Investigation of Water Molecular System Dynamics in the Early Stages of Amyloid Formation

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Amyloid fibrils

Width: 2-20 nm
Length: several um

Cross-β structure

Intervals of the strands: ~4.8 Å

Intervals of the sheets: 10-11 Å

Amyloid fibrils
Nucleation-dependent mechanism of amyloid formation

Onset of amyloidosis

Fraction of amyloid fibrils

Time

nucleation  elongation  equilibrium
What is crucial for nucleation?

Molecular mechanisms that dictate the nucleation are poorly understood.
Can we find any spectroscopic signals that tell us the formation of amyloid fibrils?

We performed near infrared spectroscopic analysis to monitor water molecular system dynamics during the amyloid fibril formation reaction.
Near Infrared Spectroscopy

NIR spectroscopy is expected to be a powerful analytical technique for investigating water structures.
Experimental procedure

Sample: 3 mg/ml human insulin in 25 mM HCl, 100 mM NaCl

NIR spectrum was measured in real time

(Chatani et al., *PLoS One* 9, e101997 (2014))
Overview of NIR spectra of fibrillation sample

(I) nucleation  (II) elongation  (III) equilibrium

(combination of OH stretching and vibrating ↓)

1\textsuperscript{st} overtone of OH stretching

(combination bands related to amide groups sensitive to secondary structure of proteins)
Spectral changes in water 1st overtone
(Difference spectra obtained by Subtraction of the spectrum at 3 min)

(I) nucleation

(II) elongation

(III) equilibrium

PCA was applied to extract spectral peaks changing simultaneously with the fibrillation reaction

Wavelength (nm)
Absorbance
PC3 suggested that free water is generated transiently, and afterwards, hydrogen-bonded water increased.

PCA for water structure change – 3rd principal component (PC3) –

Decrease in hydrogen-bonded water (Increase in free water)

Increase in hydrogen-bonded water
Reproducibility of PCA results

Repeated measurements of the same reaction verified that the spectral changes were reproducible
Aquagram supported transient dissociation and subsequent development of hydrogen-bonded water networks in the nucleation phase

\[ A'_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda} \]

- \(A'_\lambda\): value of Aquagram
- \(A_\lambda\): absorbance after MSC applied on 1\textsuperscript{st} overtone
- \(\mu_\lambda\): mean of all spectra
- \(\sigma_\lambda\): SD of all spectra
- \(\lambda\): wavelength
Schematic model for transformation of water structures

Organization of nuclei may be mediated by water molecules?
The length of lag time is dependent of salt concentration
(Masuda et al., manuscript in preparation)

NIR measurement at different NaCl concentrations

NIR spectrum was monitored at three different conc. NaCl
Biphasic spectral changes in the nucleation phase

Hiramatsu et al.: poster No. 12!!

For all conditions, free water is initially formed, and afterwards, hydrogen-bonded water is formed.

Difference spectra obtained by subtraction of the spectrum at 5 min (the baseline was corrected)
The timing of the water structural changes

The hydrogen bond formation of water is a sign of nucleation?

Lag time: 28 min
50 mM NaCl

Lag time: 69 min
400 mM NaCl
NIR measurements shed light on the transformation of water structures in the fibril nucleation. Water may be used as a new biomarker for early non-invasive diagnosis of amyloid-related diseases.

Jimenez et al., PNAS, 99, 9196 (2002)