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 830 MHz solid-state NMR facility equipped with 3.2 mm MAS probe in the NHMFL (Tallahassee).

Results and Discussion

Solid-state NMR was used to investigate native β-structure in TTR amyloid. For effective solid-state NMR study, we utilized specific labeling schemes, which generate isolated ^{13}C-^{13}C dipolar-coupled spin pairs in the native β-sheets. For example, ^{13}CO-Phe and $^{13}C\alpha$-Tyr were used to express protein, which generates two dipolar coupled ^{13}CO-$^{13}C\alpha$ spin pairs at distances of 4 – 5 Å in the β-BEF strands. The dipolar-coupled spin pairs in the native state can then be probed and compared to those in amyloid using solid-state NMR experiments that can detect spin-pairs with a separation of up to 6 Å. Two dimensional dipolar-assisted rotational resonance (DARR)[1] experiments were carried out to probe the dipolar-coupled spin pairs in the native, and also in the amyloid states (Fig. 1). The two spin pairs (F33-Y69 and F95-Y69) were observed in the 2D spectrum of the native state. The cross-peaks were also detected for the amyloid sample (Fig. 1). The two cross-peaks from the dipolar-coupled spin pairs appear overlapped in the amyloid spectrum, resulting in a broader cross-peak with 4 ppm of full width at half maximum (FWHM) compared to that of the diagonal peak (2.5 ppm). The solid-state NMR results clearly indicate that TTR amyloid contains native-like BEF β-sheet structure. More detailed structural studies using 2D DARR experiments are currently underway.

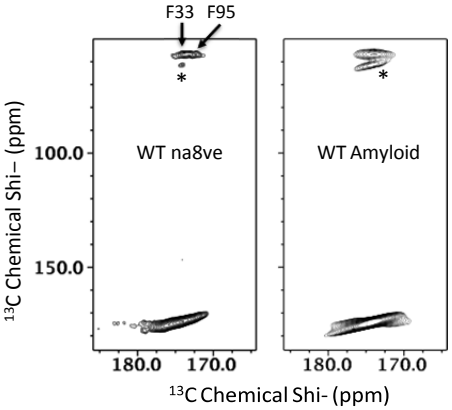

Fig.1 2D DARR spectra of native state and amyloid forms of TTR obtained using a mixing time of 500 ms at a 1H frequency of 600 MHz. A 208 X 2048 data set was acquired at spinning frequency of 17 kHz and 256 transients were averaged for each t1 increment with an acquisition delay of 2 s. * denotes spinning sidebands.

Conclusions

The TTR amyloid appears to contain extensive native-β sheet structures.

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References

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