Phosphorylation of Tau Proteins to a State Like That in Alzheimer’s Brain Is Catalyzed by a Calcium/Calmodulin-dependent Kinase and Modulated by Phospholipids*

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Calcium/calmodulin(CaM)-dependent protein kinases isolated from bovine and rat brains phosphorylated the microtubule-associated tau protein in the mode that shifts the mobility of tau in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (mode I). This mode of tau phosphorylation is the one that occurs abnormally in Alzheimer’s lesions. Purified tau protein in solution can be phosphorylated by the Ca²⁺/CaM kinases maximally to about 50% of the total tau protein. Incorporation of one phosphate group per mol of tau is sufficient to shift the protein to a slower migrating electrophoretic band. Additional phosphate incorporation into the shifted tau proteins can occur depending on protein kinase concentration. In the presence of phosphatidylserine, tau proteins were phosphorylated to an extent of 100% at a tau:phosphatidylserine ratio of 20. Phosphatidylethanolamine also stimulated tau phosphorylation by Ca²⁺/CaM kinase and phosphatidylinositol was found to be a potent inhibitor of tau protein phosphorylation. The direct observation that tau proteins interact with phospholipids such as phosphatidylethanolamine and phosphatidylinositol, resulting in a smearing of the protein band on sodium dodecyl sulfate-gel electrophoresis, supports the possibility that tau protein may interact with phospholipid membranes in vivo and that tau protein phosphorylation could be modulated by the phospholipid composition of the membranes with which tau interacts.

EXPERIMENTAL PROCEDURES

Microtubules are one of the major constituents of the neuronal cytoskeleton and play important roles in cell morphology and intracellular transport processes. In addition to tubulin and microtubule-associated protein-2, microtubules contain tau proteins (1, 2). Tau is a family of four closely related proteins of 55–68 kDa. We showed that tau can undergo two modes of phosphorylation, one which changes the electrophoretic mobilities of the tau’s (mode I) and another, which does not (mode II) (3, 4). It was clear that in vivo brain tau contains a mixture of nonphosphorylated tau and tau phosphorylated in at least mode I. The phosphorylation of tau, moreover, partially inhibits its ability to promote tubulin polymerization in vitro (3) and thus might regulate microtubule formation. Still more recently, it was reported that mode I-phosphorylated tau proteins are major components of Alzheimer paired helical filaments, and this suggested that the phosphorylation of tau proteins, at least in mode I, might be a significant factor in cytoskeletal alterations that affect neurons in Alzheimer’s disease (5, 6). Therefore, there is considerable interest in understanding the factors that might regulate tau phosphorylation in vivo and thus probably their functions. The primary focus of this article is on the phosphorylation of tau by the Ca²⁺/CaM protein kinase. This kinase catalyzes mode I phosphorylation of tau proteins in vitro, and its activity was modulated by the interaction of tau protein membrane phospholipids.

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1 The abbreviations used are: CaM, calmodulin; EGTA, (ethylenebis(oxyethylenenitrilo)tetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

17577
to rat kinase preparations (see below).

**Purification of Tau Protein Kinase from Rat Brain**—The purification procedure used was essentially that of McGuinness *et al.* (9) except that buffers D and E contained 12% glycerol, Sephacryl S400 gel filtration was replaced by Sepharose CL-4B gel filtration column, and the last concentration step of the enzyme by dialysis against a solid sucrose was omitted. The enzyme was assayed with crude tau (0.5 µM) as substrate. During all the purification steps the tau protein kinase activity was entirely dependent on Ca²⁺ and calmodulin and exhibited the same physicochemical characteristics as the Ca²⁺/CaM-dependent microtubule-associated protein-2, synapsin, or casein protein kinase (9-13).

