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A preparation of Alzheimer paired helical filaments that displays distinct τ proteins by polyacrylamide gel electrophoresis

(neurodegenerative disorders/neurofibrillary tangle/cytoskeletal proteins)

SHARON G. GREENBERG* AND PETER DAVIES

Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

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ABSTRACT **Paired helical filaments (PHFs) are prominent components of Alzheimer disease (AD) neurofibrillary tangles (NFTs). Rather than isolating NFTs, we selected for PHF populations that can be extracted from AD brain homogenates. About 50% of PHF immunoreactivity can be obtained in 27,200 × *g* supernatants following homogenization in buffers containing 0.8 M NaCl. We further enriched for PHFs by taking advantage of their insolubility in the presence of zwitterionic detergents and 2-mercaptoethanol, removal of aggregates by filtration through 0.45-μm filters, and sucrose density centrifugation. PHF-enriched fractions contained two to five proteins of 57–68 kDa that displayed the same antigenic properties as PHFs. Since the 57- to 68-kDa PHF proteins are antigenically related to τ proteins, they are similar to the τ proteins previously observed in NFTs. However, further analysis revealed that PHF-associated τ can be distinguished from normal, soluble τ by PHF antibodies that do not recognize human adult τ and by one- and two-dimensional PAGE.**

The paired helical filament (PHF) and immunologically related abnormal straight filament are prominent features of Alzheimer disease (AD). In addition to neurofibrillary tangles (NFTs), consisting of PHF aggregates, PHFs are found in dystrophic neurites of plaques and neuritic processes in the cortical neuropil (reviewed in ref. 1).

Immunochemical studies (2–4) and sequence analysis (5, 6) indicate that τ protein is a component of PHF. τ normally consists of a family of soluble, basic proteins that stabilize microtubules. Specific modifications that may alter the properties of soluble τ (τ_s) to those observed in PHF could include phosphorylation (7), ubiquinylation (8), or alternative splicing of τ mRNA.

PHFs contain "unique" (9, 10) epitopes (i.e., those not shared with normal human adult proteins) as well as epitopes shared with microtubule-associated protein 2 (MAP2) (11, 12), high molecular weight neurofilament (NF) proteins (13), and ubiquitin (14). However, MAP2 (11) and high molecular weight NF proteins (15, 16) may contain shared τ epitopes. Furthermore, PHF antibodies that do not recognize τ in normal human adults recognize τ derived from other sources (2). Therefore, the diverse antigenicity of PHFs appears to be due to the recognition of a primary τ sequence.

To further study PHF-associated τ (τ_{PHF}), it is important to analyze PHFs by PAGE. Since many investigators select for relatively insoluble preparations of NFTs, PAGE analysis of NFTs has not consistently been successful (reviewed in refs. 1 and 17). Thus, analysis of PHFs is frequently limited to immunocytochemistry or analysis of peptide fragments released from NFTs. In addition to insoluble populations of NFTs, Iqbal *et al.* (18) observed populations of PHFs that were soluble in SDS. Moreover, τ_{PHF} (4) was detected by PAGE of NFTs dispersed by sonication (18, 19). Thus, nonaggregated populations of PHFs are more amenable to analysis than NFTs. Rather than attempting to disrupt PHFs in isolated NFTs, we selected for less aggregated populations of PHFs directly from AD brain homogenates. Our results indicate that these PHFs can be analyzed by PAGE and the τ_{PHF} proteins can be distinguished from many normal τ_s proteins by one- and two-dimensional PAGE. A preliminary report of these results has been published (20).

MATERIALS AND METHODS

Tissue Source. We used postmortem tissue from 14 AD (ages 59–89) and 6 normal (ages 21–96) subjects. The samples consisted mostly of gray matter from the frontal, temporal, or parietal cortex. The diagnosis of AD was confirmed histologically by abundant plaques and/or tangles. Normal cases did not display neurofibrillary pathology in the cortex.

