

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

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Amyloid fibrils are a form of protein aggregates associated with numerous diseases including Alzheimer's, prion, and Huntington's diseases. In many cases, the formation of amyloid fibrils proceeds according to a nucleation-dependent scheme. In this scheme, the nucleation acts as a rate-limiting step controlling the overall kinetics of fibril formation, and elucidating detailed mechanisms of the amyloid nucleation is thus one of the most essential issues for understanding pathogenesis. However, much remains to be elucidated regarding how and when nuclei species which template for fibril growth emerge.

We have previously found time-dependent changes in water molecules during the fibril formation<sup>1</sup>. Here, to verify such water behaviors and examine their details, we have performed time-resolved near infrared (NIR) spectroscopic monitoring for the heat-induced spontaneous fibrillation reaction of human insulin. In this work, 3.0 mg/ml human insulin dissolved in 25 mM HCl containing 50 mM NaCl, Na<sub>2</sub>SO<sub>4</sub> or NaClO<sub>4</sub>, was used as samples, and their fibrillation reactions were started by jumping temperature of the protein solution to 75°C inside a quartz liquid sample cell with a 1-mm optical pathlength. The monitoring of the NIR transmission spectra was then executed for 90 min with a step size of 0.5 nm and data acquisition at every half minute by XDS Rapid Liquid Analyzer (Foss, Denmark).

As a result, firstly, the PCA analysis in the 2050-2350 nm spectral region revealed the  $\beta$ -sheet formation in couple with the baseline increment at 600 nm, verifying the formation of amyloid fibrils composed of cross- $\beta$  structure. When the region of 1110-1850 nm, mainly corresponding to the first overtone of water OH stretching vibrations, was focused on to analyze structural changes of water molecules, we found an increase and subsequent decrease in the free O-H at around 1410 nm and a concomitant decrease and increase in the hydrogen-bonded O-H at around 1480 nm during the nucleation phase, as revealed by the analysis of differential spectra (Figure 1; left). These changes were not observed for the reference sample without any proteins (Figure 1; right), supporting that they are specific to the fibrillation reaction.

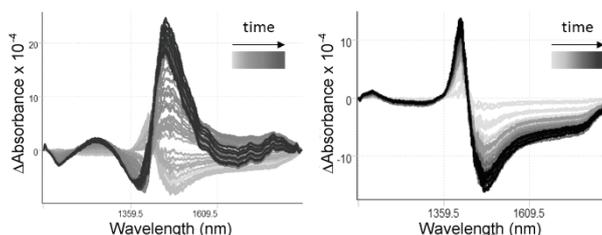


Figure 1. An example of differential spectra for the fibrillation sample (left) and the solvent (right) in the region of 1110-1850 nm.

Interestingly, such water spectral changes began during the nucleation phase irrespective of different lengths of the lag phase with different salts. This result suggests that the water structural change is a universal nature commonly observed among the nucleation of amyloid fibrils, which may lead to future early diagnosis utilizing water spectroscopic signals as a novel indicator.

## Reference:

1. E. Chatani, Y. Tsuchisaka, Y. Masuda, R. Tsenkova "Water molecular system dynamics associated with amyloidogenic nucleation as revealed by real time near infrared spectroscopy" PLoS One, **9** e101997 (2014)