Journal of Neuroscience Research 50:114–122 (1997)

Phospholipids Alter Tau Conformation, Phosphorylation, Proteolysis, and Association With Microtubules: Implication for Tau Function Under Normal and Degenerative Conditions

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Discerning the in situ functions of the microtubule-associated protein (MAP) tau is of interest both in terms of neuronal differentiation and homeostasis as well as in terms of neurodegenerative conditions such as Alzheimer's disease. In the present study, exposure to excess phosphatidyl serine (PS) for <1 min induced antigenic alterations in multiple N-terminal, C-terminal and central epitopes of purified human brain tau. Notably, "AD-like" epitopes (PHF-1, ALZ-50, AT-8) were decreased by PS; other epitopes (e.g., 5E2, Tau-1) increased and others remained relatively unchanged. Inclusion of γ-AT[^{32}P] during incubations did not reveal any contaminating kinase activity. Direct addition of chloroform:methanol (CM; the initial PS solvent) demonstrated that these changes were not derived from CM-mediated tau denaturation. Phosphatidyl choline induced similar antigenic changes, while phosphatidyl inositol did not. PS inhibited MAP-kinase generation of phospho-dependent tau epitopes and incorporation of phosphates by tau. Inclusion of PS during coincubation of tau and tubulin reduced the extent of cosedimentation of tau with MTs. Finally, PS enhanced the ability of calpain-mediated tau proteolysis. These data suggest that tau antigenicity in situ may be derived from phospholipid-dependent alterations in tau conformation in addition to tau phosphorylation state. These data further suggest that disruption of the normal association of tau with phospholipids may foster accumulation of tau and, in doing so, render tau more susceptible to hyperphosphorylation. J. Neurosci. Res. 50:114–122, 1997. © 1997 Wiley-Liss, Inc.

Key words: phosphatidyl serine; MAP kinase; paired helical filaments; neurodegeneration; Alzheimer's disease

INTRODUCTION

The in situ function(s) of the axonal-specific microtubule-associated protein tau have not been fully disclosed, but are the focus of intense study both in terms of neuronal differentiation and homeostasis, but also since tau is the major structural constituent of neurofibrillary tangles in Alzheimer's disease (for reviews, see Goedert, 1993; Mandelkow and Mandelkow, 1993; Kosik, 1993). Tau promotes microtubule (MT) assembly and renders MTs resistant to depolymerization under cell-free conditions (Cleveland et al., 1977; Dreschel et al., 1992). Tau also promotes MT stability in intact cells as evidenced by rendering MT populations more resistant to MT-depolymerizing drugs (Baas et al., 1994; Drubin and Kirschner, 1986; Shea and Beermann, 1994; Takemura et al., 1992). However, detailed analyses revealing that tau is more concentrated in the distal region of growing axons (Black et al., 1996; Brandt et al., 1995; Kempf et al., 1996; Mandell and Banker, 1996), where MTs are the most labile, have prompted the hypothesis that tau in situ must have function(s) other than promotion of MT stability, and that the likely site for such additional putative functions is the distal axon and growth cone (Black et al., 1996; Kempf et al., 1996). In this regard, recent studies have demonstrated the apparent association of tau with the plasma membrane of cultured neuronal cells (Brandt et al., 1995; Kempf et al., 1996; Shea et al., 1996a,b), where it may participate in as yet undisclosed aspects of signal transduction (Lee et al., 1996a,b). Consistent with

Contract grant sponsor: National Institute for Aging

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Received 21 February 1997; Revised 15 May 1997; Accepted 19 May 1997

such possibilities are: (1) that the association of with the plasma membrane is mediated via its N-terminal projection domain (Brandt et al., 1995), while MT-mediated association is via its C-terminus (Aizawa et al., 1988; Lee et al., 1989; Himmler et al., 1989); that (2) tau is more weakly associated with MTs than other microtubule-associated proteins (MAPs; Black et al., 1996; Kempf et al., 1996); and that (3) tau immunoreactivity is best preserved under conditions that maintain the integrity of the plasma membrane (Black et al., 1996; Kempf et al., 1996).

During continued analyses of the role of protein kinase C (PKC)-mediated signal transduction pathways in tau phosphorylation (e.g., Cressman et al., 1995a; Cressman and Shea, 1995), we noted that the presence of phosphatidyl serine (PS), a necessary cofactor of PKC, influenced the degree of tau immunoreactivity on immunoblots in control samples lacking kinase. We present data herein demonstrating that the interaction of tau with PS and other phospholipids alters tau antigenicity, its ability to undergo phosphorylation by a non-PS-dependent kinase (MAP kinase), its ability to undergo calpain-mediated proteolysis, and its ability to coassemble with MTs. The data collectively indicate that the overall conformation of tau is altered by interaction with phospholipids, and that this alteration has a major impact on tau biology.

