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Research Article

Microtubule associated protein tau binds to double-stranded but not single-stranded DNA

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Abstract. Tau, a major microtubule-associated protein of the neuron, which is known to promote the assembly of and to stabilize microtubules, has also been seen associated with chromatin in neuronal cell lines, but its role in this subcellular compartment is still unknown. In this study, the binding of tau to DNA was investigated using the electrophoretic mobility shift assay. Using polynucleotide as probe, we found that tau bound to double-stranded but not to single-stranded DNA. Formation of tau-polynucleotide complex was disrupted by alkaline pH and a high concentration of NaCl, but was not affected by dithiothreitol. Electron microscopy revealed that the protein associated with the nucleic acid in a necklacelike manner. DNA-cellulose chromatography and radioimmunodot-blot analyses showed that calf thymus histones VI-S, VII-S and VIII-S could replace both recombinant human brain tau_{352} (tau-23) and tau_{441} (tau-40) from DNA. Thus, tau appears to bind to DNA reversibly in the presence of histones.

Key words. Human neuronal tau; nuclear tau; DNA; DNA-binding protein; double-stranded DNA; electrophoretic mobility shift assay.

Tau, as a major microtubule-associated protein of the neuron [1], functions mainly in promoting the assembly and maintenance of microtubules, which are required for axonal transport and morphogenesis [2]. In a normal neuron, tau is localized in the axons and neuronal soma [3–5] and the function of tau is regulated by phosphorylation [6]. The discovery of tau as the major protein subunit of paired helical filaments/neurofibrillary tangles in Alzheimer's disease has markedly stimulated interest in understanding the structure and functions of this protein [7–9].

In addition to its major association with microtubules, tau has been found associated with ribosomes [7, 10] and has been shown to be localized in nuclei of human neuroblastoma cells, human cervical carcinoma, human macrophages, monkey kidney and PC12 cells [11–13]. Its localization has been observed at the nucleolar regions of the acrocentric chromosomes of human neuroblastoma cells, associated with both fibrillar regions of interphase nucleoli and the nucleolar organizer regions [11, 14]. Microtubule-associated proteins have been shown to have a higher affinity for DNA than for microtubules and their removal from the microtubules by DNA causes microtubule breakdown [15]. Separation of tau from LA-N-5 nuclei in the chromatin fraction has indicated the direct or indirect association of nuclear tau with DNA [16].

A potential function of neuronal tau was suggested by our recent experiments in which it increased the melting tem-

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perature of both calf thymus DNA and plasmid pBluescript-II SK [17]. Furthermore, we have shown that phosphorylated tau catalyzed by neuronal cdc2-like kinase associated with DNA in an electrophoretic mobility shift assay [18]. Similar to native tau, phosphorylated tau could increase the melting temperature of calf thymus DNA. When tau was aggregated, neither the native tau nor phosphorylated forms retained the ability to interact with DNA, suggesting that the binding of tau to DNA was in an aggregation-dependent and phosphorylation-independent manner. Here, we show that human neuronal tau associates and disassociates with DNA, and describe some properties of their interaction.

Materials and methods

Materials

Sephadex G50, Q-Sepharose and SP-Sepharose were purchased from Pharmacia; calf thymus DNA (CTDNA, readily soluble, suitable as substrate in DNase assays), DNA-cellulose (double strands), poly-Lys, histones VI-S (Lys-rich subgroup), VII-S (Lys-rich subgroup f_{2b}) and VIII-S (arginine-rich subgroup f_3) were from Sigma (St. Louis, Mo.); PolydIdC was from Boehringer Mannheim; *Eco*RI was from GIBCO; and I^{125}-labeled secondary goat anti-rabbit IgG was from NEM Life Science Products. Monoclonal antibody Tau-1 (ascites, 1:50,000 [4]) was a kind gift of Dr. Lester Binder, Northwestern University, Chicago, Ill. Other reagents used were of analytic grade without further purification. Plasmids (pEGFP-N1 and pET-15b) were from Promega. T7TsRx1 was a kind gift from Dr. Barsacchi of Pisa University [19]. T7Ts was constructed through inserting 5′ and 3′ untranslated regions of *Xenopus* β-globin mRNA into pGEM-4Z. The fragment of XRx1 (nt 250–1230), which was digested with *Eco*O109I and *Nde*I, was cloned in the *Eco*RV site of T7Ts. All DNA samples were purified according to *Molecular Cloning* [20] before use.

Expression and purification of recombinant human brain tau

The prokaryotic expression vector Prk172, bearing either tau_{352} or tau_{441}, was kindly provided by Dr. Goedert (Medical Research Council, Molecular Biology Unit, Cambridge, UK). The constructs were expressed and protein-purified as described before [21] except that the perchloric acid extraction was avoided. The phosphocellulose-purified tau was further purified on a Sephacryl 300 column. The tau fraction was detected using the Tau-1 antibody, and the peak was concentrated, dialyzed and lyophilized. All steps were carried out at 4 °C. After dialysis against 5 mM MES and 0.05 mM EGTA, pH 6.8, tau was aliquoted, lyophilized and stored at –75 °C until use. The purity of each tau was checked by SDS-PAGE which yielded a single major protein band for each tau isoform. Tau_{441} was also purified sequentially with Q-Sepharose, SP-Sepharose and Sephadex-G75 columns and the concentrations of the purified protein determined spectrophotometrically, by using $E^{280}_{mg/ml} = 0.27$ [22, 23].

