Direct interaction of soluble human recombinant tau protein with Aβ 1–42 results in tau aggregation and hyperphosphorylation by tau protein kinase II

Kenneth B. Rank[a], Adele M. Pauley[a], Keshab Bhattacharya[a], Zhigang Wang[a], David B. Evans[a], Timothy J. Fleck[a], Jennifer A. Johnston[b], Satish K. Sharma[a,*]

[a] *Pharmacia Corporation, Protein Science, 7240-267-117, Kalamazoo, MI 49007, USA*
[b] *Elan Pharmaceuticals, South San Francisco, CA, USA*

Received 7 December 2001; revised 21 January 2002; accepted 28 January 2002

First published online 13 February 2002

Edited by Jesus Avila

Abstract We report here that aggregated β-amyloid (Aβ) 1–42 promotes tau aggregation in vitro in a dose-dependent manner. When Aβ-mediated aggregated tau was used as a substrate for tau protein kinase II (TPK II), an 8-fold increase in the rate of TPK II-mediated tau phosphorylation was observed. The extent of TPK II-dependent tau phosphorylation increased as a function of time and Aβ 1–42 concentration, and hyperphosphorylated tau was found to be decorated with an Alzheimer's disease-related phosphoepitope (P-Thr-231). In HEK 293 cells co-expressing CT-100 amyloid precursor protein and tau, the release of Aβ 1–42 from these cells was impaired. Taken together, these in vitro results suggest that Aβ 1–42 promotes both tau aggregation and hyperphosphorylation.

Key words: Neurofibrillary tangle; Tau protein kinase II; Alzheimer's disease; Amyloid Aβ; Tau; Phosphorylation

1. Introduction

Neurofibrillary tangles (NFTs) and senile plaques constitute two prominent neuropathological characteristics of Alzheimer's disease (AD) [1,2]. The main fibrous component of all neurofibrillary lesions is paired helical filament (PHF) which contains predominantly the abnormally phosphorylated tau [3–8]. Tau is a microtubule (MT)-associated protein, which promotes MT assembly and stabilization [9,10]. It has been hypothesized that aberrant phosphorylation of tau leads to its dissociation from MTs and aggregation into PHF, resulting in destabilization of MTs and the death of neurons [10,11]. Recently, it has been suggested that the brain-specific tau protein kinase II (TPK II) might play an important role in the pathogenesis of AD [12–14].

Amyloid plaques are composed of a core of β-amyloid (Aβ) peptide derived from proteolytic processing of the amyloid precursor protein, APP [15]. The Aβ 1–42 peptide forms fibrils more readily than Aβ 1–40 peptide [16]. A central issue that has not been addressed concerns why NFTs and senile plaques invariably co-exist in the AD brain and what, if any, relationship exists between the two.

Our previous studies [14] support the hypothesis that the initial phosphorylation of tau by TPK II is an essential step in the detachment of tau from MTs. These in vitro results are consistent with the proposed role of TPK II in AD pathogenesis [12] as well as with studies in transgenic mice overexpressing the p25 activator protein of TPK II [13]. However, the nature of the pathological factors involved in tau aggregation and hyperphosphorylation in AD is elusive. To determine whether Aβ can alter solubility and phosphorylation characteristics of tau, we studied the in vitro effects of aggregated Aβ 1–42 on tau solubility and TPK II-mediated tau phosphorylation. Results of these studies suggest a potential role of Aβ 1–42 in tau aggregation and TPK II-mediated tau hyperphosphorylation.

2. Materials and methods

2.1. Materials

Aβ peptides were obtained from Bachem. The AT-180 antibody for phosphorylated tau was from Innogenetics. The 4G5 antibody was a monoclonal made in house and reacts with unphosphorylated and phosphorylated tau. The anti-mouse IgG-peroxidase was from Roche Molecular Biochemicals and the ECL reagents were from Amersham Pharmacia Biotech. GSK-3 (tau protein kinase I; TPK I) was obtained from New England Biolabs. Human recombinant tau 1-383 isoform and recombinant TPK II were purified as described [14]. Bovine tau was obtained from Sigma Chemical Co. Protein concentration was determined using the BCA kit from Pierce. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the procedure of Laemmli [17].

