Prion Protein Misfolding

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INTRODUCTION

The Prion Protein (PrP) belongs to the class of amyloid-forming proteins which are, in some cases, associated with certain diseases. The cellular prion protein (PrPC) is a membrane associated protein occurring in a wide range of eukaryotic cells. The wide distribution among mammalian species and the high conservation of PrPC indicates a role of general importance. However, the physiological function of PrPC is still unknown.

According to the ‘protein-only’ hypothesis [1], PrPC is able to undergo a conformational transition into an insoluble isoform known as PrPSc (‘Sc’ for ‘scrapie’) and which is thought to be the agent that causes transmissible spongiform encephalopathies (TSEs). TSEs are fatal neurodegenerative diseases, including among others Creutzfeldt-Jakob-Disease (CJD) in humans, scrapie in sheep and goats, as well as bovine spongiform encephalopathy (BSE) in cattle [2, 3]. It is the transmissibility of prion disorders which distinguishes TSEs of other protein misfolding diseases. There is evidence that the decisive process i.e., the irreversible conversion of the physiological membrane associated cellular prion protein (PrPC) into its disease-related proteinase K (PK) resistant counterpart PrPSc, initiates an ‘autocatalytic’ reaction which leads to the accumulation of amyloid in the central nervous system (CNS) and, through still unknown mechanisms, to neurodegeneration [4, 5].

A noteworthy, and heretofore unexplained, characteristic of TSEs is the existence of different strains, which can be distinguished due to specific incubation times and clinical signs in vivo [6] as well as by distinct biochemical or immunohistological characteristics in vitro [7-9]. It is still unknown how strain-specific characteristics are supposed to be transmitted by a protein itself. Structural determinants such as glycosylation are thought to be involved in strain-dependent specification of PrPSc structures and are a characteristic distribution in affected brains [10, 11]. However, the occurrence of prion strains and the protein-only hypothesis have not yet been reconciled. Despite proceeding findings in prion research, the exact mechanisms that underlie the conformational change or conversion of PrPC, as well as those that cause the typical pathological pattern of TSEs, remain an enigma [2, 12]: hence, the development of rational approaches to diagnosis and therapy are restricted [13-15].

The feature to undergo induced or spontaneous misfolding was shown to depend on structural aspects of PrPC, such as the amino acid sequence [16-19], the highly flexible amino terminal region of the protein [20] as well as secondary structure elements [21, 22] and posttranslational modification elements [11, 23]. The remarkable peculiarity of PrP to adopt several structurally favourable states requires a detailed contemplation of distinct structural parts of PrPC and their
possible role in PrPC-PrPSc interaction, misfolding and
disease transmission. Cofactors, like metal ions [24,
25] or proteins [26, 27], are also thought to be involved
in the structural determination of PrP, but the manner in
which they influence structure and interaction with
other molecules is yet to be determined. Furthermore,
whether they have an effect in preventing prion protein
misfolding is also in question.

The purpose of this review is to highlight different
sections of PrPC and their possible role in PrPC-PrPSc
interaction and prion protein misfolding. Additionally,
features of other proteins that are able to adopt
insoluble fibrillary states under certain circumstances,
are compared to PrP with regard to our understanding
of the unique characteristics of prion diseases.

STRUCTURE DETERMINATION FOR PrPC

The molecular structure of PrPC at atomic resolution
has been determined by nuclear magnetic resonance
(NMR) spectroscopy and X-ray crystallography. PrPC
consists of a long and flexible amino terminal region
spanning up to amino acid (aa) residue 121 and a
structured carboxy terminal domain. This globular
domain harbours two short sheet-forming anti-parallel
β-helices (aa 143 to 153, aa 171 to 192, aa 199 to 226;
light green) and three α-helices (helix I: aa 143 to 153; helix
II: aa 171 to 192; helix III: aa 199 to 226 in murine
PrPC) [28]. The length of the unprocessed translation
product is 256 amino acids. In the course of its transit
through the ER and Golgi apparatus, post-translational
modifications occur, such as the removal of a N-
terminal signal sequence (1-22); the formation of an
internal helix II and III stabilizing disulfide bond
(between aa 179 and aa 214); the attachment of N-
linked oligosaccharide chains (at aa 180 and aa 196);
and the replacement of the carboxy terminus (at aa
231) by a glycosylphosphatidylinositol (GPI) anchor
[29]. Fully processed (murine) PrPC therefore contains
only 209 amino acids, representing codons 23-231 of
the prion ORF. The glycosylation can be missing or
occur at either one or both sites so that cells harbour
non-, mono-, as well as diglycosylated isoforms of PrPC
(Fig. (1)).

MODEL STRUCTURES OF PrPSc

In contrast to PrPC, the three-dimensional structure
of PrPSc has not yet been fully established, since this
protein could not be purified in sufficient quantity and
quality in a soluble, non-aggregated form.

