Proposal for an inhibitor of Alzheimer’s disease blocking aggregation of amyloid-β peptides: *ab initio* molecular simulations

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**Abstract.** Aggregation of amyloid-β (Aβ) peptides is believed to play a key role in the mechanism of molecular pathogenesis of Alzheimer’s disease (AD). To inhibit the aggregation and prevent AD, numerous compounds have been synthesized. A previous experimental study elucidated that a triazine derivative AA3E2 has anti-amyloidogenic ability, while a triazine derivative AA3D2 having a different substituent has no inhibitory effect. However, the reason for this remarkable difference in the ability cannot be explained by the chemical structures of these derivatives. In the present study, we present stable structures of the solvated complexes with Aβ and AA3E2/AA3D2 obtained by classical molecular mechanics method. The specific interactions between Aβ and AA3E2/AA3D2 in the complexes are investigated by *ab initio* fragment molecular orbital calculations. Based on the results obtained, we attempt to propose new potent inhibitors for the Aβ aggregation.

1. Introduction

Recently, dementia resulting from Alzheimer’s disease (AD) is a growing problem worldwide. The cause and progression of AD is considered to be associated with senile plaques in a brain [1], which are composed of the aggregation of amyloid β (Aβ) peptides. The Aβ peptides are produced by proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases [2]. Because the γ-secretase can cleave APP at its several alternative sites, the produced Aβ peptides have a variety of lengths. The most abundant Aβ peptides existing in the senile plaques are Aβ(1-40) and Aβ(1-42)[2, 3]. Each of them has 40 or 42 amino acid residues, respectively. It was found in the experiments [4-6] that Aβ(1-42) aggregates more rapidly and comprises the major component of the senile plaque in a diseased brain, in comparison with the shorter Aβ(1-40).

The aggregation of Aβ peptides induces a cascade of events leading to a death of neuronal cells in a diseased brain [7]. Because the Aβ peptides have several hydrophobic amino acid residues, the peptides form strong aggregates in water due to the hydrophobic interactions between these residues, leading to a fibril formation [8]. Therefore, it is expected that compounds having a strong binding affinity to Aβ can inhibit the Aβ aggregation and be a potent inhibitor for the amyloidogenesis and pathogenesis of AD.
Numerous compounds have been synthesized [9-12] for producing potent inhibitors for the Aβ aggregation. It was found that a triazine derivative AA3E2 has anti-amyloidogenesis ability [9, 13-15], while a triazine derivative AA3D2 having a different substituent has no inhibitory effect [9, 15]. Because AA3E2 is classified as a β-sheet breaker [16], it can disrupt the hydrophobic interactions between Aβ peptides [17-19]. As a result, AA3E2 is expected to have a remarkable effect to prevent the Aβ aggregation [15]. On the other hand, the reason why AA3D2 has no inhibitory effect is not elucidated yet. And the reason for this remarkable difference in the ability between AA3E2 and AA3D2 cannot be explained by the chemical structures (figure 1) of these derivatives.

![Figure 1](image)

Figure 1. Chemical structures of (a) AA3E2 and (b) AA3D2.

In the present study, to elucidate the difference in specific interactions between Aβ and AA3E2/AA3D2, we obtained stable structures for the solvated complexes of Aβ with these triazine derivatives, by molecular simulations based on protein-ligand docking and classical molecular mechanics (MM) methods. For the most stable structure determined by ab initio fragment molecular orbital (FMO) [20-26] calculations, the specific interactions between each amino acid residue of Aβ and AA3E2/AA3D2 were investigated at an electronic level. From the results computed, we attempted to elucidate which amino acid residues of Aβ are important for the binding between Aβ and the triazine derivative, and which parts of the triazine derivative contribute to the binding. In addition, we proposed some new compounds and investigated their binding affinity to Aβ, in order to propose new potent inhibitors for the Aβ aggregation.

