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<td>Kurisaki, Ikuo / Tanaka, Shigenori</td>
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ATP Converts Aβ_{42} Oligomer into Off-pathway Species by Making Contact with Its Backbone Atoms Using Hydrophobic Adenosine

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**Abstract**

Adenosine tri-phosphate (ATP) newly is expected to be involved in clearance of amyloid β 1–42 (Aβ42) fibril and its precursors, Aβ42 oligomer. Meanwhile the microscopic mechanism of the role in dissolving the protein aggregate still remains elusive. Aiming to elucidate the mechanism, we examined effects of ATP on the conformational change and thermodynamic stability of protomer dimer of Aβ42 pentamer and tetramer, Aβ42(9), by employing all-atom molecular dynamics simulations. We observed interprotomer twisting and intraprotomer peeling of Aβ42(9). These conformational changes remarkably accelerate dissociation of the protomer dimer. However, the presence of ATP itself has no positive effect on dissociation processes of the protomer dimer and a monomer from the dimer, indicating its irrelevance to decomposition of Aβ42 oligomer. Rather it could be supposed that ATP prevents additional binding and rebinding of Aβ42 monomers to the Aβ42 oligomer and it then converts Aβ42 oligomer into off-pathway species which is excluded from Aβ42 fibril growth processes. Interestingly hydrophobic adenosine in ATP makes contact with Aβ42(9) on their backbone atoms, with respect to both Aβ42 monomers on the edge of Aβ42(9) and dissociated Aβ42 monomers in Aβ42(9). These roles of ATP would be applied without regard to the structural polymorphism of Aβ42 fibril.
Introduction

Fibril formation of amyloid β (1–42) (Aβ42) peptide has been supposed to be the pathogenicity factor of Alzheimer disease.¹ To keep neuronal cells normal under physiological conditions, human cell intrinsically has some clearance mechanisms for those Aβ42 conformers such as Aβ42 fibrils, its precursors (Aβ42 oligomer) referred to as on-pathway species, oligomeric assembly referred to as off-pathway species and the elementary components (Aβ42 monomer and protomer) (Figure 1A and 1B).²,³ On-pathway species denote β-strand prone conformations similar to an oligomer in the fibril (see Figure 1A and 1B for example)⁴, while off-pathway species, which are not competent for binding of additional Aβ42 monomer, denote less- or non-β-strand prone conformation deviating from an oligomer in the fibril⁵.

Adenosine tri-phosphate (ATP) currently is expected to be involved in the clearance of Aβ42 fibrils. Hyman and his colleagues⁶ clarified that ATP works as hydrotrope, a small molecule to solubilize hydrophobic molecules in aqueous solutions, by employing in vitro experiments. A more recent study by Haynes et al.⁷ reported that this biological hydrotrope dissolves endogenous protein aggregates in Xenopus oocyte nucleoli. Surprisingly, ATP dissolved even thermodynamically stable protein aggregates such as preformed Aβ42 fibril mixtures⁶. This newly discovered role of ATP now draws much
attention as an essential factor to control protein aggregates in the cell. Meanwhile the microscopic mechanism for ATP to dissolve the protein aggregate is not trivial and still remains elusive.

In order to elucidate the microscopic mechanism, it is indispensable to examine effects of ATP on the elementary processes of Aβ42 fibril dissolution, Aβ42 oligomer decomposition and its conversion into Aβ42 conformers excluded from the fibril formation (Figure S1 in Supporting Information is an example to illustrate a set of the elementary processes). However, this investigation currently is challenging due to a molecular diversity of oligomers, protomers, and conformers not involved in the fibril formation8. A realistic reaction mechanism of Aβ42 fibril formation is much more intricate than schematic illustrations of the process. It is therefore beyond abilities of current experimental techniques to selectively analyze the structure of a specific Aβ42 oligomer to identify the essential steps where ATP functions in dissolving the protein aggregates.
**Figure 1.** Structure of Aβ42 oligomer. (A) Aβ42 Oligomer; (B) Aβ42 monomer conformation, referred to as LS shape; (C) Hydrogen bond formation between Aβ42 monomers; (D) and (E) hydrophobic contact and salt bridge formation between protomers, respectively. A set of atomic coordination of Aβ42 oligomer is derived from cryo-electron microscopy Aβ42 structure (PDB entry: 5OQV). In panel (A), Aβ42 nonamer, consisting of the two protomers (chains A, C, E, F and H; chains B, D, G and I), is highlighted by coloring orange. In panel (C), hydrogen bonds are shown by red dotted line. In panels (B), (D) and (E), amino acid residues involved in interprotomer interaction are emphasized on the right of the panel. Capital letters in panels denote chain IDs, which follow the original annotation given in the PDB information.
To overcome such technical difficulty, we here employ all-atom molecular dynamics simulations to specifically examine conformational change and thermodynamic stability of Aβ42 nonamer, Aβ42(9) (highlighted by orange in Figure 1A)\(^9\), a heterodimer of protomers consisting of Aβ42 tetramer and pentamer (referred to as Aβ42(4) and Aβ42(5), respectively). Aβ42(9) is similar in size to the smallest Aβ42 oligomer detected in aqueous solution\(^10\). We have supposed that the Aβ42(9) is one of minimal Aβ42 on-pathway species and examining this model is practical to elucidate the microscopic mechanism of Aβ42 fibril dissolution.

Since Aβ42 fibril earns thermodynamic stability by non-bonded interaction (Figure 1C, 1D and 1E), Aβ42 fibril formation is essentially a reversible process. It is thus supposed that clearance of Aβ42 oligomers shifts equilibrium of Aβ42 fibril formation toward that of Aβ42 fibril dissolution. Recalling reversibility of Aβ42 fibril formation, we presume two conjectures to understand a microscopic mechanism of Aβ42 fibril dissolution by ATP: (1) ATP accelerates decomposition of Aβ42 oligomers into elementary Aβ42 fibril components, Aβ42 protomer and Aβ42 monomer; (2) ATP converts Aβ42 oligomers into off-pathway species, which lack binding affinity to the Aβ42 fibril axis ends, thus being excluded from Aβ42 fibril growth processes.
We have then studied to verify our conjectures by considering the Aβ₄₂ nonamer, Aβ₄₂(9) and we have obtained an evidence supporting the second conjecture, that ATP converts Aβ₄₂ oligomer into off-pathway species.