Amyloidal fibrillar structures are characterized by
tinctorial assays using Congo Red and Thioflavin T, far
ultraviolet CD-spectra which identify β-structures and
typical anisotropic ‘cross-β’ X-ray diffraction pattern. X-
ray diffraction studies of amyloid fibres have shown that
their protofilament cores all contained a ‘cross-β’
scaffold in which β-strands are arranged
perpendicularly and β-sheets are parallel to the axis of
the fibre. The stabilization of the core structure is
basicallly provided by hydrogen bonds and other
interactions, including the polypeptide main chain.
Some amino acids at certain residues may support the
process of arranging fibrillar structures. An alternation
of polar and hydrophobic residues can result in a
formation of the same kind of β-sheet structures that
are found in amyloid fibrils [30]. Crystal structure
analysis of short oligopeptides, which are parts of
amyloid-forming proteins, revealed that segments of
four to seven amino acid residues are sufficient to form
fibrils. The assumption is that amyloid-like fibrils are
formed by two tightly interdigitating β-sheets in a
zipper-like manner, allowing nucleation to fibril forming
aggregates. Alternately, this process can also start by
the unmasking of short zipper-forming segments, which
then stack into β-sheets.

Fig. (1). Schema of the cellular mouse prion protein (left): the N-terminal signal sequence (aa 1-22) is removed during
posttranslational processing. The unstructured region (aa 23-121) harbors five octarepeats, which function as binding site for
bivalent ions, such as Cu2+. The neurotoxic peptide (aa 106-126) is show in yellow. The globular domain consists of two very
short β-strands (aa 128 to 130 and aa 160 to 162; light green) and three α-helices (aa 143 to 153, aa 171 to 192, aa 199 to 226;
red). The disulfide bond between aa 179 and aa 214 stabilizes the three dimensional structure of the protein. Two glycosylation
sites are located at aa 180 and 196, where oligosaccharide chains are linked to the polypeptide chain. A GPI anchor (aa 231)
attaches the protein to the cell membrane, as shown in the right Picture.

DeMarco, M.L., and Daggett, V. (2005). Comptes Rendus Biol., 328, 847-862.
However, on the basis of cryoelectron microscopy and by the means of structural modeling based on similar common protein structures, it has been discovered that PrP\textsuperscript{Sc} contains β-sheets in the region of aa 81-95 to aa 171, while the carboxy terminal structure is supposedly preserved. These β-sheets form a left-handed beta-helix. Three PrP\textsuperscript{Sc} molecules are believed to form a primary unit and therefore build the basis for the so-called scrapie-associated fibrils [31] (Fig. (2)).

The increase in the content of β-sheet structures results in insolubility in mild detergent fluids and causes partial resistance to enzymatic degradation of the pathogenic isoform PrP\textsuperscript{Sc}. If PrP\textsuperscript{Sc} is treated with proteolytic enzymes, only the N-terminal amino acids (aa) up to residues 81-95 (depending on the TSE agent and the proteolytic conditions) are digested [32, 33], leaving the remaining PrP\textsuperscript{Sc} reaching from aa 81-95 to aa 231. The increased resistance to proteolysis leads to an accumulation of PrP\textsuperscript{Sc}, which can be made visible by special amyloid or immunohistochemical staining. PrP\textsuperscript{Sc} is also deposited outside the cell as well as in the lysosomal-endosomal compartments within the cell [34]. The disease-causing agents are PrP\textsuperscript{Sc} aggregates that act as templates for the conversion; its catalytic activity depends on the size of the particle. PrP\textsuperscript{Sc} particles which consist of only 14-28 PrP molecules exhibit the highest rate of infectivity and conversion [35].

**PRION PROTEIN MISFOLDING DISEASES – A COMMON PATHOLOGICAL PHENOMENON**

Apart from prion diseases, there are a number of other protein misfolding diseases: Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, spinocerebellar ataxias, type II diabetes, amyotrophic lateral sclerosis, as well as diffuse Lewy body dementia and the fronto-temporal dementias. Studying the key molecular mechanisms involved in prion diseases may also help to understand these other amyloidoses. Inducible proteinopathies, such as amyloid A amyloidosis or apolipoprotein A II amyloidosis, show remarkable similarities to prion diseases [36]. Most recently Meyer-Luehmann [37] reported that in Alzheimer’s disease the exogenous induction of cerebral β-amyloidogenesis is governed by agent and host factors. The striking parallels of infectious prion disorders to the above-mentioned putatively non-infectious protein misfolding and assembly diseases make it more and more difficult to delimitate their pathological mechanisms from each other.

Of all the proteins known to undergo misfolding, the prion protein has been, and will likely continue to be, one of the most thoroughly researched. These investigations targeted the structural stability of PrP\textsuperscript{Sc} and PrP\textsuperscript{C}, or they examined the propensity of PrP\textsuperscript{C} to be folded into PrP\textsuperscript{Sc} using cell-free systems, infected cell lines or transgenic mice. Structural stability studies on PrP\textsuperscript{Sc} include partial or consecutive protein...
denaturation steps by urea or guanidinium; subsequent measurements include using a conformation dependent immunoassay (to determine the epitope accessibility to antibodies) or circular dichroism spectra. Similar experiments have also been carried out to determine the unfolding characteristics and structural stability of PrP\textsuperscript{C}. However, PrP\textsuperscript{C} conversion assays are the most frequently used approaches and include a large variety of different experimental setups.

Protein folding and hence misfolding is determined by the primary structure of a polypeptide chain, but the complex process of protein folding kinetics has been a major topic for decades and is still not completely understood. Despite numerous models for protein folding, there also exist various theories as to how misfolding could be explained.