Agarose gel retardation assays

Three structural types of plasmid pEGFP-N1, supercoiled, linearized and single-stranded DNA (ssDNA), were incubated with tau at different tau/DNA molar ratios (0, 5, 25, 125, 250, 375 and 500) in 25 mM Na_2HPO_4-NaH_2PO_4 (pH 7.2) at room temperature for 30 min. Linearized DNA was obtained by digested plasmid using restriction endonuclease *Eco*RI. Single-stranded pEGFP-N1 was prepared by heating the plasmid to 100 °C for 5 min then quenching it to 0 °C [24]. Its absorbance at 260 nm rose from 0.912 to 1.254. To examine whether interaction of tau with DNA was related to charge effect, different amounts of NaCl (0, 50, 100, 200, 300, 400, 600, 800, 1000 and 1200 mM) were added to the mixture of tau and DNA (molar ratio tau/plasmid was 500:1) and incubated at room temperature for 30 min. All samples were loaded directly onto 1% (w/v) agarose gel in 1 × TBE buffer and electrophoresed at 55 V for approximately 1.5 h.

Electrophoretic mobility gel shift assay

A polynucleotide was arbitrarily synthesized according to the DNA motif of mouse N-Oct-3 [25]. To anneal effectively, five more nucleotides were added at the two ends. 25-polynucleotide a: 5′-AGCT CTCCG TGCAT AAATA ATAGG C-3′, and 5′ labeled with digoxin. Polynucleotide b: 5′-TCGAG CCTAT TATTT ATGCA CGGAG-3′. The two strands were annealed. The competitor polynucleotides were designed as follows with the sequences: (α) 5′-GCAAG AATTC TCATG TTTGA C-3′; (β) 5′-CGAGG ATCCG GCTGC TAA-3′; (γ) 5′-GAGGA TCCTT ACCAA GGCTT GCCAA TAAAC-3′; (δ) 5′-ACCAT ATGCA CCTGC ACAGC CCTTC CC-3′ and (ε) 5′-GCATA TCCGG ATATA GTTC-3′. All these polynucleotides were synthesized by Sangon Company.

An aliquot of the dig-labeled probe (0.6 µM) was used for a binding reaction with tau protein. The probe and tau protein were incubated in a 10-µl reaction mixture that contained 20 mM Hepes (pH 7.6), 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol (DTT), 0.2% (w/v) Tween 20 and 30 mM KCl for 20 min at room temperature. The double-stranded DNA (dsDNA)-tau complexes were resolved in a nondenaturing 5% polyacrylamide gel in 0.25 × TBE running buffer (4 °C, 8 V/cm). After electrophoresis, the gels were electroblotted (250 mA, 1 h, 4 °C) onto nylon membrane, then fixed in a UV Crosslinker FB-UVXL-1000 from Fisher Scientific. NBT and BCIP (Boehringer Mannheim) were used as the colorimetric detection reagents. Unless specified other-

wise, the tau concentration used in the gel mobility assays was approximately 1.2 µM.

Electron microscopy

Fifteen microliters of pET-15b (15 µg, in 10 mM Tris-HCl, 1 mM EDTA, pH 7.2) was added to 25 µl of tau (35 µg, in 25 mM Na_2HPO_4-NaH_2PO_4, pH 7.2). The mixture was incubated [room temperatute (RT), 30 min] and then fixed with 0.3% glutaraldehyde (RT, 10 min). The samples were separated from free proteins by filtration through a 2-ml column of Sepharose CL-6B (Pharmacia Biotech) equilibrated with 10 mM Tris-HCl, pH 7.9. The filtrate was absorbed to glow-discharged thin carbon-coated 300 mesh copper grids, dehydrated through a graded water-ethanol series, fixed with 0.2% glutaraldehyde (RT, 20 min), and rotary shadow-cast with Pt. Samples were visualized using a JEOL JEM-100CX electron microscope.