Antibodies. PHF-reactive antibodies included a PHF polyclonal antibody (ICN), diluted 1:200, and a ubiquitin polyclonal antibody, UH-11 (provided by S.-H. Yen), diluted 1:100; the monoclonal antibodies (mAbs) 39, 215, 64, 322, and 636 [provided by S. H. Yen (10, 11)], NP8, and NP14 were used undiluted and mAb Alz-50 (21) was diluted 1:10. Non-PHF-reactive antibodies included an NF68 (68-kDa NF protein) mAb (Boehringer Mannheim), diluted 1:50; a β-tubulin mAb, clone 2.1 (Sigma), diluted 1:50; an amyloid precursor protein antibody (provided by S.-H. Yen), diluted 1:100; and a ferritin polyclonal antibody (Sigma) that was used to identify ferritin on immunoblots (data not shown).

Preparation of PHF-Enriched Fractions. Tissue (10–20 g) was homogenized with 10 vol of cold H buffer (10 mM Tris/1 mM EGTA/0.8 M NaCl/10% sucrose, pH 7.4) in a Teflon/glass homogenizer with a Cafrano tissue stirrer set at a speed of 5. After centrifugation at 27,200 × *g* for 20 min at 4°C, the supernatant was saved and the pellet was homogenized with 10 vol of H buffer and centrifuged at 27,200 × *g* for 20 min. The 27,200 × *g* supernatants were combined, adjusted to 1% (wt/vol) *N*-lauroylsarcosine and 1% (vol/vol) 2-mercaptoethanol, and incubated at 37°C for 2–2.5 hr while shaking on an Orbital shaker. All subsequent steps were done at room temperature. After centrifugation at 35,000 rpm in a Beckman Ti 60 rotor, for 35 min, the PHF-containing pellets were homogenized in 5–10 ml of H buffer/1% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)/1% mercaptoethanol and filtered through 0.45-μm cellulose acetate syringe-type filters; one filter was used for each 1–2 g of starting material. After the filters were rinsed, the filtrates were centrifuged at 35,000 rpm in a Ti 60 rotor for

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Abbreviations: PHF, paired helical filament; NFT, neurofibrillary tangle; AD, Alzheimer disease; NF, neurofilament; MAP, microtubule-associated protein; TCA, trichloroacetic acid; mAb, monoclonal antibody; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; τ_s, soluble τ; τ_{PHF}, PHF-associated τ.

*To whom reprint requests should be addressed.

1 hr. The PHF-containing pellet was resuspended in 3–4 ml of H buffer/0.1% mercaptoethanol and layered over a discontinuous sucrose gradient consisting of 4 ml of 50% sucrose and 3.5 ml of 35% sucrose in 10 mM Tris/0.8 mM NaCl/1 mM EGTA/0.1% mercaptoethanol, pH 7.4. After centrifugation for 2 hr at 35,000 rpm in a Beckman SW 41Ti rotor, PHFs were collected from the 35% layer and 35–50% interface with a 5-ml syringe attached to an 18-gauge 1.5-inch needle inserted through the side of the polyallomer centrifuge tube and were stored at −70°C until used for further analysis.

Filtration through 0.45-μm filters and the CHAPS wash were not done in preliminary experiments. However, filtration removed aggregates and trapped soluble proteins. While increased yields may be obtained by vigorous homogenization and/or use of less H buffer, these PHF preparations often appeared less purified and more aggregated.

Biochemical Analysis. Samples were precipitated with 10% (wt/vol) trichloroacetic acid (TCA), with 7 vol of MeOH, or with 7 vol of MeOH/0.1% TCA. PHF-enriched fractions were commonly adjusted to 1 mM phenylmethylsulfonyl fluoride prior to precipitation with TCA to prevent proteolysis. Samples precipitated with TCA did not display the 300-kDa ferritin aggregate observed in fractions precipitated with MeOH alone. Proteins were resuspended in 8 M urea/1% SDS/0.1 M Tris, pH 6.8, with or without 1% mercaptoethanol and subjected to one-dimensional PAGE according to Laemmli (22) with a slight modification. While the standard pH of running gels is 8.8, a Tris buffer of pH 8.4 was found to selectively increase the resolution of τ_{PHF}. To visualize τ_{PHF} for one-dimensional PAGE, we loaded 10–40 μg (Coomassie blue), 1.5–3.0 μg (silver stain), or 0.5–3.0 μg (immunoblots with Alz-50 or PHF polyclonal antibody) of PHF protein per lane.

