Fluorescence Properties of Amyloid-like Fibrils Formed by Self-association of Phenylalanine and Aggregation of Serum Albumins

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Outline

• Motivation
• Some facts about amyloid fibrils
• \( \pi \)-stacking interactions as a mechanism of stabilization of amyloid-like structures
• Amyloid-like structures formed of bovine serum albumin (BSA) and phenylalanine (Phe)
• Concluding remarks
• Acknowledgement
• References
Motivation

- Aggregation of proteins to amyloid fibrils is one of the causes of social important diseases – Parkinson’s [1], Alzheimer’s diseases [2], etc.

- \(\pi\)-stacking interactions between aromatic residues are one of the mechanisms that stabilize the structure of amyloid fibrils and could be revealed in the optical response (new fluorescent and/or absorption bands) [3, 4]

- The aim of this research is to investigate optical properties of amyloid-like structures formed in the model aqueous solutions (BSA, Phe, Phe-Phe)

Amyloid fibrils

- Similar morphology for different types of proteins [1]:
  - Long (length ~ μm, diameter ~ nm)
  - Unbranched
  - β-rich structures
- Strands of β-sheet are perpendicular to fiber axis
- Structure is formed via
  - hydrogen bonds [1, 2]
  - π-stacking interactions [2]

π-stacking interactions

Total energy, electrostatic, and van der Waals potential surfaces for stacked benzene dimer [1]:

The preferred parallel-stacked orientation is found to be more stable than a T-shaped structure by 0.5-0.75 kcal/mol for Phe-Phe dimers* [1].

* Here: “Phe-Phe dimer” = “a pair of aromatic rings of two monomeric Phe”
BSA aggregation to amyloid-like structures due to thermal denaturation.

Background
BSA aggregation to amyloid-like structures due to thermal denaturation

Model of aggregation

- Heating → irreversible conformational changes (α-helix → β-structure) [1]
- Incubation at 65°C → formation of amyloid-like structures [1]

Methods for investigation:
- Turbidity measurements (aggregation) [2]
- Intrinsic fluorescence: steady-state and time-resolved measurements (conformational changes)
- Fluorescence of dyes (conformational changes) [1, 2]
- New fluorescence bands (π-stacking interactions) [3]

BSA aggregation to amyloid-like structures due to thermal denaturation. Experimental data
BSA aggregation to amyloid-like structures due to thermal denaturation

Turbidity measurements

Optical density of BSA increases during incubation at 65°C due to aggregation of the protein to amyloid-like structures
BSA aggregation to amyloid-like structures due to thermal denaturation

Intrinsic Fluorescence

Mean fluorescence lifetime:
\[ \langle \tau \rangle = 6 \text{ ns (native BSA)} \]
\[ \langle \tau \rangle = 4 \text{ ns (aggregated BSA)} \]

Position of spectral maximum:
\[ \lambda = 347 \text{ nm (native BSA)} \]
\[ \lambda = 340 \text{ nm (aggregated BSA)} \]

Thermal denaturation induce conformational changes in BSA structure that are manifested in the changes of intrinsic fluorescence of the protein
BSA aggregation to amyloid-like structures due to thermal denaturation

Fluorescence of Nile Red

Nile Red:
- Structure: C$_{20}$H$_{18}$N$_2$O$_2$ [1]
- Hydrophobic dye [2]
- Quantum yield is sensitive to polarity of microenvironment [2]
- Label for conformational changes of proteins [2]

Thermal denaturation induce conformational changes in BSA structure that are manifested in the changes of binding of Nile Red

1) "Nile-red-3D-balls" by Ben Webber - Own work; 2) A. Hawe et al., Pharm. Res. 2007, 25 (7): 1487-1499
BSA aggregation to amyloid-like structures due to thermal denaturation.

