Backbone NMR assignments of the C-terminal domain of the human prion protein and its disease-associated T183A variant

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Abstract

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders associated with the misfolding and aggregation of the human prion protein (huPrP). Despite efforts into investigating the process of huPrP aggregation, the mechanisms triggering its misfolding remain elusive. A number of TSE-associated mutations of huPrP have been identified, but their role at the onset and progression of prion diseases is unclear. Here we report the NMR assignments of the C-terminal globular domain of the wild type huPrP and the pathological mutant T183A. The differences in chemical shifts between the two variants reveal conformational alterations in some structural elements of the mutant, whereas the analyses of secondary shifts and random coil index provide indications on the putative mechanisms of misfolding of T183A huPrP.

Keywords Prion protein · Protein misfolding · Transmissible spongiform encephalopathies · Structural dynamics · Pathological T183A mutation

Biological context

The misfolding and aggregation of the human prion protein (huPrP) is directly linked to the development of a group of fatal neurodegenerative disorders known as transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob disease (CJD), kuru or fatal familial insomnia (FFI) (Prusiner 1998). The native huPrP denoted as the cellular form (huPrP^C) is believed to misfold and aggregate into the disease-associated scrapie form (huPrP^{Sc}). Misfolded huPrP^{Sc} can propagate its conformational state by acting as a protein-only infectious agent (Telling et al. 1996). huPrP^C is a glycosylated protein that is bound to the plasma membrane via a C-terminal glycosylphosphatidylinositol (GPI) anchor (Aguzzi et al. 2008). The N-terminal domain of huPrP^C (residues 23–124) is intrinsically disordered and contains five copper-binding octapeptide repeats, whereas the C-terminal domain (residues 125–230, here denoted huPrP^C_{125–230}) has a globular structure, composed of three α -helical segments

(H1, H2 and H3) and two short β -strands (S1 and S2) forming an antiparallel β -sheet. The structure of huPrP^C_{125–230} has been experimentally determined by NMR (Zahn et al. 2000) and X-ray crystallography (Knaus et al. 2001).

In humans, TSEs may arise sporadically or can be associated with inherited mutations in the prion gene, which are primarily located in the C-terminal domain of huPrP, with nearly 40 TSE-associated single point mutations currently known (Minikel et al. 2016). Equilibrium unfolding experiments have determined that T183A is the most destabilizing TSE-associated mutation huPrP^C (Liemann and Glockshuber 1999), resulting in the disruption of a crucial hydrogen bond between the sidechain of T183 and backbone atoms of Y162 (De Simone et al. 2007). This mutation is linked with a phenotype of very early-onset spongiform encephalopathy (Nitrini et al. 1997). In this work, we measured the NMR resonances of T183A huPrP^C_{125–230} and compared their values with those of the wild type (WT) protein. The analysis of the chemical shifts indicates possible alterations of the native structure and dynamics associated with the pathological mutation. The differences between the NMR resonances of the two forms of the protein may suggest crucial insights into the mechanisms of onset of prion misfolding in the context of familial forms of TSE.

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Methods and experiments

Constructs for WT and T183A huPrP^C_{125–230} contained an N-terminal His-tag followed by a TEV cleavage site. Transformed *E. coli* BL21 (DE3) pLysS cells were initially grown in 2xTY medium to an OD₆₀₀ of 1.5, and then resuspended in M9 minimal medium containing 0.7 g/L ¹⁵NH₄Cl and 2.0 g/L ¹³C D-glucose. Expression was induced by addition of 1 mM IPTG and cells were harvested after 3 h of expression at 37 °C. huPrP^C_{125–230} was purified from the insoluble fraction using Ni-NTA agarose resin in a buffer containing 6 M GdHCl, 100 mM Na₂HPO₄, 10 mM reduced L-glutathione, pH 8.0. The purified fractions were refolded by a 1:20 dropwise dilution at 4 °C, into a 100 mM Na₂HPO₄, pH 7.0 buffer. Samples were then concentrated using a stirred cell, and the tag was cleaved by addition of TEV protease at a 1:10 molar ratio. Proteins were further purified by reverse nickel affinity chromatography after cleaving, followed by size exclusion on a Superdex 75 column to ensure their monomeric state.