2. Details of molecular simulations

2.1. Optimization of solvated structures of Aβ + triazine complexes

In the present study, to elucidate how the specific interactions between Aβ and triazine are changed depending on the Aβ structure, we considered two types of Aβ structures, each of which is composed of α-helix or β-sheet conformation, and their initial three-dimensional structures were obtained from Protein Data Bank (PDB). The initial structure of the α-helix Aβ (α-Aβ) was constructed based on the NMR structure (PDB ID: 1Z0Q [27]). On the other hand, as for the β-sheet Aβ (β-Aβ) structure, there is a NMR structure for only the short Aβ(1-42) peptide (PDB ID: 2BEG [28]). We thus constructed a model structure for the part of the 1-16 residues, by using homology modeling program MODELLER 9.2 [29]. The PDB structures (PDB ID: 2BEG and 1BJB [30]) were employed as template structures,
and 10 candidate structures of full-sized $\beta$Aβ(1-42) were created. These structures were compared with the NMR structure (2BEG) to obtain the root mean square deviation (RMSD) between them. Among the 10 structures, we employed the structure having the smallest RMSD value as an initial structure for $\beta$Aβ(1-42) monomer.

Aβ monomer has three His amino acid residues, and their protonation structures cannot be determined, because His has three types of the protonated structures depending on the $pK_a$ value around it. We here evaluated the $pK_a$ values of each His residue contained in Aβ by using PROPKA Web Interface 3.0 [31] and determined the His protonation based on the $pK_a$ values. His residues with $pK_a$ value larger than 6.0 have the Hip protonation, while those with $pK_a$ value smaller than 6.0 have the Hid or Hie protonation. All His residues in Aβ were found to have the Hip protonation.

We added solvating water molecules with a 8 Å layer around the Aβ monomer structure and optimized the solvated structure by the classical MM and molecular dynamics (MD) simulation program AMBER9 [32], in which the AMBER99 force field [33] was used in combinations with the TIP3P water model [34]. The threshold value of the energy-gradient for the convergence in the AMBER9 optimization was set as 0.001 kcal/mol/Å. In addition, we performed a 1ns MD simulation at 300K for the solvated structure of $\beta$Aβ monomer, to search for its stable structures widely. The snapshots obtained from the MD trajectory were classified into 10 clusters according to the RMSD value between each of the structures. We then optimized their structures by the MM method and determined the most stable structure of the solvated $\beta$Aβ monomer by the ab initio FMO calculations.

As for the triazine derivatives AA3E2 and AA3D2, we optimized their structures in vacuo by using the ab initio MO calculations. The MP2/6-31G(d,p) [35] method implemented in the ab initio MO program package Gaussian03 (G03) [36] was used. As shown in figure 1, since the derivatives have a long polar group in the molecule, their dipole moments and charge distributions are likely to depend on the direction of the polar group. To elucidate the dependence of the dipole moment on the torsion angle of the polar group, we changed the angle and investigated dipole moment and charge distribution by using the MP2/6-31G(d,p) method in G03 to determine the most stable structure.

To obtained candidate structures for the complex of Aβ with the triazine derivative, we docked the derivative to a variety of sites around Aβ using the automated protein-ligand docking program Autodock 4.2 [37]. In the docking procedure for $\alpha$-Aβ, the grid box was set as the $41.25 \times 30.0 \times 18.75$ Å$^3$ centered on the gravity center of $\alpha$-Aβ, and the spacing between the nearest neighboring grid points was set to 0.375 Å, which is the default value of Autodock 4.2. On the other hand, for the $\beta$-Aβ, the grid box and the space of grid points were set as the $50.0 \times 27.8 \times 32.6$ Å$^3$ and 0.397 Å, respectively. The structure of Aβ was fixed, and all dihedral angles of the derivatives were freely rotated in the docking procedure to search for a variety of stable configurations for the derivative docked to Aβ. The RESP charges [38] obtained by the MP2/6-31G(d,p) calculation were assigned to each atom of the derivative. We here created 1,000 candidate structures of the complex by using the genetic algorithm of Autodock 4.2. They were classified into some clusters according to the RMSD value (10.0 Å) between each of the structures created. We created 2,000 candidate structures for the $\alpha$-Aβ + AA3E2 complex, since the number of clusters for this complex obtained by AutoDock was few.

