

Tracking protein aggregate interactions

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Amyloid fibrils share a structural motif consisting of highly ordered β -sheets aligned perpendicular to the fibril axis.^{1,2} At each fibril end, β -sheets provide a template for recruiting and converting monomers.³ Different amyloid fibrils often co-occur in the same individual, yet whether a protein aggregate aids or inhibits the assembly of a heterologous protein is unclear. In prion disease, diverse prion aggregate structures, known as strains, are thought to be the basis of disparate disease phenotypes in the same species expressing identical prion protein sequences.⁴⁻⁷ Here we explore the interactions reported to occur when two distinct prion strains occur together in the central nervous system.

Diverse Misfolded Proteins in Disease

Protein misfolding and the accumulation of highly-ordered, β -sheet rich, insoluble aggregates are implicated in a diverse group of neurodegenerative diseases, including prion, Alzheimer, Parkinson and Huntington disease. In aged patients, often different aggregated proteins coexist. For example in Alzheimer disease (AD), amyloid- β plaques and tau neurofibrillary tangles often coexist. Whether the co-occurrence of multiple aggregated proteins indicates a failure in shared clearance pathways or a fibrillized protein nucleating a second protein is unclear.

In prion disease, two conformationally distinct subtypes of PrP^{Sc}, the misfolded prion protein, have been shown to co-exist in up to 50% of patients with sporadic Creutzfeldt-Jakob disease (sCJD).⁸⁻¹² Each

subtype was localized to a particular brain region, and the spatial distribution of the subtypes did not change due to the presence of the second subtype. Indeed, the two subtypes also differed in their biochemical characteristics, including the proteinase-K resistant core size,¹¹ suggesting that the subtypes were structurally different.

In addition to sporadic human prion disease, the coexistence of prion strains commonly arises when prions cross species barriers^{13,14} and may be due to PrP sequence differences between the incoming PrP^{Sc} and the host cellular prion protein, PrP^C.¹⁵ Sequence mismatches may shift the range of newly generated PrP^{Sc} conformations that can be accommodated by the initial seed template. The resulting mix of strains has implications for the treatment of infectious prion disease, as a drug may target only one strain and thus enable rarer strains to propagate.¹⁶ Strain mixtures can also have an impact on the pathogenesis, as interference between strains can alter prion replication.

Here we review the mechanisms of prion strain interference, interactions between conformationally distinct prion strains and new tools to track protein aggregates known as luminescent conjugated oligo- or polythiophenes (LCOs or LCPs).

Prion Strain Interference

Co-infection of two prion strains can result in interference where one prion strain extends the incubation period (ip) or completely blocks a second, superinfecting strain from causing disease.^{17,18} Prion interference has been described in

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mice and hamsters infected with a wide variety of strains and routes of inoculation suggesting that it is a common property of prions.¹⁸⁻²² Additionally, interference may be involved in the emergence of a dominant strain from a mixture that occurs following interspecies transmission.^{13,23-26} Interestingly, prion strain interference has been recapitulated *in vitro* using protein misfolding cyclic amplification (PMCA).²⁷

Several parameters are known to govern prion strain interference. First, the blocking strain must be infectious in order to interfere with the superinfecting strain. Treatments that destroy prion infectivity eliminate the ability of the blocking strain to interfere.²⁸ Second, the blocking-to-superinfecting strain ratio influences the interference, in that the higher the PrP^{Sc} quantity of the blocking strain, the more effectively the ip is prolonged.^{19,29} In some cases, the PrP^{Sc} from the superinfecting strain becomes undetectable.^{22,30} Third, increasing the interval between exposure to the two strains increases the interference ability of the blocking strain.^{17,18,30} Taken together, these studies suggest that replication of the blocking strain is required for strain interference to occur.³⁰ Finally, recent reports have indicated that for prion interference to occur, the blocking and superinfecting strains must infect a common population of neurons.^{22,30} Overall, the relative onset of replication of interfering strains in a common population of neurons is the critical factor that determines which prion strain will emerge.

The mechanisms of strain interference are beginning to be deciphered in studies using the HY and DY strains of TME. Animal studies utilizing the sciatic nerve route of infection have determined that HY and DY prions are transported to the same populations of neurons in the lumbar spinal cord.³¹ Inoculation of the sciatic nerve with the DY prions prior to inoculation with HY completely blocks HY from infecting the spinal cord, while inoculation of HY prions into the opposite sciatic nerve leads to HY readily infecting the spinal cord.³⁰ In these animals, the only observed difference is that when two strains are inoculated into the same sciatic nerve, neuronal deposits in the spinal cord consist of exclusively DY and lack any trace of HY PrP^{Sc}. Interestingly, HY and

DY PrP^{Sc} each typically localize to the cell surface of neurons.³² Together, these *in vivo* data suggest that (1) DY and HY PrP^{Sc} are competing for a limiting host resource, (2) HY and DY prion interference likely occurs at the neuronal cell surface and (3) an immune response to the DY prions is not involved in the interference.

