Hypothsis

Rare Large Scale Subdomain Motions in Prion Protein can Initiate Aggregation

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Abstract

The prion protein is thought to induce prion diseases by changing its conformation from the cellular form, PrP\textsuperscript{c}, into the infectious Scrapie-form, PrP\textsuperscript{Sc}. Little is known about the structural and dynamical features of this conformational change. We here introduce a novel concept that involves rare large scale motions between the subdomains \( \beta_1-\alpha_1-\beta_2 \) and \( \alpha_2-\alpha_3 \) in the carboxy-terminal, globular part of PrP. The interface between these two subdomains carries most pathogenic mutations known to be associated with prion diseases. Based on computational simulations as well as experimental results we propose that such a large scale motion subsequently destabilizes large parts of the cellular conformer PrP\textsuperscript{c}, thus, rendering it prone to structural rearrangements, including aggregation of now partially unfolded parts of the PrP sequence. We hypothesize that such large scale motions occur as a rare event even under equilibrium conditions and that the interaction of such partially destabilized PrP\textsuperscript{c}-conformers, which we named PrP\textsuperscript{c2}, contributes to the formation of pathogenic oligomeric species of the prion protein.

Key words: TSE; BSE; Prion; Conformational disease; and Folding intermediates.

Introduction

Prion diseases are a class of rare conformational diseases, which include Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and familial fatal insomnia in humans and bovine spongiform encephalopathy in cattle, Scrapie in sheep, and chronic wasting disease in deer (1). As a hallmark, amyloidogenic plaques accumulate in the brain of patients as a consequence of a conformational change of the healthy, cellular prion protein PrP\textsuperscript{c}. The exact function of PrP\textsuperscript{c} still remains unknown. PrP\textsuperscript{c} is a soluble glycoprotein attached to the cell-surface with a GPI-anchor. It contains an approximately 100 residues long amino-terminal disordered tail and a 110 residue long globular carboxy-terminal domain. The three-dimensional structure of the latter has been determined by NMR-spectroscopy and contains three \( \alpha \)-helices and a two-stranded, short antiparallel \( \beta \)-sheet (Fig. 1) (2, 3). NMR measurements of the intrinsic amide exchange rates, however, have shown that the secondary structure elements of the globular domain are rather open to exchange compared to other stably folded proteins (3, 4). The stability and structural propensities of the individual secondary structure elements of the globular domain have been characterized in detail. The secondary structure elements of PrP\textsuperscript{c} generally do not exhibit a high propensity to form secondary structures in isolated peptides (5, 6). Only helix 1 has a high intrinsic property to adopt a stable structure in solution.

Abbreviations: PrP, Prion protein; PrP\textsuperscript{c}, Cellular PrP-conformer; PrP\textsuperscript{sc}, Scrapie conformer of PrP; PrP\textsuperscript{r}, Non-native conformers of PrP; PrP\textsuperscript{c2}, Early intermediate of PrP induced by subdomain motions; PrP-NTD, Flexible and disordered amino-terminal domain PrP(23-120); PrP-CTD, Globular, carboxy-terminal domain PrP(121-230); PrP-SD1, Subdomain 1 PrP(121-163); PrP-SD2, Subdomain 2 PrP(175-230); CJD, Creutzfeldt-Jacob Disease; BSE, Bovine Spongiform Encephalopathy; and GPI, glycosphingolipid.
(7). Also, as only sequence element it does not form fibrils in peptide models. The two longer helixes display a low intrinsic tendency to populate helical conformers by themselves (8). However, they are connected by an inter-helical disulfide bridge, which contributes to the stability of this helix-loop-helix-motif. They also carry the N-glycosyl moieties attached at residues 181 and 197, which—beside a potential functional role—at least in part contribute to the structural stability of this element. Structural studies of cellular prion proteins from various species have revealed a high conservation of sequence and structure within mammals. However, studies of prions from other organisms with a lower degree of sequence similarity also revealed a high conservation of the PrP^C fold (9).

