THE EFFECT OF ERYTHROSINE B ON AMYLOID BETA AGGREGATION IN ALZHEIMER'S DISEASE

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Hyeonjin Kwon

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THE EFFECT OF ERYTHROSINE B ON AMYLOID BETA AGGREGATION IN ALZHEIMER'S DISEASE

Approved by:

Dr. SeungSoon Jang, Advisor
School of Materials Science and Engineering
Georgia Institute of Technology

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Aβ	Amyloid Beta

SUMMARY

Alzheimer's disease is known as the most common form of dementia with an increasing number of new cases each year. The promising therapeutic strategy is the reduction of neurotoxic Amyloid β (Aβ) fibrils in which aggregation causes AD. Among numerous small molecules investigated as Aβ aggregation modulator, erythrosine B (ER) has been shown a safety profile with low toxicity and high blood-brain barrier permeability[1]. Investigation into the mechanism of ER that inhibits the aggregation of Aβ was performed using MD simulation. Also by modifying ER into Eosin Y(EOY) and Fluorescein (FLN), where halogen was added and removed respectively, the mechanism of modified ER was also investigated. From the simulation, binding sites of three molecules on Aβ, the effect of the molecules of Aβ's backbone, and the conformational change of Aβ binding residues with the molecules were studied. In conclusion, the results showed that halogen plays an important role in binding to Aβ and ER contains the most affective structure to inhibit the aggregation of Aβ.

CHAPTER 1

INTRODUCTION

It is evident from various studies that the most common form of senile dementia, Alzheimer's disease (AD), is due to the accumulation of insoluble protein aggregates, composed primarily of neurotoxic Aβ[1,2,3]. Numerous small molecules have been studied for their ability to modulate Aβ aggregation and reduce neurotoxicity [1] . Erythrosine B is a well-known food dye approved by FDA and it has been observed that it inhibits the formation of Aβ fibril through in vitro experiment.

To develop the molecules as future AD therapy, however, further investigation on specific chemical molecule structures of Aβ fibril adhered to the molecule is needed. From several studies that focused on Aβ fibril destabilization, it was not clear where the molecules bind to Aβ and how the binding to the locations promotes its destabilization [1,6]. Does the adhesion to the small molecules denature the structure of Aβ? Or do the molecules bind to a specific terminus of the fibril and prevent the proteins from further polymerizing? If so, are the molecules binding to Aβ strong enough to persist for a lifetime so that the fibrils would not form ever again?

Also, it was studied that halogenation of compounds enhance inhibitory capacities of small molecules on Aβ –associated neurotoxicity[7]. Based on the study, it is necessary to find an effective molecular structure of small molecules with various modification of halogen.

The current study attempts to address these questions by investigating the mechanism of ER and modified ER on Aβ monomer. The structure of Aβ fibrils binding to the destabilizing molecules will be studied in a molecular level using computational

modeling with GROMACS 4.6.1. The study investigating the mechanism of ER and its modified molecules on Aβ aggregates will lead to helpful results that demonstrate the way to improve the candidate drug in terms of efficiency.

CHAPTER 2

LITERATURE REVIEW

Although there are some FDA approved drugs that reduce symptoms, successful treatment that stops the progression of AD has not yet been developed[2]. A common pathological hallmark of AD is the accumulation of insoluble protein, called amyloid-beta peptide (Aβ), aggregates in the brain. Significant steps in development of potential treatment for AD was to explore how soluble Aβ starts to assemble into amyloid fibrils and to find small molecules that effectively reduce toxic Aβ aggregates and cytotoxicity. One of the effective modulators is ER, which is a FDA-approved red food dye[1]. To ultimately cure AD with the effective modulators, clarification of how ER can destabilize the aggregates and reduce the cytotoxicity is needed. Yang et al., demonstrates the exploration of the mechanism for LPFFD, a beta sheet breaker, inhibiting the formation of beta sheet conformation of A-beta in water, using several computational models. The computational modeling processes in the study can be applied to exploration of ER on Aβ.

The study mainly focuses on exploring how LPFFD inhibits the formation of B-sheet conformation of Aβ at atomic level. MD simulation results are analyzed with various GROMACS tools. The stability of the complex (LPFFD and amyloid beta) was analyzed and confirmed by comparing RMSDs results and the total interaction energies between LPFFD and amyloid beta. The extent of hydrophobic burial of different regions in the amyloid beta with and without LPFFD was analyzed using SASA (Solvent Accessible Surface Area) per residue and it was confirmed that the hydrophobicity of C-terminal residues decreases with LPFFD and decreased hydrophobicity leads to stable

helix structure. By proving that the presence of LPFFD leads to less transition from alpha –helix to beta-sheet, the study successfully explored LPFFD's role as an inhibitor.