It is likely that PrP\textsuperscript{C} undergoes some intermediate states of structural organisation before finally ending up as part of an amyloid fibril. Misfolding can only take place when the native structure of a globular protein is at least partially unfolded or degraded. Beside the prion protein, there are numerous other soluble proteins which can self-assemble to an amyloidogenic state and, as far as is known, display similar features concerning their structure [38].

A certain tertiary structure of a protein represents the equilibrium of the polypeptide molecule with the chemical environment, defined by the surrounding solvent, salt concentrations and pH. The overall folding stability, i.e. the free energy term of globular proteins is in the range of one or two hydrogen bonds which allow the transition to alternative conformations in the energy landscape without the threshold of high transition energies. As a result, for several proteins, alternative conformers are now known [39, 40]: rapid refolding under physiological conditions has been shown for spider-silk proteins that form β-sheet rich fibres contingent upon the rapid decrease of sodium, increase of potassium concentration and a drop in the pH (8 – 6) [41, 42]. If buffer conditions are changed for the all helical apo-myoglobin, then β-strand containing, fibre-forming aggregates occur [43, 44]. The authors suggest that cross-β-conformation is dominated by protein main chain interactions common to different polypeptides, whereas specific side chain interactions will define the characteristic main chain fold of globular proteins. Furthermore, they conclude that evolutionary adaptation, including mutational sequence variation and molecular chaperones, suppresses amyloid formation of globular proteins in vivo. Fibril formation of PrP can also be initiated by certain buffer conditions without the requirement of an infectious PrP\textsuperscript{Sc} seed [45]. Spontaneous protein misfolding may occur more frequently under physiological conditions than is generally assumed. Cellular factors and pathways could be of major relevance in regards to disease prevention or initiation.

Chaperones may have a key role in preventing pathogenic effects of misfolding and aggregation. An interesting example is the extracellular chaperone clusterin, which inhibits amyloid formation of human lysozyme [46]. Clusterin interacts with oligomeric pre fibrillar species, which are present in the nucleation phase prior to aggregation. Apparently, these interactions support the dissociation of the prefibrillar intermediates into native monomers. As for PrP, chaperones have been shown to play an interchangeable role: certain heat shock proteins are able to promote conversion, whereas others inhibit misfolding [47]. The chaperon BIP, which is present in the endoplasmatic reticulum (ER), has been shown to bind to certain forms of PrP that were retained in the ER due to incomplete processing [27]. Within the ER, BIP is believed to maintain proper folding of PrP by binding to defective forms for an extended period of time. In this way, the defective forms can finally be degraded by the proteasomal pathway.

Due to their occurrence in amyloids, there is evidence for the assumption that nucleic acids, lipids and glycosaminoglycans (GAGs) might play a role as cofactors in amyloidogenesis. For this reason they could be a useful therapeutic target not only for prion disorders but also for other protein misfolding diseases.

The significant effects of possible cofactors have been demonstrated in numerous experiments. In an interesting study by Yin and colleagues [48], it was shown that recombinant PrP harboring different pathogenic mutations had a more exposed amino terminus and bound more strongly to glycosaminoglycans. As common components of amyloid [49] GAGs are found in PrP\textsuperscript{Sc} in vivo [50], it has been shown that they facilitate the conversion of PrP\textsuperscript{C} into PrP\textsuperscript{Sc} in vitro [51], as well as PrP-aggregation [48]. Lipids and nucleic acids also bind to PrP\textsuperscript{C} and are detectable in PrP\textsuperscript{Sc}-aggregates [52-54]; additionally, they may facilitate PrP-conversion by functioning as a scaffold that binds and concentrates PrP\textsuperscript{C} in order to provide high amounts of substrate for a conversion into PrP\textsuperscript{Sc}.

### EFFECTS OF THE PRIMARY STRUCTURE OF PRION PROTEIN ON ITS MISFOLDING PRO-PENSITY

In light of recent studies, significant differences among different species concerning the tertiary structure of PrP\textsuperscript{C} are unlikely due to a high degree of structural and organization homology between mammalian PrP sequences and structures [55]. However, there are indeed major associations between the convertibility of the various PrP\textsuperscript{C}'s and the variability of single amino acids at certain positions within PrP\textsuperscript{C}. Single amino acids at certain positions of PrP\textsuperscript{C} can have striking effects in relation to either the susceptibility to TSEs or the chance to develop inherited forms of human prion diseases. There are more than twenty mutations of the prion protein gene (\textit{pmp}) that are known to be associated with or that are directly linked to human TSEs [56]. A well known polymorphism in the human PrP gene is located at codon 129, which either encodes for methionine or
valine: it influences the susceptibility to sporadic [57] or acquired TSEs [58], as well as the age of onset of the disease [59]. It was demonstrated that this polymorphism even has an impact on some misfolding pathways in a cell-free conversion assay which was described by Baskakov [19, 60]. This assay does not initiate the folding reaction by co-incubation with PrPSc but uses certain buffer conditions that result in different types of PrP formation. Choosing an alternative pathway with partially folded human PrP allelomorphs, the valine consisting ones showed less delay in amyloid formation compared to the methionine allelomorphs either under spontaneous or seeded folding conditions. Rezaei and colleagues [61] performed unfolding experiments using different variants of ovine PrP. Polymorphisms in the sheep PrP affected thermodynamic and kinetic parameters of the unfolding as well as the refolding process. The results of the experiments indicate a molecular basis for the effects of PrP polymorphisms on the transformation of PrPC to PrPSc.