For two-dimensional PAGE (23), PHFs collected by high-speed centrifugation or proteins in 27,200 × *g* supernatants were precipitated with MeOH, resuspended in 8 M urea/0.5% SDS with or without 1% mercaptoethanol, mixed with an equal volume of lysis buffer [8 M urea/8% (vol/vol) Triton X-100/2% (wt/vol) Ampholines (1.6% pH 5–8 and 0.4% pH 3–10; LKB)] and subjected to isoelectric focusing for 6000 V·hr.

SDS/polyacrylamide gels were stained with 0.2% Coomassie brilliant blue or silver, or the proteins were transferred to nitrocellulose for immunoblot analysis (24) using the indirect peroxidase method and 4-chloronaphthol as chromagen. Quantitative dot blot analysis was performed with an ^{125}I-labeled goat anti-mouse immunoglobulin (NEN).

Samples precipitated with 10% TCA were resuspended in 8 M urea/10 mM Tris/0.1% SDS, pH 6.8, for protein determination (25). Average concentrations ± SEM (from four to six cases) are indicated in the text.

Electron Microscopy. PHFs were spotted onto Formvar-coated grids, which were then washed with water, stained with 5% uranyl acetate in 50% ethanol, and viewed with a JEOL 100 CX or Siemens (Schaumburg, IL) 102 electron microscope at 80 kV. Semiquantitative analysis involved counting the PHFs in a standard field viewed at a magnification of ×25,000. The average number of PHFs was determined from 10 regions of the grid, onto which 5 μl of sample was originally applied. The dimensions of 14 PHFs, selected at random, were determined from 38–56 measurements using photographic negatives taken at a magnification of ×100,000. The average diameters ± SD are indicated in the text. Immunogold labeling of PHF-enriched fractions was performed on Formvar-coated grids, using 10-nm-gold-conjugated secondary antibodies, diluted 1:10.

Isolation of τ_s Proteins. Tissue was homogenized in 0.1 Mes/1 mM EGTA/0.1 mM EDTA/0.5 mM $MgCl_2$/1 mM mercaptoethanol/0.8 M NaCl, pH 6.8, and centrifuged at 100,000 × *g* for 1 hr at 4°C. The supernatant was heated at 100°C for 5 min, cooled on ice, and centrifuged at 15,000 × *g* for 30 min. The heat-stable supernatant was adjusted to 2.5% perchloric acid and centrifuged at 15,000 × *g* for 15 min, and τ_s was precipitated from the supernatant by 20% TCA.

Table 1. Quantitative immunodot analysis of 27,000 × *g* supernatants (SN) and pellets

Samples	Bound secondary antibody, dpm × 10^{-6}/g of tissue (SEM)			
	Alz-50		mAb 39	
	SN	Pellet	SN	Pellet
AD (6 cases)	4.3 (0.8)	3.3 (0.7)	6.4 (1.2)	6.9 (1.4)
Normal (4 cases)	1.0 (0.3)	1.4 (0.1)	0.9 (0.4)	0.8 (0.1)

RESULTS

Extraction of PHF Immunoreactivity. Table 1 shows that ≈50% of the total immunoreactivity of the PHF-reactive mAbs Alz-50 (26) and 39 (10, 11) can be recovered in 27,200 × *g* supernatants following homogenization of AD tissue in a buffer with 0.8 M NaCl. The extraction of Alz-50 AD antigens with 0.8 M NaCl is significantly improved over methods using less NaCl (27, 28). The 0.8 M NaCl extract contains not only PHF but also τ_s. Whereas mAb 39 recognizes a "unique" PHF epitope (10, 11), Alz-50 recognizes all τ proteins on immunoblots (28, 29), including τ_s and τ_{PHF}.† However, because Alz-50 displays a higher affinity for PHFs than for *native* τ_s (31), Alz-50 reactivity appears higher in AD samples than in normals.

