Tau Protein Provides DNA with Thermodynamic and Structural Features which are Similar to those Found in Histone-DNA Complex

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Abstract. Tau protein has been proposed as a trigger of Alzheimer's disease once it is hyperphosphorylated. However, the role that native tau forms play inside the neuronal nucleus remains unclear. In this work we present results concerning the interaction of tau protein with double-stranded DNA, single-stranded DNA, and also with a histone-DNA complex. The tau-DNA interaction results in a structure resembling the beads-on-a-string form produced by the binding of histone to DNA. DNA retardation assays show that tau and histone induce similar DNA retardation. A surface plasmon resonance study of tau-DNA interaction also confirms the minor groove of DNA as a binding site for tau, similarly to the histone binding. A residual binding of tau to DNA in the presence of Distamycin A, a minor groove binder, suggests the possibility that additional structural domains on DNA may be involved in tau interaction. Finally, DNA melting experiments show that, according to the Zipper model of helix-coil transition, the binding of tau increases the possibility of opening the DNA double helix in isolated points along the chain, upon increasing temperature. This behavior is analogous to histones and supports the previously reported idea that tau could play a protective role in stress situations. Taken together, these results show a similar behavior of tau and histone concerning DNA binding, suggesting that post-translational modifications on tau might impair the role that, by modulating the DNA function, might be attributable to the DNA-tau interaction.

Keywords: DNA, DNA melting, histones, surface plasmon resonance, tau protein, thermodynamics

INTRODUCTION

Tau is a highly soluble protein devoid of any well-defined secondary or tertiary structure, as many other proteins prone to aggregation and fibrillation also involved in neurodegenerative diseases [1]. It is the main component of intracellular tangles, which

form the paired helical filaments (PHFs), the aberrant proteinaceous aggregates found associated with Alzheimer's disease [2, 3] together with the extracellular senile plaques containing amyloid- β (A β) [4]. Aggregated forms of tau protein can also be found associated to other neurodegenerative disorders known as tauopathies [5]. Tau is a microtubule associated protein. Alternative splicing gives rise to six isoforms expressed from the same gene [6]. It participates in the microtubule stabilization and organization system which regulates cellular morphogenesis, cytoskeleton functionality, and axonal transport [7–12].

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Although this protein is mainly found in the cytosol of neuronal cells, it has also been localized within the nucleus of both neuronal [13–19] and non-neuronal cells [20–23]. The capability of tau protein to interact with DNA and chromatin was initially described by Corces et al. [24] and Villasante et al. [25]. It has been repeatedly reported *in vitro* [24–30] and *in situ* in neuronal [13] and non-neuronal [20] cells. Besides microtubules and nucleus, tau protein has also been found localized at ribosomes and plasma membrane of neuronal cells [31, 32]. The post-translational phosphorylation of the different isoforms of tau together with its complex distribution in different cellular locations suggests a multifunctional role for this protein. Reports from studies on non-neuronal cells confer tau an important role in nucleolar structure organization and heterochromatinization of ribosomal genes [20], as well as an involvement in genome and chromosome stability [33]. It has been recently reported that oxidative or heat stress give rise to accumulation of dephosphorylated tau protein within the nuclei of neurons and that nuclear translocation of tau correlates with an increase of tau-DNA interaction and DNA protection from heat stress damage [13]. It seems tau-DNA interaction, both in neuronal and non-neuronal cells, has a role related to protection of genomic integrity. We present here a kinetic and thermodynamic study of tau-DNA interaction, also analyzing the effect of histone on the binding kinetics and the role of tau in DNA melting. Distamycin A, a minor groove binder, has been also used to identify the possible binding sites of tau to DNA. Our results indicate that tau and histones share several common features, supporting the idea that tau-DNA interaction could be protective and play a similar role in cell nucleus to that one displayed by histones.

