Phosphorylation of Protein Tau by Double-Stranded DNA-Dependent Protein Kinase

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Summary. Alzheimer Disease (AD) is a distinct form of dementia characterized by the occurrence of neurofibrillary tangles, neurotic plaques and loss of certain neuronal populations. The tangles are associated with the presence of abnormal proteinaceous deposits. One such protein, referred to as tau, is found to be excessively phosphorylated in AD. We demonstrate that a double-stranded DNA-stimulated protein kinase (referred to as DNA-PK) effectively catalyzes the phosphorylation of recombinant human protein τ. Moreover, in the presence of stimulatory DNA, the hyper-phosphorylation of tau is accompanied by a significant shift in its mobility on SDS polyacrylamide gels. These results suggest that DNA-PK may contribute to the pathogenesis of AD.

Senile plaques and neurofibrillary tangles, present within the hippocampal formation and spare deep grey structures, the neocortex, and cerebrovascular amyloid deposits, characterize degenerating neurons and are recognized histopathological hallmarks of AD (1-3). Amyloid deposits presumably arise from the abnormal processing of amyloid protein precursors (AAP), whereas the neurofibrillary tangles are composed of markedly insoluble paired helical filaments (4-9). Thus in addition to being valuable in the diagnosis of AD, insoluble filamentous tangles and neuritic plaques are also considered to be of fundamental importance in its pathogenesis. Because the formation of tangles is thought to be

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linked to the hyper-phosphorylation of tau and other microtubule-associated proteins (1-4, 11, 12), there have been an intense search for protein kinase activity changes in AD cells and brain tissues. A number of kinases have been shown to phosphorylate tau (12-21). In this report, we demonstrate that highly purified DNA-PK from HeLa cells effectively catalyzes the phosphorylation of recombinant tau. Moreover, abnormal phosphorylation of tau by DNA-PK, in the presence of stimulatory DNA, results in a shift in its mobility, as analyzed by SDS/PAGE.

Materials and Methods

DNA-PK was purified from the nuclear extract of HeLa cells as described previously (22). The preparation used for these studies contained a 300 kDa polypeptide as the major component on reducing SDS-PAGE. The catalytic subunit of cyclic AMP-dependent protein kinase (PKA) isolated from bovine heart was generously provided by Dr. E. Fisher (University of Washington, Seattle). Recombinant tau was purified from extracts of E.coli transformed with pET-3d plasmid according to Vulliet et al. (20).

Protein Phosphorylation. The phosphorylation of tau (0.5 μg) with PKA (150 ng) or DNA-PK (230 ng) was assayed in a reaction volume of 25 μl containing 20 mM Hepes, pH 7.4, 90 mM KOAc, 2 mM $Mg(OAc)_2$, 1.5 mM dithiothreitol, 30 μM ATP (3000-4000 cpm/pmol). Poly(dA).poly(dT) (100 ng) or synthetic double-stranded oligomer (483 ng), CTGAGGAAACGAAACCAACAGCAGTCCAAGCTCAGTCAGCAG, corresponding to interferon response element (IRE-42 mer), were also included where indicated. The reaction mixtures were incubated at 37°C for up to 60 min, stopped by the addition of an equal volume of 2 X SDS sample buffer, boiled for 5 min, and subjected to 10% or 12.5% SDS-PAGE (23). The gel was stained with coomassie blue, destained, dried, and autoradiographed using Kodak X-AR film. Alternatively, phosphorylation of tau was monitored by counting of the total trichloroacetic acid-precipitable radioactivity (24).

Results and Discussion

Several published reports prompted us to consider DNA-PK as a candidate kinase for tau. For example, evidence exists for the specific interactions between DNA and microtubule-associated proteins and tau proteins (25, 26). A deficiency of DNA repair has been reported, particularly in connection with alkylation-induced damages, in skin fibroblasts, peripheral blood lymphocytes, monocytes, and lymphoblasts derived from sporadic and familial AD

Table 1

Phosphorylation of Tau by PKA or DNA-PK

Addition	Trichloroacetic Acid-Precipitable Radioactivity[a] (pmol/assay)
Tau alone	1.83 ± 0.84
PKA alone	2.42 ± 1.73
DNA-PK alone	1.79 ± 0.17
Tau + PKA	6.01 ± 1.73
Tau + DNA-PK	6.52 ± 1.73
Tau + poly(dA).poly(dT)	2.59 ± 1.73
Tau + DNA-PK + poly(dA).poly(dT)	79.2 ± 1.73
Tau + DNA-PK + IRE-42 mer	9.21 ± 1.73

[a]Incubations were for 20 min. Data represent the average of four independent measurements. In these experiments, 1 pmol equals 3,500 cpm. A background of 1,100±240 cpm has been subtracted.

(27, 28). Mullaart et al. demonstrated an increased number of DNA breaks or alkali-labile sites in cortical nuclear DNA from patients with AD (29). DNA-PK has been shown to require DNA ends for its activity under certain circumstances (22, 30). These observations raise the possibility that the transient fragmentation of DNA, resulting from exposure of cells to DNA-damaging agents in the environment, could activate DNA-PK and thereby exert a significant and lasting influence on cellular events. Such changes could be exacerbated by DNA repair deficiency present in AD.