PHFs were enriched from 27,200 × *g* supernatants by taking advantage of the insolubility of PHFs in zwitterionic detergents (2, 19) and mercaptoethanol, filtration through 0.45-μm filters, and sucrose density centrifugation. In the presence of 0.35–0.8 M NaCl, τ_s is dissociated from microtubules and is obtained in 100,000 × *g* supernatants. Since PHFs are obtained in 100,000 × *g* pellets (2), our isolation procedure results in PHF-enriched fractions that do not contain readily soluble τ_s proteins.

Electron Microscopy. Fig. 1*A* shows a PHF-enriched fraction obtained after sucrose density centrifugation. Although relatively nonaggregated populations of PHFs were observed in all AD samples examined, the purity and yield depended upon the case and brain region used. Occasional clusters of PHFs were also detected in 4 of the 11 cases examined. Ferritin and electron-dense aggregates are the major ultrastructural contaminants of fractions from normal and AD samples. Filaments are not generally detectable in preparations from normal samples (Fig. 1*B*).

Most filaments display the characteristic morphology of two filaments wound around each other with a maximum average diameter of 22.4 ± 4.0 nm and narrowing every 65 ± 8 nm to a diameter 10.8 ± 1.5 nm. Ten- to fifteen-nanometer paired filaments without a pronounced helical turn are also occasionally observed (arrowheads). These PHFs are reactive with a PHF polyclonal antibody (Fig. 1*D*) and with mAbs Alz-50, NP8, and 64 (Fig. 1 *C*, *E*, and *F*). Thus, PHFs isolated from extracts of AD homogenates display similar dimensions and antigenic properties as PHFs in NFTs.

†The Alz-50 antigen was originally identified as a single 68-kDa soluble protein (A68) that was not associated with PHFs (21). However, other investigators (20, 26, 28, 29) have not reproduced this result. Instead, Alz-50 was found to recognize multiple proteins of 35–68 kDa that were identified as τ (28, 29). Like other τ antibodies, Alz-50 recognizes PHFs (26) and τ_{PHF} proteins observed by PAGE (ref. 20 and Figs. 3 and 4). Although A68 originally displayed properties that were distinct from those of normal τ_s and τ_{PHF} (21), the name A68 is currently being used to describe τ_s (30) and τ_{PHF} (31–33).

