Solid-State NMR Structural Studies of Transthyrentin Amyloid

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Introduction

Transthyretin (TTR) is a 55 kDa homotetrameric protein that binds and transports thyroxine in the cerebrospinal fluid and plasma. Amyloid formation of wild type and various mutant forms is associated with senile systemic amyloidosis (SSA) and familial amyloidotic polyneuropathy (FAP). Previous biophysical studies of TTR amyloidogenesis showed that the tetrameric TTR dissociates to monomers and subsequently undergoes a conformational transition to a partially unfolded intermediate, which self-assembles into amyloid. Extensive pathogenic single-point mutations observed throughout the protein accelerate the amyloid formation. The mutations may completely disrupt the native β-structure, resulting in a non-native intermolecular cross-β structure. In this study, we employ solid-state NMR to investigate structural features of TTR amyloid. The effect of the pathogenic mutation on amyloid structure will be explored, which may provide insights into molecular mechanism of TTR amyloid formation.

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 ~ 30 mg of protein in 50 mM acetate buffer (pH 4.4) containing 100 mM NaCl, 1 mM EDTA and 0.05% NaN_3 for a period of 60 days at 25 °C. One-dimensional (1D) CPMAS and 2D DARR NMR experiments were performed on a 600 MHz solid-state NMR facility equipped with 3.2 mm MAS probe in the NHMFL (Tallahassee).

Results and Discussion

The CPMAS spectrum was acquired for the native state WT TTR prepared with a selectively labeled scheme (Figure 1). The NMR resonances were assigned with double-quantum filtered NMR experiments. The 1D CPMAS NMR spectra were also collected for TTR amyloid from WT and L55P mutant form (Figure 1). Although the NMR resonances are slightly broadened for amyloid samples due to sample inhomogeneity, the chemical shift remained unchanged. These NMR spectra suggest that the native β-structure is maintained in the amyloid structure.[1] In addition, WT and L55P mutant TTR amyloids showed almost identical NMR spectra, indicating that the amyloids have a similar β-structure. More detailed structural studies using 2D DARR experiments are currently underway.

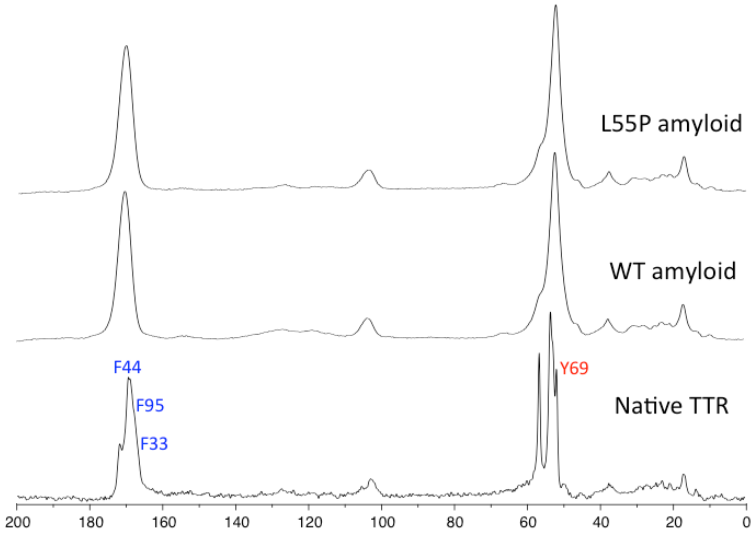

Figure 1. The ^{13}C CPMAS spectra for native state and amyloid TTR

Conclusions

The ^{13}C CPMAS spectra showed that the proposed NMR experiments are feasible for TTR amyloid samples. Multidimensional NMR experiments such as DARR experiments will provide more detailed structural features for the WT and various mutant TTR amyloids.

Acknowledgements

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References

[1] Lim, K.H., *et al*., J. Mol. Biol., **425**, 977-988 (2013).