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TOWARD A MECHANISM OF PRION MISFOLDING AND STRUCTURAL MODELS OF PrP^{Sc}: CURRENT KNOWLEDGE AND FUTURE DIRECTIONS

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Despite extensive investigation, many features of prion protein misfolding remain enigmatic. Physicochemical variables known to influence misfolding are reviewed to help elucidate the mechanism of prionogenesis and identify salient features of PrP^{Sc}, the misfolded conformer of the prion protein. Prospective work on refinement of candidate PrP^{Sc} models based on thermodynamic considerations will help to complete atomic-scale structural details missing from experimental studies and may explain the basis for the templating activity of PrP^{Sc} in disease.

For more than a decade the protein-only hypothesis of prion replication has been the prevailing theory to explain the propagation of the transmissible spongiform encephalopathies. This hypothesis asserts that a pathologic conformational variant of the prion protein, denoted PrP^{Sc}, induces endogenous natively folded prion protein, PrP^{C}, to misfold and itself become PrP^{Sc} by modification of secondary and tertiary structure in a process of template-directed misfolding. Until recently, only a handful of other proteins, mainly from yeast, demonstrated a capability like that of PrP^{Sc} to catalyze the misfolding of a protein with the same primary sequence as itself, but a growing body of evidence implicates a prion-like misfolding mechanism for many proteins involved in human neurodegenerative diseases, including tau in Alzheimer's and other tauopathies (Clavaguera et al., 2009), α-synuclein in Parkinson's disease (Desplats et al., 2009), and superoxide dismutase 1 in familial amyotrophic lateral sclerosis (L. Grad et al., personal communication). This suggests that template-directed misfolding may be a central event in the pathogenesis of many central nervous system (CNS) diseases. Unraveling the mystery of PrP^{Sc} formation and activity is therefore potentially of large consequence to human health, beyond its direct application to the (relatively rare) prion diseases. The purpose of this study is to identify physical and chemical variables that are known to influence formation of PrP^{Sc} and therefore must be considered in a comprehensive theory of prionogenesis.

At least one aspect of the $PrP^{C} \rightarrow PrP^{Sc}$ conversion reaction is understood in detail: the structure of the natively folded PrP^{C}. This structure represents a starting point for misfolding as it is the substrate on which PrP^{Sc} acts and has the same primary sequence; indeed, it is possible that some elements of the structure of PrP^{C} may not participate in the reorganization that takes place during misfolding and are conserved in PrP^{Sc}. To date, the structure of PrP^{C} has been solved by nuclear magnetic resonance (NMR) or x-ray crystallography for more than 20 different species and mutants of PrP^{C} (Lysek et al., 2005; Gossert et al., 2005; Knaus et al., 2001), all showing a highly conserved fold: of the full-length sequence comprising residues 23 to 230, the C-terminal residues from 125 to

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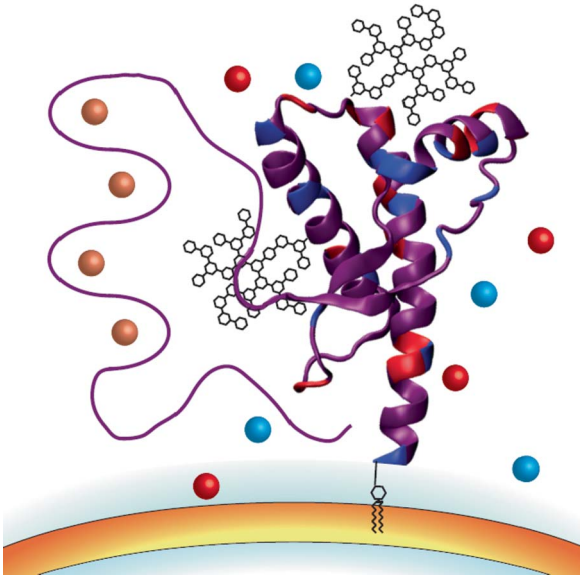

FIGURE 1. Schematic of some agents relevant to the prion misfolding process, including lipid membrane (at bottom), solution counterions (spheres surrounding the protein), the N-terminal unstructured domain (at left), copper ions (spheres associated with the octapeptide repeats in the N-terminal domain), and glycans.

