 MCB, vol.2, no.4, pp.179-190, 2005

Misfolding Dynamics of Human Prion Protein

Muhammad H. Zaman [1]

Abstract: We report the results of longest to date simulation on misfolding of monomeric human prion protein (HuPrP). By comparing our simulation of a partially unfolded protein to the simulation of the native protein, we observe that the native protein as well as native regions in the partially unfolded protein remain in the native state, and the unfolded regions fold back with increased extended (sheet and PP-II) conformations. The misfolded regions show increased basin hopping from non-helical basins while the amino acids locked in the helical conformation tend to stay locked in that conformation. Our results also validate the hypothesis that denaturation of helices and formation of a partially unfolded intermediate is required for misfolding as the native protein stayed in native conformation for the entire simulation. Finally, we also observe that there is no correlation between misfolding and the chemical identity of amino acids, as both hydrophobic and hydrophilic amino acids showed equal probability of sampling extensively from non-native conformations.

keyword: Human prion protein, Langevin dynamics, Misfolding

1 Introduction

The transformation of the prion protein (PrP^c) from its cellular form to its scrapie form (PrP^{sc}) has been linked to transmissible spongiform encephalopathies or TSEs. TSEs are a class of neurodegenerative diseases which include Creutzeldt-Jakob disease, fatala familial insomnia, Gerstmann-Strassuler-Scheinker disease, Kuru, scrapie in sheep and bovine spongiform encephalopathy (Prusiner 1991; Prusiner 1991; Prusiner 1998) . The key step in TSEs is the PrP^c to PrP^{sc} transformation which results in denaturation of helices and increases beta structure content (Caughey *et al.* 1991; Pan *et al.* 1993; Safar *et al.* 1998). The two forms have an identical amino acid composition, and the primary difference is shown to be in secondary structure of the two isoforms. It is believed that this transformation is achieved by partial unfolding of the protein, followed by misfolding of the protein into an increased beta conformation (DeMarco *et al.* 2004).

Most experimental studies on PrP^c to PrP^{sc} transformation have focused on either studying the disulfide bridge and effect of pH, or on mutation of amino acids (Hornemann *et al.* 1998; Kocisko *et al.* 1995; Maiti *et al.* 2001; Muramoto *et al.* 1996; Riek *et al.* 1998; Swietnicki *et al.* 1997). Computational efforts have also been undertaken, with simulations ranging up to 10ns on monomeric and dimeric forms of the protein studied under varying pH conditions (Sekijima *et al.* 2003). In addition the effect of mutations on different regions in the protein have also been studied extensively (el-Bastawissy *et al.* 2001; Gsponer *et al.* 2001; Okimoto *et al.* 2002; Parchment *et al.* 2000; Zuegg *et al.* 1999).

However, no computational study has, as of yet, focused on misfolding dynamics of HuPrP. In this paper, we address this issue and present a new method of studying the transformation $PrP^c \rightarrow PrP^{sc}$ by partially unfolding the protein, and then allowing it to refold. By using a mechanical force on the protein, we unfold certain regions of the protein and leave the remaining protein in the native state, hence generating a partially folded protein. As it has been reported that the transformation from the cellular form to the scrapie form proceeds by partial misfolding (DeMarco *et al.* 2004), our study aims to understand this transformation from the partially unfolded state to the misfolded state. Though, the exact nature of the partially misfolded structure is not known, our simulation is a first of its kind attempt to study the misfolding kinetics by allowing this partially folded protein to refold. This approach allows us to study the dynamics and conformations of the regions that are unfolded at the beginning of our refolding simulation, as well as to study the conformational sampling of the regions that are in the native basins at the beginning. Our 55ns simulation

[1] Whitehead Institute and Division of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139. Email: mhzaman@mit.edu

