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Chapter 6

Disruption of Metal Homeostasis and the Pathogenesis of Prion Diseases

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Abstract

Prion diseases are progressive neurodegenerative diseases that are associated with the conformational conversion of normal cellular prion protein (PrP^{C}) into abnormal pathogenic prion protein (PrP^{Sc}). PrP^{C} is a metal-binding protein that is located in the synapse and possesses the ability to bind to various metals, including Cu, Zn, Mn and Fe. Moreover, increasing evidence suggests that PrP^{C} plays essential roles in the maintenance of metal homeostasis in the synapse. Trace elements have a crucial influence on the conformational change of PrP^{C}. Given that other disease-related proteins such as β-amyloid protein and its precursor protein (APP) in Alzheimer's disease also exist in the synapse and possess a metal-binding ability, an interaction between PrP and metals and between PrP and APP, may occur in the synapse; the resulting metal homeostasis may lead to the pathogenesis of prion diseases. Here, we review our studies and other new findings that inform the current understanding of the link between trace elements and physiological functions of PrP^{C} and the neurotoxicity of PrP^{Sc}.

Keywords: Alzheimer's disease, synapse, calcium homeostasis, zinc, copper, iron, manganese

1. Introduction

Prion diseases are fatal neurodegenerative diseases, such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and Kuru in humans [1]. The common pathological hallmarks of prion diseases are the spongiform degeneration of glial cells and neurons. The accumulation of amyloidogenic prion protein (PrP) as the abnormal scrapie type isoform (PrP^{Sc}) is also

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observed in the brain of patients. Prion diseases are also called transmissible spongiform encephalopathies because their infection characteristics are caused by the activities of PrP^{Sc} in the pathogenetic tissues [2].

Although the molecular pathogenesis and transmission pathway of prion diseases are still controversial, it is widely accepted that the conformational conversion of normal cellular prion protein (PrP^{C}) into an abnormal PrP^{Sc} is the transmissible characteristic of prion diseases. Normal PrP^{C} is a 30–35 kDa cell surface glycoprotein anchored at the plasma membrane with a glycosylphosphatidylinositol (GPI) domain. PrP^{C} is ubiquitously expressed in the body and notably expressed in the brain. Both PrP^{C} and PrP^{Sc} have the same characteristic chemical modification of the same primary sequence. However, PrP^{C} differs from PrP^{Sc} in terms of resistance to protease digestion, a high content of β-sheet secondary structure and the propensity to form insoluble amyloid fibrils. When the misfolded PrP^{Sc} enters into the body via the ingestion of contaminated food, etc., the protease-resistant PrP^{Sc} can aggregate, resulting in fibril formation that in turn promotes other PrP^{C} molecules in the brain to misfold and aggregate. These lines of evidence suggest that the conformational change of PrP is crucial for the pathogenesis of prion diseases. Thus, prion diseases are included in the category of protein-misfolding diseases (conformational diseases), along with Alzheimer's disease (AD), triplet repeat diseases and dementia with Lewy bodies (DLB) [3]. All of these diseases share common properties, such as the deposition of disease-related proteins (amyloids) and in the exhibition of neurotoxicity. The disease-related proteins, which are termed amyloidogenic proteins, include β-amyloid protein (AβP) in AD, prion protein in prion diseases, polyglutamine in triplet repeat disease and α-synuclein in DLB. Although their primary sequences are identical, all of these proteins form insoluble fibril-like structures (amyloid fibrils) with β-pleated sheet structures. Furthermore, all of these amyloidogenic proteins possess the ability to bind trace metals [4].

In the brain, considerable amounts of trace elements such as iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) exist, as well as other ubiquitous elements such as sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg). The concentration and the distribution of each metal differ across brain regions [5, 6]. These trace elements are essential for the normal brain functions. However, an excess of these metals are neurotoxic. Thus, their concentration and chemical form are strictly regulated.

