FEBS Letters 379 (1996) 222–226 FEBS 16593

Distribution of isoforms of the microtubule-associated protein tau in grey and white matter areas of human brain: a two-dimensional gelelectrophoretic analysis

Carsten Janke[a], Max Holzer[a], Joachim Klose[b], Thomas Arendt[a,*]

[a]*Paul Flechsig Institute of Brain Research, Department of Neurochemistry, University of Leipzig, Jahnallee 59, 04109 Leipzig, Germany*
[b]*Institute of Human Genetics, Freie Universität Berlin, Germany*

Received 6 November 1995; revised version received 21 December 1995

Abstract The microtubule-associated protein tau in human brain consists of six molecular isoforms derived from a single gene by alternative mRNA-splicing and further modified by posttranslational processing. In the present study, the distribution of tau isoforms in grey and white matter of human temporal cortex was investigated by two-dimensional gelelectrophoresis. More than 80 isoforms were detected. The pattern of isoforms obtained after treatment with alkaline phosphatase was still more complex than those of recombinant tau, indicating that posttranslational modifications other than phosphorylation contribute to the molecular heterogeneity of tau. The tau isoform D according to Goedert [1] containing four tubulin-binding regions shown to promote tubulin polymerisation most efficiently was present in higher amounts in white as compared to grey matter. The pattern of isoform distribution was not significantly altered in Alzheimer's disease. It is concluded that molecular isoforms that differ in their tubulin-binding characteristics are differentially distributed in subcellular neuronal compartments and/or neuronal types.

Key words: Microtubule-associated protein; Tau protein; Two-dimensional gel electrophoresis; Isoform; Alzheimer's disease; Quantitative analysis; Regional distribution

1. Introduction

Tau is a cytoskeletal protein that regulates stability and dynamics of microtubule by direct interaction with tubulin. In the adult human brain, six different tau isoforms are generated by alternative mRNA-splicing. These isoforms differ by the presence of either three or four copies of a highly conserved 18-amino acid repeat separated from one another by 13–14-amino acid inter-repeat domains in the carboxy terminus and the insertion of one or two N-terminal 29-amino acid motifs [1]. The N-terminal inserts largely consist of acidic amino acid residues, while the carboxyl terminus which possesses tubulin-binding activity has a strong basic bias [2]. Isoforms with three tubulin-binding motifs containing either none, one or two N-terminal inserts are designated according to the nomenclature of Goedert [1] as A, B and C, respectively. The corresponding isoforms with four tubulin-binding motifs are referred to as D, E and F [1,3].

The binding of tau to microtubules is physiologically modulated by its phosphorylation state [4,5]. Highly phosphorylated tau species, such as those present in paired helical filaments (PHFs), one of the major molecular hallmarks of Alzheimer's disease (AD), are largely prevented from the efficiently binding microtubules [6,7]. The pathological phosphorylation state of tau under this condition is, therefore, believed to lead to an impaired microtubular organisation and eventually to a pathologically disturbed axonal transport [8].

On the longest human tau isoform, 19 potential in vivo phosphorylation sites have up to now been identified. Most of them are serine or threonine residues, followed by proline [9]. This phosphorylation of tau is probably the most important modification of tau which contributes to its molecular heterogeneity. As phospho-isoforms of tau differ with respect to both electrophoretic mobility and pI, they can be separated on SDS-PAGE and by isoelectric focusing [4]. By 2D gel analysis as many as 60 different isoforms have been detected in adult mouse brain [10]. In the present study, the distribution of molecular isoforms and the contribution of phosphorylation to their molecular heterogeneity was studied in the soluble tau fraction of grey and white matter of temporal cortex. Brains from controls and patients with AD were analysed.

2. Materials and methods

2.1. Brain tissue

Post-mortem human brain tissues from the temporal cortex (Brodmann area 22) of five normal brains (controls) and six brains affected by AD [11,12] were investigated in the present study. Age (control: 72.6 ± 16.6; AD: 84.3 ± 5.8 years), premortem severity index (control: 2.3 ± 0.8; AD: 2.4 ± 0.9) [13] and postmortem delay (control: 54.5 ± 15.8 h; AD: 59.1 ± 29.3 h) did not significantly differ between controls and cases of AD (Student's *t*-test, $P > 0.1$). Severity of AD assessed according to the Reisberg scale [14] ranged from GDS 2 to 7.

