Aggregation of a Crucial Fragment of the Tau Protein: Implications for Alzheimer’s Disease

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Outline

- Introduction to the tau protein and its role in Alzheimer’s Disease
- Evidence of aggregation
- Experimental procedure
- Preliminary results
- Conclusions and future work
The Tau Proteins

- Abundant in central nervous system
- Microtubule-associated proteins (MAPs)
  - Promote assembly of tubulin into microtubules
  - Provide structural support for assembled microtubules
- Six tau isoforms ranging from 352 to 441 amino acids long
  - Either three or four repeat regions that bind microtubules in the C-terminal half (R)
  - Either zero, one, or two amino acid insert regions in the N-terminal half (N)
  - Named accordingly (4R/2N isoform)
Primary Structure of Tau (4R/2N)

Proline-rich region has additional microtubule interactions and phosphorylation sites

N-terminal region may interact with other elements of the cytoskeleton

C-terminal repeat region binds microtubules
Alzheimer’s Disease

• One of many neurodegenerative tauopathies
  • Down’s syndrome
  • Creutzfeldt-Jakob disease

• Characterized by the accumulation of two different types of insoluble proteins:
  • Extracellular plaques of amyloid protein
  • Intracellular lesions of tau protein
    • Lesions comprised of abnormally- or hyper-phosphorylated tau
    • Hyperphosphorylation results in self-assembly into tangles or lesions of paired helical filaments (PHFs)
Alzheimer’s Disease

Two possibilities for harmful effects of tau lesions:

- **Loss of positive function**
  - Inability to bind microtubules diminishes nerve function

- **Gain of toxic function**
  - Lesions are harmful to brain function

If the tau gene is suppressed in laboratory mice, other proteins bind the microtubules instead, with no loss of nerve function. This suggests a **gain of toxic function**.

Human Tau 441

MAEPRQEFEVMEDHAGTYGLGDRKDQGGGYTMHQDQEGD
TDAGLKESEPLQTPTEDGSSEEPGSETSDAKSTPTAEVDVTAPL
VDEGAPGKQAAAYQPHTEIPEGTTAEEAGIGDTPSLEDEEAAG
HVTQARMVSKSKDGTGSDDKKAKGADGKTKIATPRGAAPP
GQKGQANATRIPAKTPPAKTPPSSSEPPKSGDRESGYSSPG
GSPGTPGSRSRTSPSLPTPTPPTREPKKVAVVVRTPPKSPSSAKS
RLQTAPVPMPDLKNVKSIGSTENLKHQPGGGKVIINIKNKD
LSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDSLKVTSKCGS
LGNIIHHKPGGGGVQEVKSEKLDKDFKDRVQSKIGSLDNITHVPG
GGNKKEIETHKLTFRKRENAKAEMKTDHGEAEIVYKSPVVSNGDTSPR
HLSNVSTGSIDMVDSQPQLALTALADEVSASLAKQGL

C-terminal repeat regions that bind microtubules
Six residues shown to be essential for aggregation

Human Tau 441

MAEPRQEFVMEHAGTYGLGDRKDQGGGYTMHQDQEGD
TDAGLKEPLQTPTEDGSEEPGSESTDAKSTPTAEDVTAPL
VDEGAPGKQAAAAPHTEIPEGTTAAEEAGIGDTPSLEDEAAG
HVTQARMVSKSKDGGTSDKKEAKGADGKTKIATPRGAAPP
GQKGQANATRIPAKTPPAPKTPPSGGEPPKSGDRSGYSSPG
GSPGTPGSRSRTPSLPTTPTREPKKVAVVRTPKPSPSSAKS
RLQTAPVPMPLKNVKSIGSTENLKHQPGGGKVIINKKLD
LSNVQSKCGSKDNIKHVPQGGGSVQIVYKPVDSLKVTSDKCS
LGNIHHPGGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPG
GGNKKIEKTHKLTFRENAKAKTDHGAEIVYKSPVVSGBPDTSPA
HLSNVSTGSIDMVDSQPLATLADERVSASLAKQGL

13 residues studied for completeness
13 residues between 2\textsuperscript{nd} and 3\textsuperscript{rd} repeat regions
Aggregation of 13mer

From Dr. John Lew’s group

- Add Thioflavin S, a fluorescent dye
  - Binds well to structured aggregates and PHFs
  - Binds poorly to monomers or unstructured aggregates
  - Only fluoresces when bound
- Heparin induces aggregation
  - The peptide will aggregate without heparin, but much more slowly
- Monitoring fluorescence gives a measure of aggregation
  - Very sensitive technique
Intermediates? Extent of aggregation?
Experimental Methods

Electrospray Ionization Drift Cell Mass Spectrometer

- ESI Source
- Ion Funnel
- Drift Cell
- MS
- Detector

Diagram:
- nano-ESI
- Ion Funnel
- Drift Cell
- Quadrupole
- Detector
Experimental Methods

- Pulse of ions separated by shape
- ATD
- Arrival time

~5 torr He
Weak electric field

Kinetic theory:

Arrival time → Mobility → Cross section (σ)
The drift cell can also separate oligomers with the same charge state.

If a dimer$^{+2}$ was exactly twice as big as a monomer$^{+1}$, the ions would have the same mobility and exit the cell at the same time.

Generally, there are favorable interactions between the two molecules that pack a dimer closer together than the sum of two monomers.

