

Tau protein binds single-stranded DNA sequence specifically – the proof obtained in vitro with non-equilibrium capillary electrophoresis of equilibrium mixtures

Svetlana M. Krylova^a, Michael Musheev^a, Razvan Nutiu^b, Yingfu Li^b,
Gloria Lee^c, Sergey N. Krylov^{a,*}

^a Department of Chemistry, York University, Toronto, Ont., Canada M3J 1P3

^b Departments of Biochemistry and Chemistry, McMaster University, Hamilton, Ont., Canada L8N 3Z5

^c Departments of Internal Medicine, University of Iowa, 200 Hawkins Drive, Iowa City, Iowa 52242, USA

Received 13 December 2004; revised 6 January 2005; accepted 13 January 2005

Available online 28 January 2005

Edited by Jesus Avila

Abstract Tau is a microtubule-associated protein, which plays an important role in physiology and pathology of neurons. Tau has been recently reported to bind double-stranded DNA (dsDNA) but not to bind single-stranded DNA (ssDNA) [Cell. Mol. Life Sci. 2003, 60, 413–421]. Here, we prove that tau binds not only dsDNA but also ssDNA. This finding was facilitated by using two kinetic capillary electrophoresis methods: (i) non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM); (ii) affinity-mediated NECEEM. Using the new approach, we observed, for the first time, that tau could induce dissociation of strands in dsDNA by binding one of them in a sequence-specific fashion. Moreover, we determined the equilibrium dissociation constants for all tau–DNA complexes studied. © 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Tau protein; Protein–DNA interaction; Alzheimer disease; Non-equilibrium capillary electrophoresis of equilibrium mixture; ssDNA-binding protein

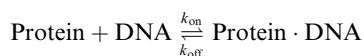
1. Introduction

Tau proteins are microtubule-associated proteins expressed in neurons and oligodendrocytes [1]. Their major known physiological function is to promote and maintain microtubular integrity, which is required for axonal transport and morphogenesis [2]. Tau is also involved in a number of pathological conditions, such as Alzheimer and Parkinson diseases, although the exact molecular mechanisms of this involvement are still debated [3–5]. Intriguingly, microtubule-associated

proteins have been shown to have a higher affinity to DNA than to microtubules [6]. Due to its potentially important role in neuronal physiology and pathology, this phenomenon has also been studied in detail for tau [7–9]. It is known that tau can bind RNA and interfere with microtubular assembly [10,11]. It has also been reported that microtubule-associated protein tau binds double-stranded DNA (dsDNA) [12]. These observations were made with electrophoretic mobility shift assay (EMSA) on slab gels, a conventional method in studies of protein–DNA and protein–RNA interactions. With its limited sensitivity and non-quantitative nature, classical slab-gel EMSA imposes serious limitations on such studies.

We recently introduced three new capillary electrophoresis methods for quantitative studies of protein–DNA interactions: (i) non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) [13]; (ii) affinity-mediated NECEEM [14], and sweeping capillary electrophoresis (SweepCE) [15]. The three methods can be described by a broad term of kinetic capillary electrophoresis (KCE).

NECEEM can be considered as a *quantitative gel-free* EMSA. Briefly, the protein and DNA are mixed and allowed to reach the dynamic equilibrium



The equilibrium mixture contains three components: free protein, free DNA, and a protein–DNA complex. A short plug of the equilibrium mixture is injected into the capillary and the three components are separated under non-equilibrium conditions (in a run buffer that does not contain the protein or DNA). As the result of electrophoretic separation, the complex is no longer in equilibrium with free protein and free DNA, and it dissociates exponentially with a unimolecular rate constant k_{off} . If fluorescence detection is used and only DNA is fluorescently labeled (labeling of DNA can be typically done in the way that it does not affect protein–DNA interaction), then a typical electropherogram consists of three characteristic features: two peaks and a single-exponential curve between the peaks. The area under one peak corresponds to the equilibrium fraction of free DNA, while the sum area under the exponential curve and the second peak corresponds to the equilibrium fraction of the complex. The ratio of the two areas is found from the electropherogram and used for the calculation of the equilibrium dissociation

*Corresponding author.

E-mail address: skrylov@yorku.ca (S.N. Krylov).