Materials and Methods

Setup of amyloid-β (1-42) nonamer systems

We used the cryo-electron microscopy (cryo-EM) structure for amyloid-β (1-42) nonamer (PDB entry: 5OQV⁹). Nε protonation state was employed for each of histidine residues, and all carboxyl groups in aspartate and glutamate residues were set to deprotonated state. Employing the Aβ₄₂(9) structure, we prepared five Aβ₄₂(9) systems, whose annotations and molecular components are summarized in Table 1. According to the study of Patel et al., Aβ₄₂ fibril is partly dissolved under physiological concentration of ATP-Mg²⁺ (8 mM). To discuss the effect of ATP-Mg²⁺ concentration, we examined five different conditions for ATP-Mg²⁺ concentration: 0, 8, 16, 32 and 64 mM. The detailed description for system setup is illustrated in Supporting Information (see SI 1).
Table 1. Molecular components of Aβ42 nonamer systems.

<table>
<thead>
<tr>
<th>system</th>
<th>concentration of ATP-Mg$^{2+}$ [mM]</th>
<th>number of molecule</th>
</tr>
</thead>
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<tr>
<td></td>
<td>ATP</td>
<td>Mg$^{2+}$</td>
</tr>
<tr>
<td>No ATP-Mg$^{2+}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8 mM ATP-Mg$^{2+}$</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>16 mM ATP-Mg$^{2+}$</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>32 mM ATP-Mg$^{2+}$</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>64 mM ATP-Mg$^{2+}$</td>
<td>64</td>
<td>40</td>
</tr>
</tbody>
</table>

To calculate the forces acting among atoms, AMBER force field 14SB$^{11}$, TIP3P water model$^{12,13}$, and JC ion parameters adjusted for the TIP3P water model$^{14,15}$ were applied for amino acid residues, water molecules, and ions, respectively. Besides, the force field parameter sets developed by Meagher$^{16}$ and Bradbrook$^{17}$ were applied for adenosine triphosphate (ATP)$^{18}$ and Mg$^{2+}$, respectively. Molecular modeling of each Aβ42(9) system was performed using the LEaP modules in AmberTools 17 package$^{19}$.

**Simulation setup**

Molecular mechanics (MM) and molecular dynamics (MD) simulations were performed under the periodic boundary condition with GPU-version PMEMD module in AMBER 17 package$^{19}$ based on SPFP algorism$^{20}$ with NVIDIA GeForce GTX1080 Ti.
Electrostatic interaction was treated by the Particle Mesh Ewald method, where the real space cutoff was set to 9 Å. The vibrational motions associated with hydrogen atoms were frozen by SHAKE algorithm through MD simulations. The translational center-of-mass motion of the whole system was removed by every 500 steps to keep the whole system around the origin, avoiding an overflow of coordinate information from the MD trajectory format. These simulation conditions referred above were common in all of the simulations discussed in this manuscript.

**Unbiased molecular dynamics simulation**

Following temperature relaxation NVT simulations, tens of nanosecond NPT simulations (300 K, 1 bar) were performed and used for following analyses. The system temperature and pressure were regulated with Berendsen thermostat\textsuperscript{21} with a 5-ps of coupling constant and Monte Carlo barostat with attempt of system volume change by every 100 steps, respectively. A set of initial atomic velocities was randomly assigned from the Maxwellian distribution at 0.001 K at the beginning of the NVT simulations. The time step of integration was set to 2 fs. The further details are shown in Supporting Information (see SI 2).
**Steered and umbrella sampling molecular dynamics simulations**

Dissociation processes of Aβ42 monomer or Aβ42 protomer were simulated by combining a steered molecular dynamics (SMD) simulation with umbrella sampling molecular dynamics (USMD) simulations. SMD was employed to dissociate an Aβ42 monomer or protomer from the remaining part of Aβ42(9). 0.25-ns SMD simulation was carried out under NPT condition (300 K, 1 bar), where the system temperature and pressure were regulated by Langevin thermostat with 1-ps\(^{-1}\) collision coefficient, and Monte Carlo barostat with attempt of system volume change by every 100 steps, respectively. The value of reaction coordinate was gradually changed through the SMD simulations by imposing the harmonic potential with the force constant of 100 kcal/mol/Å\(^2\).

Then, certain numbers of snapshot structures were extracted from the SMD trajectory and employed for USMD windows. Following temperature relaxation simulations, several nanosecond NVT USMD simulations (300 K) were performed for each of the USMD windows (Tables S1 and S2 in Supporting Information for Aβ42 protomer dissociation and Aβ42 monomer dissociation, respectively). The system temperature was regulated using Langevin thermostat with 1-ps\(^{-1}\) collision coefficient. Each of the last 1-ns USMD trajectories was used to construct a potential of mean force. The reaction
coordinates chosen are discussed in Results and Discussion section. The further details are illustrated in Supporting Information (see SI 3).

**Trajectory analyses**

Interatomic distance, dihedral angle and root mean square deviation (RMSd) were calculated with the cpptraj module in AmberTools 17 package\(^{19}\). We calculated RMSd to the cryo-EM Aβ42(9) structure\(^9\) using the backbone heavy atoms (i.e., C\(\alpha\), N, C and O). Besides, solvent accessible surface area (SASA) is calculated with NACCESS software\(^{22}\). We identify an atomic contact between an ATP molecule and an amino acid residue when the minimum interatomic distance between them was shorter than 4.0 Å. Salt bridge formation is identified when a distance between a nitrogen atom in a basic residue and an oxygen atom in an acidic residue is smaller than 3.2 Å.

Potential of mean force (PMF) was calculated with Weighed Histogram Analysis Method (WHAM)\(^{23,24}\) by using each set of USMD trajectories. Statistical errors of PMF values, \(\sigma_{PMF}(\xi)\), were estimated by employing bootstrapped sampling\(^{25}\):

\[
\sigma_{PMF}(\xi) = \left[ \left( N_b - 1 \right)^{-1} \sum_{k=1}^{200} \left( W_{b,k}(\xi) - \left\langle W_{b}(\xi) \right\rangle \right)^2 \right]^{1/2} \tag{1}
\]

Here, \(N_b\), \(\xi\), and \(W_{b,k}(\xi)\) denote the number of bootstrapped sampling, the reaction coordinate and the value of \(k\)th bootstrapped potential of mean force at each point of
$\xi$, respectively. $\langle W_h(\xi) \rangle$ is average over all $W_{h,k}(\xi)$, where $k$ ranges from 1 to 200.

