an affinity column coupled with the antibody against a C-terminal peptide of γ-tubulin. The protein complex was eluted as described in the Materials and Methods Section and analyzed by (A) 8% SDS. Lane 1: marker, lane 2: purified γ-tubulin complex. B: Electron micrograph of a field of negatively stained γ-tubulin complexes. The purified γ-tubulin complex was fixed and stained as described and viewed at 40,000 magnification (scale bar represents 100 nm). Inset: The magnified image of a single γ-tubulin complex at 100,000×.

To know the details of the structure of the complexes, the purified complex was examined by electron microscopy. A ring structure of ~25 nm diameter was observed (Fig. 1B) which showed the presence of compartments at higher resolution (Fig. 1B, inset).

The mass of the components of the γ-tubulin complex were further confirmed by MALDI-TOF (data not shown).

IDENTIFICATION OF THE HIGH MOLECULAR WEIGHT PROTEINS IN BRAIN γ-TUBULIN COMPLEX

The high molecular weight bands were further resolved in a 5% gel where four protein bands were seen above 212 kDa (Fig. 2A), bands

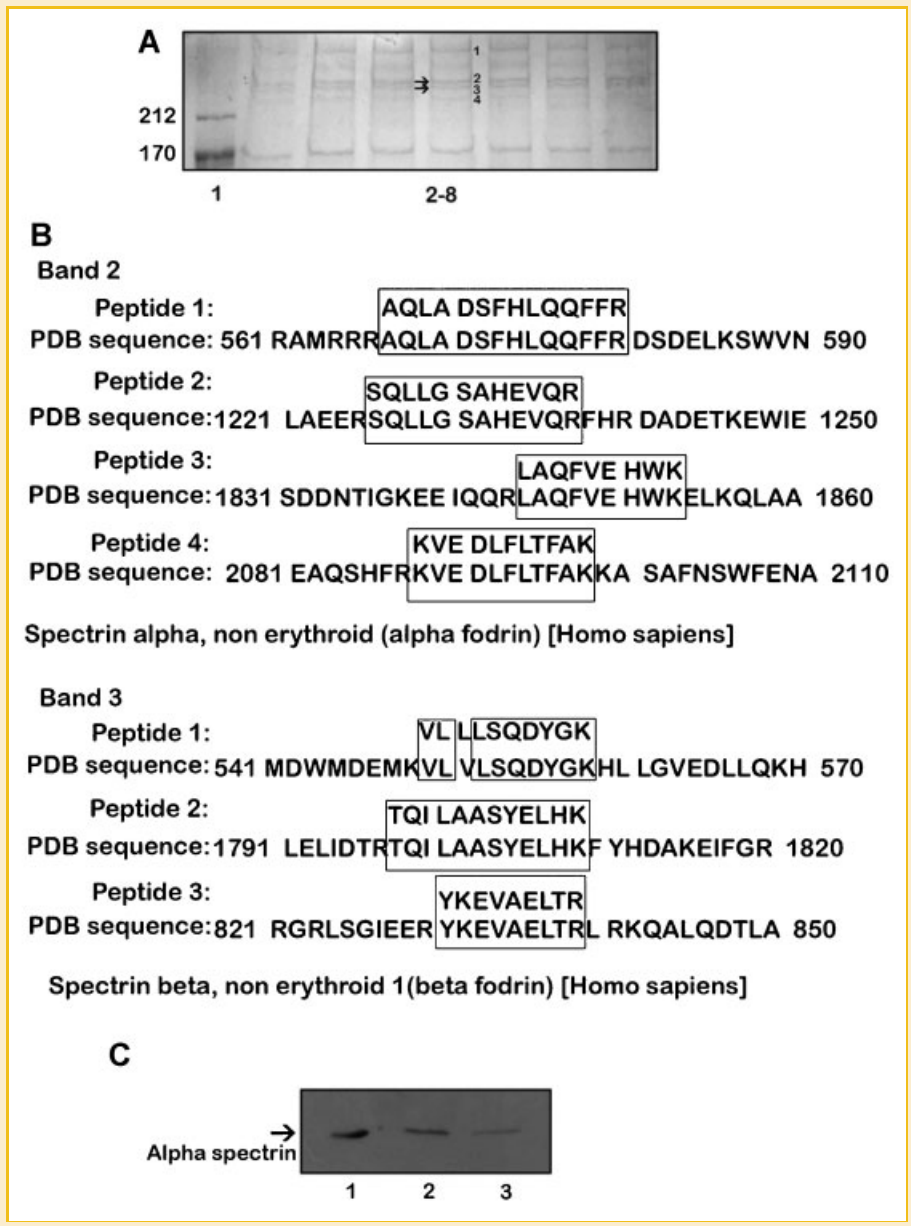

Fig. 2. Analysis of the high molecular weight bands. A: 5% SDS–PAGE. Lane 1: marker, lanes 2–8: eluted γ-tubulin complex fractions. B: Sequence analysis. The peptides obtained by in gel trypsin digestion of the protein bands shown with arrows in (A) were sequenced by LC/MS/MS and compared with the PDB sequences of α and β spectrin. C: Western blot of the purified γ-tubulin complex using anti-α-spectrin antibody. Lanes 1–3: Fractions of the eluted complex.