MATERIALS AND METHODS

Cell-Free Incubation and Phosphorylation of Tau

Human brain tau (generous gift of Drs. Marc Mercken and Ralph Nixon) was isolated by affinity chromatography as described previously (Cressman et al., 1995a). Consistent with previous studies (e.g., (Garver et al., 1994; Matsuo et al., 1994), tau in our hands isolated under these conditions displays moderate reactivity with so-called ''AD-like'' phospho-epitopes, including PHF-1 and ALZ-50, and a marginal degree of reactivity with AT-8. Tau (5 ng/ml or 300 ng/ml) was diluted in 20 mM Tris (pH 7.5) containing 5 mM EDTA, 2 mM PMSF and 50 μg/ml leupeptin with or without 0.5 mg/ml phosphatidyl serine (PS; obtained from UBI, Lake Placid, NY) then immediately mixed with Laemmli buffer on ice; the entire mixing process took less than 1 min.

Stock solutions of PS, phosphatidyl choline (PC) and phosphatidyl inositol (PI), commercially obtained in chloroform:methanol, were treated as described previously (Baudier and Cole, 1987; Baudier et al., 1987). Chloroform:methanol was slowly evaporated by blowing a gentle stream of nitrogen gas across the surface of the solution. The resulting phospholipid-containing dry sediment was resuspended in 50 mM Tris-HCl (pH 7.4) to yield a concentration of 10 mg/ml phospholipid, which was then sonicated and added directly to tau incubation mixtures. To control for the potential of residual chloroform:methanol inducing tau denaturation, 1–4× of the corresponding amount of chloroform:methanol lacking phospholipids was evaporated as described above, Tris buffer added, the tube sonicated, and added to tau incubation mixtures.

Tau (5 ng/ml) with or without 0.5 μg/ml PS was incubated with or without recombinant MAP kinase (75 activity units as defined by the manufacturer; UBI) at 37°C for 24 hr in 20 mM Tris (pH 7.5) containing 100 μM ATP, 5 mM $CaCl_2$, and 100 mM $MgCl_2$. For autoradiographic analysis, 250 nCi g-AT[P^{32}] (DuPont, Boston, MA) was included in the above reaction. Reactions were terminated by addition of concentrated sample buffer on ice, and samples were subjected to gel electrophoresis followed by autoradiographic or immunoblot analysis.

Cell-Free MT Assembly Assays

Twice-cycled bovine brain tubulin free of MAPs (Shea and Fischer, 1996) was incubated for 30 min at 37°C with and without 5 ng/ml purified tau and 0.5 μg/ml PS in 100 mM PIPES (pH 6.6) containing 1 mM EGTA and 1 mM $MgCl_2$, then centrifuged at 100,000 × *g* for 30 min at 37°C. Resultant pellet and supernatant fractions were subjected to immunoblot analysis with 5E2 and a monoclonal antibody (Sigma Immunochemicals, Inc., St. Louis, MO) directed against α-tubulin (Shea and Fischer, 1996).

Cell-Free Calpain-Mediated Proteolysis

Purified tau (5 ng/ml) in 20 mM Tris (pH 7.5) was incubated at 37°C with m calpain (Sigma) and 2 mM $CaCl_2$ as described previously (Shea et al., 1996a) in the presence and absence of 0.5 mg/ml PS. Samples were withdrawn at 0–30 min and added to concentration Laemmli buffer on ice.

Electrophoresis and Immunoblot Analysis

Samples were subjected to SDS-gel electrophoresis and either dried and placed against X-ray film to generate autoradiographs or transferred to nitrocellulose in a Hoefer Transphor apparatus. Nitrocellulose replicas were blocked with normal goat serum, then incubated overnight at 4°C with one or more of the following phospho-dependent and independent monoclonal antibodies (below) that collectively span the length of the tau molecule. Replicas were rinsed and incubated for 2 hr at room temperature with a 1:5,000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG and visualized as described (Shea et al., 1996a). Semiquantitative densitometric analysis was carried out using NIH Image (version