**Protein Phosphorylation Assay**—The standard protein assay for tau phosphorylation by the rat brain kinase contained 20 mM Tris-HCl, pH 7.5, 3 mM MgCl₂, 0.2 mM CaCl₂, 50 µM [γ-³²P]ATP. After preincubation for 30 sec at 30°C, the reaction was initiated by the addition of [γ-³²P]ATP and terminated by addition of an SDS-stop solution (5% SDS, 5% β-mercaptoethanol, 25% glycerol, 0.5 M Tris, pH 6.8) and heated for 2 min at 100°C. The proteins were separated by 0.1% SDS, 10% polyacrylamide gel electrophoresis. The gels were dried and the phosphorylated protein detected by autoradiography. The phosphorylated proteins were cut out of the gels and then dried and the phosphorylated protein detected by autoradiography. The gels were cut out of the gels and then dried and the phosphorylated protein detected by autoradiography. The stoichiometry of phosphorylation was calculated by use of the specific activity of the ATP and the amount of protein loaded on the gel.

**Preparation of Phospholipid Solutions**—Phospholipid solutions were prepared by slowly evaporating chloroform from commercially prepared phospholipid solutions in a stream of nitrogen gas. Dry phospholipids were resuspended in 20 mM Tris buffer, pH 7.5, by vortex agitation and sonicated for 5-10 min. The solutions were stored at 4°C and were sonicated once more prior to each use.

**RESULTS**

**Phosphorylation of Bovine Tau Protein by a Ca²⁺/Calmodulin-dependent Protein Kinase Purified from Bovine Brain**—Tau protein directly extracted from brain tissue contains eight components which usually migrate as seven bands in SDS-PAGE (3, 4). After treatment with alkaline phosphatase, they are converted to a four-band pattern that we call, respectively, r₁ to r₄, from the fastest to slowest (8). The change in electrophoretic mobility of the dephosphorylated tau proteins upon dephosphorylation must reflect conformational changes between the phosphorylated proteins and their dephosphorylated counterparts, since phosphorylation (in mode II) is possible without change in mobility (8).

We searched among known protein kinases for one that might mimic the tau phosphorylation that was proven to occur in vivo; i.e. mode I that caused the conformational electrophoretic migration of tau. The calcium/phospholipid-dependent protein kinase (protein kinase C) proved able to phosphorylate tau protein *in vitro*, but phosphorylation of tau by this kinase failed to change the electrophoretic mobility (8) and therefore did not represent mode I. The cAMP-dependent protein kinase did not substantially phosphorylate tau protein *in vitro*. We turned our attention to Ca²⁺/calmodulin-dependent kinase activities, since Shulman (10) reported that tau proteins are good substrates for such a kinase purified from rat brain.

A Ca²⁺/calmodulin-dependent protein kinase was first purified from bovine brain using tau protein as exogenous substrate to monitor the kinase activity during the preparation procedure (see "Experimental Procedures"). The enzyme preparation obtained after affinity chromatography on calmodulin-Sepharose had a low specific activity when compared to rat brain preparation (see below) and was heavily contaminated by a protein of 68 kDa. A striking feature of the Ca²⁺/CaM kinase is autophosphorylation which requires both calcium and calmodulin (12, 14, 15). Incubation of our enzyme preparation under conditions of Ca²⁺/CaM-dependent auto-phosphorylation resulted in phosphorylation of a single 60-kDa protein as revealed on the autoradiogram of the SDS-PAGE.