Several other in vitro conversion experiments also showed the correlation between the PrP amino acid sequence and the convertibility of PrPC into PrPres. Depending on the genotype, ovine PrP was converted into its PK-resistant counterpart in a cell-free assay, using sheep PrPSc as seed for the conversion reaction [62]. Ovine PrP genotypes, which are not susceptible to classical scrapie in vivo also failed to be converted to PrPres in vitro. Another cell-free assay in which bacterially expressed PrPC was co-incubated with PrPSc from mouse scrapie brains [63] was used to demonstrate that a single amino acid substitution within a mouse-ovine chimeric PrPSc results in an inconvertible mutant of the previous convertible molecule [64].

The aforementioned matters in regards to the PrP amino acid sequence raise the question of how a single, or simply a few, amino acid residues at certain sequence positions can have such striking effects on the convertibility without changing the globular structure of PrPC. Basically, two types of PrP conversion can be distinguished – induced misfolding and spontaneous, or non-seeded, PrPSc or PrPres forming. The latter is seen in inherited human TSEs, whereas the induced misfolding needs an infection to begin, e.g. through the oral intake of infected tissues. The misfolding kinetics of both processes can be similar. As indicated by experiments in vitro, an initial lag phase is followed by a growth period of rapid fibril formation [65]. The addition of prion particles during the lag phase shortens the same and is known as seeding. A physiological equilibrium of PrPSc and PrPC could be destabilized by either exogenous infectious agents (acquired TSE) or a high amount of spontaneously misfolded endogenous PrPSc (spontaneous disease). The incubation period or the time until the onset of the disease therefore depends on the stability of this equilibrium. Certain amino acid constellations tend to induce the misfolding more often than other sequences and subsequently lead to fibril formation as seen in susceptible PrP genotypes.

It has been demonstrated by NMR spectroscopy that some disease related mutations of the human PrPC are located in a part of the protein that is involved in the maintenance of the hydrophobic core in the fibril [66]. Amino acid mutations therefore do not necessarily alter the stability of PrP but might have some local effects on the protein interactions which are required for oligomerization into fibrillar species. The exposure of hydrophobic regions in intermediate states during protein folding could increase the tendency towards aggregation, and subsequently initiate – at a certain stage – the misfolding cascade, which ultimately leads to disease. Hydrophobic interactions play a crucial role in the formation of β-sheets, as they bring fragments of a polypeptide chain in close proximity to each other [67]. Additionally, Kutznetsov and Rackovsky [68] showed that disease-promoting mutations in the human PrPC had a statistically significant tendency towards increasing local hydrophobicity with a possible change in interactions between PrP molecules and/or between PrP and hypothetical cofactors that might initiate subsequent fibril formation.

**STABILITY AND CONVERSION PROPENSITY OF THE CARBOXY-TERMINAL PRION PROTEIN**

In contrast to the flexible N-terminal part of PrPC, structural details of the C-terminal globular domain are described for many species [28, 69, 70]. The overall structure of this region of PrPC is very similar in most species. The superposition of various three-dimensional PrPC structures, based on polypeptide backbone atoms of mammalian PrPSc, reveals only minor differences between the tertiary structures and major similarities in the secondary structures. These secondary structure elements have been the focus of a large number of conversion assays and computer-based molecular dynamics simulations [71-74]. The influence of certain amino acid residues within the α-helices or β-strands, as well as the deletion of secondary structure elements have been found to inhibit the conversion reaction in some experiments [74, 75] or have had an effect on the cellular localization of PrP [74].

Helices II and III are anti-parallel orientated and connected by a short loop. Their structural stability is supported by a disulfide bridge, which is parallel to both sheet-forming β-strands. The superpositioning and comparison of several mammalian PrPC structures reveal that these α-helices and the β-sheet form the rigid core of the globular assembly. In contrast, helix I shows a more mobile positioning due to the long loop connections to corresponding secondary structure elements. The amino acid sequence composition of helix I is exceptional as it is the most hydrophilic α-helix of all known protein structures [76]. This possibly indicates a specific function in protein-protein interactions for this helix. In line with this assumption, recently published data show that helix I promotes...
aggregation of PrP but is not converted into β-strands [77].

A computational comparative analysis of PrP and Doppel, which is a structurally similar protein and which gene is located in the close vicinity of the prion gene, focussed on so-called chameleon sequences in both proteins [68]. Chameleon sequences are polypeptide fragments that can, experimentally, adopt both α-helical and β-strand conformations depending on the environmental conditions [78]. Other than PrP, Doppel, which is not able to undergo misfolding, contains much shorter chameleon fragments. Interestingly, the most conserved part of PrP in all species contains an unusually long chameleon fragment, located in an unusually flexible sequence context between amino acid residues 114 and 125 [68]. Additionally, this 12-mer is a highly conformational variable polypeptide compared to other sequences of the same length. According to protein databank analysis, the mature PrP (without N- and C-terminal signal peptides) shows the highest conformational variability among all sequences that contain chameleon segments of 10-14 residues. The authors also found that the amino acids seen in the fragment between PrP residues 114-125 are involved in the formation of intermolecular complexes and possess high binding potential in other proteins. The results of this study concerning the highly conserved part of PrP, which contains the chameleon sequence, are in line with the results of Nguyen [79] and Zhang [80]. The researchers showed that peptides of this part of the PrP can adopt both α-helical or β-strand conformation. Interestingly, the peptide spanning aa 106-126 has shown to be neurotoxic [81].