Reaction rate, $k_{TST}$, is estimated by using Eyring’s transition state theory:

$$k_{TST} = \frac{k_B T}{h} \exp \left( -\frac{\Delta F^\dagger}{k_B T} \right)$$  \hspace{1cm} (2)

Here, $\Delta F^\dagger$, $h$, $k_B$ and $T$ denote an activation barrier height, Planck constant, Boltzmann constant and a temperature of system, respectively. Reaction time scale, $\tau_{TST}$, is defined as the inverse of $k_{TST}$. $\Delta F^\dagger$ is defined as $F(\xi_0') - F(\xi_0)$, where PMF has local minimum at $\xi_0$, and gradient of PMF turn from positive to negative values at $\xi_0'$, which is greater than $\xi_0$.

Molecular structures were illustrated using Visual Molecular Dynamics (VMD).26 A density distribution of ATP was calculated with the cpptraj module in AmberTools 17 package19 and visualized using Volmap plugin of VMD26. Error bars are calculated from standard error and indicate 95% confidence interval if there is no annotation.

**Results and Discussion**

**ATP makes contact with Aβ42 nonamer on its backbone atoms**

We examined conformational fluctuation of Aβ42 nonamer, Aβ42(9), under thermal equilibrium by employing one hundred sets of 60-ns unbiased NPT MD simulations for each Aβ42(9) system. Figure 2A shows time-course change of averaged RMSd for Aβ42(9)
(RMSd analyses for each Aβ42 monomer are discussed in Figure S2 in Supporting Information). For each of the five systems, it can be considered that the values reach equilibrium after 40 ns.

Meanwhile, the number of atomic contact between ATP and Aβ42(9) also reaches an equilibrium value after 40 ns (Figure 2B and 2C), for each of the four Aβ42(9)-ATP-Mg²⁺ systems. The number of these contact increases with ATP concentration as expected. Considering the equilibrium of these two quantities, we employed partial MD trajectories in the period after 40 ns, and analyzed conformational properties of Aβ42(9) under thermal fluctuations.
**Figure 2.** Time-course analyses of RMSd and atomic contact between ATP and Aβ_{42} nonamer. (A) RMSd; (B) The number of amino acid residues making contact with ATP molecules; (C) The number of ATP molecules making contact with amino acid residues. Black line for No-ATP-Mg^{2+} system; blue line for 8 mM ATP-Mg^{2+} system; green line for 16 mM ATP-Mg^{2+} system; orange line for 32 mM ATP-Mg^{2+} system; red line for 64 mM ATP-Mg^{2+} system. Time domain assumed as convergence is indicated by the red dotted rectangle.

ATP contact with Aβ_{42}(9) implies functional similarity between ATP and morin. Morin is one of wine-related polyphenols and has potential to destabilize preformed Aβ_{42} fibrils. The earlier simulation study by Lenkul proposed the atomistic mechanism for the role of morin in Aβ_{42} fibril destabilization: it binds to the fibril axis ends of Aβ_{42} oligomer and prevents an additional Aβ_{42} monomer from binding to the oligomer. Recalling the role of morin in Aβ_{42} oligomer, we then analyzed ATP contact on the
backbone atoms (N, C and O) for the Aβ42 monomers at the fibril axis ends (chain D, E, F and G), employing the one hundred 60-ns unbiased NPT MD simulations for 64 mM ATP-Mg²⁺ system as representative. Concentration of 64 mM is c.a. 10-fold greater than that under physiological condition. The concentration of Aβ42 monomer in the simulation box is c.a. 1 mM, while experimental Aβ42 monomer concentration is up to 0.1 mM 29. Then, we employed this system considering that our simulation condition is similar to experiment condition in the ratio between ATP and Aβ42 monomers.

Table 2 summarizes the number of atomic contact between ATP and Aβ42 monomers at the fibril axis ends, the chains D, E, F and G shown in Figure 1A (time-course analyses of the atomic contact are discussed in Figures S3 and S4) and the remaining five monomers located at the middle of the protomers, the chains A, B, C, H and I. For each of panels, we assumed convergence of the value after 40 ns. 1.8 or more residues in each of the four Aβ42 monomers make contact with ATP on their backbone atoms, while c.a. one ATP is involved in contact with backbone atoms of each of the monomers. Since ATP partially covers the fibril axis ends, it could have potential to prevent additional monomer binding to the fibril axis end of Aβ42 oligomers as well as morin.

The number of atomic contact between ATP and Aβ42 monomers at the fibril axis ends are greater than that at the middle of the protomers. Nonetheless ATP could not
preferentially make contact with Aβ_{42}(9) backbone atoms at the fibril axis end. The middle part of Table 2 shows the SASA-normalized number of amino acid residue contact with ATP. These values are not necessarily higher in the monomers at the fibril ends. This observation suggests non-site-specific interaction between ATP and Aβ_{42}(9).

Taken together, the observations for ATP contacts at the fibril axis end could support our second conjecture in the point that Aβ_{42} oligomers are excluded from the fibril growth process by ATP contact.

**Table 2.** Atomic contact between ATP and backbone atoms of Aβ_{42} monomer at the fibril axis end for 64 mM ATP-Mg^{2+} system.

<table>
<thead>
<tr>
<th>part of ATP</th>
<th>chain ID*</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td></td>
<td>0.5</td>
<td>0.9</td>
<td>0.7</td>
<td>2.7</td>
<td>0.6</td>
<td>2.7</td>
<td>0.6</td>
<td>1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>adenosine</td>
<td></td>
<td>0.4</td>
<td>0.9</td>
<td>0.6</td>
<td>2.6</td>
<td>0.6</td>
<td>2.6</td>
<td>0.6</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>tri-phosphate</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

| ATP contact with amino acid residues (normalized with SASA and multiplied by 10^{3}) |
| ATP            |           | 2.2 | 4.0 | 2.9 | 3.1 | 3.0 | 2.4 | 2.4 | 3.1 | 3.0 |
| adenosine      |           | 2.0 | 3.8 | 2.7 | 3.0 | 2.9 | 2.2 | 2.2 | 2.9 | 2.5 |
| tri-phosphate  |           | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

| ATP contact with amino acid residues |
| ATP            |           | 0.3 | 0.6 | 0.5 | 1.3 | 1.2 | 1.1 | 1.3 | 0.5 | 0.7 |
| adenosine      |           | 0.3 | 0.6 | 0.4 | 1.2 | 1.1 | 1.0 | 1.2 | 0.4 | 0.6 |
| tri-phosphate  |           | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 0.3 | 0.1 | 0.1 | 0.1 |

*Chain IDs follow the original annotation given in the PDB information for Aβ_{42} structure
by cryo-electron microscopy (PDB entry: 5OQV) and Aβ42 monomer chain at the fibril axis end is highlighted by boldface.