To test the possibility that tau is phosphorylated by DNA-PK, highly purified HeLa cell DNA-PK was incubated *in vitro* with recombinant human tau, using [γ-^{32}P] ATP as the phosphate donor. For comparison, the phosphorylation of tau by PKA was also assayed. Kinase activity was monitored by precipitating phosphorylated proteins in the reaction mixtures with cold trichloroacetic acid and quantifying the radioactivity. Results in Table 1 show that tau is phosphorylated to a similar degree by PKA or DNA-PK. In the

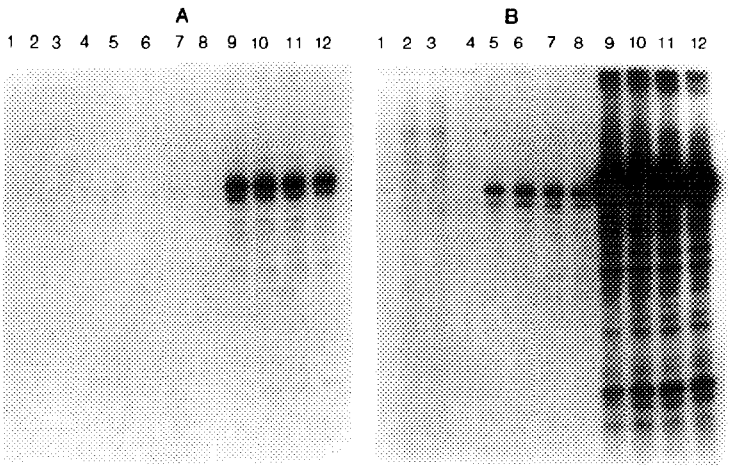

Figure 1. Phosphorylation of Tau by Purified DNA-PK. To determine dsDNA-dependent phosphorylation of tau, synthetic poly(dA).poly(dT) (0.1 μg) was added to the reaction mixtures, which were incubated at 37°C for 5, 10, 20, and 60 min. They were stopped with SDS-sample buffer, boiled for 5 min, and subjected to 10% SDS-PAGE. The gel was stained with 0.1% Coomassie blue, destained, dried, and autoradiographed. Lanes 1-4, DNA-PK alone; lanes 5-8, DNA-PK + tau; lanes 9-12, DNA-PK + tau + poly(dA). poly(dT). Lanes 1, 5, 9, 5 min incubation; lanes 2, 6, 10, 10 min incubation; lanes 3, 7, 11, 20 min incubation; lanes 4, 8, 12, 60 min incubation. Panels A and B represent results obtained following a 30 min and a 15 h exposure of the autoradiogram.

presence of poly(dA).poly(dT), phosphorylation of tau by DNA-PK was stimulated 12-fold. The addition of synthetic double-stranded oligomer, however, was only marginally effective in stimulating the phosphorylation of tau by DNA-PK and may reflect the reduced efficiency (or the relative inability) of the shorter oligomer to promote the co-localization of DNA-PK and substrate tau on DNA.

The kinetics of tau phosphorylation by DNA-PK, in the absence and presence of stimulatory poly(dA).poly(dT), is presented in Figure 1 which shows that tau is rapidly phosphorylated. Assuming a molecular weight range of 45,000-62,000 for protein tau (31), the molar ratio of phosphate incorporated into tau by activated DNA-PK is calculated to be between 7-10, highly suggestive of its hyper-phosphorylation.

Furthermore, Figure 2 shows that phosphorylation of tau by DNA-PK induced shifts in the mobilities of the tau protein that resembled those occurring in vivo (32) and more particularly, in

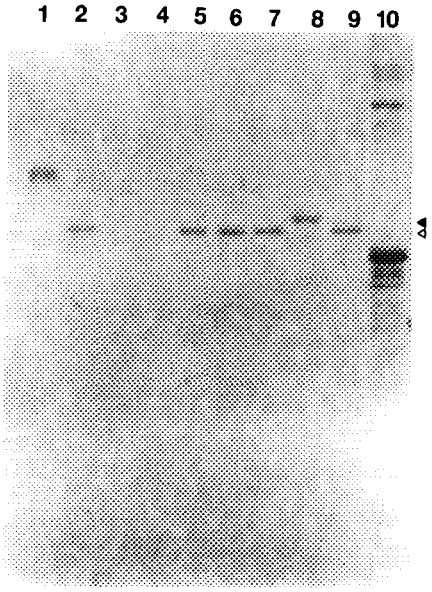

Figure 2. A Coomassie Blue-stained gel which showed the effect of Tau Phosphorylation by DNA-PK on the electrophoretic mobility of Tau. Conditions were identical to those described in legends to Table 1 except that the incubation time was increased to 40 min. Lane 1, marker bovine serum albumin (67-kD); lane 2, tau alone; lane 3, PKA alone; lane 4, DNA-PK alone; lane 5, tau + PKA; lane 6, tau + DNA-PK; lane 7, tau + poly(dA).poly(dT); lane 8, tau + DNA-PK + poly(dA).poly(dT); lane 9, tau + DNA-PK + IRE-42 mer; Lane 10, markers phosphorylase (97-kD) and actin (43-kD).

degenerative neurons of Alzheimer's brain (1-4). Thus, the faster moving electrophoretic species characteristic of non-phosphorylated tau (shown by the open triangle) was shifted to a slower electrophoretic band (shown by closed triangle) upon its phosphorylation by active DNA-PK.

In summary, DNA-PK has been shown to covalently modify tau by phosphorylation in a DNA-dependent manner. Given that a number of other protein kinases are also reported to phosphorylate tau (12-21), it is important to determine whether the sites of modification of tau by these kinases are identical to or different from those effected by DNA-PK. Such studies are currently in progress.

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