Comparative Vibrational Spectroscopy of
Intracellular Tau and Extracellular Collagen I
Reveals Parallels of Gelation and Fibrillar Structure

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Abstract

The N-terminal tau (2-19) peptide undergoes gelation, syneresis and aggregation over a period of years. These changes may be approximated on a shorter time scale by agitation and partial dehydration. The anomalously enhanced (229 nm.) ultraviolet resonance Raman (UVRR) imide II band reveals a common structural feature for gels of non-dehydrated tau (2-19) and collagen I, and insoluble paired helical filaments (PHFs) and collagen I, of weak hydrogen bonding at proline carbonyls. Anomalous UVRR enhancement of amide bands at 229 nm. results from gel structure, as demonstrated by increased amide absorption at the red edge for tau (2-19) gel, and implies the involvement of water in gel structure. In aged, dehydrated tau (2-19) gel, proline carbonyls becomes nonbonded and tyrosine becomes deprotonated, as demonstrated by UVRR spectroscopy. The Fourier transform infrared (FTIR) amide I band shows that antiparallel $\beta$-sheet structure increases with syneresis in the tau (2-19) hydrogel. The comparison of FTIR results for PHFs with collagen I gel and polyproline demonstrates that the secondary structure of PHFs is polyproline II. One implication of this assignment is that hydrophilic tau fibrillization is thermodynamically driven by the entropy gained as hydrogen-bonded water is freed, as for collagen I. The FTIR results also show that peptide domains culled from a longer protein do not necessarily fold into identical secondary structures. A pathological, sequential mechanism of gelation, syneresis and fibrillation for tau in AD is suggested, and supported by the observation of amorphous tau plaque development and fibrillation in vivo.

Abbreviations:  AD, Alzheimer's disease; CD, circular dichroism; DMF, dimethylformamide; DMSO, dimethylsulfoxide; FTIR, Fourier transform infrared; PII, polyproline II; PHF, paired helical filaments; TEM, transmission electron microscopy; UVRR, ultraviolet resonance Raman
Introduction

The cytomatrix is a living, insoluble, thixotropic gel (1) that is dynamic because its structure may change from fluid (sol) to elastic (gel) and back again; the filamentous network is constantly changing (2). Its constituent, organized structures are polymeric microtubules, actin microfilaments and intermediate filaments (2). The assembly of this gel network and the composite fibrils is assisted through interactions with a number of other proteins. Within the set of microtubule assembling proteins, tau is of particular interest.

In healthy neurons, tau, through its control of the reversible polymerization of microtubules, participates in the extension of both axons and neuritic processes, also known as neural growth cone development, and in the development of neural polarity (3). In a post-translationally modified state, however, tau is responsible for several neurodegenerative diseases or tauopathies, one of which is Alzheimer's Disease (AD), characterized by intraneuronal inclusions of phosphorylated tau that are initially amorphous. Over time, the intraneuronal inclusions of tau isoforms (4-6) self-assemble into paired helical filaments (PHF's) (7,8); these are responsible for the neurofibrillary tangles found in AD.

Braak and Braak have demonstrated the correlation between cognitive decline and the development of the tau plaques (9-11). The progression of neural destruction in AD begins with loss or retraction of distal dendrites, accompanied by the formation of neuropil threads and an incipient neurofibrillary tangle in the soma, followed by loss of proximal dendrites, increased tau aggregation and destruction of the soma (11). One of the central questions regarding the development of AD and other tauopathies is the significance of the development of the neurofibrillary plaques: are they merely markers of the progress of the disease, or are they, in fact, the causative agent of neuronal death (12)?
Within the cytomatrix, actin microfilaments, keratin intermediate filaments and tau microtubules cooperate in control of several cell activities including shape and cell membrane adhesion (13). Cellular growth and differentiation results in the build-up of tensile and compressive forces within the cytomatrix (14,15). This cytoskeletal tension is transmitted to binding sites in the inert extracellular matrix via transmembrane integrin receptors (16) and other focal adhesion proteins (17). In fact, cell shape is controlled through a delicate balance of signal (18) and adhesive factors (19) between the cytomatrix and the extracellular matrix.

Cell adhesion substrates include extracellular matrix proteins such as collagens (19,20). The most abundant protein of the extracellular matrix is collagen I, a fibrillar triple helical protein that forms gel networks in irregular connective tissue (21-23). Tau, like collagen I, is very hydrophilic (24), and shares other characteristics: both are proline-rich, nonglobular, self-assemble into fibrils, and are found in vivo as several isoforms.

The spectroscopic and electron microscopic results presented here demonstrate other common features of structure and behavior between collagen I and tau (2-19) peptide and full length protein: precedence of a gel phase leading to fibril formation, the involvement of proline residues in weak hydrogen bonding of both the gel matrix and fibril, and a polyproline II secondary structure (PHFs). These results are discussed with respect to the cytoskeletal changes that might ensue from the generation of pathological intraneuronal compressive forces by a collagen-like gel and fibril (12).

Materials and Methods

Lithium thiocyanate, glycerol, bovine skin gelatin, type B and insoluble bovine Achilles tendon collagen, type I were purchased from Sigma-Aldrich (St. Louis, MO), and used without further purification.
**Peptide Synthesis.** N-terminal tau (2-19) peptide (primary sequence AEPRQEFVME
AGTYG) and tau (309-26) (primary sequence VYKPVDLSKVTSKCGSLG), a segment from the tau protein pseudorepeat region, were synthesized using solid phase peptide synthesis methodology on an ABI 433A peptide synthesizer at a 0.1 mM scale by the standard protocol for Fmoc chemistry (25). The reagents piperidine, diisopropylethylamine, N-methylpirrolidone, dichloric methane, Fmoc amino acids and preloaded resin, and a HOBT/HBTU* activation kit were purchased from Applied Biosystems (Foster City, CA). Peptide resin was cleared and deprotected by a mixture of tetrafluoroacetic acid, water, phenol and triisopropylsilane in a ratio of 88:5:5:2 for two hours. The peptide was precipitated by ether, washed and lyophilized. The peptide was characterized by MALPI TOFMS high pressure liquid chromatography (Voyager DE STR, PerSeptive Biosystems, Foster City, CA) using a C<sub>8</sub> reverse phase column (HP1090, Hewlett Packard Co.) and amino acid analysis (Hewlett Packard Amino Quant).

For the C-terminally biotinylated tau (2-19), Fmoc ε-aminocaproic acid (Bachem California, Inc., Torrance, CA) was inserted as a biotin linker during automatic peptide synthesis. After deprotection, the Fmoc group resin was washed with dimethylsulfoxide (DMSO); 0.244 g. (1 mmol) of (+)-biotin (Sigma-Aldrich, St. Louis, MO) was dissolved in 5 ml. of a 1:1 solution of dimethylformamide (DMF) and DMSO. To this was added 2.1 ml of a 0.45 M HOBT/HBTU activation solution and 3 ml of diethyl ether. The resulting activated biotin solution was added to 0.1 mmol of the Fmoc group resin, and stirred overnight. The resulting resin was washed successively with a 1:1 solution of DMF and DMSO, DMF and dichloromethane to remove the excess biotin. The biotinylated resin was cleared as above.