MATERIALS AND METHODS

Chemicals/reagents

DNA and histone from calf thymus, DNA oligonucleotides, gold, poly-L-Lysine, and heparin were from Sigma (St. Louis, USA). DNA oligonucleotides were dissolved in distilled water to 1 mg/mL. Distamycin A hydrochloride was purchased from SINUS Biochemistry & Electrophoresis GmbH (Heidelberg, Germany) and solubilized in Hepes buffer; final concentration was determined by using $\epsilon_M = 34000 \text{ M}^{-1} \text{ cm}^{-1}$ at 303 nm. Unless otherwise indicated, all experiments were carried out in a neutral running buffer composed of 10 mM Hepes, 0.1 M NaCl, pH 7.

Short DNA oligonucleotides for tau interaction assays

Two sequences of 20 nucleotides were selected from the 5'-flanking region of the mitogen-activated protein kinase 14 (MAPK14) gene, named as MAPK1 and MAPK2. 5'-CAAAAGCAGTGGGGTCTGAA-3' and 5'-GATCTGGAGTGGGACTTAGG-3' are the sequences for the forward strands of MAPK1 and MAPK2, respectively. The forward twenty-mer 5'-GAGGAGGAGTGGGGAAGTGC-3' was selected upstream of the glycogen synthase kinase 3 beta (GSK3 β) gene which, as well as MAPK14 gene, encodes for a kinase involved in tau hyperphosphorylation. These sequences contain a central decamer which shows at least 80% homology with the TP53 HSE decamer 5'-GGATTGGGGT-3', a heat shock element of tumor protein p53 which has been reported as a site wherein A β binds as a potential transcription factor [34]. Searching a similar behavior in tau protein, we used these sequences for tau-DNA interaction assays. A twenty-mer upstream of APPHSE (amyloid precursor protein heat shock element) gene, 5'-GCTCTCGACTTTTCTAGAGC-3', was chosen as a negative control due to its low (less than 50%) homology to the TP53 HSE decamer.

Tau purification

The longest human tau isoform, htau 42 clone, was kindly provided by M. Goedert (MRC, Cambridge, UK). Tau purification protocol is based on the procedure described by Lindwall [35] with some modifications. Htau 42 was expressed in *E. coli* BL21 (D3) cells and was induced by adding IPTG to a final concentration of 0.4 mM for 2 h. Bacteria were suspended in sonication buffer (100 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.5 mM MgCl₂, 1 mM EGTA) in the presence of protease inhibitors and then sonicated in ice. Bacterial lysates were centrifuged at 20000 g for 10 min and the supernatant obtained was boiled for 10 min and centrifuged at 20000 g for 30 min. HClO₄ was then added to the supernatant to a final concentration of 2.5% (v/v) and after incubating on ice for 15 min, it was centrifuged at 35000 g for 45 min at 4°C using a Beckman JA25.50 rotor. A saturated solution of (NH₄)₂SO was added to the supernatant to a final concentration of 50% and kept on ice during 1 h. After a centrifugation at 35000 g for 30 min at 4°C, the pellet was suspended in 13 ml of sonication buffer. HClO₄ was added again to a final concentration of 2.5% (v/v) and it was centrifuged at 35000 g for 30 min

at 4°C. Glycerol was added to the supernatant to a final concentration of 25% and it was centrifuged at 35000 *g* for 15 min at 4°C using the same rotor. The pellet was suspended in 1 ml of dialysis buffer and was dialyzed overnight at 4°C against 10 mM Hepes, 100 mM NaCl, and 1 mM EDTA, pH 7; then pure tau protein was stored at -20°C. Once defrosted, tau protein was stored at room temperature over different incubation periods indicated for each experiment. The isolated protein was characterized by electrophoresis, UV-absorbance spectroscopy, and western blot. Tau protein concentration was obtained by using an extinction coefficient $\epsilon_M = 7700 \text{ M}^{-1} \text{ cm}^{-1}$ at 280 nm [36]. UV spectrum confirmed tau purity, since DNA concentration represented less than 1% of the sample.