The representative structures in each cluster were fully optimized by the AMBER9-MM method. To consider the solvation effect on the complex properly, we added water molecules with a 8 Å layer around the complex and optimized the solvated structure by using AMBER9 [32], in which the AMBER99 [33] and TIP3P [34] force fields were assigned for the complex and water molecules, respectively. The threshold value of the energy-gradient for the convergence in the AMBER9 optimization was set as 0.001 kcal/mol/Å. Finally, total energies of these optimized structures were calculated accurately by the ab initio MP2/6-31G method in FMO [20-26], and the most stable structure of the solvated Aβ + triazine complex was determined.

2.2. Ab initio FMO calculations for solvated Aβ + triazine complexes

The electronic properties for the most stable structure of the solvated Aβ + triazine complex were investigated by the ab initio FMO calculation [20-26], to elucidate which amino acid residues in Aβ
are important for the specific binding between Aβ and triazine derivative. In the FMO method, the target molecule is divided into units called “fragment”, and the electronic properties of the target molecule are estimated from the electronic properties of the monomer and dimers of the fragments. The specific interactions between the fragments can be investigated from the interaction energies obtained by the FMO calculation.

In the previous docking study [15], the candidate structures for the complex of Aβ with the triazine derivative were created by using a new generation of Autodock, and a scoring function was used for determining the most preferable structure among the candidate structures. On the other hand, in the present study, we performed the \textit{ab initio} FMO calculations for all the candidate structures to determine the most stable structure among them more accurately. In addition, from the results of the FMO calculations, it can be elucidated what amino acid residues of Aβ are important for the specific interactions between Aβ and the triazine derivative.

In the present FMO calculations, to investigate the relative stability among the solvated structures of the Aβ + triazine complexes, the number of solvating water molecules was set as 723 for all the complexes. Each amino acid residue of Aβ, triazine derivative and each water molecule were assigned as a fragment, because this fragmentation enables us to evaluate the interaction energies between the amino acid residue of Aβ, triazine derivative and solvating water molecules. The \textit{ab initio} MP2/6-31G method was employed to investigate accurately the π-π stacking, NH-π and CH-π interactions between the amino acid residues of Aβ and the triazine derivative. We used the FMO calculation program ABINIT-MP Ver.4.3 [39]. In addition, by considering water molecules explicitly, we attempted to elucidate the influence of solvating water molecules on the specific interactions. In order to investigate the binding energy between Aβ and the derivative, the solvated Aβ + triazine structure was divided into the following four structural domains: Aβ + triazine complex containing solvating water molecules (Complex), Aβ containing solvating water molecules (Aβ + water), the triazine derivative containing solvating water molecules (Ligand + water) and solvating water molecules (Water). From total energies (T.E.) obtained by the FMO calculations, the binding energy (B.E.) between Aβ and the triazine derivative mediated by solvating water molecules was estimated as

\[
\text{B.E.} = -\text{T.E. (Complex)} + \text{T.E. (Aβ + water)} + \text{T.E. (Ligand + water)} - \text{T.E. (Water)}.
\]

3. Results and discussion

3.1. Optimized structures of solvated Aβ + triazine complexes

Based on the NMR structure of α-Aβ (PDB ID: 1Z0Q [27]), we constructed its solvated structure and optimized it by the AMBER9-MM method. To check the validity of the optimized structure (figure 2(a)), it was compared with the NMR structure. The RMSD between them is 1.0 Å, indicating that the optimized structure of the solvated α-Aβ is comparable to its NMR structure.