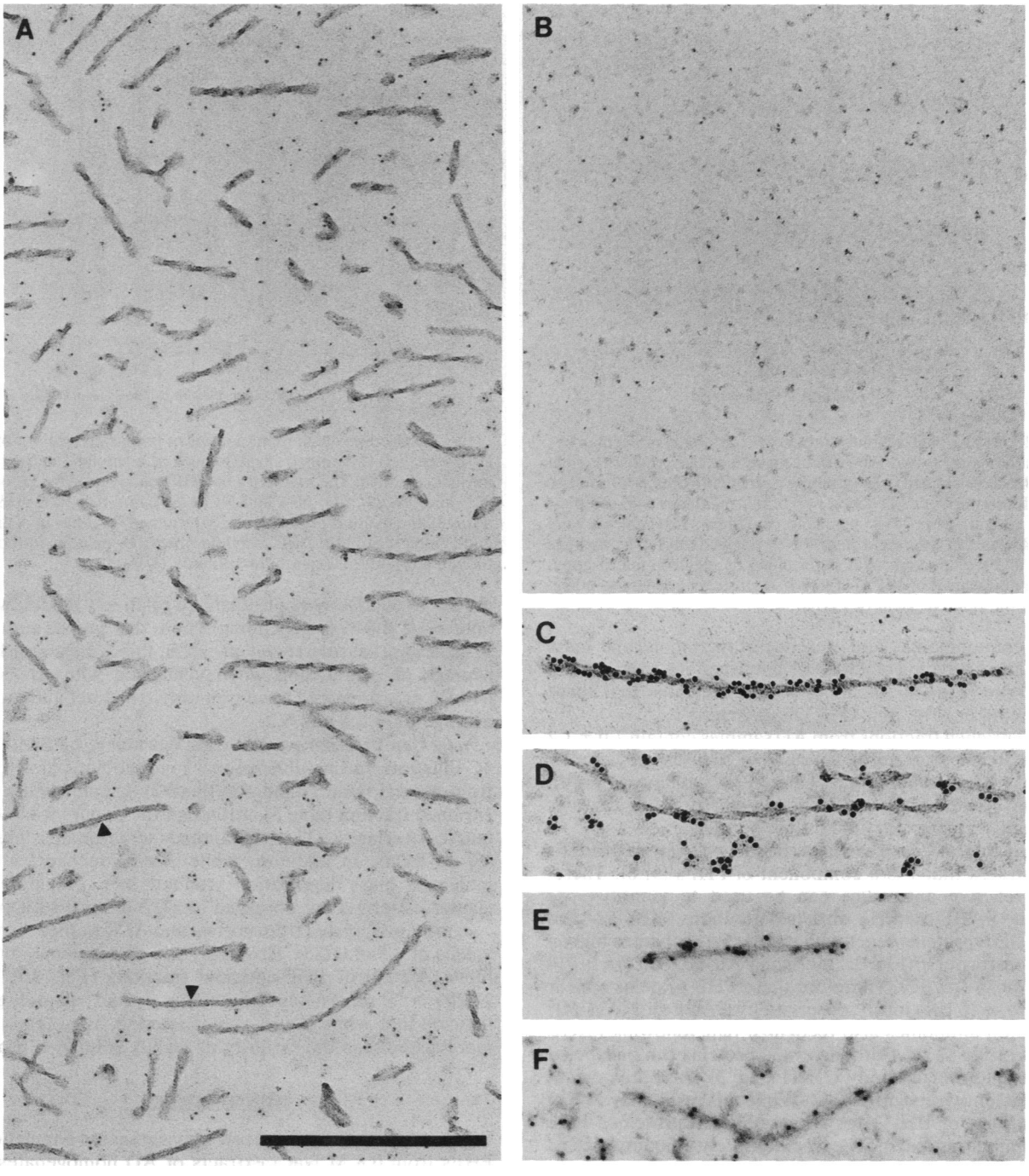

FIG. 1. Electron microscopy of a sucrose density gradient fraction isolated from an AD sample (*A*) reveals PHFs and a few abnormal paired filaments without a pronounced helical turn (arrowheads). Filaments are not observed in similar fractions from normal brain (*B*). Ferritin is observed in preparations from AD and normal brain tissue. Immunogold labeling illustrates that PHFs are immunodecorated by incubation with a PHF polyclonal antibody (*D*) or with mAb Alz-50 (*C*), NP8 (*E*), or 64 (*F*). (Bar = 0.5 μm.)

However, these populations of PHFs are not identical. For example, PHFs in Fig. 1*A* do not display the distinct "fuzzy coat" observed in NFTs (see ref. 5). Moreover, although many NFTs remain structurally intact following treatments with SDS and guanidine, dilution with an equal volume of 2% SDS or 8 M guanidine hydrochloride results in 71–96% fewer PHFs (five AD cases) compared to controls. While SDS and guanidine may not completely dissociate PHFs to individual subunits, these PHFs appear relatively sensitive to denaturants.

Protein Composition. Coomassie blue-stained (Fig. 2*A*) or silver-stained (Fig. 2*B*, lanes 1–5) gels containing PHF-enriched fractions from AD samples consistently contain two to five proteins of 57–68 kDa, of which a 64-kDa protein is the most prominent. The relative abundance of the 57- to 68-kDa PHF proteins appears to correspond to the relative yield of PHFs observed by electron microscopy from different AD cases.

