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Truncated prion protein PrP226* - A structural view on its role in amyloid disease

Valerija Kovač [a, *], Blaž Zupančič [b], Gregor Ilc [b, c], Janez Plavec [b, c, d], Vladka Čurin Šerbec [a]

[a] Department for the Production of Diagnostic Reagents and Research & R&D Service, Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, SI-1000 Ljubljana, Slovenia
[b] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[c] EN-FIST Centre of Excellence, Dunajska 156, SI-1001 Ljubljana, Slovenia
[d] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

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ABSTRACT

In the brain of patients with transmissible spongiform encephalopathies, besides PrP^{Sc} aggregates, deposition of truncated PrP molecules was described. Jansen et al. reported two clinical cases with deposition of C-terminally truncated PrP, one of them ending with Tyr226. We have previously described the discovery of monoclonal antibody V5B2 that selectively recognizes this version of the prion protein, which we called PrP226*. Using monoclonal antibody V5B2 we showed that accumulation of PrP226* is characteristic for most types of human and animal TSEs. Its distribution correlates to the distribution of PrP^{Sc} aggregates. To gain insight into the structural basis of its presence and distribution in PrP aggregates, we have determined the NMR structure of recombinant PrP226*. The structure of the protein consists of a disordered N-terminal part (residues 90–125) and a structured C-terminal part (residues 126–226). The C-terminal segment consists of four α-helices and a short antiparallel β-sheet. Our model predicts a break in the C-terminal helix and reorganized hydrophobic interactions between helix α_3 and $\beta_2-\alpha_2$ loop due to the shorter C-terminus. The structural model gives information on the possible role of the protein in the development of amyloid disease and can serve as a foundation to develop tools for prevention and treatment of prion diseases.

1. Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of infectious neurodegenerative diseases, which are caused by self-propagation of prion proteins (PrPs). The characteristic of all prion diseases is the misfolding of normal cellular prion protein, PrP^{C}, into the pathological prion isoform, PrP^{Sc} [1]. The polypeptide chains of PrP^{C} and PrP^{Sc} have identical amino acid sequence and post-translational modifications, but differ in the three-dimensional structure. While it has been shown that PrP^{C} is rich in α-helixes and has little β-sheet, PrP^{Sc} is predicted to consist almost entirely of β-sheet [2]. In mammals, PrP is mainly expressed as a glycoprotein, anchored to the cell surface by a glycophosphatidylinositol (GPI) linkage [3]. The role of GPI anchor in PrP^{Sc} replication and propagation remains unclear [4,5], but it has been shown on models of transgenic mice, expressing only anchorless PrP, that it accelerates the formation and propagation of prions [3,6–10] and further increases the risk for transmission of prion disease among species [11].

In the brain of patients with TSEs, e.g. in sporadic Creutzfeldt-Jakob disease (sCJD) and Gerstmann-Sträussler Scheinker syndrome (GSS), deposition of truncated PrP molecules was described, besides PrP^{Sc} aggregates [12–15]. Our group described and characterized monoclonal antibody (mAb) V5B2, which selectively recognizes the C-terminally truncated form of PrP ending with Tyr226, PrP226* [16,17]. We have shown that mAb V5B2 discriminates between Creutzfeldt-Jakob disease (CJD) and non-CJD human brain tissue [16,18,19]. We found that accumulation of PrP226* in the brain is characteristic for most types of human TSEs, like GSS, sCJD, variant Creutzfeldt-Jakob disease (vCJD), fatal familial insomnia (FFI) and also animal TSEs, like bovine spongiform encephalopathy (BSE) and scrapie [15,18,19]. Interestingly the distribution of PrP226* correlates with the distribution of PrP^{Sc} aggregates, making it a surrogate marker for PrP^{Sc} [19]. Jansen et al.

* Corresponding author.
E-mail address: valerija.kovac@ztm.si (V. Kovač).

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reported two clinical cases of GSS with deposition of C-terminally truncated PrP, one of them ending with Tyr226 [15]. The study serves as confirmation that PrP226* alone can cause the formation of disease *in vivo*.

The correlation between PrP226* and amyloid plaques suggests that the truncated protein has an important role in the development of amyloid diseases. Increasing evidence show that truncated PrP variants are important propagators of fibrillization. We recently substantiated the role of PrP226* and similar truncated molecules in a study on protein stability and fibrillization [20]. According to our results PrP226* was thermodynamically destabilized at neutral and acidic pH and showed propensity for *in vitro* conversion. Although the truncation destabilized the molecule, PrP226* converted with longer lag time of fibrillization than WT at acidic pH. Here we present the first high resolution NMR structure of a C-terminally truncated form of prion protein. The three-dimensional structure of PrP226* explains the role of truncation of the prion protein in conversion propensity and destabilization, and indirectly its role in amyloid disease propagation.

2. Materials and methods

2.1. Plasmid construction

The plasmid for expression of human prion protein (HuPrP) PrP226* was constructed using utilizing primers 5′- CGGGATCCT-TAATGGTGATGATGATGGTGACCACCGTAATAGGCCTGAGATTCCCT-3′ and 5′-GGAATTCCATATGGGTCAAGGAGGTGGCACC-3′ and HuPrP (90-231, M129) as template. The DNA product was then inserted into a pET-11a vector (Novagen). The cloned DNA sequences were verified by sequencing.

