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Investigating the Modulation of Aggregating Amyloid Beta 40

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Investigating The Modulation of Aggregating Amyloid-Beta 40

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Abstract

Amyloid beta protein has been linked to the formation of Alzheimer's disease in patients.[1] Plaques form from amyloid beta fibrils. The formation of these plaques between neural connections in the brain are associated with Alzheimer's disease.[2] The reduction of the formation of fibrils can be linked to utilizing protein mimics. The protocols that are used to reproduce the simulation of amyloid beta in the brain can be very important. Also, the structure of the protein mimic that is being used to inhibit the formation of fibrils can determine how the amyloid beta plaques are reduced.

The structure of sequence KLLFFLFFLLK peptoid was synthesized to test with amyloid beta. The amyloid beta must first be monomerized to the desired monomer 40 or 42 which are believed to be the main amino acid residues associated with the formation of plaques.[3] This was accomplished through both 1,1,1,3,3,3 hexaprop-2-flouro treatment and fast protein liquid chromatography. The peptoid was synthesized by hand, purified by high pressure liquid chromatography, and tested by matrix-assisted laser desorption/ionization.

Background

The amyloid beta protein (Aβ) has been shown to be deposited in the brain in patients with Alzheimer's disease (AD).[1] More particularly, Aβ has been shown to be involved in almost all cases involving an AD patient. AD not only affects the patient's life, but also everyone that is involved in a patient's life or even people who interact with the patient. Today approximately 40% of people aged 65 and older have AD, and this number is only projected to increase.[1] AD is expected to at least double in the next 35 years.[1]

Aβ starts out in monomeric form in the brain.[2] For some people, the protein begins to misfold and begins to aggregate.[2] Eventually the aggregates form fibrils and plaques, which are associated with AD.[1] These plaques, which contain Aβ with 40 and 42 amino acid residues, accumulate between neural cells and were once believed to be the main cause of AD.[3] More recent studies have shown that soluble oligomers are more likely the toxic Aβ conformation.[1] A thorough understanding of the aggregation process (Figure 1) will allow for better design of AD therapeutics.

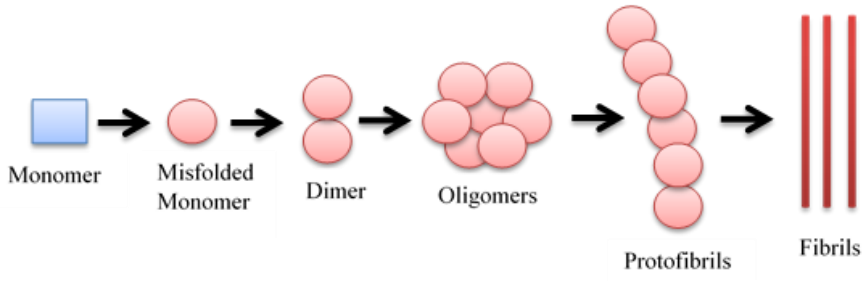


Figure 1: Illustration of the formation of fibrils in the brain from the amyloid beta precursor protein.[6]

Work in the Servoss lab focuses on using non-natural peptide mimics, peptoids, to for disease detection and treatment. A peptoid is different from a peptide because the functional group is located on the amide nitrogen rather than the alpha carbon (Figure 2).[5] The main physiological difference in these two is that a peptoid is not vulnerable to degradation in the body making it possible to be used for drug delivery.[5] Peptoids are synthesized following a submonomer protocol, shown in Figure 3. This process allows for the inclusion of a large diversity of side chains through amine displacement.

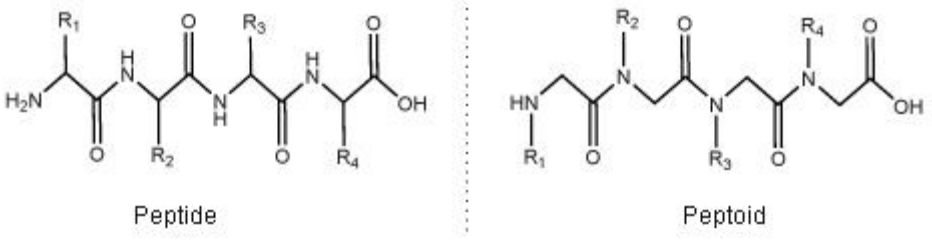


Figure 2: Image showing the difference between a peptoid and peptide.[6]