Surface plasmon resonance

Plasmon resonance has been obtained as proposed by Kretschmann and Raether [37], following the description made by Liedberg et al. [38]. This technique allows the monitoring of macromolecular interactions taking place at the interface between a solid surface and a buffered solution. The home-made experimental set-up has been described in previous reports [39–41]. One of the two interacting partners is immobilized on a gold surface, while the second one is flowed through a cell, one wall of which is the metallic solid surface. In order to avoid the direct contact of biological molecules with gold, the solid surface is covered by a three layer structure composed of polylysine-heparin-polylysine (PHP) [39]. This structure houses the immobilized partner by interacting with polylysine. It is stable at neutral pH and is easily rebuilt by changing the pH value from neutral to basic, therefore allowing for consecutive experiments using the same experimental set-up in different interaction conditions. Interaction of the flowing macromolecule through the cell with the immobilized macromolecule on this PHP structure results in an increased reflectance value, therefore supplying a mean to monitor the interaction in real time. A p-polarized radiation of 632.8 nm from a He-Ne laser was used as a source of light. The light reflected is received by a photodiode, and then transferred to a computer.

Electron microscopy

Transmission electron microscopy was carried out in a JEOL 1200 EX electron microscope. Samples were placed on carbon-collodion grids and stained with uranyl acetate.

Agarose gel retardation assays

Complementary strands of GSK3 β and APPHSE 20-mers were incubated together for 10 min at room temperature and after annealing, tau or histones were added at 1:5 mass ratios. Protein-DNA complexes were incubated for 1 h. All samples were diluted in 10 mM HEPES buffer, 100 mM NaCl pH 7, loaded onto 2% (w/v) agarose gel in 1 $\times$ TAE running buffer and electrophoresed at 80 V for approximately 45 min.

Melting of DNA

The melting processes of DNA and ds-oligonucleotides were investigated by following the absorbance at 260 nm. A Perkin Elmer spectrophotometer equipped with a thermostated set of twin cells was used. A cell contained the sample to melt. Temperature control was carried out in the second twin cell. Temperature was increased at a rate of one degree per minute. This rate was sufficient to get equilibrium absorbance values in the melting cuvette. Sigmoidal curves representing the increasing absorbance as a function of temperature were fitted to the Boltzmann Function by using ORIGIN 6.1 software:

$$A = [(A_1 - A_2)/(1 + \exp(T - T_m)/\Delta T)] + A_2 \quad (1)$$

Where A stands for the absorbance at 260 nm, while A₁ and A₂ stand for the 260 nm absorbance limit values at low and high temperature respectively. T represents temperature and T_m stands for the melting temperature. This is the temperature at which half of the double stranded DNA has melted into single strands. The value of ΔT constant determines the width or cooperativity-degree of the sigmoidal curve. The lowest its value is, the highest cooperativity results. Assuming a two states model for the double-helix melting process, the equilibrium constant, K_m, of the double strand to single strand transition would be:

$$K_m = (A - A_1) / (A_2 - A) \quad (2)$$

Substituting equation (1) into equation (2) yields the following equation for K_m as a function of temperature:

$$K_m = \exp[(T - T_m)/\Delta T] \quad (3)$$

Use of Van't Hoff equation on equation (3), yields the enthalpy change, ΔH° , of the melting process:

$$\begin{aligned}\Delta H^\circ &= RT^2 d(\ln K_m)/dT \\ &= RT^2 d[(T - T_m)/\Delta T]/dT = RT^2/\Delta T \quad (4)\end{aligned}$$

The change in the Gibbs Function, ΔG° , can be obtained from equation (1):

$$\Delta G^\circ = -RT \ln K_m = -RT(T - T_m)/\Delta T \quad (5)$$

Finally,

$$\begin{aligned}\Delta S^\circ &= (\Delta H^\circ - \Delta G^\circ)/T \\ &= (RT/\Delta T) + [R(T - T_m)/\Delta T] \quad (6)\end{aligned}$$

At the melting temperature, T_m :

$$\begin{aligned}K_m &= 1; \Delta G^\circ = 0; \Delta H^\circ = RT_m^2/\Delta T \\ \text{and } \Delta S^\circ &= RT_m/\Delta T\end{aligned}$$