Through the analysis of molecular docking and MD simulation of LPFFD on the Aβ, the study found out several results that support its conclusion; (1) LPFFD binds to Aβ (1-42) via hydrogen bonds that stabilize the complex; (2) C-terminus of Aβ no longer have conformational change from alpha-helix to beta-sheet with LPFFD; (3) LPFFD decreases the hydrophilicity of C-terminus, which helps preventing aggregation of Aβ. With these supporting results, the study concluded that LPFFD can inhibit the conformational transition from α-helix to β-sheet structure of the C-terminus of Aβ (1-42).

The study provides specific computational models to explore the role of LPFFD on Aβ and showed the analysis of simulation. The main focus of our research is also exploring the mechanism of ER on Aβ to study ER's molecular characteristics such as binding sites on Aβ and the conformational change in Aβ's backbone, thus the computational model applied to the study can be used in our research.

CHAPTER 3

METHODS AND MATERIALS

MD Simulation

To construct molecular structures of Aβ, ER, EOY, and FLN, *Cerius 2* was used. *Jaguar* was used to give charge to each molecule, setting B3LYP for functional theory, 6-31G** for basis, Mulliken for charge analysis, and total charge is -2. With AutoDock, binding sites and binding energy of three molecules were found. GROMACS 4.6.1 MD package was run for 40ns with force field, GROMOS9653A6. All molecule structures were visualized with PyMOL. SPC was used for water, nose-hoover for thermostat, PME for electrostatic, and double for precision. 150mM of NaCl is used for simulation concentration.

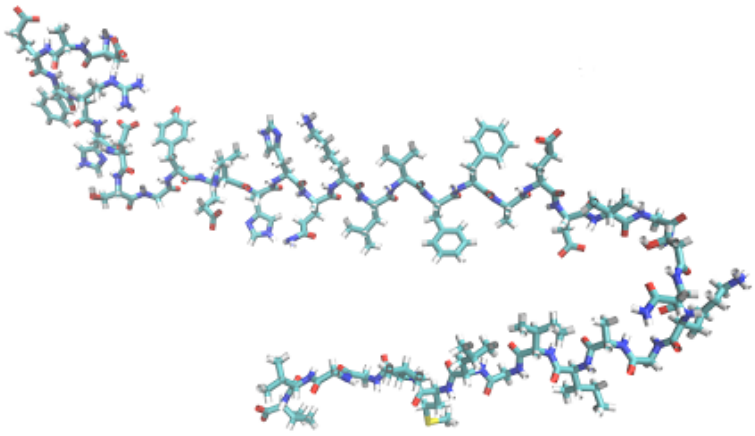

Figure 1. Sequence of Aβ (1-40) extended by Cerius 2 and visualized by PyMOL

Simulation Analysis

Root Mean Square Deviation (RMSD) was performed in GROMACS 4.6.1 to see the change in the structure of backbone of Aβ. The best-fit line was performed after

simulating 80000 ps. To compare conformational difference of Aβ with ER, EOY, and FLN, Solvent Accessible Surface Area calculation was done in GROMACS 4.6.1 as well.

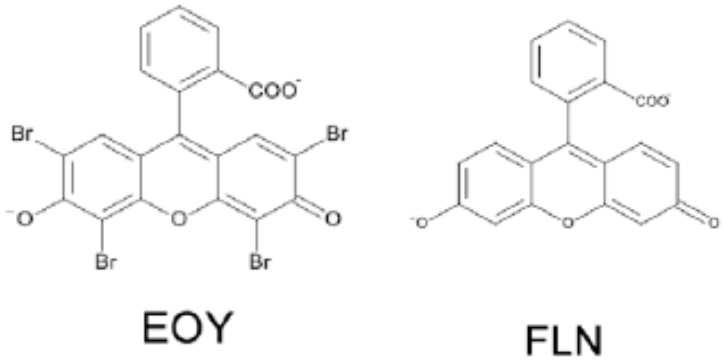

Figure 2. Molecular Structure of Eosin Y and Fluorescein modeled by Cerius 2

CHAPTER 4

RESULTS

Binding sites of ER and modified ER on Aβ

In order to figure out approximate binding sites of ER, EOY, and FLN on Aβ, AutoDock simulation was performed. AutoDock was run three times with three combinations; Aβ with ER, Aβ with EOY, and Aβ with FLN. The binding sites were obtained with residue numbers, and binding energies of each molecule were calculated as well.