NMR experiments were performed in ¹H, ¹³C and ¹⁵N labelled samples, in a buffer containing 100 mM Na₂HPO₄, 10 % D₂O, pH 7.0 at 289K. Spectral assignment of the backbone resonances was achieved using a combination of ¹H-¹⁵N HSQC, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH, HNCO and HN(CA)CO spectra as previously done (Fusco et al. 2012). Experiments were performed using an 18.8 T (800 MHz ¹H frequency) Advance III HD Bruker Spectrometer. The spectra were processed and analysed using NMRPipe (Delaglio et al. 1995) and CCPNAnalysis (Vranken et al. 2005). Chemical shift analysis was performed using the CSI 2.0 web server (Hafsa and Wishart 2014).

Extent of assignments and data deposition

NMR spectra of both WT and T183A variants of huPrP^C_{125–230} were acquired at 289K and pH 7.0. Under these conditions we could assign 95 and 89 ¹H-¹⁵N correlations for WT and T183A huPrP^C_{125–230}, respectively, out of a possible 103 non-proline residues (Fig. 1). Both constructs showed the spectral properties typical of structured proteins as indicated by the dispersion of resonances in the ¹H-¹⁵N HSQC spectra. In particular, the spectrum of WT huPrP^C_{125–230} is consistent with previously published data, suggesting that the protein is in the native state under these experimental conditions (Zahn et al. 2000). Samples of the WT huPrP^C_{125–230} could be concentrated above 300 μM without loss of NMR signal over time or visible precipitation in the NMR tube. By contrast, T183A huPrP^C_{125–230}

Resonances missing from the assigned spectra of WT huPrP^C_{125–230} mostly correspond to the loop connecting the β -strand S2 and the α -helix H2, spanning residues 164 to 171, whose signals are expected to broaden due to conformational exchange (Damberger et al. 2011). In addition, other missing resonances were associated with residues F175 and R220. In the case of T183A WT huPrP^C_{125–230}, several more residues were found to lack NMR peaks in the spectra. The missing S2-H2 region extended from residues 162 to 171. Other undetected resonances included residues M129, G131, F175, I182, V209 and M213. The spectral properties of T183A huPrP^C_{125–230} therefore suggested enhanced conformational exchange in this protein construct.

We analysed the chemical shift values to calculate the random coil index (RCI) (Berjanskii and Wishart 2005). This analysis generated RCI profiles that are generally in line with the structural elements of huPrP^C_{125–230} (Fig. 2a), suggesting that the native fold is preserved in both WT and T183A. However, in the loop regions of T183A huPrP^C_{125–230}, we found a sharp increase in the RCI values, indicating an enhancement of the flexibility and structural dynamics as a result of the pathological mutation. We also evaluated the secondary ¹³C $_{\alpha}$ chemical shifts of both variants, which are sensitive probes of secondary structure elements in proteins (Wishart and Sykes 1994). Secondary ¹³C $_{\alpha}$ chemical shifts were calculated as the experimental chemical shifts minus the random coil values (calculated using the PROSECCO server, Sanz-Hernandez and De Simone 2017), resulting in similar profiles across large portions of the PrP sequence in the two variants. Some differences, however, were observed in the C-terminal region of the α -helix H2, with reduced secondary shifts in the T183A variant with respect to the WT protein, suggesting a lower content of α -helix. The destabilisation of the α -helix H2 ranges residues 184–194, suggesting that the mutation of T183 has downstream effects of structural destabilisation into the sequence. By contrast, no significant alterations of secondary chemical shifts were found in correspondence of the α -helix H1 (residue 144–155), which is prone to misfolding once detached from the native environment (Camilloni et al. 2011). The difference in secondary shifts between the two variants are clearer when plotting the raw differences of between ¹³C $_{\alpha}$ and ¹H^N chemical shifts (Fig. 2c, d) showing a localised perturbation in the region 184–194 of the protein.

In summary, the NMR assignments of WT and T183A huPrP^C_{125–230} under native conditions reveal noticeable differences in the conformational properties of the protein, featuring enhanced structural dynamics in the case of the pathological mutant. The availability of these assignments will allow further studies at high-resolution characterisation of the misfolding mechanisms in this familial form of TSE.

Fig. 2 **a** Random coil index (Berjanskii and Wishart 2005) along the sequence of WT (black) and T183A (red) hPrP^C_{125–230}. The sequence and secondary structure schematic are displayed on the top. **b** Secondary ¹³C $_{\alpha}$ chemical shifts for WT (black) and T183 (red) constructs. **c**, **d** Raw ¹³C $_{\alpha}$ (**c**) and ¹H^N (**d**) chemical shift differences between WT and T183A hPrP^C_{125–230}

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Data availability The assignments have been deposited to the BMRB under the accession codes: 50,527 (WT) and 50,528 (T183A).

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