2.2. Cloning, expression and purification of the truncated forms of tau

The tau construct containing the four repeat (4R) domains of tau (244Q–390A) was cloned by inserting it in the pET 23a vector as an *Nde*I/*Eco*RI fragment and transforming it into competent TOP 10 cells from Invitrogen. Plasmid DNA was isolated from the correct clone and used to transform competent BL21(DE3) cells from Stratagene. The *Escherichia coli* cells were grown to an A_{600} of 0.6 and induced with 1 mM IPTG for 3 h in NS-85 medium [14]. Cells were collected by centrifugation and the pellets stored at −80°C and used for immobilized metal affinity chromatography purification as described elsewhere [14]. The tau construct containing the N-terminal domain of tau (1M–238S) with an octa-histidine C-terminal tag was cloned by inserting it in-frame behind the T7 tag of the pET 23a vector as a *Bam*HI/*Eco*RI

*Corresponding author. Fax: (1)-616-833 1488.
E-mail address: satish.k.sharma@am.pharmacia.com (S.K. Sharma).

Abbreviations: NFT, neurofibrillary tangles; MT, microtubule; TPK II, tau protein kinase II; TPK I, tau protein kinase I; AD, Alzheimer's disease; PHF, paired helical filament; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; SPA, scintillation proximity assay

0014-5793/02/$22.00
PII: S0014-5793(02)02376-1

fragment. Subcloning, expression and purification were done as for the 4R tau.

2.3. Transfection of mammalian tissue culture cells and Aβ detection

HEK 293 cells (ATCC, CRL-1573) were transfected with expression constructs using the Lipofectamine Plus reagent (Gibco/BRL). The CT-100 was expressed from the pcDNA3.1 vector (Invitrogen); the construct encodes amino acids 596–696 of the APP696 isoform and uses the methionine before the beta cleavage site. Tau cDNA (1–383 isoform) vector for transfection into HEK 293 cells contained a GFP tag at its C-terminus [18]. Cells were seeded in tissue culture plates to a density of 70–80% confluence. A six-well plate of cells was transfected with 8 μg of each DNA in Optimem (Gibco/BRL); Optimem was added to a total volume of 8 ml, distributed 1 ml per well and incubated for 4 h. The transfection medium was replaced with Dulbecco's modified Eagle's medium, 10% fetal bovine serum, Na-pyruvate, with antibiotic/antimycotic and the cells were incubated under normal conditions (37°C, 5% CO_2) for 48 h. The conditioned medium was removed to polypropylene tubes and stored at −80°C until assayed. Conditioned media samples were assayed for Aβ by sandwich enzyme-linked immunosorbent assay (ELISA) and immunoblotting with antibodies 6E10 (Senetek), biotinylated Rb208 for Aβ 1–40, and Rb165 for Aβ 1–42 (P.D. Mehta, NYS Inst.).

2.4. Tau hyperphosphorylation in the presence of Aβ

The filter-binding assay [14] was performed by incubating 2 μM tau with 3.6 nM TPK II for 10 min (or the time indicated) at 37°C in a total volume of 15 μl containing 40 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mg/ml bovine serum albumin and 1 μCi [γ-^{33}P]ATP. Aβ peptides were added at 10× concentrations to the assay to achieve the final concentration indicated.

Gel-shift assays were carried out by incubating 3 μM tau with 50 nM TPK II and/or 167 U/ml TPK I for the indicated time at 37°C in a total volume of 20 μl containing 40 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, and 1 mM ATP. Aβ peptides were added at 10× concentrations to the assay to achieve the final concentration indicated. Following incubation the samples were electrophoresed on 12% SDS–PAGE and stained with Coomassie blue R-250. For Western blotting of Aβ-mediated hyperphosphorylated tau, AT-180 antibody was used at a 1:5000 dilution followed by goat anti-mouse IgG-peroxidase used at a 1:10 000 dilution as the secondary antibody. The blots were developed using the ECL protocol.

2.5. Tau aggregation assays

When tau aggregation was studied by SDS–PAGE, 2 μM tau was incubated with the indicated amount of Aβ 1–42 for the indicated time at 37°C in a total volume of 100 μl containing 40 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT. Aβ peptides were added at 10× concentrations to the assay to achieve the final concentration indicated. Samples were centrifuged at 12 000×*g* in a microcentrifuge for 5 min. The supernatants were removed, and the pellets washed once with 40 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT and centrifuged again. The pellets were resuspended in 100 μl buffer, electrophoresed on 12% SDS–PAGE next to the corresponding supernatant, and stained with Coomassie blue R-250. Tau bands were analyzed by densitometry. Phosphorylated tau used to study aggregation in the presence of Aβ 1–42 was produced by incubating 27 μM tau in the presence of 100 nM TPK II for 4 h at 37°C containing 40 M Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, and 1 mM ATP. The sample was subsequently dialyzed into 50 mM Tris, pH 8.0, 1 mM DTT.

