

Rapid Alzheimer-like phosphorylation of tau by the synergistic actions of non-proline-dependent protein kinases and GSK-3

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Abstract Tau protein from Alzheimer disease (AD) brain is phosphorylated at eleven Ser/Thr-Pro and nine Ser/Thr-X sites. The former sites are phosphorylated by proline-dependent protein kinases (PDPKs), the latter by non-PDPKs. The identities of both the PDPKs and non-PDPKs involved in AD tau hyperphosphorylation are still to be established. In this study we have analyzed the interactions between a PDPK (GSK-3) and several non-PDPKs (A-kinase, C-kinase, CK-1, CaM kinase II) in the phosphorylation of one isoform (tau 39) of human tau. We found that the rate of phosphorylation of tau 39 by GSK-3 was increased several-fold if tau were first prephosphorylated by the non-PDPKs. Further, several Alzheimer-like epitopes in tau can be induced only slowly after phosphorylation of tau by GSK-3 alone. After a prephosphorylation of tau by the non-PDPKs, however, the rate of induction of these epitopes by GSK-3 is increased several-fold. These results suggest that one role of non-PDPK-catalyzed phosphorylation is the modulation of PDPK-catalyzed phosphorylation of tau in AD brain.

Key words: GSK-3; Tau protein; Protein kinase; Alzheimer's disease; Paired helical filament

1. Introduction

The paired helical filaments (PHFs) found in the brain of patients with Alzheimer disease (AD) are composed primarily of the microtubule-associated protein tau [1]. Compared to tau from normal brain, PHF-tau is in a hyperphosphorylated state [2]. The mechanism(s) leading to and the kinases responsible for the hyperphosphorylation of PHF-tau have not yet been identified.

One approach to identify the responsible kinases is to use purified tau as a substrate for known protein kinases *in vitro*. A comparison of the sites phosphorylated by individual kinases with those known to be phosphorylated in PHF-tau should help in the identification of possible candidate kinases. Twenty phosphorylation sites have recently been identified in PHF-tau. Eleven of these sites are on Ser/Thr-Pro motifs and are therefore phosphorylated by proline-dependent protein kinases

(PDPKs). The other nine sites are on Ser/Thr-X motifs and are probably phosphorylated by non-PDPKs [3]. Hence both PDPKs and non-PDPKs may participate in the hyperphosphorylation of PHF-tau.

The *in vitro* phosphorylation of tau by both PDPKs and non-PDPKs has been studied. The PDPKs include MAP kinase [4,5], cdk2 kinase [6], cdk5 [7], and GSK-3 [8–10]. The non-PDPKs include cyclic AMP-dependent protein kinase (A-kinase) [11,12], protein kinase C [12–14], calcium/calmodulin-dependent protein kinase II (CaM kinase II) [12,15], calcium/calmodulin kinase Gr [12], casein kinase 1 (CK-1) [12], and casein kinase-2 (CK-2) [12,16].

Several phosphorylation-dependent antibodies have been shown to react with PHF-tau. These include SMI31 (recognizes P-Ser-396, P-Ser-404) [17], SMI34 (recognizes phosphorylated epitopes on either side of the microtubule-binding domains of tau) [17], PHF-1 (recognizes P-Ser-396) [18], and M₄ (recognizes P-Thr-231) [19], among others. Antibodies that recognize dephosphorylation epitopes in tau include Tau-1 (recognizes dephosphorylated Ser-199 and Ser-202 [20]), 102C (recognizes dephosphorylated Ser-46) [21], and SMI33 (recognizes dephosphorylated Ser-235 [17], among others. The above Alzheimer-like epitopes can be induced after phosphorylation of normal tau by various PDPKs *in vitro* [4,6–8,10]. Some of these epitopes are also induced after phosphorylation of tau by non-PDPKs [22].

In this study we have investigated how a PDPK may interact with non-PDPKs to produce the hyperphosphorylated state of PHF-tau. In this respect, we have analyzed how the specificity of GSK-3 (a PDPK) is affected when the initial phosphorylation state of tau is altered by various non-PDPKs. We found that after such prephosphorylation of tau by non-PDPKs (A-kinase, CK-1, CaM kinase II) the rate at which several Alzheimer-like epitopes on tau can be induced by GSK-3 is greatly increased.