In contrast, the pathogenic conformer, PrP^Sc, is likely to exist in several different conformations that are the structural basis of the different prion strains observed (10-13). In general, PrP^Sc-conformers have decreased helix content and an increased content of β-sheet structure compared to PrP^C. Frequently, PrP^Sc forms amyloidogenic deposits, which are insoluble in non-denaturing solvents and resistant to protease treatment to varying degrees (14, 15). Recently, evidence has been presented that soluble oligomeric states of PrP^Sc are the actual pathogenic agent (16). Such species may actually be structural intermediates between PrP^C and PrP^Sc, commonly termed PrP^*. At present time no detailed structures of PrP^Sc—or PrP^*-like intermediates—are known, nor is the mechanism of the pathogenic transconformation reaction. The best model available today is based on cryo-electron microscopy of ordered two-dimensional arrays of prions. Modelling based on the low resolution structure suggests that the two carboxy-terminal helices remain structurally largely unchanged with their interhelical disulfide bond left intact (17, 18), while the N-terminally adjacent residues 90-165 form a left-handed β-helical structure (19-21).

However, because prion diseases manifest in different prion strains, which are attributed to different conformations of PrP^Sc, several pathways for prion generation and propagation have to exist. Knowledge of the transconformation pathway is mandatory for understanding the mechanism behind prion diseases and would open new avenues for therapy, such as targeting aggregation initiation sites, sites of structural instability, or intermediate states during structural rearrangement for drug interaction. In particular early events that would allow interaction with the folded globular domain of PrP^C are of interest in this context.

**Existing Hypothesis**

The protein-only-hypothesis states that the infectious agent is the prion protein itself and that no viruses or other infectious agents are involved (15). In recent years, convincing evidence has been presented for the support of this hypothesis. Thus, it is now widely accepted that PrP^Sc propagates from cellular PrP^C by interaction with non-native conformations, either PrP^Sc particles or partially folded intermediate states termed PrP^*. At present it is unclear under which conditions PrP^Sc is initially formed, if other proteins such as the postulated protein “X” or low molecular weight components such as lipids or glycan play a role in this pathogenic conformational change and contribute to the conformational heterogeneity observed...
for different prion strains (22-25). A pivotal question is how the infectious PrPsc particles that start this chain reaction are generated. Up to now, different theories on prion propagation have been put forward by Prusiner and Lansbury, which have been extensively reviewed by Eigen (26-29). In brief, the two theories assume that PrPsc is generated from monomeric PrPc by direct contact with PrPsc acting in a chaperone-like manner. While the cooperative Prusiner model assumes that the chemical equilibrium is favouring PrPsc over PrPc with several molecules of PrPsc required for the conversion, the Lansbury model of ‘linear crystallisation’ assumes PrPc to be favoured over PrPsc, which only becomes stable when a certain number of PrPsc molecules oligomerize into the catalytically functional nucleus. Numerical modelling of both theories demonstrated that prion propagation by infection is possible within the observed latency intervals while rendering spontaneous occurrence of TSE quite improbable. However, these theories do not include a molecular description of the structural events underlying prion conversion, which is critical for its overall understanding. In the following we wish to extend the existing theories concerning the initial steps of the conversion process.

New Hypothesis for the Early Steps of Conversion

We propose that rare large scale internal motions in PrPc are taking place between the putative subdomains β1-α1-β2 (subdomain 1) and α2-α3 (subdomain 2) of the globular part of the cellular prion protein. This is an extension of the existing hypothesis and provides a structural model for the initial step of the mechanism of prion transconformation. Under equilibrium conditions, rare subdomain motions lead to a small subpopulation of structurally altered, presumably destabilized states, which we term PrPc'. We propose that an opening motion between the two subdomains exposes a large buried surface in the protein, which interestingly contains a large number of pathogenic mutations, e.g., Asp178Asn (FFI, CJD), Val180Ile (CJD), Thr183Ala (dementia), Phe198Ser (GSS), Val210Ile (CJD), and Gln217Arg (GSS) (30). Loss of tertiary contacts, which may be promoted by the mutations, consequently leads to structural destabilization and increased flexibility in the now separated subdomains. Given the molecular structures of PrPc and the model of PrPsc, such an increase in disorder is an essential prerequisite for a large scale structural conversion. Although we here propose that PrPc' is present under equilibrium conditions, it may well be that the formation of such conformers is accelerated by yet unknown factors. Also, such factors may influence the fine balance within the conformational ensemble, which may be of importance for the structural variability observed between prion strains.

