On the Stability of Helix 1 of the Human Prion Protein

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Abstract

According to the protein only hypothesis, the conversion of the cellular isoform of the prion protein PrP^C into a pathogenic isoform designated PrP^{Sc} is the fundamental event in the pathogenesis of transmissible spongiform encephalopathies. Recent structural models of PrP^{Sc} (Wille, 2002) show that the most profound conformational change during this conversion reaction is found in the region 144-154 (helix 1 in PrP^C), which undergoes transconformation from α-helix to β-sheet. Therefore, helix 1 is a potential target for drugs inhibiting the pathogenic conversion reaction.

Utilizing short synthetic peptides covering helix 1 and adjacent sequences of the human prion protein, we investigated the intrinsic conformational preferences of this sequence and the dependency of the stability of helix 1 on sequential context and solvent conditions by means of NMR and CD spectroscopy and pattern searches. Helix 1 is shown to be remarkably stable, we could not observe any extended conformers being populated under the solvent conditions used in this study. The high stability of helix 1 might be important for stabilizing the cellular isoform of the prion protein and preventing the conversion to PrP^{Sc} under non-pathological conditions.

Introduction

The conversion of the soluble, predominantly α-helical form PrP^C of the prion protein to a insoluble, aggregation-prone isoform PrP^{Sc}, which is made up mostly of β-sheets, is thought to be the key event in the pathogenesis of the transmissible spongiform encephalopathies. According to a recent structural model of PrP^{Sc} [Wille et al., 2002], helices 2 and 3 retain their helical conformation during this transition, whereas helix 1 and the NH_2-terminal domain of PrP^C are incorporated into a left-handed β-helix.

Undergoing the most profound conformational change of all secondary structure elements of PrP^C, helix 1 may play an important role for the initiation and propagation of the $PrP^C \rightarrow PrP^{Sc}$ conversion. Understanding the conformational preferences of the sequence underlying helix 1 therefore might provide insights in the mechanistics of the prion conversion process, as well as presenting valuable targets for a pharmacological stabilization of the cellular prion protein PrP^C aimed at preventing the conformational transition to the pathogenic isoform.

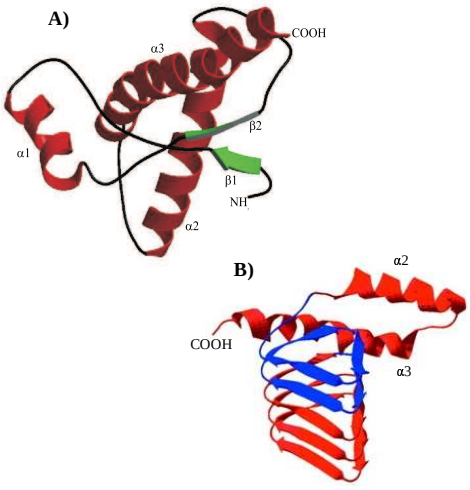

Figure 1: *A) Structure of human PrP^C (Zahn et al., 2000) B) model for human PrP^{Sc} (Wille et al., 2002)*

Methods

We characterized different peptides derived from the human prion protein encompassing helix 1 by NMR spectroscopy and computational methods. Peptides were chemically synthesized and protected by an acetyl group at the NH_2-terminus and an amide group at the COOH-terminus. We recorded homonuclear ^{1}H-TOCSY, ^{1}H-DQF-COSY and ^{1}H-NOESY experiments on Bruker AMX400 and DRX600 spectrometers. Assignment of proton resonances was achieved using the main-chain directed strategy by Wüthrich (1986). Secondary structure elements were identified by the method of Wishart (1992).

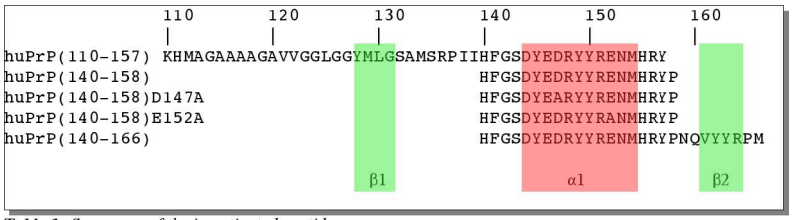

Table 1: *Sequences of the investigated peptides*

Results and Discussion

Context dependence

At pH 4.5, $^1H_\alpha$ chemical shift analysis showed no difference in helix population for the peptides huPrP(110-157), huPrP(140-158) and huPrP(140-166). All peptides showed helical conformation for the region 144-154, corresponding to helix 1 in the full length prion protein. No other secondary structure elements were populated under these conditions. As the stability of helix 1 exhibited no dependence on the flanking sequences, further investigations were conducted using the shortest and best soluble peptide, huPrP(140-158).