Another exciting outcome of the computational examination by Kuznetsov and Rackovsky was that only helix I lacks a chameleon sequence in contrast to helix II and III of PrP, which contain a chameleon hexamer (helix II) or a pentamer (helix III). The authors further show that helix I has a low β-strand propensity, especially when compared to helix II, which has a significant high propensity to β-strand conformation. Since helix I is not essential for prion infectivity [82], and it retains its α-helical conformation under a wide range of denaturing conditions [76, 83], it can be concluded that helix I does not unfold until the late states of structural transition, which occur in other parts of the PrP under the influence of global conformational rearrangements [76]. Due to the high conformational flexibility seen between residues 114-125 and with regards to the high β-strand propensity of helix II, it can be assumed that only moderate changes in the environmental conditions or interactions can induce misfolding of PrP and subsequent fibril formation.

The crystal structure of human PrP is the only example of a homodimeric PrP-structure [84]. In the homodimer, both α-helices I are in close anti-parallel orientation, allowing side chain contacts between the monomers. Other contacts in this homodimer are observed for the C-terminal parts of helices II, which form a new, short, anti-parallel β-sheet. This allows the reorientation of helices III for the dimer formation. The newly formed β-sheet is a possible initiation of α-β transition for the oligomerization of PrP [85].

A comparison of monomeric PrP structures with the prion-like protein Doppel [86] reveals a major deviation for the last two turns of helix II, where Doppel shows a strong kink. Consequently, the C-terminal two turns are positioned closer to the adjacent helix III. This could allow a more compact intramolecular interaction in Doppel, and it could explain why amyloidic misfolding of this protein is unknown.

**Fig. (3).** a) Cartoon plots of helices II and III of superimposed structures of rabbit-PrP (red), mouse-PrP (blue) and mouse-Doppel (yellow). The C-terminal turn of helix-II and the adjacent loop connection of rabbit-PrP deviate significantly to mouse-PrP and follow more the conformation of the kinked helix II of mouse-Doppel.

b) Co-tracing of helices II and III of PrP from rabbit-PrP (red), mouse-PrP (blue) and mouse-Doppel (yellow). Superposition based on Cα-positions with PrP-mouse as a target.
Among various species of laboratory animals, the rabbit is a rare example of a TSE-resistant subject. Recently, the three-dimensional structure of rabbit PrP\textsuperscript{C} was determined by NMR spectroscopy (pdb entry code 2fj3). The aforementioned structural assumption—regarding helix II in Doppel—could be an explanation for the stability of rabbit PrP\textsuperscript{C}, which also shows a C-terminal distortion of helix II (Fig. 3).

The NMR structure of elk (Cervus elaphus nelsoni) PrP displays a species-specific characteristic in the region of aa 166-175. This loop links the second β-strand with the second α-helix. In contrast to other mammalian species, this loop is exceptionally well-defined in elk [87]. The homologous region is flexibly disordered in other mammalian PrP\textsuperscript{C} species such as mice, bovines and humans. By substituting certain amino acids in the corresponding region of the mouse PrP\textsuperscript{C}, it has been shown that the rigidity of this loop results from the presence of asparagine at residue 170 in combination with threonine at residue 174. Although both amino acid side chains apparently do not interact with PrP\textsuperscript{C}, they have an additional long range effect on α-helix III, which is better defined in the presence of asparagine and threonin than in wild-type mouse PrP\textsuperscript{C}. Whether the rigid loop confers TSE susceptibility or pathological consequences remains to be examined [88].

THE ROLE OF THE AMINO-TERMINAL REGION DURING PRION PROTEIN MISFOLDING

The amino terminal region of PrP\textsuperscript{C} residues 23-120 (which make up nearly the complete first half of the amino sequence of full-length matured PrP\textsuperscript{C}) is unstructured [89] due to a high degree of main chain flexibility. Enzymatic degradation studies and transgenic mouse studies showed that the amino acids stretching from 23-89 are disposable in terms of generating infectious prions. Transgenic mice that express a truncated version of PrP\textsuperscript{C} that lacks the octapeptide region remain susceptible to prion infection [90], although disease progression is slowed down. The alteration of metal ion binding has been observed in human prion diseases [91]. This stretch harbours a region of octapeptide sequences with the ability to bind Cu\textsuperscript{2+} ions cooperatively [92], and it has been reported that incubation with copper ions at concentrations as low as 50 µM renders full length PrP\textsuperscript{C} PK resistant [93]. This is in line with the observation that preferential Cu\textsuperscript{2+} coordination by His96 and His111 induces beta-sheet formation in the unstructured amyloidogenic region of the prion protein [94].

This ability of PrP\textsuperscript{C} to bind metal ions is also seen in a non-vertebrate PrP-like molecule, termed "similar to prion protein" or StPrP [95]. Even though the metal ion binding site of StPrP consists of less amino acid repeats than the one seen in mammalian PrP\textsuperscript{C}, it is able to bind Cu\textsuperscript{2+} as effectively as human PrP does. Using various PrP fragments and spectroscopic techniques, it has been shown that two Cu\textsuperscript{2+} ions bind to two binding sites centred at His111 and His96.