Support For The Hypothesis

In the following we will list seven major arguments in favor of the hypothesis based on biophysical, biochemical, genetic, and computational studies

1) The experimentally determined structure of PrPc and the available model of PrPsc differ in a way that requires a substantial amount of local un- and refolding for the prion conversion. A recent structural model for PrPsc, which is based on low resolution cryo-electron crystallography, requires that the entire PrP-SD1 (β1-α1-β2) of PrPc adopts a completely different tertiary fold, while PrP-SD2 (α2-α3) remains largely unchanged (19, 20) with the disulfide linkage left intact (17, 18). The necessary structural rearrangement may be explained within the hypothesis presented. Further evidence for large scale structural conformations — although of different origin as the motions proposed here — comes from the experimental X-ray structure of a domain-swapped cellular PrP dimer (31). In this structure helix 3 of one monomer flips 180° and binds in native-like manner to the now vacant position of the other monomer.
II) NMR studies of the cellular conformations of prion proteins show a highly flexible disordered amino-terminal tail and globular, yet not very stable, carboxy-terminal domain. The latter conclusion, which provides strong support for our hypothesis of large scale subdomain motions in PrP, arises from the low number of long-range distance constraints for PrPC and from dynamic studies on the exchange behavior of amide protons (4). In PrPC, an average of only 1.7 long-range contacts per residue, which define tertiary interactions in the calculated structure, could be found, indicating a certain degree of conformational flexibility (32). The amide hydrogen-exchange protection factors observed for the prion protein are strongest near the structurally important disulfide bridge and are overall low compared to the protection factors found in stable proteins (33). This points towards structural variability that allows water molecules to access even well structured elements in PrP and perform proton-exchange. Furthermore, NMR-relaxation measurements have identified slow chemical exchange processes on the micro- to milli-second timescale in PrP, which can typically be attributed to complex motions associated with conformational changes (34).

III) High-pressure NMR studies reveal a small population of a different conformation. In particular, it could be shown that helices α2-α3, which are linked by a disulfide bridge, suffer from structural destabilization with increasing pressure (35, 36). It could be shown that this conformational state is present at equilibrium at concentrations of approximately 1%. Residual loss of structure in helices α2-α3, which form PrP-SD2, would subsequently lead to reduced stability in the interface between subdomains PrP-SD1 and PrP-SD2, thus leading via subdomain-motions to the formation of the PrP* intermediate proposed here. However, the observed destabilization of helices α2-α3 can also be explained by a reversed event, namely loss of tertiary contacts to helix 1. This is the proposed first step in our hypothesis and is experimentally supported (5, 37). Apetri et al. could show by thermodynamic and kinetic analysis that disease associated, destabilizing mutations have a much higher portion of intermediates, such as PrP*, under equilibrium conditions than the wild-type protein, also providing support for our hypothesis (see also next section). In addition a very recent NMR-study of prion protein

Figure 2: (A) shows the location of subdomains 1 and 2 in spacefilling representation in PrP* (3). It can clearly be seen that pathogenic mutations, shown as blue ball-and-stick models, are buried in the interface between the two subdomains. Subdomain 1 (β1-α1-β2) is shown in green, subdomain 2 (α2-α3) is colored olive, the connecting loop between β2 and α2 is colored gray. (B) depicts a schematic model of a PrP* intermediate. Destabilizing, pathogenic mutations in the subdomain-interface are shown as ball-and-stick models with color coding corresponding to the energy loss due to the mutation as reported by Liemann and Glockshuber (dark blue: ΔΔG = 78 kJ mol⁻¹, pale blue = 4 kJ mol⁻¹) (38). The PrP* model shown here corresponds to early intermediates found in unfolding MD-simulation of wild-type and two mutant prion proteins.
IV) **Fixing the position of subdomains 1 and 2 relative to each other prevents prion oligomerization.** This important evidence for our hypothesis, which postulates structural rearrangement involving motions of PrPSD1 (β1-α1-β2) relative to PrPSD2 (α2-α3), comes from recent mutation experiments, in which the position of both subdomains was fixed by introducing a disulfide bridge. While placement of the disulfide-bridge between other secondary structure elements did not prevent prion formation, the above disulfide linkage prevented prion protein conversion (Human Rezaei: personal communication, 2005). This observation provides strong evidence that the subdomain motion proposed in this paper is a necessary requirement for the PrP to PrPSC conversion.

V) **The subdomain interface between β1-α1-β2 and α2-α3 contains numerous pathogenic mutations.** A number of mutations associated with human TSE are buried in the SD1-SD2 interface in PrP and in fact have a destabilizing effect on the prion protein [Asp178Asn (FFI, CJD), Val180Ile (CJD), Thr183Ala (dementia), Phe198Ser (GSS), Val210Ile (CJD), and Gln217Arg (GSS); see Fig. 2] (37, 39). Apetri and Surewicz have shown that destabilization of the PrP-SD1-PrP-SD2 interface consequently leads to an increase fraction of intermediates, presumably PrPSC, in the structural ensemble (37).