2.2. Prion protein expression and purification

Plasmid pET11a encoding PrP226* was transformed into competent *E. coli* BL21 (DE3). Protein expression and purification was conducted as previously described [20–24]. For isotope labeling, 4 g/L $^{13}C_6$-glucose and 1 g/L ^{15}N-ammonium chloride (Cambridge Isotope Laboratories) were added. The isolated inclusion bodies were solubilized in of 6 M GndHCl and purified on a 5-mL FF Crude HisTrap column (GE Healthcare) Refolding was performed by dialysis against refolding buffer (20 mM sodium acetate and 0.005% NaN3 (pH 4.9)). Purified protein was analyzed by SDS PAGE under reducing conditions and Western blot.

2.3. NMR spectroscopy and structure calculations

All NMR experiments on 0.9 mM (^{13}C, ^{15}N)-doubly labeled PrP226* protein were performed on a Varian VNMRS 800 MHz NMR spectrometer (Varian) as previously described [21–24]. All recorded spectra were processed by NMRPipe software [25] and analyzed with Sparky [26] and CARA [27]. The initial structure calculations were performed using CYANA 3.0 [28]. The automatic NOE assignment procedure [29] yielded 2236 distance restraints for PrP226* (Table S1). The 20 conformers of the protein with the lowest residual target function values were energy-minimized in a water shell with YASARA program suite (http://www.yasara.org) [30]. The final ensemble of the 20 lowest-energy structures of PrP226* exhibited good convergence and very high definition. A validation procedure using PROCHECK-NMR [31] and WhatIF [32] demonstrated that the final family of 3D structures of PrP226* offers a good geometry and side chain packing.

3. Results

3.1. Sequence-specific NMR resonance assignment

The signals of PrP226* backbone amide resonances in the (^1H,^{15}N)-HSQC (heteronuclear single-quantum coherence) fingerprint spectrum were well-resolved suggesting that the protein adopts a globular fold and is suitable for structural determination (Fig. S1). Sequence-specific assignments of backbone ^1H, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and ^{13}CO protein resonances with the use of standard triple resonance HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH NMR experiments. The assignment was additionally confirmed by analysis of sequential and medium-range NOEs in 3D ^{15}N-edited NOESY-HSQC experiment. An almost complete assignment was obtained for each backbone nucleus with the exception of Arg164, Glu168, Tyr169, Asn171 and Phe175 for which backbone ^1H,^{15}N cross-peak could not be observed, most probably due to the line broadening caused by slow conformational exchange. A large majority of ^1H and ^{13}C resonances in aliphatic side chains were assigned using HAHB(CO)NH, HCCH-TOCSY, (H)CCH-TOCSY, ^{13}C-edited NOESY-HSQC. Aromatic ^1H and ^{13}C resonances were also assigned using a ^{13}C-edited NOESY-HSQC 3D experiment. Almost all side chain resonances were assigned with the exception of εCH groups of His140 and His177 and δCH group of His177. The overall completeness of resonance assignments was 94.7%. The side chain amide groups were assigned for almost all asparagine and glutamine residues. The presence of a disulfide bond between Cys179 and Cys214 was confirmed on the basis of their C_β chemical shift values [33], δ 40.6 and 42.0 p.p.m., respectively. NOE distance restrains were derived from 3D ^{15}N-edited and ^{13}C-edited NOESY-HSQC experiments.

3.2. Three-dimensional structure of PrP226*

The molecular structure was determined using 152 torsion angle and 2236 upper distance restraints of which there were 526 intra-residual, 633 sequential, 562 medium and 515 long-range NOE distance restraints. The complete structural statistics is summarized in Table S1. The r.m.s.d. calculated on the ensemble of 20 structures was 0.93 ± 0.25 Å for ordered backbone atoms and 1.41 ± 0.22 Å for ordered heavy atoms. We used the model closest to the mean of the whole ensemble for all pictures and comparisons to the wild type prion protein (WT PrP). The Ramachandran plot appearance gives us 89.3, 9.4, 1.2 and 0.1% for most favored, additionally allowed, generously allowed and disallowed regions respectively.

Our computational model of PrP226* has an unstructured N-terminal part (residues 90–125) and a structured C-terminal part (residues 126 - 226), of which the part from residues 165–174 is a loop (β_2–α_2 loop), that does not have a single energetically favored orientation among the calculated 20 energetically most favored structures.

The C-terminal segment consists of four α-helices and a short antiparallel β-sheet (Fig. 1a). α_1 spans from Asp144 to Glu152 and is followed by a hydrogen bonded turn between Asn153 – Arg156. Residues Asp178 – Thr193 form α_2, whereas Lys194 forms a hydrogen bonded turn. α_3 consists of residues Glu200 – Tyr218. We did not observe any *d(i,i+3)* NOE signals for residues Tyr219 and Arg220, that is why those residues are depicted as a hydrogen bonded turn and not as a classical α-helix. Residues Glu221 – Tyr225 are denoted as helix α_4 and the protein terminates with Tyr226 that forms a hydrogen bonded turn. We propose that reorganized hydrophobic interactions between helix α_3 and β_2–α_2 loop are a consequence of the C-terminal helix breakage.