Abbreviations: EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; EMSA, electrophoretic mobility shift assay; IPTG, isopropyl beta-D-thiogalactopyranoside; KCE, kinetic capillary electrophoresis; NECEEM, non-equilibrium capillary electrophoresis of equilibrium mixtures; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); PMSF, phenylmethylsulfonyl fluoride; SSB, single-stranded DNA binding protein; SweepCE, sweeping capillary electrophoresis; IS, internal standard

constant, K_d . Fitting the exponential curve with a single-exponential function reveals the value of k_{off} (k_{off} can be also found by the analysis of the areas). The bimolecular rate constant of complex formation can then be calculated as $k_{on} = k_{off}/K_d$. Uniquely, kinetic and equilibrium binding parameters can be obtained from a single electropherogram.

To explain the rationale for affinity-mediated NECEEM, we have to emphasize that NECEEM requires good separation of free DNA from the protein–DNA complex. If the separation is poor, the accuracy of the method with respect to the determination of rate constants and equilibrium constants decreases. Affinity-mediated NECEEM is based on the insight that adding to the run buffer a background affinity agent which can bind free DNA but not the protein–DNA complex can improve the separation by changing the mobility of free DNA while not affecting that of the complex. In our study showing the proof of principle, we demonstrated affinity-mediated NECEEM of protein–ssDNA pairs by using ssDNA-binding protein (SSB) from *Escherichia coli* as a background affinity agent in the run buffer [14]. To extend affinity-mediated NECEEM to studies of dsDNA–protein interactions, a suitable dsDNA-binding protein needs to be found to serve as a background affinity agent instead of SSB. Hypothetically, affinity-mediated NECEEM can also be realized with a protein-binding affinity agent, such as an antibody, instead of a DNA-binding agent, provided that the agent binds the protein but does not bind the protein–DNA complex.

SweepCE was introduced very recently as the only non-stopped-flow method for directly measuring k_{on} of protein–DNA interactions [15]. Conceptually, in SweepCE, the capillary is pre-filled with DNA and the inlet of the capillary is inserted into the solution of the protein, the interaction with which is to be studied. When electrophoresis starts, the fast-moving protein is mixed with a slowly moving DNA in a continuous mode and “sweeps” DNA upon protein–DNA complex formation. The shape of the concentration profile of swept DNA is very sensitive to k_{on} and, thus, its analysis provides accurate information on the value of k_{on} .

The three KCE methods constitute a comprehensive toolset for measuring kinetic and equilibrium parameters of protein–DNA complexes [13–16]. Moreover, they facilitate a number of other applications ranging from quantitative analyses of proteins, DNA, and RNA [14] to selection of aptamers [17].

In this work, we used NECEEM and affinity-mediated NECEEM (with SSB as a background affinity agent) to prove that despite previous reports, tau protein binds both dsDNA and ssDNA. In addition, we found that tau can induce dissociation of strands in dsDNA by binding one of them in a sequence-specific fashion. We also determined equilibrium dissociation constants for all tau–DNA complexes studied.

2. Materials and methods

2.1. Chemicals and materials

Single-stranded DNA binding protein from *E. coli* and buffer components were from Sigma–Aldrich (Oakville, ON, Canada). Monoclonal mouse anti-tau-1 antibody and goat anti-mouse IgG–HRP conjugated secondary antibody were from Chemicon International (Temecula, CA, USA). pET-3d prokaryotic expression plasmids containing the complete coding sequences of tau isoforms 381 and 410 were obtained as described in detail elsewhere [18]. Protein Assay kit was from Bio-Rad Laboratories (Mississauga, ON, Canada). A mono S HR 5/5 HPLC column was from Amersham Biosciences (Piscata-

```

ssDNA1: Fluorescein-5' -GCGGAGCGTGGCAGG-3'
ssDNA2: Fluorescein-5' -AACGAGAAGCGGATCATACAT-3'
ssDNA3: Fluorescein-5' -CTTCTGCCGCCCTCCTTCC-3'
dsDNA1: Fluorescein-5' -GCGGAGCGTGGCAGG-3'
          3' -CGCCTCGCACCGTCC-5'
dsDNA2: Fluorescein-5' -AACGAGAAGCGGATCATACAT-3'
          3' -TTGCTCTTCGCGCTAGTGTA-5'
dsDNA3: Fluorescein-5' -CTTCTGCCGCCCTCCTTCC-3'
          3' -GAAGACGGCGGAGGAAGG-5'