2.2. Preparation of soluble tau-protein

White and grey matter of the temporal cortex was carefully dissected and homogenised in 3 volumes of ice-cold MES buffer [0.1 M 2(*N*-morpholino)ethanesulfonic acid, pH 6.5, 1 M NaCl, 0.5 mM $MgCl_2$, 2 μl/ml Triton X-100, 1 mM EGTA, 1 mM EDTA, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 μg/ml *N*-*p*-tosyl-L-lysine chloromethyl ketone, 1 μg/ml aprotinin]. The 100,000 × *g* supernatant (30 min, 4°C) was supplemented with 2-mercaptoethanol to a final concentration of 5 μl/ml and boiled for 5 min. After centrifugation (50,000 × *g*, 20 min, 4°C), the supernatant was adjusted to 25 μl/ml $HClO_4$ and incubated on ice for 15 min. After another centrifugation (50,000 × *g*, 20 min, 4°C), the supernatant was precipitated with 60 mg/ml trichloroacetic acid for 20 min on ice. The pellet (50,000 × *g*, 20 min, 4°C) containing enriched tau-protein was washed twice with ice-cold methanol. Protein content was determined in 96-well polystyrene microtiter plates according to Lowry et al. [15].

2.3. Treatment with alkaline phosphatase

Tau protein was dissolved to a final concentration of 2 mg protein/ml in phosphatase buffer (0.1 M Tris-HCl, pH 8.4, 0.15 M NaCl, 2 mM

*Corresponding author. Fax: (49) (341) 2114492.

SSDI 0014-5793(95)01522-1

$ZnCl_2$, $MgCl_2$, 4 mM phenylmethylsulfonyl fluoride, 10 μg/ml pepstatin, 10 μg/ml leupeptin, 10 μg/ml *N*-*p*-tosyl-L-lysine chloromethyl ketone, 10 μg/ml aprotinin). 18 U/ml bacterial orthophosphoric-monoester phosphohydrolase from *E. coli*, alkaline optimum Type III-S (alkaline phosphatase, EC 3.1.3.1.; Sigma) were added and samples were incubated for 1, 2, 4 or 8 h at 67°C. Control incubations were performed in the presence of 0.2 M sodium pyrophosphate. Incubation was stopped by adding 0.2 mg/ml ice-cold trichloroacetic acid (20 min), precipitated protein was pelleted (50,000 × *g*, 20 min, 4°C) and washed twice with ice-cold methanol.

2.4. Electrophoretic separation of tau isoforms

Two-dimensional gel electrophoresis was used for separation of single tau-isoforms. Separation in the first dimension was performed as non-equilibrium pH-gradient electrophoresis (NEPHGE) according to O'Farrell et al. [16]. 1 mm tube gels containing 37.84 mg/ml acrylamide, 2.16 mg/ml *N,N'*-methylene bisacrylamide (Bis) (4% T, 5.4% C), 9.2 M urea, 5 μl/ml Triton X-100 and 40 μl/ml Pharmalyte 3–10 (Pharmacia) were cast to a length of 60 mm. Samples were dissolved to a final concentration of 1 μg/μl protein in 9.5 M urea, 5 μl/ml Triton X-100, 20 μl/ml Pharmalyte 3–10 (Pharmacia), 10 mM dithiothreitol (DTT). The tubes were loaded at the anode with 30 μl sample, overlaid with 3 M urea, 3.3 μl/ml Pharmalyte (Pharmacia) and run in a Mini-Protean II tube cell (BioRad) with 2.5 mM H_3PO_4 for the anode compartment and 50 mM L-lysine solution for the cathode compartment. The electric current was regulated as follows: 15 min at 300 V, 15 min at 500 V and 90 min at 750 V at 10–12°C. Gels were ejected from the tubes and incubated for 10 min in 62.5 mM Tris-HCl buffer, pH 6.8, containing 100 μl/ml glycerol, 20 μg/ml sodium dodecyl sulfate (SDS) and 50 μl/ml 2-mercaptoethanol. After incubation the gels were either stored at −20°C or directly used for separating the proteins in the second dimension.

Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) was performed according to Laemmli [17], either on Minigels (Mini-Protean II; BioRad) or on 160 × 200 mm vertical gels (Protean IIxi; BioRad), containing 97.3 mg/ml acrylamide and 2.7 mg/ml Bis (10% T, 2.7% C), 375 mM Tris-HCl, pH 8.8 and 1 mg/ml SDS. One or two NEPHGE gels were loaded on Minigels or large slab gels. Molecular weight standards and tau probes for control incubation on Western blots were loaded into extra slots.

Optimal 2D-resolution of tau was obtained with 4% (w/v) pH 3–10 ampholyte gels. Ampholytes with a more basic pH range prevented the most acidic isoforms from migrating into the gel, whereas a more acidic ampholyte composition caused an exclusion of the basic isoforms. Moreover, resolution in NEPHGE was temperature-dependent. Optimal resolution was obtained at 10–12 °C. Higher temperatures resulted in stretching the pH-gradient while lower temperatures compressed the banding pattern. For designation of isoforms derived by alternative mRNA splicing the nomenclature given by Goedert [1] was used.

Total soluble proteins were extracted from brain tissue, separated in large 2D-gels (first dimension 400 mm, second dimension 300 mm) and detected by silver staining using the methods described by Klose and Kobalz [18].

2.5. Western blotting

Proteins, separated on one- or two-dimensional gels were transferred onto Polyscreen PVDF transfer membranes (DuPont) using a water-cooled tank blot transfer cell (Trans-blot cell; BioRad; 50 V 3 h). The blot buffer contained 48 mM Tris, 39 mM glycine, 0.13 mM SDS and 200 μl/ml methanol [19]. Marker strips were stained with 1 mg/ml Coomassie brilliant blue in 100 μl/ml acetic acid, 500 μl/ml methanol) and destained twice with 100 μl/ml acetic acid, 500 μl/ml methanol. Immunoblots were blocked with 10 mg/ml gelatine for 1 h. After two washing steps in phosphate-buffered saline (PBS: 10 mM Na_2HPO_4/NaH_2PO_4, pH 7.4, 150 mM NaCl) containing 1 μl/ml Tween 20, blots were incubated for 1 h in the presence of one of the following primary antibodies, diluted in PBS, containing 0.5 μl/ml Tween 20: monoclonal antibodies (mab) anti-tau 1 (Boehringer Mannheim; 1:10,000), mab HT 7 (Innogenetics; 1:4,000), mab AT 8 (Innogenetics; 1:4000) and the polyclonal antibody BR 134 (courtesy of M. Goedert; 1:2000). Blots were washed twice (5 min) and incubated with the biotinylated sheep anti-mouse antibody (Amersham; 1:2000; 50 min) or biotinylated donkey anti-rabbit antibody (Amersham; 1:1000; 1 h) for primary monoclonal and polyclonal antibodies, respectively. Following two washing steps (5 min), immunoreactivity was visualised by an avidin-peroxidase complex (ExtrAvidin; Sigma; 1:1000) and 3,3'diaminobenzidine/$NiCl_2$.