2. Experimental

2.1. Materials

The human tau clone, tau 39 (kindly provided by M. Goedert), encodes for the tau isoform 3L that has three tandem C-terminal repeats plus a 58-amino acid insert near the N-terminal end of the polypeptide [23]. This tau was subcloned into the *Nde*I and *Eco*RI sites downstream of the T7 RNA polymerase promoter of the expression plasmid pRK172 [24]. The recombinant plasmid was transformed into *E. coli* BL 21 (DE3) cells [25] and screened by restriction enzymes. The cells were grown to an optical density of 0.6–1.0 at 600 nm. Expression was induced by adding 0.4 mM isopropyl thio- β -D-galactoside. After shaking at 37°C for 5 h, the cells were collected by centrifugation and tau purified essentially as described in [26] with the following modifications. The cells were lysed by ultrasonication in 80 mM Mes (pH 6.8) containing 5 mM dithiothreitol. After centrifugation at 120,000 $\times g$ tau was isolated from the extract by phosphocellulose chromatography

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Abbreviations: PHF, paired helical filament; A-kinase, cyclic AMP-dependent protein kinase; CaM kinase II, calcium/calmodulin-dependent protein kinase II; C-kinase, calcium/phospholipid-dependent protein kinase; CK-1, casein kinase-1; CK-2, casein kinase-2; Gr kinase, calcium/calmodulin-dependent protein kinase from rat cerebellum; GSK-3, glycogen synthase kinase-3; MAP kinase, mitogen-activated protein kinase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PDPK, proline-dependent protein kinase.

[27], heated in a boiling bath for 5 min followed by extraction in 2.5% perchloric acid [1].

CK-1, CK-2 [28] and GSK-3 [29] were purified from bovine brain. The GSK-3 preparation contained both the α and β isoforms in the ratio 3:2. CaM kinase II [30] and C-kinase [31] were purified from rat brain. CaM kinase II was a gift from Brad McDonald, Burroughs-Wellcome Laboratories; C-kinase was generously supplied by V. Chauhan of this Institute. The catalytic subunit of A-kinase, calmodulin and goat anti-mouse IgG conjugated to alkaline phosphatase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The tau monoclonal antibodies were obtained as follows: SMI31 and SMI34 were purchased from Sternberger Monoclonal Inc. (Baltimore, MD, USA); PHF-1 (1:8) and M4 [19] were gifts from S. Greenberg, Burke Rehabilitation Center, Cornell Medical College (White Plains, NY, USA) and Y. Ihara, Institute for Brain Research, University of Tokyo (Tokyo, Japan), respectively; Tau-1 was a gift from L. Binder, Molecular Geriatrics Corp. (Lake Bluff, IL, USA). (γ - 32 P)ATP was purchased from ICN Biomedicals (Costa Mesa, CA, USA).

2.2. Methods

Human tau 39 was phosphorylated by GSK-3 in a reaction mixture normally containing 0.15 mg/ml tau, 6 mM MgCl_2 , 10 mM 2-mercaptoethanol, 0.1 mM [γ - 32 P]ATP, 40 mM HEPES (pH 7.5), and GSK-3. Phosphorylation of tau by the other kinases was recently described by us [12]. Reactions were initiated at 30°C by addition of [γ - 32 P]ATP and, after the desired incubation time, aliquots removed and processed as described [32]. GSK-3 activity was measured using myelin basic protein as a substrate [33]. One unit of GSK-3 activity is defined as the amount of the kinase that will catalyze the incorporation of 1 nmol 32 P/min into myelin basic protein (1 mg/ml) at 30°C.

Immunoblotting of tau by the different tau antibodies was carried out as previously described [2]. The following dilutions of antibodies were used: SMI31 (1:75), SMI34 (1:75), PHF-1 (1:500), M4 (1:1000), and Tau-1 (1:50,000).

3. Results

3.1. Phosphorylation of tau by different kinases

We have examined the time course of phosphorylation of human tau 39 (three tandem C-terminal repeats plus a 58-amino acid N-terminal insert) by a proline-dependent protein kinase (PDPK), GSK-3, and several non-PDPKs (A-kinase, C-kinase, CK-1, CK-2, CaM kinase II) (Fig. 1A). It can be seen that either CK-1 or CaM kinase II can rapidly catalyze the incorporation of above 1 mol 32 P/mol tau in 15 min. Only a

slight additional phosphorylation (<0.2 mol 32 P) is promoted by CaM kinase II over the next 6 h. By contrast 32 P incorporation catalyzed by CK-1 is ~ 4.8 mol after 6 h and the reaction is still incomplete. 32 P incorporation catalyzed by A-kinase, C-kinase and GSK-3 occur at slower rates, and reach 1.3, 1.6, and 2.4 mol/mol tau, respectively, after 6 h incubation at 30°C. By contrast to the above five kinases human tau 39 was a very poor substrate for CK-2 (0.14 mol [32 P/mol]tau after 6 h at 30°C) (results not shown).