As for the β-Aβ structure, there are NMR structures for only the short Aβ(17-42) peptide (PDB ID: 2BEG [28]). We thus constructed 10 candidate structures for the full-length β-Aβ(1-42) peptide by using homology modeling MODELLER 9.2 [29], and chose a structure having the smallest RMSD value between the NMR structure [28]. Classical 1ns MD simulations were performed at 300K to obtain 10 clusters, and the representative structures of each cluster were selected to be optimized by AMBER9-MM. Their total energies evaluated by the \textit{ab initio} FMO calculations are listed in table 1, indicating that the cluster 1 is at least 88 kcal/mol stable compared with the other structures. Therefore, we employed the cluster 1 as a solvated β-Aβ(1-42) structure, which is shown in figure 2(b). It is noted that two hydrogen bonds are formed between the side chains of Asp23 and Lys28 residues of β-Aβ(1-42). They are likely to contribute significantly to the stability of this structure.
Figure 2. Structures of Aβ monomer optimized by AMBER99 in explicit solvating water molecules; (a) α-Aβ and (b) β-Aβ.

Table 1. Total energies (T.E.) (kcal/mol) for the β-Aβ(1-42) optimized in water by classical MM method based on AMBER99 and TIP3P force fields. The energies are evaluated by the *ab initio* MP2/6-31G method of the AMBINIT-MP 4.3 program [39].

| Cluster | T.E.     | ΔT.E. |
|---------|----------|-------|
| 1       | -52902684.1 | 0.0   |
| 2       | -52902085.8 | 598.3 |
| 3       | -52902160.9 | 523.2 |
| 4       | -52902298.5 | 385.5 |
| 5       | -52902595.6 | 88.4  |
| 6       | -52902144.5 | 539.6 |
| 7       | -52902557.9 | 126.1 |
| 8       | -52902256.5 | 427.5 |
| 9       | -52902282.7 | 401.3 |
| 10      | -52902365.7 | 318.3 |

Structures of the triazine derivatives optimized by MP2/6-31G(d,p) are shown in figures 3(a) and 3(d). To elucidate the dependence of the dipole moment on the torsion angle of the polar group X in figure 3(a), we changed the angle as shown in figure 3(b), 3(c), 3(e) and 3(f) and optimized the structures. Table 2 lists their total energies and dipole moments, indicating that the dipole moment is significantly dependent on the angle, although the total energy does not depend so much. It is thus necessary to rotate all dihedral angles of the derivatives freely in searching for a variety of stable configurations of the derivative docked to Aβ.

Table 2. Total energies (kcal/mol) and dipole moments (Debye) for the triazine derivatives optimized in vacuo by the *ab initio* MP2/6-31G(d,p) method.

| Structure       | Total energy | Dipole moment |
|-----------------|--------------|---------------|
| AA3E2 (Figure 3(a)) | -865262.2 | 2.1 |
| AA3E2 (Figure 3(b)) | -865262.0 | 2.3 |
| AA3E2 (Figure 3(c)) | -865260.6 | 3.1 |
| AA3D2 (Figure 3(d)) | -693154.2 | 2.3 |
| AA3D2 (Figure 3(e)) | -693154.2 | 2.5 |
| AA3D2 (Figure 3(f)) | -693153.6 | 2.9 |
We first docked AA3E3 and AA3D2 triazine derivatives to α-Aβ to create the candidate structures of the complex. They were grouped into 20 (AA3E2) and 17 (AA3D2) clusters according to the RMSD between them, as listed in table 3. The representative structures of each cluster were optimized by AMBER97-MM, and their total energies were accurately evaluated by the \textit{ab initio} FMO calculations to determine the most stable structure. Table 3(a) indicates that the cluster 1 and 5 are the most stable structures of the complex of α-Aβ with AA3E2 and AA3D2, respectively. Their structures are shown in figures 4(a) and 4(b). Both the derivatives bind to the region composed of the 19-42 residues of α-Aβ. In particular, the Phe19 and Phe20 of α-Aβ contribute to the binding of the derivatives.