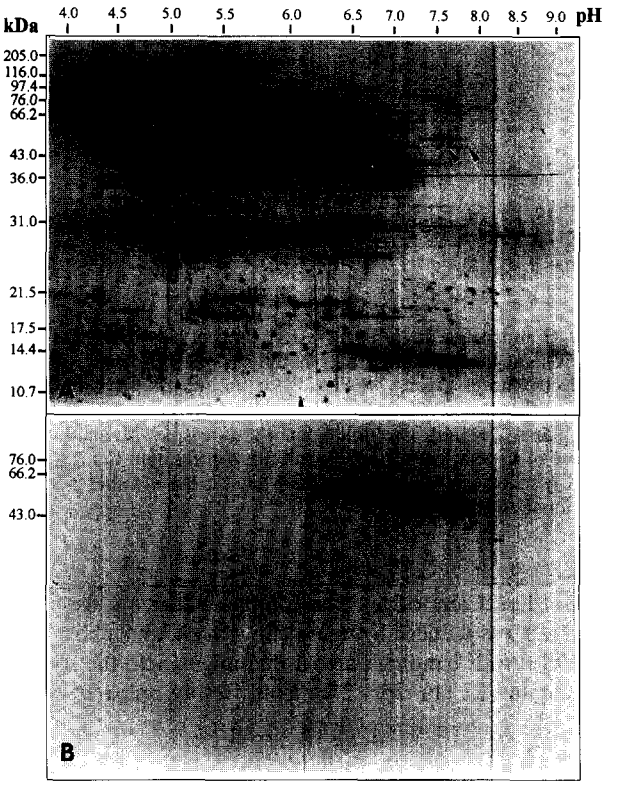


Fig. 1. High-speed supernatant of human brain tissue resolved in a large gel (300 × 400 mm) by 2D electrophoresis. (A) Silver staining; (B) Western blot probed with mab HT 7. Corresponding dots are marked by arrowheads.

For quantification of tau protein, blots were incubated with mab HT 7 (Innogenetics; 1:2000; 1 h), washed three times (10 min) and probed with ^{125}I-labelled sheep anti-mouse antibody (Amersham; 1:400; 1 h). Following three washing steps (10 min), immunoreactivity was visualised by a peroxidase-labelled rabbit anti-sheep antibody (1:1000; Dako) and 3,3'diaminobenzidine/$NiCl_2$.

2.6. Quantification of tau on immunoblots

Blotting membranes were probed with mab HT 7 and [^{125}I]anti-mouse antibody/peroxidase-labelled anti-sheep antibody. Spot groups, derived from one isoform, were cut out and quantified with a gamma-counter (multi crystal gamma counter LB 2104; Bertold). The activity (cpm) of each of the six isoforms was expressed as percentage of the total of all six isoforms.

Quantification was performed within the range of linearity between radioactivity (cpm) and the amount of tau protein blotted onto the membranes.

3. Results

3.1. Identification of tau isoforms

The protein tau identified in 2D-electrophoresis patterns of the soluble proteins of human brain tissue displayed a complex pattern consisting of more than 80 single spots at a pI range between approximately 6.1 and 8.0 (Fig. 1). In order to distinguish between molecular isoforms derived by alternative mRNA splicing and post-translational modifications, the tau pattern obtained from brain tissue was compared with that of human recombinant tau, expressed in *E. coli* (courtesy of

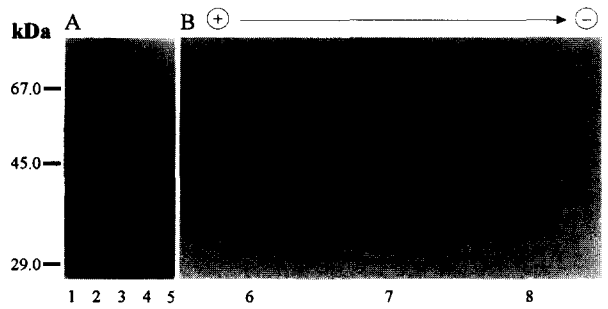
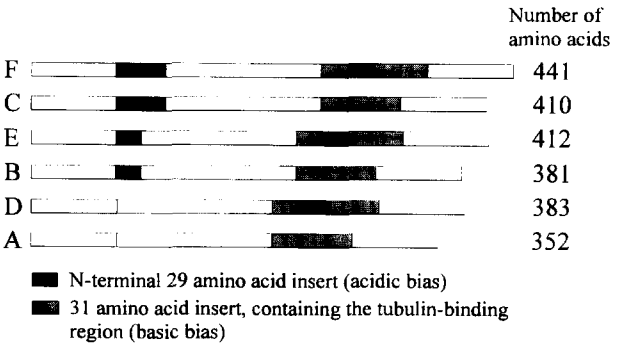


Fig. 2. Comparative analysis of Western blots of human soluble tau and recombinant tau, separated by one-dimensional SDS-PAGE (A) and 2D electrophoresis using NEPHGE for the separation in the first dimension (B). Lanes 1, 5 and 6: soluble human tau prepared from brain tissue, lanes 2, 4 and 7: soluble human tau treated with alkaline phosphatase (4 h) and lanes 3 and 8: recombinant human tau. Lanes 1 to 3 and 6 to 8 were probed with mab HT 7, lanes 4 and 5 with the polyclonal antibody BR 134.