3.2. Phosphorylation of tau by GSK-3 after an initial phosphorylation by various non-PDPKs

We have recently shown that prephosphorylation of bovine tau (a mixture of six isoforms) by A-kinase, C-kinase or CK-2 stimulated a subsequent phosphorylation by GSK-3 [34]. We have therefore examined the effect of prephosphorylation of human tau 39 on GSK-3-catalyzed phosphorylation. Tau 39 was incubated in the absence of any kinase or in the presence of A-kinase, C-kinase, CK-1, and CaM kinase as shown in Fig. 1A. After 2 h at 30°C, the reaction tubes were heated at 95°C for 5 min to inactivate the kinases. [γ - 32 P]ATP and GSK-3 were then added to the reaction tubes at 30°C and 32 P incorporation into tau quantitated. The results are shown in Fig. 1B. Prephosphorylation of tau by CK-1, C-kinase, CaM kinase II, and A-kinase stimulated 32 P incorporation catalyzed by GSK-3. After 30 min incubation with GSK-3 the level of stimulation was 1.9-, 2.9-, 5.6-, and 8.2-fold, respectively, compared to tau that was not prephosphorylated (Fig. 1B). These results indicate that prephosphorylation of human tau 39 by non-PDPKs can stimulate the subsequent level of 32 P incorporation achieved by GSK-3.

3.3. Pattern of binding of tau antibodies to different species of phosphorylated tau

Previous studies have shown that the epitopes to several tau antibodies are maximally induced only after prolonged (up to 20 h) incubation with GSK-3 [8,10]. Since prephosphorylation of tau by non-PDPKs stimulate subsequent phosphorylation by GSK-3 (Fig. 1B) we have surveyed whether the binding of

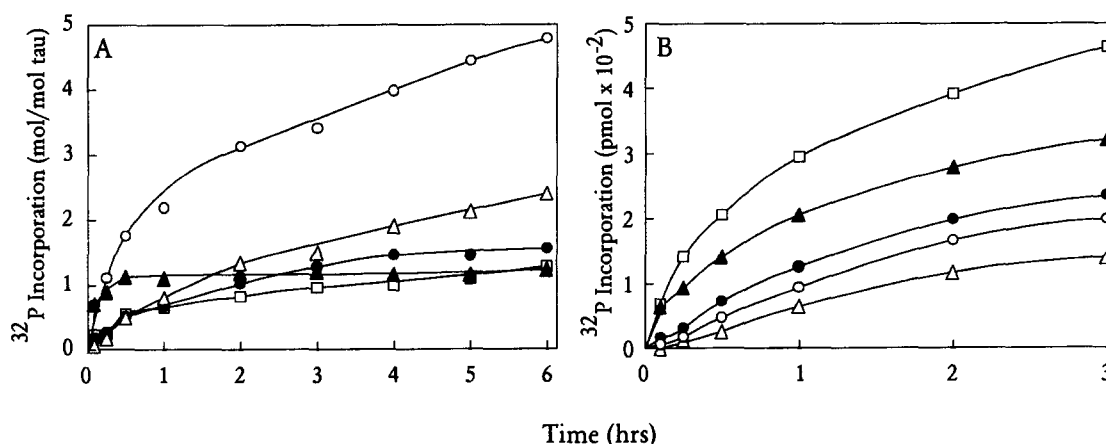


Fig. 1. Phosphorylation of human tau 39 by different kinases. (A) Comparison of tau phosphorylation by several kinases. Tau was phosphorylated by A-kinase (\square), C-kinase (\bullet), CaM kinase II (\blacktriangle), GSK-3 (\triangle), and CK-1 (\circ). (B) Use of different prephosphorylated tau species as substrates for GSK-3. Tau was incubated in the absence (\triangle) or presence of A-kinase (\square), C-kinase (\bullet), CaM kinase II (\blacktriangle), or CK-1 (\circ) and unlabeled ATP. After 2 h at 30°C, the reactions were stopped by heating at 95°C for 5 min. After removing denatured kinases (spun at $10,000\times g$ for 10 min), reaction mixtures were supplemented with [γ - 32 P]ATP and GSK-3 and again incubated at 30°C. In both A and B, aliquots of the reaction mixtures were removed and processed as described [31].