Increasing evidence suggests that trace elements are involved in the neurodegenerative pathways for prion diseases [7]. There are three possible roles for trace elements in these neurodegenerative pathways [8]: (1) supporting the "loss of the normal, protective functions of PrP^{C}"; (2) supporting a "gain of toxic functions of PrP^{Sc}"; and (3) "a combination of both." Although the physiological roles of PrP^{C} are not yet fully understood despite its wide distribution, several studies suggest that PrP^{C} regulates metal homeostasis and has antioxidant and cytoprotective effects against the neurotoxicity induced by Cu^{2+} or free radicals. Therefore, the depletion of PrP^{C} and the resulting metal dyshomeostasis may trigger the neurodegenerative processes. Moreover, PrP^{Sc} and its fragment peptides cause synaptic impairment and apoptosis in neurons or astrocytes *in vitro* or *in vivo* [9, 10]. Trace

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elements cause conformational changes in PrP and enhance its neurotoxicity. Furthermore, prion plaques in the patient brain reportedly contain low Cu and high Mn [11]. The expression level of PrP is correlated with the distribution of metals [12]. Here, we review our studies and other new findings for a current understanding of the link between trace elements and the pathogenesis of prion diseases. We also discuss the role of PrP as the regulator of metal homeostasis and the protector against neurotoxicity of β-amyloid protein (AβP) at the synapse.

2. Metal homeostasis and normal prion protein

2.1. Cu and normal prion protein

Cu is the third most abundant metal in the brain. Cu is essential for brain function and is a cofactor for numerous enzymes, including cytochrome C, superoxide dismutase, lysyl oxidase and tyrosinase. Cu is involved in Fe homeostasis as a component of ceruloplasmin. Moreover, Cu has neuroprotective activity as a component of Cu/Zn superoxide dismutase (Cu/ZnSOD), an endogenous antioxidant. Thus, Cu deficiency has adverse effects on myelination. However, Cu is a redox-active metal and exists as both oxidized Cu^{2+} and reduced Cu^{+}. Excess free Cu is toxic because it produces ROS and binds with the thiol groups of functional proteins. Cu binds to transporter proteins such as CTR1, ATP7A and ATP7B and is transported into the brain. Cu deficiency or excess due to an impairment of these transporters leads to severe neurodegenerative diseases such as Wilson's disease or Menkes disease.

Recent studies suggest that Cu has modulatory effects on neuronal information processes [13–15]. Intracellular Cu accumulates in synaptic vesicles and is then released into the synaptic clefts during neuronal excitation at the concentration about 15–100 μM. These characteristics are quite similar to Zn. The released Cu reportedly influences various receptors, including *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, AMPA-type glutamate receptor and GABA receptor to modulate the neuronal activity [16].

The link between Cu and prion diseases were first reported by Brown et al. in 1997 [17]. They demonstrated that the levels of Cu in the brains of PrP-knockout mice are significantly decreased compared to those levels in normal mice. The activity of Cu-dependent enzymes was also reduced in PrP-null mice. As shown in **Figure 1**, PrP^{C} contains 208 amino acid residues and possesses a highly conserved octarepeat domain composed of multiple tandem copies of the eight-residue sequence PHGGGWGQ in its N-terminal (**Figure 1**). Jackson et al. reported that PrP^{C} binds to four Cu atoms in its octarepeat domain and binds to two Cu atoms in addition to two histidine (His) residues, His^{96} and His^{111} [18]. They also demonstrated that other metals including Zn^{2+}, Mn^{2+} and Ni^{2+} bind to these binding sites with lower affinities compared to Cu^{2+}. Extended X-ray absorption fine structure (EXAFS) spectroscopy demonstrated that Cu binds His residues in the octarepeat domain [19]. Moreover, Valensin et al. reported that His^{111} in the neurotoxic fragment PrP106-126 has the ability to bind Cu^{+} and Ag^{+} [20].