**Fig. 1.** The effects of calmodulin and calcium on phosphorylation of tau proteins by a bovine brain kinase. Dephosphorylated tau proteins (4 µg) were incubated for 15 min at 30°C with 20 mM Tris-HCl, pH 7.5, 36 µM [γ-³²P]ATP, (1000 cpm/pmol), 18 mM MgCl₂, and 20 µl (about 40 µg/ml) of the kinase in a total volume of 55 µl. The indicated additions were present in the following final concentrations: calmodulin (CaM), 1.5 µM; total calcium, 45 mM; EDTA, 5 mM. The reaction was stopped by adding 15 µl of SDS buffer (10% SDS, 10% glycerol, 0.5 M Tris-HCl, pH 6.8) and boiled for 5 min. Samples were electrophoresed on 10% SDS-polyacrylamide gels.
The Effect of phosphorylation of tau proteins by the bovine brain Ca\(^{2+}\)/calmodulin-dependent protein kinase on the electrophoretic mobilities of tau protein combinations. Lane 1, mixture of r\(_2\) and r\(_4\); lane 2, mixture of r\(_2\) and r\(_4\); lane 3, r\(_2\); lane 4, r\(_4\); lane 5, crude \(\tau\) proteins. The samples of dephosphorylated tau were incubated with Ca\(^{2+}\)/CaM kinase (+) for 20 min at 30 °C with 20 mM Tris-HCl, pH 7.5, 36 \(\mu\)M [\(\gamma\)-\(^{32}\)P]ATP, 18 mM MgCl\(_2\), and 30 \(\mu\)l of the purified kinase in a total volume of 55 \(\mu\)l in the presence of 1.5 \(\mu\)M calmodulin and 45 \(\mu\)M calcium. The reference samples (−) were incubated in Tris-HCl buffer without kinase. The reaction was stopped by adding 15 \(\mu\)l of SDS buffer and boiling for 5 min before electrophoresis on 10% SDS-polyacrylamide gels. The gel was stained with Coomassie Blue (A) and dried before autoradiography (B). The different tau protein species are numbered \(T_1\), \(T_2\), and \(P_i\); refers to mode I-phosphorylated tau protein.

Fig. 3. Time course of \(\tau_2\) phosphorylation by the bovine brain Ca\(^{2+}\)/calmodulin-dependent protein kinase. Purified \(\tau_2\) protein was incubated at 30 °C with the kinase under the conditions described in Fig. 1. A and B represent, respectively, the Coomassie Blue staining and autoradiography of the same gel.

Polypeptide bands proved that no more than one phosphate group was incorporated per mol of tau (data not shown).

More surprising was the observation that even prolonged incubation of tau proteins with the kinase failed to shift all the tau protein to an apparent higher molecular weight, suggesting that a subpopulation (about half) of tau could not be phosphorylated (Figs. 2 and 3). Measurement of inorganic phosphate after ashing the protein (16) showed that the tau preparation used in this study was almost completely dephosphorylated, and thus the CaM kinase-resistant tau protein did not represent a phosphorylated subpopulation of tau. The other possibility was that the low specific activity of the bovine kinase preparation we used was not sufficient to allow complete tau phosphorylation. Therefore, since rat brain had a higher specific activity than bovine brain (12), we decided to examine the rat brain enzyme.

Phosphorylation of Bovine Brain Tau Protein by a Ca\(^{2+}\)/Calmodulin-dependent Protein Kinase Purified from Rat Brain—The purification procedure used for the rat brain CaM kinase was essentially that of McGuinness et al. (9). Tau proteins were used as exogenous substrate to monitor the kinase activity. The enzyme migrated as two distinct polypeptide bands in SDS-polyacrylamide gel electrophoresis having apparent molecular masses of 50 and 60 kDa. Autophosphorylation of the enzyme resulted in a mobility shift of the 50- and 60-kDa substrates to higher apparent molecular weights with a concomitant decrease in Coomassie Blue staining intensity of the polypeptide bands (12). The specific activity of the rat brain enzyme preparation was about 1000-fold that of the bovine preparation.