The peptoid JPT1 (Figure 4) has been shown in laboratory practices to modulate the formation of $A\beta_{40}$ plaques.[4] The sequence of JPT1 is based on that of the KLVFF, the hydrophobic core of Aβ that has been found to be essential for aggregation.[7] In addition, JPT1 contains chiral, aromatic side chains that help to stabilize helical secondary structure. The helical secondary structure formed allows for interaction between the peptoid and the beta sheets in Aβ. Aggregation assays with JPT1 showed that it decreases the time to aggregate formation while also decreasing the total number of aggregates formed. Current work in the Servoss lab is focused on determining the mechanism by which JPT1 modulates Aβ aggregation, as well as studying different peptoid sequences.[4]

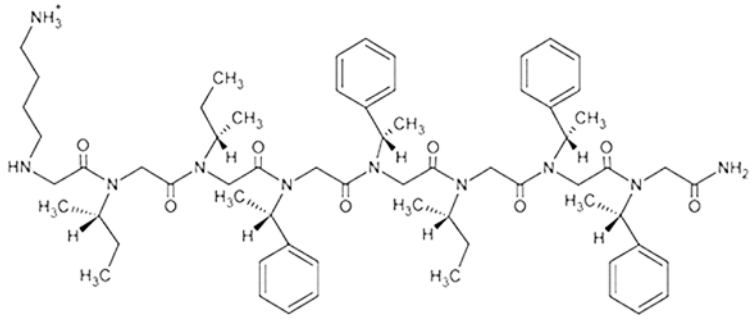


Figure 3: Molecular structure depiction of JPT 1 showing the chiral, aromatic side chains[3].

Motivation

The project focuses on studying Aβ in the presences and absences of novel protein mimics. The known function of JPT 1 will be taken and analyzed to try to produce a peptoid sequence that could possibly improve upon the inhibition of Aβ fibril formation.

The sequence of the synthesized peptoid is based on JPT 1. It contains the same structure, but is a-chiral and is increased in length. The sequence of the structure is KLLFFLFFLLK (Figure 4). Where K is lysine, L is leucine, and F is phenylalanine. The peptoid contains aromatic side chains and is hydrophobic in the center. The peptoid is extended to see the effects of a longer peptoid and the effects of the a-chirality on the aggregation of Aβ when combined with the synthesized peptoid.

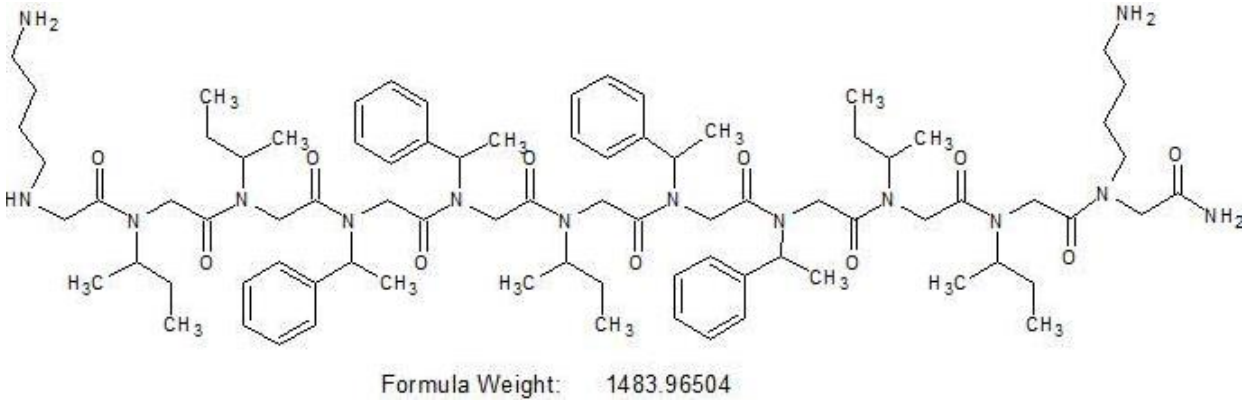


Figure 4: Molecular structure of peptoid that was synthesized drawn in ChemDraw™.

Results and Discussion

To begin the Aβ must be monomerized. This process can prove more difficult than expected based on the unreliability of biological substances, such as Aβ. Originally, I began with dissolving the Aβ in an organic solvent called 1,1,1,3,3,3-Hexaprop-2-flouro (HFIP) and allowing it to slowly evaporate overnight. The amount of HFIP used was based on the original concentration of Aβ stated from the respective peptide company. After completing this process, a control test of the HFIP treated Aβ was done. The control test done was based on the fluorescence when tested with Thioflavin T. The amount of fluorescence observed by a fluorimeter is related to the amount of Aβ that has formed fibrils. The control test done on this lot of Aβ did not perform as expected. This was concluded to be because the concentration given by the company was not the correct concentration.

