Detecting Amyloidogenesis: Developing Probes to Label Pre-formed Amyloid Protein Fibrils

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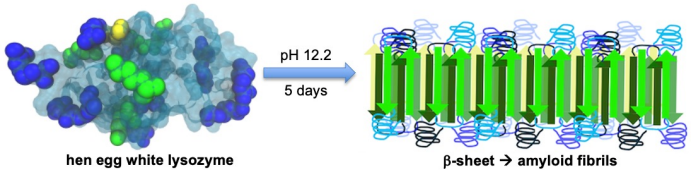
Q1: Research Question

Alzheimer's and Parkinson's Disease are progressively degenerative conditions caused by amyloidosis of proteins. Similarly, systemic amyloidosis due to human lysozyme affects essential organs, such as the liver and kidneys. I wanted to identify new fluorescent probes of amyloid fibrils that could lead to earlier diagnosis and improved patient care.

Protein aggregation to form fibrils involves burying hydrophobic groups of the protein inside of fibrils to form a hydrophobic core (below). I hypothesized that the more hydrophobic the components are within the probes, the better they will fluoresce with the amyloid fibrils.



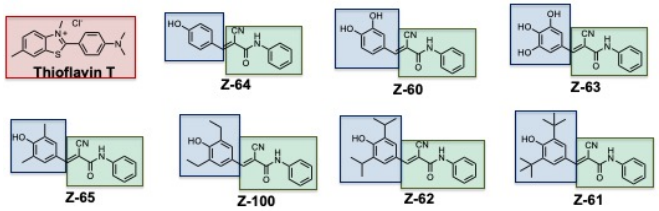
Q2: Methodology

1) Amyloid fibrils of hen egg white lysozyme (HEWL) were formed by treatment with pH 12.2 buffer for 5 days; TEM confirmed fibril formation.

2) Fibrils samples were treated with the known probe thioflavin T (ThT) or the Z compounds below. Sample fluorescence was monitored on a plate reader. Fluorescence microscopy and TEM were used to image the fibrils.

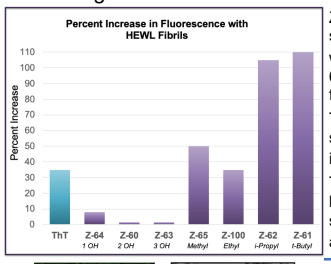
3) A rottlerin assay (known to dissolve fibrils) was used to determine if the Z compounds can be used to monitor fibril disassembly.

4) The Z compounds were evaluated to see if they can also fluorescently labeled α-synuclein amyloid fibrils.



Q3: Data Analysis and Results

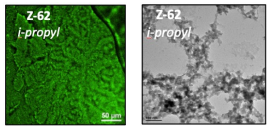
Figure 1



Z compounds with hydrophobic groups showed an increase in fluorescence with the HEWL amyloid fibrils, with Z-61 and Z-62, having ~3-times better fluorescence than ThT (Figure 1).

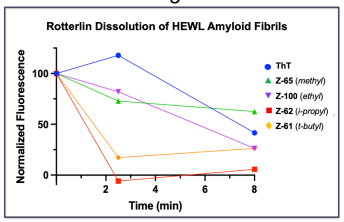
The Z compounds with 1-3 OH groups showed little or no fluorescence increase with HEWL amyloid fibrils.

The larger alkyl groups (*i*-propyl and *t*-butyl) were more effective than the smaller alkyl groups (methyl and ethyl) at binding to HEWL amyloid fibrils.



Fluorescent microscopy (left) confirmed strongly fluorescent fibrils after treatment with the probes. TEM (right) also confirmed that fibrils were still present after treatment.

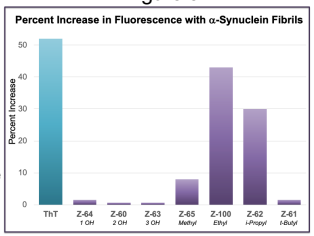
Figure 2



Rottlerin is known to rapidly dissolve HEWL amyloid fibrils. The four hydrophobic Z compounds and ThT all showed a drop in fluorescence at 2 and 8 minutes after adding rottlerin to HEWL amyloid fibrils (Figure 2).

Two Z compounds showed a fluorescence increase with α-synuclein amyloid fibrils, with Z-100 performing best (Figure 3).

Figure 3



Q4: Interpretation and Conclusion

- Overall, my hypothesis was confirmed with HEWL amyloid fibrils; the four compounds with the largest increase in fluorescence (Z-61, 62, 65, 100) had the hydrophobic groups, and the more hydrophilic compounds (Z-60, 63, 64) showed limited or no fluorescence with fibrils.
- My hypothesis was partially confirmed with α-synuclein fibrils. Two of the lesser hydrophobic compounds (Z-62 and Z-100) had an increase in fluorescence with these fibrils, whereas the more hydrophobic Z-61 and Z-65 were not very effective. α-Synuclein amyloid fibrils are known to be less hydrophobic than fibrils of HEWL, so this may explain the differences.
- My findings also showed that compound Z-61 and Z-65 were more specific for HEWL fibrils, which could be very useful to probe specific diseases caused by lysozyme amyloid fibrils, such as systemic amyloidosis.
- In the future, I would like to try other amyloid fibrils and see if this research can be mobilized for cell-based imaging experiments.