Infectious Protein Particle Prions

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Abstract: Transmissible spongiform encephalopathies (TSEs), otherwise known as prion disorders, are fatal diseases causing neurodegeneration in a wide range of mammalian hosts, including humans. The causative agent prions are thought to be composed of a rogue isoform of the endogenous prion protein (PrP). Prion diseases or TSEs are a group of rare but fatal neurological disorders that affect humans and animals. Whether sporadic, inherited or acquired, these illnesses generally correlate with the accumulation of misfolded PrP in the brain and the appearance of widespread neurodegeneration after long incubation times. The classical histopathological landmarks of TSEs are spongiform vacuolation, neuronal loss and astrocytic gliosis, whereas the main clinical manifestations in humans include progressive dementia, cerebellar ataxia and myoclonus. Interestingly, although such symptoms are also observed in more common neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD); prion diseases have received special attention because of their infectious nature and the associated risk of epidemics.

Key words: Prion Diseases • Misfolded • Neurodegeneration • Spongiform Vacuolation

INTERODUCTION

Prion, an abnormal form of a normally harmless protein found in the brain that is responsible for a variety of fatal neurodegenerative diseases of both animals and humans called transmissible spongiform encephalopathies. In the early 1980s the American neurologist Stanley B. Prusiner and colleagues identified the “proteinaceous infectious particle,” a name that was shortened to “prion” (Pronounced “pree-on”). Prions can enter the brain through infection, or they can arise from mutations in the gene that encodes the protein. Once present in the brain prions multiply by inducing benign proteins to refold into the abnormal shape. This mechanism is not fully understood, but another protein normally found in the body may also be involved. The normal protein structure is thought to consist of a number of flexible coils called alpha helices. In the prion protein some of these helices are stretched into flat structures called beta strands. The normal protein conformation can be degraded rather easily by cellular enzymes called proteases, but the prion protein shape is more resistant to this enzymatic activity. Thus, as prion proteins multiply they are not broken down by proteases and instead accumulate within nerve cells till destroying them. Progressive nerve cell destruction eventually causes brain tissue to become filled with holes in a sponge like, or spongiform, pattern.

In 1982, Prion protein isoform PrPSc was identified as the major constituent of infective fractions purified from hamster brain homogenates [1]. Subsequent characterization revealed that the pathogenic protein was host-encoded and not the product of a viral gene, as it had been assumed previously [2, 3]. It was also established that PrPSc is post-translationally derived from PrP [4]. Thus, although the two isoforms differ greatly in their spatial conformation [5], they have the same amino acid sequence and are encoded by the same single-copy gene, Prnp [6]. Under normal conditions, PrPSc is a glycoprotein tethered to the outer plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. Its unique molecular structure, studied by nuclear magnetic resonance (NMR) and crystallographic techniques, can be roughly divided into two halves: a flexible N-terminal domain rich in repetitive motifs and a C-terminal globular domain containing a characteristic array of three α-helices and two β-sheets. At the centre of the polypeptide chain,
a short hydrophobic stretch connects the two major domains [7, 8]. In contrast, attempts to resolve the molecular structure of PrPSc have not been successful.

During the past two decades, a previously unknown mechanism of disease has been described in humans and animals. Several fatal illnesses often referred to as the prion diseases and including scrapie of sheep are caused by the accumulation of a post translational modified cellular protein. Indeed, the hallmark of all prion diseases whether sporadic, dominantly inherited, or acquired by infection is that they involve the aberrant metabolism and resulting accumulation of the prion protein (Table 1) [9, 10]. The conversion of PrPc (The normal cellular protein) into PrPSc (The abnormal disease-causing isoform) involves a conformation change whereby the alpha-helical content diminishes and the amount of beta sheet increases [11]. This structural transition is accompanied by profound changes in the properties of the protein: PrPc is soluble in non-denaturing detergents, whereas PrPSc is not [12] and PrPc is readily digested by proteases, whereas PrPSc is partially resistant [2].