In contrast, the triazine derivatives can dock to a variety of sites of β-Aβ, in the present docking process. As listed in table 3(b), there are 29 (AA3E2) and 43 (AA3D2) clusters of the candidate structures for the β-Aβ + triazine complexes. The most stable structures determined by the FMO calculations are shown in figures 4(c) and 4(d). The binding site of the triazine derivative on the β-Aβ differs significantly between AA3E2 and AA3D2. AA3E2 binds to the region near to the residues 17-21 and 35-41, while AA3D2 binds to the region of the residues 17-21 and 40-41. In the previous study [40], the hydrophobic region of the residues 16-20 (KLVFF) in Aβ were elucidated to be essential for the aggregation of Aβ. Our present molecular simulations indicate that both AA3E2 and AA3D2 can interact with these hydrophobic residues and can prevent the aggregation of Aβ peptides.

\textbf{Figure 3.} Structures of triazine derivatives optimized by \textit{ab initio} MP2/6-31G(d,p) method; (a)-(c) AA3E2 and (d)-(f) AA3D2.
### Table 3. Total energies (T.E.) (kcal/mol) for the Aβ + triazine complexes optimized in water by classical MM method based on AMBER99 and TIP3P force fields. The energies were evaluated by the \textit{ab initio} ABINIT-MP 4.3 method.

| Cluster | (a) α-Aβ + AA3E2/AA3D2 complex | (b) β-Aβ + AA3E2/AA3D2 complex |
|---------|---------------------------------|---------------------------------|
|         | AA3E2 | AA3D2 | T.E. | △T.E. | T.E. | △T.E. | T.E. | △T.E. | T.E. | △T.E. |
| 1       | -54309479.3 | 745.3 | -54307862.7 | 208.6 | -5430930.9 | 258.1 |
| 2       | -54309252.7 | 226.6 | -54307726.4 | 344.9 | -5430949.7 | 753.4 |
| 3       | -54309201.9 | 277.4 | -54307654.0 | 417.3 | -5430954.0 | 394.1 |
| 4       | -54309272.7 | 206.6 | -54307893.2 | 178.1 | -5430975.0 | 473.1 |
| 5       | -54309331.5 | 147.9 | -54307476.5 | 594.8 | -5430990.0 | 345.2 |
| 6       | -54309309.2 | 170.1 | -54307557.9 | 513.4 | -5430803.8 | 444.3 |
| 7       | -54309397.7 | 81.6 | -54307433.6 | 637.7 | -5430942.7 | 634.0 |
| 8       | -54309340.9 | 138.4 | -54307976.3 | 95.0 | -5430658.0 | 590.2 |
| 9       | -54309278.4 | 200.9 | -54307553.2 | 518.1 | -5430600.4 | 647.7 |
| 10      | -54309204.1 | 272.5 | -54307570.6 | 500.7 | -5430612.9 | 118.5 |
| 11      | -54309333.4 | 145.9 | -54307653.4 | 417.9 | -5430833.2 | 414.9 |
| 12      | -54309343.9 | 135.4 | -54307396.7 | 674.6 | -5430619.8 | 49.7 |
| 13      | -54309270.6 | 208.8 | -54307861.4 | 209.9 | -5430566.9 | 683.2 |
| 14      | -54309261.5 | 217.9 | -54307663.5 | 407.8 | -5430979.1 | 451.0 |
| 15      | -54309261.4 | 217.9 | -54307715.4 | 355.9 | -5430769.6 | 478.6 |
| 16      | -54309211.1 | 268.2 | -54307729.2 | 342.1 | -5430601.6 | 236.5 |
| 17      | -54309391.8 | 87.5 | -54307823.0 | 248.3 | -5430689.9 | 558.3 |
| 18      | -54309206.9 | 272.4 | -54307489.9 | 581.4 | -5430986.9 | 461.2 |
| 19      | -54309339.3 | 140.1 | -54307986.1 | 85.2 | -5430602.3 | 165.9 |
| 20      | -54309277.2 | 202.1 | -54307867.0 | 204.3 | -5430603.6 | 194.6 |
| 21      | - - - - | - - | -54307717.0 | 354.3 | -5430942.6 | 305.5 |
| 22      | - - - - | - - | -5430601.2 | 470.1 | -5430541.7 | 830.4 |
| 23      | - - - - | - - | -54307396.7 | 674.6 | -5430619.8 | 49.7 |
| 24      | - - - - | - - | -54307517.3 | 533.6 | -5430722.4 | 525.7 |
| 25      | - - - - | - - | -54307788.8 | 182.5 | -5430551.5 | 732.6 |
| 26      | - - - - | - - | -54307553.6 | 317.7 | -5430777.3 | 470.8 |
| 27      | - - - - | - - | -54308071.3 | 0.0 | -5430749.1 | 499.0 |
| 28      | - - - - | - - | -54307279.7 | 791.6 | -5430752.7 | 522.4 |
| 29      | - - - - | - - | -54307426.8 | 644.5 | -5430530.8 | 717.4 |
| 30      | - - - - | - - | - - | - - | - - | - - |
| 31      | - - - - | - - | - - | - - | -5430644.2 | 204.0 |
| 32      | - - - - | - - | - - | - - | -5430609.1 | 150.0 |
| 33      | - - - - | - - | - - | - - | -5430666.6 | 581.5 |
| 34      | - - - - | - - | - - | - - | -5430683.7 | 564.4 |
| 35      | - - - - | - - | - - | - - | -5430874.9 | 373.3 |
| 36      | - - - - | - - | - - | - - | -5430611.8 | 136.3 |
| 37      | - - - - | - - | - - | - - | -5430887.7 | 360.4 |
| 38      | - - - - | - - | - - | - - | -5430616.8 | 86.3 |
| 39      | - - - - | - - | - - | - - | -5430835.9 | 412.2 |
| 40      | - - - - | - - | - - | - - | -5430815.9 | 432.2 |
| 41      | - - - - | - - | - - | - - | -5430562.6 | 627.5 |
| 42      | - - - - | - - | - - | - - | -5430544.0 | 807.9 |
| 43      | - - - - | - - | - - | - - | -5430579.7 | 668.5 |
3.2. Specific interactions between Aβ and triazines in the solvated complexes

