Terminal capping of an amyloidogenic Tau fragment modulates its aggregation propensity

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Abstract:
The aberrant aggregation of protein into oligomers and eventually into amyloid fibrils is associated with more than 50 human diseases. Usually, a small fragment of a big protein still keeps similar aggregation propensity, which makes it an ideal model to study protein aggregation. Paired helical filament 6 (PHF6) is part of Tau protein which drives the formation of amyloid fibrils. One important factor that is overlooked when using peptide fragment to study full length protein is the charges on the termini of these proteins. In this study, bead-string model and Monte Carlo simulation were applied to study the aggregation propensity of four PHF6 with different capping. The average number of contacts atoms were calculated for amino acid in different spots of PHF6 and for 4 different types of amino acids: positively charged, negatively charged, hydrophobic and hydrophilic. No obvious differences in aggregation propensity among the 4 PHF6 peptides under this study. Bead-string model isn't ideal to study peptide aggregation in this case.

Introduction:

Proteins misfolding into oligomers and later into amyloid fibrils are associated with many neurodegenerative diseases. The primary structure of protein is important in the formation of amyloid fibrils. In many cases, a small segment of a big protein is able to self-aggregate into amyloid fibrils and drive the whole protein to aggregate. Therefore, understanding the aggregation of protein fragment is important in the study of amyloid diseases.

Many factors will affect the aggregation propensity of a protein fragment such as primary structures, side chain interactions and solvent condition. However, the effect of the termini of a peptide fragment is overlooked. The charges on the termini will change the overall charge, hydrophobicity, electrostatic interactions, and the propensity of the peptides toward secondary structure. Therefore, understanding the effect of terminal charge can help us choose a better peptide fragment model to study full length peptide aggregation.

Paired helical filament 6 (PHF6) is an amyloidogenic peptide fragment of microtubule associated Tau protein. Tau protein can form intracellular neurofibrillary tangles, which is the pathological hallmark of Alzheimer's disease. Full-length Tau protein is intrinsically disorder and has four imperfect repeats (R1-R4) in the C-terminal domain. R3 is the only repeat that appears in all Tau isoform and affect Tau's ability to form tangle. A recent study shows that PHF6 from the R3 forms the first β -strand of Tau filament core, which indicates that PHF6 plays an important role in Tau aggregation. Most researches study PHF 6 and Tau under different solution condition, but the influence of the termini charges is overlooked. Therefore, studying the aggregation propensity of PHF6 with four different termini charges is needed to understand the effect of termini charges in Tau aggregation.

Method:

Four PHF6 peptides (Table.1) with different termini charges were studied using bead-string model and Monte Carlo simulation. Each amino acid and termini were considered as a bead. Four different kinds of beads were used to represent four different kinds of amino acids, which are positively charged, negatively charged, hydrophobic and hydrophilic.

Table 1. PHF6 under study

Annotation	Primary Structure
PHF6	$^{+}H_3N$-VQIVYK-COO^{-}
Ac-PHF6	CH_3CONH-VQIVYK-COO^{-}
PHF6-NH_2	$^{+}H_3N$-VQIVYK-$CONH_2$
Ac-PHF6-NH_2	CH_3CONH-VQIVYK-$CONH_2$

For two amino acids that are connected to each other, Hooke's law (1) was used to describe the covalent bond. For non-covalent interactions, screen Coulomb potential (2) was used between charged amino acids. Lennard-Jones potential (3) was used between hydrophobic-hydrophobic interactions and Weeks- Chandler-Andersen (WCA) potential (4) was used between hydrophilic-hydrophilic interaction and interactions between different types of amino acids.