The Effect of Ca\(^{2+}\)/CaM Kinase Concentration on Tau Protein Phosphorylation—Fig. 4 shows the Coomassie Blue staining of the SDS-polyacrylamide gel and the corresponding autoradiogram used to study the effect of increasing rat Ca\(^{2+}\)/CaM kinase concentration on \(\tau_2\) phosphorylation. As observed with the bovine Ca\(^{2+}\)/CaM kinase, phosphorylation of \(\tau_2\) by the rat enzyme induced a shift of the phosphorylated \(\tau_2\) species to an apparently higher molecular weight compared to its dephosphorylated counterpart. As the protein kinase concentration was increased from 0.6 to 13 \(\mu\)g/ml, there was a progressive increase in \(^{32}\)P incorporation into \(\tau_2\) protein, as revealed on the autoradiogram of the gel. However, the Coomassie Blue staining clearly shows that phosphate incorporation at high kinase concentrations was essentially all in the shifted phosphorylated \(\tau_2\) species and that a constant subpopulation of \(\tau_2\) remained unphosphorylated. In addition to the mobility shift noted earlier, high levels of kinase produced superphosphorylated species of tau with progressively lower mobilities. This superphosphorylation of \(\tau_2\) was not observed with the bovine enzyme, probably because of the low specific activity of the preparation. The superphosphorylation of \(\tau_2\) protein results ultimately in a smear of the protein band on SDS-polyacrylamide gel.

Fig. 5 shows the time course of \(\tau_2\) protein phosphorylation by the rat Ca\(^{2+}\)/CaM kinase at two different enzyme concentrations, i.e. 0.9 and 9 \(\mu\)g/ml. As expected from the previous results, there were large differences in stoichiometry of \(\tau_2\) phosphorylation between the two kinase concentrations used. Since the percentages of unphosphorylated \(\tau_2\) protein as shown on the Coomassie Blue staining of the gel remain nearly identical whatever the kinase concentrations used (Fig. 4A), the differences in stoichiometry mainly reflect differences in \(\tau_2\) superphosphorylation. At low kinase concentrations, after 5-min incubation, the stoichiometry was about 0.5 mol of \(^{32}\)P/mol of \(\tau_2\) protein and corresponds to the monophosphorylation and shift of about 50% of the \(\tau_2\) protein to slower mobility on the SDS-polyacrylamide gel (Fig. 5, inset). This percentage of shifted tau protein species did not significantly change as the incubation time was increased, and therefore the apparent linear increase in the stoichiometry of phosphorylation of \(\tau_2\) between 5 and 50 min might probably reflect occurrence of \(\tau_2\) superphosphorylation. Thus, as previously observed with bovine brain Ca\(^{2+}\)/CaM kinase, the rat
The effect of phosphatidylserine on \( \tau_1 \) phosphorylation by the Ca\(^{2+}\)/CaM kinase is shown in Fig. 6. As observed with \( \tau_2 \) protein, phosphatidylserine stimulated \( \tau_1 \) phosphorylation with a shift of all the protein to lower mobility on SDS-PAGE (Fig. 6, IIA), in comparison to the phosphorylation of only half the \( \tau_1 \) in the absence of this phospholipid (IA). As expected, the presence of phosphatidylserine doubled the amount of incorporated \( ^{32}P \) as shown on the corresponding autoradiograms (IB and IIB).

The effect of phosphatidylserine on the rate and extent of phosphorylation of purified \( \tau_2 \) protein was examined at various concentrations of the phospholipid and \( \tau_2 \) protein. Fig. 7 shows that phosphatidylserine, in addition to stimulating \( \tau_2 \) protein phosphorylation in a \( \tau_2:PS \) ratio-dependent manner, inhibited \( \tau_2 \) phosphorylation at higher concentrations. The maximal rate of tau phosphorylation was at a \( \tau_2:PS \) ratio of about 20. These results suggested an effect of phosphatidylserine on tau protein rather than on activation of the kinase.

The Effect of Other Phospholipids on Tau Protein Phosphorylation—Various phospholipids were compared to phosphatidylserine at similar concentrations (70 \( \mu \)g/ml) for their ability to stimulate the phosphorylation of purified \( \tau_1 \) and \( \tau_2 \) proteins by Ca\(^{2+}\)/CaM kinase. Figs. 8 and 9 show the Coomassie Blue staining of SDS-polyacrylamide gels (A) and their corresponding autoradiogram (B) used to study the effects of various phospholipids on \( \tau_2 \) and \( \tau_2 \) phosphorylation. Figs. 8C and 9C show the effects of phospholipids on \( \tau_1 \) and \( \tau_2 \) mobility in the absence of kinase. In the conditions used here, phosphorylation of tau in the absence of phospholipids resulted in the phosphorylation of approximately 50% of tau protein.

