Simulating and Modeling Tau Protein Aggregation

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Abstract

Alzheimers and many other neurodegenerative diseases are associated with the pathological aggregation of tau protein in the human brain. The mechanism by which tau aggregates into fibrils and neurofibrillary tangles (NFT) remains unclear.[1-8] To further understand this fibrillization process, we studied the fibrillization of L-Phenylalanine. Others have shown that Phenylalanine aggregates comparably to tau into paired helical filaments (PHFs) in de-ionized water at room temperature.[6] Transmission electron microscopy (TEM) was used to confirm the formation of such Phenylalanine fibrils. Samples of varying concentrations were also tested with SDS gel electrophoresis and OD spectroscopy to assess the effectiveness of these methods in quantifying aggregation.

Properties of Tau Protein

- Sequence of 758 AA's →
- 135 potential phosphorylation sites
  - (79 Ser, 6 Tyr, and 50 Thr)
- Microtubule-Associated Protein (MAP)
- Has 6 isoforms:
  - 3 isoforms with 3 MTBRs (tau 3R) and 3 isofrms with 4 MTBRs (tau 4R)

- [VQIVYK] = PHF6, a sequence of tau that aggregates into amyloid fibrils the same as the whole[3-5]
- [F] = Phe, shown to aggregate into amyloid fibrils as well[6]

MAEPRQE-[F]-EVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDA
GLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQ

PHTEI PEGTTAEEAGIGDTPSLEDEAAGHVTQEPESGKVVQEG-[F]-LRE
PGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEGGRHAPELL
KHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKAS

PAQDGRPPQTAAREATSIPGFPAEGAIPLPVD-[F]-LSKVSTEIPASEPDGP
SVGRAKGQDAPLEFTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEAR
GPSLGEDTKEADLPEPSEKQPAAAPRGKPVSRVPQLKARMVSKSKDGTGSD
DKKAKTSTRSSAKTLKNRPCLSPKHPTPGSSDPLIQPSSPAVCPEPPSSPKY
VSSVTSRTGSSGAKEMKLKGADGKTKIATPRGAAPPGQKGQANATRIPAKTP
PAPKTPPSSGEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVA
VVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTENLKHQPGGGKVQIIN

KKLDLSNVQSKCGSKDNIKHVPGGGS-[VQIVYK]-PVDLSKVTSK

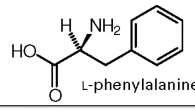
CGSLGNIHHKPGGGQVEVKSEKLD-[F]-KDRVQSKIGSLDNITHVPGGGN

KKIETHKLT-[F]-RENAKAKTDHGAE IVYKSPVVSGDTSPRHLSNVSSTGS
IDMVDSPQLATLADEVSASLAKQGL

Quantifying [F] Fibrillization

Four techniques commonly used to measure tau polymerization:[10]

1) Thioflavin S (ThS) flourescence
2) electron microscopy
3) centrifugation
4) laser scattering



Since we were studying Phenylalanine aggregation as a model system, as opposed to the tau protein, we explored other methods to quantify fibrillization:

OD Spectroscopy @ 600 nm

Time (min)	F (1 mM)	F (6 µM)	Blank (Control)
0	0.003	0.001	0
15	0.005	0.005	-0.002
30	0.001	0	0.002
45	0.009	0.011	0.003
60	0.013	0.004	0
75	0.01	0.007	0.002
90	0.007	0	0
105	0.01	0.008	0.002
120	0.008	0.001	0.001

Solution samples of 1 mM and 6 µM were prepared with L-Phenylalanine and water and then incubated for 2 hours at 25 °C. The optical density at 600 nm of these samples was then measured every 15 minutes for 2 hours. The hypothesis was that as time progressed, we would see an increase in optical density as the Phenyalanine fibrillized. We did not see an increasing trend as expected.

SDS Gel Electrophoresis

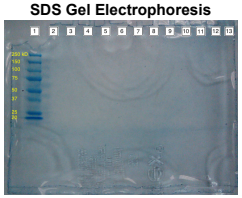


Figure 3: Column 1 contained the control scale and columns 3,5,7,9,11,12, and 13 contained samples of concentration 6µM, 12µM, 50µM,100µM, 30mM, 60mM, and 120 mM each incubated . The hypothesis was that SDS gel would separate aggregated fibers of different lengths. It was expected that samples of higher concentration would have longer fibers and a difference would be detected by SDS. The gel was run for 45 min at 109 V and stained with Coomassie to view bands. The gel did not detect any fibers in any of the concentrations (hence you see no bands in any columns besides the control.