**SASA denotes solvent accessible surface area [Å²] and are calculated for main chain atoms (Ca, C, N, O and H) in each monomer.

It should be noted that ATP contact with these backbone atoms is mostly due to the hydrophobic adenosine (Table 2). Amino group and hydroxyl groups in adenosine play significant roles in stable contact with Aβ42 monomer on their backbone. This issue also will be addressed with regard to an Aβ42 monomer dissociation process in the later part of this section.

**Aβ42 nonamer shows interprotomer twisting and intraprotomer peeling under thermal fluctuation**

In order to analyze conformational fluctuation of Aβ42(9) in atomistic details, we defined three structure descriptors typical of Aβ42(9), the interprotomer twisting angle (θτ), the intraprotomer peeling distances (d4τ and d5τ) and the interprotomer center of gravity distance (d4τ+5), which are explained in Figure 3A, 3B and 3C, respectively. The superscripts ‘4’ and ‘5’ denote Aβ42(4) and Aβ42(5), respectively. They were calculated for each of all the snapshots structures.
**Figure 3.** Schematic illustration for the structural descriptors of Aβ_{42} nonamer. (A) interprotomer twisting angle; (B) intraprotomer peeling distances; (C) interprotomer center of gravity distance. \( \bar{X}_m^n \) denotes center of gravity, which is calculated for the \( m \) C\( \alpha \) atoms of the \( n^{th} \) residues in the \( m \) mer protomer in the Aβ_{42} nonamer. A blue ball represents center of gravity.

Table 3 gives averaged values of the structure descriptors calculated for each of the five systems, and also those for the cryo-EM structure\(^9\) as the reference. Each of the systems shows remarkable difference in twisting angle, compared with the cryo-EM structure. Meanwhile, both the values of peeling distances and those of interprotomer center of gravity distance are similar between the cryo-EM structure and each of the systems.
Table 3. Statistical analyses of structure descriptors and number of interprotomer salt bridge formation

<table>
<thead>
<tr>
<th>system</th>
<th>twisting angle [deg.]</th>
<th>peeling distance [Å]</th>
<th>center of gravity distance [Å]</th>
<th>number of salt bridge formation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>4 mer</td>
<td>5 mer</td>
<td></td>
</tr>
<tr>
<td>Reference†</td>
<td>174.4</td>
<td>11.5</td>
<td>11.5</td>
<td>30.3</td>
</tr>
<tr>
<td>No ATP-Mg(^{2+})</td>
<td>197.3 ± 9.4*</td>
<td>12.1 ± 2.2</td>
<td>12.3 ± 1.5</td>
<td>32.5 ± 0.5</td>
</tr>
<tr>
<td>8 mM ATP-Mg(^{2+})</td>
<td>197.4 ± 10.6</td>
<td>12.3 ± 2.7</td>
<td>12.4 ± 1.6</td>
<td>32.5 ± 0.5</td>
</tr>
<tr>
<td>16 mM ATP-Mg(^{2+})</td>
<td>197.3 ± 9.5</td>
<td>12.2 ± 2.6</td>
<td>12.4 ± 1.6</td>
<td>32.5 ± 0.6</td>
</tr>
<tr>
<td>32 mM ATP-Mg(^{2+})</td>
<td>197.9 ± 11.2</td>
<td>12.0 ± 2.4</td>
<td>12.5 ± 1.8</td>
<td>32.5 ± 0.5</td>
</tr>
<tr>
<td>64 mM ATP-Mg(^{2+})</td>
<td>198.3 ± 11.2</td>
<td>12.1 ± 2.0</td>
<td>12.5 ± 1.8</td>
<td>32.5 ± 0.5</td>
</tr>
</tbody>
</table>

†Aβ\(_{42}\) nonamer resolved by cryo-electron microscopy (PDB entry: 5OQV).

*Error indicates 95% confidential interval, where error value is estimated from standard deviation.

The remarkable change for the twisting angle could be explained by absence of atomic contact of Aβ\(_{42}(9)\) with remaining part of Aβ\(_{42}\) amyloid fibril (see Figure 1A, the left illustration). Under the experimental condition, each Aβ\(_{42}\) monomer on the edge of protomer (Chains D, E, G or F) makes atomic contact with the neighboring monomer which is not provided in the PDB’s atomic coordinate file (see Figure 1A, the left illustration). Meanwhile, Aβ\(_{42}(9)\) in our simulation has no atomic contact with any other Aβ\(_{42}\) monomers (see Figure 1A, the right illustration). Loss of atomic contact with
neighboring monomer possibly makes the two protomers reorient to earn energetic stabilization as Aβ42(9) in aqueous solution. This observation is supported by the increased number of salt bridge between the protomers. (Table 3).

According to the analyses discussed above, we found conformational fluctuation of Aβ42(9) with regard to the twisting angle and peeling distances within a period of tens of nanoseconds. Meanwhile, in terms of these structure descriptors, each conformational characteristic of Aβ42(9) under thermal fluctuation is similar among the five systems (see Table 3) so that we could say that it is an intrinsic property of Aβ42(9) with no regard to the presence of ATP molecules.

To precisely examine influence of twisting and peeling in the following Aβ42(9) dissociation simulation, the MD simulation-derived snapshot structures were classified into 12 groups, by employing the twisting angle (θ_r) and the two peeling distances (d_p^4 and d_p^5) as classification parameters.

We use the following symbols (−, +, R and L) in classification of Aβ42(9) conformation. Minus sign (−) means that a structural descriptor has a value close to the averaged one: the value fall within range of the average value ±2 × S.D. Meanwhile the other three signs denote that a structural descriptor has values away from the averaged one: the value is found out of range of the average value ±2 × S.D. Cross (+) is for peeling in Aβ42(4)
and that in Aβ42(5). ‘R’ and ‘L’ are for the deviation in counterclockwise and clockwise directions with regard to twisting angle, respectively.

A class is defined as combination of three letters: the first, second and third letters are for twisting, peeling of Aβ42(5) and peeling of Aβ42(4). For example, R−+ is assigned to a conformation which shows counterclockwise twisting and peeling of Aβ42(4). Occupancies of classes are given in Tables S3-7 in Supporting Information, for each of the Aβ42(9) systems, indicating negligible effects of ATP on repertoire of Aβ42(9) conformations.