M. Goedert, Cambridge). The complex pattern of freshly prepared tau was reduced by treatment with alkaline phosphate to an SDS-PAGE banding pattern that aligns with the six bands of recombinant tau (Fig. 2A). Both freshly prepared tau and tau treated with alkaline phosphatase showed additional bands at a molecular weight range below the smallest recombinant tau isoform. These low molecular weight bands react with the mabs anti-tau 1 and HT 7 but fail to stain with the antibody BR 134 which specifically recognises the C-terminus of tau (Fig. 2A). Thus, they are likely to represent proteolytic degradation products. The 2D pattern of tau protein obtained after phosphatase incubations in the presence of 0.2 M pyrophosphate was similar to that of the untreated material.

By 2D electrophoresis, the mixture of the six human recombinant tau isoforms (courtesy of M. Goedert, Cambridge) was resolved into a pattern of six spots (Fig. 2B, sample 8), each representing a tau-isoform derived from a different mRNA. Migration properties of the recombinant proteins in 2D electrophoresis were related to charge and molecular weight of the inserts which correspond to the alternatively spliced exons in the sequence of tau [2]. Isoforms containing four tubulin-binding regions migrated closer towards the alkaline site compared to their molecular counterparts with only three tubulin-binding regions (compare D/A, E/B, F/C). While this finding matches the basic bias of the additional tubulin-binding domain, the acidic bias of the alternatively spliced N-terminal inserts led to an increase in acidity of the isoforms containing these region(s). In the pH-gradient of the 2D pattern, isoforms, containing the



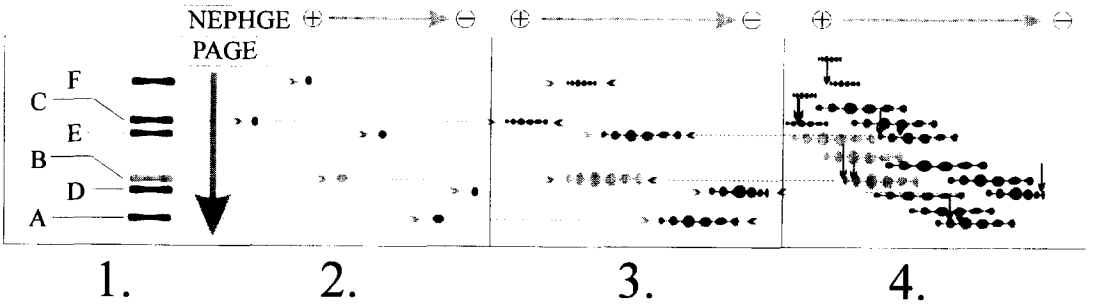


Fig. 3. Synopsis of the pattern of human molecular tau-isoforms identified after two-dimensional separation. Spots, belonging to the same molecular isoform are drawn with the same shade of grey. The diagram above shows the exon pattern of the different splicing products of human tau which lead to the molecular heterogeneity of the protein (after Goedert, 1993 [1]).

(1) *The six recombinant isoforms of human tau, separated by SDS-PAGE.* The isoforms, symbolised in this drawing, are in a non-phosphorylated form (classification of isoforms according to Goedert, 1993 [1]).

(2) *The six recombinant isoforms, resolved by two-dimensional gel electrophoresis.* Each spot was assigned to a single isoform by alignment with the SDS-PAGE bands (compare 1).

(3) *2D pattern of a dephosphorylated preparation of human tau.* This pattern is similar to the pattern of the recombinant isoforms (compare 2).

(4) *2D pattern of human tau.* Compared to the pattern of dephosphorylated human tau, this pattern contains spot groups that differ in apparent molecular weight from the proteins that were present in the 2D pattern of recombinant and dephosphorylated tau (compare 2, 3). Those additional bands were identified as phosphoisoforms of tau, because they disappeared after dephosphorylation. The way in which the pattern of human tau is hypothesised to be changed by dephosphorylation (compare 3) is indicated by black arrows.

same number of tubulin-binding regions differ in their acidity due to their number of N-terminal inserts (compare A/B/C and D/E/F).