230 possess a globular fold that contains a small antiparallel β-sheet and three α-helices, while the N-terminus does not have a well-defined secondary structure (Figure 1). As the prion protein departs from the PrP^{C} structure during misfolding, however, its geometry becomes increasingly ambiguous. Studies of the misfolding reaction in a diverse range of circumstances revealed the importance of solution conditions and other biomolecules, each of which provides some insight into the mechanism of PrP^{Sc} production.

pH AND ACIDITY

It has been known for some time that acidic conditions facilitate prion misfolding. The effect of an acidic environment is to change the protonation state of ionizable side chains, increasing the net positive charge on PrP and changing the network of salt bridges that help to hold secondary structural elements of the protein together in the native fold. This may lower the energetic barrier to internal reconfiguration of the protein necessary as part of misfolding. DeMarco and Daggett (2005) provide a more extensive discussion of the role of acidity in prion formation.

SALT CONCENTRATION

The presence of solution counterions is necessary for efficient conversion to the misfolded form. As the concentration of salts rises, the Debye length for screening of electric fields in solution decreases, so that like charges can be brought into closer proximity while incurring a lesser energetic cost. Since the prion protein carries a large positive charge (+11) at neutral pH, the cost of putting two PrP molecules in spatial contact is high in the absence of screening by solution counterions. The Debye length, a measure of the effective range of electric fields in a solution due to screening by charged solutes, is approximately 8 Å at an ionic strength of 150 m*M*. This attenuates the repulsion between distant like charges in the protein while nonetheless projecting an electric field into the nearby space that may mediate the initial recognition of PrP^{C} by PrP^{Sc}, drawing the molecules together so that other shorter range effects like van der Waals forces may augment the interaction. Anionic substances like pentosan polysulfate demonstrated in vitro and in vivo efficacy at reducing the rate of misfolding (Doh-ura et al., 2004). Interestingly, when recombinant human PrP is denatured with urea in a salt-free environment, the protein merely unfolds; in the presence of urea and even low concentrations of NaCl (50 m*M*), oligomerization, and an increase in β-sheet content are observed (Morillas et al., 2001). This supports the conclusion that screening of electrostatic interactions between PrP molecules is a prerequisite to conversion.

LIPID ASSOCIATION

PrP^{C} is held to the outer leaflet of the plasma membrane by a glycophosphatidylinositol (GPI) anchor at its C-terminus in the physiologic state, but Stewart and Harris

(2003) showed that a novel transmembrane form of PrP can arise in which residues 111 to 134 span the plasma membrane, and residues 34 to 94 are able to anchor PrP^{C} to synthetic sphingolipid-cholesterol-rich raft-like liposomes even without the GPI anchor (Baron & Caughey, 2003). Interaction with anionic lipid membranes was shown to induce β-sheet structure and promote aggregation (Sanghera et al., 2009). Lipids were also found associated with PrP^{Sc} in samples purified from infected hamster brain (Riesner, 2003). A general feature of protein amyloid is an increased exposure of hydrophobic groups that are sequestered in the protein interior in the native state, so the presence of lipids in PrP^{Sc} may help to lessen the energetic unfavorability of surface hydrophobic groups. It was postulated that the thermostability of PrP^{Sc} is due to the strength of protein/lipid associations in the aggregate (Gale, 2007), but lipids (mainly sphingomyelin, α-hydroxycerebroside, and cholesterol) are believed to comprise only around 1% of the mass of purified prions (Klein et al., 1998); this would entail approximately one lipid molecule for every one or two PrP monomers. High titer, more infectious samples contained about 40 PrP to 1 lipid molecule. Nonetheless, lipids may serve a specific physical role in the conversion process, perhaps by shielding exposed hydrophobic regions of PrP. No current PrP^{Sc} models or conversion mechanisms explicitly consider the contribution of lipids, but their potential role in stabilizing regions of PrP^{Sc} or one of its intermediates is important to consider.