Then, the HFIP treated Aβ was tested using a Nanodrop™. This was used to back calculate what the actual concentration of the Aβ. Using the new concentration and the old concentration two vials of Aβ were monomerized by HFIP and then tested. This still did not show the expected results.

With this information the HFIP treated Aβ was resuspended. A more reliable technique to monomerize the substance was now to be used. The resuspended Aβ was monomerized by use of a fast protein liquid chromatograph (FPLC). The first run of the process did not have the suspected peaks where the correct monomers were to be collected. The FPLC was then looked at further and assumed to have the incorrect superloop in place for this type of injection. The new superloop was cleaned and placed in the FPLC to complete the monomerization. A new vial from the same lot of Aβ was injected into the system. The results returned peaks that were similar to that of what the actual monomerized Aβ should be. However, the number of fractions collected were not enough to run any full test including multiple concentration and a control on the substance.

Next, a new lot of Aβ was obtained. The original lot used was deemed to be unsuitable for use. This lot was monomerized with HFIP treatment. A control test was run on the monomerized Aβ and gave expected results. This can now be used for experiments that can give results based on the formation or lack thereof of Aβ fibrils.

With the successfully monomerized Aβ; experiments could now be conducted. A new peptoid was synthesized. The synthesis is completed by a hand synthesis protocol. After synthesizing, the peptoid is cleaved from the resin using a Rotovap™. This is done to remove the tert-Butyl carbamate (BOC) protection groups that are attached to the peptoid while synthesizing. Using the cleaved version of the peptoid it was purified through preparative high pressure liquid chromatography (HPLC). The fractions from the peaks were tested for weight

using Matrix-assisted laser desorption/ionization (MALDI). One of the peaks was then determined to be the same mass as the synthesized peptoid (Figure 7).

