The Microtubule-associated Protein Tau Forms a Triple-stranded Left-hand Helical Polymer*

(Received for publication, June 18, 1991)

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High resolution transmission electron microscopy (TEM) has shown that bovine tau are 2.1 ± 0.2-nm diameter filaments which are triple-stranded left-hand helical structures composed of three 1.0 ± 0.2-nm strands. The reported amino acid sequence of human and bovine tau have been computer processed to predict secondary structure. Within the constraints imposed by the images, the secondary structure models and other structural information have been used to calculate tau's maximum and minimum length. The length calculations and secondary structure form the basis for image interpretation. This work indicates that each ~1.0-nm strand is a tau polypeptide chain and that the ~2.1-nm filament is composed of three separate tau chains (τ). Bovine tau length measurements indicate that tau trimer filaments are generally longer than a fully extended tau monomer. These measurements indicate that each trimer, τ3, is joined with other trimers to form long tau polymers, (τ3)n. An inverse temperature transition has been found in the circular dichroism spectrum of tau indicating that its structure is less ordered below 20 °C and more ordered at 37 °C. The implications of this phenomenon with respect to tau's temperature-dependent ability to reconstitute microtubules is discussed and a mechanism for the possible abnormal aggregation of tau into neurofibrillary tangles in Alzheimer's disease is proposed.

In neurons, the microtubule-associated proteins, MAP-2 and tau, are found in the dendrites and the axons, respectively (Binder et al., 1985; Peng et al., 1986; Kosik and Finch, 1987). Stability to heat and solubility in perchloric acid form the basis for tau's isolation (Grundke-Iqbal et al., 1986a; Lindwall and Cole, 1984b). The cDNA-predicted amino acid sequence of mouse tau (Lee et al., 1988), bovine tau (Himmler et al., 1989; Himmler, 1989), and human tau (Goedert et al., 1989) have been reported. All of these reports indicate that tau is a family of proteins derived from a single gene and that the heterogeneity in the amino acid chain length is due to alternative RNA splicing (Himmler, 1989). Bovine tau has a sequence of 448 amino acids (46,332 daltons) with variable deletions that can reduce its length by as much as 146 amino acids (Himmler et al., 1989; Himmler, 1989). Human tau has a sequence of 441 amino acids (45,850 daltons) with deletions of 29, 31, 58, and as many as 89 amino acids (Goedert et al., 1989). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of tau produces four bands ranging from 55,000 to 62,000 daltons (Cleveland and Cole, 1984b), with the phosphorylated bands shifted to what appear to be higher molecular weights. Fully denatured tau has a higher apparent molecular weight than a fully denatured equivalent standard globular protein marker by SDS-PAGE. Tau is much less hydophobic than globular proteins (see "Results and Discussion"). binds less negatively charged SDS, and runs more slowly in an electric field applied to a polyacrylamide gel.

The structure of tau was first studied by ultracentrifugation (Cleveland et al., 1977b). This work suggested that it was a rod-shaped molecule with an axial ratio of 20:1. More recently (Hagestedt et al., 1989), paracrystals of phosphorylated and nonphosphorylated tau have been reported. Phosphorylated tau was 90-95 nm in length and 3-6 nm in diameter whereas nonphosphorylated tau was 69-75 nm in length. An even shorter length of 30 nm was reported for undamaged tau indicating that it is an extremely flexible molecule. Tau was also studied in relation to microtubules, and its length was found to be 56.1 ± 14.1 nm (Hirokawa et al., 1988). No reference was made in this work to tau's phosphorylation state.

The study of freeze-dried vertically platinum-carbon (Pt-C) replicated isolated bovine tau was undertaken to characterize tau's structure with high resolution transmission electron microscopy (TEM) (Ruben, 1989). Since tau has been found in both Alzheimer neurofibrillary tangles and in paired helical filaments (Grundke-Iqbal et al., 1986a, 1986b; Ihara et al., 1986; Nukina and Ihara, 1986; Yen et al. 1987; Goedert et al., 1988, 1989; Wischik et al., 1988; Lee et al., 1991), the study of tau's normal structure had to preceed TEM studies of neurofibrillary tangles and paired helical filaments so that their structural relationship to tau could be properly accessed in subsequent work.

In the present study isolated bovine tau preparations are shown to contain 2.1 ± 0.2-nm filaments. It is shown that tau is triple-stranded and left-hand helical. Using the amino acid
sequence of bovine tau and/or human tau, computer programs have been used to predict protein secondary structure under "Results and Discussion." Limits imposed by the tau images and circular dichroism estimates of α helix and β structure were used to temper these secondary structure predictions. The secondary structure, even with its limited accuracy, made it possible to identify the three separate strands as different tau sequences.

