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Solid-state NMR structural studies of the fibril form of a mutant mouse prion peptide $PrP_{89–143}$(P101L)

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Abstract

The peptide fragment 89–143 of the prion protein (carrying a P101L mutation) is biologically active in transgenic mice when in a fibrillar form. Injection of these fibrils into transgenic mice (expressing full length PrP with the P101L mutation) induces a neurodegenerative prion disease (Kaneko et al., J. Mol. Biol. 295 (2000) 997). Here we present solid-state NMR studies of $PrP_{89–143}$(P101L) fibrils, probing the conformation of residues in the hydrophobic segment 112–124 with chemical shifts. The conformations of glycine residues were analyzed using doubly $^{13}C=O$ labeled peptides by two-dimensional (2D) double-quantum correlation, and double-quantum filtered dephasing distance measurements. MQ-NMR experiments were carried out to probe the relative alignment of the individual peptides fibrils. These NMR studies indicate that the 112–124 segment adopts an extended β-sheet conformation, though not in a parallel, in register alignment. There is evidence for conformational variability at Gly 113. DQ correlation experiments provide useful information in regions with conformational heterogeneity.

Keywords: Prion; Solid-state NMR; Double-quantum NMR; Multiple-quantum NMR; Alignment; β-helix

1. Introduction

The accumulation of protein aggregates in the human body is believed to underlie a significant number of diseases [1–4]. These include transmissible spongiform encephalopathies (prion diseases Creutzfeld Jacob disease (CJD), bovine spongiform encephalopathy (BSE), etc.), type II diabetes, Alzheimer's and Parkinson's diseases. At least 16 different proteins or polypeptides have been associated with these "amyloid" diseases [1]. A common feature of amyloid diseases is the conversion of soluble, often globular proteins to fibrillar forms that are insoluble aggregates. There is increasing evidence that early soluble aggregates rather than fibrils are the species toxic to cells [2], but all of the proteins involved do also form fibrillar aggregates. Spectroscopic and fiber diffraction data indicate that peptides in these fibrils are predominantly in β-sheet conformations arranged such that the backbones of β-strands run perpendicular to the fibril axis (termed cross-β structure)[5]. Various biophysical studies have shown that fibrils formed from different peptides have common structural properties, being straight and unbranched with diameters of 40–120 Å, although there is no similarity in amino acid sequence or native structure of the various amyloid forming proteins.

Knowing the detailed structure of different peptides in these amyloid fibrils is of importance in investigating the

Abbreviations: PrP, prion protein; MQ, muliple quantum; DQ, double quantum; CJD, Creutzfeld Jacob disease; BSE, bovine spongiform encephalopathy; Aβ, amyloid beta peptide; DRAWS, dipolar recoupling with a windowless sequence; CPMG, Carr–Purcell–Meiboom–Gill; EM, electron microscopy

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mechanism of amyloid formation, and thus numerous structural studies of fibrils have been carried out. However, fibrils are insoluble, non-crystalline materials to which X-ray crystallography and solution state NMR cannot be applied. A variety of solid-state NMR techniques have been developed and applied to amyloid fibrils, and have successfully addressed some of the fundamental issues [6–10]. For example, multiple quantum (MQ) experiments [11] have been used to determine the relative alignments of the individual β-strands to be parallel or not, evidence for each has been found for different peptides in fibrils. Regions of non-β-strand conformations have also been identified [12]. Recently, a detailed structural model for the amyloid beta peptide (Aβ)-amyloid (1–40) fibril was proposed based on extensive solid-state NMR studies [13]. In this model the 40 residue peptides have the first 10 residues disordered, residues 12–24 and 30–40 as β-strands, and connecting residues 25–29 forming a turn. The resulting 'hairpin' peptides are hydrogen bonded and associate along the fibril axis with individual peptide 'hairpins' running parallel to each other. To understand what features of peptides in fibrils are general it is important to compare this structure of Aβ with those formed by peptides from different amyloidogenic proteins.