To elucidate the binding affinity between Aβ and the triazine derivatives, we evaluated the binding energy between Aβ and AA3E2/AA3D2 for the most stable structures (figure 4) determined by the FMO calculations. Table 4 lists the total energies and the binding energies estimated from these total energies. The AA3X2 derivative is our newly proposed derivative, whose structure and binding affinity to Aβ will be described in Section 3.3. Table 4 indicates that the binding energies between α7Aβ/β7Aβ and AA3E2 are larger than those for the Aβ + AA3D2 complexes, being consistent qualitatively with the experimental results [9, 15] showing the larger effect of AA3E2 than AA3D2. In addition, it was found that the binding energy between β-Aβ and the triazine derivative is larger than that for the α-Aβ + triazine complex. Therefore, β-Aβ is expected to bind the triazine derivatives more strongly than α-Aβ.

To elucidate the specific interactions between Aβ and the triazine derivatives, we furthermore investigated the interaction energies (I.E.) between each amino acid residue of Aβ and AA3E2/AA3D2 for the most stable structures of the complexes, by using the \textit{ab initio} FMO method. As shown in figures 5(a) and 5(b), Phe20 of α-Aβ interacts most strongly with both the triazine derivatives AA3E2 and AA3D2, indicating that Phe20 is important for the binding between α-Aβ and the derivatives. Because the compounds having large binding affinity to the region of the 16-20 residues of Aβ are believed to inhibit the aggregation of Aβ peptides, AA3E2 and AA3D2 are expected to have the ability of inhibiting the aggregation of α-Aβ peptides. Therefore, our present result is consistent with the experiment results [9, 13-15] showing that AA3E2 prevents the Aβ aggregation.