Among the twelve classes, the four (−−−, −++, L−−, R−−) occupy c.a. 90% of snapshot structures for each of the five systems. Besides, these four classes contain an average conformation (−−−) and typical ones which show significant deviations of intraprotomer peeling, interprotomer clockwise twisting and interprotomer counterclockwise twisting (−++, L−− and R−−, respectively). We here thus supposed that considering the four classes is enough to examine the influence of conformational change of Aβ42(9) on dissociation processes.

We defined a representative structure for each of the four class by using the score function:
We considered this function to evaluate structural deviation of an MD-derived Aβ42(9) conformation from the averaged one of the corresponding ATP-Mg$^{2+}$ system. As addressed above, the twisting angle, peeling distances of Aβ42(4) and Aβ42(5) are denoted by $\theta_r$, $d_{p}^4$ and $d_{p}^5$. Subscripts Ave and SD mean average and standard deviation, respectively (specific values for each ATP-Mg$^{2+}$ system are shown in Table 3). The representative structure of —— and those of the other three correspond to the smallest and largest values of the score function, respectively. The former is supposed to take an average structure, while each of the latter three is supposed to take a structure with relatively large deviation with regard to these structure descriptors.

These four representative structures were employed for the following dissociation simulations of Aβ42(9) (Figure 4A-D, and Table 4). They were derived from 64 mM ATP-Mg$^{2+}$ system. This is because values of twisting angle and peeling distances are relatively large compared with the other four systems (see Tables S3-7 in Supporting Information), which are expected to lead to apparent effects of conformational difference from —— on dissociation processes.

\[
Score(\theta_r, d_p^4, d_p^5) = \left| \frac{\theta_r - \theta_{r,Ave}}{\theta_{r,SD}} \right| + \left| \frac{d_p^4 - d_{p,Ave}}{d_{p,SD}} \right| + \left| \frac{d_p^5 - d_{p,Ave}}{d_{p,SD}} \right| \tag{3}
\]
Figure 4. Representative conformations of Aβ42 nonamer in aqueous solution. A conformation annotation is given on the top of each panel. (A) Standard conformation (−−−); (B) Peeling on both protomer (−++); (C) Twisting toward left side (L--); (D) Twisting toward right side (R--). The cryo-electron microscopy Aβ42 nonamer structure (PDB entry: 5OQV), colored in transparent blue, is superposed on each representative conformation.
Table 4. Values of the structural descriptors and number of interprotomer salt bridge formation for the four representative conformations

<table>
<thead>
<tr>
<th>conformation †</th>
<th>twisting angle [deg.]</th>
<th>peeling distance [Å]</th>
<th>center of gravity distance [Å]</th>
<th>number of interprotomer salt bridge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 mer</td>
<td>5 mer</td>
<td></td>
</tr>
<tr>
<td>− − −</td>
<td>198.3</td>
<td>12.1</td>
<td>12.5</td>
<td>31.8</td>
</tr>
<tr>
<td>− ++</td>
<td>191.8</td>
<td>17.6</td>
<td>32.2</td>
<td>35.7</td>
</tr>
<tr>
<td>L − −</td>
<td>183.9</td>
<td>13.9</td>
<td>9.2</td>
<td>31.7</td>
</tr>
<tr>
<td>R − −</td>
<td>255.9</td>
<td>11.3</td>
<td>14.1</td>
<td>32.3</td>
</tr>
</tbody>
</table>

†— for standard conformation; −++ for peeling on both protomer conformation; L−− for twisting toward left side conformation; R−− for twisting toward right side conformation.

Protomer dissociation proceeds through conformational change

We discuss effects of ATP on Aβ42(9) decomposition by considering Aβ42(9) protomer and monomer dissociation processes through the remaining parts of this section. We here address the former, Aβ42(9) protomer dissociation process. No ATP-Mg$^{2+}$ and 64 mM ATP-Mg$^{2+}$ systems were reconstructed with the four representative conformations and examined hereafter (the details are given in SI 3 in Supporting Information). Here the 64 mM ATP-Mg$^{2+}$ condition was chosen, expecting that the effect of ATP on Aβ42(9) is
emphasized by enhancing Aβ42(9)-ATP interaction more than in the system under physiological condition.

We first considered No ATP-Mg$^{2+}$ system. Aβ42(9) protomer dissociation process is simulated by a steered MD (SMD) method, where the interprotomer center of gravity distance ($d_{4-5}$) is employed as the reaction coordinates (see Figure 3C) and steered to increase by 60 Å in each SMD simulation. We observed that each of the protomers dissociates without apparent structural deviations, except for −++ for the No ATP-Mg$^{2+}$ system (see Figure S5 in Supporting Information). The structural deviation for −+++ is explained by the fluctuation of peeling distances, although the distances still satisfy the criteria of −++ (see Figure S6 in Supporting Information). We then suppose that structural deviation observed in the SMD simulations for −+++ are essentially irrelevant for the following analyses. This observation similarly holds for the 40 mM ATP-Mg$^{2+}$ system, so that we here address it in advance (see Figures S6 and S7 in Supporting Information).

As shown in Figure 5A-D, each of the PMFs has one activation barrier toward the dissociation direction, appearing to be up-hill. The PMF for −+++ is different from those for the other three in the position of minimum. This is simply because a positions of center of mass of −+++ is shifted due to peeling. This difference changes appearance of the PMF subtly but is not essential in the following discussion.
Figure 5. Potential of mean force of Aβ42 protomer dissociation for No ATP-Mg²⁺ and 64 mM ATP-Mg²⁺ systems. (A) Standard conformation (−−−−); (B) Peeling on both protomer conformation (−+++); (C) Twisting toward left side conformation (L−−−); (D) Twisting toward right side conformation (R−−−). A conformation annotation is given on the top of each panel. Error bar indicates 95% confidential interval. Black lines for No ATP-Mg²⁺ system; red lines for 64 mM ATP-Mg²⁺ system.

The PMF for −−−− shows the highest activation barrier, 19.1 kcal/mol of the four systems. Meanwhile those for −+++ , L−−− and R−−− are 12.4, 15.3 and 14.3 kcal/mol,
respectively. It can be supposed that \( A\beta_{42}(9) \) conformational changes from \( --- \) accelerate protomer dissociation reaction with lower activation barrier. This could be due to weakening interprotomer interaction, which acts between N-terminal of one protomer and C-terminal of the other (see Figure 1E). Actually, we can find apparent reduction of salt bridge formation between the protomer in \( +++ \), \( L--- \) and \( R--- \), compared with \( --- \). The number of interprotomer salt bridges is 24 as for \( --- \), while it is reduced to 17 or lesser with regard to the remaining three (Table 4).