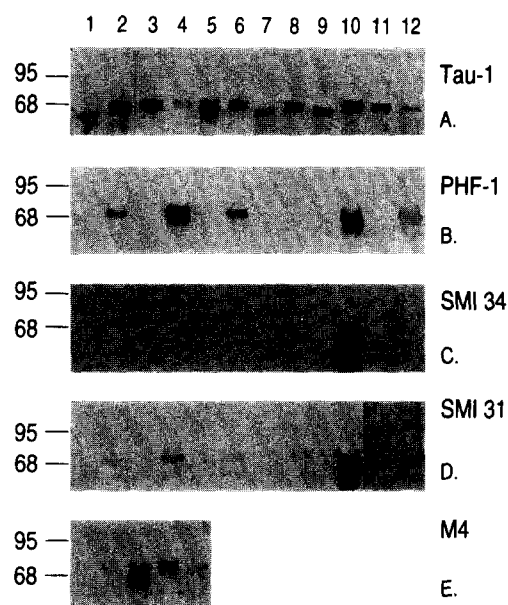


Fig. 2. Pattern of binding of tau antibodies to different species of phosphorylated tau. Non-phosphorylated tau (lane 1) or phosphorylated tau (lanes 2–12) were immunoblotted with Tau-1 (A), PHF-1 (B), SMI34 (C), SMI31 (D), and M4 (E). Tau was phosphorylated by GSK-3 (lane 2), A-kinase (lane 3), A-kinase + GSK-3 (lane 4), C-kinase (lane 5), C-kinase + GSK-3 (lane 6), CK-2 (lane 7), CK-2 + GSK-3 (lane 8), CK-1 (lane 9), CK-1 + GSK-3 (lane 10), CaM kinase II (lane 11), or CaM kinase II + GSK-3 (lane 12). Tau was phosphorylated for 2 h at 30°C when each kinase was used alone. When a combination of kinases was used, phosphorylation was for 2 h by the first kinase followed by heating at 95°C for 5 min. The second kinase (GSK-3) was then added and phosphorylation continued at 30°C for 1 h. In panel E, tau was not phosphorylated (lane 1) or phosphorylated by A-kinase + GSK-3 (lane 2), CK-1 + GSK-3 (lane 3), CaM kinase II + GSK-3 (lane 4), or GSK-3 (lane 5).

different phosphorylation-dependent tau antibodies are also enhanced (or inhibited) under these conditions. For these studies we prepared three categories of phosphorylated tau: (a) tau phosphorylated by the five non-PDPKs; (b) tau phosphorylated by GSK-3; (c) tau phosphorylated by GSK-3 after an initial phosphorylation by the non-PDPKs. The binding of several tau antibodies to these different phosphorylated tau species was then analyzed by immunoblotting (Fig. 2). Phosphorylation of the epitope to Tau-1 is known to inhibit binding of this antibody [8,10,20]. The binding of different phosphorylated tau species to Tau-1 is shown in Fig. 2, panel A. Compared to non-phosphorylated tau (lane 1), the binding of Tau-1 was significantly inhibited only after tau was first prephosphorylated by A-kinase (lane 4) or CaM kinase II (lane 12). Phosphorylation by the other kinases, acting singly or in combination, did not significantly affect binding of Tau-1 (Fig. 2, panel A). The antibodies PHF-1 (panel B), SMI34 (panel C), SMI31 (panel D) and M4 (panel E) recognize only phosphorylated epitopes on tau [17–19]. None of these antibodies were found to bind to non-phosphorylated tau (Fig. 2, panels B–E, lane 1). PHF-1 (Fig. 2, panel B) recognized tau that was phosphorylated by GSK-3 alone (lane 2), or by a combination of A-kinase + GSK-3 (lane 4), C-kinase + GSK-3 (lane 6), CK-1 + GSK-3 (lane 10), and CaM kinase II + GSK-3 (lane 12). SMI34 recognized mainly tau that was phosphorylated by the combined actions of CK-1 and GSK-3 (Fig. 2, panel C, lane 10).

With SMI31 (Fig. 2, panel D) the highest level of binding was achieved when tau was phosphorylated by a combination of CK-1 + GSK-3 (lane 10). Binding of the antibody M4 (Fig. 2, panel E) was promoted when tau was phosphorylated by GSK-3 alone (lane 5) or a combination of CK-1 + GSK-3 (lane 3) and CaM kinase II + GSK-3 (lane 4). These results suggest that the epitopes of various tau antibodies become good substrates for GSK-3 after tau is first prephosphorylated by a non-PDPK.

3.4. Rapid induction of epitopes by a combination of kinases

From the results of Fig. 2, the epitopes of various tau antibodies become better substrates for GSK-3 when tau is first prephosphorylated by A-kinase, CK-1 or CaM kinase II but not by C-kinase or CK-2. We have further explored this interaction between GSK-3 and non-PDPKs by analyzing the time course of inhibition (Tau-1) or enhancement (PHF-1, SMI31, SMI34, M4) of the binding of tau antibodies to different species of phosphorylated tau. Fig. 3 shows the results achieved with Tau-1. A significant decrease in Tau-1 binding occurs only after 21 h of phosphorylation of tau either by GSK-3 alone (Fig. 3, panel A) or a combination of CK-1 + GSK-3 (Fig. 3, panel C). A decrease in Tau-1 binding is observed after only 1 h phosphorylation of tau by a combination of either A-kinase + GSK-3 (Fig. 3, panel B) or CaM kinase II + GSK-3 (Fig. 3, panel D). However, whereas A-kinase + GSK-3-catalyzed phosphoryla-