Most cases of prion disease are sporadic; that is, they arise spontaneously for no known reason. More rarely prion disease is inherited due to a faulty gene, or acquired by medical procedures, transfusions, or contaminated food. Sporadic and inherited prion disease occurs worldwide in all populations. The incidence of sporadic Creutzfeldt-Jakob disease (CJD) is around 1 per million of the population per annum; males and females are equally affected. The incidence of the various acquired prion diseases, however, is more localised to specific groups and populations. Diseases caused by prions that affect humans include: Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia and kuru. Prion diseases affecting animals include scrapie, bovine spongiform encephalopathy (Commonly called mad cow disease) and chronic wasting disease of mule deer and elk. For decades physicians thought that these diseases resulted from infection with slow-acting viruses, so-called because of the lengthy incubation times required for the illnesses to develop. These diseases were and sometimes still are, referred to as slow infections. The pathogenic agent of these diseases does have certain viral attributes, such as extremely small size and strain variation, but other properties are atypical of viruses. In particular, the agent is resistant to ultraviolet radiation, which normally inactivates viruses by destroying their nucleic acid.

Prions are unlike all other known disease-causing agents in that they appear to lack nucleic acid—i.e., DNA or RNA—which is the genetic material that all other organisms contain. Another unusual characteristic of prions is that they can cause hereditary, infectious and sporadic forms of disease—for example, Creutzfeldt-Jakob disease manifests in all three ways, with sporadic cases being the most common. Prion proteins can act as infectious agents, spreading disease when transmitted to another organism, or they can arise from an inherited mutation. Prion diseases also show a sporadic pattern of incidence, meaning that they seem to appear in the population at random. The underlying molecular process that causes the prion protein to form in these cases is

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unknown. Other neurodegenerative disorders, such as Alzheimer disease or Parkinson disease, may arise from molecular mechanisms similar to those that cause the prion diseases.

**Characteristics of Prions:** PrP \(^{Sc}\) is the major and very probably the only, component of the infectious prion particle. PrP\(^{Sc}\) formation is a post-translational process involving only a conformational change in PrP\(^{C}\) [11, 13]. Molecular modelling studies predicted that PrP\(^{C}\) is a four-helix bundle protein containing four regions of secondary structure, denoted H1 through H4 [14, 15]. Fouriertrans form infrared (FTIR) and circular dichroism (CD) studies showed that PrP\(^{C}\) contains about 40% alpha helix and little beta sheet, consistent with the structural predictions [11, 16]. Subsequent nuclear magnetic resonance (NMR) studies of a synthetic PrP peptide containing residues 90 to 145 provided good evidence for H1 [17]. This peptide contains residues 113 to 128, the most highly conserved residues among all species studied [15, 18]. When the peptide is extended to include alpha helix A, this forms the central domain of PrP\(^{C}\) (approximately residues 95 to 170) that binds to PrP\(^{Sc}\) during the formation of nascent PrP\(^{Sc}\) [19]. This domain shows higher homology between cattle and humans than between sheep and humans, which raises the possibility that prion transmission from cattle to humans may occur more readily than from sheep to humans [20].

**What Causes Prion Disease?** Prion diseases are associated with the build-up in the brain (And some other organs) of an abnormal or 'rogue' form of a naturally occurring cellular protein, known as the prion protein. The rogue protein results from a change in shape of the normal prion protein. Once formed in the body these rogue proteins recruit and convert more of the normal prion protein into the abnormal form, setting off a kind of chain reaction which leads to a progressive accumulation of the rogue protein. In the normal course of events, once they have served their purpose, prion proteins are broken down by enzymes in the body. The abnormal prions however are more resistant to this process; so they accumulate and cause damage in the brain, which interferes with normal brain functioning. All forms of the disease are thought to be associated with an incubation period. This is a clinically 'silent phase' during which replication of the rogue protein is thought to be taking place.

The function of the normal form of the protein remains unclear, though it is thought they may possibly play a role in the transport of messages between specific brain cells (Synaptic transmission).

**Genetic Susceptibility:** At present the most important and well defined genetic factor which influences the susceptibility of an individual to developing prion disease relates to a common variation in the prion protein gene itself. At a particular position in the prion gene known as codon 129, there are two possible genetic types, which in turn specify the body to produce different amino acids at this position. These amino acids are called methionine and valine, or M and V for short. In most countries, MM and MV frequencies in the population are roughly equal (40-50%). It has been known for some years that individuals, who are MV, are at much less risk of developing prion disease than are MM or VV individuals. All definite cases of vCJD have been MM.

**Prion Disease and Infection Control:** Prion disease is not contagious; there is no evidence to suggest it can be spread from person to person by close contact. Once a person has developed prion disease, central nervous system tissues (Brain, spinal cord and eye tissue) are thought to be extremely infectious. However this is only relevant for those handling infected tissue directly, which does not include carers looking after a person with the disease. Infectivity in the rest of the body varies in different types of prion disease but is generally much less than in brain tissue. People with any form of prion disease are requested not to be blood or organ donors and are requested to inform their doctor and dentist prior to any invasive medical procedures or dentistry.