marked as 1, 2, 3, and 4. The high molecular weight proteins observed in the purified complex were not reported in the γ-tubulin complex of any of the species such as *Drosophila melanogaster*, *Xenopus*, *Aspergillus nidulans*, mouse and human cultured cells [Zheng et al., 1995; Akashi et al., 1997; Murphy et al., 1998; Oegema et al., 1999; Murphy et al., 2001]. In HEK cells, a 244 kDa protein was predicted from the cDNA of GCP6, although the molecular weight of GCP6 observed by SDS–PAGE was 219 kDa [Murphy et al., 2001]. An earlier report from brain, however, showed the presence of a protein band of about 250 kDa [Detraves et al., 1997].

The two major high molecular weight bands (band 2 and 3, shown with arrow in Fig. 2A) were subjected to in-gel trypsin digestion and analyzed by LC/MS/MS. The amino acid sequences of four peptides of the protein from band 2 and 3 peptides from band three were determined. The peptide sequences were submitted to UniProt knowledgebase database. The sequences were searched for similarity profiles with related mammalian sequences using BLAST search. Matches were found for all the four peptides of band 2 with spectrin α nonerythroid brain (*Homo sapiens*) (SwissProt accession no. Q13813) and with β spectrin nonerythroid 1 (*Homo sapiens*) (SwissProt accession no. Q01082) for all the three peptides of band 3. The peptide sequences and the regions of sequence similarities in α and β spectrins are shown in Figure 2B. The eluted fractions of the γ-tubulin complex on a 5% gel could also recognize an antibody

against α spectrin by Western blotting (Fig. 2C). This confirmed the presence of spectrin in brain γ-tubulin complex.

SPECTRIN IS A SPECIFIC COMPONENT OF γ-TUBULIN COMPLEX

Since spectrin is also an integral protein of brain, we wanted to check whether spectrin is indeed coming as a γ-tubulin complex interacting protein or it was a contaminant in our preparation. To verify this, the affinity purification was performed in presence of 0.6 M NaCl (Fig. 3A). A colloidal Coomassie-stained gel showed that in this condition also, the complex contained all the bands including spectrin. To further confirm the specific interaction between spectrin and γ-tubulin complex, immunoprecipitation of the complex was performed using protein A agarose coupled to either spectrin, γ-tubulin, or Bid antibody (negative control) or protein A agarose beads alone. The protein A-agarose beads were preincubated with 10% BSA followed by the coupling with the respective antibodies. The S_{12} pellet dissolved in PEM buffer was immmunoprecipitated with these antibody coupled beads and immunoblotted. Figure 3B shows the presence of spectrin and γ-tubulin coprecipitated in the blot. There were no bands from the Bid antibody coupled beads or protein A agarose alone indicating that spectrin is specifically bound to γ-tubulin complex. This confirmed that spectrin indeed is an interacting component of the γ-tubulin complex in brain tissues.

The immunoprecipitation was also performed for the S_{12} lysate which was dissolved in the presence of 0.6 M NaCl and this also showed the presence of both spectrin and γ-tubulin (Fig. 3C). This further confirmed the specificity of spectrin as a component of the γ-tubulin complex from brain and the high-affinity binding of spectrin to the complex.