$$u(r_{ij}) = \frac{1}{2} k (r_{ij} - r_0)^2 \qquad (1)$$

$$\frac{\lambda_B q_1 q_2}{r_{ij}} \exp\left[\frac{-r_{ij}}{\lambda_D}\right] \qquad (2)$$

$$4\times 5\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right] \qquad (3)$$

$$4\times \varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right] \qquad (4)$$

k represents bond strength and r0 represents equilibrium bond length, which are set to 236 and 3.5, respectively. r_{ij} represents the distance between particle *i* and *j*, and σ is the particle diameter (set equal to 2.5 Å based on the average size of amino acids within the peptide backbone not including the R group). λ_D is the screening length, while λ_B is the Bjerrum length (7 Å in water at room temperature), and q_1 and q_2 represent the residue charges (set to ±1). The well depth ε was set to 1 for the WCA potential while the L-J potential used to describe the interaction between hydrophobic residues in water involved an ε of 5 because the contact energies of two hydrophobic residues has been shown to range from approximately -3 to -7 k_BT with a tryptophan-tryptophan contact roughly equal to -5 k_BT. To determine the screening length, a physiologic salt concentration (10 mM NaCl) was assumed to calculate a λ_D using equation 5. [1]

$$\lambda_D(nm) = \frac{0.304}{\sqrt{I(M)}} \quad (5)$$

To save computational time, a cut-off of 2.5 σ was used for all kinds of interaction and a cut-off of 5 was used to calculate contact numbers.

25 peptides with 6 amino acids and two termini each were placed into a cubic box with length L = 8.5 to achieve a density of 0.8. The system was first minimized for 1000 steps using conjugate-gradient method, and then equilibrate for 50000 steps using Monte Carlo simulation until the contact number conversed. After the equilibration, the system underwent production for 50000 steps where the average of contact numbers was calculated.

Result and Discussion:

The average contact numbers of all four peptides are listed in table 2. Those number are very similar, indicating that under this simulation condition, the aggregation propensity of these four peptides can be differentiated or the production time was not long enough to differentiate the peptides. If we consider the minor differences, the aggregation propensity order will be: Ac-PHF6-NH_2 > PHF6 > PHF6-NH_2 ≈ Ac-PHF6.

Table 2. Average Contact Number of Peptides under Study

Peptides	Average Contact Number
PHF6	26.0
Ac-PHF6	25.9
PHF6-NH_2	25.9
Ac-PHF6-NH_2	26.1

The average contact number for an amino acid at a specific position are calculated in Table 3. There are no significant differences between different positions either. One interesting thing is that for PHF6, the contact numbers are bigger at the end of the peptide, while for the fully capped PHF6, the contact numbers are bigger in the middle of the peptides.

Table 3. Average Contact Number of a Beads at a Particular Spot (from N termini to C termini)

Peptides	0	1	2	3	4	5	6	7
PHF6	26.5	26.4	26.5	26.2	26.4	26.2	26.6	26.6
Ac-PHF6	26.5	26.0	26.5	25.9	26.6	26.1	26.3	26.1
PHF6-NH_2	26.5	26.0	26.5	25.9	26.6	26.1	26.3	26.1
Ac-PHF6-NH_2	26.4	26.3	26.8	26.6	26.6	26.6	26.4	26.2

The average contact number of peptides of four different kinds are listed in table 4. PHF6-NH_2 and Ac-PHF6-NH_2 doesn't have negatively charged residue, so the number is not available. Similarly, there is no significant difference between each peptide. The average contact number

of positively and negatively charged residues are similar, but the hydrophilic residues have average contact number slightly higher than that of hydrophobic residues.

Table 4. Average Contact Number of a Particular Types of Beads

Peptides	Positive	Negative	Hydrophobic	Hydrophilic
PHF6	26.2	26.3	26.0	26.2
Ac-PHF6	26.3	26.1	26.3	26.8
PHF6-NH_2	26.5	NA	26.3	26.7
Ac-PHF6-NH_2	26.1	NA	26.6	26.9

Conclusion:

The aggregation propensity of four PHF6 with different charges on termini were studied using bead-string model and Monte Carlo simulation. Fully capped Ac-PHF6-NH_2 seems to aggregate the most and hydrophilic residues have more interaction over hydrophobic residues. However, there is no statistically significant difference between these peptide which indicates that this simulation condition might not be propriate to study the aggregation propensity of peptides with different termini charges.

1. Serra Elliott, *Effect of Amino Acid Type on Peptide Structure*, 2012 (previous student's project)