PMCA recapitulates strain interference between HY and DY prions *in vitro* similar to what is observed *in vivo*, indicating that the competing factors are present in this system.²⁷ These *in vitro* studies suggest that prion strain interference is not due to DY PrP^{Sc} rapidly converting all of the available PrP^C to PrP^{Sc}. After one round of PMCA, PrP^{Sc} consistently accumulates to higher levels in the HY compared to DY seeded reactions, consistent with the strain-specific *in vivo* rates of PrP^{Sc} accumulation.²⁷ If DY PrP^{Sc} were converting all of the available PrP^C to PrP^{Sc} in the PMCA reaction, then DY and HY PrP^{Sc} abundance would be predicted to be equal. A possible explanation is that DY PrP^{Sc} sequesters PrP^C or another required cofactor (e.g., RNA, glycosaminoglycans), rendering it unavailable to HY PrP^{Sc}.³³⁻³⁶ This is consistent with the observation that a vast excess of DY PrP^{Sc} compared to HY PrP^{Sc} is required for interference.

LCPs Discriminate Distinct Prion Conformations *In Vitro* and *In Vivo*

Methods to visualize and track HY and DY or other prion strains in tissue sections have been limited. Small hydrophobic molecules such as thioflavin T (ThT) or Congo red have been used to detect protein aggregates having an extensive cross β -pleated sheet conformation. However, these conventional dyes are unable to distinguish heterogeneous populations of aggregated proteins because they are sterically rigid. A new class of amyloid ligands, the LCPs, consists of a polythiophene backbone that freely rotates and allows multiple rotational (torsion) angles between the thiophene rings.^{37,38} When bound to protein aggregates, the thiophene rings lock into a specific planarity and provide a direct correlation between the LCP geometry and the light emitted. Protein-bound LCPs with maximum emission at shorter wavelengths suggests that the

polythiophene backbone is nonplanar and LCPs are loosely packed, whereas a shift toward longer wavelengths suggests that the backbone is planar and LCPs are tightly packed.^{39,40} Thus the LCPs provide structural insights regarding the morphology of bound protein deposits and can be used as a complementary technique to conventional staining protocols for characterizing protein aggregates.

In vitro, the LCPs bound to recombinant protein fibrils of the same protein can yield distinct spectral profiles. This was shown with recombinant mouse prion protein (mPrP) that had been converted into two chemically identical types of amyloid fibrils using varying conditions for fibrillation.⁴¹ The two fibril types could be distinguished by different PTAA emission spectra. The same procedure also distinguishes A β ₁₋₄₂ fibrils grown under different conditions *in vitro*.⁴²

LCPs have been additionally tested on brain sections of mice infected with different prion strains.^{41,43} We applied the anionic LCP polythiophene acetic acid (PTAA) to frozen brain sections and measured different spectral emission signatures depending on the strain. By calculating ratios of the intensity of the emitted light at certain wavelengths, prion aggregates associated with distinct prion strains were easily distinguished from each other, verifying the usefulness of spectral properties of LCPs for labeling specific strains. PTAA also binds to *de novo* prion aggregates, which do not stain with thioflavin T nor Congo red, indicating that LCPs could be used to identify a subset of protein deposits normally undetectable by conventional methods.⁴⁴

Heterogenic protein aggregates are also found in other proteinopathies, such as AD or systemic amyloidoses. Upon application of several LCPs to transgenic mouse models having AD pathology, a striking heterogeneity in the characteristic plaques composed of the amyloid β (A β) peptide was identified. LCP staining of A β deposits in brain tissue sections revealed different sub-populations, observed as protein deposits with different spectral emissions.⁴² Furthermore, PTAA could be used to spectrally separate A β deposits associated with the Swedish and Arctic mutation as compared to the Swedish mutation

in the amyloid precursor protein (APP), indicating that different point mutations of the amyloidogenic protein can give rise to various morphologies of protein deposits. The spectral profile from PTAA was also used for the structural sub-typing of systemic amyloidoses and similar to observations in earlier studies of prion deposits, some tissue samples exhibit mixtures of amyloid having separate PTAA spectra.⁴⁵

LCPs in Studies of Prion Strain Mixtures

We recently characterized interactions that occur between prion strains *in vivo* using the LCPs.⁴⁰ Three prion strains were used that had been previously characterized in mice histologically and biochemically: mouse-adapted chronic wasting disease (mCWD), natural sheep scrapie (mNS) and bovine spongiform encephalopathy (mBSE). Each individual strain and mixtures of two strains were intracerebrally inoculated into mice. The strain mixture consistently led to a delay in the incubation period as compared to that of the most rapid strain. Nevertheless, the lesion profile revealed that the affected regions essentially encompassed the combined sites of the two individual strains. The only exception was the mNS/mBSE strain mixture, in which interference occurred in a single region, the hippocampus, where mNS no longer accumulated.