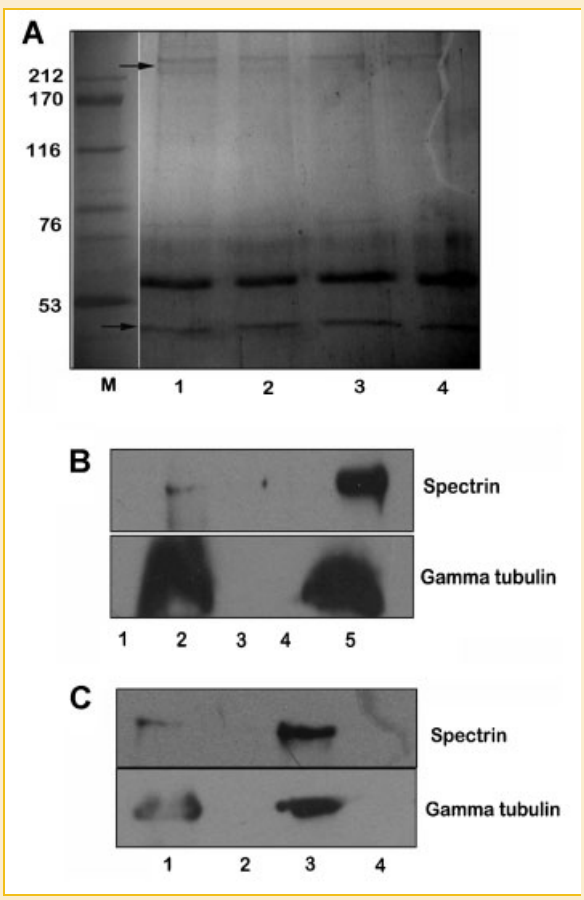

Fig. 3. Specificity of spectrin in the γ-tubulin complex. The ammonium sulfate precipitated pellet of brain homogenate was immunoprecipitated with spectrin, γ-tubulin, or Bid antibody coupled to protein A agarose blocked with 10% BSA or 0.6 M NaCl. The pelleted beads were subjected to immunoblot with the corresponding antibodies. A: 8% SDS–PAGE. Lane 1, 2: γ-tubulin complex fractions eluted in PEM buffer containing 0.6 M NaCl, lane 3, 4: γ-tubulin complex fractions eluted in PEM buffer. The arrows indicate the putative spectrin and γ-tubulin bands. B: Immunoprecipitation in the presence of 10% BSA. Lane 1: marker, lane 2: beads coupled to γ-tubulin antibody, lane 3: protein A agarose beads alone, lane 4: beads coupled to Bid antibody, lane 5: beads coupled to spectrin antibody. C: Immunoprecipitation in the presence of 0.6 M NaCl. Lane 1: Beads coupled to γ-tubulin antibody, lane 2: protein A agarose alone, lane 3: beads coupled to spectrin antibody, lane 4: marker.

SPECTRIN ASSOCIATION TO γ-TUBULIN COMPLEX IS TISSUE SPECIFIC

The presence of spectrin was then checked in other tissues as spectrin was not reported as a member of the γ-tubulin complex in any other tissues so far. An HEK cell lysate was prepared and subjected to immunoaffinity purification using the same antibody column. Figure 4A,B shows that at a similar concentration to brain extract, HEK cell lysate did not contain spectrin as a component of γ-tubulin complex as seen in both the colloidal Coomassie-stained gel and blot, although it showed most of the other members reported earlier in the gel. γ-Tubulin complex was also purified from the tissue homogenates of sperm, uterus, and liver. The purified complexes were then checked for the presence of spectrin. Equal amount of proteins were loaded and only the brain γ-tubulin complex detected nonerythroid spectrin along with γ-tubulin in an immunoblot (Fig. 4C). The γ-tubulin complexes eluted from different tissues including brain were also probed with antibody against erythroid spectrin to check whether complexes from these tissues contain this spectrin which is the common isoform of spectrin in these tissues. However, none of the complexes from these tissues showed the presence of erythroid spectrin (Fig. 4D). The lack of association of either isoform of spectrin with γ-tubulin complexes from other tissues indicates that nonerythroid spectrin in brain γ-tubulin complex could have a special role.

CO-LOCALIZATION OF NONERYTHROID SPECTRIN AND γ-TUBULIN COMPLEX IN BRAIN TISSUE

To check whether the association of spectrin and γ-tubulin complex had physiological significance, the proteins were immunostained in brain tissue sections prepared from the cortical region. Figure 5 shows the co-localization of the proteins mainly in the extranuclear region.

DISCUSSION

The relatively new member of the tubulin super family, γ-tubulin, is now accepted as the major mediator of microtubule nucleation.

