Structure-function aspects of prion proteins

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Prions diseases are fatal neurodegenerative disorders resulting from conformational changes in the prion protein from the normal cellular form, PrP^{C}, to the infectious scrapie isoform, PrP^{Sc}. High resolution structures for PrP^{C} are now available, and biochemical investigations are shedding light on the nature and determinants of the conformational transition. Together, these studies are beginning to provide a framework to describe structure-function relationships of the prion protein.

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Abbreviations

CJD	Creutzfeldt-Jakob disease
GPI	glycophosphatidyl inositol
PrP	prion protein
PrPC	cellular prion protein
PrP^{Sc}	scrapie prion protein

Introduction

For most globular proteins, chain folding results in a structure with a stable conformation. When globular proteins fold incorrectly or undergo a conformational change as a result of an amino acid mutation, chemical modification, change in the environment, or other unknown factors, the proteins often are degraded or they can aggregate and form amyloid plaques. Characterization of the conformational properties of peptides and proteins at the atomic level is challenging using experimental means and becomes particularly difficult when the process involves aggregation. For example, whereas one may be able to investigate the structure of a peptide or protein in solution under a particular set of conditions, changes in the conditions necessary to elicit a conformational change relevant to amyloidosis generally lead to aggregation and insolubility, such that different experimental techniques must be employed to study the two endpoints. This is difficult enough, but it is next to impossible to characterize the conformational transitions between these endpoints. Add to this the fact that the biological function is unknown, so that a biochemical assay for activity — the hallmark of the native state — is unavailable. Study of the prion protein (PrP) suffers from such issues.

Prions are transmissible pathogens that cause rare and fatal neurodegenerative diseases of the central nervous system in humans and animals [1,2]. In humans, prion diseases fall into four classes: kuru; Creutzfeldt-Jakob disease (CJD); Gerstmann-Sträussler-Scheinker disease; and fatal familial insomnia [3]. These diseases can be sporadic, inherited, or infectious (or iatrogenic) disorders. The inadvertent transmission of CJD to approximately 50 individuals receiving human growth hormone derived from cadavers and a few cases from human gonadotropin [4] provides strong support for biotechnology in general and the recombinant production of protein therapeutics in particular. In addition, the study of prion diseases has taken on new urgency given the presumed transmission of a bovine prion disease, Bovine Spongiform Encephalopathy, to humans leading to a new variant of CJD [5–8].

Prion diseases differ from other infectious diseases in that the pathogen is a proteinaceous particle, and the essential component of prions is the scrapie prion protein (PrP^{Sc}). PrP^{Sc} is chemically indistinguishable from the normal cellular prion protein (PrP^{C}) [9]; however, their secondary and tertiary structures differ [10,11]. The biological function of PrP^{C} is not precisely known but its expression is highest in neuronal cells [12]. It is a sialoglycoprotein bound to the plasma membrane of cells by a glycophosphatidyl inositol (GPI) anchor. PrP^{Sc} generally, though not in all cases, accumulates in the extracellular space as amyloid plaques in the brains of humans and animals with prion diseases [3].

Difficulties with the purification of PrP, as well as its instability and proclivity to aggregate, have prevented the use of high-resolution techniques for structure determination of full-length PrP^{C} and PrP^{Sc} until very recently. Fourier transform infrared and circular dichroism spectroscopy studies indicate that PrP^{C} is highly helical (42%) with virtually no β-sheet structure (3%), and, in contrast, PrP^{Sc} contains a large amount of β-sheet structure (43%) and less helical structure (30%) [10,11,13,14]. These results, together with those of structure prediction and peptide studies [11,15–17] led to the suggestion that a conversion of α-helices to β-sheets may occur upon formation of PrP^{Sc} from PrP^{C}. While we do not yet have detailed and unambiguous structures for PrP^{C} and PrP^{Sc}, three NMR structures of PrP^{C} have appeared in the past year [18,19••,20].

