Long Range DARR ^{13}C-^{13}C Correlation Experiments on HuPrP23-144 Fibrils

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Introduction

Prion protein (PrP) is a member of a class of commonly misfolded proteins whose improper folding has been implicated in a variety of diseases ranging from dementia to heart disease. PrP can convert into β-sheet rich aggregate structures, the accumulation of which is associated with transmissible spongiform encephalopathies (TSEs), a group of fatal neurodegenerative disorders. TSEs affects both human and animals, resulting in fatal diseases such as Creutzfeldt-Jakob disease (humans), mad cow disease (bovines), and scrapie (sheep). The neurotoxic structures associated with TSE can be propagated in normally folded prion protein via contract with the misfolded protein, indicating that the ability to fold is associated with the structure of the prion protein aggregates. Understanding the detailed structures of these misfolded aggregates will play a vital role in determining how the neurotoxic folds can be transferred from protein to protein and may assist in designing novel drugs which prevent such misfolding. NMR provides a valuable tool for determining the structures of these proteins on an atomic level.

Experimental

Using the 900 MHz NMR system at the National High Magnetic Field Laboratory we acquired ^{13}C-^{13}C DARR correlation spectra of four selectively ^{13}C labeled truncated prion protein samples containing PrP residues 23-144 (HuPrP23-144). These samples were generated using 1,3 -^{13}C glycerol or 2 -^{13}C glycerol and contain either 100% of the labeled protein, or a sample diluted with 75% natural abundance PrP in order to obtain only intra-molecular contacts.

The DARR ^{13}C-^{13}C correlation experiments were conducted using relatively long mixing times (500 ms and 100 ms) on the 900 MHz instrument equipped with the low electric field probe optimized for ^{13}C detection. The experiments on the 2-^{13}C glycerol labeled PrP sample and on both dilute samples were conducted with the probe set to double resonance, while our 900 MHz experiments on the 1,3 -^{13}C glycerol sample were conducted with the probe in triple resonance mode. This setting resulted in approximately half the signal to noise for this sample.

Results and Discussion

Our DARR experiments provided numerous short and long range crosspeaks between different regions of the structured fibril core. A series of correlations between residues 117-119 and 134-137 indicates the possibility that the peptide contains a turn that puts these regions in proximity. These contacts were observed both in samples that were fully labeled and samples where the labels were diluted to 33% indicating that they arise from contacts within a single subunit of the fibril.

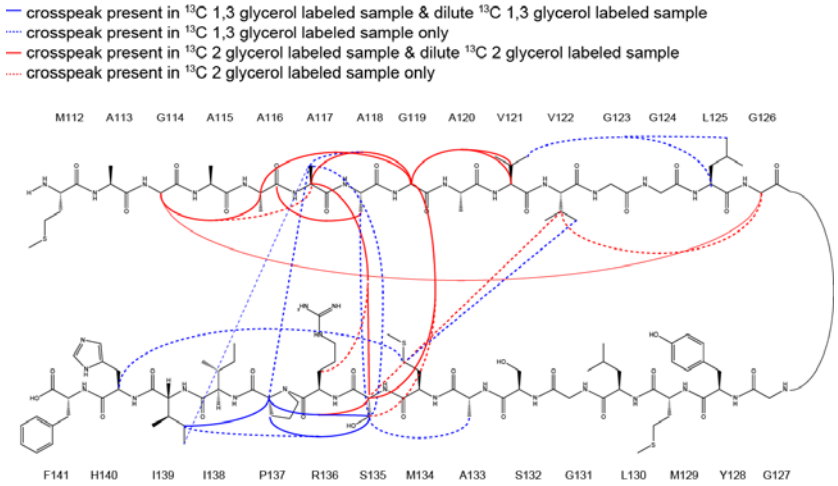

Fig.1 Cartoon representation of unambiguously assigned long range crosspeaks from DARR ^{13}C-^{13}C correlation experiments of HuPrP23-144 fibrils.

Conclusions

Our data provides new constraints for the structure of the core region of the human prion protein, and indicates the presence of a turn in the structured region.

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