According to the heights of activation barriers calculated above, the time scale of protomer dissociation approximately ranges from milliseconds to tens of second (Table 5). This time scale is greater than that of relaxation of ATP contact with \( A\beta_{42}(9) \), tens of nanoseconds (see Figure 2B and 2C). Under such a circumstance, ATP contact with \( A\beta_{42}(9) \) probably is relaxed in advance of \( A\beta_{42} \) protomer dissociation.
Table 5. Physicochemical characterization of potential of mean force of Aβ42 protomer dissociation for No ATP-Mg^{2+} and 64 mM ATP-Mg^{2+} systems. Free energy barrier, reaction rate calculated with Eyring’s transition theory and reaction time are denoted by $\Delta F^\ddagger$, $k_{TST}$ and $\tau_{TST}$ (inverse of $k_{TST}$), respectively.

<table>
<thead>
<tr>
<th>Conformation</th>
<th>$\Delta F^\ddagger$ [kcal/mol]</th>
<th>$k_{TST}$ [s$^{-1}$]</th>
<th>$\tau_{TST}$ [s]</th>
<th>$\Delta F^\ddagger$ [kcal/mol]</th>
<th>$k_{TST}$ [s$^{-1}$]</th>
<th>$\tau_{TST}$ [s]</th>
</tr>
</thead>
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<tr>
<td>-- --</td>
<td>19.1 (1.2)</td>
<td>7.2E-02</td>
<td>1.4E+01</td>
<td>21.7 (1.8)</td>
<td>1.0E-03</td>
<td>9.7E+02</td>
</tr>
<tr>
<td>-- ++</td>
<td>12.4 (1.5)</td>
<td>5.5E+03</td>
<td>1.8E-04</td>
<td>11.4 (1.4)</td>
<td>3.2E+04</td>
<td>3.1E-05</td>
</tr>
<tr>
<td>L --</td>
<td>15.3 (0.8)</td>
<td>4.4E+01</td>
<td>2.3E-02</td>
<td>17.8 (1.6)</td>
<td>6.8E-01</td>
<td>1.5E+00</td>
</tr>
<tr>
<td>R --</td>
<td>14.3 (0.9)</td>
<td>2.5E+02</td>
<td>4.0E-03</td>
<td>14.1 (1.8)</td>
<td>3.4E+02</td>
<td>2.9E-03</td>
</tr>
</tbody>
</table>

*Value inside parentheses denotes 95% confidential interval.

†— for standard conformation; --++ for peeling on both protomer conformation; L-- for twisting toward left side conformation; R-- for twisting toward right side conformation.

Considering these two molecular events different in the time scale, we simulated protomer dissociation after relaxation of ATP contact with Aβ$_{42}(9)$ (Figure 6) (see SI 3 in Supporting Information for computational details) and calculated the PMFs of 64 mM ATP-Mg$^{2+}$ system (Figure 5A-D). The height of an activation barrier is reduced by conformational change from ——, being similar to the cases of the No ATP-Mg$^{2+}$ systems
Meanwhile, we cannot find significant difference in height of activation barrier between PMFs in the absence of ATP and that in the presence of ATP. The timescale of protomer dissociation still is between milliseconds and tens of second.

**Figure 6.** Spatial distribution of ATPs around Aβ₄₂ nonamer under 64 mM ATP-Mg²⁺ condition. Locations with density > 0.0075 Å⁻³ is drawn with red wire frame.

These PMF analyses indicate that ATP has no remarkable influence on Aβ₄₂(9) protomer dissociation reaction, so that we could not obtain evidences supporting our first conjecture. Nonetheless, physicochemical insights derived from the PMF analyses still are worth discussing in the context of thermodynamic stability of Aβ₄₂(9) oligomer. Recalling the heights of activation barriers, the time scale of Aβ₄₂(9) protomer
dissociation approximately ranges from milliseconds to tens of second (Table 5), and then is similar to those of protein-protein dissociation processes previously reported\textsuperscript{30-32}. Considering that Zhang and colleagues\textsuperscript{33} reported that a protein complex dissociation undergoes rotational motion in the initial step, it could be said that Aβ\textsubscript{42}(9) should assume R– or L– at the beginning of protomer dissociation process under thermal fluctuation. According to these observations, Aβ\textsubscript{42}(9) as Aβ\textsubscript{42}(5)-Aβ\textsubscript{42}(4) complex possibly is similar to biologically functional protein-protein complexes in thermodynamic stability.

Meanwhile, more elongated Aβ\textsubscript{42} oligomers possibly show greater thermodynamic stability. It is presumed that elongation of Aβ\textsubscript{42} oligomer accompanies enthalpy gain and entropy loss to earn thermodynamic stability. It would result in restriction of configurations of the protomers, then suppressing the rotational motion between a pair of protomers as represented by R– and L–. Suppressing such structural changes would practically keep an activation barrier of the protomer dissociation higher. This observation seems to be worth studying to obtain deeper insights into a microscopic mechanism of Aβ\textsubscript{42} fibril formation process, although it is beyond the scope of this study. We then leave it as the future problem.

ATPs make contact with partially dissociated Aβ\textsubscript{42} monomer and
**convert Aβ₄₂ oligomer into off-pathway species**

Next, we examined the effect of ATP on Aβ₄₂ monomer dissociation. In Aβ₄₂ monomer dissociation SMD simulations, the distance between center of gravity of the monomer in Aβ₄₂(5) (Chain F, referred to as Aβ₄₂(F) hereafter) and that of the remaining part of the pentamer (Chains A, C, E and H, referred to as Aβ₄₂(ACEH)) is employed as the reaction coordinate (Figure 7). A value of the reaction coordinate was steered to increase by 40 Å in each SMD simulation.

**Figure 7.** Schematic illustration of reaction coordinate for monomer dissociation simulation. $\bar{X}_m^n$ denotes center of gravity, which is calculated for the C$_\alpha$ atoms of a set of Aβ₄₂ monomer chain, $m$. The monomer undergoing dissociation is distinguished by coloring yellow. Bold capital letters beside Aβ₄₂ pentamer denote chain ID, which follows original annotation given in PDB information of cryo-electron microscopy Aβ₄₂ structure
(PDB entry: 5OQV). Blue balls represent center of gravity for each descriptor.