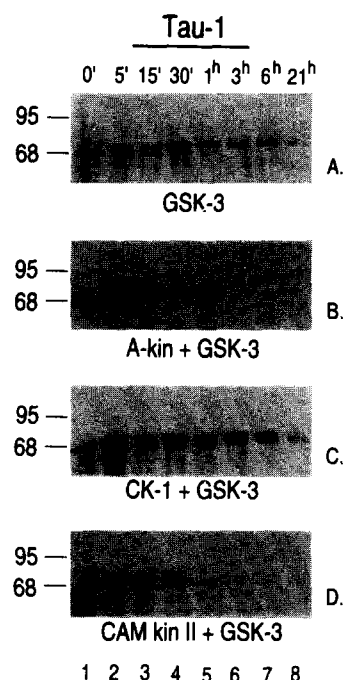


Fig. 3. Time course of inhibition of Tau-1 binding by different combination of kinases. Tau was not prephosphorylated (A) or was prephosphorylated for 2 h by A-kinase (B), CK-1 (C), and CaM kinase II (D). All samples were then heated at 95°C for 5 min, transferred back to 30°C and GSK-3 added to all tubes. Aliquots of the reaction mixtures were then removed at 0 min (lane 1), 5 min (lane 2), 15 min (lane 3), 30 min (lane 4), 1 h (lane 5), 3 h (lane 6), 6 h (lane 7), and 21 h (lane 8). All samples were then immunoblotted with Tau-1. From the results of Fig. 2 prephosphorylation of tau either by C-kinase or CK-2 did not significantly promote inhibition of Tau-1 binding or increase of PHF-1, SMI31, SMI34, M4 binding (below) and were therefore not tested further.

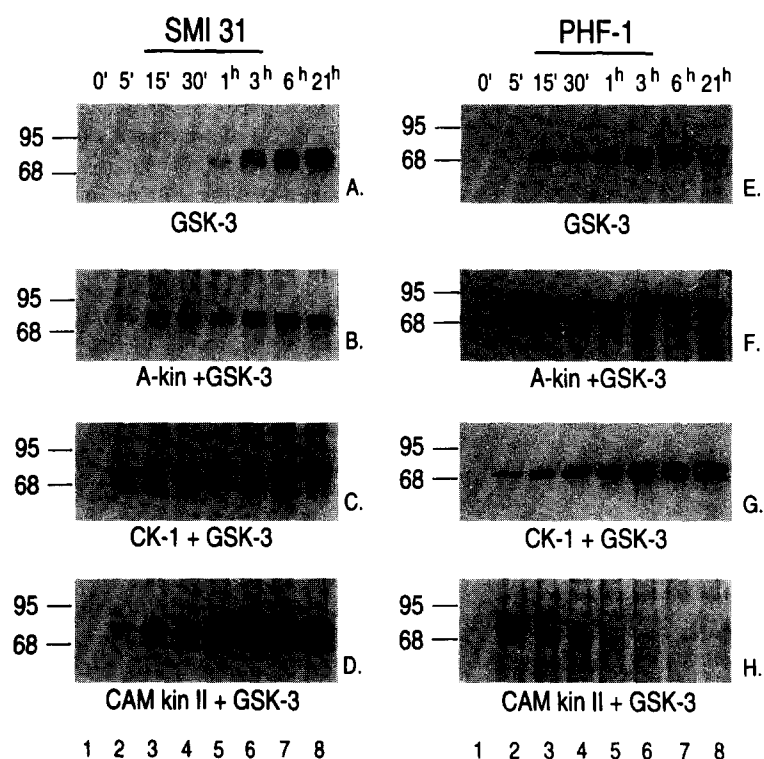


Fig. 4. Time course of induction of SMI31 and PHF-1 binding by different combination of kinases. Tau that was not prephosphorylated (A,E) or was prephosphorylated by A-kinase (B,F), CK-1 (C,G), and CaM kinase II (D,H) was then further phosphorylated by GSK-3. Aliquots of the reaction mixtures were removed at different times and immunoblotted either with SMI31 (A–D) or PHF-1 (E–H). Lane numbers and phosphorylation times are the same as in Fig. 3.

tion completely abolished Tau-1 binding after only 3 h (Fig. 3, panel B), such binding only slowly disappeared after phosphorylation of tau by CaM kinase II + GSK-3 and is still apparent after 21 h (Fig. 3, panel D).