MATERIALS AND METHODS

Bovine Tau Preparation for TEM—The bovine tau was isolated by heat treatment of three cycle microtubules at pH 2.7, followed by extraction in 2.5% perchloric acid according to Grundke-Iqbal et al. (1986a). These preparations generally revealed in SDS-PAGE three to four bands in the 48,000-62,000-dalton range shown in Fig. 1 (lane 1) and no bands corresponding to microtubule-associated protein 2 (MAP-2). The line at the top of lane 1 is the protein entrance to the gel. Western blots of bovine tau (Fig. 1, lane 2) were developed with monoclonal antibody Tau-1 (Binder et al., 1985) using avidin biotin reagents of Vector (Burlingame, CA) according to Grundke-Iqbal et al. (1986a). Three samples were measured and averaged to yield 78 ± 9 (n = 3) nmol of phosphate/mg of protein (Iqbal and Grundke-Iqbal, 1990).

The tau used for freeze-drying and vertical Pt-C replication in a mixed state of phosphorylation was at a concentration of 100 μg of protein/ml in 0.15 M NaCl, pH 7, and was deposited on the surface of a 13-nm disc with a 0.1-μm porosity (mixture of cellulose acetate and cellulose nitrate) from Millipore Corporation (type: VC, catalog no. WP01300). This sample was washed with distilled water at 18–20 °C, blotted with ashless filter paper to remove excess water, and frozen in liquid propane. It was then freeze-dried in the modified acetate and cellulose nitrate) from Millipore Corporation (type: VC, (1969). This helical coil (β spiral) has a -1.66-nm diameter (could be 0.5 nm less, see Table III footnotes) with a pitch of 0.945 nm (Chang and Urry, 1989). The circular dichroism spectra were recorded as a function of temperature at 5° intervals from 10 to 80 °C. The sample was equilibrated at each temperature for 15–20 min before two separate CD spectra were recorded and averaged. The circular dichroism equipment and methods have been described before (Clardelli et al., 1988).

Circular Dichroism—The bovine tau prepared for circular dichroism was 160 μg of protein/ml in 25 mM sodium phosphate, pH 6.5, and was also in a mixed state of phosphorylation averaging 78 ± 9 (n = 3) nmol of phosphate/mg of protein or 3.1 ± 0.4 (n = 3) phosphates/384 amino acid tau (average number of amino acids in tau due to alternative RNA splicing, see table IV) as measured by Iqbal and Grundke-Iqbal (1990). The circular dichroism spectra were recorded as a function of temperature at 5° intervals from 10 to 80 °C. The samples were equilibrated at each temperature for 15–20 min before two separate CD spectra were recorded and averaged.

RESULTS AND DISCUSSION

TEM of Isolated Bovine Tau Protein

EXAMINATION OF THE REPLICAS OF TAU PREPARED ON 0.1-μM FILTER DISCS

Examination of the replicas of tau spread on 0.1-μM filter discs reveals the presence of long narrow filaments that are 2.1 ± 0.2 nm in diameter (2.7 nm with 0.6 nm of Pt-C coating; Ruben, 1989) (Fig. 2a). The length of the filaments are 120, 300, and 800 nm as far as they can be measured on the surface of the 0.1-μM Millipore filter. The filter surface without any tau present does not contain any of these 2.1-nm filaments. In Fig. 2b, sections of tau filaments extending across the holes in the filter were examined at high magnification. Although the filament in panel A was measured as 2.2 nm (2.8

FIG. 1. SDS-PAGE of bovine tau. Sodium dodecyl sulfate-polyacrylamide gel patterns of isolated bovine tau protein: lane 1 (0.75 μg of protein) stained with silver; lane 2 (0.055 μg of protein). Western blot developed with monoclonal antibody Tau-1 (0.1 μg of IgG/ml). The standards used for molecular mass gel markers were as follows: phosphorylase b (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α-chymotrypsinogen (25.7 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.3 kDa).
crossing the axis in a left-hand helical direction (see arrows). These strands average 1.0 ± 0.2 nm in diameter (1.6 nm with 0.6 nm of Pt-C coating). In panel B three 1.0 ± 0.2-nm strands are left-hand wrapped around a 2.1 ± 0.2-nm filament axis. In panel C, a 2.1 ± 0.4-nm filament shows a left-hand helical substructure. Finally, in panel D, three strands, 1.0 ± 0.3 nm, cross the filament axis in a left-handed direction. Fig. 2b indicates that tau protein is a triple-stranded left-hand helical filament with a 2.1 ± 0.2-nm diameter which is composed of three 1.0 ± 0.2-nm strands.