To determine whether strains were colocalizing, frozen brain sections from mice infected with a mixture were stained with PTAA. The mCWD/mBSE mixture showed no evidence of interaction between aggregates, although both strains were present as seen by their characteristic plaque morphologies and the PTAA emission spectra.

PTAA bound to mCWD plaques emits light with a maximum intensity at ~565 nm and appears yellow, whereas PTAA bound to mNS emits light with a maximum intensity at ~595 nm and appears red. Surprisingly, the mCWD/mNS mixture led to the formation of hybrid plaques, wherein the plaque core was consistently composed of yellow mCWD, bordered by the red aggregates of mNS. The existence of the mCWD plaques may have facilitated mNS deposition by providing a

scaffold, although hybrid plaques did not accelerate the mNS infection. We then investigated whether the hybrid plaques were composed of homotypic fibrils of one strain or heterotypic fibrils containing both strains. If the fibrils were homotypic, we would expect that on second passage, we would again observe the hybrid plaques containing each strain with its characteristic PTAA emission spectra. This was indeed the case as hybrid plaques were again observed. Thus, with three different strain combinations, interactions varied from strain inhibition (mNS/mBSE) no interaction (mBSE/mCWD), or hybrid plaque formation (mCWD/mNS) (Fig. 1).

Specificity of Aggregation Among Other Misfolded Proteins

Interactions between pre-formed aggregates have also been studied in yeast prions.^{46,47} Yeast that propagate two different protein aggregates frequently showed aggregate co-localization, even when the proteins did not seed the fibrilization of each other. Although the proteins co-localized, they did not seem to coassemble, indicating that the proteins more efficiently converted a homologous

rather than a heterologous protein. This colocalization does not indicate any promotion of aggregate formation and is consistent with our observations of colocalization in the prion strains where there was no disease acceleration. Rajan and colleagues also studied the aggregation of unrelated proteins expressed in the same cell. Here they found that the aggregates were homogenous, indicating that aggregation of single proteins was exquisitely specific.⁴⁸

Conclusion

Prion strain mixtures are common in certain sporadic and infectious prion diseases, as most recently described in chronic wasting disease (CWD) in mule deer.¹⁵ Yet detecting a second strain can be a challenge since one prion strain may mask a second strain in western blot or immunohistochemical assays. Although prion strains have been shown to interfere or aggregate with a second strain, other interactions may be possible, such as the formation of heterotypic fibrils. Staining with the conformationally sensitive LCPs presents a powerful technique for exploring the range of possible interactions

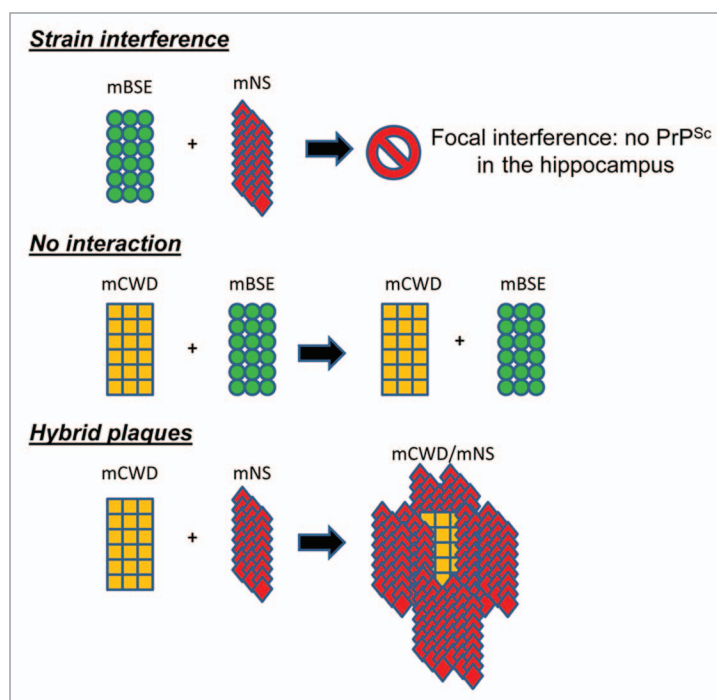


Figure 1. This schematic depicts interactions that have occurred in the brain among distinct prion strains as reported in reference 41.

between protein aggregates. Finally, the LCPs can provide insights into the structural arrangements of protein aggregates and can be used to track aggregates in vivo in real time.⁴⁹

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