Fig. 4 shows the time course of induction of the SMI31 (panels A–D) and PHF-1 (panels E–H) epitopes. Phosphorylation of tau with GSK-3 alone requires greater than 6 h phos-

phorylation to achieve maximal binding of either antibody (panels A and E). However, both of these epitopes are rapidly induced by GSK-3 if tau is first prephosphorylated by A-kinase (panels B and F) or CaM kinase II (panels D and H). Prephosphorylation of tau by CK-1 rapidly induced the SMI31 epitope (panel C) but only slightly improved binding of PHF-1 compared to that promoted by GSK-3 alone (compare panels

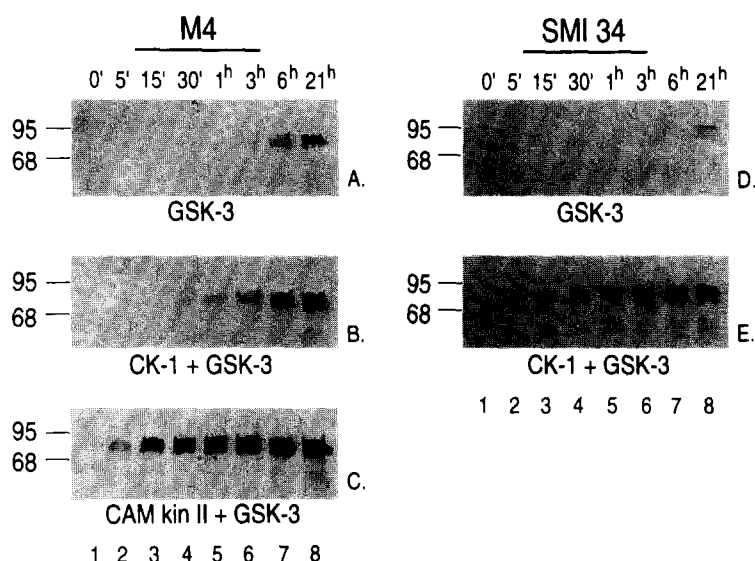


Fig. 5. Time course of induction of M4 and SMI34 binding by different combination of kinases. Tau that was not prephosphorylated (A,D) or was prephosphorylated by CK-1 (B,E), and CaM kinase II (C) was then further phosphorylated by GSK-3. Aliquots of the reaction mixtures were removed at different times and immunoblotted with M4 (A–C) or SMI34 (D–F). Lane numbers and phosphorylation times are the same as in Fig. 3.

E and G). Among the three non-PDPKs (A-kinase, CK-1, CaM kinase II), prephosphorylation of tau by CK-1 and CaM kinase II are best for rapid induction of the SMI31 and PHF-1 epitopes, respectively, by GSK-3. Both epitopes are maximally induced in about 1 h under these circumstances compared to 21 h needed for their maximal induction by GSK-3 alone (Fig. 4, compare panels A,C, and E,H, respectively). In only 5 min prephosphorylation of tau by CK-1 (and to a lesser degree, A-kinase and CaM kinase II also) induce binding of SMI31 several-fold. Under these conditions, GSK-3 alone only minimally induce this epitope (compare lane 2 in panels A and C). A similar conclusion is also true for induction of the PHF-1 epitope when tau is prephosphorylated by CaM kinase II (compare lane 2 in panels E and H, Fig. 4).

Fig. 5 shows the time course of induction of the M4 (A–C) and SMI34 (D–E) epitopes. Both of these epitopes are maximally induced only after 21 h phosphorylation with GSK-3 alone (Fig. 5, panels A and D). As shown previously (Fig. 2, panel C) recognition of the SMI34 epitope by GSK-3 is promoted when tau is first prephosphorylated mainly by CK-1. With GSK-3 alone significant binding of SMI34 required about 6 h incubation (Fig. 5, panel D). After prephosphorylation by CK-1, significant binding of this antibody was detected after only 5 min incubation, but required ~6 h to reach a maximum. Prephosphorylation of tau with either CK-1 (panel B) or CaM kinase II (panel C) increased the rate and extent of binding of M4 to tau. For this purpose CaM kinase II is better. Binding of M4 is increased several-fold over control after only 5 min phosphorylation with CaM kinases II + GSK-3. Maximal binding is reached in ~1 h compared to ~21 h when GSK-3 alone is used (Fig. 5, compare panels A and C). The results outlined in Figs. 3–5 indicate that after tau is first prephosphorylated by a non-PDPK (A-kinase, CK-1, CaM kinase II, depending on epitope being tested) the epitopes of several antibodies can be rapidly induced (SMI31, SMI34, PHF1, M4) or inhibited (Tau-1) by GSK-3 compared to tau that is not prephosphorylated.

4. Discussion

In the present study we have analyzed the phosphorylation of one isoform of human tau – tau 39. This isoform has three (of four) tandem C-terminal repeats and a 58-amino acid insert near the N-terminal end of the molecule [23]. CK-1 and CaM kinase II can rapidly phosphorylate this tau isoform, whereas phosphorylation catalyzed by A-kinase, C-kinase, CK-2, and GSK-3 proceed at a slower rate. A prior phosphorylation of tau by CK-1, C-kinase, CaM kinase II, and A-kinase was found to stimulate a subsequent phosphorylation catalyzed by GSK-3. C-kinase [34], A-kinase, CK-1, and CK-2 [35], have all been previously shown to modulate GSK-3 activity by prephosphorylation of protein substrates. This is the first report, however, of such modulation by CaM kinase II.