**Preparation of Tau (2-19) Samples.** Freeze-dried tau (2-19) was dissolved in deionized water to a concentration of 6 mM and stored undisturbed at 4°C for five years. The tau (2-19) was found to have gelled after this time; UVRR results were recorded on the undiluted gel. Typically,
polyanions such as perchlorate (0.2M) (26,27) are added as internal peak calibration standards in the acquisition of UVRR data. However, polyanions are known catalysts of *in vitro* PHF formation (28,29), and so an internal standard was not added to the aged tau (2-19) gel. The gel was cloudy after curing, and so an absorption spectrum could not be recorded. When the gel was pipetted into a quartz tube, the gel became less viscous, exhibiting a gel phenomenon known as shear thinning; upon standing the gel recovers its original viscosity (30). The gel was subsequently dissolved and diluted to 4 mM by addition of a 100 µl aliquot of 9.1 M lithium thiocyanate. The UV laser beam (1.8 mW) that was incident on the quartz NMR tube holding the dissolved tau (2-19) sample during the UVRR experiment caused the formation of a translucent tau film on the tube. The remaining tau solution was removed from the quartz tube, and UVRR results were recorded for the film alone. The tau film was found to be soluble in 3 M lithium thiocyanate solution. The tau (2-19) solution decanted from the quartz tube was spun at high speed (estimated as 1 kHz) and the resulting aggregated tau (2-19) was pelleted. The aggregate was resuspended in deionized water, and UVRR results were likewise recorded for it.

A 10 mM solution of lyophilized tau (2-19) with biotin on the C-terminus was freshly prepared in deionized water for UVRR spectroscopy.

In trials to reproduce the UVRR results of the cured tau (2-19) gel on a shorter time scale, a fresh aliquot of lyophilized tau (2-19) was dissolved in deionized water at the gelation concentration of 10 mM and stored undisturbed at 4°C. This aliquot gelled within two months. A UVRR spectrum of this gel was acquired periodically up to nine months of age. Additional 10 mM tau (2-19) aliquots were subject to different treatments: 1. heating in a sealed ampule to 95°C for five days, 2. vigorous stirring at room temperature for 19 hours, 3. vigorous stirring
followed by partial drying under a nitrogen stream, 4. adjusting the pH to 10.5 and 5. dissolution in 10 mM sodium chloride (31).

**Preparation of PHF Samples.** Immunoaffinity-purified PHFs (32,33) at 2 mg/ml in Tris buffered saline (10 mM Tris, 0.15 M sodium chloride, pH 7.4) were a gift of Dr. Peter Davies. PHF's dissolved in 9 M LiSCN were dialyzed exhaustively against distilled water, and recovered as a solid by drying under a nitrogen gas stream at room temperature prior to incorporation in a KBr pellet. Additionally, it was found that PHFs dissolved in 9M LiSCN, as for tau (2-19) in LiSCN, formed a translucent film when exposed to the UV laser beam.

**Preparation of Collagen Samples.** Insoluble bovine collagen type I was suspended in Tris buffered saline at a concentration of 25 mg/ml. A bovine skin gelatin gel was prepared by dissolution in deionized water and in D2O at a 50 mg/ml concentration at ca. 60°C with stirring.

**Negative stain electron microscopy.** Solid tau (2-19) samples, gel and aggregate, were transferred to grids, and negatively stained with 1% uranyl acetate. The grids were then blotted dry before immediately observing in a JEOL 100CX II transmission electron microscope (JEOL USA, Inc.) at 80 kv.

**UVRR Spectroscopy.** An argon ion laser system, described elsewhere (34), was used to generate continuous wave, 228.9 nm. light at an incident power of 1.8 mW. The samples were chilled to (10°C ± 4°C), rastered and spun in order to minimized UV-induced photo damage. The UVRR spectra are an average of a set of three minute acquisitions, collected over a 805 cm⁻¹ to 1680 cm⁻¹ frequency window; see Figures for numbers of acquisitions. The frequency scale of the spectra is calibrated against the peaks of two reference solvents, indene and toluene, and is accurate to ± 1cm⁻¹. The software program Grams/32 AI, version 6.00 (Galactic Industries Corp.,
Salem, NH) was used to determine peak heights (full width at half maximum), and for curve fits to complex bands. Fitting parameters were not restricted.

**FTIR Spectroscopy.** FTIR spectra were acquired on a Nicolet (Madison, WI) Magna 600 FTIR spectrometer. Samples were prepared either as potassium bromide pellets or as smears on zinc selenide windows. For the tau (2-19) samples, the peptide concentration was 10 mM in D$_2$O. Protein samples embedded in KBr were not subjected to potentially denaturing high pressure as the pellets were formed using a screw-type press, which allows only for pressures applicable by hand-held wrenches. The number of accumulated scans varied; instrumental resolution was set to 4 cm$^{-1}$.

**Results**

**UVRR Spectroscopy of Tau (2-19) Peptide Phases.** UVRR results (229 nm excitation) for the five-year old tau (2-19) peptide in four different phases are given in Figure 1. The spectral window has been truncated to the 1100 - 1680 cm$^{-1}$ region since the bands of interest are concentrated here. Figure 1, spectrum a shows the UVRR results for five-year old, cured tau (2-19) gel while results for solution phase tau (2-19) with the C-terminally linked biotin, which prevents aggregation, are given in Figure 1, spectrum c. The UVRR difference spectrum between the cured gel (Fig. 1, spectrum a) and solution phase (Fig. 1, spectrum c) of tau (2-19) is given in Fig. 1, spectrum b, where the 1616 cm$^{-1}$ peaks were normalized before spectral subtraction. Addition of lithium thiocyanate (final thiocyanate concentration = 3 M), a known disrupter of water structure (35), to the cured tau (2-19) gel yields a non-cloudy liquid, implying tau dissolution. The UVRR spectrum for the thiocyanate solution of the cured tau (2-19) gel is given in Figure 1, spectrum d. The UVRR result for the tau (2-19) aggregate, described in "Methods and Materials," is given in Fig. 1, spectrum e. The result for the tau (2-19) film, also
described above, is superimposable on those for the tau (2-19) aggregate (Fig. 1, spectrum e), and so is not shown.