VI) **PrPSC-like properties as well as infectivity could be produced from PrP under partially denaturing condition in vitro (40-42).** These findings provide convincing evidence that PrPSC-like conformers are highly unlikely to be created directly from the cellular conformer. In fact, NMR-studies of PrPC show that the molecule stays monomeric even at milli-molar concentrations (3). Instead, partially disordered states that have the required conformational plasticity are needed. Most likely, denaturing conditions exploit natural weaknesses in the structure thereby promoting subdomain motions leading to PrPSC. Thus, it may be assumed that under mild denaturing conditions PrPSC (or intermediates even further downstream in the conversion process) are increased in their population. This would significantly reduce the time required for the conversion reaction in nature and finally enable production of infectivity in the test tube. Indeed, Baskakov and colleagues have shown that the shortest lag time for spontaneous fibril formation is found under partially denaturing conditions with a urea concentration close to the middle point of PrPSC-unfolding (43). Moreover, the increase of 1-anilinonaphthalene-8-sulfonate fluorescence excited by energy transfer from a single tryptophan together with a slight increase in the hydrodynamic radius and with no change in the circular dichroism at moderate urea concentrations was interpreted by these authors as loss of tertiary contacts in PrPSC, which causes increased flexibility while the secondary structure content is essentially unchanged (43).

VII) **Subdomain motions are detectable in equilibrium as well as unfolding simulations of cellular prion protein.** Molecular dynamics simulation carried out by Daggett and colleagues under equilibrium conditions provide microscopic insights into the dynamic behaviour of PrP (32). In particular, these simulations revealed a dissociation of helix 1 from the remainder of the proteins, which is a logical first step in the dissociation of subdomains β1-α1-β2 and α2-α3. Importantly, it was found that this event is reversible and that it occurs only in a fraction of the trajectories providing support that this is a rare motion. Due to the limitations in
computational modelling it is unlikely to observe the entire subdomain motion within simulation times available today under equilibrium condition. However, this can be overcome by simulation of the unfolding of PrPC using a simplified solvent model and high temperature. In a series of several independent unfolding simulations on wild-type as well as mutant prion proteins it could indeed be shown that the same motions found under equilibrium conditions can be seen in the beginning of the unfolding of PrP (H. Sticht et al., unpublished data).

**Consequences of the Hypothesis**

As explained in the following, the proposed structural mechanism for the initial steps in prion conversion, which involved rare large scale motions between subdomains in the folded part of PrPC leading to increased populations of structurally destabilized, flexible prion conformations, can explain both, spontaneous generation as well as infection mediated replication of PrPSc. Importantly, it is not in conflict with the previously proposed models of prion replication and rather represents a mechanistic extensions of both, the Prusiner and the Lansbury model.

For the understanding of the consequences of our hypothesis it is important to consider the interaction of the various prion conformers with other prion conformations. For clarity, we define two different chemical equilibria for the prion conversion: A first monomeric unfolding-refolding equilibrium influenced by rare conformational subdomain motions, in which W is the stable, native conformer, and a second equilibrium considering interaction of PrF with other PrP conformers, which can be of different nature (e.g., PrPC, PrPSc, or PrPSc), leading to disease associated aggregation and where the oligomeric form, PrPSc, is the most stable form.

I) PrPC $\leftrightarrow$ PrPC*, monomeric unfolding found in healthy organisms,

II) PrPC* + PrP $\leftrightarrow$ PrPSc, aggregation in diseases organisms with PrPSc being an oligomeric species depending on the nature of PrP.

In equilibrium I, which is also found in the healthy organism, PrPC is the native conformer in a monomeric folding-unfolding equilibrium. It refers to a simple conformational change – in particular a rare reversible partial unfolding event of PrPC to PrPC*. In the healthy organism and without contact with other proteins, monomeric PrPC is the energetically preferred – thus native – conformer and no pathogenic PrPSc is formed. However, in a different chemical environment, e.g., caused by the contact with other molecules, the energy landscape can change such that new, polymeric conformers – such as PrPSc – become energetically preferred. As pointed out above there is experimental evidence that (i) a small fraction of partially disordered conformers exists at equilibrium (see point III above), and that (ii) the equilibrium is on the side of PrPC, because PrPC can reversibly be folded back from denaturing condition when intermolecular contacts are avoided (44).