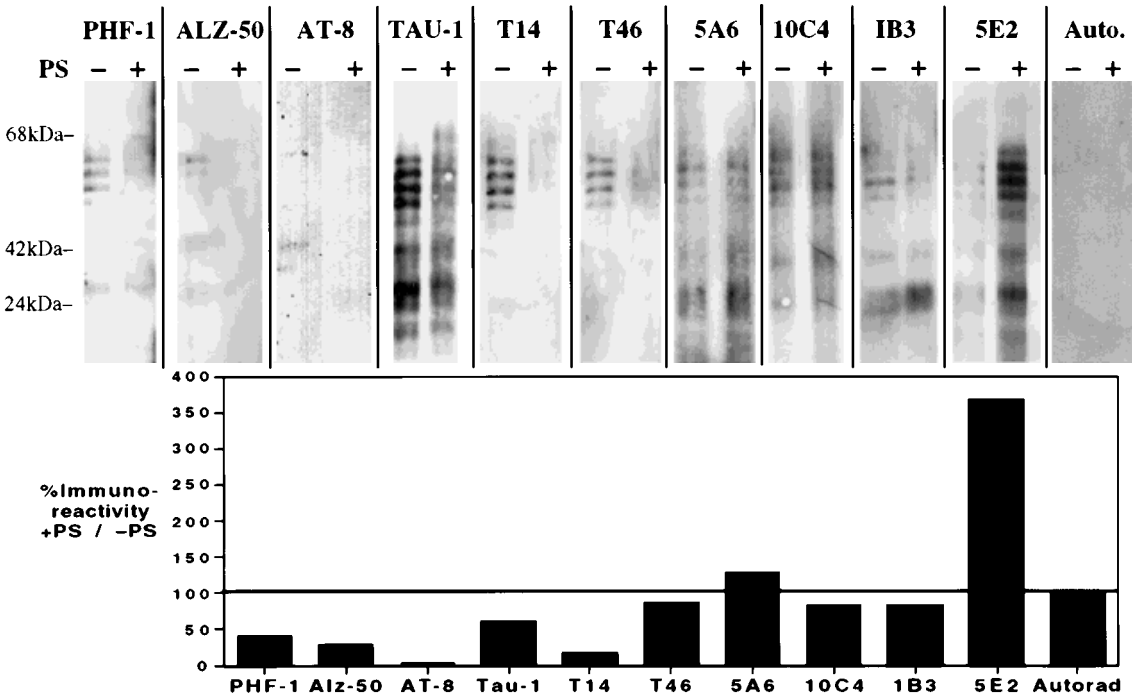

Fig. 1. Phosphatidyl serine alters tau antigenicity in the absence of detectable kinase activity. Immunoblot analyses of 5 ng/ml human brain tau incubated for approximately 30 sec in the presence (+PS) or absence (−PS) of 0.5 mg/ml phosphatidyl serine (PS) prior to SDS gel-electrophoresis and immunoblot analysis with various tau antibodies as indicated. An additional sample incubated with g-AT[P^{32}] was subjected to autoradiography (Auto.). The accompanying graph presents the ratio of values obtained from these immunoblots and autoradiograph of samples incubated in the presence (+) and absence (−) of PS. Note that PS markedly reduced the reactivity of tau with some antibodies, including, but not limited to, the reduction of so-called ''AD-like'' immunoreactivities PHF-1, ALZ-50 and AT-8, only slightly altered others (e.g., 5A6, 10C4, 1B3, differential altered reactivity of isoforms with Tau-1), and markedly increased immunoreactivity with the phospho-independent antibody 5E2. Note further that autoradiographic analyses following incubation with g-AT[P^{32}] failed to indicate the presence of any contaminating kinase activity (compare with autoradiograph from Fig. 3).

1.57; available by anonymous FTP from *zippy.nimh.nih.gov*) following digitization of immunoblots and autoradiographs via a UMAX scanner (with transparancy adaptor) connected to a Macintosh PowerPC 7100 (e.g., Shea et al., 1996a). The density of an area of the immunoblot encompassing all reactive isoforms was circumscribed using the free-hand selection tool and the density recorded, after which the selection was shifted to an area of the immunoblot free of immunoreactive bands, and the corresponding ''background'' density recorded and subtracted from the above value to generate a net immunoreactive density. These values were exported to Excel spreadsheet software. Mean and standard error of the mean were calculated following digitization and analyses of multiple samples. All experiments were carried out at least three times. Variance in ratios of reactivity with phospholipid versus without phospholipid was 20–30% or less among replicate experiments. However, in all cases, antigenicity reported as increased by PS was always increased; that reported as decreased was always decreased, and that unaffected never showed major alterations. In all figures presenting analysis with multiple antibodies, individual samples were aliquoted into multiple lanes for immunoblot analysis with multiple antibodies in. Error bars are not reported for all graphs expressing ratios of samples ± PS since relative, rather than absolute, values are generated by comparative densitometry; values presented are in all cases representative. Statistical comparisons were carried out using Student's t test. All reagents unless indicated otherwise were obtained from Sigma Chemical Co., Inc.