By comparison, freshly prepared tau showed additional spots which differed from the pattern of recombinant tau with respect to both pI and mobility on SDS-PAGE (Fig. 2B, sample 6). After phosphatase treatment, the pattern of tau prepared from human postmortem brain was still more complex than that of recombinants (Fig. 2B, sample 7). However, only those spots remained which comigrated with the SDS-bands of recombinants but still varied in their pI. Each phosphatase-resistant band had two corresponding phosphatase-sensitive copies, which were shifted towards the acidic end of the NEPHGE gel and decreased in SDS mobility. SDS-PAGE mobility shift and acidic shift of these additional bands were interrelated. The principal findings of the 2D analysis of human soluble tau relating it to the pattern of recombinant tau are summarised in Fig. 3.

3.2. *Quantification of tau isoforms*

Preparations of soluble tau, obtained from either white or grey matter of controls and of cases with AD were resolved in 2D gels and quantified after radioactive labelling. The two isoforms characterised by two N-terminal inserts (isoforms C and F according to Goedert [1]) could hardly be discriminated by 2D separation and were thus analysed together (Fig. 4).

In the normal human brain, significant differences between the distribution of isoforms in grey and white matter were observed (Figs. 4, 5). In grey matter, the isoform B (i.e. the isoform containing three tubulin-binding regions and one N-terminal insert) was most prominent while in white matter, isoform D (i.e. the isoform containing four tubulin-binding regions and no N-terminal insert) and B, present in about equal amounts, were the most abundant isoforms, but without reaching the level of B in the grey matter. The content of isoform D in white matter was about twice as high as in grey matter. Isoforms C and F were least abundant in both grey and white matter. No significant changes of the overall pattern of isoforms were observed in AD (Fig. 5).

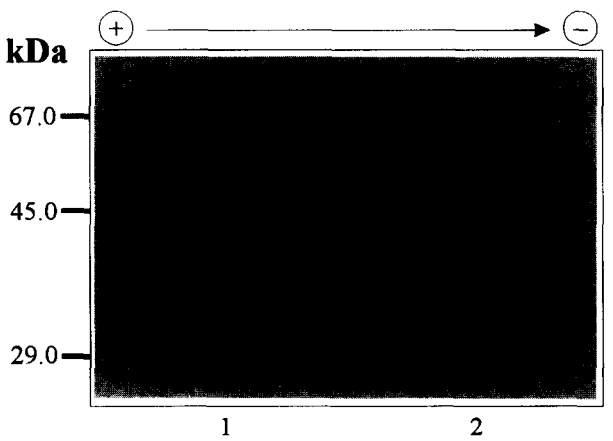


Fig. 4. Two-dimensional Western blot of soluble tau protein prepared from grey (1) and white matter (2) of normal human brain used for quantification of single isoforms. Equal amounts of purified tau were first run in NEPHGE, followed by separation in a 160 × 200 mm SDS-gel. The mab HT 7 was used as primary antibody. Note the differences in the *relative* amount of isoform D spots (arrowheads).

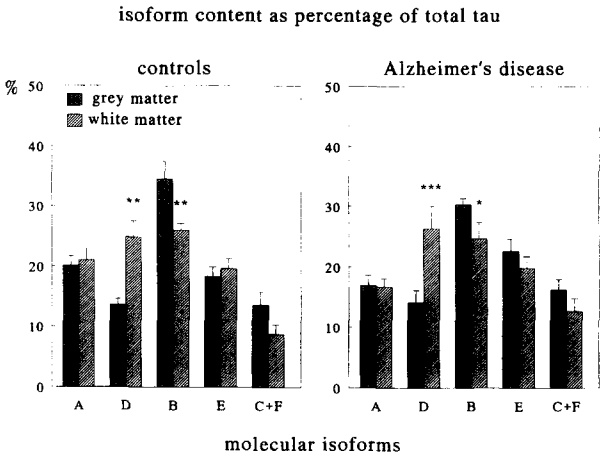


Fig. 5. Relative distribution of tau isoforms in grey and white matter of normal human brain and in AD. Data are mean (n = 5 controls and n = 6 AD cases) expressed as a percentage of total tau content (relative isoform content = cpm(isoform)/cpm(Σisoforms A–F) × 100%). Error bars indicate the data S.E.M. Isoforms were designated according to the nomenclature of Goedert [1] (see Fig. 3). Differences between grey and white matter were significant for: $*P = 0.016$, $**P = 0.008$ and $***P = 0.004$ (Mann–Whitney U-test [37]).