```

Fig. 1. DNA molecules used in this work. The dsDNA molecules were obtained by annealing the ssDNA molecules with complementary strands.

way, NJ, USA). Spectra regenerated cellulose membrane (25 000 Da MWCO) was purchased from Fisher Scientific (Markham, ON, Canada). Uncoated fused-silica capillaries were from Polymicro (Phoenix, AZ, USA). All solutions were made using the Milli-Q quality deionized water and filtered through a 0.22 μ m filter (Millipore, Nepean, ON).

Six different fluorescently labeled DNA molecules were used in this work: three ssDNA and three dsDNA (Fig. 1). Double-stranded DNA molecules were obtained by annealing ssDNA₁, ssDNA₂, and ssDNA₃ with corresponding complementary strands. Single-stranded DNA₁ was synthesized and purified as described elsewhere [14]. All other DNA strands were custom synthesized by Integrated DNA Technologies (Coralville, IA, USA).

2.2. Expression and isolation of tau 381 and tau 410

pET-3d tau plasmids were transfected into *E. coli* BL21(DE3) pLysS cells for expression. Bacteria were grown at 37 °C to a density with an absorbance of 0.6–0.7 at 600 nm measured in a cuvette with an optical path-length of 1 cm. Then, bacterial cultures were induced with isopropyl beta-d-thiogalactopyranoside (IPTG) at a final concentration of 0.4 mM for 4 h. Cells were harvested by centrifugation at 5000 \times g for 10 min at 4 °C. Pelleted cells were resuspended in the sonication buffer: 50 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) at pH 6.8 supplemented with 1 mM ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 5 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 5 mM 2-mercaptoethanol. Bacterial lysates were prepared by sonication on ice with 5-s “on”/15-s “off” intervals for a total of 15 min. Cell debris were pelleted by centrifugation at 15 000 \times g for 20 min at 4 °C. The supernatant was then incubated in a bath with boiling water for 20 min to denature most of the bacterial proteins [19]. Heat-labile bacterial proteins were removed by centrifugation at 10 000 \times g for 10 min at 20 °C. The supernatant was dialyzed two times for 2 h against 1 L of the dialysis buffer: 20 mM PIPES buffer at pH 6.8 supplemented with 1 mM EGTA, 0.2 mM MgCl₂, and 5 mM 2-mercaptoethanol. They were further purified by ion-exchange HPLC using a Mono S HR 5/5 column with a 10- μ m diameter of the particles. Separation was performed with a Hewlett-Packard 1050 series HPLC system equipped with a 280 nm UV detector. A two-buffer system was used for the purification of all tau protein samples with buffer A being 20 mM PIPES, 1 mM EGTA, 0.2 mM MgCl₂, 0.25 mM PMSF, and 5 mM 2-mercaptoethanol at pH 6.8 and buffer B being buffer A supplemented with 1 M NaCl. The best separation quality was achieved by a linear elution gradient at a flow rate of 1 mL/min. Tau protein eluted with NaCl concentrations in the range between 0.3 and 0.4 M [18]. Concentrations of tau protein were determined by the Bradford’s method [20].

2.3. SDS–PAGE and immunoblotting

The presence and purity of tau protein was analyzed by SDS–PAGE in 7–15% gradient gel. Proteins were electrophoretically transferred to the polyvinylidene difluoride membrane (Millipore, Bedford, MA) for 2 h at 300 mA, 35 V in 25 mM Tris, 192 mM Glycine, 0.1% (w/v) SDS, and 10% (v/v) methanol, pH 8.3. Non-specific binding sites were blocked with 1% non-fat dried milk in 20 mM Tris–HCl, 150 mM NaCl, and 0.1% (v/v) Tween 20, pH 7.3. The membrane was incubated with monoclonal mouse anti-tau-1 antibody for 1.5 h at room temperature. Bound antibodies were detected by goat anti-mouse IgG HRP-conjugated secondary antibody.

