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# Early structural features in mammalian prion conformation conversion

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**The conversion to a disease-associated conformer ($PrP^{Sc}$) of the cellular prion protein ($PrP^{C}$) is the central event in prion diseases. Wild-type $PrP^{C}$ converts to $PrP^{Sc}$ in the sporadic forms of the disorders through an unknown mechanism. These forms account for up to 85% of all human (Hu) occurrences; the infectious types contribute for less than 1%, while genetic incidence of the disease is about 15%. Familial Hu prion diseases are associated with about 40 point mutations of the gene coding for the PrP denominated *PRNP*. Most of the variants associated with these mutations are located in the globular domain of the protein. In a recent work in collaboration with the German Research School for Simulation Science, in Jülich, Germany, we performed molecular dynamics simulations for each of these mutants to investigate their structure in aqueous solution. Structural analysis of the various point mutations present in the globular domain unveiled common folding traits that may allow a better understanding of the early conformational changes leading to the formation of monomeric $PrP^{Sc}$. Recent experimental data support these findings, thus opening novel approaches to determine initial structural determinants of prion formation.**

**Key words:** prions, prion protein, human, pathogenic mutations, structure, molecular dynamics, nuclear magnetic resonance

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Prion diseases have attracted much attention from researchers with different scientific backgrounds and coming from various areas of expertise. Many questions still remain unanswered in the study of these rare and yet unique neurodegenerative disorders. Central to understanding the disease is deciphering the nature of the causative agent of these disorders: the prion. In fact, the mechanism by which a prion ($PrP^{Sc}$) is formed and the structure of the latter, have posed major challenges to this field. Indeed, prion research has achieved a great deal of detailed information in understanding the pathogenesis of the disease, but until now the early events leading to the conformational change harboring prions have remained elusive.[1] In an attempt to learning how the protein may undergo this conformational rearrangement, my group and the group of Paolo Carloni at the German Research School for Simulation Science, in Jülich, Germany, reasoned that some clues might come from the study of pathogenic mutants in HuPrP. At the time of beginning our work the structures of few mutants were known.[2] The structure of HuPrP was used as template for our studies.[3] We therefore performed molecular dynamics (MD) simulations for each of these mutants to investigate their structure in aqueous solution. In total, almost 2 μs MD data were obtained. The calculations were based on the AMBER(parm99) force field, which has been shown to reproduce very accurately the structural features of the wild-type HuPrP and a few variants for which experimental structural information was available.[4] All the variants present structural features different from those of wild-type HuPrP and the protective dominant negative polymorphism HuPrP(E219K). These characteristics include loss of salt bridges in the $\alpha_2$-$\alpha_3$ regions and the loss of π-stacking interactions in the $\beta_2$-$\alpha_2$ loop. In addition, in the majority of the mutants analyzed, the $\alpha_3$ helix is more flexible and the residue Tyr169 is more exposed to the aqueous solvent. The biological relevance of these findings is of utmost importance.

The presence of similar traits in this large spectrum of mutations hints to a role of these characteristics in their known capabilities to generate disease-causing properties. Overall, we concluded that the regions most affected by disease-linked mutations in terms of structure and/or flexibility might be those involved in the pathogenic conversion of $PrP^{C}$ to the scrapie form of the protein, and ultimately, in the interaction with cellular partners.

Recent reports have indicated that the alteration of *PRNP* sequence by pathological mutations is sufficient to generate prions in transgenic mice.[5] Therefore, solution-state NMR studies on PrP mutants may help identifying critical regions in $PrP^{C}$ structure involved in the conversion. The comparison between the structures of Q212P and V210I mutants with the wild-type HuPrP revealed that, although structures share similar globular architecture, mutations introduce novel local structural differences.[6] The observed variations are mostly clustered in the $\beta_2$-$\alpha_2$-loop region and in the $\alpha_2$-$\alpha_3$ inter-helical interfaces. In contrast to the wild-type protein, where the structures of Q212P and V210I mutants point to the interruption of aromatic and hydrophobic interactions between residues located at the interface of the $\beta_2$-$\alpha_2$ loop and the C-terminal end of $\alpha_3$ helix. A loss of contacts between the $\beta_2$-$\alpha_2$-loop and the $\alpha_3$ helix in the mutants results in higher exposure of hydrophobic residues to solvent. Similar findings have also been reported in the NMR structure of the E200K mutant,[7] X-ray structures of F198S and D178N mutants[8] and in independent MD studies.[9-11] In addition, in the two mutants here considered side chains of Phe141 and Tyr149 adopt different orientation. Our findings indicate that the structural disorder of the $\beta_2$-$\alpha_2$-loop together with the increased distance between the loop and $\alpha_3$ helix represent key pathological structural features and may shed light on critical epitopes on the HuPrP structure possibly involved in the conversion to $PrP^{Sc}$.

Different experimental studies suggested that the conformation of the $\beta_2$-$\alpha_2$-loop plays a role in the prion disease transmission and susceptibility. Several studies have indicated that mammals carrying a flexible $\beta_2$-$\alpha_2$ loop could be easily infected by prions, whereas prions are poorly transmissible to animals carrying a rigid loop.[12] Importantly, horse and rabbit have so far displayed resistance to prion infections and there are no reports of these species developing spontaneous prion diseases. NMR studies showed that their PrP structures are characterized by a rigid $\beta_2$-$\alpha_2$ loop and by closer contacts between the loop and $\alpha_3$ helix.[13,14] Thus, it seems that prion resistance is enciphered by the amino acidic composition of the $\beta_2$-$\alpha_2$-loop and its long-range interactions with the C-terminal end of the $\alpha_3$ helix.

Interestingly, it has been proposed a role of $\alpha_1$ helix as a promoter of $PrP^{C}$ aggregation.[15] In support, Tyr149 in $\alpha_1$ helix is part of a motif, which may be solvent exposed in $PrP^{Sc}$ and involved in structural rearrangements during fibril formation.[16] Pronounced stabilization of $\alpha_1$ helix in the protein may represent another important factor in the prevention of spontaneous $PrP^{Sc}$ formation.

Comparing the structures of the wild-type protein and the mutants enabled us to detect regions on HuPrP structure that may play a key role in the pathogenic conversion. The obtained structural data indicate that the $\beta_2$-$\alpha_2$ loop and, in particular, interactions of this loop with residues in the C-terminal part of $\alpha_3$ helix determine the extent of exposure of hydrophobic surface to solvent, and thus could influence propensity of $PrP^{C}$ for intermolecular interactions. Moreover, our results highlight the significance of the $\alpha_1$ helix and its tertiary contacts in overall stabilization of HuPrP folding.

Overall, the many features discussed here involve the most important regions that confer stability to wild-type HuPrP, although the mutations considered are different for position and characteristics. In particular, the $\beta_2$-$\alpha_2$-loop and the $\alpha_2$-$\alpha_3$ regions are the most affected in terms of structural organization and flexibility of the molecule. These two subdomains are crucial for the stability of the wild-type $HuPrP^{C}$ fold[17] and might play a prominent role in the early unfolding events leading to $PrP^{Sc}$ conversion.


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