4. Discussion

In the present study, the distribution of molecular isoforms of the microtubule-associated protein tau was investigated in the human temporal cortex by 2D-gel electrophoresis. A complex pattern of more than 80 distinct isoforms was resolved. The molecular heterogeneity of tau is due to both mRNA-processing as well as posttranslational processing [4].

For each single isoform derived by alternative mRNA-splicing a single spot or a closely related group of spots with similar SDS-PAGE migration properties were resolved from a mixture of the recombinant human isoforms and dephosphorylated human tau, respectively. A larger heterogeneity was obtained from soluble tau. Two additional bands of closely related single spots slightly decreased in SDS-PAGE mobility and shifted towards the acidic end of the NEPHGE gel were observed corresponding to each phosphatase-stable band. Since these additional bands were sensitive to phosphatase treatment, they most likely represent phospho-isoforms of tau, even if the soluble tau of human brain was demonstrated to be already dephosphorylated postmortem [20]. Similar shifts in mobility on SDS-PAGE are well-documented for in vitro phosphorylated recombinant tau [21] as well as for the abnormally high phosphorylated PHF-tau in AD [22–27]. The negative charge of the incorporated phosphate residues might decrease the pI of the isoforms, thereby giving rise to the acidic shift observed for the phosphatase-sensitive bands in the present study.

The pattern of dephosphorylated tau resolved on 2D-gels was similar, but not identical to the pattern of recombinant tau. A pI-microheterogeneity for each molecular tau-isoform still remained in the pattern of the dephosphorylated tau as compared to the recombinant protein. Posttranslational modifications other than phosphorylation, such as glycation [28], or deamination of several glutamine or asperagine residues are,

therefore, likely to contribute to the molecular heterogeneity of tau.

In the normal brain, tau is mainly localised in the axon and is thus more abundant in white matter than in grey matter areas [29]. In AD and under conditions of other neurodegenerative disorders, however, phosphorylated tau-species are also found in the somatodendritic compartment [30]. Even in the normal brain, tau phospho-isoforms are differentially distributed between the axonal and somatodendritic compartment [31]. In order to investigate whether similar compartimental differences might exist between tau isoforms irrespectively of their phosphorylation state, special attention was paid in the present study on a differential distribution of tau isoforms in the white matter and grey matter. Major differences were noted with respect to the isoforms B and D (designation according to Goedert [1]) containing three and four tubulin-binding motifs, respectively. The tubulin-binding of tau and the process of dynamic stabilisation of microtubuli largely depend on the molecular structure of the tubulin-binding domain and their flanking regions [32]. Tau isoforms with four tubulin-binding motifs assemble tubulin in vitro several times faster than three-repeat isoforms [33,34]. It was shown, furthermore, that isoform D, which was demonstrated to be highly abundant in white matter, promotes microtubule-assembly most efficiently [35].

While the tau-pool of white matter largely derives from long axons of projection neurones, such as pyramidal neurones, other compartments, such as interneurones, and the somatodentritic domain of projection neurones contribute to the tau-pool of grey matter. Based on the present results, it might, therefore, be hypothesised that axons of projection neurones contain about equal amounts of isoforms A, D, B and E, but less of isoforms C and F. On the contrary, isoform B, containing three tubulin-binding regions, might be the most abundant tau isoform of interneurones. This conclusion is supported by the previous finding of a differential expression of tau-isoforms in long-axon and short-axon neurones. While mRNA for tau isoforms containing either three or four tubulin-binding regions was detected in pyramidal cells of the cerebral cortex and the hippocampal formation, hippocampal granule cells contained exclusively mRNA encoding three-repeat isoforms [36]. It remains to be investigated whether this differential distribution of tau-isoforms might causally be related to the high vulnerability of certain long axon projection neurones for 'paired-helical-filament' tau in AD.

Acknowledgements: We gratefully thank M. Goedert, Cambridge, for his gift of a mixture of the recombinant human tau-proteins and antibody BR 134 and C. Moeller, Leipzig for help in mathematical problems. This study was supported by the Bundesministerium für Forschung und Technik (01 ZZ 91032.7).