Antibodies Utilized in This Study

Multiple antibodies were utilized that collectively span the entire tau molecule and react with the following epitopes: PHF-1 (generous gift of Dr. S. Greenburg), which recognizes ser 396 and 404 when phosphorylated; AT-8 (Immunogenetics) and Tau-1 (Sigma) which recognize ser-202 when phosphorylated and nonphosphorylated, respectively; ALZ-50 (generous gift of Dr. P. Davies), a conformation-dependent antibody that recog-

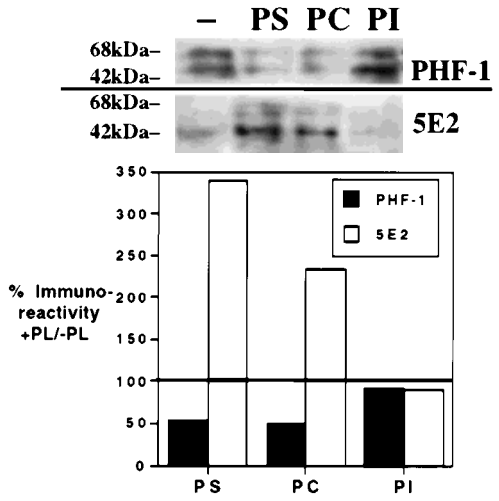

Fig. 2. Multiple phospholipids alter tau antigenicity. Immunoblot analyses of 5 ng/ml human brain tau incubated for approximately 30 sec in the absence (−) or presence of 0.5 mg/ml PS, phosphatidyl choline (PC) or phosphatidyl inositol (PI), prior to SDS gel-electrophoresis on mini-gels and immunoblot analysis with PHF-1 or 5E2 as indicated. The accompanying graph presents the ratio of values obtained from these immunoblots and autoradiograph of samples incubated in the absence (−) and presence of each phospholipid species as indicated. Note that PC, like PS, reduces PHF-1 immunoreactivity and increases 5E2 immunoreactivity (see also Fig. 1), while PI does not appreciably alter either of these immunoreactivities from phospholipid-free control samples.

nizes the N-terminus; 5E2 (generous gift of Dr. Ken Kosik), N- and C-terminal monoclonal antibodies T14 and T46 (respectively; generous gifts of Dr. Virginia Lee); 5A6 (N-terminal to exon 2); 10C4 (C terminal to exon 11) and 1B3 (which recognizes an undefined epitope within the central portion of the tau molecule; monoclonal antibodies 5A6, 10C4 and 1B3 were generous gifts of Dr. Gail Johnson); and JM (generous gift of Dr. Marc Mercken), a rabbit polyclonal antibody prepared against the purified human tau utilized in this study.

RESULTS

Phospholipids Alter Tau Antigenicity

Mixing of PS with purified human brain tau at a PS:tau ratio of 100:1 for approximately 30 sec prior to SDS gel-electrophoresis altered tau immunoreactivity towards epitopes spanning the entire tau molecule (Fig. 1). Notably, certain ''AD-like'' phospho-epitopes (Alz-50, PHF-1 and AT-8) were markedly reduced by PS. Overall immunoreactivity towards an antibody that recognizes a nonphosphorylated epitope (Tau-1) was decreased, yet an additional isoform, migrating at approximately 68 kDa, appeared following incubation with PS. While phospho-epitopes such as PHF-1 were reproducibly reduced (by 40 ± 10%, mean ± standard error of the mean, n = 3) following incubation with PS, by contrast, overall immunoreactivity towards the phosphate-independent epitope 5E2 was increased (330 ± 20%, mean ± standard error of the mean, n = 3) following incubation with PS (Fig. 1). Several other phospho-independent epitopes were unchanged by PS; these latter findings confirm that the reduction of ''AD-like'' tau immunoreactivity was not due to prevention of entry of tau into gels or a generalized interference with tau immunoreactivity. Autoradiographic analysis of samples incubated with g-AT[P^{32}] did not reveal any residual/contaminating kinase activity (Fig. 1). Increasing the amount of tau relative to PS to yield a PS:tau ratio of 1.7:1 under these conditions eliminated the influence of PS on tau antigenicity with most antibodies, and markedly reduced the impact of PS on 5E2 immunoreactivity (not shown). All subsequent analyses of the influence of PS on tau were carried out at the above higher phospholipid:tau ratio (100:1::phospholipid:tau).

Antigenic changes were not induced when tau was exposed to the putative residual chloroform:methanol resulting from nitrogen evaporation of up to four volumes of the original amount of phospholipid-free chloroform: methanol (not shown). These findings confirmed that the observed alterations in tau antigenicity were mediated by phospholipids themselves and not any putative chloroform: methanol residue. Increasing the concentration of SDS utilized in sample preparation for electrophoresis from 1% to 5% did not reverse the effects of PS on tau as ascertained by maintenance of an increase in 5E2 immunoreactivity (not shown). Effects similar to those observed with PS were also induced by PC but not by PI (Fig. 2).