General Mechanism for Tau Aggregation

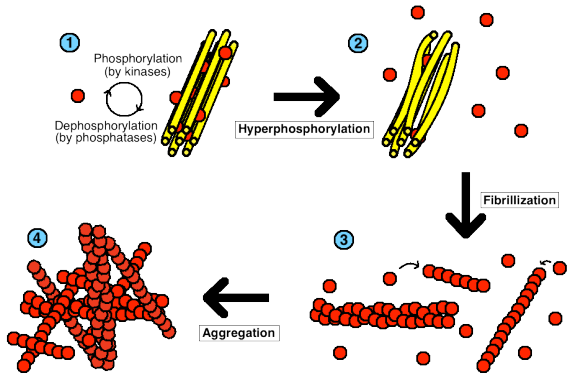
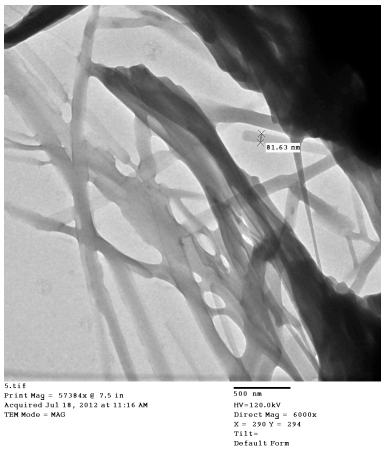


Figure 1: This represents what is currently considered the process of Tau aggregation in Alzheimers Disease. **1)** Shows the normal case, where tau proteins (red circles) are in a dynamic state of binding and unbinding to microtubules of CNS axons (yellow rods) by phosphorylation and dephosphorylation. **2)** Hyperphosphorylation causes a majority of tau proteins to unbind from the microtubules, compromising the axon's stability and structure. **3)** The high concentration of unbound tau leads to fibrillization into paired helical and single filaments (PHFs and SFs). **4)** The fibers then aggregate into hydrophobic neurofibrillary tangles (NFTs), which compose lesions in diseased brains.[1,5,8]



• **Figure 2:** TEM image of a 120 mM sample of Phenylalanine dissolved in ddH2O. The sample had been incubating at room temperature for 6 days prior to imaging. A 10 µL aliquot was placed on a 200 mesh copper grid. Once dry it was then stained with 2% uranyl acetate and dried. Images were taken using a JEOL JEM-2010 electron microscope operating at 120 kV.

Computational Model in Progress

$$nA_1 \underset{k_{n-}}{\overset{k_{n+}}{\longleftrightarrow}} A_n \qquad A_1 + A_i \underset{k_{e-}}{\overset{k_{e+}}{\longleftrightarrow}} A_{i+1}$$

$$\frac{dc_1}{dt} = -2(k_{n+}c_1^2 - k_{n-}c_2) - \sum_{i=2}^{N}(k_{e+}c_1c_{i-1} - k_{e-}c_i)$$

$$\frac{dc_2}{dt} = (k_{n+}c_1^2 - k_{n-}c_2) - (k_{e+}c_1c_2 - k_{e-}c_3)$$

$$\frac{dc_i}{dt} = (k_{e+}c_1c_{i-1} - k_{e-}c_i) - (k_{e+}c_1c_i - k_{e-}c_{i+1})$$

$$\vdots$$

$$\frac{dc_N}{dt} = (k_{e+}c_1c_{N-1} - k_{e-}c_N)$$

- Based on the work of Congdon et al. (9)
- Models tau monomers polymerizing into fibers of length n
- Assumes reversible association of monomers with rate constants k_{n+} , k_{n-} , k_{e+} , and k_{e-}
- Sets up a system of N differential equations, where each equation is the concentration of fiber length *i*

Future Focus:

- The solution is a matrix of Conc. values where each row represents C's at a give time and each column represents C's of different fiber lengths.
- Congdon et al. studied the frequency of C's of different fiber lengths, but we would like to focus on how the maximum length N affects the time of aggregation and equilibrium

Fiber length →
Time ↓

$$\begin{bmatrix} C_{1,t=0} & C_{2,t=0} & C_{3,t=0} & \cdots & C_{n,t=0} \\ C_{1,t=1} & C_{2,t=1} & C_{3,t=1} & \cdots & C_{n,t=1} \\ \vdots & \vdots & \vdots & & \vdots \\ C_{1,tf} & C_{2,tf} & C_{3,tf} & \cdots & C_{n,tf} \end{bmatrix}$$

Discussion

Tau has 5 phenylalanine AA's so understanding the interactions that cause Phenylalanine to aggregate could lead to better understanding of how tau as a whole aggregates. Association of Beta sheets has been implicated in formation of protein aggregates and fibrils and aromatic AA's, specifically adjacent Phe AA's, are known to accelerate amyloid assembly.[6]

TEM microscopy confirmed that Phenylalanine aggregates into fibrils. However, OD spectroscopy and SDS Gel techniques proved unsuccessful. Factors that could have caused these results include sample concentrations, incubation time, and breaking up of fibrils when transferring solution. In the future it would be valuable to conduct a more in-depth study how fiber length is dependent on concentration and incubation time to compare with the computational model above. It would also be valuable to further study if and how sequences, such as PHF6, would aggregate differently if Phenylalanine AA's are included.

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