The replicas of tau spread on mica discs revealed a wide range of lengths of tau filaments (Fig. 3) The longest filament lengths were 2030 nm (n = 2) with other lengths of 1740 (n = 2), 1600, 1380, 1330, 1240, 914, 682 nm (n = 2), and smaller.

**The Structure of Tau by Circular Dichroism**

The CD spectrum in Fig. 4a shows a large trough in the spectrum at 197 nm which is identified as a coil polypeptide conformation (Greenfield and Fasman, 1969; Johnson, 1987). Because bovine tau is mainly coil conformation (60–66%) the empirical formulas for calculating α helix and β-sheet are not considered accurate in quantitating these secondary structures. These formulas are only reliable when these structures are in abundance (Taylor and Kaiser, 1987). Nonetheless, these formulas were used on the 10, 40, or 80 °C CD spectra with nearly identical estimates of α helix (10–12%) and β structure (24–28%) which were also similar to the room temperature values reported by Cleveland et al. (1977b). The CD spectrum of bovine tau in Fig. 4a was taken as a function of temperature. Fig. 4b shows that the mean residue ellipticity (θ) at 197-nm increases by 52% from 10 to 80 °C indicating that with increasing temperature random coil is disappearing and is being replaced with a more ordered secondary structure.

**Interpretation of Tau Images Using the Secondary Structure of Bovine and Human cDNA-derived Amino Acid Sequences**

The long filamentous properties of tau suggest that it should have an unusual distribution of hydrophobic and helicophilic amino acids in comparison to globular proteins. In Fig. 5a, the hydrophathic index is plotted against the amino acid sequence in human tau (Goedert et al., 1989). A similar plot for bovine tau (not shown) looked almost identical to human tau. The tubulin-binding domains of tau, the four 31-amino acid repeats from about amino acid 244 to 368 are less hydrophilic than the sequence from 1 to 244 and not as hydrophobic as

**Fig. 3. The length of bovine tau on mica.** Tau in 5 mM Tris, pH 7.0, was freeze-dried and replicated as described under "Materials and Methods." The irregular thickness of the tau filaments does not contain any of the tau fine structure of Fig. 2b, and the filaments appear to be coated with condensed buffer. Panel a, tau length of 2,050 nm; panel b, tau length of 1,235 nm; panel c, tau length of 2,088 nm; panel d, tau length of 1,378 nm; and panel e, tau length of 1,740 nm. × 29,000.
Fig. 4. Panel a, CD spectrum of bovine tau. Circular dichroism spectrum of 160 μg/ml of bovine tau in 25 mM sodium phosphate, pH 6.5, as a function of temperature from 10 to 70 °C at 10 °C intervals. The curve for 80 °C was very close to that at 70 °C, and for figure clarity it was not included. The mean residue ellipticity (θ) was based on the bovine tau sequence and an average amino acid molecular weight of 103.4. This bovine tau has a mixed phosphorylation state averaging 3.1 ± 0.4 (n = 3) phosphates/384 amino acid tau (average tau sequence with deletions, Table IV) (Iqbal and Grundke-Iqbal, 1990). Panel b, inverse temperature transition in bovine tau. The ellipticity at 197 nm as a function of temperature from 10 to 80 °C at 5 °C intervals for the bovine tau in panel a. The progressive increase at 197 nm with increasing temperature indicates that random coil is being replaced with a more ordered structure. This figure indicates that bovine tau undergoes an inverse temperature transition. Most proteins heated from 10 to 90 °C show an increasing negative ellipticity at 197 nm or increasing random coil secondary structure.