As expected, in the presence of phosphatidylserine and diacylglycerol or phosphatidylserine alone, essentially 100% of tau protein was phosphorylated. The tau protein mobility in SDS-gel electrophoresis was not changed in the presence of these phospholipids if the kinase was omitted (Figs. 8C and 9C).

The presence of phosphatidylethanolamine shifted all the tau protein to lower mobility and like phosphatidylserine, increased \( ^{32}P \) incorporation into tau protein. However, the phosphorylated tau bands appeared much more diffuse on the autoradiogram. The phosphorylated \( \tau_2 \) protein even resolved into two distinct phosphoproteins when electrophoresis was extended (not shown). Since it was also observed that phosphatidylethanolamine in the absence of kinase significantly smeared the tau protein band on SDS-PAGE (Figs. 8C and 9C), whereas phosphatidylserine did not, it is impossible to say whether or not there were different modes of tau phosphorylation in the presence of the two phospholipids.

Phosphatidylinositol was found to be a potent inhibitor of tau protein phosphorylation and appeared to have the same effect as phosphatidylethanolamine on the mobility of tau in

**Fig. 5.** Time course of \( \tau_2 \) phosphorylation by the rat brain Ca\(^{2+}\)/calmodulin-dependent protein kinase: the effect of phosphatidylyserine. Purified \( \tau_2 \) protein (0.35 \( \mu \)M) was incubated with rat brain Ca\(^{2+}\)/CaM kinase (0.9 \( \mu \)g/ml in the absence (I) and presence (II) of 150 \( \mu \)g/ml phosphatidylyserine or with the kinase at a concentration of 13 \( \mu \)g/ml in the absence of phosphatidylyserine (III). The inset shows the Coomassie Blue staining of the 10% polyacrylamide, 0.1% SDS-gel electrophoresis, representing 5-min incubation of \( \tau_2 \) (0.3 \( \mu \)M) with the kinase (0.9 \( \mu \)g/ml) in the absence (I) and presence (II) of 86 \( \mu \)g/ml phosphatidylyserine; lane C is the reference \( \tau_2 \) sample incubated in Tris-HCl buffer.

**Fig. 6.** Time course of \( \tau_1 \) phosphorylation by the rat brain Ca\(^{2+}\)/calmodulin-dependent protein kinase: the effect of phosphatidylyserine. Purified \( \tau_1 \) protein (0.20 \( \mu \)M) was incubated with rat brain Ca\(^{2+}\)/CaM kinase (0.9 \( \mu \)g/ml) in the absence (I) or presence (II) of 150 \( \mu \)g/ml phosphatidylyserine. IA, IIA, IB, and IIB were from a single gel stained with Coomassie Blue (A) and a single autoradiogram (B); the panels were separated to simplify their presentation.
Mode I Phosphorylation of Tau Proteins

FIG. 7. The effect of phosphatidylserine concentration on the extent of phosphorylation of purified \( \tau_2 \) protein. Purified \( \tau_2 \) protein at a concentration of 3 \( \mu M \) (○) or 0.5 \( \mu M \) (□) was incubated for 5 min with rat brain Ca\(^{2+}\)/CaM kinase in the presence of increasing amounts of phosphatidylserine.