One reasonable therapeutic strategy would be to stabilize the structure of PrP\(^{C}\) by binding a drug; another would be to modify the action of proteinX, which might function as a molecular chaperone. It remains to be determined whether a drug that binds to PrP\(^{C}\) at the protein X binding site would be more efficacious than a drug that mimics the structure of PrP\(^{C}\) with basic polymorphic residues that seem to prevent scrapieand CJD. Because PrP\(^{Sc}\) formation seems limited to caveolae-like domains [21], drugs designed to inhibit this process need not penetrate the cytosol of cells, but they must be able to enter the CNS. Alternatively, drugs that destabilize the structure of PrP\(^{Sc}\) might also prove useful.

As prions cannot be completely destroyed by conventional sterilisation procedures, transmission has also occurred inadvertently through the use of surgical instruments previously used during neurosurgery on a person with sporadic prion disease. Current Department of Health guidelines are that all surgical instruments used on medium or high infectivity tissues in a patient with suspected prion disease are quarantined and not re-used unless an alternative diagnosis is confirmed. Instruments used on patients with known prion disease are not reused.
Fish Prion Proteins: PrP genes from land vertebrates have typically been cloned using conventional procedures such as cDNA hybridization and PCR screening. In contrast, fish PrP genes could not be isolated with these methods due to their large DNA sequence divergence. The first fish PrP homologue was identified in *Fugu rubripes* by searching genome databases for ORFs encoding key structural features of PrPs [22]. Subsequent cloning and sequencing of PrP genes in salmon, trout, carp, stickleback, sea bream and zebra fish [23-26] revealed that bony fish possess two PrP orthologs, PrP-1 and PrP-2, which probably arose during a fish-specific genome duplication [27]. Northern and Western Blot analyses indicated that the fish proteins are expressed at particularly high levels in adult fish brains, as well as in muscle, skin, heart and gills [23]. Although quite variable in size and sequence, PrP-1 and PrP-2 present characteristic features of mammalian PrPs: an N-terminal signal peptide conserved N-glycosylation sites, two cysteine residues that form a disulphide bond, a central hydrophobic stretch and the C-terminal signal for the attachment of a GPI-anchor. An intriguing feature of fish PrPs is the presence of a highly conserved motif of 13 amino acids in length between the repetitive region and the hydrophobic stretch [23]; the structural or functional importance of this motif is not evident from its sequence.

The repetitive domains of fish PrPs are easily recognizable but at the same time clearly different from those of tetrapod PrPs. For example, while a constant number of nearly identical octarepeats is typical for mammals (Or hexarepeats in birds); in fish the number and length of degenerate repeats vary from species to species. Nevertheless, all PrP repeats can be considered variations of two basic types of repeats (A and B), which evolved differentially in every vertebrate class [23]. The sequence of the globular domain also has diverged considerably between fish and mammals, owing to high rates of amino acid substitutions, insertions and deletions. Notably, these changes predominantly affect residues outside key structural motifs and are not expected to affect the globular fold [23].

Apart from PrP-1 and PrP-2, other genes partially resembling PrP have been reported in fish. Originally described as PrP-like and Shadoo [28, 29], they are also known as PrP-1-rel and PrP-2-rel because of their genomic location, directly downstream of PrP-1 and PrP-2, respectively [23]. The encoded products are GPI-anchored polypeptides of only 150-180 amino acids in length, expressed in brain tissues and containing the characteristic PrP hydrophobic stretch. Further similarities to PrPs include a β-strand motif in PrP-rel-1 identical to the first β-strand of PrP and the presence of degenerated B-and A-like repeats in PrP-rel-2. This situation is reminiscent to that of Prnp and its genomic neighbour Doppel. Thus, PrP-rels, like Doppel, may have arisen from the tandem duplication of an ancestral PrP gene and then diverged as a result of high substitution rates and the differential loss of motifs. Interestingly, although PrP-rels are predicted to be unstructured proteins, their mammalian homologue Shadoo has recently received attention because it appears to influence biological and pathogenic activities of PrP in vivo [30, 31].

Risk of Prion Transmission to Fish: According to the aforementioned arguments, fish, poultry and pigs represent potential candidate hosts for prion infection. In order to assess the risk of prion transmission to fish, five main parameters should be considered: 1. The potential use of prion-contaminated MBM as a nutritional supplement in aquaculture, 2. the consumption of infected farmed fish by humans, 3. The use of feeds or other products (*i.e.* gelatin, milk replacers) derived from TSE-affected fish for mammalian or piscine nutrition, 4. The use of fish meals cross-contaminated with MBM in mammalian diets and 5. The escape of infected fish, or the release of infected waste from aquaculture facilities into the marine environment.