Secondary structure predicting programs are useful for showing trends in protein structure, even though they are no better than 63% (Garnier et al., 1979; Kabsch and Sander, 1983) to 70% accurate (Chou and Fasman, 1979). Estimates from our circular dichroism measurements and Cleveland et al. (1977b) suggest that tau is 10–12% α helical and 20–27% β conformation whereas the Garnier program (Garnier et al., 1978) suggests that tau contains 31% α helix and 37–39% β conformation (Tables I and II). It is unlikely that triple stranded tau (~2.1-nm diameter) contains β-sheet since each sheet strand could contain only two amino acid chains roughly 1.0–1.3 nm × 0.3–0.6 nm in cross-section to approximate the 1.0 ± 0.2-nm strands. Second, it is difficult to understand how these strands could remain as separate strands with unfulfilled hydrogen bonds on both sides of a strand. Third, β-sheet is a fully extended conformation (0.3–0.35-nm spacing between amino acids) which would not produce an elastic tau as described by Hagestedt et al. (1989). Finally it has been shown that β-sheet can have a right-handed helical twist with a pitch as short as 9.2–9.6 nm (Fraser and Macrae, 1974; Fraser et al., 1971; Stewart, 1977), but it is hard to understand how these two amino acid strands could cross the 2.1 ± 0.2-nm filament axis at 5.4-nm intervals and maintain their β-sheet structure and their separate strand identity. The β structure in tau is more likely to take the form of β-turns also called β-bends or reverse turns.

**Tau Lengths Calculated Using Its Secondary Structure and an Idealized Periodic Conformation**

The analysis of the amino acid sequence is consistent with tau's filamentous conformation. The images in Figs. 2 and 3 reinforce this prediction, and in conjunction with the previous analyses, the evidence suggests that the three strands are separate tau monomers in an extended filamentous conformation. Assuming that each 2.1 ± 0.2-nm filament is composed of three adjacent tau monomers (τ₃), the longest and the shortest tau monomer strand lengths can be calculated (see "Materials and Methods"). The longest tau axial lengths in the triple helix filaments are 106–117 nm (112 nm for 0.325-nm amino acid spacing) or 104–116 nm (110 nm for 0.325-nm amino acid spacing) for bovine tau (448 amino acids, 42 β-turns) and human tau (441 amino acids, 42 β-turns), respectively. Assuming that the full-length tau and tau with deletions are equally represented then the longest average length of human tau is 96.2 nm and the longest average length of bovine tau is 95.8 nm. In Table III, the longest measured porcine tau monomer paracrystalline length was reported as 90–95 nm. Clearly the tau monomer length within a triple helix model can accommodate the longest measured tau monomer.

The shortest tau lengths were calculated by assuming that the amino acid sequence has the axial spacings of an α helix. This first calculation is shown in Table IV along with the length calculation for the β spiral conformation first described in elastin by Urry et al. (1969). This helical coil (β spiral) has a ~1.66-nm diameter (its diameter could be 0.5 nm less, see Table III footnotes) with a pitch of 0.545 nm. In the α helix the amino acid chain would follow a path ~1.16-nm long/0.54-nm pitch. The length calculations for these two models is shown in Table IV. Neither of these models by themselves yields lengths close to 30 nm. Since tau contains ~20% α helix and ~38% β-turn structure, and ~42% β spiral residues (excludes β-turns) (Table II), a composite model (containing the aforementioned secondary structure) shortest length was calculated in Table IV. The composite model gives estimates of tau’s monomer length slightly shorter than 30 nm. The shortest paracrystalline length reported for porcine tau in Table III is 30 nm which can be accommodated by the tau monomer length in the triple-helical tau polymer model. A nonphosphorylated porcine tau length of 69–75 nm (Hagesstedt et al., 1989) or the other porcine tau length of 56.1 ± 14.1 nm (Hirokawa et al., 1988) have also been reported (Table...
Elastin's Similarities to Tau Suggest That Tau Shortens from 20 to 37 °C and Becomes Elastic—Elastin is filamentous,
The MAP Tau Forms a Three-stranded Left-hand Helical Polymer

Predicted $\beta$-turn and $\alpha$ helical regions of human tau

Four consecutive amino acids are underlined showing the location in the human tau sequence of the predicted $\beta$-turns from the Chou and Fasman (1979) computer program. The $\beta$-turn probability map is shown in Fig. 5b. The predicted sequences of a helix are in boldface letters in the sequence. Although these sequences (24 residues) frequently overlap $\beta$-turns (1-4 residues), we have chosen to ignore the incompatibility of these two structures and report the results of the Garnier et al. (1978) program. In compiling Table II, we have retained all the $\beta$-turn predictions at the expense of $\alpha$ helix except for the one at residue 3-6 which was omitted. If $\alpha$ helix sequences are 4 residues or fewer it has been assumed arbitrarily that they are not likely to retain this configuration. $\beta$ sheet secondary structure is assumed to be incompatible with the 1.0-nm strand diameter in the tau images (see “Discussion”). Secondary structure which is neither $\beta$-turn or $\alpha$ helix we assume to be coil or $\beta$ spiral. This same approach was used in judging secondary structure for all the proteins listed in Table II.

