# INVESTIGATIONS OF AMYLOIDOGENIC INTERMEDIATE STATES

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## Introduction

An accumulation of insoluble protein aggregates (amyloids) in the human body is believed to lead to degeneration of the brain, pancreas, heart, kidneys, and other tissues, ultimately being fatal to the organism. The amyloid-forming process involves conformational changes from soluble polypeptides to intermediate states and their self-assembly into insoluble aggregates. It is, therefore, of critical importance to identify and characterize the intermediate states involved in the amyloidogenesis to understand the amyloid-formation mechanism.

In this project, we have investigated beta-amyloid peptide (Aβ) peptides associated with Azheimer's diseases. Recently, we observed conformational transitions of initially unfolded Aβ (1-40) peptides to a β-sheet like monomeric intermediate state under amyloidogenic conditions (high temperatures) by using CD spectroscopy.[1] Thus residue-specific characterizations of the structural changes were carried out with high-field NMR facilities.

## Experimental

Two-dimensional $^{1}H/^{15}N$ HSQC NMR experiments were initially performed at variable temperatures (5 – 45 $^{o}C$) to probe the conformational changes on a 720 MHz instrument (Fig. 1). A very low Aβ (1-40) peptide concentration of 20 μM sample was used to prevent oligomerizations at the amyloidogenic experimental conditions with ~ 12hrs experimental time for the each HSQC spectrum.

## Results and Discussion

The low temperature HSQC spectrum at 5 $^{o}C$ is almost identical to those obtained in the previous NMR study, corresponding to unstructured peptides. As the temperature was increased, certain NMR resonances were gradually broadened and the signal intensities were decreased (Fig. 1), presumably due to extensive conformational transitions. Interestingly, mainly non-hydrophobic residues [7 – 9 (DSG), 13 and 15 (HQ), 20 – 21 (FA), 25 – 27 (GSN), and glycines in the C-terminus (29, 33, 37, 38)] were showed to undergo more significant conformational fluctuations than other residues. This indicates that the Aβ peptides may adopt a partly β-sheet conformation mainly through the above residues under the amyloidogenic conditions (high temperatures), resulting in the CD spectra corresponding to a more ordered state of the peptides. More quantitative analyses of the conformational transitions will be carried out in a near future by using relaxation dispersion NMR experiments.

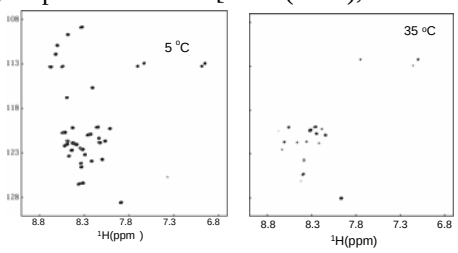


**Fig. 1** HSQC spectra at different temperatures

## Conclusions

Residue-specific information as to the conformational changes from non-amyloidogenic unfolded to amyloidogenic states of the Aβ peptide was obtained with the variable temperature HSQC experiments. Additional experiments including dynamic experiments and residual dipolar coupling (RDC) measurements are, therefore, under investigations for more detailed studies of the amyloidogenic states.

## Acknowledgements

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## References

[1] Lim, K.H., *et al*., submitted.