2.4. NECEEM and affinity-mediated NECEEM

NECEEM and affinity-mediated NECEEM of tau protein–DNA complexes were performed with a Beckman P/ACE MDQ instrument (Mississauga, ON, Canada) with fluorescence detection. A 488-nm line of an Ar-ion laser was utilized to excite fluorescence of the fluorescein label on DNA molecules. Tau had no fluorescent label and thus it was undetectable in the NECEEM electropherograms. Uncoated fused-silica capillaries of 50 cm in length with an inner diameter of 75 μm and outer diameter of 375 μm were used in all experiments. The distance from the capillary inlet to the detector was 40 cm. The electrophoresis run buffer was 25 mM sodium tetraborate at pH 9.4. For affinity-mediated NECEEM, the run buffer was supplemented with 100 nM SSB. The samples were injected into the capillary by a 5-s pressure pulse of 3.44 kPa; the length and the volume of the corresponding sample plug were 6.42 mm and 28 nL, respectively. Electrophoresis was carried out by an electric field of 400 V/cm with a capillary temperature biased at 20 ± 0.2 °C. The capillary was rinsed with the electrophoresis run buffer for 2 min prior to each run. At the end of each run, the capillary was rinsed with 100 mM HCl for 1 min, 100 mM NaOH for 1 min, followed by a rinse with deionized water for 1 min. Rinsing was driven by a constant pressure of 137 kPa.

2.5. Tau–DNA equilibrium mixtures

Unless otherwise stated, equilibrium mixtures were prepared by mixing tau protein with DNA in the incubation buffer (50 mM Tris-base, 5 mM MgCl_2 , pH 8.3) followed by overnight incubation at room temperature.

2.6. K_d calculation

K_d values for tau–DNA interaction were calculated from NECEEM electropherograms as described in details elsewhere [13]. The procedure was slightly modified to be applicable to on-column detection of the CE instrument used in this work: peak areas in electropherograms were divided by corresponding migration times.

3. Results and discussion

3.1. Preamble

DNA molecules used in this study were those used by us in other studies; there was no bias in the choice of sequences. All six DNA molecules studied in this work were fluorescently labeled (see Fig. 1), while tau was not. Therefore, NECEEM electropherograms contain peaks of free DNA and tau–DNA complexes but not that of free tau. NECEEM electropherograms also contain a peak of fluorescein, which was used as an internal standard (IS) to control the reproducibility of NECEEM analyses. The peak of the IS is ignored in the presentation and discussion of the results.

3.2. Tau binding to ssDNA

First, we studied the interaction of tau 381 with ssDNA₁. With growing concentration of tau in the equilibrium mixture, the peak of tau–ssDNA₁ complex increases, while that of free ssDNA₁ decreases (Fig. 2A). Interestingly, the exponential line between two peaks has low intensity; the area under the exponential line is smaller than the peak of the complex. It suggests that the rate constant of complex dissociation is lower than the reciprocal migration time of the complex, $k_{\text{off}} < 10^{-3} \text{ s}^{-1}$. The peaks are separated by less than 1 min. Although this separation window is relatively large comparing to the peak widths of 3 s, using other DNA molecules could shift the peak of the complex closer to that of free DNA and thus potentially obscure the analysis. Therefore, we decided to use affinity-mediated NECEEM, with SSB as a background affinity agent