Transgenic mice expressing a prion protein with up to eight extra octapeptide repeat insertions suffered from a spontaneous non-infectious accumulation of PK resistant PrP in the brain [96], while transgenic mice expressing a bovine PrP with five octapeptide repeats displayed a reduced susceptibility to BSE infection [97]. The role of metal ion binding in these pathologies is still not fully determined. Due to the redox-properties of Cu\textsuperscript{2+}-ions, oxidative stress is thought to induce prion misfolding. However, this assumption is still lacking solid experimental data.

Cell-free conversion of a N-terminally truncated, ovine-mouse chimeric, bacterially expressed PrP\textsuperscript{B} by mouse-passaged BSE resulted in two PK resistant PrP\textsuperscript{res} fragments with a difference of about 1 kDa in their molecular mass [98]. In contrast to mouse-passaged BSE, mouse scrapie Me7, 22A or 87V induced a conversion into only one detectable PK resistant PrP\textsuperscript{res} fragment. These results show that the flexible N-terminal region might support a specific docking of PrP\textsuperscript{C} to PrP\textsuperscript{Sc}. Depending on the structure of the PrP\textsuperscript{Sc} seed, the truncation of amino terminal parts of PrP\textsuperscript{C} can hinder either the binding of PrP\textsuperscript{Sc} in general—which leads to a decrease of conversion efficiency—or it inhibits a specific binding of PrP\textsuperscript{Sc}, which results in the conversion of PrP\textsuperscript{C} into differently shaped and sized PK resistant PrP\textsuperscript{res} fragments. How Cu\textsuperscript{2+} and other metal ions influence the flexibility of the N-terminal part and subsequently the interaction between PrP\textsuperscript{C} and PrP\textsuperscript{Sc} in vivo and in vitro in a strain-specific fashion remains an interesting topic that has not yet been determined.

Many studies focus on the amino terminal part in order to define its relevance for the physiological function of PrP and its conversion into PrP\textsuperscript{Sc}. Various aspects of transgenic mice with diverse deletions within the N-terminus, as well as truncated forms of recombinant PrP\textsuperscript{C}, have been researched. Even though PrP-knockout (PrP\textsuperscript{0/0}) mice do not show remarkable deficits due to the lack of PrP\textsuperscript{C} expression, the deletion of residues 105-125 of PrP\textsuperscript{C} leads to neonatal lethality in transgenic mice [81]. The authors assume a neuroprotective function of PrP\textsuperscript{C}, especially for the region between residue 105 and 125, which may be associated with the signal transduction in order to avoid cellular death; it may be converted to a neurotoxic signal by truncation of certain parts of PrP\textsuperscript{C}. Neurotoxic effects, along with neurodegenerative disease, were also observed in transgenic mice expressing other N-terminally truncated forms of PrP\textsuperscript{C} [99].

Doppel, the downstream prion-like protein that shows similarities to the C-terminal domain of PrP\textsuperscript{C} but lacks the N-terminal part [100], also causes neurodegenerative dysfunction with massive Purkinje cell loss if overexpressed in transgenic PrP\textsuperscript{0/0} mice [100]. Since the reintroduction of a prnp transgene abrogates the disease [101], it is likely that PrP\textsuperscript{C} functions as neuroprotective molecule. It would be interesting to know if similar effects are provided by the
neurotoxicity could also be demonstrated express PrPC [104]. Because the prion fragment 106-characteristic are restricted exclusively to cells which referred to as neurotoxic peptide. These toxic This part of the prion protein is therefore commonly are more toxic than larger amyloid fibrils [109].

authors assume that by diminishing fibril formation, the experiment with a mutant form of PrP 106-126. The form amyloid fibrils but at the same time increased PrP 106-126 methionines reduced the propensity to cytotoxicity. A similar result was shown in another Bergström [109] showed that the oxidation of human PrP 106-126 methionines reduced the propensity to form amyloid fibrils but at the same time increased cytotoxicity. A similar result was shown in another experiment with a mutant form of PrP 106-126. The replacement of glycine at residues 114 and 119 by alanine led to the inability of the peptide to build fibrils but it nevertheless increased cytotoxicity [110]. The authors assume that by diminishing fibril formation, the peptides could be available as soluble oligomers, which are more toxic than larger amyloid fibrils [109].

CONCLUDING REMARKS

Protein misfolding remains a conundrum, although there are plenty of research methods that aim to resolve this question. Structural aspects of the prion protein, including the amino acid sequence, secondary structural elements and post translational modifications have been taken into consideration with regard to the conformational transition of the protein. The ability of prions and other misfolding proteins to adapt more than one stable conformation has brought up numerous questions concerning the mechanisms of protein folding, unfolding and misfolding. There is no doubt that not only the intrinsic properties of a polypeptide chain determine its three dimensional structure, but also the multiple environmental influences such as the cellular milieu which contribute to the conformation of a protein.

The development and establishment of new research methods and tools over the last decades have opened up possibilities to investigate and understand the nature of protein folding and misfolding. Discovering the mechanisms behind this complex process of conformational transition will establish new prospects for combating the increasingly common and most devastating protein misfolding diseases.