Given the lack of concrete structural information for PrP^{Sc}, and to some extent PrP^{C} as well as any intermediates between them, and the fact that the biological function of the protein is unknown, a detailed, conventional account of structure–function aspects of prion proteins is not possible. This is a fascinating system, however, and if we broaden the scope to include pathological processes, the structural information now available from NMR and other biochemical studies provides some clues regarding the conversion process, species barriers, strain differences, and possible therapeutic and diagnostic agents for prions. This

review focuses on the advances in these areas over the past year. Given space constraints, the reader is referred to the following recent reviews for discussion of papers prior to 1997 and material that falls outside of the scope of this review [1–3,21,22••,23–25].

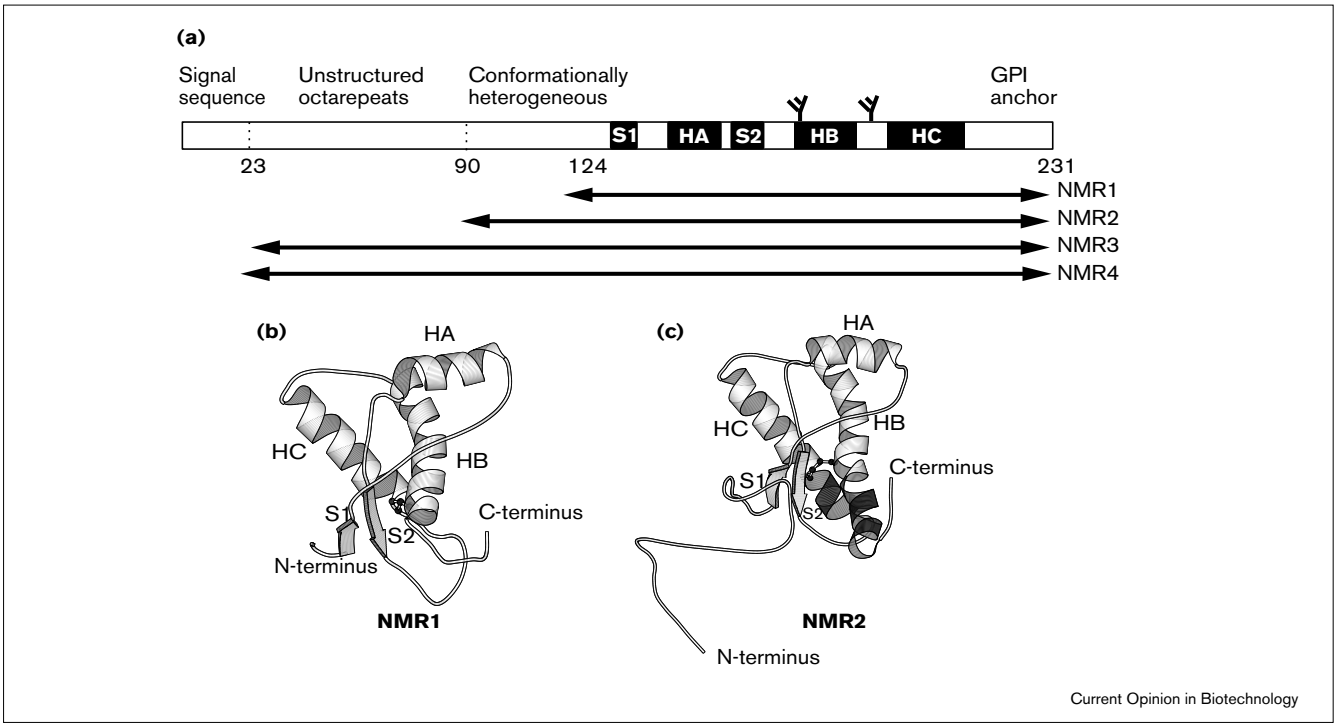
The structure of PrP^C

The conformational plasticity of the prion protein has been demonstrated by a variety of techniques for both fragments and the full-length protein. Zhang *et al.* [26] have focused on a 142-residue fragment of the Syrian hamster sequence, PrP(90–231), corresponding to the whole of the protein apart from the signal sequence and octarepeat region, which includes all residues involved in the conversion of $PrP^C \rightarrow PrP^{Sc}$ (Figure 1). This protein adopts two different states rich in α-helical structure. One is found between pH 5–8 and displays NMR chemical shift dispersion and a cooperative thermal unfolding transition consistent with an ordered native state. In contrast, the other helical conformation found at pH 2 has the characteristics of a molten globule or other partially folded state. At elevated temperatures a more stable β-sheet rich state is formed. Similar results were obtained by Swietnicki *et al.* [27] for the same fragment of the human sequence, which undergoes the pH-dependent conformational change in the region of pH 4.4–6. The ΔG° for unfolding at pH 5 is 2.4 kcal $mole^{-1}$ [27] and the helical content is estimated to drop by approximately 5–6 residues based on a change in the ellipticity measured by circular dichroism of 1000 deg cm^2 $dmol^{-1}$ compared to pH 7.2 [26].