![Image of the page]
The MAP Tau Forms a Three-stranded Left-hand Helical Polymer

### TABLE III

Filament length measurements and structural properties

| Protein and source | Unphosphorylated* | Fully phosphorylated* or longest | Partially/ or undefined state of phosphorylation | Filament quaternary structure and diameter | Shortening of length, with inverse temperature transition, temperature increased from 20 to 40 °C |
|--------------------|-------------------|---------------------------------|-----------------------------------------------|------------------------------------------|----------------------------------------------------------------------------------|
| Tau                |                   |                                 |                                               |                                          |                                                                                  |
| Bovine             |                   |                                 |                                               |                                          |                                                                                  |
| Porcine            |                   |                                 |                                               |                                          |                                                                                  |
| Crayfish           |                   |                                 |                                               |                                          |                                                                                  |
| MAP-2              |                   |                                 |                                               |                                          |                                                                                  |
| Porcine            |                   |                                 |                                               |                                          |                                                                                  |
| Crayfish           |                   |                                 |                                               |                                          |                                                                                  |

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| Protein and source | Unphosphorylated* | Fully phosphorylated* or longest | Partially/ or undefined state of phosphorylation | Filament quaternary structure and diameter | Shortening of length, with inverse temperature transition, temperature increased from 20 to 40 °C |
|--------------------|-------------------|---------------------------------|-----------------------------------------------|------------------------------------------|----------------------------------------------------------------------------------|
| Tau                |                   |                                 |                                               |                                          |                                                                                  |
| Bovine             |                   |                                 |                                               |                                          |                                                                                  |
| Porcine            |                   |                                 |                                               |                                          |                                                                                  |
| Crayfish           |                   |                                 |                                               |                                          |                                                                                  |
| MAP-2              |                   |                                 |                                               |                                          |                                                                                  |
| Porcine            |                   |                                 |                                               |                                          |                                                                                  |
| Crayfish           |                   |                                 |                                               |                                          |                                                                                  |

**TABLE IV

Calculated tau lengths assuming tau model in Fig. 6a

| Protein and source | Extended length | a Helix (62% α helix and 38% β-turns) | β Spiral* (β spiral with 38% β-turns) | Composite model* |
|--------------------|-----------------|--------------------------------------|--------------------------------------|-----------------|
|                    | 0.325 nm/amino acid | nm | nm | nm |
| Bovine tau         | Full-length (448 amino acids) | 112 | 52 | 28 | 32.5 |
|                    | Average length (584 amino acids) | 95.8 | 44.4 | 24.1 | 27.8 |
| Human tau          | Full-length (441 amino acids) | 110 | 51.1 | 27.6 | 32 |
|                    | Average length (396 amino acids) | 96.2 | 45.8 | 24.8 | 28.7 |

* β spiral incorporates the β-turns within the spiral. According to the original definition this model would be 100% β spiral.

** The length of the composite model is calculated assuming that the shortest tau would contain 38% β-turns, ~42% β spiral residues (coil) not including the β-turns, and ~20% α helix. According to the definition of β spiral, this model would be ~80% β spiral, and ~20% α helix.

by similar changes in amino acid composition.

** Microtubule Assembly and Stability—**Since the circular dichroism of tau demonstrates a progressive increase in mean residue ellipticity (θ) at 197 nm with increasing temperature from 10 to 80 °C, tau, like elastin and the elastin-like pentapeptide polymer, undergoes an inverse temperature transition (Fig. 4, a and b) which starts in a less ordered extended state at 10 °C and is transformed to a shortened more ordered state at 37 °C (Table III) and at higher temperatures with an upper limit of ~80 °C. This process has important implications for
The MAP Tau Forms a Three-stranded Left-hand Helical Polymer

**Table V**

| Protein and source | No. of amino acids (full sequence) | NH$_2$ terminal charged residues/100 amino acids | COOH terminal charged residues/100 amino acids |
|--------------------|-----------------------------------|-----------------------------------------------|-----------------------------------------------|
| Tau Human$^a$      | 441                               | 24 acidic/9+ basic                             | 16+ basic/13 acidic                           |
| Bovine$^b$         | 448                               | 27 acidic/4+ basic                             | 17+ basic/12 acidic                           |
| MAP-2 Mouse$^c$    | 1828                              | 19 acidic/13+ basic                            | 17+ basic/10 acidic                           |

$^a$ Goedert et al., 1989  
$^b$ Himmler et al., 1989.  
$^c$ Lewis et al., 1988.