Material in the stacking gel and proteins of 400, 180–220, 110, and 22–55 kDa are also commonly observed in PHF-enriched fractions. However, many of these proteins represent aggregates or degradation products of the 57- to 68-kDa PHF proteins (34). While some of the high molecular mass material represents insoluble PHFs (18), the significant amount of silver-stained material (Fig. 2*B*) is not as apparent in Coomassie blue-stained gels (Fig. 2*A*). Therefore, some of the high molecular mass material may represent nonprotein-

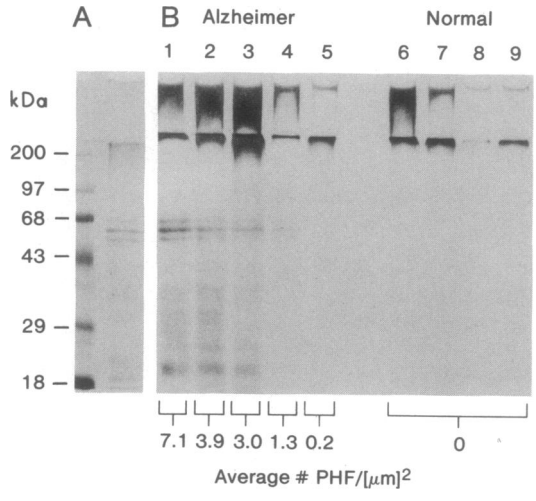

FIG. 2. (*A*) Coomassie blue-stained gel containing molecular size markers (left lane) and a PHF-enriched fraction from AD tissue (right lane). (*B*) Silver-stained gel containing equivalent volumes of sucrose density fractions from AD (lanes 1–5) or normal (lanes 6–9) samples. The AD patients were 74–85 years old, and the normal subjects were 96, 85, 45, and 21 years old (lanes 6–9, respectively). The average number of PHFs per μm^2 was determined by electron microscopy. Relative abundance of the 57- to 68-kDa proteins appears to correspond to the relative yield of PHFs.

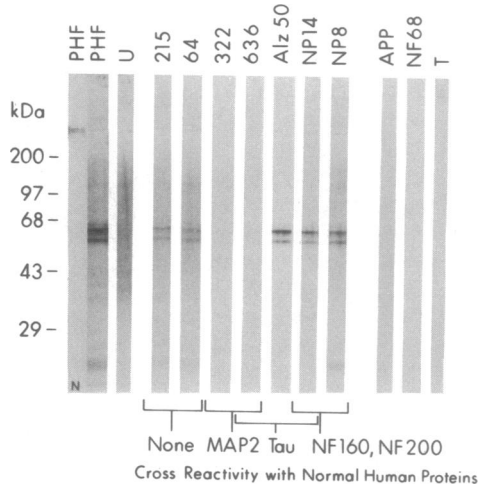

FIG. 3. Composite of immunoblots of preparative gels containing PHF-enriched fractions from AD cases or a similar fraction from a normal case (N). The reactivity of PHF-reactive mAbs 39, 64, 215, 322, and 636 (10, 11), NP8 and NP14 (15), and Alz-50 (28, 29) with normal human adult proteins was determined by other investigators and is indicated here. APP, amyloid precursor protein antibody; T, tubulin antibody; U, ubiquitin antibody (UH-11).

aceous contaminants. The presence of high molecular mass material and proteins of 18–20 kDa in fractions from normal brain tissue (Fig. 2*B*, lanes 6–9) also indicates that these components may be non-PHF contaminants.

PHF-enriched fractions from AD samples contain 7.0 ± 1.2 μg of protein per gram of tissue (n = 6 cases) and similar fractions from normal samples contain 2.8 ± 0.6 μg/g (n = 4). The total yield of protein in fractions from AD cases is significantly higher ($P < 0.05$) than normals.

Immunological Crossreactivity. Sequence analysis of PHFs demonstrated that τ is a component of PHFs (5, 6). Therefore, τ-reactive antibodies can be used to identify τ_{PHF} following PAGE of PHF-enriched fractions. The 57- and 64-kDa PHF proteins react with mAbs that recognize human adult isoforms of τ (Fig. 3), including Alz-50 (28, 29), NP14 (15), and 636 (10, 11). Therefore these PHF proteins appear similar to τ_{PHF} previously observed by PAGE of NFTs (4).