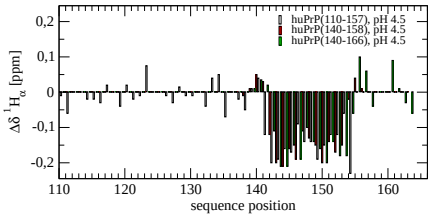

Fig. 2: *$^1H_\alpha$ chemical shifts indicate helical conformation for residues 144-154*

Electrostatic contributions to stability

Helix 1 contains several elements which may be involved in stabilizing its helical conformation: N144 may act as an N-cap, a cluster of negatively charged residues at the NH_2-terminus, as well as a cluster of positive charges at the COOH-terminus stabilize the helix macro dipole. Furthermore, two pairs of oppositely charged residues in i, i+4 spacing (D147-R151; R148-E152) might form stabilizing intra-helical salt bridges (Fig. 3).

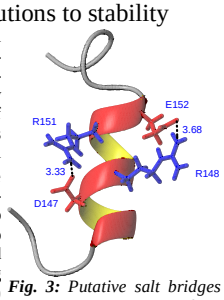

Fig. 3: *Putative salt bridges in helix 1 (coordinates from 1QM0.pdb)*

We probed for the role of these putative salt bridges in stabilizing helix 1 by introducing the mutations D147A and E152A, respectively, in the peptide huPrP(140-158). Both mutant peptides retained their helical conformation at pH 4.5, the effects of the mutations were strictly local. The E152A mutation led to a local decrease in helix population (Fig. 4). As an E to A exchange should generally lead to a stabilization of about 0.3 to 0.6 kcal/mol in helices (Pace, 1998), this hints to the existence of a stabilizing effect in the wildtype, the loss of which could not be compensated by the higher helix propensity of A. The D147A mutation, in contrast, exhibits a small local increase in helix stability. The stabilizing effect of the amino acid exchange should amount to 0.6-1.1 kcal/mol, which should cause a greater increase in helix population than observed. Again, this points to a stabilizing role of the wildtype D147, which most likely can be attributed to the D147-R151 salt bridge.

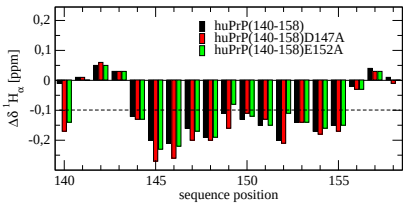

Fig. 4: $^1H_\alpha$ secondary structure shifts for the D147A and E152A mutants

Bioinformatics

The importance of the putative salt bridges for helix stability was further corroborated by computational methods. We performed a search for the particular pattern of charged residues over a filtered PDB (Pattern *[DE]-X-X-[DE]-[RK]-X-X-[RK]* and permutations) and evaluated the occurrence of this pattern in regular secondary structure elements. The results show a clear preference of this arrangement of charged residues for helical conformations, with the strongest helical preference in the center of the pattern. (Fig. 5).

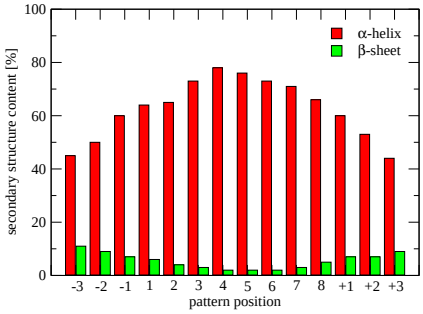

Fig. 5: *Helix and sheet propensities of residues forming the investigated patterns*

Aromatic contributions to stability

Interestingly, huPrP(140-158) retains a significant population of helical conformers in the presence of 30% acetonitrile or 1M urea, and even at pH 2.0, where most of the described electrostatic interactions should be abolished due to protonation of the acidic residues. Helix stabilization under this extremely acidic pH could arise from aromatic interactions, such as a edge-to-face stacking between Y145 and Y149 present in the full length prion protein. In this context, it should be noted that the aromatic residues in helix 1 are possible binding sites for anti-prion compounds like quinacrine (Frank, Schwarzinger, Ziegler, Rösch, unpublished results).

Conclusions

In conclusion, helix 1 of the human prion protein proved to be remarkably stable under a wide range of conditions. The peptide huPrP(140-158) retained a significant amount of helical population under extremely acidic pH, as well as in presence of 1M urea or 30% acetonitrile. This high stability can be attributed to a combination of electrostatic and aromatic side-chain interactions. In particular, the charge-charge interactions between D147-R151 and R148-E152 act as a major stabilizing force. This could also be corroborated by a pattern search over known protein structures, which showed a high preference of this charge pattern for helical structures. Due to its high stability, it seems unlikely that helix 1 takes part in the early stage of the conformational transition of PrP^C to PrP^{Sc}. In contrast, the high helix propensity of the sequence 144-145 of huPrP might act as a barrier preventing spontaneous transconformation of PrP^C. Further stabilization of helix 1 by suitable ligands might therefore constitute a viable target for the pharmacological treatment of prion diseases.

References

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