Another major finding of this study is that prephosphorylation of tau 39 by non-PDPKs permits a subsequent phosphorylation by GSK-3 to rapidly phosphorylate some of the same epitopes seen in PHF-tau. Previous studies (reproduced here with GSK-3) have shown that prolonged incubation (16–20 h) of tau with MAP kinase [4,5], GSK-3 [8,10], cdk2, and cdk5 [7] are required to maximally induce the binding of several of the phosphorylation-dependent tau antibodies used here. Phosphorylation of protein in cells is a relatively rapid event (sec-

onds to minutes). Hence, the prolonged time of incubation required to induce maximal binding of the different tau antibodies suggests that the epitopes to these antibodies are poor substrates for GSK-3, MAP kinase, cdk2 and cdk5. The results presented in this study suggest that the epitopes to the various tau antibodies become very good substrates for GSK-3 (and maybe other PDPKs) after an initial phosphorylation of tau by a non-PDPK. Under these conditions, significant enhancement of binding of several of the antibodies occur relatively rapidly. For instance, after prephosphorylation of tau by CaM kinase II, GSK-3 can increase the binding of PHF-1 and M4 antibodies several-fold in only 5 min. Without such prephosphorylation significant binding of these antibodies is only observed after 30–60 min incubation with GSK-3. Similarly, the time required for maximal binding of these antibodies is decreased from ~21 h (no prephosphorylation) to ~1 h (with prephosphorylation).

Our results also indicate that prephosphorylation of tau by a non-PDPK can differentially affect the induction of the different tau antibody epitopes. For instance, prephosphorylation of tau by CaM kinase II is best for rapidly inducing the PHF-1 and M4 epitopes but not the SMI31 or SMI34 epitopes (CK-1 is best in both cases). Similarly, prephosphorylation of tau by A-kinase promote an increased subsequent rate of induction of the SMI31 and PHF-1 epitopes by GSK-3. Such prephosphorylation, however, did not promote induction of either the SMI34 or M4 epitopes. In addition, even though prephosphorylation of tau by C-kinase increased subsequent ³²P incorporation by GSK-3, it only minimally affected the rate of induction of several epitopes by GSK-3. These findings suggest that after phosphorylation by a non-PDPK such as A-kinase, tau may undergo a conformational change. Such conformational change may promote binding of some antibodies (such as PHF-1), may inhibit binding of others (such as Tau-1), whereas the binding of still others (such as SMI34) may remain unaffected.

The results of this study suggest a possible dual role for the participation of non-PDPKs in the hyperphosphorylation of PHF-tau. Such hyperphosphorylation has been shown to occur almost equally at Ser/Thr-Pro and Ser/Thr-X sites [3]. Hence, the first possible role of non-PDPKs (their numbers and identities are still obscure) will be to phosphorylate the Ser/Thr-X sites found in PHF-tau. The second possible role of non-PDPKs is a direct consequence of the first. After tau is phosphorylated by non-PDPKs its conformation would be so changed as to modulate the specificities of subsequent phosphorylation by PDPKs (in a similar way as shown in this study). Modulation of tau hyperphosphorylation by PDPK–PDPK interactions are also possible. Prior phosphorylation of tau by TPK II/cdk5 (a PDPK) has been reported to increase the extent of a subsequent phosphorylation catalyzed by TPKI/GSK-3 (another PDPK) [36]. Unlike our study, however, prolonged incubation of the prephosphorylated tau with GSK-3 was still required to observe such increased level of phosphorylation [36]. It is not known whether prephosphorylation of tau by TPK II/cdk5 can increase the rate at which Alzheimer-like epitopes in tau are induced by GSK-3 in a way similar to that observed with non-PDPKs in this study.