The peak frequency of the vibrational bands for the UVRR results of Fig. 1 are summarized in Table 1. As expected for excitation at 229 nm, the solution phase UVRR spectrum for biotin-linked tau (2-19) in Fig. 1, spectrum c exhibits strong vibrational bands for the single tyrosine residue: Y8a at 1616 cm\(^{-1}\) (ring C-C stretch), a Y8b shoulder at 1600 cm\(^{-1}\), Y9a at 1178 cm\(^{-1}\) (ring C-H bend) and Y7a at 1208 cm\(^{-1}\) (C\(_{\beta}\)-C\(_{\gamma}\) stretch) (36-39). Less intense bands are also seen at 1238 cm\(^{-1}\), 1551 cm\(^{-1}\) and 1579 cm\(^{-1}\).

The UVRR results for the cured, tau (2-19) gel in Fig. 1, spectrum a display not only the aforementioned tyrosine peaks but also strongly---and unexpectedly---enhanced amide bands at 1238 cm\(^{-1}\) (C\(_{\alpha}\)H mode), 1298 cm\(^{-1}\) (Amide III, Am III), 1384 cm\(^{-1}\) (Amide S), 1550 cm\(^{-1}\) (Amide II, Am II) and 1579 cm\(^{-1}\) (Am II) (36,40). A very weakly enhanced imide II band at 1445 cm\(^{-1}\), due to a carbonyl stretching mode of the single proline, is also seen (41). The presence of this band is also not expected as proline is resonantly enhanced at a shorter wavelength, ca. 218 nm. The shoulder at 1218 cm\(^{-1}\) is an unassigned amide mode that has also been found in the UVRR spectrum (229 nm.) of neat N-methylacetamide at -30°C (Juszczak, unpublished data).

The difference spectrum shown in Fig. 1, spectrum b clarifies the amide band positions for the five-year old, cured tau gel results in Fig. 1, spectrum a. It also reveals a residual peak at 1600 cm\(^{-1}\), which is attributed to deprotonation of some unspecified population of tyrosines within the gel rather than to the single phenylalanine, because of the predominance of tyrosinate peaks in Fig. 1, spectra d,e and the presence of a weak Y9a' band at 1168 cm\(^{-1}\) (42,43). The presence of tyrosinate also suggests that the 1579 cm\(^{-1}\) band contains a tyrosinate Y8b'
The 1218 cm$^{-1}$ shoulder of Fig. 1, spectrum a is revealed in the difference spectrum, b of Fig. 1. Amide I bands, which appear at or above a frequency of ca. 1630 cm$^{-1}$ are not seen (37,44).

The addition of lithium thiocyanate to the cloudy, cured tau (2-19) gel resulted in a clear solution, and yielded the UVRR results shown in Fig. 1, spectrum d. The spectrum is now less complex: tyrosine bands are now shifted to tyrosinate positions. The Y8a' band lies at 1602 cm$^{-1}$ and the Y8b' cm$^{-1}$ band, at 1583 cm$^{-1}$; low frequency Y9a' and Y7a' bands are poorly resolved. The 1555 cm$^{-1}$ band is attributed to amide II. The 1583 cm$^{-1}$ band may also contain a weak amide II component although a three component curve fit (data not shown) to this high frequency cluster of bands (1555-1602 cm$^{-1}$) yielded a reduced $\chi^2 < 1$, suggesting that both amide II and Y8b' bands do not contribute.

The UVRR results for the tau (2-19) aggregate are shown in Fig. 1, spectrum e; the tau (2-19) film results are similar (data not shown). The configuration of high frequency bands (1555-1602 cm$^{-1}$) for the tau peptide aggregate is the same as for the dissolved tau gel (Fig. 1, spectrum d). At the low frequency end of the spectrum, however, bands are better resolved.

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1 The tyrosinate Y8b' mode is reported (43. Fodor, S., Copeland, R., Grygon, C., and Spiro, T. (1989) J Am Chem Soc 111, 5509-5518) as occurring at 1558 cm$^{-1}$ while the data there within shows that it is actually at ~1585 cm$^{-1}$. The phenylalanine F8b mode is reported at 1593 cm$^{-1}$ and the F8a, at 1604 cm$^{-1}$ (42. Asher, S., Ludwig, M., and Johnson, C. (1986) J Am Chem Soc 108, 3186-3197) while tyrosinate Y8a' is reported at 1601 cm$^{-1}$. Since the tyrosinate Y8b' mode is very similar to the F8b mode, Y8b' would therefore be expected at ~1590 cm$^{-1}$ and not at 1556 cm$^{-1}$ as reported. The data there within shows, in fact, a shoulder at ~1590 cm$^{-1}$ on the Y8a' band.
Peaks appear at 1168 cm\(^{-1}\) and 1182 cm\(^{-1}\); these are assigned as Y9a’ and F9a, respectively. A weak amide III band appears at 1302 cm\(^{-1}\), and a weak imide II band is found at 1445 cm\(^{-1}\).

**Electron Microscopy of Tau (2-19) Phases.** Electron micrographs at 20,000 magnification of the tau (2-19) aggregate and five-year old gel in the presence of uranyl acetate stain are shown in Figure 2. The aggregate, Fig. 2a, does not stain, indicating that water is excluded from the structure while the cured gel, Fig. 2b, is stained; water permeates the gel structure.

**UVRR Spectroscopy of Tau (2-19) Gels Alternately Processed.** Clearly, tracking the structural change evident in the five year old tau (2-19) gel at earlier time points is of interest. UVRR results for 10 mM samples of tau (2-19) under varying solution conditions and processing methods are given in Figure 3. The high frequency quartet of peaks from the UVRR results of the five year old, cured tau gel are reproduced in Fig. 3, spectrum a for ease of comparison. The UVRR results for tau (2-19) vigorously stirred for 19 hours at room temperature are shown in Fig. 3, spectrum b. This treatment yielded a cloudy gel. The tyrosine Y8a peak at 1615 cm\(^{-1}\) and Y8b peak at 1597 cm\(^{-1}\) are clearly present; a very weak amide II band at 1555.5 cm\(^{-1}\) is also evident. Curve fitting results to the broad Y8 band envelope showed that additional bands, evident as shoulders in Fig. 3, spectrum b, are located at 1603 cm\(^{-1}\) and 1584 cm\(^{-1}\) (Data not shown). These latter bands have counterparts in the results for the five year old tau gel, and became more explicit after gel drying, discussed below. Heating tau (2-10) to 95°C for five days yielded a Y8 band with low frequency shoulders of greater intensity, and a Y8a band slightly upshifted to 1613 cm\(^{-1}\). These spectroscopic results approach those of the five year old tau gel, but the physical appearance of the gel was yellow, suggesting oxidation. The UVRR results for a 10 mM tau (2-19) solution at pH 10.5 are given in Fig 3, spectrum d. Here, the tyrosinate Y8a’ peak appears at 1604 cm\(^{-1}\), close to the 1600 cm\(^{-1}\) tyrosinate peak of the five year old tau gel (Fig. 3, spectrum a), and a Y8b’ shoulder is located at 1585 cm\(^{-1}\). The amide II band is stronger than
those found in the UVRR results for the heated or stirred tau gels (Fig. 3, spectra c and b, respectively).