Although prior to 1997, the NMR structure determined by Wuthrich and co-workers [28] for the murine PrP fragment containing residues 121–231 (Figure 1, NMR1), PrP(121–231), represented a tremendous advance in the area of prion research. At the time, the structure was rightly criticized as being potentially irrelevant by virtue of being a fragment, as previous studies demonstrate that smaller fragments of PrP adopt a variety of conformations depending on solution conditions. The new structures reported in 1997, however, confirm the overall structural features of the PrP(121–231) fragment and support the contention that these residues constitute an autonomous folding unit. One of the newer structures is of Syrian hamster PrP(90–231), NMR2 [19••]. The other two structures are full-length versions of these two fragments, comprising all residues including the amino-terminal octarepeats [18,20]. The important structural attributes of the fragments are described below, followed by a discussion of the full-length proteins.

The two primary PrP fragments upon which the structures of the full-length proteins are based are given in Figure 1. The overall folds of the two average NMR structures are

Figure 1



Structural features of PrP^C. **(a)** Secondary structure and other structural attributes of the prion protein are labeled along the sequence. The branched symbols in helix B (HB) and the neighboring turn represent the N-linked glycosylation sites. The various fragments used for the NMR studies are indicated by the arrows: NMR1 [28], NMR2 [29], NMR3 [18], and NMR4 [20]. Ribbon traces of the NMR1 and NMR2 structures are given in **(b)** and **(c)**, respectively. The dark portions of the helices in NMR2 correspond to extensions relative to NMR1. The molecular images were created using Molscript [57].

similar, as would be expected for sequences exhibiting >90% homology. They contain three well-resolved helices and a short two-stranded β-sheet. Differences are evident: helix B is six residues longer and helix C is 10 residues longer in the PrP(90–231) structure (Figure 1). This widespread increase in helical content is consistent with the pH-dependent conformational behavior described above; the PrP(121–231) mouse structure was determined at pH 4.5 in water [28] and the structure of hamster PrP(90–231) was at pH 5.0 in acetate buffer [19••]. In addition, however, the differences could be caused by the differences in the sequence or the lengths of the fragments. The short and slightly variable β-sheet is similar in the two structures. In contrast, the loop between S2 and helix B (Figure 1) is disordered in the smaller fragment but gives rise to many medium- and long-range NOEs in the PrP(90–231). So, overall the structures are very similar and would appear to be part of a continuum, with the James *et al.* PrP(90–231) [19••] conformation being more structured and presumably closer to the physiological form of PrP^C.

An obvious difference between the two fragments is the amino-terminal extension on the James *et al.* [19••] structure (Figure 1). This region is particularly interesting because it is implicated in the conversion of $PrP^C \rightarrow PrP^{Sc}$. It is conformationally heterogeneous and overall disordered by NMR, but note that both of these fragments were studied at a pH falling within the pH-dependent conformational transition described above and also that the protein undergoes rapid interconversion between a weak dimer and monomer [19••]. As such, even the longer fragment may not be entirely representative of the conformation under physiological conditions. Part of this region (residues 109–121 [H1]) was predicted to be helical [15] and it does indeed form a helix under different solvent conditions [15,17,29]. Furthermore, the last four residues of the predicted H1 helix display some protection to isotopic exchange (H/D) with solvent and the α-proton and α-carbon chemical shifts for residues 90–127 are consistent with weak α-helical content. There are nine nonsequential NOEs in this region, implying that some structure exists at least transiently; however, this is in marked contrast to the other three well-ordered helices. In any case, the work from James' group provides some evidence for weak helical structure in this region of the protein and structural information for the protein at higher pH is needed.