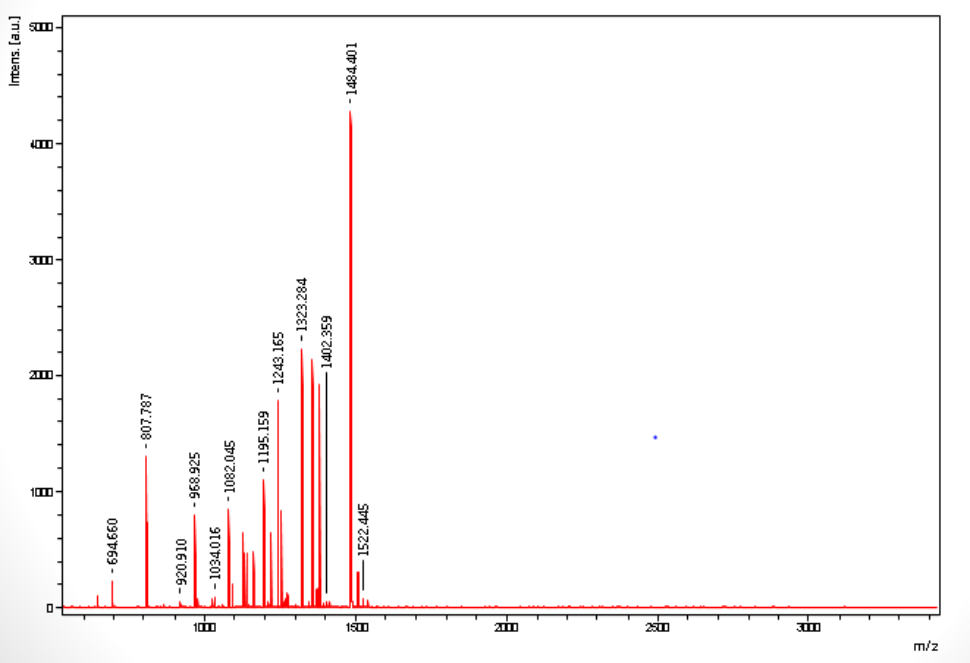
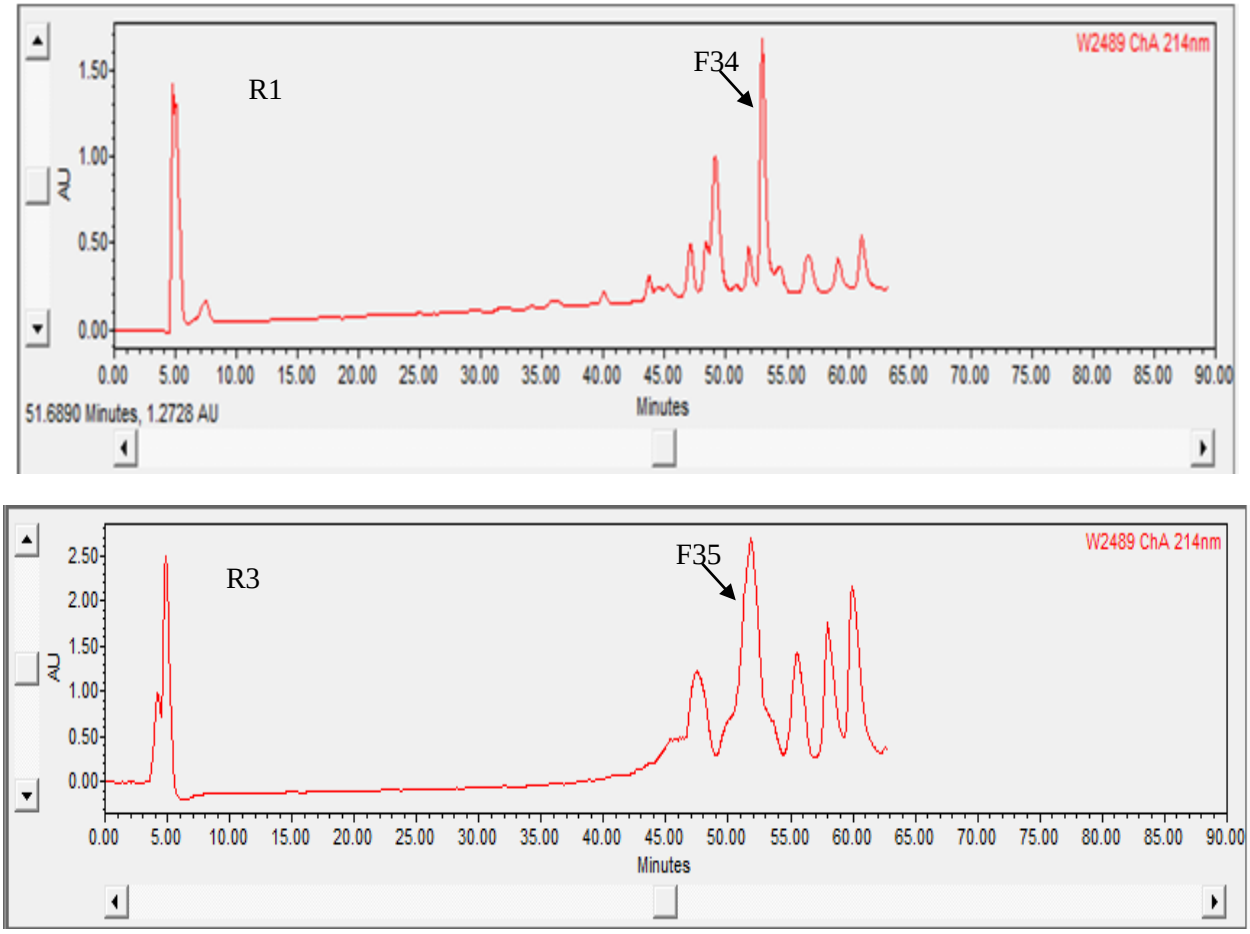


Figure 7: MALDI illustration of crude peptoid, before purification. The mass of 1484.401 is thought to be the peptoid.

More of the peptoid was then desired to be purified. The peptoid was cleaved and purified. This did not give expected results. This was concluded to be due to the cleavage lasting too long and removing side chains from the peptoid. The process was then completed again and conformed through MALDI to have produced the peptoid of the correct mass (Figure 8). This purified liquid is then combined by using analytical HPLC. This was used to ensure that the combination will not include any outlying fractions that are not of the correct mass.