In order to determine the age at which a tau (2-19) gel stored at 4°C attains the spectroscopic markers of the five year old tau gel (Fig. 1, spectrum a), a solution at the gelation onset concentration of 10 mM, was prepared. UVRR spectra of this tau gel were taken at the two, five, seven, eight and nine months of age time points; the results are superimposable, and the result at the nine months of age time point is shown in Figure 4, spectrum a. The nine month old tau gel is clear in physical appearance. The tyrosine Y8 band (Fig. 4, spectrum a) has the shape and frequency characteristic of solution phase tau (2-19) (Fig. 1, spectrum c). However, a broad, fairly intense imide II peak (1465 cm⁻¹), indicating weak hydrogen bonding at the proline carbonyl (41) also appears (Fig. 4, spectrum a). It is relatively more intense than the imide II band appearing in the results for the five year old gel (Fig. 1, spectrum a). Thus, the UVRR results for the nine month old gel indicate structural differences relative to the tau (2-19) gel similarly processed (quiescent, stored at 4°C) but allowed to age for years. The UVRR results in Figure 4, spectrum b are for the tau (2-19) gel processed by vigorous stirring for 19 hours at room temperature (Fig. 3, spectrum b); note the lack of imide II band at ca. 1465 cm⁻¹. It is reproduced for comparison with the UVRR results for the tau (2-19) gel, which was similarly stirred and subsequently dehydrated, shown in Fig. 4, spectrum c. (This gel was also very cloudy; the significance of which will be discussed below.) The low frequency peaks of the tyrosine Y8 band at 1597 cm⁻¹ and 1584 cm⁻¹, and the amide II band at 1555 cm⁻¹ have gained intensity vis-à-vis the UVRR results for the tau gel which has only been stirred (Fig. 4, spectrum b). Here, too, the imide II band is absent. Curve fit results to the Y8 band reveal an additional peak at 1603 cm⁻¹. Thus, the cloudy physical appearance of the stirred, dehydrated tau gel
together with the incipient quartet of UVRR peaks (Fig. 4, spectrum c) indicate that vigorous, prolonged stirring followed by dehydration puts the tau (2-19) peptide gel on the path to spectroscopic equivalence with the five year old tau gel (Fig. 1, spectrum a).

**FTIR Spectroscopy of Tau (2-19) Gels Alternately Processed.** In order to ascertain the presence of secondary structure in the tau (2-19) gels, FTIR results were acquired for a one week old tau (2-19) gel, non-stirred process, stored at 4°C (Fig. 5, spectrum a) and for a tau gel vigorously stirred for 18 hours, followed by dehydration (Fig. 5, spectrum b). Both gels were prepared in D₂O under a nitrogen-purged, water-free atmosphere. The amide I region, which reports on secondary structure, is shown. The nonambiguous features of the amide I band for the tau (2-19) gel produced by the non-stirred process (Fig. 5, spectrum a) are a fairly sharp peak at 1628 cm⁻¹, a strong shoulder or buried peak at 1673 cm⁻¹ and a low frequency shoulder at 1609 cm⁻¹; these peak values were obtained by curve fitting (Data not shown). The amide I region for the stirred, dehydrated tau gel (Fig. 5, spectrum b) has a similar contour, but the obvious peaks (1601 cm⁻¹, 1620 cm⁻¹, 1672 cm⁻¹; curve fitting results, not shown) are more intense, better defined and down-shifted relative to the non-stirred gel results. Inverted second derivatives of these FTIR amide I bands were also calculated, and are shown below each FTIR result. The second derivative peaks are in good agreement with those obtained by curve fitting, albeit frequency up shifted a few wave numbers relative to peaks predicted by curve fitting.

**UVRR Spectroscopy of Tau (2-19) and Collagen I Gels.** As comparison between a well-characterized protein and one that is not can often provide insight into the nature of the latter, a spectroscopic comparison of UVRR results for the non-stirred tau (2-19) gel at two months of age (Fig. 6, spectrum b) is made with those for a nonaged bovine skin collagen I gel, (Fig. 6, spectrum a). As for the tau (2-19) peptide gels, peaks appear in the bovine collagen gel UVRR
results that are not resonantly enhanced with 229 nm excitation for proteins in aqueous solution. Most noticeable is the very strong imide II peak at 1467 cm\(^{-1}\). As collagen isoforms are \(~25-30\%\) proline, a large absorption cross section predicts a strong imide II peak. The Y8 tyrosine band shape, with a peak at 1615 cm\(^{-1}\), is typical of solution phase results. The bovine collagen gel spectrum has an amide II band at 1555 cm\(^{-1}\), which is conspicuously absent in the nonstirred, two month old tau gel. This difference will be important to the discussion of the PHF secondary structure, below.

**UVRR Spectroscopy of PHFs and Insoluble Collagen I.** PHFs represent the endpoint in a transition from molecular disorder to order. Like the N-terminal tau (2-19) gel (Fig. 2b), they are stained by uranyl acetate; water is incorporated in their structure (7). The UVRR result for PHFs, composed of full-length tau isoforms, is given in Figure 7, spectrum b along with result for insoluble bovine Achilles tendon collagen type I (Fig. 7, spectrum a). Both spectra show a tyrosine Y8a band at 1616 cm\(^{-1}\) and a strong broad, imide band at 1466-68 cm\(^{-1}\). For full length tau isoforms, the proline content is \(~10-12\%\) whereas the proline content of collagen type I isoforms is \(~25\%\); thus the area of the imide II band scales accordingly. An amide II band at 1555 cm\(^{-1}\) is strongly enhanced in the PHF spectrum, and weakly enhanced in the collagen type I results. A weak and broad amide III band at 1305 cm\(^{-1}\) is found in the PHF results (Fig. 7, spectrum b). Lastly, a broad, weak amide S band appears at \(~1380\) cm\(^{-1}\) in both spectra.