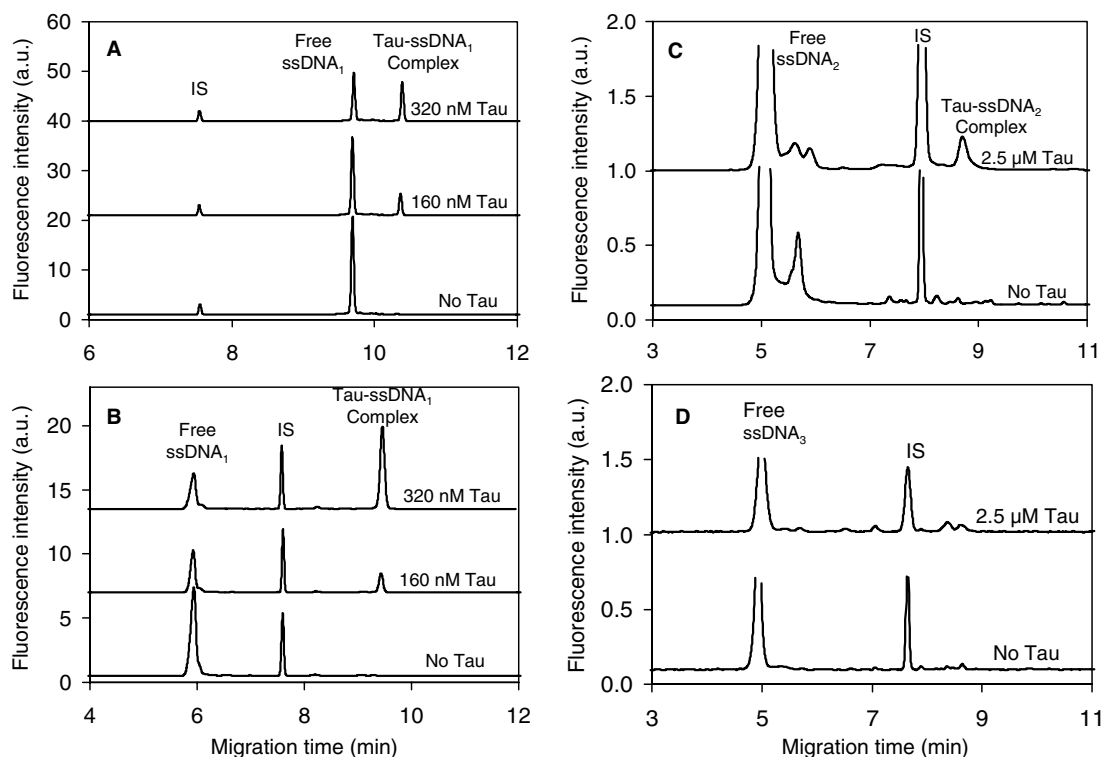


Fig. 2. Binding of tau 381 to different ssDNA molecules monitored with NECEEM and affinity-mediated NECEEM. Panels A and B show the tau–ssDNA₁ interaction data for NECEEM and affinity-mediated NECEEM, respectively. Panels C and D show affinity-mediated NECEEM data for tau interaction with ssDNA₂ and ssDNA₃, respectively. The concentration of DNA in all experiments was 80 nM; the concentrations of tau 381 are shown in the panels next to corresponding electropherograms. The run buffer for NECEEM was 25 mM sodium tetraborate at pH 9.4; the run buffer for affinity-mediated NECEEM was the same but supplemented with 100 nM SSB. The electropherograms are offset along the vertical axis for clarity of presentation.

Table 1
Equilibrium dissociation constants for interaction between tau protein and DNA

	K_d (μM)					
	ssDNA ₁	ssDNA ₂	ssDNA ₃	dsDNA ₁	dsDNA ₂	dsDNA ₃
Tau 381	0.19 ± 0.01	40 ± 4	>200	Binding to tau dissociates dsDNA ₁	1.7 ± 0.2	0.5 ± 0.1
Tau 410	0.35 ± 0.03	40 ± 10	>200		0.9 ± 0.1	0.3 ± 0.1

present in the run buffer, for our further study of tau–ssDNA interactions. In affinity-mediated NECEEM, the peak of free DNA is considerably shifted to the left, while that of tau–DNA complex experiences only a slight shift. The resulting separation window exceeds 3 min (Fig. 2B).

Second, we used affinity-mediated NECEEM to study the interaction of tau 381 with ssDNA₂ and ssDNA₃. The complexes of tau with ssDNA₂ (Fig. 2C) were present in the amount much lower than those of tau with ssDNA₁. The complexes of tau with ssDNA₃ were undetectable with the used concentration of tau (Fig. 2D). The K_d value of the tau–ssDNA₁ complex is lower than those of the tau–ssDNA₂ and the tau–ssDNA₃ complexes by more than two orders of magnitude (Table 1). The affinity of tau to ssDNA is Mg^{2+} dependent, which was confirmed by suppressed complex formation in the absence of Mg^{2+} in the incubation buffer. The difference in the affinity for the three ssDNA molecules indicates that tau binds ssDNA in a sequence-specific fashion.

