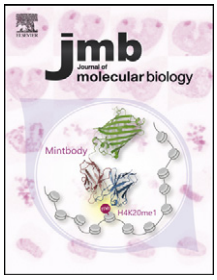

Pathogenic Mutations within the Disordered Palindromic Region of the Prion Protein Induce Structure Therein and Accelerate the Formation of Misfolded Oligomers

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Abstract

Little is understood about how the intrinsically disordered N-terminal region (NTR) of the prion protein modulates its misfolding and aggregation, which lead to prion disease. In this study, two pathogenic mutations, G113V and A116V, in the palindromic region of the NTR are shown to have no effect on the structure, stability, or dynamics of native mouse prion protein (moPrP) but nevertheless accelerate misfolding and oligomerization. For wild-type moPrP, misfolding and oligomerization appear to occur concurrently, while for both mutant variants, oligomerization is shown to precede misfolding. Kinetic hydrogen–deuterium exchange–mass spectrometry experiments show that sequence segment 89–132 from the NTR becomes structured, albeit weakly, during the oligomerization of both mutant variants. Importantly, this structure formation occurs prior to structural conversion in the C-terminal domain and appears to be the reason that the formation of misfolded oligomers is accelerated by the pathogenic mutations.

Introduction

The prion protein is a Glycosylphosphatidylinositol-anchored protein, present primarily in the mammalian brain, which is responsible for the transmission and pathogenesis of a group of neurodegenerative disorders known as transmissible spongiform encephalopathies [1,2]. Conformational conversion of the native, monomeric cellular prion protein (PrP^C) into an aggregated form (PrP^{Sc}) is associated with disease pathology and neurodegeneration [3]. PrP^C has an unstructured N-terminal region (NTR; residues 23–120) and a structured C-terminal domain (CTD; residues 121–231) [4]. The structure of PrP^{Sc} remains poorly defined, but it has been shown that residues 90–230 form the protease-resistant core of PrP^{Sc} [2,5].

Despite the well-known role of the conformational conversion of PrP^C in prion pathogenesis, the mechanism of this conversion and the final structure of the misfolded PrP are poorly understood. Generally, prion diseases occur spontaneously or are transmitted from individuals infected with prion disease. Nevertheless, familial forms of prion diseases can occur, due to specific mutations in the *PRNP* gene that codes for the prion protein. These disease-causing mutations are localized mainly in helix 2 (α2) and helix 3 (α3) of the structured CTD and in sequence segment 105–126, known as the middle hydrophobic core region of the unstructured NTR [6]. Several pathogenic mutations found in the CTD reduce the thermodynamic stability and increase the native state dynamics of the prion protein [7–9]. However, other pathogenic mutations like E199K, V209I, and T189V (mouse numbering; mouse numbering is used throughout this article), which occur in the α3 region, do not affect the stability of PrP [8]. Hence, it appears that the destabilization of PrP^C, leading to the formation of aggregation-competent intermediates, may not be a general mechanism for the formation of PrP^{Sc} [8].

In the unstructured NTR of the prion protein, the middle hydrophobic region is the main hotspot for mutations associated with familial forms of prion diseases. This region has been shown to play an important role in the conformational conversion of the prion protein [10,11]. In particular, a palindromic sequence in this segment spanning residues 111–120 (VAGAAAAGAV) has been implicated to play a role in the assembly of fibrils and in the structural changes accompanying prion conversion [12–14]. Additionally, it appears that this segment is essential

for the productive association of PrP^C with PrP^{Sc}, which leads to prion propagation in animals [15]. In the middle hydrophobic region, the P104L, G113V, A116V, and G130V mutations have been linked to the Gerstmann–Sträussler–Scheinker (GSS) syndrome [3,6]. However, the mechanism by which these mutations lead to prion pathogenesis remains unclear [16].