**FTIR Spectroscopy of PHFs, Collagen I and Polyproline.** FTIR results were acquired for two forms of PHFs; these are presented, along with results for other illustrative proteins, in Figure 8. Results for freshly isolated PHFs in KBr pellet are given in Fig. 8, spectrum a. The peaks, located at 1294 cm\(^{-1}\), 1404 cm\(^{-1}\), 1460 cm\(^{-1}\), 1552 cm\(^{-1}\) and 1631 cm\(^{-1}\), are remarkably sharp; this is a consequence of the limited conformational motion possible in the solid state. Curve fits to the amide I (1631 cm\(^{-1}\)) and amide II (1552 cm\(^{-1}\)) bands are included, but the amide I results will
be focussed on; it is composed of a Lorentzian band at 1631 cm\(^{-1}\) and a Gaussian at 1665 cm\(^{-1}\). A small peak at 1746 cm\(^{-1}\) has been magnified for comparative purposes, and will be discussed below. The amide I region for three proteins is given in Fig. 8, spectra b. These include amide I results for PHF’s recovered from dissolution in LiSCN, with a broad Lorentzian peak (1631 cm\(^{-1}\)) corresponding to that for freshly isolated PHFs (Fig. 8, spectrum a); a single broad amide I band at 1643 cm\(^{-1}\) for polyproline and a similar Lorentzian-shaped amide I band at 1653 cm\(^{-1}\) for bovine skin gelatin. The 1746 cm\(^{-1}\) peaks for the recovered PHF and polyproline results have been magnified. The high frequency region of the FTIR spectrum for insoluble bovine Achilles tendon collagen is given in Figure 8, spectrum c. Although this sample was similarly embedded in KBr pellet, the amide I band at ca. 1650 cm\(^{-1}\) is not as sharp as that for the PHFs (Fig. 8, spectrum a); the amide I band for collagen is clearly composed of two Lorentzian peaks, determined from curve fitting and centered at 1645 cm\(^{-1}\) and 1686 cm\(^{-1}\). Another feature of the bovine collagen FTIR spectrum is the strong, sharp band at 1746 cm\(^{-1}\), which may be attributed to type I \(\beta\)-turns (45) but has also been found in the FTIR amide I band for other proteins with PII secondary structure (46).

Discussion

Water and Hydrogen Bonding in the Tau (2-19) Gel Network. By definition, a gel is a disordered network of molecules linked through some type of intermolecular bonding (47,48). For a gel in a water-rich environment, which is called a hydrogel, water participates in hydrogen bonding through bridging of charged groups (30,49,50). Self-assembled hydrogels, such as the tau (2-19) peptide gel, are characterized by the property of shear thinning, as observed here, wherein the gel viscosity decreases upon application of a force, such as that imposed by pipetting, and increases once the force is removed (30). This property—the ease of breaking and reforming of the intermolecular gel structure—also supports the idea that water participates in
intermolecular hydrogen bonding. The positive staining of the five year old tau (2-19) gel with aqueous uranyl acetate (Fig. 2b) also indicates that it is a hydrogel and supports the role of water as a bridging element in maintaining gel structure. Lastly, the disappearance of the amide bands with dissolution of the five year old tau gel by the addition of a high concentration of lithium thiocyanate (Fig. 1d), which is know to disrupt water structure (35), further supports this interpretation.

The disappearance of the amide bands from the UVRR results for the tau (2-19) film and aggregate grown from the lithium thiocyanate solution (Fig. 1, spectrum e) suggests a water-excluded environment for the amides, as found in globular proteins, under these processing conditions; the lack of uranyl acetate staining seen in the transmission electron microscopy (TEM) image of the tau (2-19) aggregate (Fig. 2a) affirms this interpretation.

The Evidence for Water-Bridged Amide Hydrogen Bonding. The anomalous appearance of the amide and imide II vibrational bands in the UVRR results for the tau (2-19) gels (Fig. 1, spectrum a and Fig. 4, spectra a,c) implies heterogeneous broadening at the red edge of the corresponding absorption bands and/or a red shift in the absorption maxima such that they are on resonance with the 229 nm exciting laser beam. A comparison of far UV absorption spectra for a six month old gel phase tau (2-19) (non-stirred process), a solution phase tau (309-26) and solution phase tau (2-19) with C-terminally linked biotin, shown in Figure 9, supports this idea: the gel phase tau (2-19) is greatly red-edge broadened relative to tau (309-26) and noticeably broadened relative to the biotin-linked tau (2-19). Furthermore, the UVRR results presented in Fig. 1 demonstrate that water plays a role in the tau gel structure and that it is responsible for the anomalous appearance of imide and amide bands with 229 nm excitation. The relatively simple UVRR result for the biotinylated tau (2-19) peptide in aqueous solution (Fig. 1, spectrum c) is similar to those for other peptides reported in the literature where a longer wavelength has been
used for excitation (51,52). If the criterion for the red-edge broadening and/or red shift of the maximum of the amide absorption band (Fig. 9) was only the ordering of water via hydrogen bonding about exposed amides and carbonyls, then the UVRR results of these solution phase peptides, with 229-240 nm excitation, would similarly show strongly enhanced amide vibrational bands. Nor can intermolecular amide-carbonyl hydrogen bonding account for the amide absorption red shift as resonantly-enhanced amide bands for the secondary structural elements, α-helices and β-sheets, of globular proteins are optimally enhanced at ~210 nm. (53), and 229 nm.-excited UVRR results for globular proteins are dominated by vibrational bands of the aromatic residues, tyrosine and tryptophan (54-57).

Interpretation of the Tau (2-19) UVRR Results

Presence of Amide S Bands Signals Lack of α-Helices. The appearance of an amide S band (~1384 cm⁻¹) in an UVRR spectrum signals the absence of α-helical structure (40,58). The amide III mode frequency has recently been shown to depend on not only the amide backbone dihedral angle, ψ (59), but also on φ (60). Additionally, the amide III mode is coupled to the CH bending motions of the amide S band through the NH bending mode (40,58). These vibrations mix when the dihedral angle, ψ, takes a value characteristic for β-sheet structures, polyproline II helices and random coils, i.e., ψ ~ 120°. At a ψ value characteristic of α-helices, ψ ~ 60°, the vibrations do not mix and the amide S band does not appear (58). As an amide S band is evident in the UVRR results for the five year old (Fig. 1, spectrum a), and to a lesser extent, the two month old (Fig. 6, spectrum b) and the stirred, dehydrated (1404 cm⁻¹; data not shown) tau (2-19) gels, α-helical structure is not incipient in the tau (2-19) gel curing process.

Weak Hydrogen Bonding at the Tau (2-19) Proline Residue. Takeuchi and Harada (41) found that the frequency of the imide II carbonyl-nitrogen stretch mode is sensitive to the strength of
hydrogen bonding at the carbonyl. For a non-bonded carbonyl, the mode frequency is \( \approx 1445 \text{ cm}^{-1} \) whereas a strongly hydrogen bonded carbonyl mode appears at \( \approx 1485 \text{ cm}^{-1} \). As the imide II band position is 1445 cm\(^{-1}\) for both the cured tau (2-19) gel (Fig. 1, spectrum a) and the tau (2-19) aggregate (Fig. 1, spectrum e), the prolyl carbonyl in these phases is predicted to be non-bonded. For the tetra peptide, tuftsin, the imide II band appears at 1466 cm\(^{-1}\), indicating a weaker hydrogen bond strength than that found for X-proline dipeptides hydrogen bound to water, \textit{i.e.}, 1475 cm\(^{-1}\) (41). The imide II band for the two month old tau (2-19) hydrogel (Fig. 6, spectrum b) also appears at 1466 cm\(^{-1}\); its broadness suggests a spread in the range of C=O hydrogen bond strengths. A hydrogen bond strength somewhat less than that for C=O bound to water would be expected for a C=O bound to a bridging water (C=O \(-\text{H-O-H}\)). There is no clear imide II band in the UVRR results for the stirred, dehydrated tau (2-19) gel (Fig. 4, spectrum c).