RESULTS

Tau-DNA interaction probed by surface plasmon resonance

The tau-DNA interaction can be monitored by flowing tau on a DNA activated sensor surface (Fig. 1A) and also by flowing DNA on a tau activated sensor surface (Fig. 1B). In both cases, the total reflectance increase due to the tau immobilization results to be close to that corresponding to polylysine. This means that, according to the reported thickness value of the polylysine layer [39], the increased reflectance observed when tau is flowed either on DNA or polylysine must correspond to the building of a tau layer composed of unfolded monomers or small oligomers of tau about 1 nm thick. Tau-DNA interaction when both partners are in solution can also be observed by means of the sequestration experiment described in Fig. 1C and D: panel C shows how tau flowed through the sensor cell binds easily to a heparin activated sensor surface. However, when tau was flowed in the presence of DNA, which cannot bind heparin because of electrostatic repulsion, there was no tau immobilization, probably due to the sequestration of tau by DNA once a complex was formed between them.

Electron microscopy

In Fig. 2A, fresh tau protein formed small round beads-like particles, which show sizes consistent with the layer formed on the sensor surface, and also with the size of tau protein preparations measured by means

Fig. 1. DNA-tau protein interaction. A) Tau protein binding to DNA immobilized on the sensor surface. Solutions were flowed at the times indicated by arrows: P, 40 μg/ml polylysine. DNA, 8 μg/ml DNA. Tau, 16 μg/ml tau protein. Tau protein was prepared from a stock solution which was one-day-old after defrosting. B) DNA binding to tau protein immobilized on the sensor surface. Solutions flowed at the times indicated by arrows: P, 40 μg/ml polylysine. Tau, 8 μg/ml tau protein. DNA, 8 μg/ml DNA. Tau protein was prepared from a stock solution which was three-days-old after defrosting. C) Tau protein binding to a heparin activated sensor surface. Solutions flowed at the times indicated by arrows: H, 20 μg/ml heparin. Tau, 20 μg/ml tau protein; Panel D: H, 20 μg/ml heparin. Tau + DNA, a 20 μg/ml tau protein and 16 μg/ml DNA mixture incubated for 1 h. Tau protein was used a few minutes after defrosting a stock at -20°C .

Fig. 2. Electron micrographs of tau protein and the result of DNA-tau protein interaction. The following samples (20 μl) were placed onto the carbon-collodion grids. A) 8 $\mu\text{g/ml}$ tau protein solution prepared from an eight-day old 0.8 mg/ml stock solution at room temperature. B) 10 $\mu\text{g/ml}$ tau protein solution prepared from a six-months old, 0.8 mg/ml, stock solution at room temperature. C) 8 $\mu\text{g/ml}$ DNA. D) A mixture of 8 $\mu\text{g/ml}$ DNA and 8 $\mu\text{g/ml}$ tau protein from the same stock used for (A). E, F) Two different micrographs of a mixture of 8 $\mu\text{g/ml}$ DNA and 10 $\mu\text{g/ml}$ tau from the same stock solution used for (B). Sample shown in (F) was incubated at 37°C for 15 days before taking the image. G) The tau-DNA complex observed from a bacterial lysate in which DNA was not eliminated. H) Digital amplification of view shown in (G). Scale bars: G = 40 nm; H = 30 nm.

of AFM a few hours after defrosting the protein [42]. On the other hand, tau protein aged for several months (Fig. 2B) shows larger structures which may derive from the association of oligomeric forms. Tau-DNA interaction was firstly studied in Fig. 2D, where small round-shaped forms of tau appeared bound to DNA after incubating fresh tau with calf thymus DNA. In order to get an *in situ* tau-DNA complex we carried out a new purification expressing tau in *E.coli*: this time, we isolated tau-DNA complex from the bacterial lysate without separating DNA from tau protein. In this case, the complex can be seen as a clear beads-on-a-string structure, similar to the one formed between DNA and histones in eukaryotic cells (Fig. 2G, H). When exogenous DNA is added to an aged tau sample (Fig. 2E), tau oligomers associate reaching sizes beyond 100 nm, and it is possible to find massive complexes in which tau gets entangled around DNA. Nevertheless, after incubating this sample at 37°C for two weeks, these complexes acquired a new organization (Fig. 2F), which resembles the beads-on-a-string structure shown in Fig. 2G and H.