It has been difficult to study how mutations that occur in the unstructured NTR affect the global structure of PrP, as well as the conformational conversion of PrP, due to the intrinsically disordered nature of the NTR [4]. Several effects of these mutations have been reported. The A116V mutation has been reported to facilitate the formation of a transmembrane form of PrP, which leads to neurodegeneration without any detectable accumulation of PrP^{Sc} [17,18]. In fact, a recent study has shown that this mutation enhances ion-selective channel formation by the mouse prion protein (moPrP) [19]. The P101L and P104L mutant variants have been reported to form fibrils with an N-terminally extended amyloid core [20]. An *in vivo* study has reported that the G113V and A116V mutations lead to the formation of a protease-sensitive PrP conformation with a high degree of neurotoxicity [21]. The mechanisms by which mutations in this region affect the conformational conversion of PrP remain, however, unclear. Clearly, it has become important to study the effects of mutations in the unstructured NTR, particularly in the palindromic sequence, to obtain a better understanding of its role in the conformational conversion of PrP.

Results and Discussion

The pathogenic mutations G113V and A116V do not affect the structure and stability of native monomeric moPrP

In the current study, the effects of two GSS syndrome-associated pathogenic mutations, which are found in the palindromic region of PrP, G113V, and A116V (Fig. 1), on the structure, stability, and misfolding of moPrP were characterized. Figures 2 and 3 show that the G113V and A116V mutations did not affect the structures and stabilities of moPrP. The circular dichroism (CD) spectra (Fig. 2a), the Fourier transform infrared (FTIR) spectra (Fig. 3), and the dynamic light scattering (DLS) profiles (Fig. S1) of wild-type (wt), G113V, and A116V moPrP were found to be very similar to each other. Denaturant-induced equilibrium unfolding studies indicated that the three moPrP variants had identical thermodynamic stabilities at pH 4 (Fig. 2b) and at pH 7 (Fig. 2d) and had identical midpoints for their thermally induced unfolding transitions at pH 4 (Fig. 2c). Hence, the G113V and A116V mutations in the unstructured NTR did not, unsurprisingly, affect the global stability of moPrP [22].

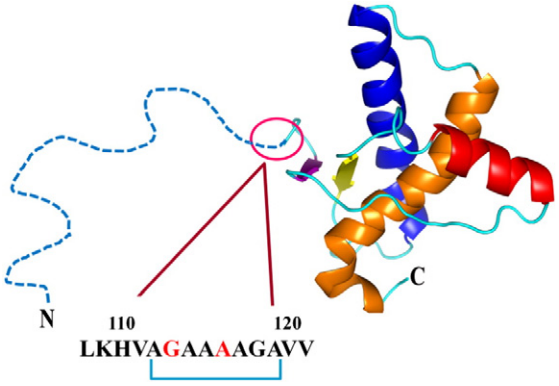

Fig. 1. Structure of the full-length (23–231) moPrP. The CTD of the prion protein was drawn using PyMOL and the PDB file 1XYX. The NTR (23–120) is known to be unstructured in the full-length protein and is drawn as a random coil. The mutation sites are marked in the sequence.

In an earlier study [23], another nearby pathogenic mutation, P101L, in the NTR had also been shown to not affect the structure and dynamics of moPrP. It became important to determine whether the G113V and A116V mutations had an effect on misfolding and aggregation, even though they did not affect global structure and stability.

The pathogenic mutations G113V and A116V accelerate the formation of misfolded oligomers

The prion protein is known to misfold and oligomerize not only on the cell surface but also in the endocytic pathway, where it encounters low pH [24]. In earlier studies [25,26], it had been shown that moPrP remains native at pH 4 but becomes prone to misfolding because the critical residue His186 has become protonated [27]. Misfolding and oligomerization can then be triggered by the addition of salt [25]. In this study, the kinetics of misfolding of wt, G113V, and A116V moPrP were monitored by the measurement of CD change, and the kinetics of oligomerization were monitored by size-exclusion chromatography (SEC) at pH 4, upon the addition of 150 mM NaCl (Fig. S2). It should be noted that the formation of misfolded oligomers at low pH correlates well with the propensity to get prion disease [28]. Such oligomers have been shown to be capable of disrupting membrane structure [25] and also to be capable of forming worm-like amyloid fibrils [25,29].

As shown previously, the kinetics of CD-monitored conformational change and SEC-monitored oligomerization of 100 μM wt MoPrP were identical at pH 4 in the presence of 150 mM NaCl (Fig. 4a). The observed rate matched to that reported earlier [25]. In contrast, in the case of both 100 μM G113V and A116V moPrP, the rate of SEC-monitored oligomerization was twofold higher than the rate of CD-monitored