METAL IONS

PrP^{C} contains 4 octapeptide repeats from residues 56 to 87 that have an affinity for copper (Cu). This lies outside the protease K-resistant core of PrP^{Sc} but may nonetheless participate in its toxicity through free radical production, loss of a physiologic Cu-binding function, or oxidation of side chains in PrP (Colombo et al., 2009; Stahl et al., 1993; Cannello et al., 2008). An NMR structure of the octapeptide repeat from residues 61 to 84 bound to pentosan polysulfate was recently released showing a series of loosely defined loops with hydrophobic exposed tryptophans (Taubner et al., 2009). Interestingly, Cu may also influence the protease resistance of PrP^{Sc} molecules (Nishina et al., 2004), and PrP^{Sc} formed in a Cu-free environment was 20 times more susceptible to proteinase-K digestion; addition of Cu restored normal protease resistance.

GLYCANS

There are N-linked glycans attached to asparagines 181 and 197 of PrP^{C}, each with a molecular mass of approximately 5 kDa. During conversion glycans may present steric restrictions to the approach of PrP^{Sc}, as it is a bulky multimolecular aggregate, and may also reduce or eliminate certain structural transitions. Unglycosylated, monoglycosylated, and diglycosylated chains are detected in approximately equal quantity in PrP^{Sc}, although there is some variation in glycosylation patterns between different prion strains (Priola & Vorberg, 2002). Other sugar molecules separate from the glycans are found in scrapie aggregates and appear to contribute to the resilience of prion rods (Riesner, 2003). Thermodynamically, the presence of the sugars enhances the stability of protein regions near their attachment site by approximately 1 kcal/mol (unpublished calculations), primarily by reducing accessible states and thus the configurational entropy of the unfolded chain, but this may or may not be a significant effect of glycosylation. Glycans in other proteins were recently found to increase protein stability by up to 3 kcal/mol (Hanson et al., 2009). Molecular dynamics (MD) simulation of PrP^{C} with chitobiose glycosylation demonstrated a minimal effect on the protein's overall structure, but the absence of glycosylation did result in an observed rise in β-content (Zhong & Xie, 2009). Removal of PrP glycosylation by the mutations D181Q and D197Q in a human astrocytoma cell line resulted in an increased rate of apoptosis (Chen et al., 2007), but this is believed to be the result of a Bcl2-associated

mechanism rather than protein misfolding. The balance of current evidence is that glycans are not a central actor in misfolding but are useful in constraining models of PrP^{Sc}, which must accommodate the large volume of the glycans presumably by projecting them into solution.

PROTEIN–PROTEIN INTERACTIONS

It was postulated that another protein different from PrP^{C} or PrP^{Sc}, a so-called "Protein X," may facilitate unfolding of PrP^{C} and its subsequent conversion to PrP^{Sc}. Various roles were ascribed to Protein X, including physiologic endoproteolytic cleavage of PrP^{C} (Hachiya et al., 2007) or binding to a discontinuous epitope on the surface of PrP^{C} (Kaneko et al., 1997). However, in the absence of successful isolation of Protein X and increasing success in generating infectious prions from purified PrP^{C} (Deleault et al., 2005), it seems unlikely that an accessory protein is a necessary contributor to the misfolding process; indeed, it is possible that no such protein exists. The sufficiency of PrP^{C}, PrP^{Sc}, nonprotein biomolecules, and solvation conditions to account for prion misfolding simplifies substantially the considerations in establishing the mechanism of prionogenesis.

INTERACTIONS BETWEEN THE N- AND C-TERMINAL DOMAINS

Since only residues 125–230 adopt a folded conformation in PrP^{C} and residues 90–230 participate in the protease-resistant core of PrP^{Sc}, most attention has focused on structural transitions involving the C-terminal part of the protein. However, the N-terminal unstructured domain is capable of exerting a subtle but detectable influence on the C-terminal domain: Deletion of residues 34 to 99 resulted in formation of protease resistant PrP in an in vitro cell-free conversion assay with accessible protease cleavage sites at residues 130 and 157 (Lawson et al., 2004). Similarly truncated PrP was also observed in brain homogenates from mice exposed to mouse-passaged hamster scrapie, raising the possibility that the N-terminal domain of PrP is involved in the barrier to prion disease transmission between species (Lawson et al., 2004). Even in the native state, antibody binding to an epitope near the N terminus can promote loosening of the C-terminal structured domain (Li et al., 2009), supporting the notion of a significant if inconstant association between the N- and C-terminal domains of PrP^{C} that may modulate misfolding.