Distamycin A effect

Distamycin A is an antibiotic with anti-cancer activity [43]. It binds to the minor groove of DNA double helix, therefore displacing transcription factors and altering genetic expression. Experiments of electrophoretic mobility shifting assay have been reported showing that tau protein may compete with Distamycin A for DNA binding [30]. Figure 3A shows how distamycin A gives rise to a strong inhibition of tau binding on DNA immobilized on the sensor surface. These results strongly support the reported conclusion that tau protein binds to the minor groove of DNA. Nevertheless, it can be observed how a residual binding capability remains (Fig. 3A, curve b), suggesting the possibility that additional structural domains on DNA may be involved in tau interaction. In agreement with this observation, Fig. 3B shows how the binding of tau to a single DNA strand immobilized on the sensor surface is not inhibited by the presence of Distamycin A. Essentially the same time course for the tau-ssDNA interaction is observed both in the absence and in the presence of Distamycin A (Fig. 3B, curves d and e). Altogether, these results show that tau protein binds DNA in a complex manner involving the minor groove of the DNA double helix as well as structural DNA components unrelated to the double helix structure.

Fig. 3. Effect of Distamycin A on the interaction of tau with double- and single-stranded DNA. A) Tau binding to a dsDNA activated surface in the absence (a) and in the presence (b) of 0.125 mM Distamycin A. Tau protein solution was flowed at 10 $\mu\text{g/ml}$ in running buffer. Calf thymus DNA at 8 $\mu\text{g/ml}$ was used as a source of dsDNA. Tau protein solutions were used within a 3- to 7-day time range after defrosting. B) Tau binding to an ssDNA activated surface in the absence (d) and the presence (e) of 0.125 mM Distamycin A. Tau protein solution was flowed at 10 $\mu\text{g/ml}$ in running buffer. The sensor surface was activated by flowing a 6 $\mu\text{g/ml}$ solution of ssGSK3 β oligonucleotide. When added, distamycin A was present both in the running buffer to activate the DNA surface and in the sample containing tau protein. Tau protein solutions were used within a 3- to 7-day time range after defrosting.

Histone effect

DNA is condensed within the nucleus forming chromatin, a complex in which eight histone subunits are wrapped twice around DNA. Extremely sensitive machinery is indeed involved in regulating the chromatin structure during gene transcription. We have found that, at least *in vitro*, histones bound to DNA do not prevent tau from binding to the histone-DNA complex. As it can be seen in Fig. 4, the binding of tau to DNA is not blocked by the presence of histone. A DNA solution incubated for one hour with calf thymus whole histone was immobilized on a heparin activated sensor surface. A tau solution flowed then through the sensor cell led to a reflectance increase, denoting the

Fig. 4. Binding of tau to the Histone-DNA complex. Calf thymus DNA ($8 \mu\text{g/ml}$) was incubated with histone ($8 \mu\text{g/ml}$) for 1 h. After that time, the mixture was injected through a heparin activated sensor surface, H, at the time indicated by C. Once the reflectance signal leveled off, a tau protein solution ($10 \mu\text{g/ml}$) was added. The inset shows the first-order fitting of increasing reflectance due to the tau binding to the Histone-DNA complex.

tau protein interaction with the immobilized histone-DNA complex. The pseudo-first order analysis of the reflectance time course (inset in Fig. 4) yielded a second order constant, $k_2 = 0.76 \cdot 10^6 \text{ M}^{-1} \text{ min}^{-1}$, which is about one order of magnitude lower than the rate constant obtained for the interaction of tau with immobilized DNA (Fig. 1A), $k_2 = 4.9 \cdot 10^6 \text{ M}^{-1} \text{ min}^{-1}$. These results suggest that the presence of histone, although decreases tau-DNA affinity, does not preclude the interaction between them.

Effect of tau on the DNA melting thermodynamics

Polynucleotide denaturation was followed by monitoring the 260 nm absorbance increase which, after heating, follows the double-stranded helix melting into two random coil strands due to the unstacking of bases. The melting process was observed to be reversible for the MAPK1 oligonucleotide. As can be seen in Fig. 5, two successive thermal denaturations show the same melting profile, indicating the full reversibility of the denaturation-hybridization process. The thermodynamic analysis of melting, following the procedure described under Materials and Methods, by fitting the experimental points to equation (1) and (4), renders the melting temperature, T_m , width values, ΔT , and melting enthalpy values, ΔH_m , included in Table 1.