Fluorescent band at 420 nm

Intensity of fluorescent band with spectral maximum at ~420 nm increases during aggregation of BSA to amyloid-like structures.
BSA aggregation to amyloid-like structures due to thermal denaturation

Conclusions

• BSA forms amyloid-like structures upon incubation at 65°C (turbidity measurements, literature [1-3])

• BSA conformation altered due to thermal denaturation (intrinsic fluorescence and fluorescence of Nile Red)

• Intensity of fluorescent band with spectral maximum at ~420 nm enhances during formation of amyloid-like structures

Phe and Phe-Phe dipeptide self-association to amyloid-like structures.

Background
Phe and Phe-Phe dipeptide self-association to amyloid-like structures

Model of self-association

Chemical structure of Phe [1]

Chemical structure of Phe-Phe dipeptide [3]

AFM image of twisted fibrillar aggregates of Phe-Phe. Inset is a part of the twisted fibril and its schematic diagram [3]

Representative snapshot of the filamentous structure obtained by molecular dynamics simulations (cyan - Phe molecules, yellow spheres - counterions, gray surface - van der Waals envelope of aromatic rings) [2]

Supramolecular array of Phe-Phe through intermolecular H-bond and aromatic-aromatic interactions (black lines - H-bonds, green lines - the shortest C-C distances for aromatic-aromatic interactions) [3]

Phe and Phe-Phe dipeptide self-association to amyloid-like structures. Experimental data
Phe and Phe-Phe dipeptide self-association to amyloid-like structures

Scheme of the experiment

Chemical structure of Phe:  

\[
\text{Chemical structure of Phe: } \begin{array}{c}
\text{Phe} + \text{ddH}_2\text{O} \\
\text{Centrifugation} \\
\text{Supernatant liquid} \\
\text{Possible precipitate} \\
\text{Sample 1} \\
\text{Time (~1d)} \\
\text{No visible structures} \\
\text{Centrifugation} \\
\text{Sample 2} \\
\text{Supernatant liquid} \\
\text{Possible precipitate} \\
\end{array}
\]

Chemical structure of Phe-Phe dipeptide:  

\[
\text{Chemical structure of Phe-Phe dipeptide: } \begin{array}{c}
Phe-Phe + \text{ddH}_2\text{O} \\
\text{Centrifugation} \\
\text{Supernatant liquid} \\
\text{Possible precipitate} \\
\text{Sample 1} \\
\text{Time (~2h)} \\
\text{A small aliquot was dissolved in ddH}_2\text{O} - \text{Sample 1} \\
\text{Visible structures} \\
\text{Centrifugation} \\
\text{Sample 2} \\
\text{Supernatant liquid} \\
\text{Possible precipitate} \\
\text{Centrifugation} \\
\text{Precipitate} \\
\end{array}
\]
Phe and Phe-Phe dipeptide self-association to amyloid-like structures

Optical density measurements

Optical density of Phe and Phe-Phe increases at long wavelengths due to formation of amyloid-like structures and decreases at short wavelengths due to precipitation.
Phe and Phe-Phe dipeptide self-association to amyloid-like structures

Fluorescent band at 420 nm

Intensity of fluorescent band with spectral maximum at ~420 nm increases during self-association of Phe and Phe-Phe to amyloid-like structures.
Conclusions

• Both free Phe and Phe-Phe dipeptide form amyloid-like structures as a result of self-association in aqueous solution (turbidity measurements, visible aggregates, literature [1, 2])

• Intensity of fluorescent band with spectral maximum at ~420 nm enhances during formation of amyloid-like structures

Conclusions
Concluding remarks

• Intensity of fluorescent band with spectral maximum at ~420 nm enhances during formation of amyloid-like structures by thermally aggregated BSA, self-associated free Phe and Phe-Phe dipeptide in aqueous solution

• π-stacking interactions seems to stabilize amyliod-like structures mentioned above [1]

• Fluorescent band with spectral maximum at ~420 nm is similar to collagen fluorescence band [2] which structure seems to be stabilized by π-stacking interactions too

Enhancement of fluorescent band with spectral maximum at ~420 nm during formation of amyliod-like structures could be explained by presence of π-stacked aromatic amino acids

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