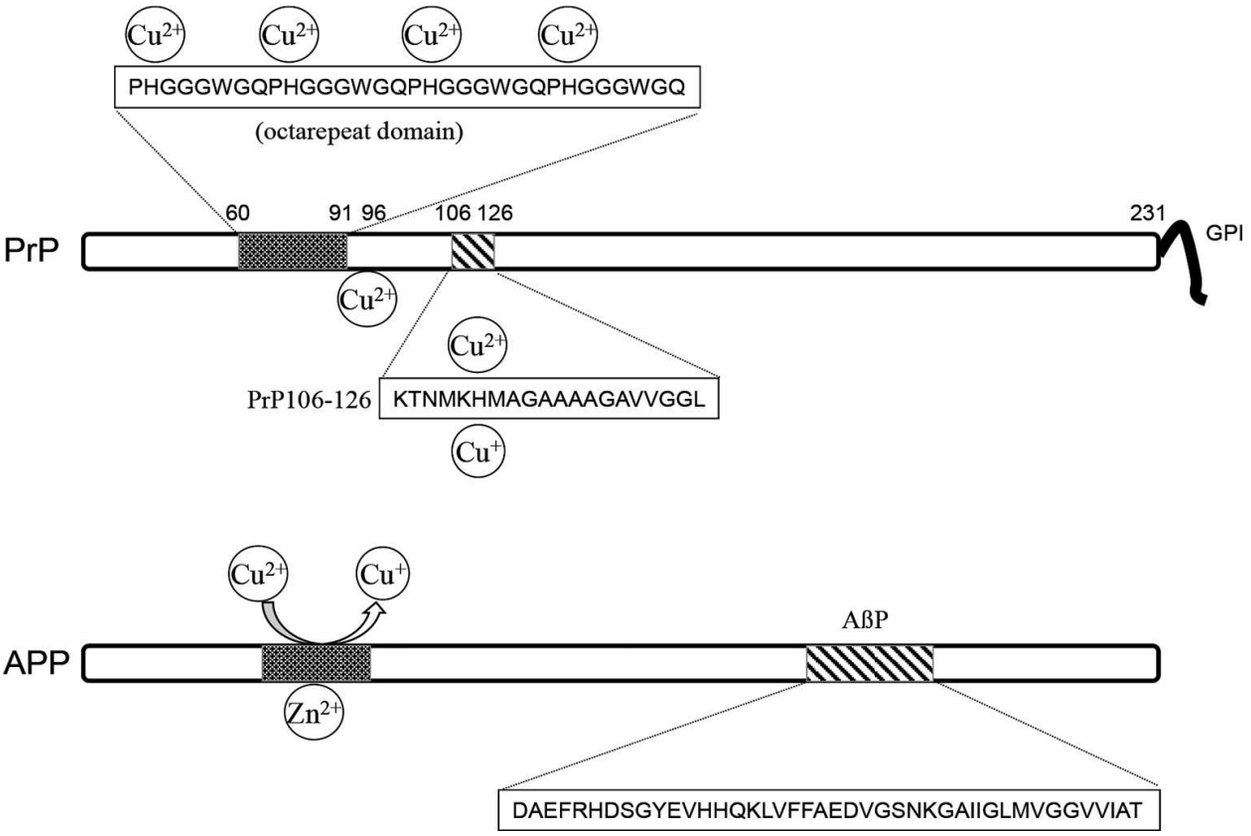


Figure 1. The structure and the metal-binding properties of prion protein and APP.

PrP^C reportedly transports Cu from the extracellular space to the intracellular space via endocytosis and thus regulates the intracellular concentrations of Cu [21]. Furthermore, PrP possesses or modulates Cu/ZnSOD activity in the brain and plays roles in the cellular resistance to oxidative stress [22]. Indeed, PrP-deficient neurons exhibit a lower glutathione activity and susceptibility to hydrogen peroxide [23]. Recent studies suggest that PrP^C regulates the excitability of NMDA-type glutamate receptor in a Cu-dependent manner [24]. Moreover, Cu^{2+} influences the gene expression and cellular trafficking of PrP [25]. PrP^{Sc}-infected cells exhibit decreased Cu^{2+} binding. The Cu-deficient condition due to a mutation of ATP7A delays the onset of prion diseases. These results indicate that the regulation of Cu homeostasis is involved in the physiological roles of PrP and its mechanism of infection and neurodegeneration.

These characteristics of PrP^C are similar to those of the AD-related protein AβP (**Figure 1**). AβP is a small peptide of 39–43 amino acid residues, which results from a cleavage of a large precursor protein (APP; amyloid precursor protein). The conformational change of AβP and its neurotoxicity play central roles in the pathogenesis of AD [26]. APP has distinct binding domains for Cu, Zn and Fe. APP binds to Cu with its N-terminal and converts Cu^{2+} into Cu^{+} [27]. Cu influences the expression and the dimerization of APP and the trafficking of APP from the ER to the neurites. Moreover, Cu promotes the production of AβP.

2.2. Zn and normal prion protein

Other metals are also associated with prion diseases. Among them, Zn^{2+} has the next highest binding affinity to PrP^{C} compared to Cu^{2+}. Zn is the second most abundant element in the brain. Zn is essential for most organisms and plays important roles in various physiological functions such as mitotic cell division, immune system functioning and synthesis of proteins and DNA [28]. Moreover, Zn acts as a cofactor to more than 300 enzymes or metalloproteins. Recent studies have revealed that Zn signaling plays crucial roles as a second messenger in various human biological systems. Thus, Zn deficiency in children results in dwarfism, delayed mental and physical development, immune dysfunction and learning disabilities. Zn deficiency also produces learning disorders, taste disorders and odor disorders in adults.