The dissociated Aβ42(F) shows a partially or fully unfolded conformation (Figure 8). We suppose that it could reflect structural properties of Aβ42 monomer under Aβ42 oligomer formation condition. Each Aβ42 monomer in an oligomer assumes thermodynamically unstable LS shape conformation (see Figure 1B). Actually, this conformation has no α-helices and no intramolecular β-sheets so that it earns energetic stability by forming hydrogen bonds with neighboring Aβ42 monomers (see Figure 1C). It is then supposed that Aβ42 monomer folding into the LS shape conformation is coupled with its binding to Aβ42 oligomer and its reverse reaction, Aβ42 monomer dissociation, is coupled with unfolding of LS shape conformation.

We first analyzed the PMFs of Aβ42(9) monomer dissociation in absence of ATP-Mg²⁺ (Figure 9A-D). The PMFs for −−− and R−− are different from those for −+++ and L−− with respect to concerning the steep slopes from the minima. Nonetheless, these PMFs are similar in their physicochemical characters. They have no intermediate states and thus essentially have each one activation barriers toward the dissociation direction, appearing to be up-hill with no regard to conformations of Aβ42(9).
Figure 8. Ensemble of snapshot structures of monomer dissociated Aβ42 nonamer, obtained from independent twelve steered MD simulation for No ATP-Mg²⁺ system of standard conformation (---), which is similar to cyro-EM structure (PDB entry: 5OQV). The monomer undergoing dissociation is distinguished by coloring yellow.

According to the heights of activation barriers calculated above, the time scale of Aβ42 monomer dissociation approximately ranges from sub-microseconds to milliseconds (Table 6), thus being greater than that of relaxation of ATP contact on Aβ42(9) (see Figure 2B and 2C). We then have performed Aβ12 monomer dissociation simulations under the condition that ATP contact with Aβ42(9) has been relaxed (see Figure 6), as is the case of the protomer dissociation simulations. Interestingly, addition of ATP suppresses Aβ42
monomer dissociation. Each of the PMFs for 64 mM ATP-Mg\(^{2+}\) system has the activation barrier whose height is larger than 18.2 kcal/mol (Figure 9A-D). According to the heights of the activation barriers, the time scale of monomer dissociation is in range of seconds or longer (Table 6).

Figure 9. Potential of mean force of A\(_{42}\) monomer dissociation for No ATP-Mg\(^{2+}\) and 64 mM ATP-Mg\(^{2+}\) systems. (A) Standard conformation (-----); (B) Peeling on both protomer conformation (——+); (C) Twist toward left side conformation (L——); (D) Twist
toward right side conformation (R−−). Error bar indicates 95% confidence interval. Black lines for No ATP-Mg$^{2+}$ system; red lines for 64 mM ATP-Mg$^{2+}$ system.

**Table 6.** Physicochemical characterization of potential of mean force of Aβ$_{42}$ monomer dissociation for No ATP-Mg$^{2+}$ and 64 mM ATP-Mg$^{2+}$ systems. Free energy barrier, reaction rate calculated with Eyring’s transition theory and reaction time are denoted by $\Delta F^\ddagger$, $k_{TST}$ and $\tau_{TST}$ (inverse of $k_{TST}$), respectively.

<table>
<thead>
<tr>
<th>Conformation</th>
<th>No ATP-Mg$^{2+}$</th>
<th>64 mM ATP-Mg$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta F^\ddagger$ [kcal/mol]</td>
<td>$k_{TST}$ [s$^{-1}$]</td>
</tr>
<tr>
<td>−−−</td>
<td>13.7 (1.8)</td>
<td>6.9E+02</td>
</tr>
<tr>
<td>−++</td>
<td>11.6 (2.3)</td>
<td>2.1E+04</td>
</tr>
<tr>
<td>L−−</td>
<td>8.1 (1.8)</td>
<td>7.4E+06</td>
</tr>
<tr>
<td>R−−</td>
<td>17.2 (3.8)</td>
<td>1.8E+00</td>
</tr>
</tbody>
</table>

*Value inside parentheses denotes 95% confidence interval.

†= for standard conformation; ++ for peeling on both protomer conformation; L= for twisting toward left side conformation; R= for twisting toward right side conformation.

In contrary to our first conjecture, ATP rather suppresses Aβ$_{42}$ monomer dissociation. It can thus be supposed that Aβ$_{42}$ fibril dissolution under the presence of ATP is due to
molecular processes except for acceleration of Aβ42 oligomer decomposition. On the other hand, there still is room to consider our second conjecture, because our analyses showed the capability of ATP to exclude Aβ42 monomers from the Aβ42 fibril formation process (see Table 2 and the related discussion). We then examine the effects of ATP on Aβ42(F) under dissociation conditions by keeping our second conjecture in mind in the following.

Suppression of Aβ42(F) dissociation observed above possibly is due to ATP contact with Aβ42(F). As addressed above, there are a certain amount of ATPs making stable contact with Aβ42(9) (see Figure 2B and 2C, and Figure 6). A part of such ATPs could interfere with Aβ42(F) dissociation by anchoring the monomer around the oligomer. We then speculate the presence of ATP which makes steady contact with both Aβ42(F) and the remaining of the Aβ42(9).

Meanwhile, it is possible to consider the effect of ATP on exclusion of dissociated Aβ42 monomers from Aβ42 oligomer formation process. Assuming ATP contact with dissociated Aβ42 monomer on the backbone atoms (N, C and O), such a contact competes with intermonomer hydrogen bond formation and refolding to LS shape conformation (see Figure 1B and 1C) to interfere with rebinding of the dissociated monomer to the Aβ42 oligomer. The above assumption seems physicochemically reasonable because we already observed that ATP makes contact with Aβ42 monomers at the fibril axis ends on
their backbone atoms. ATP could make contact with (partially) unfolded Aβ42(F) similarly. We then made a speculation that ATP stably makes contact with Aβ42(F) on the backbone atoms.

To verify the speculation, we performed fifty unbiased 40-ns NPT MD simulations starting from Aβ42(9) with (partially) dissociated Aβ42(F) and analyzed ATP contact on the monomer. Recalling the similarity in physicochemical characteristics of Aβ42 monomer dissociation PMF among the four Aβ42(9) 64 mM ATP-Mg2+ systems (see Figure 9A-D), we considered the standard Aβ42(9) system (——) as representative.