References

[1] Goedert, M. (1993) TINS 16, 460–465.
[2] Crowther, R.A. (1993) Curr. Opin. Struct. Biol. 3, 202–206.
[3] Goedert, M., Crowther, R.A. and Garner, C.C. (1991) TINS 14, 193–199.
[4] Butler, M. and Shelanski, M.L. (1986) J. Neurochem. 47, 1517–1522.
[5] Lindwall, G. and Cole, R.D. (1984) J. Biol. Chem. 259, 12241–12245.
[6] Baudier, J. and Cole, D. (1987) J. Biol. Chem. 262, 17577–17583
[7] Gustke, N., Steiner, B., Mandelkow, E.M., Biernat, J., Meyer, H.E., Goedert, M. and Mandelkow, E. (1992) FEBS Lett. 307, 199–205.
[8] Bramblett, G.T., Goedert, M., Jakes, M., Merrick, S.E., Trojanowski, J.Q. and Lee, V.M.-Y. (1993) Neuron 10, 1089–1099.
[9] Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Titani, K. and Ihara, Y. (1995) J. Biol. Chem. 193, 823–829.
[10] Larcher, J. C., Boucher, D., Ginzburg, I., Gros, F. and Denoulet, P. (1992) Dev. Biol. 154, 195–204.
[11] Khatchaturian, Z.S. (1985) Arch. Neurol. 42, 1097–1105.
[12] McKhann, G., Drachmann, D., Folstein, M., Katzmann, R., Price, D. and Stadlan, E.M. (1984) Neurology 34, 939–944.
[13] Monfort, J.C., Javoy-Agid, F., Hauw, J.J., Dubios, B. and Agid, Y. (1985) Brain 108, 301–313.
[14] Reisberg, B., Ferris, S.H., deLeon, M.J. and Crook, T. (1982) Am. J. Psychiatry 139, 1136–1139.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. (1977) Cell 12, 1133–1142.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Klose, J. and Kobalz, U. (1995) Electrophoresis 16, 1034–1059.
[19] Towbin, H., Stehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4354–4356.
[20] Matsuo, E.S., Shin, R.W., Billingsley, M.L., Van deVoorde, A., O'Connor, M., Trojanowski, J.Q. and Lee, V.M.-Y. (1994) Neuron 13, 989–1002.
[21] Litersky, J.M., Scott, C.W. and Johnson, G.V.W. (1993) Brain Res. 604, 32–40.
[22] Flament, S., Delacourte, A., Hémon, B. and Défossez, A. (1989) J. Neurol. Sci. 92, 133–141.
[23] Goedert, M., Spillantini, M.G., Cairns, N.J. and Crowther, R.A. (1992) Neuron 8, 159–168.
[24] Greenberg, S.G. and Davies, P. (1990) Proc. Natl. Acad. Sci. USA 87, 5827–5831.
[25] Ksiezak-Reding, H. and Yen, S.-H. (1991) Neuron 6, 717–728.
[26] Lee, V.M.-Y., Balin, B.J., Otvos, L. and Trojanowski, J.Q. (1991) Science 251, 675–678.
[27] Brion, J.P., Hanger, D.P., Couck, A.M. and Anderton, B.H. (1991) Biochem. J. 279, 831–836.
[28] Ledesma, M.D., Bonay, P., Colaço, C. and Avila, J. (1994) J. Biol. Chem. 269, 21614–21619.
[29] Binder, L.I., Frankfurter, A. and Rebhun, L.I. (1985) J. Cell Biol. 101, 1371–1378.
[30] Kowall, N.W. and Kosik, K.S. (1987) Ann. Neurol. 22, 639–643.
[31] de Ancos, J.G. and Avila, J. (1993) Biochem. J. 296, 351–354.
[32] Gustke, N., Trinczek, B, Biernat, J., Mandelkow, E.M. and Mandelkow, E. (1994) Biochemistry 33, 9511–9522.
[33] Goedert, M. and Jakes, R. (1990) EMBO J. 9, 4225–4230.
[34] Goode, B.L. and Feinstein, S.C. (1994) J. Cell Biol. 124, 769–782.
[35] Scott, C.W., Klika, A.B., Lo, M.M.S., Norris, T.E. and Caputo, C.B. (1992) J. Neurosci. Res. 33, 19–29.
[36] Goedert, M., Spillantini, M.G., Potier, M.C., Ulrich, J. and Crowther, R.A. (1989) EMBO J. 8, 393–399.
[37] Mann, H.B. and Whitney, D.R. (1947) Ann. Math. Stat. 18, 50.