# Protease-Resistant Prion Protein Formation in Cell-Free Systems

**Aakshi Sati***

*Department of Life-science, Graphic Era University, India*

***Corresponding Author****

*Aakshi Sati*
*Department of Life-science, Graphic Era University, India*
*E-mail: Aakshi.kan7@gmail.com*



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## Abstract

The endogenous protease-sensitive prion protein (PrP-sen) of the host is changed to an aberrant pathogenic version with a characteristic partial protease resistance in transmissible spongiform encephalopathies (TSE) or prion disorders (PrP-res). PrP-res can directly trigger this conversion of PrP-sen, according to studies with cellfree reactions. This PrPres-induced conversion reaction is highly specific in ways that could account for TSE species barriers, polymorphism barriers, and strains at the molecular level. This reaction has been detected in TSEinfected brain slices as well as in mainly pure PrP-sen and PrP-res reactants. The binding of PrP-sen to polymeric PrP-res and a conformational shift that results in incorporation into the PrP-res polymer appear to be involved in the conversion pathway.

**Keywords:** Protease • Spongiform • Transmissible encephalopathies • Insoluble protein

## Introduction

In all transmissible spongiform encephalopathies, unusually protease-resistant prion protein (PrP-res) accumulates (TSE). PrPres resistance varies depending on the TSE strain and host species, but TSE-associated PrPres are far more resistant to proteinase K than the regular PrP isoform. The many aberrant TSE-associated variants of PrP-res (e.g. PrPSc, PrPCJD, and PrPBSE) form insoluble aggregates and have greater beta sheet content than PrP-sen, in addition to having increased protease resistance [1]. PrP-res generation is implicated in many forms of evidence as a crucial mechanism in TSE pathophysiology and TSE agent replication. PrP mutations appear to cause abnormal PrP-sen behaviour and spontaneous conversion to more proteaseresistant forms in the case of rare familial TSE disorders (see other chapters for review). As indicated by the 1 per million annual prevalence of sporadic CJD in humans, spontaneous conversion happens rarely, if at all, in hosts with wild type PrP-sen. The production of neurotoxic PrP-res from wild type PrP-sen after infection of hosts with TSE agents is far more common in animals. Studies using tissue culture cells and animals have revealed a great deal about the cell biology of PrP-res production and its relationship to TSE pathogenesis; these topics are covered in more detail elsewhere [2]. Studies in cell-free systems have allowed researchers to look at PrP-res production under considerably more controlled and defined conditions. In vitro studies like these have shed light on the mechanism of PrP-res generation, as well as the molecular underpinnings of TSE agent replication, strain propagation, and species barrier effects. Most notably, these investigations have demonstrated that, as previously anticipated, PrP-res can directly drive PrP-sen to PrP-res conversion via a mechanism so specific that it could theoretically account for the majority of TSE clinical symptoms.

### PrP-res formation via self-seeding

Most protein-only TSE infectious agent models propose that the putative infectious protein, PrP-res, interacts directly with its normal, host-encoded counterpart, PrP-sen, to convert it to PrP-res. It might then propagate in the host without the need for a nucleic acid specific to the drug [3-5]. The ability of PrP-res to convert PrP-sen to PrP-res (converting activity) was first proven by combining PrP-res purified from scrapie-infected brain tissue with immune precipitated 35S-PrP-sen and seeing that 35S-PrP-sen was changed into 35S-PrP-res. In the absence of PrPres, or in the presence of another form of amyloid (Alzheimer's beta), this conversion was not detected. In addition, in the reaction with PrP-res, additional tagged proteins were not transformed to PK-resistant versions. As a result, the conversion response is PrP-res dependent and PrP-specific. Because, although partial, reversible unfolding of PrP-res boosts conversion efficiency, more complete irreversible denaturation reduces converting activity, the converting activity is dependent on the specific conformational structure of PrP-res. Further research into the influence of denaturants on PrP-res converting activity has revealed that maintaining the native folding of a Cterminal domain (16 kDa in the aglycosyl structure) is critical for refolding and converting activity recovery after denaturant dilution. Denaturation of this important C-terminal region resulted in significant decreases in both converting activity and scrapie infectivity.

### Conversion vs. binding

PrP-res-induced PrP-sen to PKresistant PrP-sen conversion has now been observed in multiple laboratories [6]. However, the authors of one of the resulting papers preferred to refer to it as a binding phenomenon rather than conversion. In the conversion process, the PrP-sen precursor binds to the PrP-res aggregate, which we believe is an important aspect of the conversion mechanism. However, not all PrP-sen binding or aggregation culminates in the change to the PK-resistant state seen in PrP-res. Although PK completely digests PrP-sen, it only eliminates about 67 residues on average. The N-terminus of each monomeric unit of the PrPres aggregate forms Protease-Resistant Prion Protein in Cell-Free Systems, resulting in a 6-7 kDa downward shift in their apparent molecular weight in SDS-PAGE gels. This form of PK resistance is not attributable to nonspecific sequestration of full PrP molecules within aggregates that are not pierced by PK because virtually all of the PrP-res molecules are similarly exposed to PK and similarly truncated. When 35S-PrP-sen is incubated with high molar excesses of a synthetic PrP peptide fragment, nearly full-length PrP molecules remain following PK treatment, as has been found when 35S-PrP-sen is incubated with large molar excesses of a synthetic PrP peptide fragment [7]. The N-terminal residues of both PrP-res molecules and the 35S-PrP-res products of the conversion reaction are partially exposed, indicating that the monomers units were incorporated into highly organised polymeric structures like amyloid fibrils. PrPsen can thus not only attach to PrP-res, but also change it from a PK-sensitive to a partially PK-resistant state, as seen in TSE brain-derived PrP-res. Riesner and colleagues discovered that treating SDS-solubilized, alpha helical, and PKsensitive PrP27-30 with acetonitrile causes aggregation of PrP and an increase in its total PK-resistance and beta sheet content without restoring scrapie infectivity or fibrils [8]. This could be an example of a PrP aggregated and PK-resistant version that isn't PrPSc, as previously stated. They believe that our PrPres-induced conversion of 35S-PrP-sen to 35S-PrP-res could be explained by a similar seemingly nonspecific aggregation mechanism. However, there is a significant difference between their observation and ours.