Both NMR groups have followed up on their fragment studies with experiments on the full-length PrP protein, PrP(23–231) or PrP(29–231) [18,20]. In essence, these investigations have confirmed the previous studies: the core of the molecule comprised of the three helices and a short β-sheet is retained and the rest of the molecule is disordered and/or highly mobile. New structures, *per se*, were not determined and the flexible amino-terminus is represented schematically on the framework provided by the fragment studies. While these studies have provided little new structural data, they are important controls as one must always question the relevance of data obtained from fragments. The most striking new result of these studies is the marked difference in flexibility and dynamic behavior between the amino-terminus and the folded core of the molecule, as probed by the heteronuclear ^{15}N-^{1}H NOE and described by the related ^{15}N-^{1}H correlation time. These studies conclusively demonstrate the plasticity of the amino-terminal region of the prion protein.

Function of PrP^C

It has been difficult to pinpoint the biological function of PrP^C and studies of knockout mice have been inconclusive or at odds (see [30] and references therein). Currently, the most promising hypothesis is that PrP^C binds copper and is involved in copper transport and metabolism [31]. This suggestion is intriguing given that the copper-binding proteins superoxide dismutase, monoamine oxidase, and the amyloid precursor protein have been implicated in the pathogenesis of familial amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease, respectively. Recent studies by Miura *et al.* [32] indicate that the isolated unstructured octarepeat region of PrP^C (residues 23–90 with the repeating sequence PHGGGWGQ; single letter amino acid code) forms a helical structure upon binding copper.

A 32-residue peptide with the octarepeat sequence promotes the survival of PrP knockout cerebellar cells, which are sensitive to copper toxicity and oxidative stress [33]. Furthermore, *in vivo* studies by Brown *et al.* [34••] indicate that wild-type mouse brains have a much higher level of membrane-associated copper than PrP-deficient mice, which have a correspondingly higher concentration of serum copper. Cleavage with phosphatidylinositol-specific phospholipase C to remove PrP^C from the cell surface showed that the copper was localized to PrP^C, or possibly some other protein with a GPI anchor. These findings and others presented by Brown *et al.* [34••] suggest that PrP^C is involved in regulation of copper levels as a 'sink' or shuttle to intracellular compartments. Neurons are sensitive to perturbations in copper content and in this regard it is interesting that PrP knockout mice have brains with reduced activity of copper-dependent superoxide dismutase [35]. Structural information for PrP^C with bound copper is needed to explore further structure-activity relationships.

Conversion of $PrP^C \rightarrow PrP^{Sc}$

Various low resolution structural methods provide evidence for a conformational change linking PrP^C and PrP^{Sc}, but localization of the change along the sequence has proved difficult. Peretz *et al.* [36••] have recently produced antibodies to the amino-terminal region (residues 90–120) that recognize PrP^C but do not bind PrP^{Sc}. In contrast, an epitope at the carboxy-terminus is accessible in both forms. The authors argue that the major conformational change occurs at the amino-terminus, which is consistent with the plasticity of this region. But, it remains

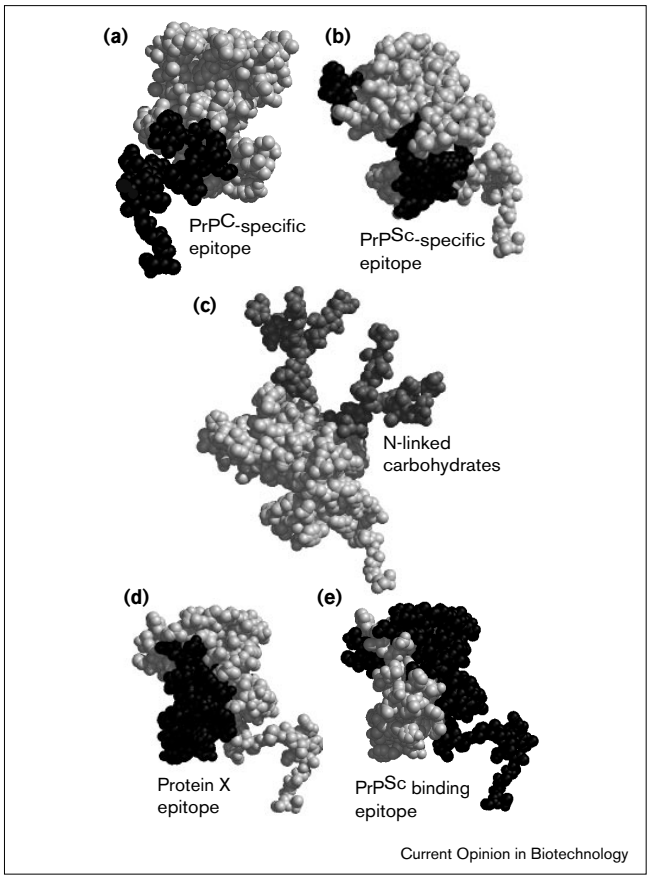
a possibility that the amino-terminal epitope does not change conformation and instead no longer binds because the surface is unavailable in the aggregate due to intermolecular packing in that region. It is unlikely, however, that PrP^{C} would retain a disordered amino-terminus in PrP^{Sc} as even amorphous aggregates typically display specific packing arrangements [37,38•]. PrP-directed antibody studies have recently produced another exciting finding — a PrP^{Sc} specific antibody [39••]. This antibody recognizes a discontinuous epitope, which could form a continuous surface following a conformational transition or upon complexation (Figure 2). This antibody works across species lines, opening up the possibility of a diagnostic test to detect PrP^{Sc} in humans, blood products, food products, animals, and so on.