IMPLICATIONS FOR PrP^{Sc} STRUCTURE

Extensive characterization of PrP^{Sc} was undertaken in an attempt to understand the structural features that enable it to catalyze PrP^{C} misfolding, but despite this effort there remains considerable disagreement about even basic features of the PrP^{Sc} fold. As shown in Figure 2, several different structural models of PrP^{Sc} have been promulgated based on a range of theoretical and experimental data, the three most prominent of which are:

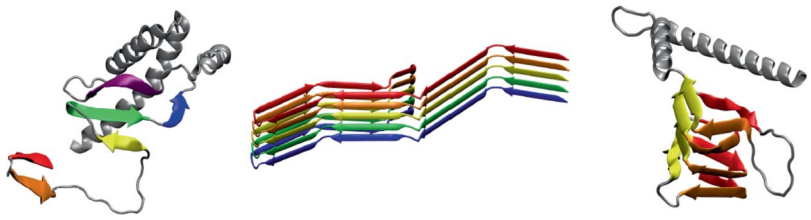

FIGURE 2. Three current models of PrP^{Sc}: extension of the native β-sheet (DeMarco & Daggett, 2007) (one monomer of residues 90–230 shown), a parallel in-register β-sheet (Cobb et al., 2007) (5 monomers of residues 160–220 shown), and a β-helix (Govaerts et al., 2004) (one monomer of residues 90–230 shown). The structures presented here comprise a small part of the total misfolded aggregate. Note the large qualitative differences between these models.

- A β-helix based on fiber diffraction and electron microscopy studies of brain-derived PrP^{Sc} (Wille et al., 2002, 2007; Govaerts et al., 2004).
- A parallel in-register β-sheet determined from spin labeling and electron paramagnetic resonance studies of recombinant misfolded PrP (Cobb et al., 2007).
- An extension of the native β-sheet from immunologic studies (Cohen, 1999) and molecular dynamics simulations at low pH (DeMarco & Daggett, 2007).

The lack of agreement on even basic features of the PrP^{Sc} fold may derive in part from an inherent heterogeneity in misfolding: Although there is one native structure for PrP^{C}, there may be multiple misfolded structures, each arising from a local energetic minimum in the conformation space of PrP. Environmental conditions, including the presence of PrP^{Sc} seeds of a particular subtype, and variation in the PrP primary sequence may steer PrP^{C} molecules into one of a range of misfolded geometries. Since each study described above employed a different methodology for generating PrP^{Sc}, it is possible that each one accessed a different stable misfolded conformer, some or all of which may be relevant in disease. This is supported by the well-known existence of strains in prion disease with distinct neuropathological features (Hill et al., 2000). In this sense the search for the *PrP^{Sc} structure*, while not futile, may yield a plurality of valid possibilities rather than a single definitive answer. Recent atomic-resolution structural studies of peptide amyloids (Nelson et al., 2005) and yeast prions (Krishnan & Lindquist, 2005) also provide collateral information that should be considered in formulating proposed PrP^{Sc} structures.

To date the experimentally derived PrP^{Sc} models have not been subject to a theoretical analysis of their thermodynamic stability, which aside from confirming their plausibility may help to refine important details like side-chain configurations and that are beyond the precision of the experimental evidence. Computational tools to separately calculate the enthalpic and entropic contributions to the free energy of a protein structure were previously developed (Guest et al., 2009; Guest et al., 2010) and applied to describe local unfolding of PrP^{C}, and use of the same techniques on models of PrP^{Sc} will identify regions of stability and instability. Moreover, reconciliation of these models with all available biochemical and immunological information on PrP^{Sc} has not been comprehensively attempted, so this body of data will be used to suggest modifications that may better account for all observed characteristics of PrP^{Sc}. A complete picture of PrP^{Sc} structure and function remains a distant goal, but a combination of empirical and theoretical techniques offers the best hope of unravelling this central mystery of neurodegenerative disease.

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