**Interpretation of the Tau (2-19) FTIR Results.** The amide I band results for the one week old tau (2-19) gel, non-stirred process (Fig. 5, spectrum a) and for the stirred, dehydrated tau (2-19) gel (Fig. 5, spectrum b) demonstrate that within the disordered gel network, reorganization of peptide chains to a recognizable secondary structure is occurring. Gel networks are known to be dynamic structures where reorganization processes may occur over time (61). The more sharply defined peaks for the stirred, dehydrated tau gel (Fig. 5, spectrum b) relative to those for the non-stirred tau gel (Fig. 5, spectrum a) suggest that the former gel possesses greater secondary structural organization. The cloudy physical appearance of the stirred, dehydrated tau gel results from a refractive index difference created by an incomplete phase separation of the peptide into discrete islets in a gelation process known as syneresis (61); these islets of concentrated peptide may facilitate the development of secondary structure.

The two most prominent peaks in the amide I band for the stirred, dehydrated tau gel (Fig. 5, spectrum b) are located at 1620 cm\(^{-1}\) and 1672 cm\(^{-1}\). The frequency position of these bands
and their splitting (52 cm\(^{-1}\)), resulting from transition dipole coupling of nearest neighbor peptide groups, strongly suggests the inception of an antiparallel β-sheet structure in the gel (45,62). As FTIR peaks below 1620 cm\(^{-1}\) have been noted for β-sheet (63), the strong shoulder in the amide I results for the stirred, dehydrated gel is also ascribed to β-sheet structure. The remaining disordered fraction of peptide in the gel undoubtedly yields an FTIR signal; the broad, unresolved absorbance region between the 1620 cm\(^{-1}\) and 1672 cm\(^{-1}\) peaks is assigned to this fraction.

A parallel assignment of amide I peaks can be made for the non-stirred tau gel (Fig. 5, spectrum a): antiparallel β-sheet structure for the 1628 cm\(^{-1}\) and 1673 cm\(^{-1}\) peaks, β-sheet structure for the 1609 cm\(^{-1}\) shoulder and disordered gel matrix to the region between the 1628 cm\(^{-1}\) and 1673 cm\(^{-1}\) peaks. Significantly, the non-stirred tau gel remained transparent so that syneresis has not taken place. In the absence of localized concentrations of peptide, the development of secondary structure is not as readily facilitated, and thus amide I peaks are weaker for the non-stirred tau gel.

Changes in the Tau (2-19) Gel Structure with Time. The UVRR and FTIR results for the tau (2-19) gels show that the involvement of proline in gel structure decreases, while the development of β-sheet secondary structure increases, with the onset of syneresis, mechanical agitation followed by dehydration, or gel aging. In the UVRR results for the optically clear, nonstirred, nine month old (Fig. 4, spectrum a) and two month old (Fig. 6, spectrum b) tau (2-19) gels, a proline band at 1465 cm\(^{-1}\) unexpectedly appears, indicating a carbonyl hydrogen bond strength somewhat less than what is expected for H-bonding to water (41) while tyrosine peaks are like those expected for solution phase. In the FTIR results for the clear, non-stirred, one week old tau gel (Fig. 5, spectrum a), the inception of β-structure is indicated, but peaks are not as well-defined as for the optically cloudy gel (Fig. 5, spectrum b).
In the UVRR results for cloudy, stirred and dehydrated gels of low age (Fig. 4, spectra b,c), the proline peak is absent while the intensity of the low frequency bands in the tyrosine/amide II quartet increases. At the extreme of five years of age, a non-stirred yet cloudy tau (2-19) gel (Fig. 1, spectrum a) has a fully-developed quartet of bands at high frequency, with both tyrosine and tyrosinate bands present, an imide II band at 1445 cm\(^{-1}\) for the nonbonded prolyl carbonyl, and an amide S band that precludes $\alpha$-helical structure. In the limit of aggregate formation (Fig. 1, spectrum e), UVRR data for tau (2-19) aggregates shows that the prolyl carbonyl remains nonbonded and that the single tyrosine is deprotonated. The UVRR results imply that only the structure of the optically transparent tau (2-19) gels parallels that of PHFs in that the imide II band frequency for both proteins shows proline carbonyl hydrogen bonding.

*Comparative Spectroscopic Analysis of PHFs.* Collagens are a complex, multi-isoform set of generally nonglobular proteins containing stretches of proline residues which *may* (e.g., collagen I) or *may not* (e.g., collagen VI) be arranged in a periodic repeating motif, with N- and C-terminal propeptide sequences having no periodic repeat sequence of amino acids, and containing water-bridged interamide hydrogen bonds (64-66). Collagen I in the fibril state is a coiled coil of three collagen I isoforms with a polyproline II (PII) secondary structure (64,66). Subsequent to proteolysis by procollagen peptidases, which removes frayed sequences at the termini (67,68), the primary structure of the collagen chains consists overwhelmingly of a three amino acid repeat of the type X-Y-Gly, where X is often proline and Y, hydroxyproline (64,66). The PII structure of collagen isoforms is stabilized by stereochemical interactions and does not require *intrachain* hydrogen bonds (69). X-ray diffraction results show that the *interchain* interactions, however, are stabilized by both interamide hydrogen bonds and water-bridged hydrogen bonds (64,66); main chain carbonyls are also hydrogen bonded to water molecules (66). Numerous collagen isoforms exist (65), and although domains of PII structure are often proline-rich, the prolines are not
necessarily arranged in a regular sequence pattern as in collagen I (See, for example, the human collagen VI alpha 1 chain). This, of course, is the case for the proline-rich domain of the tau isoforms. Indeed, Ramachandran has shown that all amino acids can be accommodated in the PII conformation (64). Thus, the comparison between the proline- and water-rich structure of collagen I and the tau PHF is valid, and supported both by the similarity of the UVRR results given in Figure 7 and the correspondence of FTIR peaks in Figure 8.