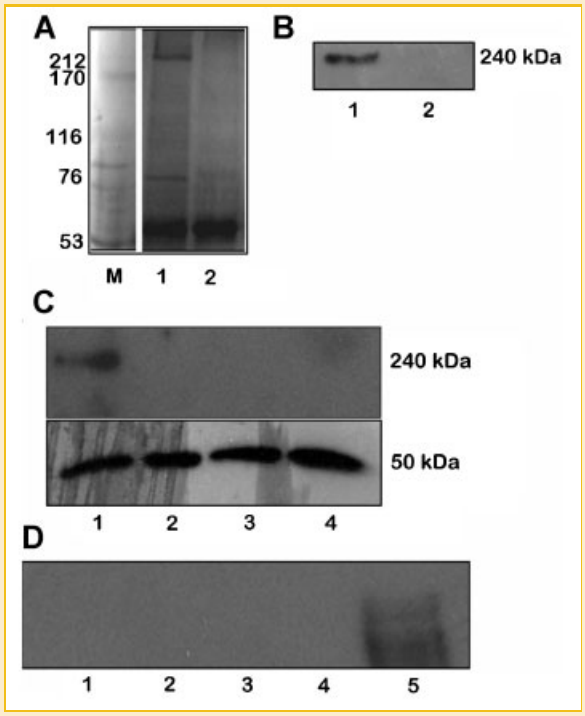

Fig. 4. Tissue specificity of spectrin association to γ-tubulin complex. γ-Tubulin complex was eluted using immunoaffinity purification from brain ammonium sulfate precipitated protein extract, HEK cell lysate, sperm, uterus, and liver tissue extracts as described in the Materials and Methods Section. γ-Tubulin complex fractions were subjected to (A) 5% SDS–PAGE (B) immunoblot using antibody against nonerythroid spectrin. Lane 1: from brain extract, lane 2: from HEK cell lysate. Two micrograms of the complex were loaded in both cases. C: Immunoblot of different γ-tubulin complexes probed with nonerythroid spectrin antibody lane 1: brain tissue, lane 2: sperm tissue, lane 3: uterus tissue, lane 4: liver tissue. D: Immunoblot using antibody against erythroid spectrin. Lane 1: brain tissue, lane 2: sperm tissue, lane 3: uterus tissue, lane 4: liver tissue, lane 5: RBC lysate showing the presence of erythroid spectrin. Equal amounts of the purified γ-tubulin complexes from various tissues were loaded in all lanes.

γ-Tubulin mediates nucleation in conjugation with some other proteins, with which it exists as a complex [Zheng et al., 1995; Oegema et al., 1999]. Microtubule nucleation preferably initiates from the MTOCs, which in case of higher organisms, is the centrosome. However, there are reports that in the absence of available centrosomes or in certain organisms, nucleation can happen from other sites, for example, from plasma membrane in plants [Murata et al., 2005] and drosophila [Mogensen and Tucker, 1987] and from Golgi membrane [Chabin et al., 2001]. Evidences from immunocytochemistry experiments show that the major amount of γ-tubulin is present in the centrosome. However, a considerable amount is present in the cytoplasm also. The relevance of the presence of γ-tubulin complex in the cytoplasm has been a topic of debate for quite some time. It is anticipated that an equilibrium exists between the cytoplasmic and centrosomal γ-tubulin and there is evidence that γ-tubulin is recruited more in the centrosome during the onset of mitosis [Khodjakov and Rieder, 1999], but it is not well known how the complex gets transported to the centrosome. However, in the last few years, reports are coming up to show the involvement of some proteins like NEDD1, pericentrin, CDK5RAP2 in the anchoring of the γ-tubulin complex to the centrosome [Zimmerman et al., 2004; Haren et al., 2006; Fong et al., 2008]. All the γ-tubulin complex proteins that were found in the cytoplasmic complex were also identified in the centrosome. γ-Tubulin complex has been purified from the cytoplasm of different tissues and organisms and many of them were shown to nucleate microtubules in vitro. It can thus be anticipated that there could be one or more controlling factors in the cytoplasm which prevent the complex to nucleate from cytoplasm. These factors possibly got lost during purification of the cytoplasmic γ-tubulin complex from tissues reported earlier. It could be expected that the control mechanism is governed by the anchoring or transport of the complex to the centrosome.

Brain tissues contain the largest amount of microtubules whose functions include the targeting of neuronal components, intracellular transport, and cellular movement through axons and dendrites. In this study, we have observed that, in the brain cytosol, γ-tubulin exists as a ring complex with internal compartments, whose diameter and structure are similar to the complexes reported earlier from other tissues.

