Searching for Factors Involved in Misfolding of the PrP^{C} via In-silico Techniques

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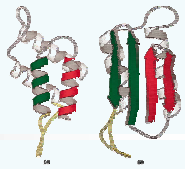


Background and Motivation

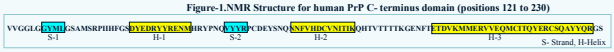
Prion protein misfolding is responsible for the Transmissible Spongiform Encephalopathies (TSE) [1]. Experimental investigations suggest that the pathogen of prion diseases is characterized by the unfolding of normal prion (PrP^{C}) followed by misfolding into an infectious scrapie isoform (PrP^{Sc}) [2].

We present novel in-silico results that identify potential factors that predispose PrP^{C} to misfold into PrP^{Sc} using computational methods. In-silico exploration of possible factors involved in the misfolding process is a crucial first step in understanding and (eventually) treating TSEs, as the number of possible factors is literally astronomical. In-silico techniques identify candidate factors for further wet-lab investigations, providing an enormous gain in efficiency compared to manual wet-lab searches through all the possible factors.

Related Work

NMR experimental methods have determined that the human PrP (huPrP) sequence of the C-terminus domain consists of three alpha-helices (Helix-1, Helix-2, Helix-3) and two beta-strands (Strand-1, Strand-2) as shown below:

Figure-1.NMR Structure for human PrP C- terminus domain (positions 121 to 230)



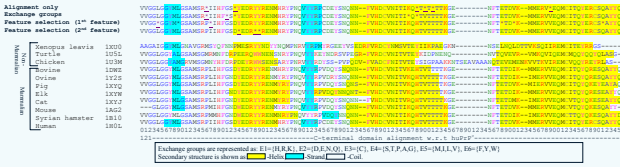
Recent models for the pathologically misfolded form of huPrP [3] show that helix-1 region is unstable and has to unfold during the conformational transition, but the most recent results provide strong evidence that helix-1 is not converted into a β-sheet during the aggregation of PrP^{C} to PrP^{Sc}[4]. Additionally, the PopMusic algorithm, which performs computer-aided single site mutation analysis, identified that the most structure-stabilizing point mutations are located in helix-2 region [5].

Several known inheritable prion diseases are related to distinct point mutants within human PrP^{C}. For example D178N, E200K and V210I cause CJD (Creutzfeldt-Jacob disease) and Y145W, F198S and Q217R cause GSS (Gerstmann-Straussler-Sheinker) in humans [6,7]. The effect of A117V mutation in humans, which is related to GSS, was identified with the use of molecular dynamics and quantum chemical calculations [8]. Western blot analysis revealed that V180I mutation in humans can prevent pathogenic form [9]. Recently, an evolutionary computation based approach that was applied to human helix-1 and used single point mutation analysis concluded that N153W mutation results in high increase in the helix-1 stability (content), also potentially preventing the pathogenic conformational change [10].

Methods and Results

Our research is based on a novel approach that contrasts mammalian and non-mammalian PrP^{C} proteins. Unlike mammalian prions, non-mammalian prions do not cause prion diseases. We have undertaken a systematic comparison of mammalian vs. non-mammalian PrP sequences (hereafter "the contrasts"), with a focus on the C-terminus domain. We used an array of computational techniques including multiple sequence alignment, exchange group similarities, feature selection methods and the AGADIR [11] helix-stability function.

Alignment based analysis



The findings of our analysis are shown as '*' with an underline in the above figure and details are given below:

➢Alignment method identified six distinct point mutations that differentiate the contrasts: P137M, D144P, H187Y, V189I, T191P, and V210I

➢V210I, which is associated with CJD in humans, was identified using the alignment

➢Exchange groups (conservative replacement through evolution) with alignment, identified five distinct point "mutations" that differentiate the contrasts: $E_1$137E_5, $E_1$142E_2, $E_2$144E_4, $E_1$185E_5, and $E_1$187E_6.

[E_1 ={H,R,K}-red, E_2={D,E,N,Q}-green, E_3={C}-gray, E_4={S,T,P,A,G}-blue, E_5={M,I,L,V}-black, and E_6={F,Y,W}-pink]

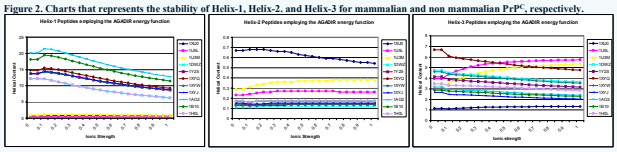
➢Feature selection method identified that Lucine and Tryptophan residue positions (i.e. entries in the composition moment vector) also differentiate the contrasts.

➢Positions 145 and 200 were identified as significant locations; from the literature, mutation Y145W is associated with GSS, and E200K is associated with CJD.

Helix stability based analysis

Helical fragments in the C-terminus domain were extracted from mammalian and non-mammalian prions. We used the AGADIR function to compute helical stability of these sequence segments under the standard conditions of pH 4.5, temperature 290K, ionic strength [0.0-1.0]M and peptides with acetylated N-terminus and amidated C-terminus. Charts I, II and III (see Figure 2) show the helix stability of helix1, helix2 and helix3 at different ionic strength levels.

Figure 2. Charts that represents the stability of Helix-1, Helix-2, and Helix-3 for mammalian and non mammalian PrP^{C}, respectively.



Our analysis shows that:

➢Stability of the helix-1 region is relatively high and higher in mammalian prions than in non-mammalian prions.

➢Stability of helix-2 is very low compared with helix-1 and helix-3 in mammalian prions. In addition, stability of helix-2 is more than twice as high in non-mammalian vs. mammalian prions.

➢Helix-2 is much less stable compared with helix-3 and thus is likely to be the most susceptible to form β-sheets in the PrP^{Sc}.

Conclusions and Future Work

Our results, which were compared with previously reported research, confirmed some existing findings. Additionally, we uncovered several new hypotheses:

➢Analysis with the use of exchange groups and alignment revealed three potential mutations: P137M, D144P and H187Y. The residues on these positions are conserved and their exchange groups are different between mammalian and non-mammalian PrP^{C}, even despite relatively low sequence homology among the non-mammalian prions.

➢Stability of helix-2 and helix-3 are very low compared with helix-1 in both mammalian and non-mammalian prions. In addition, helix-2 is much less stable compared with helix-3 and thus is likely to be the most susceptible to form β -sheets in the PrP^{Sc}.

➢Using feature selection methods, we found that the absence of Lucine in helical regions of mammalians is also a major distinction from the non-mammalian prions.

All of these new findings are candidate factors for conformational change from PrP^{C} to PrP^{Sc}. They are a new set of hypotheses that should be investigated via wet-lab experimentation or (at a minimum) molecular dynamics simulations. We plan to extend this research by applying multi-point mutation analysis and development of a method for prediction of the most likely β-sheet locations in the PrP^{Sc}.

Acknowledgements and References

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