ER had three different binding sites with average binding energy -5.53 kcal/mol. EOY is binding to two different residues with average energy -5.95 kcal/mol. FLN showed the highest binding energy, -6.65 kcal/mol, with two different binding sites. (Figure 3, Table 1).

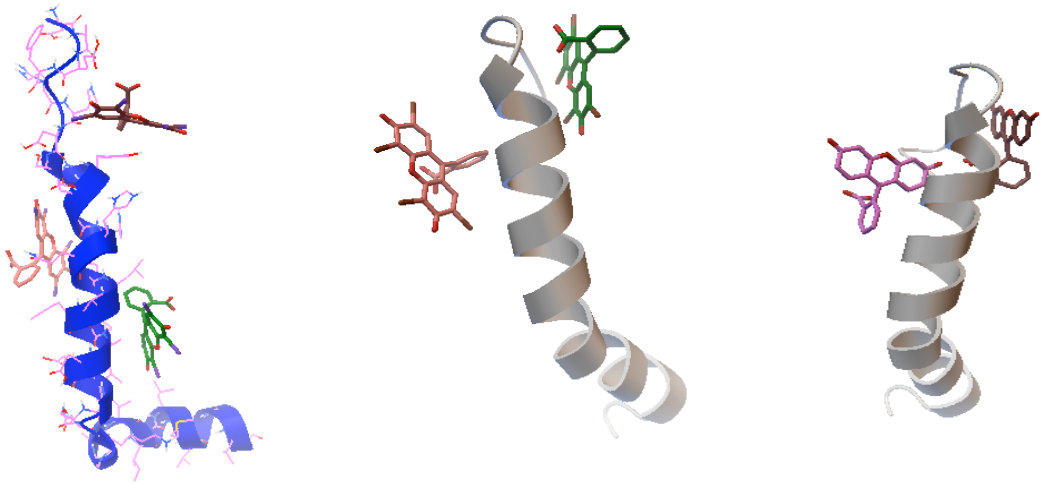

Figure 3. Binding sites of ER, EOY, and FLN on Aβ obtained from AutoDock simulation. A) Three binding sites of ER on Aβ, B) Two binding sites of EOY on Aβ, C) Two binding sites of FLN on Aβ.

Molecule	Molecule model	Binding Energy (kcal/mol)
ER	Brown	-5.8
	Pink	-5.5
	Green	-5.3
EOY	Red	-6.0
	Green	-5.9
FLN	Pink	-6.9
	Brown	-6.4

Table 1. Binding Energy of ER, EOY, and FLN

RMSD Analysis

Based on GROMACS simulation done for 40 ns, Root Mean Square Deviations (RMSD) of Aβ only, Aβ with ER, Aβ with EOY, and Aβ with FLN were calculated in GROMACS and the average value of RMSD for each group are shown in Table 2. Note that Aβ only has the lowest value with -6e-6±0.7235 nm and Aβ with ER has the highest value with 5e-6±0.7651 nm.