Micro-chromatography of DNA-cellulose

DNA-cellulose (0.1 g) was resuspended in a standard buffer containing 10% (v/v) glycerol, 10% (w/v) BSA, 1 mM EDTA, 50 mM NaCl, 1 mM DTT, and 20 mM Tris-HCl (pH 7.5). Prior to use, it was kept at 4 °C overnight as described elsewhere [26]. It was then washed (1000 rpm, 4 °C, 2 min) with the same buffer five times (1 ml each) in an Eppendorf tube and then suspended in the same buffer to a final volume of 2.5 ml, in which DNA-cellulose contributed ~0.5 ml to the volume. Approximately 200 µg tau/0.625 ml was added to the DNA-cellulose suspension, with an occasional shake, at 37 °C for 30 min to mix tau with DNA. The DNA-cellulose was spun down and washed with the Tris-HCl buffer four times (1 ml each). Each wash was assayed by immunodot-blot [27] to confirm the removal of any unbound tau before the DNA-cellulose suspension was divided into 25-µl aliquots in Eppendorf tubes. Histones (1 mg/ml) VI-S, VII-S and VIII-S were individually dissolved in phosphate buffer (25 mM Na_2HPO_4-NaH_2PO_4, pH 7.5) before use. DNA-cellulose (25 µl) was incubated with 50 µl of 1 mg/ml histone at 37 °C for 30 min, with an occasional mixing with a Vortex mixer. The sample was then spun down and the supernatant was assayed for tau by radioimmunodot-blot. At the same time, elution by poly-Lys was used as a control.

Radioimmunodot-blot

Radioimmunodot-blot was performed as described previously [27]. Five microliters of sample (containing 0.1–2.0 ng tau) was applied onto the nitrocellulose membrane before being dried at 37 °C for 1 h. Then it was blocked in 20 mM Tris-HCl buffer (pH 7.5), containing 150 mM NaCl and 5% BSA. Rabbit antibody R134d to tau (1:5000) was employed as the primary antibody, sequentially reacted with an I^{125}-labeled secondary antibody (anti-rabbit, 1:100). The radioimmunoreactivity of the blots was visualized and quantitated using a Phosphorimager (Fujifilm BAS-1500) and TINA 2.0 software (Raytest Isotopenmeßgeräte).

Results

Tau binds to double-stranded but not single-stranded plasmid DNA in a nonsequence-specific manner

To confirm whether tau binds to DNA and to understand the characteristics of such binding, the gel retardation assay (electrophoretic mobility shift assay, EMSA) was used. A distinct retardation of the plasmid, with the DNA mobility depending on the tau/plasmid (pEGFP-N1) molar ratio (fig. 1A, B) was observed in agarose gels. When the molar ratio was around 250, the retardation became clearly detectable. Similar results were obtained using plasmid T7TsRX1 and pET-15b (data not shown). On the other hand, no retardation was detected in the gel when ssDNA was incubated with tau (fig. 1C) or dsDNA was incubated with BSA (fig. 1A–D). These findings suggested that tau bound to dsDNA, either supercoiled, relaxed closed-circular or linear plasmid, but not to ssDNA. The high molar ratio of tau binding to DNA, as mentioned above, showed that the binding might not be sequence specific. Tau may bind to DNA by a charge effect. This was confirmed by increasing the NaCl concentration to interfere with the retardation of the plasmid (fig. 1E). When the NaCl concentration was around 300 mM, the DNA became slightly retarded. When the NaCl reached 800 mM, the sample did not retard.

Interaction of tau with double-stranded probe

Since the size of the DNA fragment is normally kept below ~250 base pairs to enable clear distinction of the probe from any complexes, the plasmids (pEGFP-N1, 4733 bp; pET-15b, 5708 bp; T7TsRX1, 4002 bp) were larger than the size suitable for the gel shift assay. Thus, we employed a labeled polynucleotide probe for further studies (see below). We arbitrarily prepared a pair of polynucleotides (25 bp, complementary to each other) labeled with digoxin and then annealed them as a probe. The interaction of tau with double-stranded nucleic acids was examined by EMSA. Tau was added in increasing concentrations (0.06–1.8 µM) to a constant amount of the digoxin-labeled DNA probe (0.6 µM, fig. 2A). The retardation of electrophoretic mobility of the double-stranded polynucleotide was found to be more distinguishable as the tau concentration increased. We analyzed the intensity of both the unretarded and retarded bands using Advanced American Biotech Imaging and calculated the ratio of free DNA probe over the total probe at each tau concentration. The molar ratio of tau and DNA for half DNA binding to tau was approximately 1.5 (the

