Abstract

Prion diseases are a group of fatal neurodegenerative diseases caused by the scrapie form of prion protein, PrP^{Sc}. Prion protein (PrP) is bound to the cell by a glycosylphosphatidylinositol (GPI) anchor. The role of GPI-anchor in PrP^{Sc} replication and propagation remains unclear. It has been shown that anchorless and truncated PrP forms accelerate the formation and propagation of prions *in vivo* and further increase the risk for transmission of prion diseases among species. Jansen et al. reported two clinical cases with deposition of C-terminally truncated PrP, ending with Tyr225 and Tyr226, respectively (1). We have previously described the discovery of a monoclonal antibody V5B2 that selectively recognizes the version of the prion protein that ends with Tyr226 and named it PrP226*. Using monoclonal antibody V5B2 we showed that accumulation of PrP226* is characteristic for most types of human and animal TSEs.

To gain insight into the structural basis of PrP226* 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 2-strand antiparallel β-sheet. In comparison to the wild type form, our model predicts a break in the C-terminal helix, reorganized hydrophobic interactions between helix α_3 and β_2-α_2 loop and greater exposure of hydrophobic amino acids to solvent due to the shorter C-terminus.

To further explain the role of the anchorless protein PrP226* in the development of prion diseases, we have determined its' thermodynamic properties and analyzed the kinetics of conversion into amyloid fibrils. Since PrP226* is not the only C-terminally truncated PrP variant responsible for development of disease, we have prepared four other PrP similar to PrP226*. We prepared PrP224*, PrP225*, PrP227* and PrP228*, ending with Ala224, Tyr225, Gln227 and Arg228, respectively. According to our results, thermodynamic and kinetic properties of all the PrP variants are affected both by pH and truncation. We have shown that the shortest variant was the most destabilized and converted faster than other variants in acidic pH. Other variants converted with longer lag time of fibrillization than wild type PrP despite comparable or even decreased stability in acidic pH.

Our results indicate that even the change in length for 1 amino acid residue can have a profound effect on *in vitro* conversion of PrP. The structural model of PrP226*, together with

other results, we have obtained information on the possible role of PrP226* and similar truncated proteins in the development of amyloid disease and can serve as a basis to develop tools for prevention and treatment of prion diseases.

Keywords: prion protein, PrP2226*, NMR structure, fibrillization