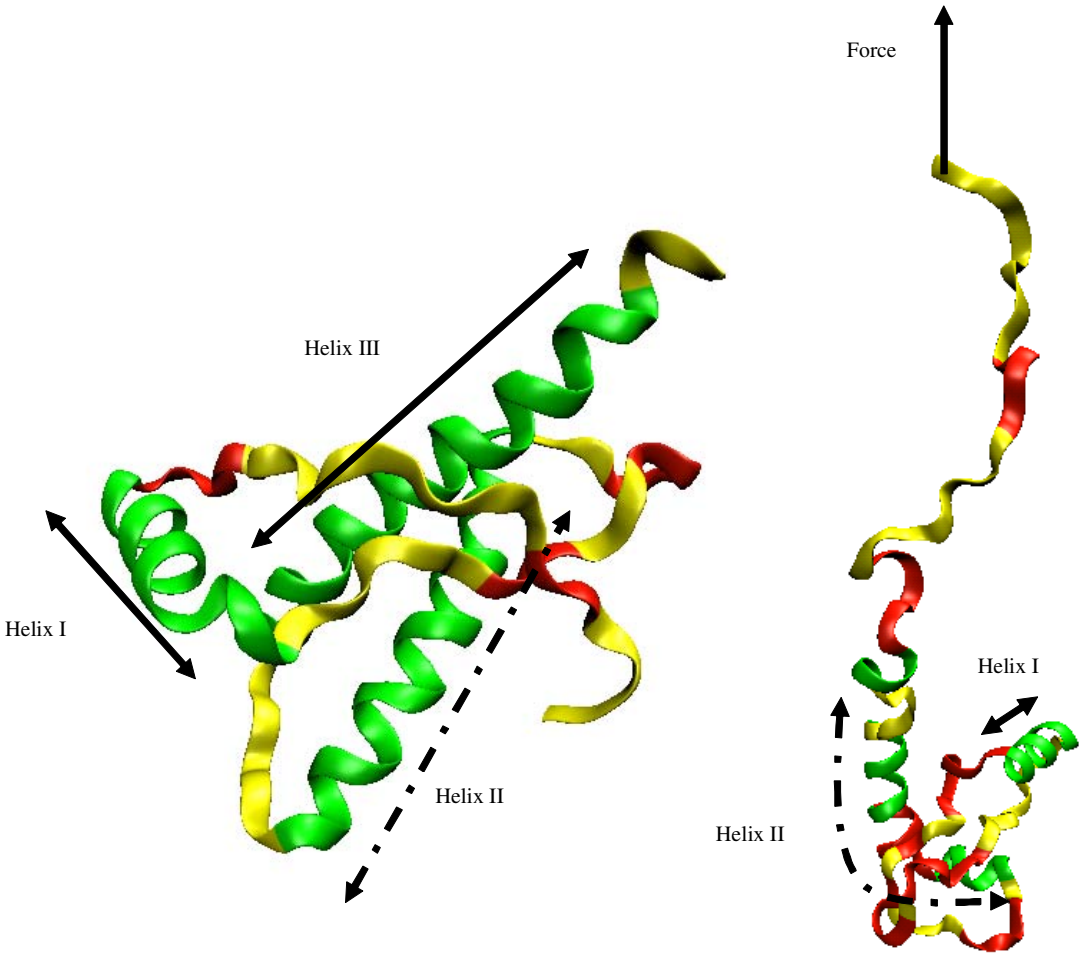

Figure 1 : HuPrP native and partially unfolded protein. The left figure shows the native structure used for our control simulation and the right structure is the initial structure used for our partially unfolded protein simulation (generated after pulling the C-term of the protein with 100pN for 100ps while keeping the N-term fixed). The three helices in the left figure are marked by arrows and the direction of the force applied on the protein for 100ps is also shown. The partially unfolded protein is unfolded in the helix III region, whereas it is partially in the native state in the helix I region. The helix II region, remains mainly helical.

is the longest reported simulation to date on monomeric HuPrP. In addition, we also carry out a control simulation, where unperturbed HuPrP molecule is studied for 20 ns to provide rigorous tests for the use of our model, force field and to provide a comparison with the contacts and conformations sampled by the partially folded protein. In addition the control simulation tests whether the protein can go from the native to misfolded state without any external perturbation or without the formation of an intermediate.

We hope that the results of our long-time simulation will provide new insight into the PrP^{c} to PrP^{sc} transformation and will serve as a basis for a wide variety of new computational and experimental endeavors to understand the TSEs.

2 Methods

2.1 Simulation Methods

Computer simulations were performed on monomeric HuPrP (pdb code: 1QM2) (Zahn *et al.* 2000) using modified TINKER (Ponder 1999; Shen *et al.* 2002), employing langevin dynamics algorithm using an implicit solvent as reported by Shen et al (Shen *et al.* 2001). The simulations were performed using Garcia's modified Amber 94 (Garcia *et al.* 2002), and were carried out

at 298 K. The implicit solvent algorithm used has been shown to produce excellent agreements with both experimental results as well as explicit solvent simulations. The langevin dynamics algorithm has been discussed in detail by Shen et al and Zaman et al (Shen *et al.* 2002; Zaman *et al.* 2003; Zaman *et al.* 2003). The simulations were carried out using a 2 fs time step with the results recorded every 10ps.

2.2 Generation of Partially Unfolded Structure

In order to study the refolding kinetics of the partially folded protein, we generated a partially unfolded structure that was in part completely stretched out and in part natively helical. This was achieved by pulling the C-term of the protein away from the N-term at constant force of 100 pN for 100ps. This stretching resulted in unfolding majority of the protein except a central helical region (residues 172-195) which stayed in a helical conformation, and parts of the helix near the N-term which also stayed natively helical (Fig. 1). As it is believed that the transformation of PrP from the cellular form to the scrapie form results in denaturation of helices, we focus our attention to the three main helical regions in the native protein. The first helical region in the native structure is formed between amino acids 144-154, the second helix between amino acids 172-195 and the final helix is formed between amino acids 204-226. Upon stretching the protein and generating an unfolded structure, the first helix was partially unfolded, the second one remained more or less intact whereas helix III, which is closest to the C-term, was completely unfolded. Thus our stretched unfolded protein had native like regions as well as completely stretched out regions. This method allowed us to study the conformations of the natively helical structures, as well as unfolded regions (that were helical in the native state) within this new partially unfolded protein. The refolding simulation of this protein was carried out for 55ns at 298 K and took nearly 4 months to complete on IBM PIV cluster.

2.3 Control Simulation

In order to justify the use of the specific force field (Garcia's modified Amber 94) used in our simulation, as well as to see whether the native structure underwent a transition to a misfolded conformation without intermediate formation, we carried out LD simulation on native HuPrP under exactly similar conditions as the partially unfolded protein for 20 ns. This also allowed us to study the conformational preferences and dynamics for the two processes simultaneously and quantify the differences between the two systems as they sampled the conformational space.

2.4 Analysis

Conformational analysis of the protein was carried out by using the DSSP algorithm (Kabsch *et al.* 1983). The basin hopping probabilities were carried out using the methods outlined by Zaman et al. We used Mathematica 4.1 (Wolfram Inc.) to quantify and represent the conformational preferences of individual amino acids in our simulation. The simulated protein structures were generated using VMD 1.8.2 (Humphrey *et al.* 1996).