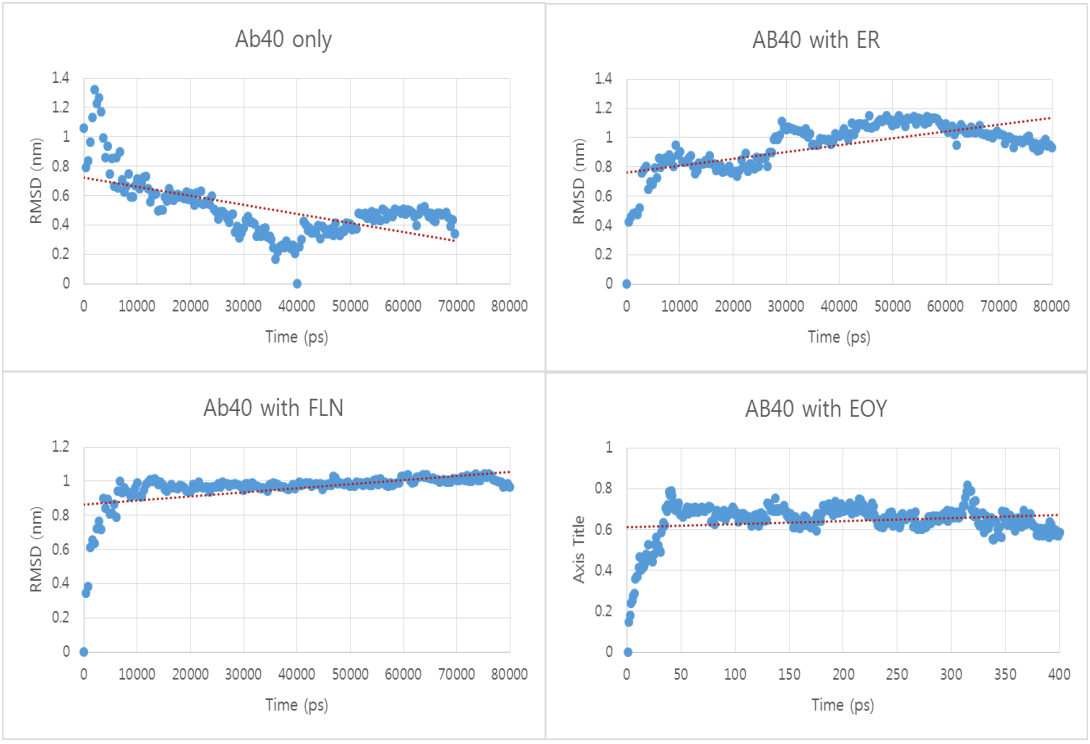

Figure 4. RMSD plotted over 8000 ps

Groups	Aβ only (nm)	Aβ with ER (nm)	Aβ with FLN (nm)	Aβ with EOY (nm)
RMSD	-6E-6±0.7235	5E-6±0.7651	2E-6±0.8635	1E-6±0.6134

Table 2. RMSD for Aβ only, Aβ with ER, Aβ with FLN, and Aβ with EOY

CHAPTER 4

DISCUSSION

Data of binding affinities of each molecule obtained by AutoDock shows that Aβ with FLN has the highest binding energy compared to Aβ with ER and Aβ with EOY. As mentioned above, halogen has high binding affinity to Aβ[7]. However, FLN that was modified by removing all halogen from ER showed higher binding energy with average of -6.65 kcal/mol than ER (-5.53 kcal/mol) and EOR (-5.95 kcal/mol) with iodine and bromine respectively. This indicates that it may not necessarily mean that having halogen increases binding affinity to Aβ.

RMSD represents the measure of the average distance between atoms or molecules. RMSD analysis was done to see how much three molecules deformed Aβ's backbones by changing the distance between Aβ peptides. The results showed that the presence of drug with Aβ showed the positive trend of RMSD but Aβ itself had the negative trend. The negative trend indicates that the average distance between the monomers is getting closer and Aβ monomers are forming aggregates. On the other hand, the positive trend of ER, EOY, and FLN shows that they have increased the distance, causing some deformations of the backbone.

As Figure 4 is shown above, RMSD analysis on Aβ with FLN shows that even though the fit line has the positive trend, it is almost constant with a little fluctuation. However, Aβ with ER and Aβ with EOY had some fluctuation on the graphs with the positive trends. The fact that the overall trend of Aβ with FLN is constant demonstrates that FLN molecule does not induce enough deformation of the backbone to inhibit the aggregation. Compared to ER and EOY that had iodide and bromine as halogen, FLN

was the molecule from which iodide was removed. This proves that halogenation enhances inhibitory capacities of the molecules[7].

CHAPTER 6

CONCLUSION AND FUTURE WORK

In this experiment, the investigation has established that ER molecule with halogen has more potential of efficient inhibition of Aβ aggregation. The results In case of FLN where all halogen were removed, it did not induce significant amount of deformation of Aβ, indicating that halogen should be added to small molecules that are targeting the Aβ aggregation. Specific residues of Aβ where each molecule binds to are important data that can be used to calculate solvent accessible surface area in the future, which represents the conformational change of the protein's residues when interacted with the molecules.

For future study, RMSD analysis should be performed longer than 8000 ps. Even though the analysis showed differences on the effects of three molecules on the deformation of Aβ, longer simulation would make it possible to verify whether the molecules keep deforming the protein over the duration and to investigate the pattern of the deformation caused by the molecules as time goes on. Furthermore, to clarify that molecules that contain halogen are more capable of inhibiting the aggregates, various modifications of halogen in ER structure needs to be studied finding the effective structure of ER for the inhibition.

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