The presence of tau affects the thermodynamics of DNA melting. Figure 5 shows two consecutive melting experiments done in the presence of tau. Analysis

Fig. 5. Effect of tau protein on the melting thermodynamics of dsMAPK1 oligonucleotide. A) The melting of $0.6 \mu\text{M}$ ds-oligonucleotide in the absence (empty squares) and in the presence (full squares) of $6.7 \mu\text{g/ml}$ tau protein. B) The second melting of samples described in panel A. In all figures, points correspond to experimental measurements. Continuous lines correspond to equation [1] fitting.

of both melting profiles as described above reveals that, while the melting temperature remains practically unchanged, the presence of tau protein produces a decrease in the steepness of the melting profile (Table 1). According to equation (4), it means that a lower ΔH value is involved in the denaturation process which becomes less cooperative.

In order to study the effect of the presence of tau on the melting denaturation of large nucleic acid molecules, calf thymus DNA solutions were previously heated at 80°C in a very fast process to produce a partial melting of the dsDNA. After cooling and annealing, a second full melting was carried out, both in the absence and in the presence of tau (Fig. 6A). The results of thermodynamic analysis are included in Table 1. Also in this case, the presence of tau caused a loss of cooperativity in the melting profile. Similarly to MAPK1 oligonucleotide, no change in melting temperature was observed under our buffer conditions (10 mM Hepes, 0.1 M NaCl, pH 7). Our results concerning melting temperatures of nucleic acids, as well as the influence

Table 1
Thermodynamic analysis of melting data

Nucleic acid	ΔT	$\Delta H/kJ$	T_m / K
Oligonucleotide MAPK1 (First melting)*	2.6 ± 0.1	355 ± 13	333.4 ± 0.1
Oligonucleotide MAPK1 (Second melting)*	2.58 ± 0.06	355 ± 8	332 ± 0.1
Oligonucleotide MAPK1 (First melting) –Tau*	3.4 ± 0.1	270 ± 8	332.1 ± 0.1
Oligonucleotide MAPK1 (Second melting) –Tau*	2.8 ± 0.1	331 ± 12	333.9 ± 0.1
Calf thymus DNA**	2.48 ± 0.17	421 ± 29	354.3 ± 0.3
Calf thymus DNA – Tau**	3.2 ± 0.2	327 ± 19	354.7 ± 0.4
Oligonucleotide MAPK2***	2.06 ± 0.06	447 ± 11	332.7 ± 0.1
Oligonucleotide MAPK2-Histone (1 : 4)***	3.1 ± 0.1	294 ± 10	332.7 ± 0.1
Oligonucleotide MAPK2-Histone (1 : 2)***	3.96 ± 0.23	231 ± 14	332.2 ± 0.2
Oligonucleotide MAPK2-Histone (1 : 1.5)***	3.5 ± 0.2	257 ± 15	330.6 ± 0.2

*Experiments described under legend of Fig. 5. **Experiments described under legend of Fig. 6A. ***Experiments described under legend of Fig. 6B.

Fig. 6. Effects of tau and histone on the DNA melting. A) Melting profiles of calf thymus DNA (10 µg/ml) in the absence (empty squares) and in the presence (full squares) of tau protein at 10 µg/ml. DNA solutions were previously heated at 80°C in a very fast process in order to produce a partial melting of the dsDNA. After cooling and annealing, a second full melting was carried out, both in the absence and in the presence of tau. B) Effect of histones on the melting profiles of MAPK2 ds-oligonucleotide (8 µg/ml). Different curves correspond to: a) control without histone; b, c, and d stand for a mass relation histone:oligonucleotide 1 : 4, 1 : 2, and 1 : 1.5 respectively.

of tau on this parameter, are in contrast with those reported by Hua and He [27]. These authors report low values for the melting temperature of DNA in the absence of tau, and an important increase induced by the presence of tau. It is well known that ionic strength has a strong influence on the melting temperature of nucleic acids. The low ionic strength and high pH used by Hua and He (10 mM Tris, pH 8) may be at the origin of this different behavior reported for DNA.