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REFERENCES

[1] Prusiner, S.B. (1982) Science, 216, 136-144.
[2] Chesebro, B. (2003) Br. Med. Bull., 60, 1-20.
[3] Dormont, D. (2002), FEBS Lett., 529, 17-21.
[4] Adjou, K.T., Deslys, J.P., emainay, R. and Dormont, D. (1997) Trends Microbiol., 5, 27-31.
[5] Modler, A.J., Fabian, H., Sokolowski, F., Lutsch, G., Gast, K. and Dammenschun, G. (2004) Amyloid, 11, 215-231.
[6] Bessen, R.A. and Marsh, R.F. (1992) J. Gen. Virol., 73(Pt 2), 329-334.
[7] Grotzschel, A., Buschmann, A., Eiden, M., Ziegler, U., Luken, G., Erhardt, G. and Groschup, M.H. (2005) J. Vet. Med. B, 52, 55-63.
[8] Schoch, G., Seeger, H., Bogousslavsky, J., Tolnay, M., Janzer, R.C., Aguzzi, A. and Glatzel, M. (2006) PLoS Med., 3, e14.
[9] Vorberg, I. and Priola, S.A. (2002) J. Biol. Chem., 277, 36775-36781.
[10] DeArmond, S.J., Sanchez, H., Yehiely, F., Qiu, Y., Ninchak-Casey, A., Daggett, V., Camerino, A.P., Cayetano, J., Rogers, M., Groth, D., Torchia, M., Tremblay, P., Scott, M. R., Cohen, F. E. and Prusiner, S. B. (1997) Neuron, 19, 1337-1348.
[11] Rudd, P.M., Merry, A.H., Wormald, M.R. and Dwark, R.A. (2002) Curr. Opin. Struct. Biol., 12, 578-586.
[12] Cecili, F. and Pergami, P. (2001) Curr. Protein Peptide Sci., 2, 191-204.
[13] Crosses, E.A. (2004) Expert Opin. Pharmacother., 5, 2391-2396.
[14] Koster, T., Singh, K., Zimmermann, M. and Grusy, E. (2003) J. Vet. Pharmacol. Ther., 26, 315-326.
[15] Rossi, G., Salmona, M., Fortoni, G., Bugiani, O. and Tagliavini, F. (2003) Clin. Lab. Med., 23, 187-208.
[16] Campbell, S., Dannhe, U. and Telling, G. (2000) Archiv. Virol., 87-94.
[17] Tahiri-Alaoui, A., Gill, A.C., Disterer, P. and James, W. (2004) J. Biol. Chem., 279, 31390-31397.
[18] Vorberg, I., Groschup, M.H., Pfaff, E. and Priola, S.A. (2003) J. Virol., 77, 2003-2009.
[19] Baskakov, I., Disterer, P., Breydo, L., Shaw, M., Gill, A., James, W. and Tahiri-Alaoui, A. (2005) FEBS Lett., 579, 2589-2596.
[20] Lawson, V.A., Priola, S.A., Meade-White, K., Lawson, M. and Chesebro, B. (2004) J. Biol. Chem., 279, 13689-13695.

[21] Winklhofer, K.F., Hesse, J., Helfer, U., Reinjens, A., Muranyi, W., Moarefi, I. and Tatzelt, J. (2003) J. Biol. Chem., 278, 14961-14970.

[22] Iovino, M., Falconi, M., Petruzziello, R. and Desideri, A. (2001) J. Biomed. Struct. Dynamics, 19, 237-246.

[23] Rudd, P.M., Wormald, M.R., Wing, D.R., Prusiner, S.B. and Dwark, R.A. (2001) Biochemistry, 40, 3759-3766.

[24] Choi, C.J., Kanthasamy, A., Anantharam, V. and Kanthasamy, A.G. (2006) Neurotoxicology, 27, 777-787.

[25] Gonzalez-Iglesias, R., Pajares, M.A., Ocal, C., Espinosa, J.C., Oesch, B. and Gasset, M. (2002) J. Mol. Biol., 319, 527-540.

[26] Hachiya, N.S., Imagawa, M. and Kaneko, K. (2007) Med. Hypotheses, 68, 670-673.

[27] Jin, T., Gu, Y., Zanussi, G., Sy, M., Kumar, A., Cohen, M., Gambetti, P. and Singh, N. (2000) J. Biol. Chem., 275, 38699-38704.

[28] Riek, R., Homemann, S., Wider, G., Billiet, M., Globischuber, R. and Wuthrich, K. (1996) Nature, 382, 190-192.

[29] Campagna, V., Samartaro, D. and Zurzolo, C. (2005) Trends Cell Biol., 15, 102-111.

[30] Broome, B.M. and Hecht, M.H. (2000) J. Biol. Chem., 296, 961-968.

[31] Govaerts, C., Wille, H., Prusiner, S.B. and Cohen, F.E. (2004) Proc. Natl. Acad. Sci. USA, 101, 8342-8347.

[32] Chen, S.G., Zou, W., Parchi, P. and Gambetti, P. (2000) Arch. Virol., 209-216.

[33] Hayashi, H.K., Yokoyama, T., Takata, M., Iwamaru, Y., Imamura, M., Ushiki, Y.K. and Shinagawa, M. (2005) Biochim. Biophys. Res. Commun., 328, 1024-1027.

