Solvent and mutation effects on the nucleation of amyloid β-protein folding

Presented by
Rusty A. Stough
June 14, 2007
Background on Alzheimer’s

• Progressive mental disorder
  – Confusion
  – Memory Failure
  – Disorientation
  – Restlessness
  – Agnosia
  – Speech disturbances
  – Inability to carry out purposeful movement
Understanding Alzheimer’s

- Amyloid cascade hypothesis
  - the deposition of amyloid fibrils is the seminal event in the pathogenesis of Alzheimer’s disease
  - Proposed in the 1990’s
Amyloid $\beta$–protein Theory

• Developed around the millennium
• Studies suggest that amyloid $\beta$–protein could be responsible for Alzheimer’s
  – Non-toxic in monomeric form
  – Inhibiting amyloid–protein polymerization into oligomers could prove to be an effective treatment
  – For this to occur there needs to be a better understanding of amyloid $\beta$–protein
Experimental difficulty

- Process is solvent dependent
  - Alpha-helical in ionic solution
  - Helixes and Beta sheets in aqueous solution
Stability and Folding

- Solution state NMR and diffusion ordered spectroscopy show variation in anionic strength in the buffer shifts equilibrium between monomer and oligomer.
- Amino acid structure at specific sites influences ability to form oligomer.
- Ile-41 – Ala-42 responsible for biophysical behaviors of $\text{A} \beta_{1-42}$ and $\text{A} \beta_{1-40}$.
- Oxidation of Met-35 affects $\text{A} \beta_{1-40}$ but not $\text{A} \beta_{1-42}$. 
**Structural Basics**

- A protease resistant segment has been found
  - Ala-21 – Ala30
  - Decapeptide shows same resistance
- A loop that is stabilized by hydrophobic interactions in the Val-24 – Lys28 region exists
- A high degree of flexibility in the termini
- Electrostatic interactions between the charged groups
  - Glu-22, Asp-23, and Lys-28 that modulate the stability of the folded structure
Purposes of the experiment

- Test whether the stability of the Val-24 –Lys-28 loop persists in all simulations
- To determine the effects of solvent alterations on the folding dynamics
- To investigate changes in dynamics caused by amino acid substitutions
- Study the dynamics of a monomer with a specific mutation
Molecular Dynamic Simulations

• Long-time MD simulations of monomer
  – In water at normal and reduced density
  – Normal density with dissociated salt ions
  – Mutated in normal density

• All atoms with potential energy given by CHARMM-27 force field were considered

• TIP3P model for water molecules

• Same temperature as *in vitro* tests
Molecular simulations cont’d

• Solvated each monomer
  – 43 angstrom cube of water
  – Around 2500 water molecules

• 25 dissociated molecules of NaCl put in resulting in a system of around 2400 water molecules
Structural Determinants

• 2 quantities used to characterize the structure of protein
  – Distance between the two alpha Carbon atoms of Ala-21 and Ala-30
  – The radius of gyration

\[ R_g^2 = \frac{\sum_i m_i (|\mathbf{r}_i - \mathbf{r}_c|)^2}{\sum_i m_i} \]
Results

- Distances found to be fluctuating
- Events lasted for extended periods of time with small fluctuations are seen

Table 1. Total accumulated times of events in each of the five trajectories

<table>
<thead>
<tr>
<th>Time/event</th>
<th>[RC]</th>
<th>[P1]</th>
<th>[P2]</th>
<th>[DU]</th>
<th>[RCS]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total time, ns</td>
<td>102.6</td>
<td>65.0</td>
<td>83.6</td>
<td>80.0</td>
<td>145.0</td>
</tr>
<tr>
<td>Events, ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S^*$</td>
<td>37.1</td>
<td>45.9</td>
<td>27.0</td>
<td>12.6</td>
<td>34.0</td>
</tr>
<tr>
<td>$R^* (4,8)$</td>
<td>35.2</td>
<td>46.6</td>
<td>21.4</td>
<td>9.6</td>
<td>30.8</td>
</tr>
<tr>
<td>$R^* (2,8)$</td>
<td>7.6</td>
<td>8.1</td>
<td>5.9</td>
<td>0.4</td>
<td>19.0</td>
</tr>
<tr>
<td>$R^* (3,8)$</td>
<td>5.9</td>
<td>14.7</td>
<td>1.7</td>
<td>1.7</td>
<td>18.0</td>
</tr>
</tbody>
</table>
Fig. 1. Distances between Cα atoms of Val-24 and Lys-28 [R(4,8)], charged atoms of Glu-22–Lys-28 [R(2,8), black], Asp-23–Lys-28 [R(3,8), gray], and radius of gyration $R_g$ as a function of time for each trajectory. All of the distances are measured in Å.
Temporal overlap

• Each overlap divided into 2 columns
  – One for each of the events
• The first overlap column shows that $S^*$ and $R^*$ (4,8) are correlated
  – Proximity of Val-24 and Lys-28 linked to hydrophobic interaction

<table>
<thead>
<tr>
<th>Table 2. Percentage of overlap between pairs of events per trajectory</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overlaps</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Trajectory</strong></td>
</tr>
<tr>
<td>[RC]</td>
</tr>
<tr>
<td>[P1]</td>
</tr>
<tr>
<td>[P2]</td>
</tr>
<tr>
<td>[DU]</td>
</tr>
<tr>
<td>[RCS]</td>
</tr>
</tbody>
</table>
Secondary Structure

- The pi-helix correlates with lowered values of all the $R(2,8)$ $R(3,8)$ and $R(4,8)$ distances
  - Similar correlation found during helix formation
  - Helices formed in both [P1] and [P2] formed under a pre-existing $R^*(4,8)$ event
Discussion and conclusions

• For five trajectories hydrophobic events predominate over electrostatic events
  – Hydrophobic caused by packing of isopropyl groups
  – Highly correlated with smaller value for radius of gyration
• Loop is formed in reduced density water
• In normal density water SBs play a prominent role in stabilization of loop