Quantifying Inhibition of Proteasomal Activity by Tau Fibrils

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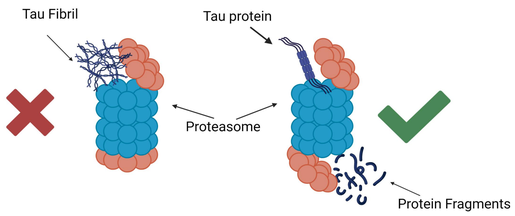
Abstract

Alzheimer's disease (AD), a neurodegenerative disease, is often caused by a build-up of Tau fibrils in the brain. In a normally functioning brain, proteasomes, large protein complexes, degrade misfolded proteins. However, in AD, misfolded Tau proteins stick together, leading to the creation of fibrils[1]. These are not able to be degraded by the proteasome, which contributes to their build-up inside neurons[1]. AD is worsened because the proteasome is not able to function properly, leading the fibrils to spread throughout the brain causing brain tissue to decrease in mass significantly[2]. In this research, we focused on Tau fibrils and how to increase proteasomal activity by degrading fibrils through sonication.

Hypothesis

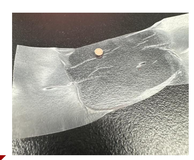
- If Tau fibrils are sonicated, then they will become smaller relative to the amount of time they are sonicated.
- Tau fibrils will inhibit the proteasomal activity depending on their size and quantity



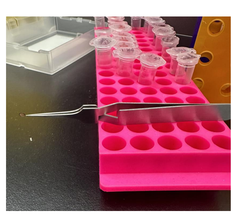
Preparing Samples for Negative Staining

1. Put droplet of sample onto a grid and wait 3 minutes

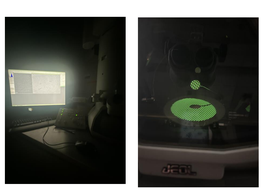
2. Dry the sample from the grid then drop the grid on a stain droplet and wait 2 minutes



3. Wipe stain from the grid and let dry for 10 minutes



4. Use transmission electron microscope to look at the sample



Breaking Down and Observing Fibrils

Sonication

The method used to break down fibrils was sonication. The water bath sonicator sent sound waves through water in order to break down the fibrils. Fibrils were divided into three categories: no sonication, one hour of sonication, and two hours of sonication.

Proteasome Activity Assay

The activity assay analyzes the amount of fluorescent activity produced during the reaction. We put our three samples (no sonication, one hour of sonication, and two hours of sonication) with proteasome into a plate with two controls: proteasome alone and Mg132 (a known inhibitor of proteasome) alone. Once in the tray, we add a substrate to each well. The cleavage of the substrate results in a fluorescence signal which is translated into proteasomal activity.

Transmission Electron Microscopy

The transmission electron microscope (TEM) works by sending electrons through a grid with a sample on it. The TEM displays the image of fibrils on a computer screen. After using the TEM, it is possible to measure the length of fibrils as well as observe the quantity of fibrils from each interval of sonication.

Results

Figure 1: TEM images at each hour of sonication

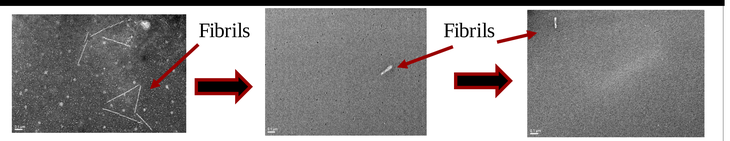


Figure 2: Activity assay of sonicated fibrils

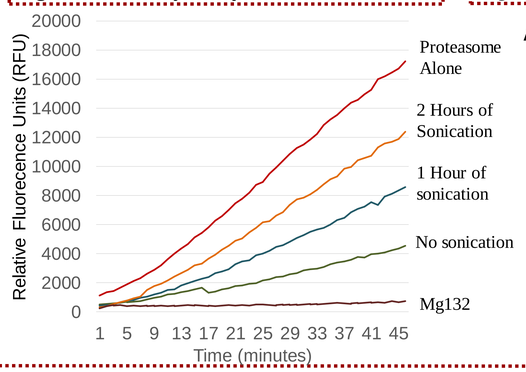
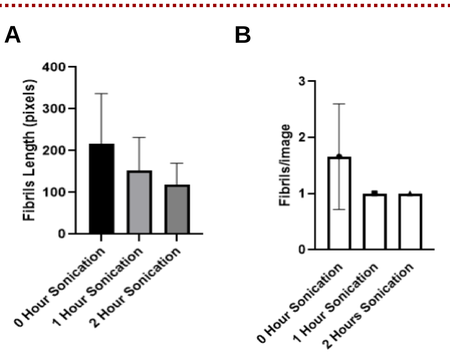


Figure 3: Effects on fibrils at each hour of sonication



1: Transmission electron microscope (TEM) images of fibrils at different sonication intervals. No sonication has many long fibrils while images of one hour and two hours of sonication have a single small fibril. **2:** Proteasome alone is a control of the highest possible RFU . Mg132 is a known inhibitor of proteasome which is why it is used as a control for lowest RFU. The longer the sonication time the more RFU because the shorter fibrils are able to be degraded by the proteasome. **3A:** With each additional hour of sonication the fibrils significantly decreased in pixel length. **3B:** When there was no sonication there was an average of 1.66 fibrils per image while there was an average of 1 fibril per image for the sonicated samples. When fibrils are sonicated, it is observed that there are fewer fibrils per image.

Summary

- Increased time of sonication leads to fibrils becoming shorter in length and fewer in quantity.
- Longer sonication times show a significant increase in proteasomal activity.
- As fibrils become shorter in length and fewer in quantity, the proteasomal activity is higher.

Conclusion

- By using the TEM, it is observed that longer sonication time leads to shorter and fewer fibrils.
- Our observations from the activity assay supports our hypothesis because it shows how proteasomal activity is higher with increased time of fibril sonication.
- The combination of the use of the TEM and the activity assay shows that as fibrils are shorter and less concentrated, the proteasomal activity greatly increases.

Future Directions

- This observation could be further supported with longer sonication times in addition to a ratio of how long it takes to find the fibrils on the TEM to show an improved representative quantity.
- Since our research shows shorter fibril length in fewer quantities increases proteasomal activity, the next step would be to find a therapeutic chemical that could disaggregate the fibrils.

References

1. *What happens to the brain in Alzheimer's disease?* (n.d.-b). National Institute on Aging. https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease#:~:text=In%20Alzheimer's%20disease%2C%20however%2C%20abnormal,the%20sthe ynaptic%20communication%20between%20neurons.
2. *Alzheimer's Disease fact Sheet*. (n.d.). National Institute on Aging. https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet

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