Ubiquitin antibodies also recognize τ_{PHF} following PAGE analysis of NFTs (8). Although τ_{PHF} proteins can be labeled with the ubiquitin antibody UH-11 (Fig. 3), this antibody did not consistently recognize τ_{PHF}. While this result may reflect a low affinity of UH-11 for immunoblotted proteins, these PHF populations may not be as extensively ubiquinylated as NFTs.

The 57- and 64-kDa τ_{PHF} proteins react with a PHF polyclonal antibody and mAbs that recognize unique PHF epitopes (mAbs 215 and 64), epitopes shared with high molecular mass NF proteins (NP8 and NP14), and weakly reactive epitopes shared with MAP2 (mAbs 322 and 636) (Fig. 3). The 68-kDa τ_{PHF} protein, when present, also reacted with PHF antibodies. Therefore the 57- to 68-kDa τ_{PHF} proteins contain the same antigenic determinants as PHFs.

Some PHF antibodies also recognize aggregates and degradation products of the 57- to 68-kDa τ_{PHF} proteins. By contrast to PHF antibodies, antibodies that recognize tubulin, NF68, or the amyloid precursor protein do not recognize τ_{PHF} proteins. In similar fractions from normal tissue, the PHF polyclonal antibody recognizes 400- and 18- to 20-kDa proteins but does not label proteins of 57–68 kDa.

Two-Dimensional PAGE. Two-dimensional PAGE of PHF-enriched fractions visualized by silver stain (Fig. 4*A*) or immunoblot analysis using Alz-50 (Fig. 4*B*) demonstrates that the 57- to 68-kDa τ_{PHF} proteins are relatively acidic, with pI values of 4.8–6.6 depending upon the preparation. The 57-kDa τ_{PHF} has an average pI ≈ 6.0, the 68-kDa τ_{PHF} has an average pI ≈ 5.9, and when visualized with Alz-50, the 64-kDa τ_{PHF} appeared to consist of two distinct variants with average pI ≈ 5.8 and 6.2.

τ_{PHF} Can Be Distinguished from Normal τ_s by PAGE. Fig. 4*C* illustrates a one-dimensional immunoblot (Alz-50) of τ_s from normal (lane 1) and AD (lane 2) samples and a PHF-enriched fraction (lane 3). Although the apparent molecular masses overlap, τ_s (47–63 kDa) tends to be smaller than τ_{PHF} (57–68 kDa). In addition, while Alz-50 recognizes lower molecular mass degradation products in τ_s preparations, a similar pattern is not observed in PHF-enriched fractions.

τ_s proteins isolated from normal and AD samples display pI values of 6.5–8.0 (35). However, two-dimensional immunoblots (Alz-50) of PHF-enriched fractions (Fig. 4*B*) and a 27,200 × g supernatant containing τ_s and τ_{PHF} (Fig. 4*D*) indicate that while the pI values overlap, τ_{PHF} tends to be more acidic than the majority of readily soluble τ_s proteins.

DISCUSSION

We have enriched for relatively nonaggregated populations of PHFs from 0.8 M NaCl extracts of AD homogenates. Our results suggest that ≈50% of PHF immunoreactivity can be extracted by this method. The extracted PHF proteins may be derived from neuronal processes of the neuropil, loosely associated PHFs in NFTs, or dissociated PHF. Unlike PHFs in NFTs, these PHFs do not display a distinct "fuzzy coat," appear to be sensitive to SDS and guanidine, and can be susceptible to proteolysis (unpublished observation). While NFTs may contain additional modifications or associated proteins responsible for crosslinking PHFs, the PHFs isolated in this study are similar in structure and many antigenic properties to PHFs in NFTs.

PHF epitopes containing N-terminal regions of τ may be removed during the preparation of SDS-insoluble NFTs (6). Similarly, while Alz-50 does not recognize SDS-treated NFTs (21, 26), it recognizes PHFs in tissue sections (26) and PHFs isolated in this study. Therefore, our isolation procedure appears to preserve antigenic determinants that may be lost during the isolation of SDS-insoluble NFTs.