Drift times: \( \text{trimer}^{+3} < \text{dimer}^{+2} < \text{monomer}^{+1} \)
Computational Methods

- Molecular dynamics
- Simulated annealing
- 100-200 candidate structures
- Projection cross section of each structure
Experimental Conditions

- 13-residue peptide with capped ends
  - N-terminal acetylation
  - C-terminal amidation
- 1 mM ammonium acetate buffer
- 100-500 μM tau
- 10-50 μM heparin (10:1 tau:heparin)
- Variations in waiting time and temperature
Results

Tau without heparin

Tau with heparin
Results: $m/z = 1520$

With and without heparin

$SVQIVYK^+PVD-LSK^+$

$[\text{tau}]^{+1}$

Different conformations of the same species, or different species?
Results: m/z = 1520

- Peak assignments: injection energy studies
  - Higher injection energy: larger species are dissociated by the force of entering the drift cell
  - Relative intensity of peaks shifts towards smaller species (monomers) at high injection energy, larger species (trimers) at low injection energy
Results: m/z = 1520

Injection Energy

40 V

50 V

60 V

70 V

80 V

90 V
Results: m/z = 1520

Peak Height vs Injection Energy

Trimer peak most prevalent at lower injection energy as expected
Results: m/z = 1520

With and without heparin

\[
\begin{align*}
226 \text{ Å}^2 & \\
255 \text{ Å}^2 & \\
318 \text{ Å}^2 &
\end{align*}
\]

\[\text{SVQIVYK}^+\text{PVD}^-\text{LSK}^+\]

\([\text{tau}]^{+1}\]

Calculate cross sections for every species with a charge of +1
Calculations: m/z = 1520

Experimental cross sections: 226, 255, 318 Å²

Cross section vs Energy

SVQIVYK+PVD-LSK+

[tau]+1

Average of lowest 10 kcal: 316 Å²
Lower limit: 299 Å²
Upper limit: 345 Å²

σ = 317.5 Å²
Results: m/z = 1520

SVQIVYK+PVD-LSK+

[tau]+1

Exp: $\sigma = 318 \text{ Å}^2$

Calc: $\sigma = 316 \text{ Å}^2$
Results: m/z = 1520

$\text{SVQIVY} \text{K}^+ \text{PVDLSK}^+$

$\text{SVQIVY} \text{K}^+ \text{PVDLSK}^+$

$[2\text{tau}]^+^2$

Calculate cross sections for every species with a charge of +2

Double the charge, double the cross section
Calculations: m/z = 1520

Experimental cross sections: 451, 510 Å²

Cross section vs Energy

[2tau]+²

Average of lowest 20 kcal: 487 Å²
Lower limit: 458 Å²
Upper limit: 543 Å²

σ = 494.7 Å²
Results: m/z = 1520

With and without heparin

\[
\begin{align*}
& SVQIVYK^+PVDLSK^+ \\
& SVQIVYK^+PVDLSK^+ \\
\end{align*}
\]

\([2\text{tau}]^+\]

Exp: \(\sigma = 510 \, \text{Å}^2\)

Calc: \(\sigma = 487 \, \text{Å}^2\)
Results: m/z = 1520

With and without heparin

\[\text{SVQIVYK}^+\text{PVDLSK}^+\]
\[\text{SVQIVYK}^+\text{PVDLSK}^+\]
\[\text{SVQIVYK}^+\text{PVDLSK}^+\]

\[\text{[3tau]}^+^3\]

Exp: \(\sigma = 677 \text{ Å}^2\)
Results: $m/z = 760$

$[\text{tau}]^{+2}$

SVQIVYK$^+PVDLSK^+$

Tau without heparin

Exp: $\sigma = 328$

Wider than one conformation
Results: m/z = 760

\[ \text{[tau]}^+^2 \]

\text{SVQIVYK}^+\text{PVDLSK}^+

Tau without heparin

Exp: \( \sigma = 328 \)

Tau with heparin after several weeks

Exp: \( \sigma = 270, 345 \)
Calculations: \( m/z = 760 \)

Experimental cross sections: 270, 328, 345 Å\(^2\)

Cross section vs Energy

\[ \sigma = 327.0 \text{ Å}^2 \]

Sequence: SVQIVYK+PVDLSK+

\([\text{tau}]^+2\)

Average of lowest 10 kcal: 324 Å\(^2\)

Lower limit: 302 Å\(^2\)

Upper limit: 383 Å\(^2\)
Results: m/z = 760

\[\text{[tau]}^{+2}\quad SVQIVYK^{+}PVDLSK^{+}\]

Tau without heparin

Exp: \(\sigma = 328\)
Calc: \(\sigma = 324\)

Tau with heparin after several weeks

Exp: \(\sigma = 540, 345\)
Results: $m/z = 760$

- $+1$: 318
- $+2$: 328
- 510
- 540
Conclusions

- Adding heparin has no immediate observable effect on the sample.
- Peaks in ATD of m/z = 1520 suggest aggregation before addition of heparin.
- Concentrations of tau and buffer seem to have no effect.
- Due to the drastically different timescale (10 minutes vs 7 weeks) the two observed aggregation events appear to be different.
Future Work

- Calculations for the \([3\text{tau}]^{+3}\) and \([2\text{tau}]^{+4}\) species
- Reproduce additional peak in ATD of \(m/z = 760\)
- Look at binding of Thioflavin S with the tau peptide
- Other aggregation inducers
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Experimental Methods

Varying the drift voltage yields a plot of arrival time vs P/V.

The slope of this line is inversely proportional to the ion's reduced mobility, $K_0$.

$$t_d = \frac{L}{v_d} = \frac{L}{K \cdot E} = \frac{L^2 T_0}{K_0 P_0 T V}$$

The mobility of the ion is related to its collision cross section, $\Omega$.

$$K_0 = \frac{3e}{16N_0} \left(\frac{2\pi}{\mu kT}\right)^{1/2} \frac{1}{\Omega}$$