Thus far, PrP^{C} and PrP^{Sc} have been considered bare proteins, but much work is being performed to elucidate the importance of the carbohydrate groups and the GPI anchor. For example, Lehmann and Harris [40] have shown that the N-linked oligosaccharide chains protect PrPC from conversion to PrP^{Sc}. It is not yet clear whether this is a direct effect or merely due to the added bulk of the sugars (Figure 2), which can decrease flexibility, increase stability, and deter aggregation in other systems [41].

Previous studies have shown that the formation of PrP^{Sc} is a post-translational process occurring after PrP^{C} reaches the plasma membrane. Kaneko *et al.* [42] have now demonstrated that efficient production of PrP^{Sc} is associated with GPI linked-targeting to cholesterol-rich caveolae-like domains. Chimeric constructs to replace the GPI anchor with different carboxy-terminal transmembrane domains prevent the formation of PrP^{Sc}. Daude *et al.* [43], however, have found that the conversion process can occur earlier in the endoplasmic reticulum for mutant forms of PrP. Information about the processing and trafficking of PrP takes on special importance in the case of some heritable prion diseases in which neurodegeneration occurs in the absence of PrP^{Sc} accumulation.

Hegde *et al.* [44••] have characterized a particular transmembrane form of PrP that confers severe neurodegeneration in mice with the characteristics of prion diseases but without amyloidosis. The transmembrane form spans the membrane near the amino-terminus (approximately residues 110–135). This transmembrane form was also found in the brain of a patient afflicted with Gerstmann–Straüssler–Scheinker (Ala117→Val mutation), where plaques and protease-resistant PrP^{Sc} could not be detected. Furthermore, studies of small amino-terminal peptides by Pillot *et al.* [45] indicate that a peptide comprised of residues 118–135 induces liposome fusion and the maximum effect is obtained when it is α-helical. Thus, neurotoxicity in these systems may be due to destabilization of cell membranes. These results and a variety of others presented by Hegde *et al.* [44••] suggest that prion diseases are heterogeneous and that some familial prion diseases displaying neurodegeneration in the absence of plaques may be due to expression of the transmembrane form of the protein.

Figure 2



Structural features of the surface of PrP^{C}. In all cases NMR2 is displayed. **(a)** The amino-terminal region representing a PrP^{C}-specific antibody binding site [36••] is highlighted (residues 90–120). **(b)** The discontinuous PrP^{Sc}-specific antibody binding site [39] is displayed (residues 142–148, 162–170, 214–226). **(c)** N-linked carbohydrates at Asn181 and Asn197 were modeled to correspond to the predominant defucosylated oligosaccharide present in PrP^{Sc} reported by Endo *et al.* [55] (Alonso and Daggett, unpublished data). **(d)** The protein X binding site is highlighted, residues 160–180 and 205–231. **(e)** The PrP^{Sc} binding site is believed to lie within residues 90–144, 180–205. The figures were created using UCSF MidasPlus [58].