Figure 4. Most stable structures of the Aβ + triazine complexes optimized by AMBER99 in water; (a) α-Aβ + AA3E2, (b) α-Aβ + AA3D2, (c) β-Aβ + AA3E2 and (d) β-Aβ + AA3D2 complexes.
Table 4. Total energies for solvated Aβ + triazine complexes (Complex), solvated Aβ (Aβ + water), solvated triazine derivative (Ligand + water) and solvating water molecules (Water), and estimated binding energies between Aβ and triazine derivative evaluated by the \textit{ab initio} FMO calculations.

| Complex          | Total energy (kcal/mol) | Binding energy (kcal/mol) |
|------------------|-------------------------|---------------------------|
|                  | Complex | Aβ + water | Ligand + water | Water  |
| α-Aβ + AA3E2     | -45309479.3          | -44445619.8          | -35407403.8 | -34543755.9 | 31.7  |
| α-Aβ + AA3D2     | -4537585.6           | -44445523.9          | -35235485.6 | -34543437.2 | 13.4  |
| α-Aβ + AA3X2     | -45431979.1          | -44445425.8          | -35529840.4 | -34543320.2 | 33.1  |
| β-Aβ + AA3E2     | -45308071.3          | -44444212.3          | -35405061.6 | -34541240.0 | 37.4  |
| β-Aβ + AA3D2     | -45136248.1          | -44444220.9          | -35233431.0 | -34541438.6 | 34.9  |
| β-Aβ + AA3X2     | -45430838.0          | -44444250.9          | -35528131.1 | -34541586.0 | 42.5  |

The interacting structures between the residues of α-Aβ and AA3E2 were investigated in detail to elucidate which part of AA3E2 and which residues of α-Aβ contribute to the binding between α-Aβ and AA3E2. As shown in figure 6(a), AA3E2 interacts with Phe19 and Phe20, in addition, the hydrocarbon chain of AA3E2 contacts with Val24 of α-Aβ. This contact does not occur in the Aβ + AA3D2 complex (figure 6(b)), because AA3D2 does not have the hydrocarbon chain. As indicated in figure 5(a), AA3E2 has also interacts strongly with Ala42 of α-Aβ. The terminal NH$_2$ group of AA3E2 forms hydrogen bonds with the oxygen atom of Ala42 backbone via a water molecule, as shown in figure 6(a). These specific hydrogen bonds contribute to the larger binding energy between α-Aβ and AA3E2 listed in table 4.

As for the β-Aβ + triazine complexes, the specific interactions between β-Aβ and the triazine are remarkably different for AA3E2 and AA3D2, as indicated in figures 5(c) and 5(d). AA3E2 interacts strongly with Val36 and Leu34, while AA3D2 interacts with Phe19, Val18, Val40 and Ile41. All of these residues are hydrophobic. In the β-Aβ + AA3E2 complex shown in figure 6(c), AA3E2 forms hydrogen bonds with Leu34 and Val36 of β-Aβ. In the β-Aβ + AA3D2 complex, AA3D2 forms hydrogen bonds with Phe19 and Ile41 of β-Aβ, as shown in figure 6(d). The interactions between AA3D2 and Val18/Val40 are hydrophobic interactions. The β-Aβ has more extended structure in comparison with the α-Aβ as shown in figures 2(a) and 2(b). As a result, the side chains of each residue in β-Aβ are distributed more sparsely than those in α-Aβ. Accordingly, the triazine derivatives can contact more closely with β-Aβ, resulting in the larger binding energy compared with α-Aβ, as listed in table 4.
Figure 5. Interaction energies between triazine derivative and each amino acid residues of Aβ; (a) α-β + AA3E2, (b) α-β + AA3D2, (c) β-β + AA3E2 and (d) β-β + AA3D2.
Figure 6. Interacting structures in Aβ + triazine complexes; (a) α-Aβ + AA3E2, (b) α-Aβ + AA3D2, (c) β-Aβ + AA3E2 and (d) β-Aβ + AA3D2.
3.3. Proposal for a potent inhibitor for $\text{A}\beta$ aggregation

It is elucidated from the present simulations that AA3E2, which has a remarkable effect to prevent the $\text{A}\beta$ aggregation [9, 13-15], interacts strongly with the hydrophobic amino acid residues of $\text{A}\beta(1-42)$ and that the hydrocarbon chain of AA3E2 contributes to the interactions. In order to strengthen these interactions, we designed a novel potent compound AA3X2 shown in figure 7, by adding a hydrocarbon chain to AA3E2. The binding energy and the specific interactions between AA3X2 and $\text{A}\beta$ were investigated by the $\textit{ab initio}$ FMO calculations.