3 Results

3.1 Control Simulation and Simulation Stability

Fig. 2 shows that even after 20 ns of simulation, the PrP^c protein stayed in native conformation. The native structures before and after 20ns are superimposed (Fig. 2b) and show that our choice of the force field was reasonable and the protein does not denature under native conditions. There is no significant global structural change in the protein before and after the 20 ns simulation, suggesting that the protein is inherently stable and does not transform into any isoform under native conditions.

The simulation of the partially unfolded structure also shows convergence as after 17ns the protein has nearly the same radius of gyration as the native protein (Fig. 2c). The partially unfolded protein does not show signs of any further collapse and remains within the same radius of gyration for the remainder of the simulation.

3.2 Helical Region within the unfolded structure

The comparison between preference for a certain Ramachandran basin between the native and the partially unfolded protein is shown in a color-coded map in Fig. 3. Our simulation for the native protein was 20ns long while the dynamics for the partially unfolded protein were studied for 55 ns. As there is no divergence in the native structure even after 20 ns (Fig. 2b), we believe that our native state simulation is long enough to compare the native state dynamics with that of the partially unfolded protein.

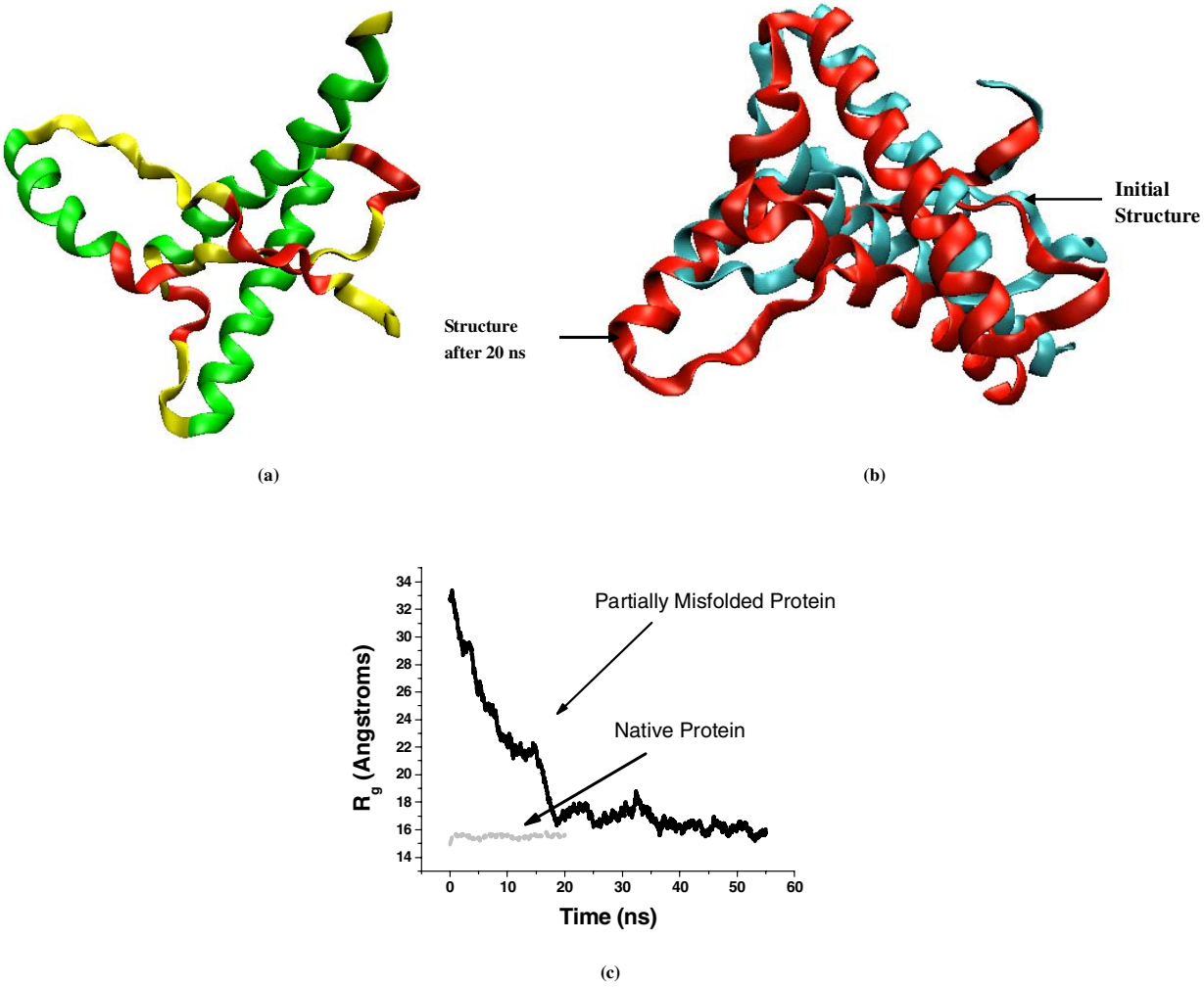

Figure 2 : Control simulation and radii of gyration. a) The control simulation results after 20 ns. b) The native protein at the beginning and end of 20ns simulation superimposed on each other. The structure at 0 ns and at 20ns are marked by arrows. There are statistically insignificant differences between the two structures, confirming that the protein stays in native conformation. c) Radii of gyration for the partially folded and the native protein. The partially unfolded protein has a radius of gyration in the same region as the native protein after $\sim$17 ns.