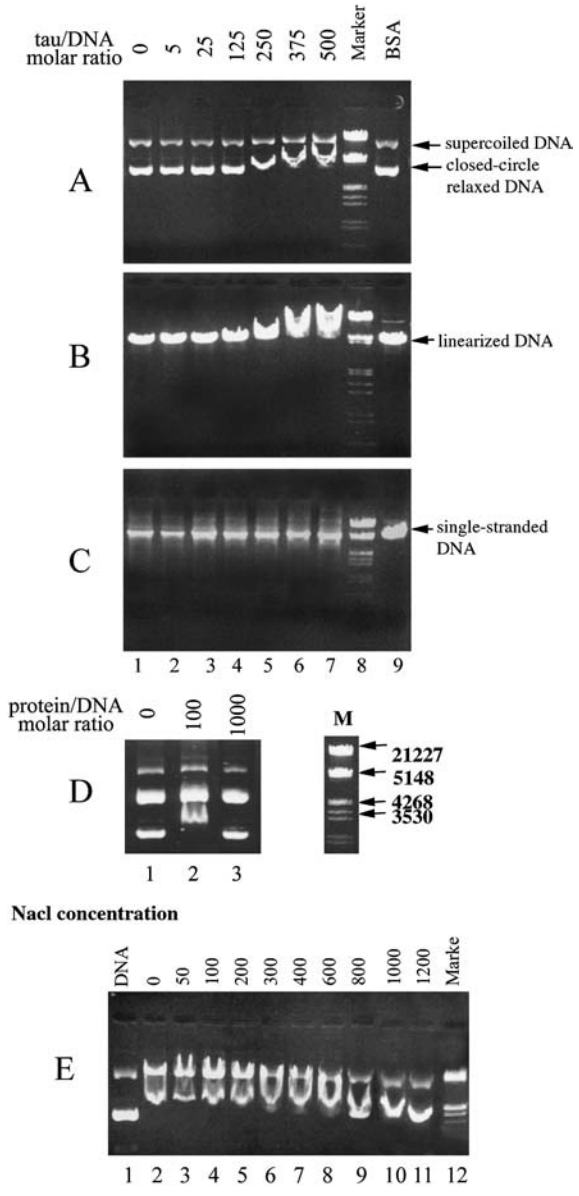

Figure 1. Gel retardation assay of protein-DNA binding. Recombinant tau_{441} or BSA was incubated with plasmid DNA (pEGFP-N1, 4733 bp; T7TsRx1, 4002 bp) at different molar ratios of protein/DNA (as indicated above each gel lane) in 25 mM Na_2HPO_4-NaH_2PO_4 (pH 7.2, at RT for 30 min). Aliquots were then electrophoresed in a 1% (w/v) agarose gel and the DNA bands were visualized by the AmpGene Gel 1000 Gel Imaging system. (*A*) Interaction of different molar ratios of tau and plasmid pEGFP-N1 containing supercoiled DNA and closed-circular relaxed DNA. (*B*) Different molar ratios of tau and linearized pEGFP-N1 (by *Eco*RI). (*C*) Different molar ratios of tau and single-stranded pEGFP-N1. Lanes 1–7 represent different molar ratios of tau and three types of pEGFP-N1 DNA (0, 5, 25, 125, 250, 375 and 500, respectively); lane 8, marker (*λ*DNA*Hin*dIII + *Eco*RI); lane 9, BSA incubated with DNA (molar ratio 1000) used as control. (*D*) T7TsRx1 alone (lane 1), with tau (lane 2) and with BSA (lane 3). (*E*) Gel retardation assay of tau-dsDNA interactions in the presence of different concentrations of NaCl. Lane 1, plasmid DNA; in lanes 2–11, tau was incubated with plasmid DNA (molar ratio 500:1) in the presence of various concentrations of NaCl (0, 50, 100, 200, 300, 400, 600, 800, 1000 and 1200 mM, as final concentration, respectively); lane12, *λ*DNA/*Hin*dIII + *Eco*RI used as molecular marker.

tau concentration was around 0.9 μM with DNA concentration 0.6 μM; fig. 2B). In this case, one would have thought that each additional bound molecule should cause further retardation. According to some reports [28], the absence of intermediate bands may imply that the binding was cooperative.

The complex was formed when tau and the double-stranded polynucleotide were mixed together (fig. 2C). A 40-fold excess of polydIdC could eliminate the binding of dsDNA to tau (lane 3), suggesting that tau interacted with dsDNA in a non-sequence-specific manner. Moreover, as described above, tau protein bound to both pET-15b and T7TsRX1, without any complementary and similar sequences compared to the 25-bp probe. Nevertheless, formation of the complex appeared to depend on the double-stranded nucleotide chain, since none of the several ssDNA polynucleotides could prevent the complex formation, even when added at a 100-fold excess (lanes 4–8). The failure of tau to bind to the single-stranded polynucleotide revealed that the double strands were a precondition for tau interaction with DNA.

To determine the time kinetics of the formation of the tau-DNA complex, incubations were performed for various periods of time at room temperature. The results of the EMSA revealed that the minimum time required for tau to interact with DNA was not more than 10 s. Incubations at different time intervals from 10 to 3600 s did not result in a change in the intensity of the complex band (data not shown), suggesting that the complexes were formed within 10 s.

Effects of tau concentration, DTT and pH on interaction of tau with dsDNA

Interactions of tau with dsDNA were examined in solutions at different pH, ranging from 1.5 to 13. No significant change in the affinity of tau interaction with dsDNA by EMSA was detected between pH 1.5 to 10.5 (fig. 2D). At pH higher than 10.5, the interaction was reduced. The weak tau-DNA interactions at the high pH were probably due to the strong basic pI of tau.

The effect of the reducing agent DTT on formation of the tau-dsDNA complex was detected by incubating tau with dsDNA at different concentrations of DTT (0.1–100 mM) (fig. 2E). Up to 50 mM DTT, tau-dsDNA interactions were not affected. Even at 100 mM, the inhibitory effect of DTT on the formation was not marked. Therefore, the reduced thiol group was barely involved in binding activity of tau to dsDNA, although tau_{441} contained two Cys residues (Cys-291 and Cys-322).