### TSE strains with self-propagating PrP-res conformations as a possible basis

Species tropism, incubation duration, clinical illness, neuropathological symptoms, and PrP-res distribution in brain tissue can all be used to differentiate TSE agent strains. TSE strains have been found in isogenic hosts in large numbers. This result presents an interesting challenge to the protein-only concept for infectious agents: it necessitates that the "inheritance" or propagation of agent strain differences be mediated by stable variations in PrP-res structure rather than mutations in an agent-specific nucleic acid. Different TSE strains have been linked to structural variations in PrP-res [9]. The various types of PrP-res associated with the

hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy are particularly noteworthy (TME). PK cleaves these PrP-res forms differently, despite the fact that they are both generated from Syrian hamster PrP. This indicates that they differ in conformation rather than covalent structure, which FTIR research has validated. Furthermore, when incubated with hamster PrP-sen molecules, HY and DY PrP-res faithfully stimulate the synthesis of strain-specific PrP-res conversion products, propagating themselves in a nongenetic manner. These findings were the first to show that strain-specific PrP-res polymers with the same amino acid sequence but distinct 3-D structures or conformations can self-propagate. This is consistent with the idea that PrP-res polymer self-propagation represents a molecular underpinning for scrapie strains. A recent study found that injecting agents generated from different forms of familial CJD into mice resulted in the accumulation of PrP-res with seemingly unique conformations, supporting this theory [10].

# Conclusion

At this time, it's reasonable to pose a few key questions: Is protease resistance acquired by PrP owing to conformational change, polymerization, or both? And, in terms of TSE pathogenesis and transmission, which feature of aberrant PrP is most important? Is PrP-res the agent of transmission? Is it true that the most pathogenic and neurotoxic forms of PrP are also the most transmissible (assuming that any form of PrP is transmissible in and of itself)? Finally, is the sequence of events and rate-limiting processes in the presumed spontaneous production of wild type PrP-res from mutant PrP-sen in familial TSE disorders the same as the induced formation of wild type PrP-res following TSE infection? The ultimate answers to these concerns aren't evident yet, but there are a few key aspects to consider. Although the characteristic partial PK-resistance of PrP-res appears to genuinely reflect a specific aberrant conformational and/or aggregation state that varies from PrP-sen in the majority of cases, this feature is not shared by all disease-associated variants of PrP. Because mammals lack PK, there's no reason to believe that all forms of pathogenic PrP must be PK-resistant in order to cause disease. However, PrP-res' survival as a possible transmissible agent and accumulation as a pathogenic material in the host would presumably be aided by some form of global proteolysis resistance. Even overexpression of wild type PrP-sen can cause neurological illness, implying that pathogenic (but not necessarily transmissible) PrP accumulations can be achieved without PK-resistance in some cases. Because no one has clearly documented the existence of a monomeric form of PrP that is both rich in beta sheet and PK-resistant, the only conclusion that can be drawn is that all PrP-res forms that have been adequately well characterised are both high in beta sheet and multimeric. Because conformational change and polymerization cannot be reliably separated in time, it is impossible to say which parameter is most important in TSE pathogenesis and transmission. It's important to remember that not all PrP aggregates are PK-resistant, high in beta sheet, and related with infectivity, and that not all PK-resistant, high beta sheet aggregates are the same or linked with infectivity. Although binding appears to be a requirement for PrP-induced conversion, not all binding of PrP-sen to preexisting PrP-res aggregates results in conversion to PrP-res. These observations highlight the fact that only a certain style of PrP polymerization/aggregation is associated with PrP-res' characteristic partial PK-resistance and the presence of TSE infectivity. Given that this is a correlation, it is critical to examine if the production of PrP-res (alone) results in new TSE infectivity. With so many variables and complexities, it's tempting to look for a great unifying pathogenic mechanism that would account for all conceivable illness states connected to PrP conformation, aggregation state, and sequence perturbations and accidents. However, the processes and properties of PrP that might explain TSE transmissibility differ from those that explain pathogenesis. Furthermore, the ostensibly spontaneous conversion of mutant PrP-sen molecules to PrP-res in familial TSE disorders could differ mechanistically and cell biologically from the PrP-res-induced conversion of wild type PrP-sen in infectious TSEs.

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