Phosphatidyl Serine Alters the Ability of Tau To Be Phosphorylated by a Nonphospholipid-Dependent Kinase

MAP kinase phosphorylates tau under cell-free conditions and, in doing so, alters its antigenicity (see references in Introduction). We examined the influence of PS on the ability of tau to serve as a substrate for MAP kinase under cell-free conditions. Immunoblot analysis of samples incubated in the absence of PS confirmed a MAP kinase-mediated increase in PHF-1, ALZ-50 and AT-8 immunoreactivity, while corresponding autoradiographic analysis of samples incubated with g-AT[P^{32}] confirmed MAP kinase-mediated tau phosphorylation (Fig. 3). However, the ability of MAP kinase to confer PHF-1, ALZ-50 and AT-8 immunoreactivity to tau, and to phosphorylate tau, was markedly impaired by coincubation with PS (Fig. 3). Notably, the activity of MAP kinase (or, at the very least, that commercially available isoform used in this study) is not directly affected by phospholipids (S. Pelech, personal communication).

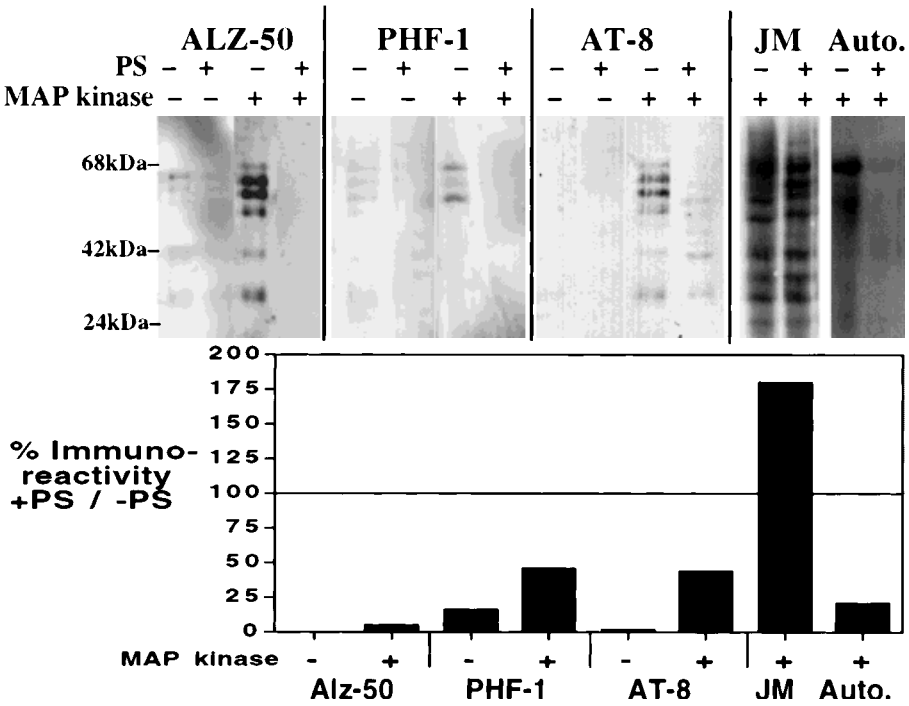

Fig. 3. Phosphatidyl serine alters the ability of tau to be phosphorylated by microtubule-associated protein (MAP) kinase. Immunoblot analyses of 5 ng/ml human brain tau incubated with (+) or without (−) 0.5 μg/ml PS was incubated with (+) or without (−) MAP kinase for 24 hr in the presence of g-AT[P^{32}] prior to SDS gel-electrophoresis followed by transfer to nitrocellulose, immunoblot analysis with various tau antibodies, and subsequent autoradiography of the dried immunoblot. The portion of the immunoblot reacted with polyclonal antibody JM was used to generate the corresponding autoradiograph (Auto.). The accompanying graphs presents the percent of immunoreactive or autoradiographic density conferred upon tau by MAP kinase in the presence of PS versus that in the absence of PS (i.e., [densitometric value +MAP kinase, +PS]/[densitometric value +MAP kinase, −PS]). Note that in the absence of PS, MAP kinase increases tau immunoreactivity with PHF-1, Alz-50 and AT-8, and phosphorylates tau as evidenced by the incorporation onto tau of radiolabeled phosphate groups, while PS attenuates these phenomena. Note further that, like monoclonal antibody 5E2, immunoreactivity of tau with polyclonal JM is markedly enhanced by PS. While the overall pattern of tau immunoreactivity revealed by polyclonal antibody JM resembles that of the monoclonal antibodies used herein, JM reveals additional minor tau species; these may represent breakdown products.

Phosphatidyl Serine Alters the Ability of Tau to Associate With MTs Under Cell-Free Conditions

Tau was incubated with purified bovine brain tubulin under conditions that promote MT assembly, after which MTs were sedimented by high-speed centrifugation (100,000 × *g*, at 37°C) for 15 min. As previously demonstrated (Shea and Fischer, 1996), a significant amount of tau was cosedimented with these newly formed MTs (Fig. 4). Inclusion of PS during coincubation of tau and tubulin did not significantly reduce the extent of MT formation, but did reduce the extent of cosedimentation of tau with newly formed MTs (Fig. 4). PS did not induce tau sedimentation in the absence of tubulin under these conditions (not shown); in this regard, while PS has been shown to promote polymerization of tau into filaments in previous studies (Binder et al., 1996; Kuret et al., 1996) as well as in our hands (to be published), the length of incubation required for significant tau filament formation significantly exceeds that utilized in the present study to assay for cosedimentation of tau with MTs (not shown, see Binder et al., 1996).