[34] Borchelt, D.R., Taraboulos, A. and Prusiner, S.B. (1992) J. Biol. Chem., 267, 1689-1695.

[35] Studier, J.R., Raymond, G.J., Hughes, A.G., Race, R.E., Hayashi, H.K., Yokoyama, T., Takata, M., Iwamaru, Y., Silveira, J.R., Raymond, G.J., Hughson, A.G., Race, R.E., DeArmond, S.J. (1989) J. Biol. Chem., 264, 777-787.

[36] Wopfner, F., Weidenhofer, G., Schneider, R., von Brunn, A., Gilch, S., Schwarz, T.F., Werner, T. and Schatzl, H.M. (1999) J. Mol. Biol., 289, 1163-1178.

[37] Kovacs, G.G., Laszlo, L., Bakos, A., Marinovits, J., Bishop, M.T., Strobel, T., Vajna, B., Mitrova, E. and Majtenyi, K. (2005) Neurology, 65, 1666-1669.

[38] Palmer, M.S., Dryden, A.J., Hughes, J.T. and Collinge, J. (1991) Nature, 352, 340-342.

[39] Collinge, J. (2001) Ann. Rev. Neurosci., 24, 519-550.

[40] Baker, H.E., Poultier, M., Crow, T.J., Frith, C.D., Lofthouse, R. and Ridley, R.M. (1991) Lancet, 337, 1286.

[41] Borchelt, D.R., Taraboulos, A. and Prusiner, S.B. (1992) J. Biol. Chem., 277, 237-246.

[42] Sunde, M., Serpell, L.C., Bartlam, M., Fraser, P.E., Pepys, M.B. and Blake, C.C. (1997) J. Mol. Biol., 273, 729-739.

[43] Santini, S. and Derreumaux, P. (2004) Macromol. Biosci., 4, 183-188.

[44] Fandrich, M., Fletcher, M.A. and Dobson, C.M. (2001) Nature, 410, 507-511.

[45] Sunde, M., Serpell, L.C., Bartlam, M., Fraser, P.E., Pepys, M.B. and Blake, C.C. (1997) J. Mol. Biol., 273, 729-739.

[46] Chesebro, B. (2004) J. Biol. Chem., 279, 2379-2389.

[47] Bascak, K., Ziegler, J., Sticht, H., Marx, U.C., Muller, W. and Rosch, P. (2001) J. Mol. Biol., 316, 2581-2589.

[48] Rezaei, H., Choiset, Y., Eghiaian, F., Treguer, E., Mentre, P., Debey, P., Groslaude, J. and Haertle, T. (2002) J. Mol. Biol., 322, 799-814.

[49] Hornemann, S., Schorn, C. and Wuthrich, K. (2004) J. Vet. Med. B, 51, 279-286.

[50] Hornemann, S., Schorn, C. and Wuthrich, K. (2004) Proc. Natl. Acad. Sci. USA, 101, 11667-11671.

[51] Barrow, C.J., Yasuda, A., Kenny, P.T. and Zagorski, M.G. (1992) J. Mol. Biol., 225, 1075-1093.

[52] Kuznetsov, I.V., Aagaard, C., Menti, P., Wider, G., Billiet, M., Homemann, S., Globischuber, R. and Wuthrich, K. (1998) Proc. Natl. Acad. Sci. USA, 95, 11667-11672.

[53] Calzolai, L., Lysek, D.A., Perez, D.R., Guntert, P. and Wuthrich, K. (2005) J. Mol. Biol., 343, 3869-3877.

[54] Watzlawik, J., Skora, L., Frense, D., Griesinger, C., Zweckstetter, M., Schulz-Schaeffer, W.J. and Kramer, M.L. (2003) J. Biol. Chem., 278, 26445-26450.

[55] Chesebro, B. and Caughey, B. (2001) Trends Biochem. Sci., 26, 323-444.

[56] Cornaglia, S., Schorn, C. and Wuthrich, K. (2004) J. Mol. Biol., 343, 3869-3877.

[57] Chesebro, B. and Caughey, B. (2001) J. Biol. Chem., 276, 5108-5113.

[58] Bascak, K.I.V., Aagaard, C., Menti, P., Wider, G., Billiet, M., Homemann, S., Globischuber, R. and Wuthrich, K. (1998) Proc. Natl. Acad. Sci. USA, 95, 11667-11672.

[59] Barrow, C.J., Yasuda, A., Kenny, P.T. and Zagorski, M.G. (1992) J. Mol. Biol., 225, 1075-1093.

[60] Kuznetsov, I.V., Aagaard, C., Menti, P., Wider, G., Billiet, M., Homemann, S., Globischuber, R. and Wuthrich, K. (1998) Proc. Natl. Acad. Sci. USA, 95, 11667-11672.

[61] Barrow, C.J., Yasuda, A., Kenny, P.T. and Zagorski, M.G. (1992) J. Mol. Biol., 225, 1075-1093.

[62] Kuznetsov, I.B. and Rackovsky, S. (2004) Protein Sci., 13, 3230-3244.

[63] Kuznetsov, I.V., Legname, G., Baldwin, M.A., Prusiner, S.B. and Cohen, F.E. (2002) J. Biol. Chem., 277, 21140-21148.

[64] Kuznetsov, I.B. and Rackovsky, S. (2004) Proc. Natl. Acad. Sci. USA, 101, 514-526.