Prion diseases are different from other amyloid diseases in that pathogenic (also called scrapie or PrP^{Sc}) forms of the prion protein (PrP) can transmit the disease from one animal to another [14]. Many efforts have been made to identify crucial residues of the protein involved in conformational conversion and infectivity. Mature PrP in cells is found as a 209 amino acid protein after processing removes signal sequences. It was shown that the *N*-terminal 'octa-repeat' region (implicated in copper binding and perhaps native function of PrP) could be proteolytically removed from the pathogenic forms without affecting infectivity [15]. Mice expressing PrP starting at residue 89 have normal susceptibility to prions and the disease progression is as in wild-type mice. A further truncation from the *N*-terminus to residue 146 resulted in mice that cannot be infected, indicating that some residues in the 89–145 segment are required for the conformational change to the infectious, scrapie form [16]. A 'mini-prion' containing residues 89–140 linked to 176–231 was shown to induce the neurodegeneration characteristic of the prion diseases in transgenic mice expressing this construct. Recently, a 55 residue peptide from the mouse PrP, carrying a proline to leucine mutation at residue 101, MoPrP(89–143, P101L) which we will abbreviate PrP^{*}_{89-143}, was shown to form amyloid fibrils in vitro. This peptide induces prion disease in transgenic mice (expressing full length PrP with the P101L mutation) when the mice are inoculated with fibrils [17]. The 55 residues contained in PrP^{*}_{89-143} are thus believed to play a central role in the conformation change from normal protein to the pathogenic forms.

^{13}C CP MAS NMR experiments have already been used to investigate secondary structure of the fibrillar, pathogenic ('infectious') and randomly aggregated ('non-infectious') forms of PrP peptides [18]. It was shown that the fibrillar form has a predominantly β-sheet conformation, while a considerable population of helical conformers exists in the randomly aggregated form. In this report, more extensive solid-state NMR studies on a particularly hydrophobic segment (residues 112–124) of the PrP^{*}_{89-143} peptide in fibrils are presented. Our experiments were designed to be able to detect residues that adopt turn conformations, as are predicted in β-helical models for this segment of PrP [19,20], and which have been observed in the 40mer Aβ peptide fibrils [12]. To obtain conformational information while avoiding spectral overlap and assignment issues, isotope labels were incorporated so that two adjacent ^{13}COs, one $^{13}C_{\alpha}$, and one Ala $^{13}C_{\beta}$ label were used in each peptide, Fig. 1. The secondary structure information was derived from ^{13}C isotropic chemical shifts for the $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ labels. Two-dimensional (2D) double-quantum correlation NMR, exploiting the chemical shift anisotropy (CSA) of the two adjacent CO carbons, [21] was used to probe the structure at glycines, for which chemical shift analysis alone is less reliable. Dipolar dephasing NMR experiments were also carried out to estimate distances between the CO carbons. Probing glycines was viewed as important because of their greater conformational flexibility and common role in forming turns. MQ NMR experiments [22] were also performed on Ala $^{13}C_{\beta}$ labels to probe the relative alignment of the peptide backbones. The solid-state NMR results are discussed with respect to β-helix structural models.

GQGGGTHNQWNKLSKPKTNMKHM**AGAAAAGAVVGGL**GGYMLGSAMSRPIIHFGSD

isotopic label combinations used:

peptide 1. $^{13}C_{\alpha}$ A112; $^{13}C_{\beta}$ A114; ^{13}C=O A117 and G118

peptide 2. ^{13}C=O A112 and G113; $^{13}C_{\beta}$ A117; $^{13}C_{\alpha}$ L124

peptide 3. $^{13}C_{\alpha}$ A115; $^{13}C_{\beta}$ A116; ^{13}C=O G122 and G123

Fig. 1. Sequence of PrP(89–143)P101L (residue 101 as outline). The segment 112–124 that is the focus of this work is underlined. Sites probed with specific isotope labels in this work shown in black bold letters, and sites probed in previous work shown in bold gray letters.