We showed that purified tau protein in solution can be phosphorylated by Ca\(^{2+}\)/CaM kinase maximally to about 50\% of the total tau protein. Incorporation of one phosphate group/mol of tau shifted the protein to a lower migrating electrophoretic band. For a number of reasons, we believe that there is physiological significance in the phosphorylation of tau by the Ca\(^{2+}\)/CaM kinase. First, the mode of phosphorylation produced by this kinase is the one most clearly shown to exist in vivo (3, 4). Second, the endogenous state of phosphorylation of tau in normal brain is a balance between nonphosphorylated and phosphorylated forms (3, 4). Third, the level of phosphorylation is known to affect the ability of tau to promote microtubule formation (3). Fourth, the phosphorylation by the Ca\(^{2+}\)/calmodulin-dependent kinase appears to produce a conformational change in tau, and it seems likely

FIG. 8. The effect of various phospholipids on the phosphorylation and electrophoretic mobility of purified \( \tau_2 \) protein. Purified \( \tau_2 \) protein (0.3 \( \mu M \)) was incubated 15 min with various phospholipids at similar concentrations (~70 \( \mu g/ml \)) in the absence (C) or presence (A and B) of rat brain Ca\(^{2+}\)/CaM kinase (0.9 \( \mu g/ml \)) and subjected to 10\% polyacrylamide, 0.1\% SDS-gel electrophoresis. A and B represent, respectively, the Coomassie Blue staining and autoradiography of the same gel. PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidic acid; DG, diacylglycerol.

FIG. 9. The effect of various phospholipids on the phosphorylation and electrophoretic mobility of purified \( \tau_2 \) protein. Purified \( \tau_2 \) protein (3 \( \mu M \)) was incubated 5 min with various phospholipids at similar concentrations (~70 \( \mu g/ml \)) in the absence (C) or presence (A and B) of rat brain Ca\(^{2+}\)/CaM kinase (0.9 \( \mu g/ml \)) and subjected to 10\% polyacrylamide, 0.1\% SDS-gel electrophoresis. A and B represent, respectively, the Coomassie Blue staining and autoradiography of the same gel. PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidic acid; DG, diacylglycerol.

DISCUSSION
changes in the phosphorylation of tau proteins. The Ca\(^{2+}\) produced by phosphorylation of purified tau with the Ca\(^{2+}/CaM\) kinase is enhanced by the anolamine and calmodulin-dependent kinase is one enzyme that could mediate such changes because it appears to act as a molecular switch that remains active long after an initial triggering event. Ca\(^{2+}\) and calmodulin stimulate autophosphorylation of the enzyme and incorporation of 3–30 phosphate groups/holoenzyme. The phosphorylated form that results from autokatalysis becomes autonomous, being completely independent of Ca\(^{2+}\) and calmodulin. Such properties allow the enzyme to remain active long after the decay of an initial Ca\(^{2+}\) signal. Dephosphorylation by a distint phosphatase is required to turn off the enzyme. An imbalance of the autophosphorylation–phosphatase system involving either an increase in kinase or a decrease in phosphatase activity that normally would control autophosphorylation of the enzyme might be responsible for the abnormal phosphorylation of tau protein in Alzheimer’s neurons. In this respect, it is interesting to note that Saitoh and Dobkins (17) reported the increase in phosphorylation of a Mr 60,000 protein in brain from patients with Alzheimer’s disease. This protein is a minor component of the cytosolic extract of Alzheimer’s brain, and the authors suggested that it may correspond to a Mr 60,000 protein kinase. Although it could well be coincidence, we note that our Ca\(^{2+}/CaM\) kinase purified from bovine brain also migrated as a single Mr 60,000 polypeptide upon auto-phosphorylation as well as the \(\beta\) subunit of the rat enzyme. Comparison of the antigenic properties of the two proteins might be a first step in testing for a common identity of these proteins.

The finding that purified tau protein in solution could only be phosphorylated to a limit of approximately 50% suggests that some interaction between tau proteins can occur that prevents phosphorylation of half of it. Whether these interactions require phosphorylation of the first half of tau protein or are independent of previous phosphorylation is not known at present.