The human body contains approximately 2 g of Zn. In the brain, Zn is concentrated in the regions such as cerebral cortex, amygdala, hippocampus, thalamus and olfactory cortex. Zn in the brain firmly binds to metalloproteins or enzymes. However, a substantial fraction (approximately 10% or more) of Zn either forms free Zn ions (Zn^{2+}) or is loosely bound. This Zn fraction is histochemically detectable via staining with chelating reagents [29]. In the presynaptic vesicles of excitatory glutamatergic neurons, the chelatable Zn is stored and is secreted into synaptic clefts together with glutamate during neuronal firings. The concentration of this secreted Zn is estimated to be 1–100 μM [30, 31]. Secreted Zn^{2+} modulates overall brain excitability by binding with *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, GABA receptors and glycine receptors. The secreted Zn^{2+} is critical for neuronal communication, synaptic plasticity and memory formation [32]. Indeed, Zn^{2+} in the hippocampus is essential for the induction of long-term potentiation (LTP), which is a form of synaptic information storage that has become a well-known paradigm for the mechanisms underlying memory formation.

There are two factors involved in the maintenance of Zn homeostasis, metallothioneins and Zn transporters. Metallothioneins are ubiquitous metal-binding proteins with 68 amino acids that bind seven metal atoms (including Zn, Cu, Cd, etc.) via 20 cysteine residues. There are three types of metallothioneins, MT-1, MT-2 and MT-3. MT-1 and MT-2 are ubiquitously expressed throughout the entire body, whereas MT-3 is primary localized in the central nervous system.

Zn transporters also control Zn homeostasis by facilitating Zn influx and efflux [33]. There are two types of mammalian Zn transporters, ZnT transporters and Zrt-, Irt-like protein (ZIP) transporters. ZnT transporters are involved with the solute carrier (*SLC30*) gene family and decrease intracellular Zn via a facilitation of Zn efflux from cells. There are 14 types of ZnT transporters in mammals, including ZnT-1 and ZnT-3, which are colocalized with chelatable Zn in the brain. ZnT-1 is a membrane protein with six transmembrane domains and is widely distributed in mammalian cells. ZnT-1 has a pivotal role in Zn efflux and protects against excess Zn. ZnT-3 is localized to the membranes of presynaptic vesicles, transports Zn into synaptic vesicles and maintains high Zn concentrations in the vesicles.

ZIP transporters are another type of Zn transporter encoded by *SLC39* genes. ZIP transporters increase cytosolic Zn by promoting transport from extracellular to intracellular compartments.

Fourteen ZIP genes have been identified in mammals and the ZIP transporters are localized to the cell membranes or to the membranes of the Golgi apparatus or ER. These transporters control Zn influx into subcellular organs. The impairment of Zn transporters results in severe diseases such as Ehlers-Danlos syndrome.

The concentration of Zn in the brain is much higher compared to Cu; therefore, Zn^{2+} may influence PrP^C binding to Cu [34]. Bioinformatics analysis has revealed evolutionary similarities between prion genes and gene-encoding ZIP transporters [35, 36]. Among 14 transporters, the sequential similarities exist between PrP^C and ZIP5, ZIP6 and ZIP10. Taylor et al. reported that ZIP6 and ZIP10 form heteromers similar to PrP structures and influences cell migration [37]. PrP^C colocalizes with ZIP5 and forms dimers [38]. These findings strongly suggest that PrP^C plays important roles in the neuronal regulation of Zn. Watt et al. reported that PrP^C-enhanced cellular uptake of Zn via binding with the AMPA-type glutamate receptor and that PrP^C acts as Zn sensor in the synapse [39]. Indeed, PrP facilitates Zn influx into the brain, regulates Zn homeostasis and attenuates Zn-induced neurotoxicity.

2.3. Fe and prion protein

Fe is the most abundant metal in the brain as well as in the entire body. Fe is essential for numerous biological functions as an enzyme cofactor for metabolic processes such as the oxygen transport, oxidative phosphorylation and energy transfer. Fe has critical roles in specialized brain functions such as the synthesis of dopaminergic neurotransmitters and myelination. Therefore, Fe deficiency impairs learning, especially in children or infants. The Fe deficiency impairs working ability or learning ability also in adults. However, excess Fe can generate reactive oxygen species (ROS) that damage DNA, proteins and lipids and can therefore be toxic to neurons.