Phosphatidyl Serine Alters the Ability of Tau to Undergo Calpain-Mediated Proteolysis

Consistent with previous studies (Yang and Ksiezak-Reding, 1995; Litersky et al., 1993; Johnson et al., 1989; Shea et al., 1996a), purified m calpain rapidly degraded tau under cell-free conditions (Fig. 5). PS significantly decreased the rate of calpain-mediated tau degradation. This was apparently not the result of a general inhibitory effect of PS on calpain, since we have previously demonstrated that calpain-mediated degradation of azocasein is not affected by PS (Cressman et al., 1995b; Lang et al., 1995).

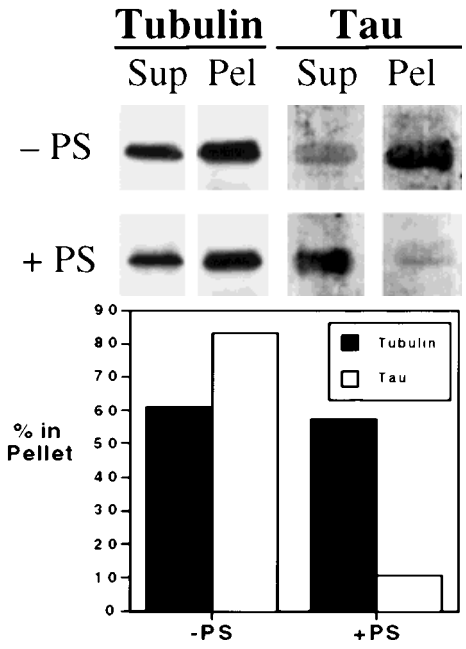

Fig. 4. PS reduces the ability of tau to cosediment with microtubules. Twice-cycled bovine brain tubulin was incubated for 30 min at 37°C with purified tau and 0.5 μg/ml PS in 100 mM PIPES (pH 6.6) containing 1 mM EGTA and 1 mM $MgCl_2$, then centrifuged at 100,000 × *g* for 30 min at 37°C. Resultant pellet (Pel) and supernatant (Sup) fractions were subjected to immunoblot analysis with 5E2 and a monoclonal antibody directed against α-tubulin. To facilitate these comparative analyses of tau distribution among supernatant and pellet fractions, digitized images of 5E2-reacted immunoblots were normalized to yield identical total tau levels before densitometry; representative normalized images are presented in this figure. The accompanying graph presents the percent of tau or tubulin sedimented in the absence (−PS) or presence (+PS) of PS. Note the apparent lack of an influence of PS on polymerization of tubulin, but a marked attenuation by PS of the amount of tau that cosediments with these newly formed microtubules.

DISCUSSION

In the present study, we have examined the interaction of tau with certain phospholipids, in particular PS. This was prompted by our serendipitous observation, during PKC-mediated tau phosphorylation under cell-free conditions, that control samples containing PS but not PKC exhibited diminished tau immunoreactivity with certain ''AD-like'' phospho-dependent tau antibodies. Subsequent examination as presented herein demonstrate that PS markedly alters tau antigenicity with a variety of antibodies that collectively span the tau molecule. Limited analyses also demonstrated that PC, but not PI, induced similar changes. Recent studies have indicated that incubation with phospholipids can induce tau polymerization (Binder et al., 1996; Kuret et al., 1996), leaving open the possibility that our observed reduction in certain phosphate-dependent immunoreactivities was derived from selective failure of a portion of tau, or particular tau isoforms, to enter the gel due to SDS-resistant polymerization. However, the observation of identical, and in some cases, increased levels of immunoreactivity with certain monoclonals indicates that similar levels of tau entered gels. These latter findings also confirm that PS did not induce generalized interference with all antibody interactions. While tau antigenic alteration in the presence of PS is evident in immunoblot analyses, it remains unclear whether or not similar alterations in tau antigenicity are reflected in immunocytochemical and immunofluorescent analyses; i.e., whether or not certain epitopes are diminished or rendered cryptic in phospholipid-rich cellular subregions and vice versa.