Formation of the tau-DNA complex

Next, we investigated by electron microscopy the binding of tau to DNA. This binding resulted in beadings of plasmid DNA (fig. 3). The protein clustered evenly along the DNA double strands like a necklace. Such a clustered

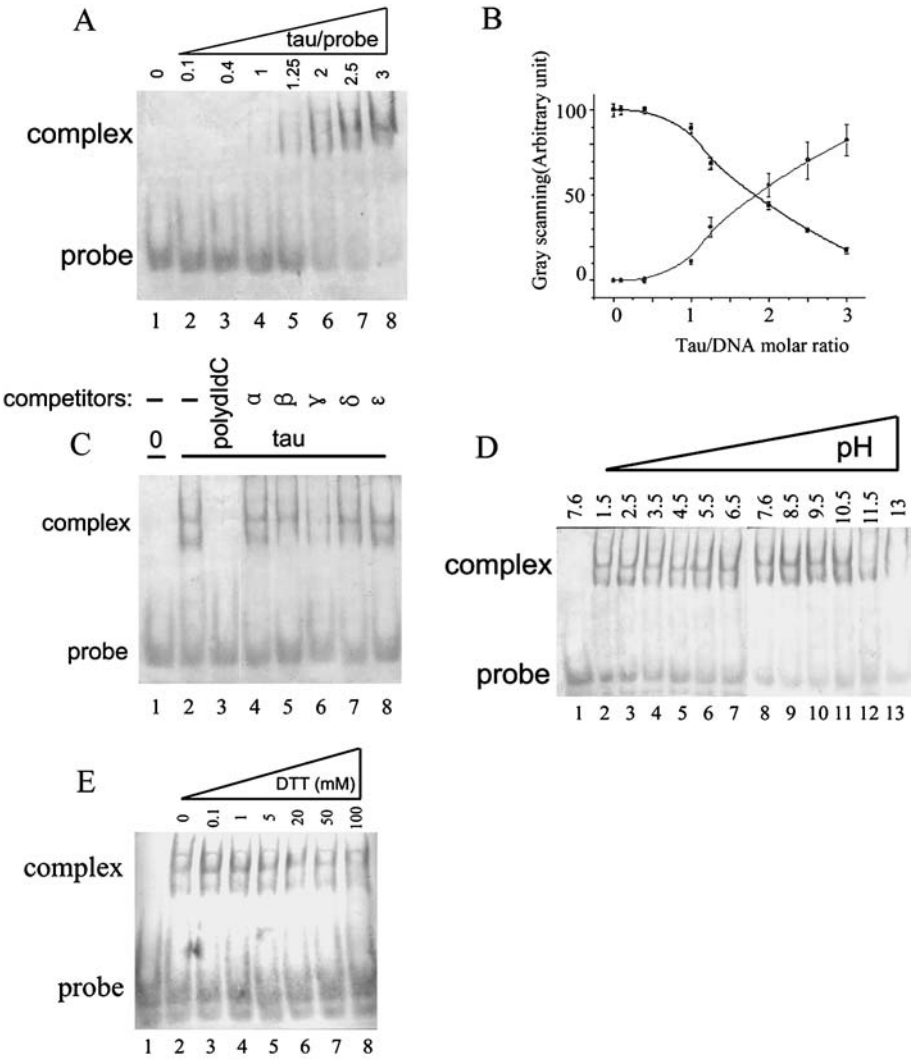

Figure 2. EMSA of tau with double-stranded probe. A polynucleotide as a probe was synthesized according to the DNA motif of mouse N-Oct-3 [25]. The labeled one was 5′-AGCT CTCCG TGCAT AAATA ATAGG C-3′ (digoxin labeled at 5′), and the two complementary single-stranded polynucleotides were made double stranded by annealing both strands. The probe and tau protein were incubated in a 10-µl reaction mixture that contained 20 mM Hepes (pH 7.6), 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, 1 mM DTT, 0.2% (w/v) Tween 20 and 30 mM KCl for 20 min at room temperature. (*A*) Tau incubated with the probe (0.6 µM) at different molar ratios. Lane 1, free probe; lanes 2–8, samples at different molar ratios (tau/DNA probe) of 0.1, 0.4, 1, 1.25, 2, 2.5 and 3, respectively. (*B*) Gray scanning of the data in *A*. Curves represent the free probe (black), and tau-probe complex (gray), respectively. **(***C*) Tau-dsDNA interactions in the presence of competitors. The competitor single-stranded polynucleotides employed were as follows: (*α*) 5′-GCAAG AATTC TCATG TTTGA C-3′; (*β*) 5′-CGAGG ATCCG GCTGC TAA-3′; (*γ*) 5′-GAGGA TCCTT ACCAA GGCTT GCCAA TAAAC-3′; (*δ*) 5′-ACCAT ATGCA CCTGC ACAGC CCTTC CC-3′ and (*ε*) 5′-GCATA TCCGG ATATA GTTC-3′. Lane 1, probe only; lane 2, probe (0.6 µM) incubated with 1.2 µM tau; lane 3, same as lane 2 plus 40-fold excess of polydIdC; lanes 4–8, same as lane 2 plus a 100-fold excess of unlabeled competitor single-stranded polynucleotide. For details of EMSA see Materials and methods. *(D)* Effects of pH on the tau-DNA interaction. Lane 1, free probe; lanes 2–13, 0.6 µM probe incubated with 1.2 µM tau in different pH buffers. The pH value is indicated above each lane. **(***E***)** Tau-dsDNA interactions in the presence of different concentrations of DTT. Lane 1, free probe; lanes 2–8, 0.6 µM probe incubated with 1.2 µM tau in the presence of various concentrations of DTT (0, 0.1, 1, 5, 20, 50 and 100 mM, as final concentration, respectively).