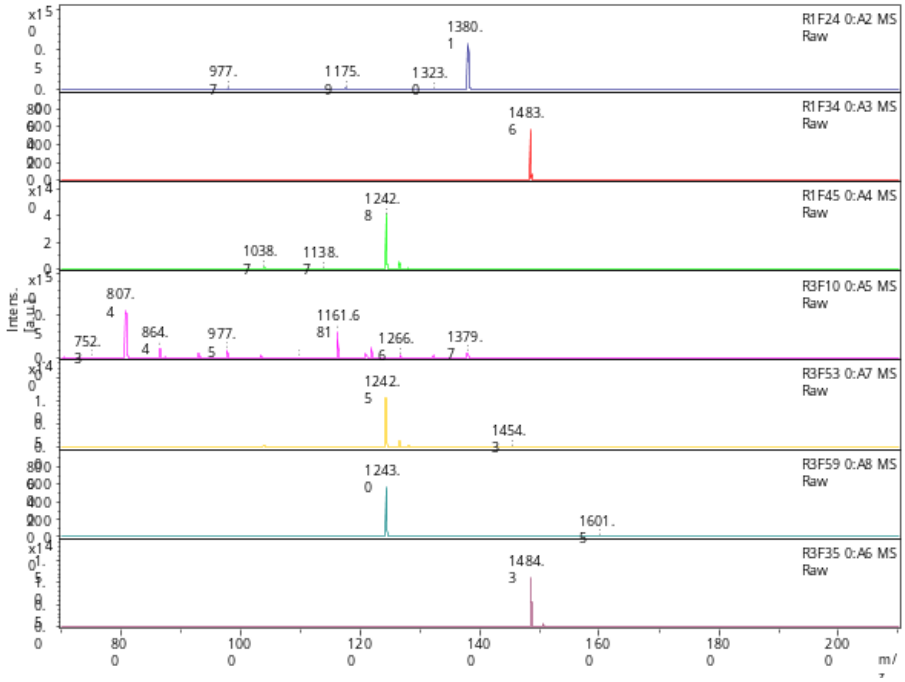


Figure 8: Shorter cleavage time HPLC peaks and MALDI mass matching.

The combined fractions were then lyophilized to reform into a solid substance that consists of only the desired peptoid. This peptoid is currently in the process of being confirmed by an outsourced lab. When the peptoid is confirmed it is to be tested with the HFIP treated Aβ at multiple concentrations to determine the inhibitory ability of the synthesized peptoid.

Conclusions

In conclusion, the protein mimic KLLFFLFFLK should be tested with the HFIP monomerized Aβ. This will lead to results on whether the peptoid will inhibit the formation of fibrils in the aggregation of Aβ.

The monomerized Aβ is in a useful form to conduct experiments. If the peptoid is confirmed to be the correct sequence the usefulness of a-chiral peptoids in the modulation of the aggregation of Aβ can be concluded.

Methods

- **1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) Monomerization:** HFIP is mixed with the Aβ protein and allowed to evaporate in order to monomerize the substance.
- **Fast Protein Liquid Chromatography:** Uses a column to separate proteins by ion exchange. The automatic capabilities of the machine make it possible to reproduce results easily.[5]
- **Peptoid Hand Synthesis:** Synthesis specific peptoid sequences on resin. This occurs by deprotecting, then adding the specified amino acid, washing to remove access, and reprotecting the chain. The end result leads to the amino acids attaching in the desired sequence if performed correctly.
- **Preparative High Pressure Liquid Chromatography:** Pumps solvent through a column in order to separate the different component of a mixture. Preparative is used to purify mixtures.
- **Matrix-assisted laser desorption/ionization:** Mass analysis involving a minimum amount of sample. Laser light is dispersed across the samples and the light that is returned can be used to determine mass of the components. [6]
- **Analytical High Pressure Liquid Chromatography:** Pumps solvent through a column in order to separate the different component of a mixture. Analytical is faster than preparative and is used to analyze rather than purify.

Acknowledgements

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References

[1] "2014 Alzheimer's Disease Facts and Figures ." *Alzheimer's Association* . Web. 10 Oct. 2014. <http://www.alz.org/downloads/Facts_Figures_2014.pdf>.

[2] Berg, Jeremy Mark, and John L. Tymoczko. *Biochemistry*. 7th ed., International ed. Basingstoke: Palgrave Macmillan, 2011. Print.

[3] Vivekanandan, Subramanian, Jeffrey Brender, Shirley Lee, and Ayyalusamy Ramamoorthy. "A Partially Folded Structure of Amyloid-Beta(1-40) in an Aqueous Environment." *National Center for Biotechnology Information*. U.S. National Library of Medicine, 25 June 2011. Web. 10 Oct. 2014. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3148408/>.

[4] Turner, J. Phillip, Tammy Lutz-Rechtin, Kelly A. Moore, Lauren Rogers, Omkar Bhave, Melissa A Moss, and Shannon L Servoss. "Rationally designed peptoids modulate aggregation of amyloid-beta 40." *ACS Chemical Neuroscience* 1 (2014): 140401181815007. Print.

[5] Madadlou, A., S. O'Sullivan, and D. Sheehan. "Result Filters." National Center for Biotechnology Information. U.S. National Library of Medicine, n.d. Web. 27 Apr. 2016.

[6] "The Protein Facility of the Iowa State University Office of Biotechnology: MALDI-TOF Mass Analysis." The Protein Facility of the Iowa State University Office of Biotechnology: MALDI-TOF Mass Analysis. N.p., n.d. Web. 27 Apr. 2016.