The fact that in the presence of phosphatidylerine, tau proteins were phosphorylated to an extent of 100% at a tau-phosphatidylerine ratio of 20 suggests that the effect of phosphatidylerine was probably through a breakdown of tau–tau interactions. The specific requirements for the primary structure at the phosphorylation site of protein substrates have been delineated for a number of protein kinases (18). In addition, substrate activity of protein may also be affected by the conformation of the protein (19, 20). Results of the present study apparently confirm the latter point. Whether the purified tau proteins in solution or bound to phosphatidylerine liposomes assume their physiological conformations is not known, and further studies are required to clarify this question. However, different conformation states of tau protein in Alzheimer’s brain compared to normal brain might also well account for the different extent of tau phosphorylation found in the two cases.

The direct observation that tau proteins can also interact with other phospholipids such as phosphatidylethanolamine and phosphatidylinositol, resulting in a smearing of the protein band on SDS-PAGE, supports strongly the possibility that tau protein may indeed interact with biological membranes in vivo. Furthermore, the fact that phosphatidylerine and phosphatidylethanolamine have effects opposite to that of phosphatidylinositol on tau phosphorylation brings out the interesting possibility that tau protein phosphorylation could be modulated by the phospholipid composition of the membranes with which tau interacts. Studies on the interactions and phosphorylation of tau with mixed phospholipid liposomes would not only illuminate the effect of the various phospholipids on tau protein phosphorylation but might shed new light on the biological function of tau protein in vivo.

**REFERENCES**

1. Cleveland, D. W., How, S. Y., and Kirschner, M. W. (1977) *J. Mol. Biol.* 116, 207–225
2. Cleveland, D. W., Hwo, S. Y., and Kirschner, M. W. (1977) *J. Mol. Biol.* 116, 227–247
3. Lindwall, G., and Cole, R. D. (1984) *J. Biol. Chem.* 259, 12241–12245
4. Lindwall, G., and Cole, R. D. (1984) *J. Biol. Chem.* 259, 5301–5305
5. Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Winstead, H. M., and Binder, L. I. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 4913–4917
6. Ihara, Y., Nukina, N., Miura, R., and Ogawa, M. (1986) *J.
Mode I Phosphorylation of Tau Proteins

Biochem. (Tokyo) 99, 1807–1910

7. Isobe, T., Nakajima, T., and Okuyama, T. (1977) Biochim. Biophys. Acta 494, 222–232
8. Baudier, J., Lee, S. H., and Cole, R. D. (1987) J. Biol. Chem. 262, 17584–17590
9. McGuinness, T. L., Lai, Y., and Greengard, P. (1985) J. Biol. Chem. 260, 1696–1704
10. Schulman, H. J. (1984) Cell Biol. 99, 11–19
11. Goldenring, J. R., Gonzalez, B., McGuire, J. S., Jr., and DeLorenzo, R. J. (1983) J. Biol. Chem. 258, 12632–12640
12. Kuret, J., and Schulman, H. (1984) Biochemistry 23, 5495–5504
13. Miller, S. G., and Kennedy, M. B. (1985) J. Biol. Chem. 260, 9039–9046
14. Lai, Y., Nairm, A., and Greengard, P. (1986) Proc. Natl Acad. Sci. U. S. A. 83, 7136–7139
15. Miller, S. G., and Kennedy, M. B. (1986) Cell 44, 861–870
16. Buss, J. E., and Stull, J. T. (1983) Methods Enzymol. 99, 7–14
17. Saitoh, T., and Dobkins, K. R. (1986) Proc. Natl Acad. Sci. U. S. A. 83, 9764–9767
18. Krebs, E. G., and Beavo, J. A. (1979) Annu. Rev. Biochem. 48, 923–959
19. Suzuki, T., and Wang, J. H. (1987) J. Biol. Chem. 262, 3880–3885
20. Johnsson, N., Van, P. N., Soling, H. D., and Weber, K. (1986) EMBO J. 5, 3455–3460