2.6. Scintillation proximity assay (SPA) for Tau:Aβ interaction

For SPA, [^{33}P]tau was added at various concentrations to 2 μM Aβ 1–42 and 5 mg/ml anti-mouse PVT SPA beads (Amersham) in a total volume of 100 μl of 40 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT for 30 min at 37°C. The samples were centrifuged at 12 000×*g* in a microcentrifuge and counted using a Packard TriCarb liquid scintillation counter. The background counts per minute (CPM) were obtained by incubating varying concentrations of [^{33}P]tau with the SPA beads in the absence of Aβ 1–42. SPA counts were plotted versus [^{33}P]tau concentration.

The data obtained from the SPA assay were analyzed according to the equation shown below, a rectangular hyperbola widely employed for analyzing solid phase-binding data [19], to determine the half-saturating level of binding (estimated dissociation constant, K_D) using the program Prism (GraphPad),

$$B = \frac{B_{max}[L]}{K_D + [L]}$$

where B is the complex concentration (in CPM), B_{max} is the maximum complex concentration and [L] is the molar concentration of tau.

3. Results

3.1. Characterization of tau and Aβ peptides

The properties of Aβ 1–42 and tau used in this study were established by a variety of techniques. The composition of Aβ 1–42 peptide was confirmed by mass spectrometry (MS). Further characterization of this peptide by thioflavine T-binding [20] and native PAGE revealed that, as expected, Aβ 1–42 peptide was predominantly aggregated. A non-aggregated form of Aβ 1–40 was used as a control and was also characterized by thioflavine T fluorescence [20]. The human tau (1–383) construct [14] was expressed in *E. coli*, purified, and characterized by MS which showed that it was devoid of any phosphorylation.

3.2. Aβ-mediated tau aggregation

As shown in Fig. 1A, Aβ 1–42-induced tau aggregation is instantaneous (lanes 3 and 4) under defined conditions. Under these conditions, at each time point the tau protein was found to be in the pellet fraction along with Aβ 1–42 which ran at 4 kDa. A dose–response study is shown in Fig. 1B. The percentage of tau protein in the pellet fraction was determined by densitometry of the SDS–PAGE (Fig. 1C). These results show that addition of Aβ 1–42 to soluble tau promotes tau aggregation in a dose-dependent manner.

The effect of Aβ 1–42 on aggregation of unphosphorylated wild-type tau was indistinguishable from TPK II phosphorylated tau (Fig. 1C). Since TPK II is a proline-directed kinase and all the proline-directed phosphorylation sites for TPK II are outside the MT-binding repeat region, it is hypothesized that Aβ 1–42 exerts its effect on tau by interacting with its MT-binding repeat domains. To test this hypothesis, 4R tau (Q244–390A) lacking the N-terminal and the C-terminal domains and N-terminal tau (M1–238S) lacking the four repeats and the C-terminal domain were prepared to study the effect of Aβ 1–42 on aggregation. Aβ 1–42-induced aggregation of 4R tau (Q244–390A) was comparable to that observed for the wild-type tau, while Aβ 1–42-induced aggregation was not observed with tau that lacked the MT-binding repeat domains (Fig. 1C). These results suggest that MT-binding repeat domains are critical for Aβ 1–42/tau interaction, while the N-terminal tau domain (1M–238S) is not essential for Aβ 1–42-induced tau aggregation.

Since human tau is known to exist in at least six various isoforms and only one recombinant isoform of tau (1–383) was used in the above studies (Fig. 1), it was of interest to determine if the observed effect of Aβ was tau isoform (1-383)-specific. Therefore, we also studied various untagged bovine tau isoforms in the presence of Aβ 1–42. The pelleted bovine tau isoforms were monitored by Western blot using 4G5 antibody to tau. These results showed that all various bovine tau isoforms were insoluble in the presence of Aβ 1–42