In contrast, equilibrium II, where several PrP molecules are in contact with each other and where the aggregated form, PrPSc, is more stable, is associated with the disease. Depending on the exact nature of PrP in Eq. [II], two different scenarios can be envisaged (45):

II.a) PrPC* + PrPC* $\leftrightarrow$ PrPSc, corresponding to slow spontaneous formation of PrPSc,

II.b) PrPC* + PrPSc $\leftrightarrow$ PrPSc, corresponding to rather fast (compared to II.a) prion propagation after infection.

In the following we will briefly discuss why these two equilibria may explain the differences in incubation time observed for spontaneous TSE (Eq. [II.a]) and for acquired TSE (Eq. [II.b]).
Because PrP<sup>sc</sup> represents a structurally destabilized, partially unfolded conformational state present at only a small fraction under equilibrium conditions, the contact of two such species is very unlikely. In numbers, assuming a 1% abundance of PrP<sup>sc</sup> would make a contact of two PrP<sup>sc</sup> molecules roughly 10,000 times less likely than the contact of two neighboring PrP<sup>C</sup> conformers (35). On the other hand, partially disordered states are well known to oligomerize into β-rich structures as a consequence of the solvent exposure of hydrophobic parts of the sequence. In the case of PrP numerous studies of peptide fragments have shown that practically every part of the sequence — except helix 1, which is very stable — has the potential to form amyloidogenic fibrils (5-7, 46-48). In particular exposure of the β-strand elements of PrP<sup>C</sup>, which is an important consequence of the here proposed rare subdomain motions within PrP<sup>C</sup>, represents a large, conformationally predetermined hydrophobic surface, which may seed further sheet formation. As a result, interaction of initially two PrP<sup>sc</sup> molecules results in the formation of a conformationally more stable state which represents the first form of a PrP<sub>Dsc</sub>-nucleus. As pointed out in the Lansbury model, and in agreement with recent experimental data, the addition of more than two monomeric units may be required to form a stable, infectious nucleus (28, 49). Indeed, occurrence of a meta-stable, highly helical prion dimer has recently been described, which converts in a longer-time scale into higher oligomeric assemblies (50). Regarding the assembly of higher oligomerization states, interaction of a putative (PrP<sup>sc</sup>)<sub>2</sub>-molecule — where two disordered conformers have to interact — with either another molecule of the same species or with other PrP<sup>sc</sup> or even PrP<sup>sc</sup> molecules is more likely to occur because now at least a meta-stable conformer, (PrP<sup>sc</sup>)<sub>2</sub> takes part in the aggregation process. Thus, based on the probability in which conformation two contacting PrP-molecules actually are, the initial contact of two PrP<sup>sc</sup> conformers is the rate limiting step. It does not involve PrP<sup>sc</sup> molecules and, therefore, can explain why TSE occurs spontaneously. Moreover, the low probability of the required PrP<sup>sc</sup> contacts may explain the extremely long incubation times. For instance, assuming again an abundance of PrP<sup>sc</sup> of 1% and that the stable nucleus is built only from contacts with PrP<sup>sc</sup>, formation of a hexameric nucleation-unit has a probability of approximately 1:10<sup>12</sup> compared to a theoretical (PrP<sup>sc</sup>)<sub>6</sub>-molecule.

The situation changes when PrP<sup>sc</sup>, which in this context is regarded as a polymeric assembly of nucleation units, is brought into the organism, e.g., by infection. Each PrP<sup>sc</sup>-particle, representing a stable oligomeric species, can now itself act as nucleus and recruit other monomeric PrP<sup>sc</sup> molecules or higher aggregates, thereof. Thus, the rate limiting step of nucleus formation is eliminated resulting in essentially linear growth behavior. Compared to equilibrium [II.a] this leads to a much faster PrP<sup>sc</sup> replication/propagation and can explain the reduced latency times observed in transmitted prion disease. The actually observed latency time will depend on the initial dose of infectious material, i.e., the number of available ends of prion fibrils, which can be further extended. Experimental support for the importance of number of available ends can be derived from the PMCA (protein misfolding cyclic amplification), which is used to amplify PrP<sup>sc</sup> for diagnostic purposes. Here, ultrasonic treatment is used to disrupt PrP<sup>sc</sup>-aggregates in order, thereby, presumably increasing the number of available interfaces required for conversion (51, 52). Further evidence is provided by Baskakov and Bocharova, who use partially denaturing conditions in an in vitro conversion assay. Based on a weak dependence of the lag time of the protein concentration they suggest a model, which incorporates a multiplication of active centers during polymerization. They propose that the partially denaturing conditions are likely responsible for fibril fragmentation, thereby increasing the number of conversion interfaces (53).