It is interesting to note that whereas the native protein and the partially unfolded protein have similar radii of gyration, the structural differences between the two proteins are in fact quite significant (Fig. 3).

In our partially unfolded protein simulation, helix I, which was partially unfolded, shows some preference towards helicity in our simulation. The region of this helix that was left unperturbed, does in fact stay mostly in helical configuration. Though we also see a slight preference for helicity in the unfolded part of helix I, yet the differences in the structural preferences between the two proteins are somewhat substantial, with the last few residues of helix I showing a strong preference for coil/ sheet region (Fig. 3b). These last few amino acids in this protein were in fact in a non-native conformation at the beginning of the simulation. Once again, this observation suggests the presence of a two-step procedure where misfolding is preceded by partial unfolding.

For helix II, which was left in essentially the native state in our partially unfolded protein, we observed that it re-

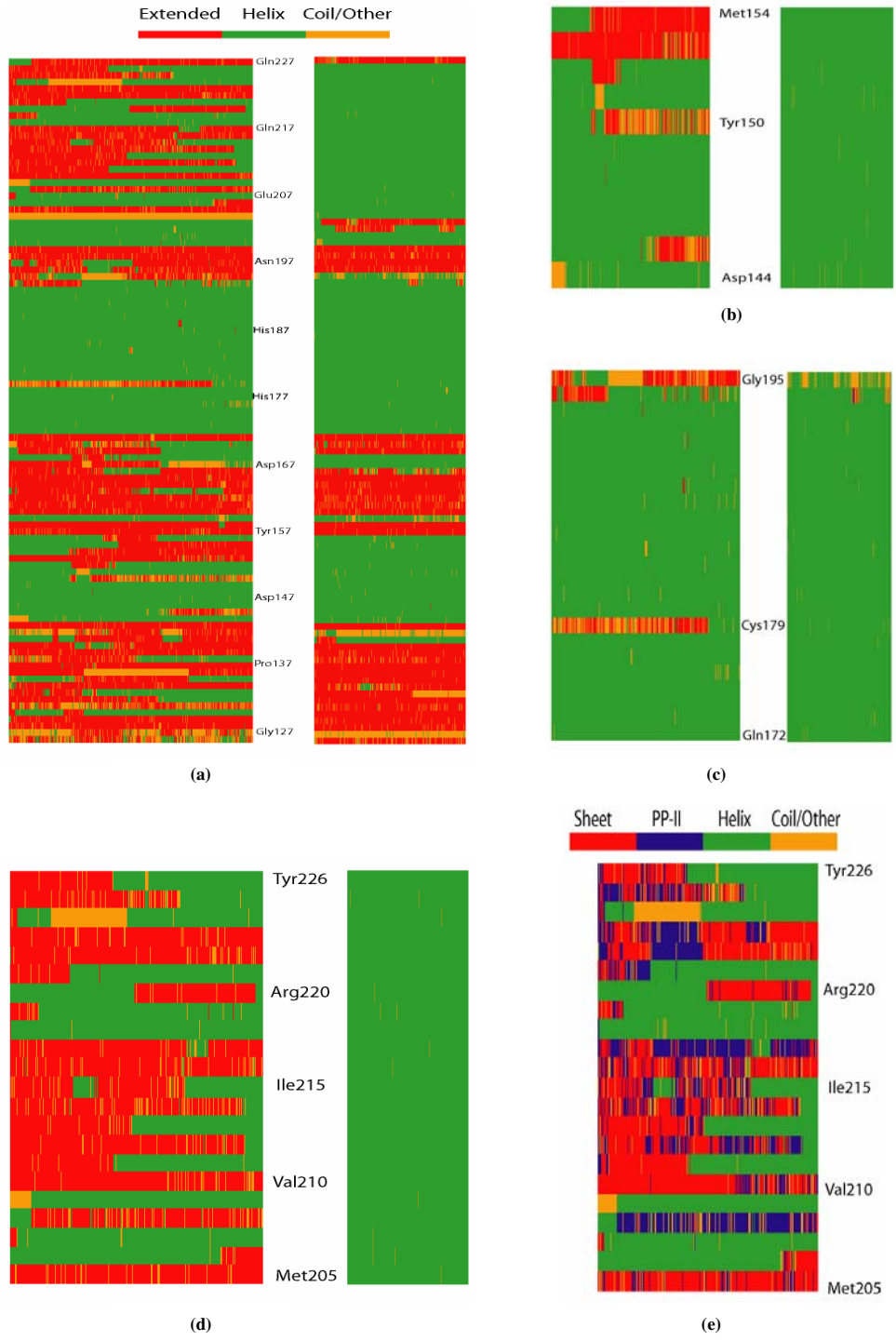

Figure 3 : Rama-Dynamics Map. a) The Rama-dynamics map is shown for the entire protein for both the native and partially unfolded protein. The x-axis represents the simulation time. b) Helix I rama-map comparison. c) The central helix rama-map comparison. d) Helix III rama-map comparison between the native structure simulation and the partially unfolded protein. e) Further distinction between PP-II and sheet basin for helix III shows frequent sampling between the two basins.