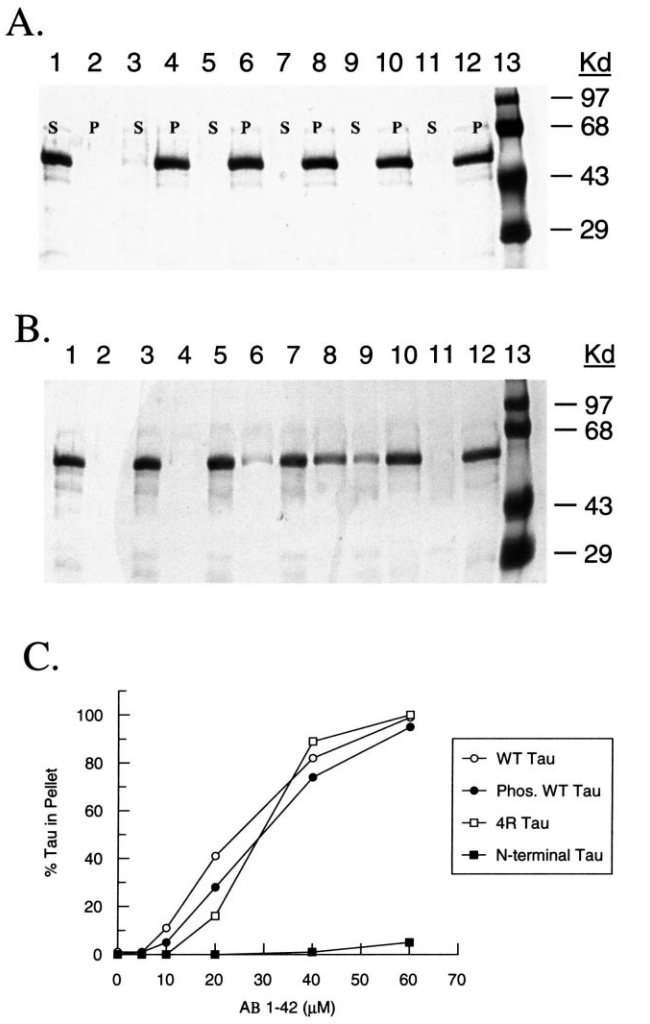

Fig. 1. A: Time course of tau aggregation in the presence of Aβ 1–42. Tau (2 μM) was incubated with 60 μM Aβ 1–42 for the indicated time and the samples centrifuged. The resultant supernatant (S) and pellet (P) were run on SDS–PAGE and Coomassie-stained. Lanes 1 and 2, tau incubated for 2 h; lanes 3 and 4, tau and Aβ 1–42 after mixing; lanes 5 and 6, tau and Aβ 1–42 incubated for 15 min; lanes 7 and 8, for 30 min; lanes 9 and 10, for 1 h; lanes 11 and 12, for 2 h; lane 13, markers. B: Tau (2 μM) was incubated with varying Aβ 1–42 for 30 min at 37°C. Odd-numbered lanes indicate supernatant fractions and even-numbered lanes the pellet fractions. Lanes 1 and 2, tau; lanes 3 and 4, tau and 5 μM Aβ 1–42; lanes 5 and 6, tau and 10 μM Aβ 1–42; lanes 7 and 8, tau and 20 μM Aβ 1–42; lanes 9 and 10, tau and 40 μM Aβ 1–42; lanes 11 and 12, tau and 60 μM Aβ 1–42; lane 13, markers. C: The gel shown in B was scanned by densitometry and the percentage of tau in the pellet was plotted versus Aβ 1–42 concentration. Also shown are dose–response curves for the aggregation of TPK II phosphorylated tau, 4R tau and N-terminal tau in the presence of varying Aβ 1–42.

in a dose-dependent manner (data not shown). Since recombinant tau (1–383) used in this work contains T7 and octa-His affinity tags, the results with bovine tau isoforms also confirm that the observed aggregation of human recombinant tau is indeed due to specific interaction between tau and Aβ 1–42.

3.3. Direct Tau:Aβ interaction by SPA

In order to find a more sensitive and direct measure of the interaction of tau with Aβ 1–42, we used a SPA. Due to the

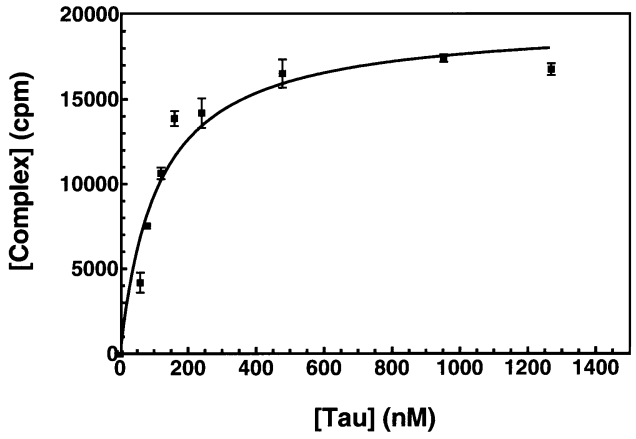

Fig. 2. Aggregation of [^{33}P]tau in the presence of Aβ 1–42. Tau:Aβ complex concentration (CPM) was plotted versus [^{33}P]tau concentration and the data fitted using the equation stated in Section 2.