The one-to-one correspondence in UVRR peaks within the 1350 to 1650 cm\(^{-1}\) frequency window (Fig. 7) suggests a similarity in secondary structure for bovine Achilles tendon collagen type I and PHFs. Solid state PHFs, reconstituted from dissolution in LiSCN (Fig. 8, spectrum b), yield a single Lorentzian amide I band when probed by FTIR spectroscopy, as do solid state polyproline and bovine skin gelatin gel. Clearly, a PII structure is indicated for the reconstituted PHFs. Freshly isolated PHFs in the solid state result in an unambiguous amide I peak at 1631 cm\(^{-1}\) as well (Fig. 8, spectrum a), and a second, broader amide I band at 1665 cm\(^{-1}\). Again, the 1631 cm\(^{-1}\) peak suggests a PII structure for the virgin PHFs; the second amide band at 1665 cm\(^{-1}\) does not necessarily indicate that a second structural element is present, as discussed below, although the FTIR band frequency is consistent with either random coil or \(\alpha\)-helical structure (63). The latter assignment seems unlikely as the amide S band in the UVRR results precludes \(\alpha\)-helical structure (Fig. 7, spectrum b).

The amide I band for insoluble bovine Achilles tendon collagen, type I (Fig. 8, spectrum c) is also resolvable into two components, yet x-ray diffraction data shows the molecular structure of collagen to be solely PII (64). In their infrared spectroscopic study of undenatured bovine Achilles tendon collagen, Susi et al. similarly recorded an amide I band resolvable into

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2 Swiss Protein Database = Swiss-Prot # P12109
two peaks, which they attributed to two populations of carbonyl groups within the structural repeat unit (70). The 1631 cm⁻¹ peak of the PHFs may similarly arise from heterogeneity in carbonyl binding.

**PII Structure predicted for PHFs.** The vibrational spectroscopic evidence presented here strongly supports the assignment of a PII structure to PHFs. Phosphotungstate staining results for the PHFs also supports the assignment of PII structure to PHFs as the fibrils have been shown to consist of subunit domains of greater and lesser stain inclusion, corresponding to fibril domains of greater and lesser hydrophilicity (7). The assignment of polyproline II secondary structure to PHFs is not excluded by Congo red staining since dense collagen fibers, known to possess polyproline II secondary structure, are also stained by Congo red (71).

**Factors Effecting Secondary Structure.** The FTIR spectroscopic results presented here show that peptide domains culled from a longer protein will not necessarily fold into the same secondary structure, and that the physical process applied to gelling peptides will effect the structure (31). The apparent discrepancy between reported CD results for various tau constructs (72-75) suggests that solution phase secondary structure can also depend on one or more factors such as protein concentration (76), time, the construct used and ionic strength (73). Also, CD and other spectroscopic techniques have demonstrated that many proteins classified as 'random coil' in fact possess PII structure (46,69,77-80). Indeed, the CD spectrum for the tau construct K12, which is reported as similar to results for full length tau, exhibits a strong, negative peak at ~198 nm. and is negative over the 190-250 nm. spectral window (76). A similar CD result has been reported for collagen I (80).

**Pathological Tau Gelation within the Cytomatrix and the Contractility of Dendrites.** Previous studies of tau fibrillation have emphasized the kinetics of the process (81,82), yet concepts from
polymer science have not been applied. As for collagen I in the extracellular matrix and actin in the cytoskeletal matrix, spontaneous fibrillogenesis or self-assembly is preceded by a gelation phase (23). Prefibrillar amorphous plaques and the masses of tangled tau fibrils seen in vivo, as well as the formation of a tau film from a dissolved PHF solution reported here, suggest a gel precursor for PHFs. Indeed, a reticulated structure, seen in the tau (2-19) gel electron micrograph in Fig. 2, spectrum b, is also seen in an image of PHFs incubated at 90°C for five minutes (73) and in that of the cytomatrix (2).

The gel state is characterized by processes that are ongoing beyond the initial gelation event. These may include reprecipitation and separation into solid-liquid or solid-solid phases (61). The five year incubation period leading to the development of syneresis within the nonstirred tau (2-19) peptide concurs with the decades timeframe for development of tau neurofibrillary tangles in vivo, supporting the idea that tau fibrillation occurs via a gel curing process. Comparison of collagen I with tau and PHFs also suggests a solution to the paradox of tau fibrillation: the hydrophilicity of tau seems to countermand its fibrillation. Yet the assembly of hydrophilic collagen I into a triple helix is spontaneous and endothermic, thermodynamically driven by the increase in entropy accompanying the loss of bound water (23).

Cellular differentiation entails a delicate balance of transmembrane signalling and adhesive forces between the cytoskeletal matrix and the extracellular matrix (83). On the outside of the cell membrane, the extracellular matrix controls the structure of the cytomatrix and therefore, controls cell shape (19), yet within the cell, the development of cellular protrusions involves rapid and reversible sol-gel transitions of the actin and cytoskeleton aided by an array of auxiliary proteins (23,84). Microtubules appear to stabilize specialized cell extensions by counterbalancing the mass force produced in the cytoskeleton (85), and are involved in actin cytoskeletal organization. (13).
The microtubules and actin microfilaments act as a single integrated lattice with built-in redundancy such that the load-bearing function of the cytomatrix may be maintained when the functioning of either element is compromised (12,19,86). Indeed, microtubule disassembly alone does not prevent dendrite growth as the use of extracellular matrix as a cell adhesion substrate, in the absence of microtubules, is sufficient to induce dendrite elongation in PC12 cells (86). Apparently, dendrite growth on extracellular matrix results in less internal cellular tension, diminishing the need for the compressive support of microtubules (86). However, cell spreading is totally halted when microtubule and actin microfilament function is simultaneously compromised (19). Since distal, followed by proximal, dendritic processes are found to retract as neurofibrillary tangles appear (11), both microtubule and actin microfilament function must be compromised in AD. This suggests a nonspecific method of action for the tau plaques.

From a purely biophysical perspective, PC12 dendrites and chick dorsal root ganglion neurons have been found to behave as viscoelastic solids under applied forces (87). Generation of low levels of cellular tension and the ensuing retraction response is the reverse of growth, which requires higher levels of cellular tension (87,88). This cellular tension model has been used to explain the complete retraction of severed dendrites in PC12 and chick sensory cells (87).

The gelation-associated processes of syneresis and fibrillation potentially generate cytoplasmic forces that are responsible for the dendritic retraction seen in AD. It is foreseen that the process is set in motion by the permeation of the cytomatrix gel by phosphorylated tau, which subsequently gels to form an enmeshed network of tau gel and cytomatrix gel. The initial effect of an increase in cytoskeletal viscosity may be to retard the sol-gel transitioning of the cytoskeletal network and therefore, to interfere with signalling pathways. Indeed, cognitive impairment in AD has been shown to precede tau fibril formation (9,10).
Subsequent tau gel syneresis, followed by fibrillation and the accompanying loss of bound water, leads to mass contraction of the tau plaque. This contraction constitutes the application of an unmitigated, pathological force on both the actin microfilaments and microtubules of the cytomatrix. Studies of the effect of compressive forces in dendrites have shown that the force applied regulates microtubule polymerization (14). An unmitigated force is expected to lead to massive microtubule depolymerization, denying specialized cell extensions the necessary mechanical support (89). The stress force generated by tau gel contraction and fibrillation may also destabilize the balance of tensile and adhesion forces across the actin-transmembrane integrin-extracellular matrix bonds. The expected outcome is dendritic retraction and cell rounding, as cytoskeletal supports of cell shape will have been compromised. The delicate balance of tensile forces between the cytomatrix and the extracellular matrix is thus destroyed by the matching of the collagogenic extracellular matrix with an intracellular collagen-like network of tau. This scenario, where cellular differentiation is reversed and cytomatrix dynamics are fouled, is predicted to lead to neuronal death.