We classified ATPs making contact with Aβ42(F) with three types: ATPs making contact with the remaining part of the Aβ42 oligomer simultaneously (ATP<sup>S</sup>; Figure 10A); ATPs making contact with other ATPs for forming cluster with the remaining of Aβ42(9) (ATP<sup>C</sup>; Figure 10B); ATPs making no contact with the Aβ42 monomers except for Aβ42(F) (ATP<sup>F</sup>; Figure 10C). Calculating the number of each of ATP types along time-course, we observed convergence after 20 ns so that we analyzed the values for the time domain between 20 and 40 ns (see Figure S8 in Supporting Information). The numbers of ATP<sup>S</sup> and ATP<sup>C</sup> are 1.6 ± 0.3 and 1.8 ± 0.5, respectively. Meanwhile, the number of ATP<sup>F</sup> is 0.6 ± 0.3. The analyses for ATP making contact with Aβ42(F) thus indicate that a certain amount of ATP makes contact with Aβ42(F) and the remaining of the Aβ42(9). These ATPs
would anchor $A\beta_{42}(F)$ around the remaining part of $A\beta_{42}$ oligomer to interfere its dissociation, then resulting in the higher activation barriers of the PMFs.

**Figure 10.** Representative illustration for classification of ATP making contact with dissociated monomer, $A\beta_{42}(F)$. (A) ATP making contacts with the remaining part of the $A\beta_{42}$ nonamer simultaneously (red stick). (B) ATPs making other ATPs forming cluster with the remaining part of $A\beta_{42}$ nonamer (green stick). (C) ATPs making no contact with $A\beta_{42}$ nonamer except for $A\beta_{42}(F)$ (blue stick). In panel B, ATP cluster is shown by lines. $A\beta_{42}(F)$ is highlighted by yellow.

Then, to verify the speculation, we analyzed ATP contact with backbone atoms (N, C and O) of $A\beta_{42}(F)$ (see Figure S9 in Supporting Information). Assuming convergence of values after 20 ns, we analyzed the values of contacts for the time domain between 20 and 40 ns. 2.4 ± 0.5 ATPs make contact with backbone atoms (N, C and O) of $A\beta_{42}(F)$. 
and 4.6 ± 0.8 amino acid residues in Aβ42(F) make contact with ATPs on their backbone atoms. This observation shows that ATPs steadily make contact with Aβ42(F) on the backbone atoms, thus verifying the speculation above. Such ATP contacts are supposed to interfere with rebinding of the dissociated Aβ42 monomer to the Aβ42 oligomer. As referred above, the role of ATP might be similar to that of morin in the point that it inhibits fibril elongation by blocking the monomer binding on the Aβ42 fibril axis end27,28.

To obtain further insights into ATP contact with the dissociated Aβ42 monomer, we examined Aβ42(F) backbone-ATP contact by distinguishing ATP parts (hydrophilic triphosphate and hydrophobic adenosine). Assuming convergence of values after 20 ns, we analyzed the values of contacts for the time domain between 20 and 40 ns (see Figure S10). 0.8 ± 0.2 and 4.2 ± 0.9 backbone atoms make contact with the hydrophilic triphosphate and hydrophobic adenosine, respectively. The relatively large number of contact with adenosine could be due to the presence of amino group of adenine and hydroxyl group of ribose. This observation suggests that the hydrophobic part of ATP mainly contributes to contact with the backbone atoms of dissociated Aβ42 monomer.

In summary, we could suppose that ATP thus shows the two opposite effects on the Aβ42 monomer dissociation process: ATPs suppress monomer dissociation while they prevent refolding of dissociated monomer into oligomer. It is of note that ATP forming
aggregates with Aβ42 oligomer make contact with dissociated Aβ42 monomer on their backbone atoms. Such an aggregate formation could prevent Aβ42 oligomer from recovering on-pathway species. We then suppose that the presence of ATP changes the thermal equilibrium of Aβ42 oligomer system from on-pathway species rich one to off-pathway species rich one. Increasing off-pathway species could practically shift thermal equilibrium of Aβ42 fibril formation toward that of dissolution. This observation supports our second conjecture that ATP converts Aβ42 oligomers into off-pathway species.

We have here proposed the conjecture which reasonably explains the role of ATP, yet it is of note that evidences we have obtained still are not sufficient to verify our conjecture. Actually our present study has not directly examined effects of ATP on unfolded Aβ42 monomer association with an oligomer or those on conversion of Aβ42 oligomer into off-pathway species. Then these important problems will be investigated in our future research.

**Concluding Remarks**

We analyzed influences of ATP on an Aβ42 oligomer model to examine our two conjectures, ‘ATP accelerates the decomposition of Aβ42 oligomers into elementary Aβ42 components’ and ‘ATP converts Aβ42 oligomers into off-pathway species’.
Recalling destabilization effects of salt on biomolecule complex formation\textsuperscript{34,35}, the first conjecture seems to be obvious and likely, but we could not obtain any evidences supporting this conjecture. Interestingly, the PMF analyses show that ATP does not accelerate either monomer or protomer dissociation processes and even suppresses Aβ\textsubscript{42} monomer dissociation. This observation suggests that ATP in aqueous solution does not have positive effects on decomposition of Aβ\textsubscript{42} oligomer.

By analyzing ATP contact with Aβ\textsubscript{42} monomer at the fibril axis ends and dissociated Aβ\textsubscript{42} monomer, we obtained such an insight that ATP contact with backbone atoms of Aβ\textsubscript{42} monomer is a critical microscopic process to understand the mechanism of Aβ\textsubscript{42} fibril dissolution under the presence of ATP. The insight supports our second conjecture, conversion of Aβ\textsubscript{42} oligomer into off-pathway species by ATP. We then consider that ATP dissolves Aβ\textsubscript{42} fibril by shifting thermal equilibrium of Aβ\textsubscript{42} oligomer system from on-pathway species rich equilibrium to off-pathway species rich equilibrium. Nevertheless, evidences obtained from the present study still are not sufficient to verify this conjecture so that we will perform additional molecular dynamics simulations for its verification in our future studies.

The molecular diversity of Aβ\textsubscript{42} fibril structure is widely known\textsuperscript{36,37}. However, the mechanism we proposed here would be applied with no regard to the structural
polymorphism of Aβ42 fibril, because it is essentially attributed to ATP contact with the backbone atoms in Aβ42 monomers and oligomers. Furthermore, recalling that Aβ42 monomer is an intrinsic disorder protein38, a similar mechanism might hold for ubiquitous dissolution of protein aggregates consisting of other intrinsic disorder proteins such as FUS family proteins39.

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website. Detailed procedures for unbiased MD, SMD and USMD simulations, Figures and Tables for analyses of these simulations.

**Acknowledgements**

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