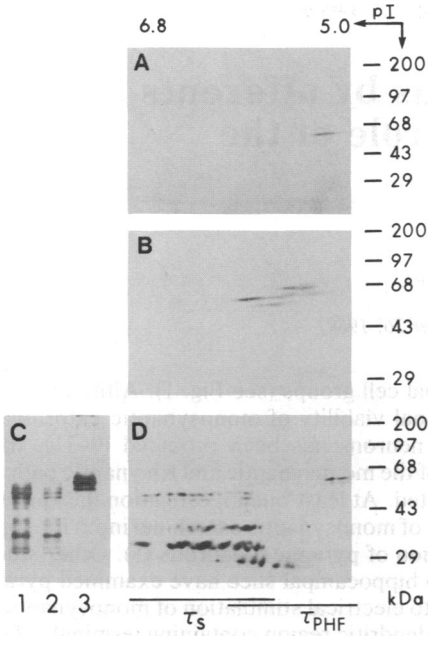

FIG. 4. (*A* and *B*) Silver stain and Alz-50 immunoblot, respectively, of two-dimensional gels containing PHFs show that the 57- to 68-kDa PHF proteins are relatively acidic. (*C*) One-dimensional Alz-50 immunoblots of normal adult τ_s (lane 1), AD τ_s (lane 2), and PHFs (lane 3) show that τ_{PHF} proteins tend to be slightly larger than τ_s. (*D*) Two-dimensional Alz-50 immunoblot of an AD 27,200 × *g* supernatant containing τ_s and τ_{PHF}. The relatively acidic τ_{PHF} proteins are selectively enriched in PHF-enriched fractions (*A* and *B*).

By selecting for relatively nonaggregated populations of PHFs from AD homogenates, we sought to overcome the problem that is commonly associated with the PAGE analysis of NFTs. Indeed, our PHF-enriched fractions contain two to five proteins of 57–68 kDa whose relative abundance appears to correspond with the yield of PHFs. As demonstrated by Alz-50 reactivity, the 57- to 68-kDa PHF proteins contain antigenic determinants shared with normal τ. Thus, the 57- to 68-kDa PHF proteins appear similar to τ_{PHF} proteins in NFT-enriched preparations (4, 18, 19).

We have extended the immunochemical analysis of PHF proteins and determined that in addition to τ (4) and possibly ubiquitin (8), the 57- to 68-kDa τ_{PHF} proteins contain unique epitopes, epitopes that are shared with high molecular mass NF proteins, and weakly reactive epitopes shared with MAP2. Moreover, like PHFs in NFTs (7), the reactivity of the 57- to 68-kDa PHF proteins with the mAb τ-1 is enhanced by treatment with alkaline phosphatase (33). Thus, the 57- to 68-kDa τ_{PHF} proteins appear to be responsible for the diverse antigenic properties of PHF.

Since τ_{PHF} displays antigenic determinants not shared with many normal human adult τ_s proteins, specific antigenic properties can be used to identify τ_{PHF} proteins. τ_{PHF} can also be distinguished from τ_s by one- and two-dimensional PAGE. Thus, τ_{PHF} proteins tend to be larger and more acidic than many τ_s proteins. Re-electrophoresis experiments have also demonstrated the capacity of the 57- to 68-kDa τ_{PHF} proteins for reaggregation (31). Therefore, determining factors that contribute to the unique properties of τ_{PHF} may establish what causes the formation of PHF.

In summary, we have further characterized some biochemical properties of the τ_{PHF} proteins. In large part, this was made possible by isolating relatively nonaggregated populations of PHFs from AD homogenates. Although the purity and yield could be improved, our general isolation procedure offers advantages over the isolation of NFTs. For example, nonaggregated PHFs appear more soluble than NFTs (17), proteins that copurify with NFTs due to entrapment are less likely to contaminate these preparations, and PHFs isolated with zwitterionic detergents appear to maintain antigenic sites that may be lost during the isolation of SDS-insoluble NFTs. Moreover, when our general isolation procedure is used, PAGE analysis of PHFs can be repeated by other investigators (33). Thus, isolation of PHFs from extracts of AD homogenates overcomes many difficulties associated with NFTs.

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