structure approximately resembled the binding manner of some specific DNA-binding proteins proposed by Travers et al. [29]. A significant length of DNA strands was organized by protein into a curved conformation (fig. 3B). This may be because the nucleotide chain of the plasmid was in a circular configuration. However, tau binding to calf thymus DNA did not result in a curved conformation of the nucleotide chain, although the combination of tau with DNA was in a necklace manner (data not shown).

The diameter of the DNA and the beads (the bound tau protein) were found to be ~9.8 ± 2.2 nm and ~25.3 ± 8.2 nm (means ± SD), respectively. These values were considerably larger than the DNA ~2 nm and ~5 nm for that of a 50-kDa protein. But these results might have been due to the rotary shadow-cast with Pt when prepar-

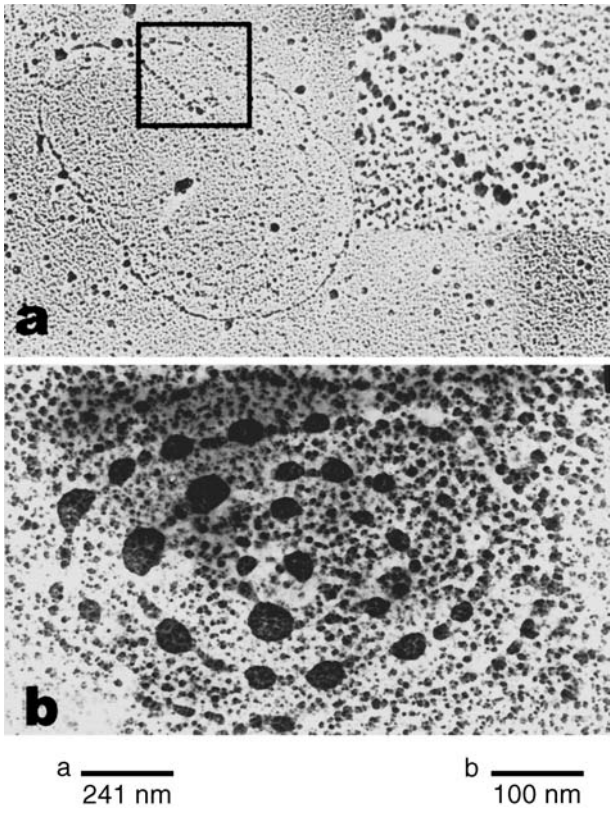

Figure 3. Visualization of tau associated with pET-15b plasmid by electronic microscopy. Fifteen microliters of pET-15b (15 µg, in 10 mM Tris-HCl, 1 mM EDTA, pH 7.2) was added to 25 µl of tau (35 µg, in 25 mM Na_2HPO_4-NaH_2PO_4, pH 7.2). The mixture was incubated (RT, 30 min) and then fixed with 0.3% glutaraldehyde (RT, 10 min). The DNA-bound tau was separated from free protein by filtration through a 2-ml column of Sepharose CL-6B (Pharmacia Biotech) equilibrated with 10 mM Tris-HCl, pH 7.9. The DNA-tau complex was absorbed to glow-discharged thin carbon-coated grids, dehydrated through a graded water-ethanol series, fixed with 0.2% glutaraldehyde (RT, 20 min), and rotary shadow-cast with Pt. Samples were visualized using a JEOL JEM-100CX electron microscope. (*a*) PET-15b as control, inset at the same as *b*. (*b*) The plasmid with tau. Scale bar: (*a*) 241 nm, (*b*) 100 nm.

ing the samples, which was used to increase both resolution and contrast. When a Pt granule deposits on a sample, it always enlarges the sample size. The apparent size of a sample would depend on the rotary angle; the smaller the angle, the more the size will be enlarged. In this study, the rotary angle was 7° so that the deposited granule enlarged the sizes of both the nucleic acid chain and protein about five times. According to Lane et al. [28], an even distribution of the protein along the DNA chains would indicate that the binding was limited or not sequence specific, which is consistent with our results described above.

Disassociation of tau from DNA

We were interested in what happened to the binding when competition proteins were present. We investigated whether tau bound to DNA could be displaced by histone. For this purpose, we used calf thymus DNA-cellulose affinity chromatography according to Alberts and Herrick [26]. Tau was overloaded to saturate the DNA linked to cellulose. After DNA-cellulose chromatography, tau in both flow-through and elution was detected by radioimmunodot-blot. The results indicated that ~46.8 µg recombinant tau_{352} or 45.7 µg tau_{441} bound per milligram DNA-cellulose (table 1).