It shall briefly be reemphasized that generation of PrP<sup>sc</sup> directly from PrP<sup>C</sup> is viewed unlikely. Therefore, PrP<sup>C</sup> has not been considered in the above equilibria leading to disease. Nevertheless, we do not exclude that direct interaction of PrP<sup>C</sup> with PrP<sup>sc</sup> or a putative protein "X" acts on the PrP<sup>C</sup>----PrP<sup>sc</sup> equilibrium; there-
by, increasing the fraction of PrP\(^C\) in the organism and in turn leading to an even increased rate of propagation in Eq. [11b]. Involvement of different PrP\(^C\) conformers in PrP\(^Sc\) formation, on the other hand, may explain the observed different conformers association with different prion strains (25, 54). Being partially disordered, PrP\(^C\) has the required flexibility to form different conformers. Partially disordered ensembles can be influenced by other factors, such as small molecules. Indeed, involvement of lipids and glycosids may influence aggregation (23, 24, 55, 56). The conformational heterogeneity of PrP\(^Sc\) may also be explained by different effects exerted by disease associated mutations. We here present a possible mode of action of the destabilizing mutations clustering in the different effects exerted by disease associated mutations. We here present a possible mode of action of the destabilizing mutations clustering in the SD1-SD2 interface. However, the existence of disease associated mutations that occur outside the subdomain interface and that do not destabilize PrP\(^C\) (37, 39), such as Pro102Leu, point towards the existence of different pathways for the prion conversion. In this context it is also of interest to have a brief look at the Met129Val polymorphism. Hosszu and colleagues have shown by NMR that this polymorphism does not affect the structural properties of PrP\(^C\) and that it likely has an influence later in the conversion mechanism (57), a view that recently gained further support (58). It appears that effect of the Met129Val polymorphism depends on its misfolding pathway. We have recently shown that substitution of Met129 by Val results in a local increase in the calculated aggregation propensity of short prion peptides (59), an effect that likely does not affect the rare subdomain motions described here, thus describing an alternative disease causing mechanism. Nevertheless, this effect can only efficiently act in a partially denatured state, such as PrP\(^C\), underlining its importance for the pathogenic prion conversion.

**Conclusion**

We have presented an extension of the available models underlying prion propagation. In particular, we propose that rare large scale motions exist between the \(\beta1-\alpha1-\beta1\) and the \(\alpha2-\alpha3\) subdomains of the folded part of cellular prion protein. Such domain motions lead to a small population of a structurally destabilized, flexible prion conformation that can refold in a different conformation depending of its oligomerization state (monomeric: PrP\(^C\)\(\leftrightarrow\)PrP\(^C\), oligomeric: PrP\(^C\)\(\leftrightarrow\)PrP\(^Sc\)). Our model provides a mechanistic description of the early steps of PrP-conversion that can be incorporated into all presently available models and provides schematic explanations for mediated infection as well as spontaneous prion propagation. Moreover, it provides a first structural model for the prion replication. Nevertheless, there are still a large number of unknowns involved, such as the possible interaction of other factors in the organism. The incorporation of destabilized, partially disordered intermediates such as PrP\(^C\) may also explain structural variability in prion strains, as the conformational equilibrium of such flexible ensembles may easily be perturbed by environmental variations. Because conformational states that are present only at low concentrations at equilibrium conditions are particularly difficult to characterize by experiment, new experimental strategies will have to be explored. Likely, a combination of biophysical techniques, such as NMR and fluorescence spectroscopy, and computational simulations can be used to detect and structurally describe intermediate states in the prion conversion. Our new model should prove helpful in the design of drugs that interfere with the conversion process and for the prediction of the pathogenic potential of known and newly discovered mutations.

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References and Footnotes

45. It shall be mentioned that – for simplicity – additional intermediates, such as higher intermediate oligomerization steps, are omitted in the subsequent equilibria. PrPSc here stands for the non-native, aggregated form. As mentioned above, its exact conformation depends on the interaction partner of PrPSc.