fact that TPK II phosphorylated tau and unphosphorylated tau seemed to show similar aggregation in the presence of Aβ 1–42 (Fig. 1C), tau was labeled using [^{33}P]ATP and TPK II. The subsequent [^{33}P]tau was purified and then incubated in the presence of Aβ 1–42 and SPA beads and the solution was centrifuged. Due to the insoluble nature of the tau/Aβ complex, the [^{33}P]tau centrifuged into a pellet came in close proximity to the pelleted SPA beads and resulted in SPA counts (Fig. 2). The low background SPA counts seen in the absence of Aβ 1–42 suggested that the SPA counts obtained were the result of an interaction between [^{33}P]tau and Aβ 1–42. By varying the concentration of [^{33}P]tau and keeping the concentration of Aβ constant (2 μM), a relative K_D of 108 ± 25 nM was calculated (Fig. 2). These SPA counts were inhibited in a dose-dependent manner when [^{33}P]tau and unlabeled tau were added together to SPA beads in the presence of Aβ 1–42 (data not shown). In contrast, soluble tau was unable to compete with preformed Aβ–[^{33}P]tau complex, suggesting Aβ promotes tau aggregation. Our results with the above two independent techniques and using purified and well characterized tau and Aβ argue against any confounding interactions of either Aβ or tau with other assay components or proteins.

3.4. Effect of co-expression of tau and CT-100 on Aβ secretion from HEK 293 cells

Recent studies show that in the absence of functional PS1, the C-terminal fragment of APP (CT-100) is accumulated in Golgi, endoplasmic reticulum, and lysosomes [21]. This APP stub may be prone to aggregation and/or hydrolysis to Aβ 1–42 through a non-specific mechanism [22]. Also, it is known that expression of CT-100 in HEK 293 cells results in secretion of both Aβ 1–42 and 1–40 [23]. Fig. 3 shows the co-expression of CT-100 with Tau-GFP in these cells prevents secretion of both Aβ 1–40 and 1–42. This does not appear to be due to lower expression of CT-100 (Fig. 3, inset) as the CT-100 expression in the presence and absence of tau overexpression is comparable. As expected, in the tau control (cells transfected with tau alone), no CT-100 was detected. These cell culture studies are interpreted to mean that in a cellular environment tau presumably interacts with Aβ and/or its CT-100 precursor.

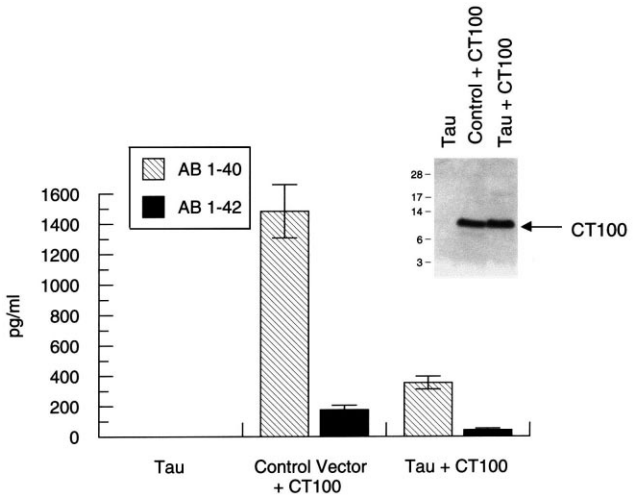

Fig. 3. Co-expression of CT-100 and tau. Tau-GFP construct [18], or a control vector (GFP) was transfected alone or in combination with CT-100 into HEK 293 cells. The resulting conditioned medium was then assayed for the presence of Aβ peptides by sandwich ELISA. Aβ 1–40 or Aβ 1–42 concentrations were plotted in pg/ml. Inset: immunoblot of the expressed CT-100 used as a standard for monitoring expression levels.

3.5. *Effect of Aβ on TPK II-mediated tau phosphorylation*

A number of recent studies are consistent with the proposed role of TPK II in AD pathogenesis [12,14]. Therefore, we studied the effect of Aβ 1–42 on TPK II-mediated phosphorylation of tau by following incorporation of ^{33}P into tau using a filter-binding assay. Fig. 4 shows a time course study demonstrating that in the presence of Aβ 1–42 there is an about 8-fold increase in the initial rate of tau phosphorylation by TPK II. Moreover, Aβ 1–42 had no stimulatory effect on mitogen-activated protein kinase or PKA phosphorylation of tau under defined conditions (data not shown).