Table 1 : Structural preference of helices in HuPrP in native and partially unfolded simulations

	Native Protein Simulation			Partially Unfolded Protein Simulation		
	Helix	Sheet/PP-II	Coil	Helix	Sheet/PP-II	Coil
Helix I	98	1	1	72	16	12
Helix II	93	6	1	90	7	3
Helix III	92	5	3	39	53	8

Table 2 : Structural preferences of hydrophobic amino acids in helices I, II and III

Residue Name and Number	Region	Percent time spent in helical basin in native state simulation	Percent time spent in helical basin in partially unfolded state simulation
Tyr-145	Helix I	99	72
Tyr-149	Helix I	99	98
Tyr-150	Helix I	99	52
Met-154	Helix I	99	29
Phe-175	Helix II	99	96
Cys-179	Helix II	98	25
Val-180	Helix II	98	96
Ile-182	Helix II	99	95
Ile-184	Helix II	98	97
Val-189	Helix II	99	96
Met-205	Helix III	99	2
Met-206	Helix III	99	93
Val-209	Helix III	99	91
Val-210	Helix III	99	2
Cys-214	Helix III	99	17
Ile-215	Helix III	99	60
Tyr-218	Helix III	99	96
Ala-224	Helix III	99	58
Tyr-225	Helix III	98	34
Tyr-226	Helix III	98	73

mained in the helical conformation throughout the simulation (Fig. 3c). It is interesting to note that with the exception of a single amino acid (Cys 179), that introduced a kink in the helix, all the other amino acids remained in helical conformation, suggesting that perhaps the native well for the amino acid is in fact deeper that previously thought. In addition, we observe that this region is robust to the change in the surroundings, as other regions surrounding this native helix alternate between coil, and extended conformations (Fig. 3a and 3c).

Finally, the most divergence between the native protein and the partially unfolded protein's simulation is observed in helix III, where the partially unfolded protein misfolded completely and predominantly preferred a coil/extended conformation, as opposed to the highly helical conformation of the native protein for the same region (Fig. 3d).

The overall change in conformation is noted in Table I, suggesting that the region comprising of helix III not only did not stay helical, but in fact the refolding increased the sheet population up to 53%. In other words, we observe that if we start with a partially unfolded structure, where helices have been denatured, the protein prefers to adopt conformations that are rich in non-helical regions. On the other hand, if the protein as a whole or parts of the protein are allowed to stay in native basins, the amino acids in the said regions tend to stay in those conformations. Thus our results show that misfolding is in fact at least a two-

Table 3 : Structural preferences of hydrophilic amino acids in helices I, II and III

Residue Name and Number	Region	Percent time spent in helical basin in native state simulation	Percent time spent in helical basin in partially unfolded state simulation
Asp-144	Helix I	97	90
Glu-146	Helix I	99	98
Asp-147	Helix I	99	97
Arg-148	Helix I	99	97
Arg-151	Helix I	97	92
Glu-152	Helix I	98	70
Asn-153	Helix I	99	4
Ser-171	Helix II	99	95
Asn-172	Helix II	98	92
Gln-173	Helix II	99	94
Asn-174	Helix II	99	91
His-177	Helix II	99	96
Asp-178	Helix II	99	93
Asn-181	Helix II	99	92
Thr-183	Helix II	99	97
Lys-185	Helix II	99	93
Gln-186	Helix II	99	98
His-187	Helix II	99	99
Thr-188	Helix II	98	98
Thr-190	Helix II	98	91
Thr-191	Helix II	99	94
Thr-192	Helix II	98	97
Thr-193	Helix II	98	97
Lys-194	Helix II	87	63
Lys-204	Helix III	99	1
Glu-207	Helix III	99	98
Arg-208	Helix III	99	7
Glu-211	Helix III	99	88
Gln-212	Helix III	99	8
Thr-216	Helix III	99	3
Gln-217	Helix III	99	5
Glu-219	Helix III	99	91
Arg-220	Helix III	99	85
Glu-221	Helix III	99	70
Ser-222	Helix III	99	7
Gln-223	Helix III	99	1

step process, with the formation of the partial unfolded state being the first and a required step for misfolding.

3.3 Hydrophobic and Hydrophilic Amino Acids conformations

In order to study the correlation between misfolding and chemical identity of amino acids, we studied the percent