We constructed the structures of $\text{A}\beta$ + AA3X2 complexes based on the $\text{A}\beta$ + AA3E2 complexes and optimized the solvated $\text{A}\beta$ + AA3X2 complexes by using the AMBER9-MM method. The total energies and the binding energies were evaluated for the optimized structures by the $\textit{ab initio}$ FMO method. As listed in table 4, AA3X2 has larger binding energy than AA3E2. Therefore, AA3X2 is expected to have larger binding affinity to $\alpha$-$\text{A}\beta$ and $\beta$-$\text{A}\beta$ and to inhibit the $\text{A}\beta$ aggregations.

To elucidate the reason for this large binding affinity, we investigated the interaction energies and the structure around AA3X2 in the $\text{A}\beta$ + AA3X2 complexes. As shown in figures 8(a) and 8(b), AA3X2 interacts more strongly with Phe20 of $\alpha$-$\text{A}\beta$ and Leu34 of $\beta$-$\text{A}\beta$, in comparison with AA3E2. Figure 9 shows the structures around AA3X2 in the $\alpha$-$\text{A}\beta$ + AA3X2 and $\beta$-$\text{A}\beta$ + AA3X2 complexes. These structures indicate the hydrogen bonds between AA3X2 and Phe20 of $\alpha$-$\text{A}\beta$ (figure 9(a)) and between AA3X2 and Leu34 of $\beta$-$\text{A}\beta$ (figure 9(b)). The comparison between the interaction energies for $\text{A}\beta$ + AA3E2 (figures 5(a) and 5(b)) and those for $\text{A}\beta$ + AA3X2 (figures 8(a) and 8(b)) elucidates that these interactions are larger for AA3X2 than AA3E2. Therefore, our proposed AA3X2 is expected to be a potent inhibitor for the $\text{A}\beta$ aggregation.

![Chemical structure of our proposed triazine derivative AA3X2.](image-url)
Figure 8. Interaction energies between AA3X2 and each amino acid residues of Aβ; (a) α-Aβ + AA3X2 and (b) β-Aβ + AA3X2.

Figure 9. Interacting structures in Aβ + AA3X2; (a) α-Aβ + AA3X2 and (b) β-Aβ + AA3X2.
4. Conclusions
We here investigated the specific interactions between Aβ(1-42) and triazine derivatives for the two types of Aβ structures as well as the two kinds of triazine derivatives (AA3E2 and AA3D2) shown in figure 1, by *ab initio* molecular simulations, in which protein-ligand docking, classical molecular mechanics and *ab initio* fragment molecular orbital methods were used. The results elucidate the following points.

1. Among the 42 amino acid residues of α-Aβ, the hydrophobic residue Phe20 is the most important for the binding between α-Aβ and AA3E2/AA3D2.
2. Leu34 and Val36 of β-Aβ interact strongly with AA3E2.
3. Val18 and Phe19 of β-Aβ interact strongly with AA3D2.
4. Hydrophobic residues of Aβ contribute mainly to its binding to AA3E2/AA3D2.
5. The hydrocarbon chain of AA3E2 enhances the interaction between AA3E2 and Val24 of α-Aβ.

Based on the above results, we furthermore proposed a novel compound AA3X2 (figure 7) and investigated its binding properties with Aβs. The binding energy evaluated indicates that AA3X2 binds more strongly to Aβ than AA3E2. Therefore, AA3X2 is expected to be a more effective inhibitor for the Aβ aggregation.

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