3.6. *Aβ-mediated TPK II phosphorylation of the AD-related tau phosphoepitope Thr-231*

In order to determine the effect of Aβ 1–42 on the extent of tau phosphorylation, a gel-shift assay was used to follow an increase in tau's apparent molecular weight following Aβ-induced phosphorylation by TPK II. As shown in Fig. 5A, a significant gel shift was observed in the presence of Aβ 1–42 (lane 5) and this effect was both dose- and time-dependent (data not shown). The control peptides, non-aggregated Aβ 1–40 or 25–35 peptide, had no effect on tau in the gel-shift assay.

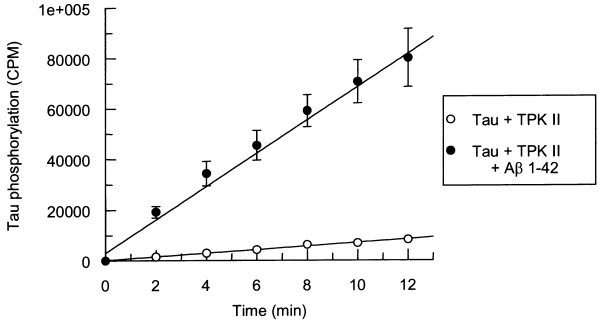

Fig. 4. Time course of the phosphorylation of tau by TPK II in the presence of Aβ 1–42. Tau (2 μM) was incubated with or without 60 μM Aβ 1–42 and 3.6 nM TPK II for various times. Phosphorylation was followed by ^{33}P incorporation using the filter-binding assay and plotted as CPM incorporated versus time. Data represent the average of three experiments.

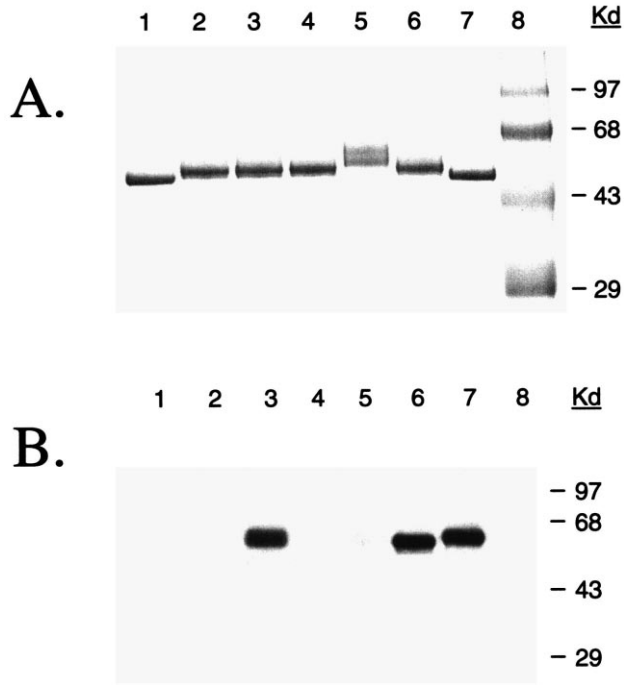

Fig. 5. A: Phosphorylation of tau by TPK II in the presence of Aβ peptides using the gel-shift assay. Lane 1, tau; lane 2, TPK II phosphorylated tau; lane 3, TPK II phosphorylated tau+Aβ 25–35; lane 4, TPK II phosphorylated tau+non-aggregated Aβ 1–40; lane 5, TPK II phosphorylated tau with Aβ 1–42; lane 6, TPK II phosphorylated tau; lane 7, unphosphorylated tau; lane 8, molecular weight markers. B: Western blot using the AT-180 antibody for phosphorylated Thr-231. Lane 1, unphosphorylated tau; lane 2, TPK II phosphorylated tau; lane 3, TPK II phosphorylated tau with Aβ 1–42; lane 4, TPK I phosphorylated tau; lane 5, TPK I phosphorylated tau with Aβ 1–42; lane 6, TPK II and TPK I phosphorylated tau; lane 7, TPK II and TPK I phosphorylated tau with Aβ 1–42; lane 8, unphosphorylated tau.

Phosphorylation of an AD-related epitope (P-Thr-231) in tau can be detected by the AT-180 monoclonal antibody, which almost exclusively recognizes PHFs from AD brain [24]. Fig. 5B shows that Aβ 1–42 stimulates phosphorylation of Thr-231 by TPK II. In contrast, Aβ 1–42 failed to stimulate tau phosphorylation of Thr-231 by TPK I (GSK-3β). The Thr-231 phosphoepitope in tau could also be generated by phosphorylation of tau in the presence of both TPK II and TPK I, suggesting a requirement for prior TPK II-mediated tau phosphorylation. Notably, a slight additive effect was observed when tau was phosphorylated by TPK II and TPK I in the presence of Aβ 1–42 (Fig. 5B), suggesting that the observed hyperphosphorylation effect is additive. Furthermore, studies with a T231A tau mutant confirmed that, in wild-type tau, stimulation of phosphorylation at Thr-231 by Aβ 1–42 was indeed due to phosphorylation at this epitope (data not shown).