Calf thymus histones VI-S, VII-S and VIII-S were then employed individually to replace the tau (either tau_{352} or tau_{441}) associated with DNA-cellulose. In all cases, the histones were able to replace either tau_{352} or tau_{441} from the nucleic acid chains (fig. 4A). Of the histones VII-S showed the greatest displacement of tau from DNA; histone VIII-S was the weakest, while poly-Lys, used as a control for a charge effect, was the most effective of all the proteins studied. The number of basic amino acids/basic charge in histones appeared to be involved in the displacement of tau from DNA. A search through PubMed GenBank (table 2) showed that the Lys-rich histone VII-S had the strongest theoretical basic pI (11.13) among the histones [31]. Furthermore, poly-Lys, which had the strongest effect on replacing tau from DNA with a pI of 12.06 was the most basic. Histones VI-S and VIII-S possessed relatively lower pIs and were less effective in replacing tau from DNA.

Next, we investigated whether tau, which could rapidly bind to DNA (see above), could also be rapidly replaced from DNA by histones. As shown in figure 5B, substitution of histones with tau_{352} was almost completed within

Table 1. Tau binding to DNA-cellulose (µg tau/mg DNA-cellulose).

Original tau	Each loading	Unbound tau	Unspecific bound	Bound tau
Tau_{352} (1 mg/ml)	100 ± 3.4	34.0 ± 6.1	19.2 ± 3.6	46.8 ± 7.2
Tau_{441} (1 mg/ml)	100 ± 11.3	42.6 ± 5.7	11.7 ± 0.5	45.7 ± 5.1

Data are from four independent experiments (means ± SD). One hundred micrograms of tau was subjected to 1 mg of calf thymus DNA-cellulose chromatography, as described by Alberts and Herrick [26], and tau concentrations were determined by a radioimmunodot-blot assay as described previously [27].

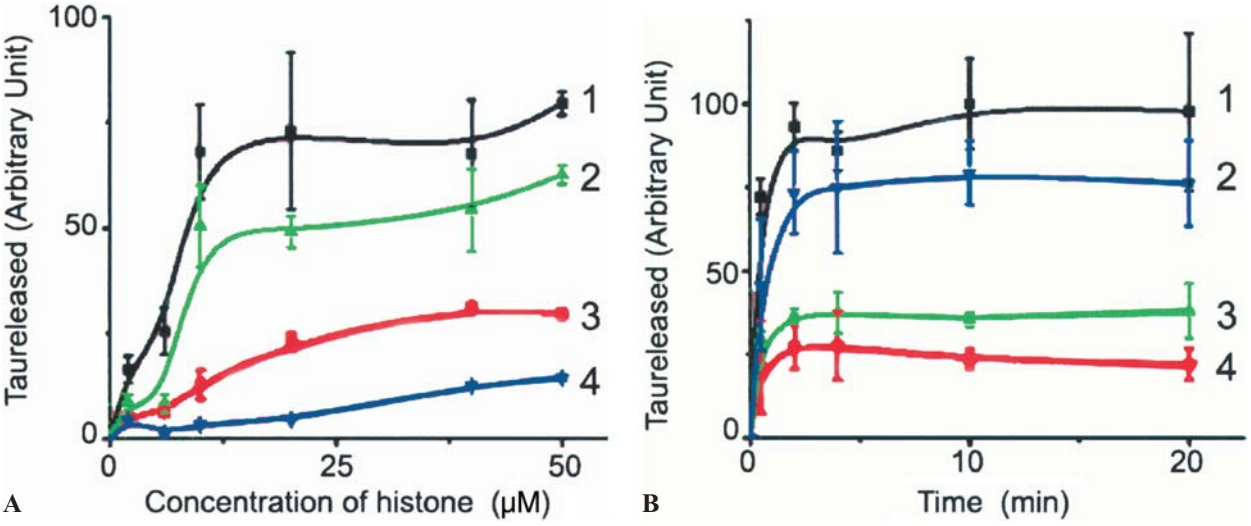

Figure 4. (*A*) Dissociation of tau from DNA-cellulose with histone or poly-lysine. One milligram of DNA-cellulose [26] was incubated with 10 µg tau_{352} in 20 mM Tris-HCl (pH 7.5), containing 10% (w/w) glycerol, 10% (w/w) BSA, 1 mM EDTA, 50 mM NaCl, 1 mM DTT at 37 °C for 30 min and washed with the same buffer (1 ml each) four times until unbound tau was negative as monitored by the radioimmunodot-blot assay. Histone at different concentrations was added to elute tau off DNA-cellulose at 37 °C for 30 min. Aliquots were taken for radioimmunodot-blot assays. Tau_{352} was replaced from DNA-cellulose by addition of Poly-Lys (curve 1), and histones VII-S (curve 2), VI-S (curve 3) and VIII-S (curve 4). (*B*) Kinetics of replacement of tau_{352} from DNA-cellulose. Conditions were the same as in *A*, except for measurements of the time course of histone replacement by tau from DNA-cellulose. Histone was used at an equal-molar ratio of tau. Curves 1–4 represent elution by poly-Lys, and histones VII-S, VI-S and VIII-S, respectively.