Fe exists in two different forms, ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}). In general, oxidized Fe^{3+} is insoluble and exists extracellularly, whereas reduced Fe^{2+} is soluble and intracellularly located. Orally administrated Fe is primarily absorbed from the gastrointestinal pathway via divalent metal transporter-1 (DMT-1) as Fe^{2+}. Once it enters the circulation, Fe^{2+} ions are oxidized into Fe^{3+} by ferroxidases such as ferritin or ceruloplasmin. Transferrin, an iron transporter protein, binds two Fe^{3+} ions. Transferrin-bound iron (Fe^{3+}) crosses the blood brain barrier (BBB) via transferrin receptors and enters into cells. Finally, Fe^{3+} is reduced into Fe^{2+} by ferrireductase and functions as a cofactor for neuronal enzymes such as tyrosine hydroxylase, which is necessary for the dopamine synthesis. Thus, Fe levels, as well as the ratio between Fe^{2+} and Fe^{3+}, are strictly regulated in normal brains.

Increasing evidence suggests that PrP^C is involved in Fe homeostasis [40]. PrP^C reportedly has ferrireductase activity and modulates the cellular uptake of Fe [41]. Tripathi et al. demonstrated that PrP induces the conversion from Fe^{3+} to Fe^{2+} and then Fe^{2+} is intracellularly transported by ZIP14/DMT-1 [42]. PrP-knockout mice exhibit altered Fe metabolism [43]. PrP is cotransported with ferritin, an iron-binding protein. Moreover, a decreased level of transferrin was observed in the cerebrospinal fluid of CJD patients [44].

2.4. Mn and prion protein

There are several studies suggesting that Mn may be a facilitator of prion diseases. Mn is an essential trace elements and crucial for various enzymes such as hydrolase, glutamine synthetase, arginase and pyruvate carboxylase [45]. However, excess Mn is neurotoxic and induces Parkinson's disease-like syndrome.

Johnson et al. investigated the levels of trace elements in prion-infected hamster brains using X-ray photoelectron emission microscopy with synchrotron radiation and found reduced Cu and increased Mn in prion protein plaques [11]. Thackray et al. reported that PrP^{C} loses its SOD-like activity when Cu is replaced with Mn [46]. Mn enhances the survival of PrP in model soils and increases its infectivity [47]. The risk of a prion disease in elk, termed chronic wasting disease, was associated with an Mg deficiency and increased Mn concentrations [48]. A recent epidemiological survey suggests a relationship between the pathogenesis of CJD and the imbalance of Mn [49]. Moreover, the impairment of Mn transporter is reportedly involved in the infection process [50].

3. Metal and PrP^{Sc} neurotoxicity

3.1. Metal-induced conformational changes of prion protein and its neurotoxicity

The conformational changes and neurotoxicity of PrP^{Sc} are central for the transmission and the pathogenesis of prion disease. To investigate the neurotoxicity of PrP^{Sc}, we and other researchers have employed synthetic fragment peptides of PrP (PrP106-126) as a model peptide of PrP^{Sc}, considering the methodological difficulties of using a whole prion protein owing to its strong infectious characteristics [51]. The structure of PrP106-126 coincides with the proposed β-sheet structures of PrP^{Sc} [52]. PrP106-126 forms aggregates with β-sheet structures as amyloid fibrils that share several characteristics of PrP^{Sc}, causes the apoptotic death of cultured neurons or glial cells and possess the ability to bind to metals including Cu^{2+} and Zn^{2+}.

We synthesized three fragment peptides: PrP77-83 (WGQPHGGGWGQPHGGG), PrP106-126 (KTNMKHMAGAAAAGAVVGGLG) and PrP144-157 (DYEDRHRENMHRY). All three peptides attenuated Cu^{2+}-induced neurotoxicity [51]. Although PrP77-83 and PrP144-157 are not neurotoxic, PrP106-126 forms β-sheet structures during the "aging" process (the incubation at 37°C for several days), as determined using the thioflavin T (ThT) fluorescence assay, far-UV circular dichroism (CD) spectroscopy and atomic force microscopy (AFM) imaging. Moreover, aged PrP106-126 exhibits enhanced neurotoxicity on primary cultured rat hippocampal neurons. Thus, we added various trace elements or metal chelators to solutions of PrP106-126 during the aging process and evaluated its conformational changes and neurotoxicity. We found that the coexistence of Zn^{2+} or Cu^{2+} during the aging process significantly attenuated the neurotoxicity of PrP106-126. Moreover, the presence of Al^{3+}, Fe^{2+} and Fe^{3+} did not result in significant changes. We also observed the oligomerization of PrP106-126 using the fluorescent changes of ThT, which binds with pleated β-sheet structures. The ThT fluorescence of solutions of aged PrP106-126 increased compared to freshly dissolved PrP106-126 solutions. The ThT