**Conclusion**

This vibrational spectroscopic comparison of tau PHFs with collagen I and polyproline has demonstrated that the secondary structure of PHFs is polyproline II. This structural comparison also leads to an explanation for the paradox of how an hydrophilic protein such as tau could spontaneously form fibrils: as for collagen I, fibrillation may be thermodynamically driven by the entropy gained as hydrogen-bonded water is freed. The N-terminal tau (2-19) peptide has been shown to form a hydrogel with distinct spectroscopic markers when aged for years. The spectroscopic characteristics of this tau peptide gel may be approximated on a shorter time scale by vigorous agitation and partial dehydration. A common structural feature for non-dehydrated tau (2-19) gel as well as PHFs, collagen I gel and insoluble collagen I is weak
hydrogen bonding at the carbonyl of proline residues. Several additional threads of evidence point to a pathological, sequential mechanism of gelation, syneresis and fibrillation for tau in AD. It is hypothesized that phosphorylated tau intercalates into the cytomatrix and enmeshes it by formation of its own gel structure. The resulting tau matrix, beyond the control of the cell, is thought to interfere with the dynamic rearrangement of the actin microfilaments, and related signalling pathways. Subsequent syneresis and fibrillation of the tau gel produces intracellular stress forces, overwhelming the supportive cell scaffolding elements of microtubules and actin microfilaments, along with their integrin-mediated bonds to extracellular collagen I. The retraction of neuronal dendrites, as found in preaptotic neurons, is predicted.
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Figure 1. UVRR spectra (229 nm) for tau (2-19) in various phases. a. five year old cured gel originally prepared at 6 mM concentration (3 x three minute acquisitions) b. difference spectrum where the minuend is the gelatin spectrum, a, and the subtrahend is c. the spectrum for the C-terminally biotinylated tau in aqueous solution (3 x three minute acquisitions). d. gelatin dissolved in 3 M lithium thiocyanate (final concentration) (4 x three minute acquisitions). e. aggregate; film spectrum is identical (Data not shown; 5 x three minute acquisitions for each).

Figure 2. Electron micrographs of tau (2-19) in two different phases, 20K magnification, 1% uranyl acetate stain: A. aggregate (negative staining) and B. aged or cured gel (positive staining).

Figure 3. Detail of UVRR results (1520-1650 cm⁻¹) for tau (2-19) under different conditions. a. five year old cured gel b. cloudy gel at 10 mM vigorously stirred for 19 hours at room temperature c. gel (10 mM) heated to 95°C for five days d. 10 mM solution at pH 10.5.

Figure 4. UVRR (229 nm.) for tau (2-19) gels formulated under different conditions. a. nonstirred, clear, nine month old gel at 10 mM stored at 4°C b. cloudy gel at 10 mM stirred vigorously for 19 hours at room temperature c. sample in b. partially dehydrated under a nitrogen stream for 40 minutes at room temperature. Curvefitting results for the high frequency peaks are shown as dashed line curves.

Figure 5. FTIR spectra of tau (2-19) gels in D₂O; 100 scans, 4 cm⁻¹ resolution with inverted second derivative spectra, below. a. one week old gel, non-stirred process and b. gel vigorously stirred for 18 hours followed by dehydrating under a nitrogen gas stream for one hour.
Figure 6. UVRR spectra (229 nm) **a.** bovine skin gelatin prepared at 50 mg/ml in deionized water (3 x three minute acquisitions) and **b.** two month old tau (2-19) gel prepared at 10 mM concentration in deionized water (1 x three minute acquisition).

Figure 7. UVRR spectra (229 nm) **a.** bovine Achilles tendon collagen type I (25 mg/ml) suspended in Tris buffered saline (3 x three minute acquisitions) and **b.** tau PHFs (2 mg/ml) suspended in Tris buffered saline (10 x three minute acquisitions).

Figure 8. FTIR spectra of nonglobular proteins, 4 cm\(^{-1}\) resolution  
**a.** PHFs, 1000 scans, potassium bromide pellet. Curves fitted to the 1552 and 1631 cm\(^{-1}\) peaks are shown as dashed lines  
**b.** polyproline, 100 scans, potassium bromide pellet, heavy solid line; paired helical filaments dialysed from LiSCN solution, 100 scans, potassium bromide pellet, solid line, and bovine skin gelatin gel in D\(_2\)O, 100 scans, dashed line.  
**c.** insoluble bovine Achilles tendon collagen, type I, 100 scans, potassium bromide pellet. Curves fitted to high frequency peaks are shown as dotted lines.

Figure 9. Absorption spectra (10 mM, 0.5 mm path; normalized at 190 nm.) for tau (309-26), solid line; tau (2-19) with C-terminally linked biotin, dashed line and 6 month old tau (2-19) gel, non-stirred process, heavy solid line.
Table 1. Vibrational Band Frequencies (cm\(^{-1}\)) with Residue Attribution for Tau (2-19) Phases*

| Cured Gel | Solution | Dissolved Gel | Film or Aggregate | Attribution\(^a\) |
|-----------|----------|---------------|-------------------|------------------|
| 1168      | 1168     | 1168          | 1168              | Y9a'             |
| 1178      | 1178     | 1182          | 1182              | Y9a              |
|           |          |               |                   | F9a              |
| 1196      | 1196     | 1196          |                   | Y7a'             |
| 1208      | 1208     |               |                   | Y7a              |
| 1218      |          |               |                   |                  |
| 1238      | 1238     | 1238          |                   | C\(_\alpha\)H    |
| 1298      | 1302     | 1302          | 1302              | amide III        |
| 1384, 1397| 1384     |               |                   | amide S          |
| 1445      | 1445     | 1445          |                   | imide II         |
| 1550      | 1551     | 1555          | 1555              | amide II         |
| 1579      | 1579     | 1583          | 1583              | Y8b', amide II, F8b |
| 1600      | 1600     | 1602          | 1602              | Y8b, Y8a', F8a   |
| 1616      | 1616     |               |                   | Y8a              |

*Corresponds to UVRR results for tau (2-19) phases given in Fig. 1a, c-d, respectively.

\(^a\)See refs. (36-43)
Comparative vibrational spectroscopy of intracellular tau and extracellular collagen I reveals parallels of gelation and fibrillar structure

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