Assuming a Zipper model for the melting transition [44], once the polypeptide chain opens at a particular point, the probability of opening at adjacent points is much higher than the probability of opening at isolated points unrelated to the first opening. This probability is defined by the nucleation parameter, which is related to the probability that unstacking of bases may occur in an isolated point of the polynucleotide chain. The lower the value of the nucleation parameter is, the lower the probability of an isolated opening is and the higher the melting cooperativity results. The loss of cooperativity defined by a wider melting profile may be interpreted therefore as an increase in the possibility of opening the double helix in an isolated point of the chain. The tau protein binding to DNA would facilitate the double helix opening. A similar behavior could be observed when the melting profile of an oligonucleotide was studied in the presence of whole histone, the protein forming chromatin with DNA. Figure 6B shows the temperature melting profile of the MAPK2 oligonucleotide in the presence of increasing concentrations of histone. While the T_m values remain essentially unchanged, the transition cooperativity becomes progressively lower with the histone concentration. The results of the thermodynamic fitting following the procedure described under Materials and Methods are included in Table 1. We must mention here that, although the melting of the MAPK2 oligonucleotide was observed to be fully reversible, in

the presence of histone a lower absorbance increase was measured with increasing temperature, together with some increase in the T_m value. This might be attributable to the plausible thermal denaturation that histone may suffer when reaching the high temperature needed for the full polynucleotide unstacking. On the contrary, the presence of tau would not induce that irreversibility due to the lack of well-defined structure and heat-stability of tau protein. In fact, a 10-minute step of boiling is included in the purification protocol of tau protein, as mentioned in Materials and Methods section.

DNA retardation assay

Figure 7 shows a DNA retardation assay, using two different kinds of oligonucleotides. Both tau and histone induce comparable DNA retardation. No stacking of oligonucleotide is observed at the top of the gel, which is indicative of the absence of large aggregates. In both cases, protein-DNA complexes can be resolved during electrophoresis pointing out that aggregates formed are similar in size.

DISCUSSION

Tau is a protein prone to aggregation and fibrillation [2, 3]. Nevertheless, the SPR results presented here show that monomers or small oligomers are the par-

ticular tau forms interacting with DNA, according to the magnitude of reflectance increase registered after flowing tau on the immobilized DNA (Fig. 1A). This interaction does not depend on the immobilization of the partners upon the surface. As it can be seen in Fig. 1D, it also takes place when tau and DNA are both in solution. Electron micrographs show that the result of this interaction is a beads-on-a-string type of structure as it can be observed in Fig. 2F–H. A similar pattern for the double helix of DNA and tau has been reported by Wei et al. [30], suggesting that tau might act on DNA as a type of chaperone. Although no large tau aggregates interacting with DNA can be observed by SPR, the electron micrographs indicate that aged tau solutions may bind to DNA forming massive huge complexes (Fig. 2E, F). These results suggest that binding of tau to DNA results in complexes of different structures depending on the aggregation state of tau.

The amino acid sequence of tau comprise an acidic amino-terminal region, the projection domain, followed by a proline rich region and the microtubule binding domain, which is composed of four imperfect residue repeats with a predominant number of basic residues, at the carboxy-terminal region. This type of bipolar structure, together with the absence of well-defined secondary structure and the proneness to aggregation, are probably at the origin of the reported promiscuity of tau as a macromolecule binder [45]. The ability of Distamycin A to inhibit the binding of tau to DNA indeed suggests, as it has been previously observed [30], that tau binds to the minor groove of DNA. As it can be seen in Fig. 3A, however, residual binding remains in the presence of Dystamycin A, therefore suggesting that, in addition to the minor groove, more elements may participate in the interaction. In fact, the binding on a single stranded DNA (Fig. 3B) supports it. It is conceivable that the polyanionic character of DNA may also contribute to the interaction. This would be in agreement with the observation previously reported that tau binds DNA in a sequence independent manner [25]. The binding to single stranded DNA is in agreement with the observation reported by Krylova et al. [29] on the ability of tau to dissociate double stranded DNA due to its ability to bind the single strand of the double helix. It is also interesting the observation that the linker histone H1 also competes with Distamycin A for binding to the minor groove in DNA [46]. This suggests that histone and tau share the minor groove as a common structural feature to bind DNA. The fact, however, that histone does not preclude the tau binding to DNA agrees with the concept that anionic phosphates on DNA may be