fluorescence of aged PrP106-126 with Zn^{2+}, Fe^{2+}, or Fe^{3+} was significantly decreased compared to aged PrP106-126 alone. In particular, the addition of Cu^{2+} dramatically decreased ThT fluorescence to the same level as fresh PrP106-126. Furthermore, aged PrP106-126 forms amyloid fibrils with distinct straight and long morphology on mica plates, as observed using AFM, although we did not observe fiber-like structures in freshly prepared PrP106-126. Moreover, aged PrP106-126 with Cu or Zn exhibited different morphological features compared to aged PrP106-126 alone. Therefore, it is possible that Cu^{2+} and Zn^{2+} influenced the β-sheet formation of PrP106-126 and thereafter attenuated its neurotoxicity. Our results are consistent with other studies that demonstrate that Cu^{2+} inhibited the ß-sheet formation of PrP111-126 or that Cu^{2+} inhibited the conformational changes of the larger fragment [53]. Thakur et al. reported that Cu^{2+} did not induce the oligomerization of PrP at physiological temperature and that Cu^{2+} may act as an attenuator in prion diseases [54].

3.2. Molecular mechanism of the neurotoxicity induced by PrP106-126

Understanding the pathway for PrP^{Sc}-induced neurodegeneration is of particular importance for identifying substances that protect against prion diseases. PrP106-126 causes adverse effects, such as the proliferation of microglia, the induction of pro-inflammatory responses, the production of reactive oxygen species (ROS) and the activation of ER stress. Our review focuses the disruption of Ca homeostasis, because Ca^{2+} ions are required for various functions of key enzymes such as kinases, phosphatases and proteases. The disruption of neuronal Ca homeostasis and alteration of intracellular calcium Ca^{2+} levels ($[Ca^{2+}]_i$) activated, various apoptotic proteins such as calpain and caspase, leading to neuronal death. The disruption of Ca homeostasis might trigger various adverse effects that are also associated with prion diseases.

Support of this idea was first demonstrated in a study of the neurotoxicity of AβP [55–57]. In 1993, Arispe et al. demonstrated that AβP(1–40), i.e., the first 40-residues of AβP, directly incorporates into artificial lipid bilayer membranes and forms cation-selective ion channels [58]. The channels, termed "amyloid channels," are giant multilevel pores and can allow a large amount of Ca^{2+} to pass through them. Their activity can be blocked by Zn^{2+} ions. We observed the appearance of amyloid channels on membrane patches from a neuroblastoma cell line (GT1-7 cells). GT1-7 cells possess neuron-like characteristics, such as the expression of neuron-specific proteins, the expression of various channels and receptors and the extension of neuritis [59]. After the administration of AβP(1–40), the current derived from the amyloid channels appeared. The activity of amyloid channels was inhibited by the addition of Zn^{2+} and recovered by the administration of *o*-phenanthroline (a Zn chelator). Based on these findings, we proposed the hypothesis termed the "amyloid channel hypothesis." This hypothesis demonstrates that the direct binding of AβPs on membranes and the subsequent disruption of Ca^{2+} homeostasis through amyloid channels might be the primary event in AβP neurotoxicity. AβP might have a similar mechanism of toxicity as that underlies the toxicity of various antimicrobial or antifungal peptides that also exhibit channel-forming activity and cell toxicity [60]. Indeed, Soscia et al. demonstrated that AβP exerts antimicrobial activity against eight common and clinically relevant microorganisms [61]. Furthermore, the presence of pore-

like structures of AβPs was demonstrated in the neuronal cell membrane of the brains of AD patients and AD-model mice [62].

