Photoreversible Control of Pre-Amyloid Oligomer Conformation

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Photoresponsive surfactants

Photocontrol of protein quaternary structure

$(\alpha$-Chymotrypsin ($\alpha$-Ch) – azoTAB)
• Analog of DTAB (C$_{15}$H$_{34}$BrN)
• Surfactants bind to proteins causing **IRREVERSIBLE** changes in conformation
  – SDS (C$_{12}$H$_{25}$NaO$_4$S) PAGE
• With azoTAB these conformation changes can be **REVERSIBLY** controlled with light
Small Angle Neutron Scattering (SANS)

- We see things because they scatter light.
- Go to smaller wavelengths (light $\rightarrow$ neutrons) to “see” smaller things.
Small Angle Neutron Scattering (SANS)

- Data fit by treating the protein as a collection of n spherical scattering centers
- Most commonly used GA_STRUCT\textsuperscript{1} and GASBOR\textsuperscript{2}

Photocontrol of $\alpha$-chymotrypsin ($\alpha$-Ch) Association

At pH 3.0
- 11.38mg/mL $\alpha$-Ch

At pH 7.2
- 11.61mg/mL $\alpha$-Ch

Graph showing the relationship between $Q/\text{Å}^{-1}$ and $L/\text{Å}$ for different concentrations of pure $\alpha$-chymotrypsin, UV and visible light conditions.
pH 3 Deconvolution

\[ I(Q) = I(0) \exp\left(-Q^2 R_g^2 / 3\right) \]
\[ M_W = \frac{I(0) N_A}{c v (\rho_P - \rho_S)^2} \]

\[ M_W = x_M W_{m,1} + (1 - x_M) W_{m,n} \]

\[ I_{mix} = I_A + I_B \]
pH 3 Deconvolution
pH 3 Deconvolution

![Graph showing distribution of P(r) for different models: pure α-chymotrypsin pH 3, 2cha PDB scaled, and dimer.](image)
pH 7 Deconvolution

\[ I(Q) \text{ / cm}^{-1} \]

\[ Q / \text{Å}^{-1} \]

\[ P(r) \]

- pure α-chymotrypsin pH 7
- 2CHA
- hexamer
**Shape Reconstruction of pure α-Ch**

<table>
<thead>
<tr>
<th>pH 3</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="6CHA" /></td>
<td><img src="image2.png" alt="2CGA" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="40 Å" /></td>
<td><img src="image4.png" alt="2CGA" /></td>
</tr>
</tbody>
</table>

6CHA & 2CGA
PDDF Analysis of the α-Ch SANS data

![Graphs showing PDDF analysis results with various concentrations and types of α-chymotrypsin complexes]
Shape Reconstruction of the α-Ch/azoTAB SANS data

<table>
<thead>
<tr>
<th>visible</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.59 mM</td>
<td>1.03 mM</td>
</tr>
<tr>
<td>4.23 mM</td>
<td>4.03 mM</td>
</tr>
<tr>
<td>6.70 mM</td>
<td>9.92 mM</td>
</tr>
<tr>
<td>pH 3</td>
<td>pH 7</td>
</tr>
</tbody>
</table>

50 Å
Importance of The “Corkscrew”

Amyloid characteristics

Congo red fluorescence

“apple green” birefringence
Reversibility using the pH indicator
Bromophenol Blue (BPB)

UV light on
Visible light on
UV light on

Absorbance 605 nm

550 600 650 700 750 800
Wavelength (nm)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Absorbance

0 1000 2000 3000 4000 5000
time (s)
**α-Chymotrypsin Conclusions**

- Monomer ↔ oligomer equilibrium depends on light
  - Visible, monomer ↔ hexamer
  - UV, monomer ↔ dodecamer
- Fraction of oligomer depends on concentration
  - ↑ concentration, ↑ oligomer fraction
- Oligomers are corkscrew like
- Hexamers associate two at a time (slightly offset) into dodecamers
- Dodecamer very similar to pre-amyloid oligomer
- Can break up dodecamer with visible light