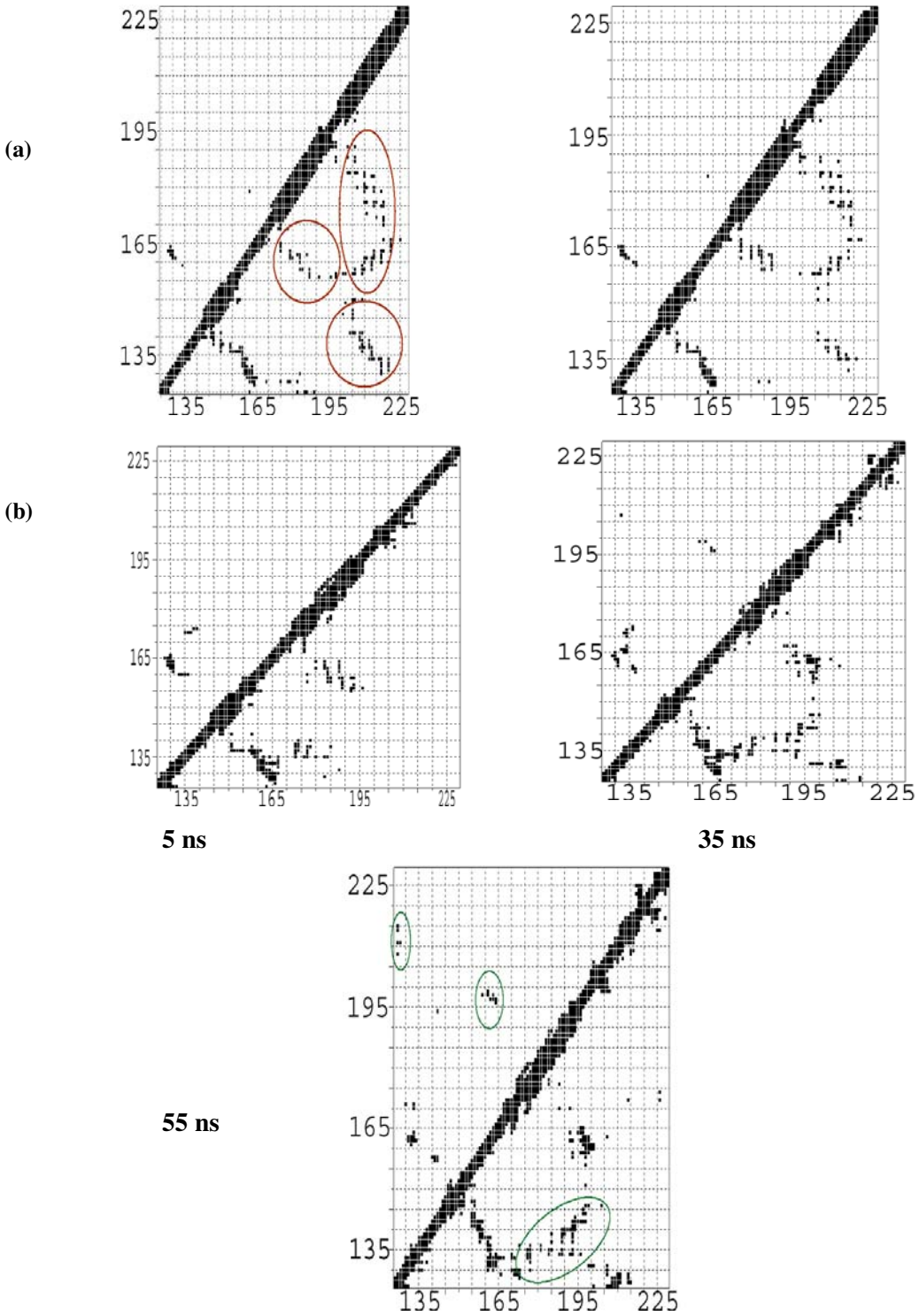

Figure 4 : Contact Map of a) native protein (top: 0 ns and bottom: 20ns) and b) partially unfolded protein at (top left: 5 ns, top right: 35ns and bottom: 55 ns). Lower right section in the contact map shows all-atom contacts while the top left section shows backbone only contacts. The native protein stays stable and only a few new contacts are formed over a 20ns period. The ovals show key contacts in the native state that are absent in the partially unfolded protein even after 55 ns. The ovals in 55ns contact map depict key contacts formed in the partially unfolded protein that are missing in the native protein.

time spent in the helical basin by each hydrophobic (or hydrophilic) amino acid in the three helices. The results of this analysis are summarized in tables II and III respectively. It is clear from the tabulated results, that misfolding is not a function of hydrophobicity or hydrophilicity. Both hydrophobic and hydrophilic amino acids in helices I and II show preference for helical conformations, with a few exceptions in helix I and one exception in helix II (Cys 179). On the other hand, in helix III, some hydrophobic amino acids show a preference for the native helical conformation, while others do not. Similar trend is observed for hydrophilic amino acids, the minor differences between the results of the two types of amino acids are statistically insignificant. This result is potentially very useful as it underlines the global nature of the transformation from PrP^{c} to PrP^{sc}, and also suggests that in fact the transformation is not a function of the misfolding of a single amino acid, or a specific type of amino acids, but is in fact a global behavior. In other words, we observe that the regions that were unfolded, refolded back with increased beta structure, whereas the regions which were natively helical in the partially unfolded protein, stayed in that conformation regardless of their amino acid composition.

The time evolution of contacts in the native and the partially unfolded protein are shown in Fig 4. The map shows that the misfolded protein is in fact quite dynamic, and whereas the central helix remains in the native conformation and samples few conformations, the other regions sample extensively from sheet/coil conformations. It is interesting to note that as the contacts evolve in the partially unfolded protein, only fewer stable helical contacts are made. This is in agreement with our previous observation that misfolded regions sample rarely from the helical basins and most contacts in the unfolded regions of the protein are formed in the sheet/turn/coil region.

4 Discussion

Using long time computer simulations of the native protein, and a partially unfolded protein, we show that the regions that are left in native conformations in the unfolded structure remain in their conformation, whereas the remaining unfolded regions tend to fold with increased sheet/turn conformation.

4.1 Dynamics and sampling of conformational state

Comparing the basin hopping of partially unfolded protein with that of the control simulation, we observe several interesting features. Firstly, we observe that the helical regions of the native protein are less dynamic in nature, and sample rarely from basins other than the helical basins. This is true for all the three helical regions of interest. On the other hand, with the exception of helix II, both helix I and helix III regions in the partially unfolded protein show a dynamical behavior. In addition, it is interesting to note that most of the regions that sample other conformations besides the helical basin are dynamic between PP-II, coil and sheet basins, and hop back and forth with high frequency (Fig. 3e). This is in sharp contrast with the dynamics in the helical basin, as the amino acids that end up in the helical basin tend to stay locked in the helical conformation for extended times and do not actively hop back and forth, regardless of whether they are in helix I, II or III. Thus the interbasin hopping between helical basin and other basins is rare and infrequent, whereas hopping between other basins occurs frequently. These results suggest that interbasin barriers are higher between helical basin and other basins whereas for other basins, they are relatively lower. Thus, based on our results, we argue that for the native protein it is highly unlikely to transform to the PrP^{sc} form without any perturbation, whereas if the barriers are lowered by change in environment (changes in pH, temperature etc) or with external force, the amino acids sample other conformations which lead to misfolding of the protein.