Increasing evidence suggests that other amyloidogenic proteins also form pores and disrupts Ca homeostasis. Lashuel et al. used electron microscopy to show that α-synuclein, a DLB-related protein with 141 amino acid residues, forms annular pore-like structures [63]. Lal et al. investigated the oligomerization and conformational changes of AβP, α-synuclein, islet amyloid peptide (amylin) and other amyloidogenic proteins using gel electrophoresis and AFM imaging [64]. Their results demonstrate that these amyloidogenic proteins form annular channel-like structures on bilayer membranes. Electrophysiological and morphological studies have revealed that PrP forms amyloid channels similar to AβP. PrP106-126 reportedly forms cation-permeable pores in artificial lipid bilayers as well as AβP [65]. Zn^{2+} also inhibited the activity of PrP channels. Kourie et al. found that PrP106-126 was directly incorporated into the lipid bilayers and formed cation-selective, Cu-sensitive ion channels and that quinacrine (a potent therapeutic drug for prion diseases) inhibited the currents induced by PrP channels [66]. Demuro et al. reported that AβP, human amylin, prion and polyglutamine increased the elevation of $[Ca^{2+}]_i$ in a conformation-dependent manner [67]. Furthermore, a recombinant PrP protein (PrP90-231) formed channels through artificial lipid bilayers [68]. PrP has microbial activity similar to AβP [69].

We observed temporal changes in $[Ca^{2+}]_i$ within GT1-7 cells using a high-resolution multisite video imaging system with fura-2 as the cytosolic free fluorescent calcium reporter probe [70]. Shortly after exposure to PrP106-126, a marked increase in $[Ca^{2+}]_i$ occurred within many neurons, as well as an increase in AβP. Moreover, scrambled PrP106-126 (a nontoxic and nonamyloidogenic analogue with the random sequence of PrP106-126) did not cause such an elevation. These findings strongly suggest that the disruption of Ca homeostasis via unregulated amyloid channels may be the molecular basis of the neurotoxicity of prion diseases and other conformational diseases.

3.3. Protective substances against PrP106-126-induced neurotoxicity

Substances that prevent the neurotoxicity of PrP^{Sc} are of particular interest for screening preventive drugs for the treatment of prion diseases. Trace elements can induce conformational changes in AβP; therefore, clioquinol (5-chloro-7-iodo-8-quinolinol), a chelator of Zn and Cu and its derivatives have been used in therapeutic trials for AD [71]. Clioquinol also affected scrapie-induced memory impairment [72] and D-(-)-penicillamine, a Cu^{2+}-specific chelator, attenuated the pathogenesis of prion diseases *in vivo* [73]. Furthermore, small peptides, such as that the β-sheet breaker peptide, inhibit the conformational changes of PrP and AβP [74]. Our survey for substances that protect against PrP106-126-induced neurotoxicity revealed that carnosine (β-alaninyl histidine) might be a candidate for treatment of prion diseases. Carnosine is a water-soluble dipeptide that is contained in mammalian muscle and brain and particularly in the olfactory bulbs [75]. Carnosine has antioxidant, anticross-linking, antiglycosylation activities and the ability to bind to metals (**Figure 2**). Carnosine inhibits the oligomerization of AβP and attenuates neurodegeneration in AD model mice [76]. We have reported that carnosine inhibits Zn^{2+}-induced neuronal death

which plays a central role in the pathogenesis of vascular-type senile dementia [77]. Considering these beneficial characteristics of carnosine, carnosine may act as a neuroprotector in the brain. Based on these findings, we published a patent for carnosine as a possible target for drugs for vascular type senile dementia [78].