With fish mariculture turning into a very important and rich protein source for humans, consumers may become concerned about the possibility of farmed fish developing prion disease, or serving as passive carriers of prion infectivity. All farmed fish receive commercial feeds containing 40-55% protein and since some of it is likely to be of animal origin, the possibility of feed contamination with mammalian prions cannot be excluded. Interestingly, in the late 1980s, total fish feed production in the UK was in the order of 75,000 tons per year, with the use of about 3750 tons of MBM in fish feed [32]. It has been suggested that the extended type A-repeat domain in sea bream PrP-1 and other fish PrPs could confer on these proteins the ability to self-polymerize and aggregate [25]. In addition, it has been noted that motifs similar to those observed in fish PrPs can also be identified in unrelated proteins such as the piscine annexin 11 and the mammalian lectin L-29 [33]. Notably, both these proteins display self-aggregation properties and annexin 11 is, like PrP, a neuro-specific membrane protein.
Transmission Studies in Commercially Important Fish Species: At present, only a few studies have explored the experimental transmission of TSEs to teleost fish. The first attempts in this direction involved the oral and parenteral inoculation of two commercially important fish species, rainbow trout and turbot, with the 139A mouse-adapted scrapie strain [34]. Although the fish displayed no clinico-histopathological signs during the three month-experimental period, a mouse bioassay revealed that they carried residual infectivity. Specifically, mice intracranially inoculated with trout intestinal extracts one day after oral challenge were positive for brain PrPSc deposition approximately 200 days post inoculation (p.i.), despite the absence of clinical symptoms. Similar results were obtained when mice were intracerebrally (i.c.) inoculated with spleen extracts from turbots, 15 days after parenteral inoculation of the fish with scrapie material. Finally, brain tissue from parenterally inoculated turbots 15 and 90 days after challenge was also able to elicit PrPSc accumulation in the brains of recipient mice, without causing clinical disease.

Recently, we reported data on the oral transmission of BSE and scrapie to gilthead sea bream (Sparus aurata) [35]. Interestingly, at two years p.i., a number of fish that had been force-fed BSE-or scrapie-infected brain homogenates developed abnormal plaque-like deposits in their brains. Specifically, the brains of two out of five fish inoculated with scrapie developed signs of abnormal protein aggregation at 24 months p.i. These aggregates were positively stained with polyclonal antibodies raised against fish PrPs, but showed no proteinase K (PK)-resistance or Congo red birefringence. The brains of the BSE-challenged fish, however, displayed a much more striking picture, having already developed the first signs of abnormal deposition at eight months p.i. A general progression in size, PK-resistance and morphological features was observed thereafter, resulting in an impressive number of aggregates in all the brain regions examined at 24 months p.i. Three out of five fish sacrificed at this time point showed 500-800 deposits per brain section each, 70-85% of which were PK-resistant and had a mean diameter of 30 μm. These aggregates were PAS-positive, congophilic and birefringent in polarized light, indicating an amyloid or amyloid-like fibrillar structure. In contrast to the TSE-challenged individuals, no signs of abnormal aggregation or any other lesions were observed in the brains of the control fish “challenged” with bovine or ovine brain homogenates prepared from healthy animals.

Altogether, the development of abnormal brain deposits in BSE-challenged sea bream constitutes an unprecedented histopathology in fish.

CONCLUSION

Understanding how PrPC unfolds and refolds into PrPSc not only has implications for interfering with the pathogenesis of prion diseases, but may open new approaches to deciphering the causes of and developing effective therapies for the more common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS). In addition, two different stable metabolic states in yeast and one in a fungus have been ascribed to prion-like changes in protein conformation. Indeed, the expanding list of prion diseases and their novel modes of pathogenesis, as well as the unprecedented mechanisms of prion propagation and information transfer, indicate that much more attention to these fatal disorders of protein conformation is urgently needed.

REFERENCES


9. Prions are defined as proteinaceous infectious particles that lack nucleic acid. PrPC is the cellular prion protein; PrPSc is the pathologic isoform. Amino-terminal truncation during limited proteolysis of PrPSc produces PrP27-30 (So named because this protease-resistant core of PrPSc migrates at 27 to 30 kD).