4.2 Is Misfolding dependent on chemical nature of amino acids

Is PrP^{c} misfolding to PrP^{sc} a function of misconformation of certain amino acids, or certain types of amino acids? Our simulations suggest that both hydrophobic and hydrophilic amino acids are equally likely to have increased sampling from non-native basins, as well as native basins, and hence it is more of a global effect than a local effect. Perturbation of the protein from the native state results in increased sampling from the non-native basins in both hydrophobic and hydrophilic amino acids with essentially equal probability. In addition, we observe that in the native protein no particular amino acid always tends to misfold, regardless of its identity or type. This implies that in fact a single point mutation is not likely to completely correct misfolding,

but does not contradict the fact that single point mutations may lead to increased beta structure formation, as has been shown experimentally as well as through computational studies (Okimoto *et al.* 2002; Riek *et al.* 1998; Sekijima *et al.* 2003).

Perturbation, PES and Prions: The purpose of our simulations of partially unfolded protein is to observe the dynamics of the misfolding process. Our results indicate that perturbing the native state of the protein increases the probability of the unfolded region to refold back with an increased sheet/coil structure. This is in sharp contrast with the native regions of the protein, which remain in their native conformations. By focusing on helical regions in the native protein, we address the issue of denaturation of helices and formation of new contacts in those regions that lead to increased sheet structure. Thus there are several interesting features to be noted here, the first one being that the perturbation of the protein or parts of the protein from the native state results in misfolding of the protein while the regions which are resistant to perturbation or are not perturbed tend to stay in the native basins. This is shown by both the helix II and parts of helix I staying in native well, as well as the control simulation showing little changes over a 20 ns long simulation. Our simulations thus suggest that only perturbations can lead to misfolding and native state conformational sampling does not lead to misfolding or increased extended structure formation. This result validates the hypothesis of formation of an "intermediate" or a partially unfolded structure that precedes the formation of misfolded protein. In other words, we note that the transformation of PrP^{c} to PrP^{sc} is at least a two-step process, with the first being the misfolding of the protein and the denaturation of helices. This observation leads to several other important questions; the first one is that does the protein undergo any sort of perturbation in vivo to go from the native to the partially unfolded state? This change in environment could be due to change in pH, increase in temperature or a mechanical perturbation. So far there is no evidence for any such perturbation; however, we hope that further experiments will test this hypothesis. Secondly, it remains to be seen whether a change in pH or a temperature jump (that results in an unfolded protein or partially unfolded protein) would result in a misfolded protein, and whether this misfolding would have a similar signature as in the case of mechanical perturbation. Finally, the nature of this intermediate state remains elusive. A detailed study on the characteristics of this state will tremendously improve our understanding of the misfolding process.

Efforts are underway in our group to computationally study the effect of chemical and thermal partial denaturation, and the refolding kinetics of HuPrP as a result of these perturbations. We also hope that these parameters are tested experimentally. Recently, it has been shown that dimerization leads to aggregation, and increases the probability for misfolding (DeMarco *et al.* 2004). The results reported in our study only address the misfolding at a monomer level, and it would be interesting to see whether mechanical, thermal and chemical perturbation in monomer induce a greater probability for aggregation and corresponding misfolding.

5 Conclusion

We report the longest simulations to date on PrP^{c} misfolding. By using a new approach of partially misfolding the protein, we study the dynamics of misfolding and compare it to the dynamics of the native protein. Our long-time simulations suggest that the native protein, as well as native structures in the partially folded protein in fact remain in the native basin, whereas the regions that are unfolded in the partially folded protein tend to refold with increased extended structure. We observe that the native protein, as well as individual regions in the native state in the partially unfolded protein are stable by themselves and an external change in environment is required to enhance their probability of misfolding. The dynamics of the misfolded regions also suggest that inter-basin hopping between non-helical basins is fast and frequent, whereas residues that sample helical basin, even if they are in the misfolded regions of the protein, tend to stay locked in helical conformations. Finally, we do not find any correlation between hydrophobicity, hydrophilicity or any other chemical identity of amino acids and misfolding, suggesting that individual mutations are unlikely to correct misfolding.

Reference

Caughey, B. W.; Dong, A.; Bhat, K. S.; Ernst, D.; Hayes, S. F. and Caughey, W. S. (1991): Secondary structure-analysis of the scrapie-associated protein Prp 27-30 in water by infrared-spectroscopy. *Biochemistry*, vol. 30, pp. 7672-7680.

DeMarco, M. L. and Daggett, V. (2004): From conversion to aggregation: Protofibril formation of the prion protein. *Proceedings of the National Academy of Sciences of the United States of America*. vol. 101, pp. 2293-2298.

el-Bastawissy, E.; Knaggs, M. H. and Gilbert, I. H. (2001): Molecular dynamics simulations of wild-type and point mutation human prion protein at normal and elevated temperature. *J Mol Graph Model*, vol. 20, pp. 145-154.

Garcia, A. E. and Sanbonmatsu, K. Y. (2002): Alpha - Helical stabilization by side chain shielding of backbone hydrogen bonds. *Proc. Natl. Acad. Sci. U S A*, vol. 99, pp. 2782-2787.

Gsponer, J.; Ferrara, P. and Caflisch, A. (2001): Flexibility of the murine prion protein and its Asp178Asn mutant investigated by molecular dynamics simulations. *J Mol Graph Model*, vol. 20, pp. 169-182.