Relative to Aβ 1–42, non-aggregated Aβ 1–40 had very little effect on tau phosphorylation (Fig. 5A) and aggregation. We also obtained a batch of preaggregated Aβ 1–40 as measured by thioflavine T fluorescence and native PAGE and studied its effect on tau phosphorylation. In contrast to

non-aggregated Aβ 1–40, the effect of aggregated Aβ 1–40 on tau phosphorylation and aggregation (data not shown) was comparable to the results obtained with aggregated Aβ 1–42 (Fig. 1). Taken together, our in vitro results suggest that aggregation of Aβ 1–42 or 1–40 is essential for its effect on tau aggregation and hyperphosphorylation by TPK II.

4. Discussion

The currently dominant amyloid cascade hypothesis for AD etiology and pathogenesis is not entirely consistent with a number of studies [25–30]. There is experimental evidence which suggests that TPK II might be involved in the formation of hyperphosphorylated tau-containing NFTs at an early stage [31]. Phosphorylation of human tau by TPK II inhibits tau's ability to promote MT assembly and TPK II-mediated phosphorylation of MT-associated tau results in tau's dissociation from the MTs and tubulin depolymerization [14]. Dysregulation of TPK II is reported to play an important role in the pathogenesis of AD [12]. These studies are consistent with the studies in transgenic mice overexpressing the p25 activator protein of TPK II [13]. Thus, in vitro studies were carried out to determine if there is a potential link between aggregated Aβ 1–42/1–40, TPK II, tau aggregation, and hyperphosphorylation. We have shown here that Aβ 1–42 promotes tau aggregates and this in turn stimulates TPK II-mediated tau phosphorylation under defined conditions. In addition, interaction of tau with Aβ 1–42 results in phosphorylation of tau at Thr-231 in the presence of TPK II (Fig. 5B) and this phosphoepitope is an integral part of hyperphosphorylated tau-containing NFTs. Since we probed only with AT-180 antibody, we cannot rule out other AD-related phosphoepitopes that might be present in tau hyperphosphorylated in the presence of Aβ 1–42 and TPK II. Although the extent of tau phosphorylation by TPK I is increased in the presence of Aβ 1–42, the phosphorylation at Thr-231 did not take place when Aβ 1–42 was incubated with tau and TPK I, suggesting that the observed stimulation of phosphorylation at Thr-231 seems to be unique to TPK II. In the context of plaques and tangles found in the AD brain [1,2], our present studies are intriguing because they show that direct interaction of tau with Aβ stimulates tau aggregation as well as the rate of tau phosphorylation by TPK II.

Recent studies [32] indicate that aggregation of tau protein may result in the earliest clinically detectable stages of dementia in AD. Thus, it is conceivable that intraneuronal accumulation of Aβ 1–42 makes an important contribution to early dementia in AD by initiating the onset of cytoskeletal disruption and tau aggregation [33]. This would be consistent with a recent study which suggests that Aβ 1–42 accelerates NFT formation in transgenic mice [34]. Thus, initial Aβ:tau interaction may set the stage for tau dysfunction and dementia.

Acknowledgements: We thank the laboratories of Jerry Slightom and Rod Mathews for DNA sequencing and MS analysis, respectively. We are also thankful to Don Carter, Bob Heinrikson, George Melchior, Mark Gurney, and Don Frail for helpful comments.