Table 2. Properties of histones in molecular masses, pI and basic amino acids.

Polypeptide	MW	pI	Arg	Lys
VI-S	14.004	10.90	12	14
VII-S	13.774	11.13	8	21
VIII-S	15.324	10.32	18	13
Poly-Lys	14.886	12.06	–	106
Tau_{352}	32.260	9.39	14	37
Tau_{441}	45.849	8.24	14	44

The sequences of histones refer to those of MedLine GenBank, according to John [30].

2 min with a molar ratio of histone/tau = 1 as determined by an immunodot-blot assay of tau release. Similar results were obtained when the tau_{441}-DNA complex was used (data not shown). These results indicated that tau could indeed be replaced rapidly.

Discussion

Tau, a major neuronal microtubule-associated protein, promotes the assembly and stabilizes the structure of microtubules [1]. Studies have shown the presence of tau in nuclei of neuronal cell lines [11–14, 16, 35], and the binding of tau to both eukaryotic and phage DNA [15]. In the present study, we have shown some properties of tau binding to DNA.

Almost all DNA-binding proteins, for example, transcription factors, can bind to a specific nucleotide motif. However, some DNA chaperones, such as HMG1 and HMG2 [33], bind to DNA cooperatively with little or no sequence specificity. Their saturated binding is at a high ratio of protein/DNA (plasmid 4363 bp; approx. 250/1), indicating that one chaperone protein associates with a 20-bp binding size [34]. Similarly, four HU monomers, the archetype of the bacterial proteins of DNA chaperone [28], interact with about 60 bp of DNA [35]. The present study showed that tau bound to CTDNA, plasmid DNA and to synthetic polynucleotides of arbitrary nucleotide sequences when they were double stranded. Direct-binding experiments show that ssDNA did not bind to tau. In addition, a 100-fold excess of several single-stranded polynucleotides did not compete for the binding of tau to dsDNA. Tau appeared to bind to dsDNA and induced DNA conformational bending. Furthermore, tau showed a strong preference for dsDNA, binding to either plasmid or CTDNA, while the concentration of NaCl affected the binding of tau and dsDNA, suggesting little specificity in the association with the nucleotide sequences. DNA chaperones are believed to interact principally in the minor groove of the double strands and thus do not bind to ssDNA [33].

The binding of tau to DNA was found to be dynamic, i.e., rapid and reversible, and histones were found to replace tau from DNA. Whether the positive charge of the basic amino acid residues contributes significantly to histone

replacement of a chaperone from DNA is not yet known. Histones, as major proteins of chromatin, contain a high proportion of basic amino acids (arginine and lysine) that probably facilitate binding to the negatively charged DNA molecule. Though histones are a unique feature of eukaryotic cells, the DNA of prokaryotes is similarly associated with proteins, some of which presumably function as histones do, packaging the DNA within the bacterial cell [36]. Tau is a basic protein [37], whose basic amino acid residues may be involved in the binding. Because of the charge effect on binding to DNA, in the present study, poly-Lys was tested to replace tau associated with DNA and was found to be more effective than any of the histones. The mechanism of replacing tau from DNA might be due to the high positive charge of histones, which enables them to competitively bind to the negatively charged double-strand nucleotidyl chains. The ability of histones to bind to DNA and replace other DNA-binding proteins from DNA might attributable to the presence of a relatively large number of Lys and Arg residues.

Immunohistochemical experiments show that tau exists in nuclei of various lines, such as human neuroblastoma and human retinoblastoma. In the nucleus, as detected by Tau-1 (a monoclonal antibody), tau localizes to the nucleolar organizer regions (NORs), as described by Loomis et al. [11]. Their work indicates that tau may function in processes not directly associated with microtubules, and that highly insoluble complexes of tau may also play a role in normal cellular physiology. However, whether or not tau is associated with DNA in mature brains in vivo needs further study. In our recent experiments, tau could increase the melting temperature of calf thymus DNA from 67 to 81 °C and that of plasmid from 75 to 85 °C [38]. Kinetic studies in thermal denaturation and renaturation of DNA incubated with tau show that tau protects DNA from thermal denaturation and improves its renaturation. Phosphorylated tau catalyzed by neuronal cdc2-like kinase can associate with DNA, as shown by EMSA. Similar to native tau, phosphorylated tau could increase the melting temperature of calf thymus DNA. Nevertheless, when tau was aggregated under near physiological conditions or treated with formaldehyde, neither native nor phosphorylated tau retained then ability to interact with DNA, suggesting that binding of tau to DNA was in an aggregation-dependent and phosphorylation-independent manner [18]. Hypothetically, since phosphorylated tau readily aggregates and polymerizes into pavied helical filaments by itself, dysfunction in the phosphorylation of tau might result in aggregated tau losing its functions to both stabilize the microtubule system and protect DNA molecules.

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