FIG. 6. Panel a, idealized periodic triple-stranded left-hand helical tau model. This tau model idealizes the structural features of tau that have been observed in Figs. 2 and 6d. The diameter of 2.1 nm is composed of three ~1.0-nm strands which cross the filament axis at ~1.8-nm intervals with a pitch of ~5.4 nm. Panel b, triple-stranded collagen model. In b the model for collagen is shown with a 1.5-nm diameter. Three 0.7-nm strands cross the axis at ~2.9-nm intervals along the axis with a pitch of 8.6 nm. The collagen strands also contain a triplet repeat motif, (Gly-X-Y), where X and Y are frequently proline and hydroxy proline. These residues form a left-handed helix with a pitch of 0.87-0.90 nm with each amino acid extending 0.296-0.30 nm axially (Ramachandran, 1963; Schulz and Schirmer, 1979). This is very near the fully extended amino acid distance of 0.33 nm (~17% longer than 0.3 nm) found in $\beta$-sheet and probably explains why rat tail collagen can only extend about 1.5-17% before it ruptures (Kastelic and Baer, 1980). Panel c, triple-stranded $\alpha$-keratin model. In panel c, the model for $\alpha$-keratin is shown with a 1.9-nm diameter. Three 0.95-nm strands cross the axis at ~6.3-nm intervals along the axis with a pitch of 19 nm. The coiled coil of three $\alpha$ helices has a diameter of 1.9-2.5 nm and is composed of three helices each 0.95-1.0 nm in diameter which left-hand twist around each other (Crick, 1953) with a pitch of 18.6-20 nm (Crick, 1953; Fraser and Cole, 1984a) and other heavily phosphorylated microtubule-associated proteins (Jameson et al., 1989) do not promote microtubule assembly at 37 °C. This suggests that the shorter, more ordered tau or MAPs at 37 °C binds more strongly than the longer less ordered state at lower temperature and that heavy phosphorylation can make tau or MAPs more hydrophilic and prevent their transition to a shorter more ordered state at 37 °C. An elastic tau is needed to stabilize very long axonal microtubules that are subject to constant bending, as at knee and elbow joints. It is unlikely that individual tau monomers with only a single binding domain could restore microtubule structure elastically after a bending insult. Each tau has a single region of three or four repeating sequences, totaling 93 or 124 amino acids, that bind to tubulin in microtubules (Aizawa et al., 1988). A tau polymer oriented axially along the microtubule would connect tau monomers which each extend over at least seven or eight (~63 nm) 8-nm tubulin dimers with binding domains approximately 13.1 or 17.5-nm long. Such an elastic tau polymer repeated around the microtubule periphery would stabilize the microtubule and maintain microtubule structure through a bending process. We are investi-
gating the prediction that tau polymer is longitudinally oriented on microtubules and that it has a functional role in the axons.

**Alzheimer Neurofibrillary Tangle Formation**

The inverse temperature transition has important theoretical implications for neurofibrillary tangle formation from tau in Alzheimer's disease. Elastin and the elastin-like pentapeptide polymer form concavates at 24–27 °C instead of promoting microtubule assembly. Shifting the coacervate phenomena to lower temperatures by 10–15 °C may require the replacement or elimination of only 1 aspartate or glutamate/100 amino acid residues (Urry, 1990). It seems probable then that some tau produced in the nerve cell body of Alzheimer's disease victims contains a slightly more hydrophobic sequence which self-aggregates and condenses into neurofibrillary tangles at 37 °C and neutral pH. This mechanism could be triggered if tau was phosphorylated in the wrong position leaving a long sequence of about 100–150 amino acids more hydrophobic. Tau from Alzheimer neurofibrillary tangles is abnormally phosphorylated (Grundke-Iqbal et al., 1988) and is unable to reconstitute microtubules at 37 °C (Iqbal et al., 1988; Nieto et al., 1990). We know that tau that is functionally active in microtubule assembly contains a number of phosphate groups (Lindwall and Cole, 1984a; Iqbal and Grundke-Iqbal, 1990). The number and location of the phosphorylation sites on both normal and abnormally phosphorylated tau are yet to be determined.

**Acknowledgments**—We thank GeoM Co. and IBR for its support and the Dartmouth Rippel Electron Microscope Facility for the use of the JEM 100CX and the modified Balzers 300. Isolation of bovine tau was carried out by T. Zaidi and M. S. Zaidi at IBR.

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