References

[1] Kosik, K.S. (1991) Trends Neurosci. 14, 218–219.
[2] Lee, V.M.-Y. and Trojanowski, J.Q. (1992) Curr. Biol. 2, 653–656.
[3] Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., Seiteberger, F., Grundke-Iqbal, I., Iqbal, K. and Wisniewski, H.M. (1989) Brain Res. 477, 90–99.
[4] Bondareff, W., Wischik, C.M., Novak, M., Amos, W.B., Kluf, A. and Roth, M. (1990) Am. J. Pathol. 137, 711–723.
[5] Goedert, M. (1993) Trends Neurosci. 16, 460–465.
[6] Greenberg, S.G. and Davies, P. (1990) Proc. Natl. Acad. Sci. USA 87, 5827–5831.
[7] Ksiezak-Reding, H. and Yen, S.-H. (1991) Neuron 6, 717–728.
[8] Lee, V.M.-Y., Balin, B.J., Otvos, L. and Trojanowski, J.Q. (1991) Science 251, 675–678.
[9] Goedert, M. and Jakes, R. (1990) EMBO J. 9, 4225–4230.
[10] Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. and Crowther, R.A. (1989) Neuron 3, 519–526.
[11] Kosik, K.S. (1990) Curr. Opin. Cell. Biol. 2, 101–104.
[12] Patrick, G.N., Zukerberg, L., Nikolic, M., De La Monte, S., Dikkes, P. and Tsai, L.-H. (1999) Nature 402, 615–622.
[13] Ahlijanian, M.K., Barrezueta, N.X., Williams, R.D., Jakowski, A., Kowsz, K.P., McCarthy, S., Coskran, T., Carlo, A., Seymour, P.A., Burkhardt, J.E., Nelson, R.B. and McNeish, J.D. (2000) Proc. Natl. Acad. Sci. USA 97, 2910–2915.
[14] Evans, D.B., Rank, K.B., Bhattacharya, K., Thomsen, D.R., Gurney, M.E. and Sharma, S.K. (2000) J. Biol. Chem. 275, 24977–24983.
[15] Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. and Muller-Hill, B. (1987) Nature 325, 733–736.
[16] Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C. and Glabe, C. (1992) J. Biol. Chem. 267, 546–554.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Nagiec, E.E., Sampson, K.E. and Abraham, I. (2001) J. Neurosci. Res. 63, 268–275.
[19] Balbona, K., Tran, H., Godyna, S., Ingham, K.C., Strickland, D.K. and Argraves, W.S. (1992) J. Biol. Chem. 267, 20120–20125.
[20] LeVine III, H. (1993) Protein Sci. 2, 404–410.
[21] Chen, F., Yang, D.S., Petanceska, S., Yang, A., Tandon, A., Yu, G., Rozmahel, R., Ghiso, J., Nishimura, M., Zhang, D.M., Kawarai, T., Levesque, G., Mills, J., Levesque, L., Song, Y.Q., Rogaeva, E., Westaway, D., Mount, H., Gandy, S., George-Hyslop, P.S.T. and Fraser, P.E. (2000) J. Biol. Chem. 275, 36794–36802.
[22] Nixon, R.A., Cataldo, A.M. and Mathews, P.M. (2000) Neurochem. Res. 25, 1161–1172.
[23] Zang, L., Song, L. and Parker, E.M. (1999) J. Biol. Chem. 274, 8966–8972.
[24] Goedert, M., Jakes, R., Crowther, R.A., Cohen, P., Vanmechelen, E., Vandermeeren, M. and Cras, P. (1994) Biochem. J. 301, 871–877.
[25] Terry, R.D. (1996) J. Neuropathol. Exp. Neurol. 55, 1023–1025.
[26] Braak, H., Braak, E., Bohl, J. and Reintjes, R. (1996) Neurosci. Lett. 210, 87–90.
[27] Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagopian, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B. and Zhao, J. (1995) Nature 373, 523–527.
[28] Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. and Cole, G. (1996) Science 274, 99–102.
[29] Holcomb, L., Gordon, M.N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.-M., Eckman, C., Younkin, S., Hsiao, K. and Duff, K. (1998) Nat. Med. 4, 97–100.
[30] Capsoni, S., Ugolini, G., Comparini, A., Ruberti, F., Berardi, N. and Cattaneo, A. (2000) Proc. Natl. Acad. Sci. USA 97, 6826–6831.
[31] Pe1, J.-J., Grundke-Iqbal, I., Iqbal, K., Bogdanovic, N., Winblad, B. and Cowburn, R.F. (1998) Brain Res. 797, 267–277.
[32] Makaetova-Ladinska, E.B., Garcia-Siera, F., Hurt, J., Gertz, H.J., Xuereb, J.H., Hills, R., Brayne, C., Huppert, F.A., Paykel,

E.S., McGee, M., Jakes, R., Honer, W.G., Harrington, C.R. and Wischik, C.M. (2000) Am. J. Pathol. 157, 623–636.
[33] Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J.P., Haroutunian, V., Buxbaum, J.D., Xu, H., Greengard, P. and Relkin, N.R. (2000) Am. J. Pathol. 156, 15–20.
[34] Lewis, J., Dickson, D.W., Lin, W.-L., Chisholm, L., Corral, A., Jones, G., Yen, S.-H., Sahara, N., Skipper, L., Eckman, C., Hardy, J., Hutton, M. and McGowan, E. (2001) Science 293, 1487–1491.