Third, we used affinity-mediated NECEEM to study the interaction of another isoform of tau protein, tau 410, with the ssDNA₁, ssDNA₂, and ssDNA₃. The electropherograms were qualitatively similar to those for tau 381 (not shown). K_d values for complexes of ssDNA with tau 410 were also similar to those for complexes of ssDNA with tau 381 (see Table 1).

3.3. Tau binding to dsDNA

Affinity-mediated NECEEM with SSB as a mediator is not applicable to studying tau interaction with dsDNA, since

SSB does not bind dsDNA. Therefore, to study tau–dsDNA interaction we used NECEEM instead of affinity-mediated NECEEM. Fig. 3 illustrates electropherograms for NECEEM of tau 381–dsDNA₁ complexes. In the absence of tau protein (line 1), dsDNA generates a single peak with a migration time of 10 min. In the presence of tau (lines 3–5), we observe 3 peaks. The peak with a migration time of 10 min was identified as free dsDNA₁ by comparing the migration time with that in the control experiment with no tau (line 1). The peak with the migration time of 8 min 20 s was identified as the tau–dsDNA₁ complex as it increases with increasing concentration of tau. The rightmost peak with a migration time of 10 min 10 s was identified as the complex of tau with ssDNA₁, which was used as one of the strands in dsDNA₁. This identification was confirmed by NECEEM of tau–ssDNA₁ mixture (line 2). The peak of tau–ssDNA₁ grows with growing concentration of tau in the same fashion as the peak of tau–dsDNA₁. This result suggests that tau can destabilize dsDNA by binding one of the strands sequence specifically. When tau interacted with dsDNA₂ and dsDNA₃; however, dissociation of the DNA hybrid and the formation of the tau–ssDNA₂ and tau–ssDNA₃ complexes were not observed, indicating that the destabilization of dsDNA is sequence-specific (a common feature for interactions between proteins and their aptamers) (Fig. 3B). The peaks assigned to free dsDNA in lines 2 and 3 cannot be composed of overlapping peaks of dsDNA and tau–ssDNA complexes for two reasons. First, the migration time of the tau–ssDNA₂ complex was 8.7 min independently on whether