We interpret these findings to indicate that even a brief (<1 min) exposure to excess PS induces an SDS-resistant conformational alteration in the tau molecule that renders certain antigenic sites cryptic, renders others more prominent, and leaves additional sites unaltered. The presence of PS also impaired the ability of MAP kinase phosphorylate tau, and in doing so, to confer PHF-1, AT-8 and ALZ-50 immunoreactivity upon tau; this finding, along with the apparent SDS resistance of the influence of PS on tau herein and previous studies (Baudier and Cole, 1987; Baudier et al., 1987), suggests that any putative phospholipid-induced alteration in tau conformation be relatively stable. In these earlier studies, only 50% of the total tau was readily phosphorylated in the absence of PS, while 100% was able to be phosphorylated in the presence of PS, leading the investigators to conclude that PS disrupted tau-tau dimers (Baudier and Cole, 1987). Moreover, in these earlier analyses, certain phospholipids also altered the electrophoretic migration of tau, indicative of a conformational change; in this regard, we note that the electrophoretic migration of Tau-1-immunoreactive tau species was altered (e.g., Fig. 1). Antigenicity, however, was not examined in the earlier study of Baudier and Cole (1987).

Previous analyses suggest that the N-terminal projection domain of tau mediates its association with the plasma membrane in intact cells (Brandt et al., 1995). While it is reasonable to conclude that the N-terminal region also mediates tau-PS associations under cell-free conditions, our antibody library revealed that PS induced antigenic changes along the entire length of the molecule, indicative of overall conformational change. Thus, we cannot be certain of the nature and extent of tau-PS associations along the molecule. This PS-induced conformational change is reminiscent of the previous demonstration of marked conformation alterations in tau molecule following phosphorylation (Hagestedt et al., 1989). This putative PS-induced conformational change exerts a major physiological impact since the ability of tau to

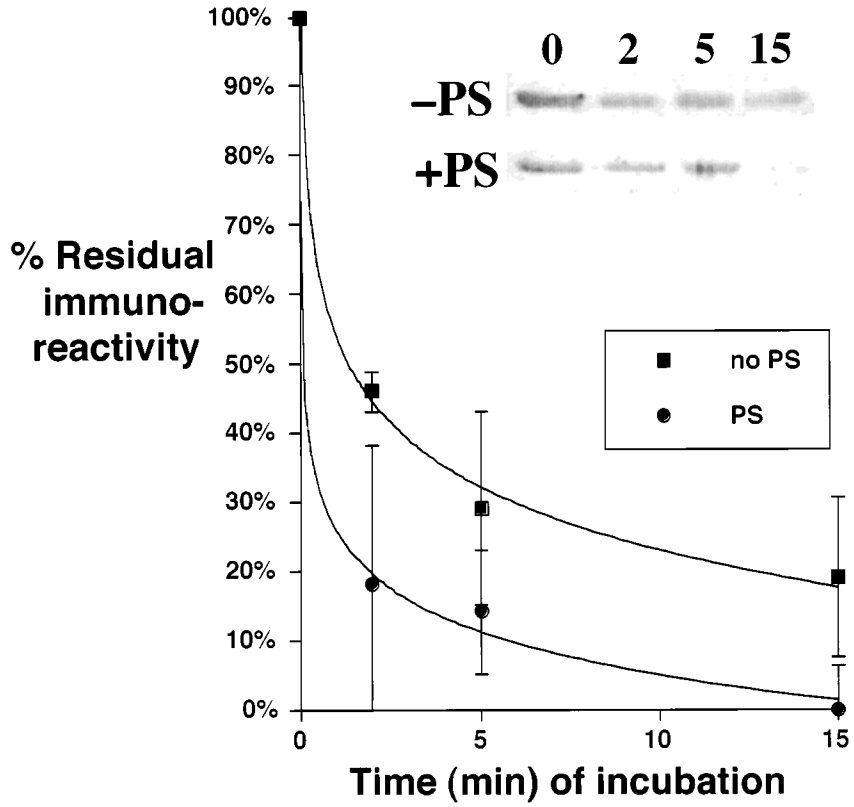

Fig. 5. PS facilitates calpain-mediated proteolysis of tau. Purified tau (5 ng/ml) in 20 mM Tris (pH 7.5) was incubated at 37°C with m calpain and 2 mM $CaCl_2$ in the presence (+PS) and absence (−PS) of 0.5 mg/ml PS. Samples were withdrawn at 0–30 min and added to concentrated Laemmli buffer on ice to stop the reaction, followed by immunoblot analysis with 5E2. To facilitate these comparative analyses of tau degradation by calpain, digitized images of 5E2-reacted immunoblots were normalized to yield identical total tau levels in the presence and absence of PS at t = 0 min; representative normalized images are presented in this figure. Values present the mean ± standard error of the mean of the percent of 5E2 immunoreactivity remaining at 5–15 min versus that at time 0 for three independent normalized experiments. Note that tau is more rapidly degraded in the presence of PS than in its absence. Values obtained at 15 min after incubation with and without PS were statistically significant ($P < 0.04$; Student's t test).

undergo phosphorylation, proteolysis and to associate with microtubules were altered by PS. Detailed studies of full-length tau and various fragments by techniques such as circular dichroism will be required to ascertain the nature and extent of alteration in tau configuration by phospholipids (e.g., Willis, 1994). It should be noted that the diminished ability of tau to be phosphorylated by MAP kinase or to associate with MTs is unlikely to be derived from simple steric prevention of these phenomena by the presence of excess PS, since this same concentration of PS enhanced tau proteolysis by calpain.

