Investigating the Transthyretin Amyloid Formation Mechanism Using Solid-State NMR

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Introduction

Transthyretin (TTR) is a homotetrameric protein with 127 amino acid residues in each monomer and is rich in β-sheet structure in which eight β-strands are arranged in a β-sandwich consisting of two β-sheets (strands CBEF and DAGH). Misfolding/unfolding of TTR to form beta sheet rich amyloid is associated with numerous amyloid diseases. It is important to understand the misfolding/amyloid formation mechanism towards the development of therapeutic strategies. Under amyloidogenic conditions (pH 4.4, 37°C) we have shown that the significant native-like structures were observed in the aggregation prone-states and in amyloid state.[1,2] We also have shown that at pH 4.4, DA substructure (fig. 1a) is disordered in pathogenic TTR variants (V30M and L55P) which exposes the A strand for intermolecular interactions to facilitate the more efficient amyloid formation than in WT.[3] The purpose of this study is to probe the DA substructure in WT and variant forms of TTR amyloids by intraresidue and sequential assignment of cross peaks by using 2D DARR and 3D NCACX experiments.

Experimental

The protein samples were expressed and purified using a bacterial expression system. The DNA plasmids (pMMHa) for TTR were kindly provided by Prof. Kelly (Scripps, CA). Amyloid samples were obtained by incubating ~ 35 mg of protein for a period of 30 days at 37 °C at pH 4.4. 2D DARR and 3D NCACX NMR experiments were performed on an 800 MHz solid-state NMR facility equipped with 3.2 mm MAS probe in the NHMFL (Tallahassee).

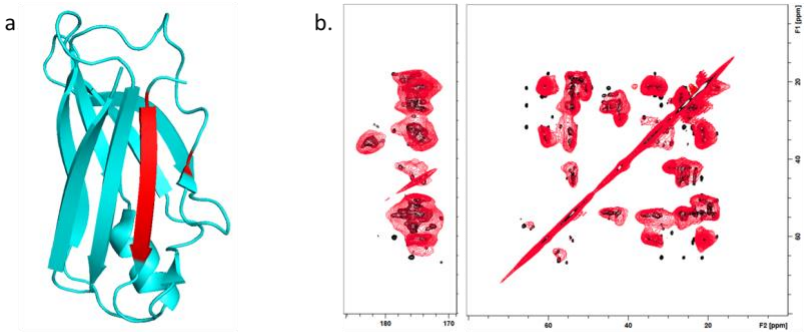

Figure 1: a. Crystal structure of TTR. ^{13}C labeled residues in strands A and D are colored red.
b. Superposition of 2D ^{13}C DARR spectra of native (black) and WT amyloid (red) forms.

Results and Conclusion

In this study, TTR was uniformly labeled with ^{15}N and sparsely labeled with ^{13}C (L, M, V, K amino acid residues) to obtain the intraresidue correlations in DA substructure. In the initial stage of study, we acquired ^{13}C-^{13}C DARR correlation spectra with a mixing time of 100 ms for WT TTR amyloid and was compared with the spectra obtained from likewise labeled native TTR (Fig. 1b). The number of overlapping cross-peaks from these spectra indicate the presence extensive native-like structural features in the WT amyloid. However, the absence of a few cross-peaks in WT amyloid spectrum is likely due to the structurally disordered regions in the amyloid state. We further intend to analyze the 3D NCACX experiment results for the cross-peak assignment and conduct the similar experiments in TTR variants to probe the structural changes in the DA substructures during the amyloid formation.

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References

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