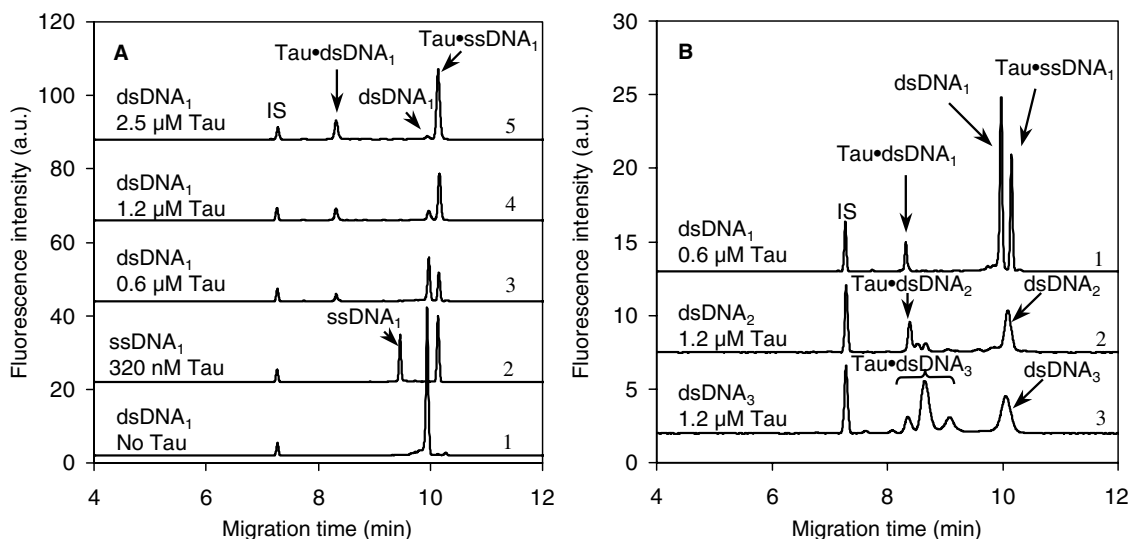


Fig. 3. Binding of tau 381 to 3 different dsDNA molecules monitored with NECEEM. Panel A shows the interaction of tau 381 with dsDNA₁. Lines 1 and 2 show two control electropherograms: dsDNA₁ with no tau protein (line 1) and tau protein with ssDNA₁, which is one of the strands of dsDNA₁ (line 2). Lines 3–5 show NECEEM electropherograms of dsDNA₁ with varying concentrations of tau. Panel B proves that interaction of tau 381 with dsDNA₁ (line 1) is different from that of tau with dsDNA₂ and dsDNA₃ (lines 2 and 3). The concentration of DNA in all equilibrium mixtures was 80 nM; the concentrations of tau 381 are shown in the figure next to corresponding electropherograms. The run buffer for NECEEM was 25 mM sodium tetraborate at pH 9.4. The electropherograms are offset along the vertical axis for clarity of presentation.

or not the buffer contained SSB (see Fig. 2C). This migration time is significantly shorter than the migration time of free dsDNA₂, which was 10.1 min (Fig. 3B, line 2). Second, we proved that tau does not form the complex with ssDNA₃ (see Fig. 2D).

The equilibrium dissociation constants for tau binding dsDNA are shown in Table 1. The value for the tau–dsDNA₁ complex is not shown because it cannot be accurately measured due to the dissociation of the strands by tau.

Finally, we studied the interaction of the other tau isoform, tau 410, with dsDNA. We found that it was qualitatively and quantitatively similar to that of tau 381.

4. Discussion

Our results of tau binding ssDNA indirectly suggest that ssDNA, when tightly bound to tau, can stabilize the tertiary structure of tau, which is believed to be natively unfolded. The results on sequence-specific binding of tau to ssDNA also suggest that tau protein may be a potentially suitable target for selection of ssDNA aptamers with high affinity and specificity. If selected, such aptamers could be used as affinity probes in analyses and, potentially, as drug candidates for treating or preventing tauopathies.

A number of studies with different cell types demonstrated that tau proteins could be found in nuclei in a complex with nuclear DNA [6,21–24]. Iqbal and co-workers [12] reported that by binding dsDNA tau increases its melting temperature. It was suggested that one of the physiological functions of tau could be the stabilization of the dsDNA helix. Our *in vitro* experiments showed an opposite effect: the melting of dsDNA duplex by tau in a sequence-specific fashion. This allows us to suggest that tau may have yet another physiological function in processes such as chromatin activation. The biological relevance of this phenomenon is still to be studied.

5. Conclusions

To summarize, this study demonstrated for the first time that tau proteins can bind not only dsDNA but also ssDNA. The affinity of tau–ssDNA interaction is sequence dependent and can be even higher than that of tau–dsDNA interaction. When tau binds dsDNA, it can destabilize the DNA hybrid by binding a single strand of the DNA hybrid. In addition to intriguing biological findings, this work demonstrates the power of KCE methods in studies of biomolecular interactions.

Acknowledgments: This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Ontario Cancer Research Network to SNK and NSERC and Canadian Institutes of Health Research to Y.L. S.N.K. and Y.L. are the holders of Canada Research Chairs.