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References

- [1] Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.-C., Zaidi, M.S. and Wisniewski, H.M. (1986) *J. Biol. Chem.* 261, 6084–6089.
- [2] Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H.M. and Binder, L.I. (1986) *Proc. Natl. Acad. Sci. USA* 83, 4913–4917.
- [3] Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Titani, K. and Ihara, Y. (1994) *Neurobiol. Aging* (in press).
- [4] Drewes, G., Lichtenberg-Kraag, B., Coring, F., Mandelkow, E.-M., Biernat, J., Goris, J., Doree, M. and Mandelkow, E. (1992) *EMBO J.* 11, 2131–2138.
- [5] Goedert, M., Cohen, E.J., Jakes, R. and Cohen, P. (1992) *FEBS Lett.* 312, 95–99.
- [6] Vulliamt, R., Halloran, S.M., Braun, R.K., Smith, A.J. and Lee, G. (1992) *J. Biol. Chem.* 267, 22570–22574.
- [7] Baumann, K., Mandelkow, E.-M., Biernat, J., Piwnicka-Worms, H. and Mandelkow, E. (1993) *FEBS Lett.* 336, 417–424.
- [8] Mandelkow, E.M., Drewes, G., Biernat, J., Gustke, N., Van Lint, J., Vandenheede, J.R. and Mandelkow, E. (1992) *FEBS Lett.* 314, 315–321.
- [9] Yang, S.-D., Song, J.-S., Yu, J.-S. and Shiah, S.-G. (1993) *J. Neurochem.* 61, 1742–1747.
- [10] Ishiguro, K., Shiratsuchi, A., Sato, S., Omori, A., Arioka, M., Kobayashi, S., Uchida, T. and Imahori, K. (1993) *FEBS Lett.* 325, 167–172.
- [11] Litersky, J.M. and Johnson, G.V.W. (1990) *J. Biol. Chem.* 267, 1563–1568.
- [12] Singh, T.J., Grundke-Iqbal, I., McDonald, B. and Iqbal, K. (1994) *Mol. Cell Biochem.* 131, 181–189.
- [13] Correia, I., Diaz-Nido, J. and Avila, J. (1992) *J. Biol. Chem.* 167, 15721–15728.
- [14] Baudier, J., Lee, S.-H. and Cole, R.D. (1987) *J. Biol. Chem.* 262, 17584–17590.
- [15] Baudier, J. and Cole, R.D. (1991) *J. Biol. Chem.* 262, 17577–17583.
- [16] Greenwood, J.A., Scott, C.W., Spreen, R.C., Caputo, C.B. and Johnson, G.V.W. (1994) *J. Biol. Chem.* 269, 4373–4380.
- [17] Lichtenberg-Kraag, B., Mandelkow E.-M., Biernat, J., Steiner, B., Schroter, C., Gustke, N., Meyer, H.E. and Mandelkow, E. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5384–5388.
- [18] Greenberg, S.G. and Davies, P. (1990) *Proc. Natl. Acad. Sci. USA* 87, 5827–5831.
- [19] Hasegawa, M., Watanabe, A., Takio, K., Suzuki, M., Arai, T., Titani, K. and Ihara, Y. (1993) *J. Neurochem.* 60, 2068–2077.
- [20] Biernat, J., Mandelkow, E.-M., Schroter, C., Lichtenberg-Kraag, B., Steiner, B., Berling, B., Meyer, H., Mercken, M., Vandermere, A., Goedert, M. and Mandelkow, E. (1992) *EMBO J.* 11, 1593–1597.
- [21] Iqbal, K., Grundke-Iqbal, I., Smith, A.J., George, L., Tung, Y.-C. and Zaidi, T. (1989) *Proc. Natl. Acad. Sci. USA* 86, 5646–5650.
- [22] Singh, T.J., Grundke-Iqbal, I. and Iqbal, K. (1994) *J. Neurochem.* (in press).
- [23] Goedert, M., Spillantini, M., Jakes, R., Rutherford, D. and Crowther, R.A. (1989) *Neuron* 3, 519–526.
- [24] McLeod, M., Stein, M. and Beach, D. (1987) *EMBO J.* 6, 729–736.
- [25] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorf, J.W. (1990) *Methods Enzymol.* 185, 60–89.
- [26] Goedert, M. and Jakes, R. (1990) *EMBO J.* 9, 4225–4230.
- [27] Weingarten, M.D., Lockwood, A.H., Hwo, S.Y. and Kirschner, M.W. (1975) *Proc. Natl. Acad. Sci. USA* 72, 1858–1862.
- [28] Singh, T.J. (1988) *Arch. Biochem. Biophys.* 267, 167–175.
- [29] Tung, H.Y.L. and Reed, L.J. (1989) *J. Biol. Chem.* 264, 2985–2990.
- [30] Ohmsted, C.-A., Jensen, K.J. and Sahyoun, N.E. (1989) *J. Biol. Chem.* 264, 5866–5875.
- [31] Huang, K.-P., Chan, K.-F.J., Singh, T.J., Nakabayashi, H. and Huang, F.L. (1986) *J. Biol. Chem.* 261, 12134–12140.
- [32] Huang, K.-P. and Robinson, J.C. (1976) *Anal. Biochem.* 72, 593–599.
- [33] Yang, S.-D. (1986) *J. Biol. Chem.* 261, 11786–11791.
- [34] Singh, T.J., Grundke-Iqbal, I. and Iqbal, K. (1994) *FEBS Lett.* (in press).
- [35] Woodgett, J.R. (1991) *Trends Biochem. Sci.* 16, 177–181.
- [36] Arioka, M., Tsukamoto, M., Ishiguro, K., Kato, R., Sato, K., Imahori, K. and Uchida, T. (1993) *J. Neurochem.* 60, 461–468.