Auxiliary factors in the conversion of $PrP^{C} \rightarrow PrP^{Sc}$

Cell-free conversion of $PrP^{C} \rightarrow PrP^{Sc}$ occurs but the efficiency is relatively low [24,46]. Therefore, there has been much speculation about co-factors or chaperones that may facilitate the process. Studies of the transmission of human prions to transgenic mice led Telling *et al.* [47] to postulate that another factor, 'protein X', modulates the interactions between PrP^{C} and PrP^{Sc} in the conversion of PrP^{C}, possibly near the carboxy-terminus. Experiments by Kaneko *et al.* [48] suggest that protein X binds to a discontinuous epitope of PrP^{C} (residues 160–180 and 205–231) (Figure 2). PrP^{Sc}, on the other hand, appears to recognize

another nearby discontinuous epitope consisting of residues 90–144 and 180–205 [20] (Figure 2).

Although these studies suggest that a protein or chemical chaperone is involved in the conversion, a definitive chaperone has not been identified. DebBurman *et al.* [49••] have recently tested the efficiency of *in vitro* conversion of $PrP^{C} \rightarrow PrP^{Sc}$ in the presence of known chaperones. In particular, GroEL promoted the conversion but only in the presence of PrP^{Sc}. Also, the conversion was more efficient in the absence of the N-linked carbohydrates, as discussed above this study provides the first evidence that molecular chaperones can regulate conformational transitions in PrP.

Species barriers and strain differences

Billeter *et al.* [50] have investigated the structural basis of the species barrier for transmission of prion diseases. Mapping the variability of 23 mammalian PrP sequences onto the NMR structure of PrP(121–231) results in three distinct clusters. One of these is the protein X binding region (Figure 2). Another cluster is near the region thought to be involved in the binding of PrP^{Sc}. This surface, in particular, appears to have the potential to discriminate between different PrP^{Sc} variants through orientationally-specific electrostatic interactions (Figure 2). The third cluster is in the core of PrP^{C} and presumably only accessible to other proteins after conversion. Similar residues have been identified by Scott *et al.* [51•], but the clustering is a bit different. More importantly, Scott *et al.* [51•] describe the development of transgenic mice susceptible to bovine prions, which may lead to a bioassay for Bovine Spongiform Encephalopathy. Along related lines, Bossers *et al.* [52] have shown that PrP polymorphisms in sheep associated with scrapie susceptibility modulate *in vitro* conversion and reproduce the expected effects on efficiency of conversion.

Another peculiarity of prion disease is strain differences in the absence of nucleic acid. This has been explained in terms of multiple conformations of PrP^{Sc}. In this model, PrP^{Sc} serves as a template for conversion such that conformational diversity leads to different forms of endogenous, newly-converted PrP^{Sc} [47]. However, the strain behavior is difficult to explain entirely by a phenomenon involving protein–protein interactions or multiple conformations of PrP^{Sc}, although there is evidence supporting this idea [46–47,53,54]. The carbohydrates may add another level of complexity to increase the diversity of PrP^{Sc}, as it is estimated that 401 different PrP glycoforms are possible [55]. PrP^{Sc} molecules from different prion strains differ in size and state of glycosylation [9]. Recent studies by DeArmond *et al.* [56•] show that different regions of the brain synthesize their own unique sets of PrP^{C} glycoforms and that homologous regions in hamsters and mice express animal species-specific PrP^{C} glycoforms. The carbohydrate groups might influence PrP^{Sc}–PrP^{C} interactions, and thus replication of PrP^{Sc}. It may be that carbohydrates, either through direct interactions involving protein recognition events or through modulation of the structure, dynamics and stability of PrP, or in other words by creation of different folded conformations or different recognition surfaces, are also involved.

Conclusions

Results from biochemical and structural studies of the prion protein in the past year are beginning to shed light on the biological function of PrP^{C}, the $PrP^{C} \rightarrow PrP^{Sc}$ conformational transition and factors that modulate the process, and the nature of strain differences and species barriers. There are still many more questions than answers, but the necessary framework for obtaining a detailed picture of structure-function relationships of the prion protein is beginning to emerge. If progress continues in this way, diagnostic and therapeutic agents for prion diseases should become a reality.

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