References

- [1] Avila, J., Lucas, J.J., Perez, M. and Hernandez, F. (2004) Role of tau protein in both physiological and pathological conditions. *Physiol. Rev.* 84, 361–384.
- [2] Johnson, G.V.W. and Hartigan, J.A. (1999) Tau protein in normal and Alzheimer's disease brain: an update. *J. Alz. Dis.* 1, 329–351.
- [3] Mandelkow, E. (1999) The tangled tale of tau. *Nature* 402, 588–589.
- [4] Hatakeyama, S., Matsumoto, M., Kamura, T., Murayama, M., Chui, H., Planel, E., Takahashi, R., Nakayama, K.I. and Takashima, A. (2004) U-box protein carboxyl terminus of Hsc70-interacting protein (CHIP) mediates poly-ubiquitylation preferentially on four-repeat Tau and is involved in neurodegeneration of tauopathy. *J. Neurochem.* 91, 299–307.
- [5] Richter-Landsberg, C. and Bauer, N.G. (2004) Tau-inclusion body formation in oligodendroglia: the role of stress proteins and proteasome inhibition. *Int. J. Dev. Neurosci.* 22, 443–451.
- [6] Corces, V.G., Manso, R., De La Torre, J., Avila, J., Nas, A. and Wiche, G. (1980) Effects of DNA on microtubule assembly. *Eur. J. Biochem.* 105, 7–16.
- [7] Hua, Q. and He, R.Q. (2002) Effect of phosphorylation and aggregation on tau binding to DNA. *Protein Peptide Lett.* 9, 249–357.
- [8] Hua, Q. and He, R.Q. (2000) Human neuronal tau promoting the melting temperature of DNA. *Clin. Sci. Bull.* 45, 999–1001.
- [9] Hua, Q. and He, R.Q. (2003) Tau could protect DNA double helix structure. *Biochim. Biophys. Acta* 1645, 205–211.
- [10] Bryan, J., Nagle, B.W. and Doenges, K.H. (1975) Inhibition of tubulin assembly by RNA and other polyanions. Evidence for a required protein. *Proc. Natl. Acad. Sci. USA* 72, 3570–3574.
- [11] Kampers, T., Friedhoff, P., Biernat, J., Mandelkow, E.-M. and Mandelkow, E. (1996) RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett.* 399, 344–349.
- [12] Hua, Q., He, R.-Q., Haque, N., Qu, M.-H., del Carmen Alonso, A., Grundke-Iqbal, I. and Iqbal, K. (2003) Microtubule associated protein tau binds to double stranded but not single-stranded DNA. *Cell. Mol. Life Sci.* 60, 413–421.
- [13] Berezovski, M. and Krylov, S.N. (2002) Nonequilibrium capillary electrophoresis of equilibrium mixtures – a single experiment reveals equilibrium and kinetic parameters of protein–DNA interactions. *J. Am. Chem. Soc.* 124, 13674–13675.
- [14] Berezovski, M., Nutiu, R., Li, Y. and Krylov, S.N. (2003) Affinity analysis of a protein–aptamer complex using nonequilibrium capillary electrophoresis of equilibrium mixtures. *Anal. Chem.* 75, 1382–1386.
- [15] Okhonin, V., Berezovski, M. and Krylov, S.N. (2004) Sweeping capillary electrophoresis: a non-stopped-flow method for measuring bimolecular rate constant of complex formation between protein and DNA. *J. Am. Chem. Soc.* 126, 7166–7167.
- [16] Okhonin, V., Krylova, S.M. and Krylov, S.N. (2004) Nonequilibrium capillary electrophoresis of equilibrium mixtures, mathematical model. *Anal. Chem.* 76, 1507–1512.
- [17] Berezovski, M., Drabovich, A., Krylova, S.M., Mushev, M., Okhonin, V., Petrov, A. and Krylov, S.N. (2005) Non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) – a universal tool for the development of aptamers. *J. Am. Chem. Soc.*, in press.
- [18] Brandt, R. and Lee, G. (1993) Functional organization of microtubule-associated protein tau. *J. Biol. Chem.* 268, 3414–3419.
- [19] Kontsejkova, E., Cattaneo, A. and Novak, M. (1995) Quick purification of recombinant human truncated tau proteins for immunoanalysis. *J. Immunol. Meth.* 185, 245–248.
- [20] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- [21] Greenwood, J.A. and Johnson, G.V.W. (1995) Localization and *in situ* phosphorylation state of nuclear tau. *Exp. Cell Res.* 220, 332–337.
- [22] Loomis, P.A., Howard, T.H., Castleberry, R.P. and Binder, L.I. (1990) Identification of nuclear tau isoforms in human neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* 8, 8422–8426.
- [23] Wang, Y., Loomis, P.A., Zinkowski, R.P. and Binder, L.I. (1993) A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. *J. Cell. Biol.* 121, 257–267.
- [24] Mei-hua, Q., Hui, L., Rui, T., Chun-lai, N., Ying, L., Bao-shan, H. and Rong-qiao, H. (2004) Neuronal tau induces DNA conformational changes observed by atomic force microscopy. *NeuroReport* 15, 2723–2727.