γ-Tubulin (48 kDa), αβ-tubulin (56 kDa), and the other components of the complex corresponding to molecular weights 75, 109, 135, 195 in the brain γ-tubulin complex are similar to those of similar molecular weight proteins found in the complexes from other sources. Here we also report the presence of four high molecular weight proteins which were not reported in γ-tubulin complex purifications from other tissues. An earlier report from mammalian brains, however, showed the presence of a 250 kDa protein in the immunoprecipitated γ-tubulin complexes [Detraves et al., 1997]. Two major protein bands of these high molecular weight proteins were identified as α and β spectrins of nonerythroid nature. Spectrin is a membrane bound protein which forms a flexible network. Since microtubule nucleation can happen from membranes in certain cases, spectrin might be involved in transporting γ-tubulin complex to cortical regions. Spectrin has also been reported to interact with centractin which is a centrosomal actin like protein [Holleran et al., 1996]. So it can also help in the anchoring of γ-tubulin complex to the centrosome through its membrane associated network. Co-localization of γ-tubulin complex with spectrin in brain tissue sections indicates the involvement of spectrin in the transport or function of γ-tubulin complex. At this point, it is not clear why the spectrin that we find is present only in the γ-TuRC of brain tissues. One special feature of brain tissue is that 10% of the brain cells are neurons which are highly flexible, fully differentiated, and microtubules in neurons are arranged into noncentrosomal nonradial arrays [Bartolini and Gundersen, 2006]. Further studies with different cell types of brain may throw light on the specific role of this protein.

CONCLUSION

The brain γ-tubulin complex was affinity purified and characterized. Electron microscopic examination showed that it had a ring structure of 25 nm diameter similar to the complexes reported from

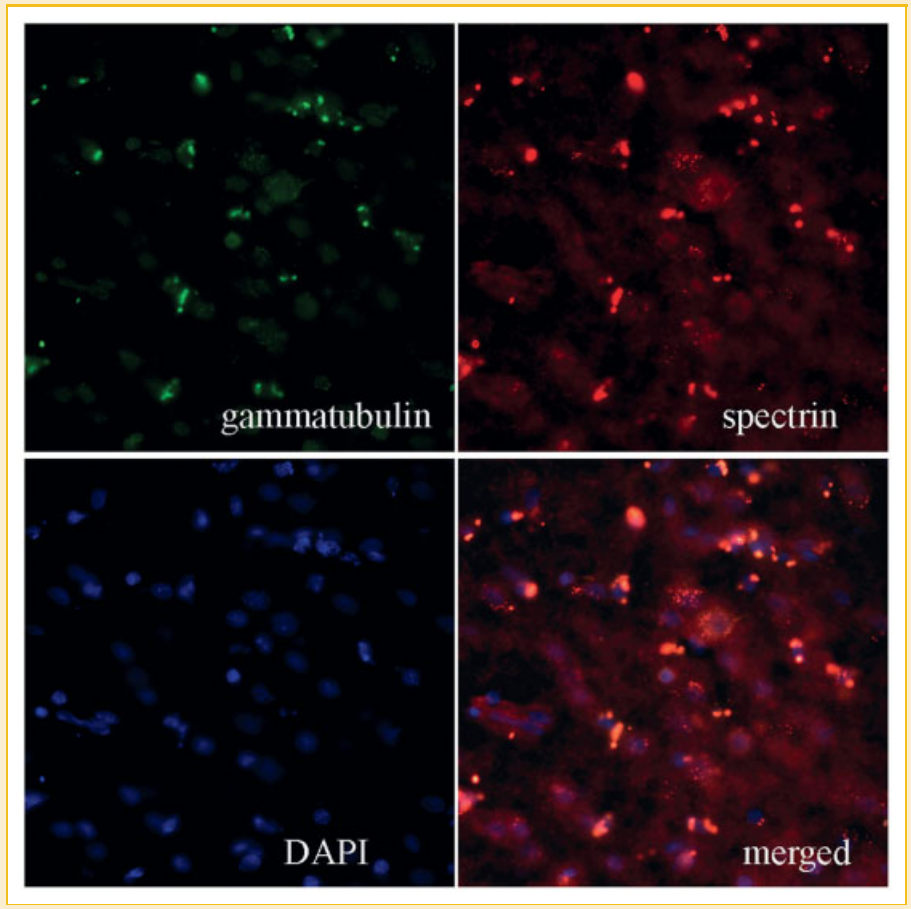

Fig. 5. Co-localization of γ-tubulin complex and spectrin in brain tissue. Brain tissue sections of 13 μm were stained with antibodies against γ-tubulin and spectrin followed by respective secondary antibodies conjugated with alexa fluors and DAPI as described in the Materials and Methods Section. Subsequently they were mounted and viewed.

other tissues. However, along with the other reported members, the complex also contained the heterodimeric protein spectrin of nonerythroid origin. The association of spectrin to the brain γ-tubulin complex was tissue specific and was not found in the complexes purified from liver, sperm, and uterine tissues or HEK cell lysate. Co-localization of γ-tubulin with spectrin in brain tissue indicates that this association is physiologically significant.

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