Diminished ability of tau to associate with MTs in the presence of PS may indicate that tau in situ is associated with either membranes or MTs, and does not simultaneously associate with both. Our demonstration of diminished calpain-mediated proteolysis in the absence of PS may relate to a previous study indicating that phosphorylation alone may not be fully responsible for conferring resistance of tau to calpain-mediated proteolysis (Yang and Ksiezak-Reding, 1995). One interpretation of reduced tau proteolysis in the absence of PS is that cytosolic tau (i.e., not associated with phospholipids or MTs) may demonstrate an increased potential for inappropriate accumulation; such accumulation, perhaps coupled with key phosphorylation events, may, under certain conditions, contribute to paired helical filament (PHF) formation (Bancher et al., 1989; Shea et al., 1996a).

While these data, taken together with previous investigations, indicate that phospholipid interaction impacts most if not all of the major aspects of tau biology thus far studied, the in situ physiological significance of the observed phospholipid-induced alteration in tau antigenicity has not been determined. However, tau immunoreactivity is localized along the plasma membrane (Brandt et al., 1995; Shea et al., 1996b). Tau in situ may associate directly with the plasma membrane phospholipids (e.g.,

Binder et al., 1996), or may do so by association with additional membrane-associated protein(s) (Brandt et al., 1995; Lee et al., 1996a,b), and, in doing so, may participate in signal transduction mechanisms (Hwang et al., 1996; Lee et al., 1996a,b). The findings of the present study are consistent with the possibility that at least some tau may associate directly with membrane phospholipids. In this regard, tau has previously been shown to associate with phospholipid vesicles (Surridge and Burns, 1994). It was further noted in these analyses that MAP2 associates with PI via its N-terminal projection domain and that this association altered the configuration of the MAP2 MT-binding domain (Surridge and Burns, 1994; Burns and Surridge, 1995). Conversely, tau did not associate with PI (Surridge and Burns, 1994; Burns and Surridge, 1995); this latter finding is consistent with our failure to observe antigenic alterations in tau following incubation with PI. The additional possibility exists that differential interaction of tau and MAP2 with axonal and dendritic membranes and/or membrane transport systems, respectively, may contribute to the differential segregation of tau and MAP2 within axons and dendrites during the development of polarity (Kosik and Finch, 1987). The ability of tau to associate with the plasma membrane via its N-terminal projection domain (Brandt et al., 1995), while it associates with MTs via its C-terminus (Aizawa et al., 1988; Lee et al., 1989; Himmler et al., 1989), leaves open the additional possibility that tau in situ may bridge the MT-membrane distance; this possibility is supported by the ability of tau to form polymers under physiological conditions in the presence of lipids (Binder et al., 1996; Kuret et al., 1996).

It has been hypothesized that (inappropriate) dissociation of tau from MT may foster tau hyperphosphorylation (Brambett et al., 1993). One interpretation of the findings in the present study suggests that tau may also be potentially at risk for inappropriate phosphorylation/conformational change following (inappropriate) dissociation from phospholipids in situ. It must further be considered that some degree of antigenicity previously attributed only to changes in tau phosphorylation state may actually reflect conformational changes brought upon by the presence or absence of phospholipids, perhaps in combination with key phosphorylation events. This latter consideration may indicate why certain epitopes, such as that recognized by ALZ-50, can be increased by phosphorylation in cell-free analyses, yet are clearly not entirely phospho-dependent (Goedert et al., 1993). Notably, PHFs, which are comprised chiefly if not entirely of tau, have been observed arising from the surface of cytomembranes (Gray et al., 1987).

Finally, although induction of epitopes such as PHF-1, ALZ-50 and AT-8 are often referred to as ''AD-like'' changes due to their similarity with tau isolated from PHFs, it must be kept in mind that such immunoreactivities are detected during normal development (Goedert et al., 1993; Pope et al., 1993, 1994; Rebhan et al., 1995). Furthermore, these ''abnormal'' sites are detectable in normal adult brain biopsy tissue, autopsy-derived fetal tissue and rapidly processed rat tissue (Garver et al., 1994; Matsuo et al., 1994). Our findings further suggest that differential association of tau with phospholipids during development, and any changes in such interactions during the postmortem interval and/or isolation, may contribute to the observed tau antigenic profile.

ACKNOWLEDGMENTS

The author is grateful to Ms. Mabel Chang for her excellent technical assistance and to Dr. Robert Lynch for helpful suggestions. This research was supported by the National Institute for Aging.

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