Hornemann, S. and Glockshuber, R. (1998): A scrapie-like unfolding intermediate of the prion protein domain PrP(121-231) induced by acidic pH. *Proc Natl Acad Sci U S A*, vol. 95, pp. 6010-6014.

Humphrey, W.; Dalke, A. and Schulten, K. (1996): VMD: Visual molecular dynamics. *J Mol Graphics*, vol. 14, pp. 33-38.

Kabsch, W. and Sander, C. (1983): Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, vol. 22, pp. 2577-2637.

Kocisko, D. A.; Priola, S. A.; Raymond, G. J.; Chesebro, B.; Lansbury, P. T.; Jr. and Caughey, B. (1995): Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. *Proc Natl Acad Sci U S A*, vol. 92, pp. 3923-3927.

Maiti, N. R. and Surewicz, W. K. (2001): The role of disulfide bridge in the folding and stability of the recombinant human prion protein. *J Biol Chem*, vol. 276, pp. 2427-2431.

Muramoto, T.; Scott, M.; Cohen, F. E. and Prusiner, S. B. (1996): Recombinant scrapie-like prion protein of 106 amino acids is soluble. *Proc Natl Acad Sci U S A*, vol. 93, pp. 15457-15462.

Okimoto, N.; Yamanaka, K.; Suenaga, A.; Hata, M. and Hoshino, T. (2002): Computational studies on prion proteins: Effect of Ala(117) → Val mutation. *Biophysical Journal*, vol. 82, pp. 2746-2757.

Pan, K. M.; Baldwin, M.; Nguyen, J.; Gasset, M.; Serban, A.; Groth, D.; Mehlhorn, I.; Huang, Z. W.; Fletterick, R. J.; Cohen, F. E. and Prusiner, S. B. (1993): Conversion of Alpha-Helices into Beta-Sheets features in the formation of the scrapie prion proteins. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, pp. 10962-10966.

Parchment, O. G. and Essex, J. W. (2000): Molecular dynamics of mouse and syrian hamster PrP: Implications for activity. *Proteins-Structure Function and Genetics*, vol. 38, pp. 327-340.

Ponder, J. W. R. S.; Kundrot, C.; Huston, S.; Dudek, M.; Kong, Y.; Hart, R.; Hodson, M.; Pappu, R.; Mooiji, W.; Loeffler, G. (1999): TINKER: software tools for molecular design. *St. Louis, MO.*, Washington University.

Prusiner, S. B. (1991): Novel properties and biology of scrapie prions. *Curr Top Microbiol Immunol*, vol. 172, pp. 233-257.

Prusiner, S. B. (1991): Prion biology and diseases. *Harvey Lect*, vol. 87, pp. 85-114.

Prusiner, S. B. (1998): Prions. *Proc. Nat. Acad. Sci. USA*. vol. 95, pp. 13363-13383.

Riek, R.; Wider, G.; Billeter, M.; Hornemann, S.; Glockshuber, R. and Wuthrich, K. (1998): Prion protein NMR structure and familial human spongiform encephalopathies. *Proc. Natl. Acad. Sci. USA*, vol. 95, pp. 11667-11672.

Safar, J.; Wille, H.; Itri, V.; Groth, D.; Serban, H.; Torchia, M.; Cohen, F. E. and Prusiner, S. B. (1998): Eight prion strains have PrP(Sc) molecules with different conformations. *Nat Med*, vol. 4, pp. 1157-1165.

Sekijima, M.; Motono, C.; Yamasaki, S.; Kaneko, K. and Akiyama, Y. (2003): Molecular dynamics simulation of dimeric and monomeric forms of human prion protein: Insight into dynamics and properties. *Biophysical Journal*, vol. 85, pp. 1176-1185.

Shen, M. Y. and Freed, K. F. (2001): Long time dynamics of met-Enkephalin: Explicit and model solvent simulations and mode-coupling theory studies. *Abstr Pap Am Chem S*, vol. 222, pp. 361-362.

Shen, M. Y. and Freed, K. F. (2002): Long time dynamics of met-enkephalin: Comparison of explicit and

implicit solvent models. *Biophysical Journal*, vol. 82, pp. 1791-1808.

Swietnicki, W.; Petersen, R.; Gambetti, P. and Surewicz, W. K. (1997): pH-dependent stability and conformation of the recombinant human prion protein PrP(90-231). *J Biol Chem*, vol. 272, pp. 27517-27520.

Zahn, R.; Liu, A.; Luhrs, T.; Riek, R.; von Schroetter, C.; Lopez Garcia, F.; Billeter, M.; Calzolai, L.; Wider, G. and Wuthrich, K. (2000): NMR solution structure of the human prion protein. *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 145-150.

Zaman, M. H.; Shen, M. Y.; Berry, R. S. and Freed, K. F. (2003): Computer simulations of Met-Enkephalin using axplicit atom and united atom force-fields: similarities, differences and suggestions for improvement." *J. Phy. Chem. B*, vol. 107, pp. 1685-1691.

Zaman, M. H.; Shen, M. Y.; Berry; R. S.; Freed; K. F. and Sosnick, T. R. (2003): Investigations into sequence and conformational dependence of backbone entropy, inter-basin dynamics and the Flory isolated-pair hypothesis for peptides. *J Mol Biol*, vol. 331, pp. 693-711.

Zuegg, J. and Gready, J. E. (1999): Molecular dynamics simulations of human prion protein: importance of correct treatment of electrostatic interactions. *Biochemistry*, vol. 38, pp. 13862-13876.