Fig. 7. Gel retardation assays with GSK3 β and APPHSE oligonucleotides in the presence of tau protein or histones. ds-DNA oligonucleotides were incubated with tau protein or histones at room temperature for 1 h prior to electrophoresis. Lanes: 1. GSK3 β 20-mer and tau protein at a mass ratio of 1 : 5, 2. Control of GSK3 β 20-mer (0.5 μ g), 3. GSK3 β 20-mer and histones at a mass ratio of 1 : 5, 5. APPHSE 20-mer and tau protein at a mass ratio of 1 : 5, 6. Control of APPHSE 20-mer (0.5 μ g), 7. APPHSE 20-mer and histones at a mass ratio of 1 : 5.

important in tau interaction, and also with the initial observation that tau binds differently to both chromatin and DNA [25].

Thermodynamic analysis of the oligonucleotide and DNA melting processes reveals that binding of tau results in a loss of rigidity of the double helix. The lower cooperativity for melting observed in the presence of tau may be interpreted, according to the Zipper model of melting [44], as an increase in the possibility of opening the double helix in isolated points along the chain, upon increasing temperature. Similar behavior could be observed when the melting profile of an oligonucleotide was studied in the presence of whole histone. The presence of histone confers flexibility to the nucleic acid structure. A similar result is obtained when the effect of tau is analyzed. Tau-DNA interaction seems to have not only thermodynamic but also structural consequences similar to those derived from the histone binding. In this sense, Fig. 7 shows a comparable DNA retardation due to either the presence of histones or tau protein. On the other hand, the similar retardation observed with both GSK3 β and APPHSE peptides is in agreement with the original observation made by Villasante et al. [25] pointing out that tau protein does not show preferential binding to specific DNA sequences. This would be in agreement with the protective role assigned to the tau-DNA interaction [13]. Given that tau is a heat stable protein, it is conceivable that the role of nuclear tau in neurons may be related to protect DNA from damage, particularly from that derived of heat stress.

The reported distribution of tau in various cellular locations suggests a multifunctional role for this protein. It has been shown to be related to nucleolar structure conformation and heterochromatinization of ribosomal genes [20] in non-neuronal cells. Although the presence of tau within the nucleus of neuronal cells has been repeatedly reported, the role that it might play there has not yet been established. The results we present here show that the binding of monomers or small oligomers of tau to DNA has features resembling the binding of histones. In this respect, the tau-DNA interaction results in a structure resembling the beads-on-a-string form produced by the binding of histone to DNA; the minor groove of DNA double helix is involved in the binding of tau, similarly to the binding of histone H1. In addition, the thermodynamic consequence of the tau binding to DNA is, similarly to the case of histone, a higher flexibility in the double helix structure. It has been recently reported that oxidative or heat stress leads to accumulation of dephosphorylated tau protein within the nuclei of neurons and that this

nuclear translocation of tau correlates with an increase of tau-DNA interaction and DNA protection from heat stress damage [13]. It is worth mentioning here that tau has been reported to inhibit the action of histone deacetylase 6 (HDAC6) on microtubules [47]. Since HDAC6 has also a nuclear localization, nuclear tau might participate in the chromatin structure regulation by modulating the histone acetylation in some conditions. Taken together, our results about the similar behavior of tau and histones concerning DNA binding; the generally accepted notion that histones, as modulators of the chromatin structure, play a regulation role in transcription; and finally, the reported role of tau protecting DNA from heat stress damage, make it tempting to speculate a connection with Alzheimer's disease. Tau hyperphosphorylation found in Alzheimer's disease might impair the cytosol-nucleus translocation, therefore preventing tau from binding DNA with the consequent failure in the protection of DNA from heat stress damage or impairing the role that, by modulating the closing and opening of the double helix of DNA, might be attributable to tau protein in transcription regulation.

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