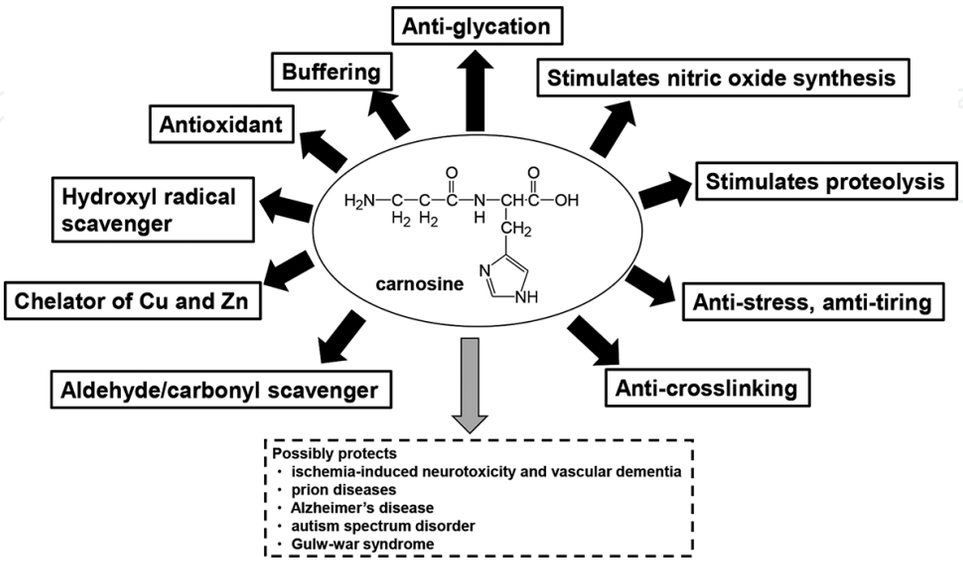


Figure 2. Structure and roles of carnosine.

4. Hypothesis: link between trace elements in the pathogenesis of prion diseases

4.1. PrP^C as a controller of metal homeostasis at the synapse

Considering the results of our study together with those of the other studies, we propose the following hypothetical mechanism regarding the role of PrP and the neurodegeneration processes underlying prion diseases (**Figure 3**). PrP^C and other various proteins including Alzheimer's APP, receptors, such as the NMDA-type glutamate receptor or AMPA-type glutamate receptor, are colocalized at the synapse. Synapses are critical nodes for the processing of neural information and the memory formation in neural networks. Thus, disorders of the synapses are the primary symptoms of neurodegenerative diseases. APP is localized to the presynaptic region of synapses and AβP is secreted into synaptic clefts in the presence of neuronal stimuli [79]. Meanwhile, PrP^C is localized to the postsynaptic membrane and is coupled to glutamate receptors [24]. Both of PrP^C and APP are metal-binding proteins and regulate metal homeostasis. The synaptic cleft is considered to be cylindrical with a radius of 120 nm and a height of 20 nm; it composes ~1% of the extracellular volume and ~20% of the total brain volume [80]. Zn and Cu are released into this small compartment at the micromolar levels. Therefore, APP and PrP^C likely interact with each other in this small compartment, which is filled with Zn and Cu.

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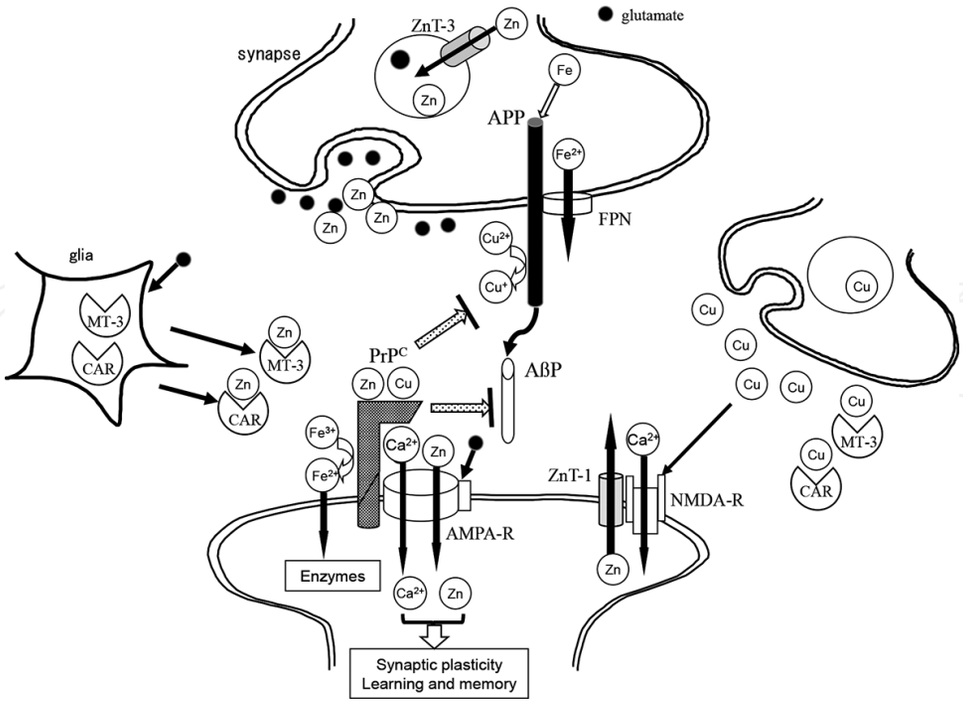


Figure 3. Hypothetical model of the physiological roles of normal prion protein at the synapse. Zn^{2+} and glutamate accumulate in synaptic vesicles and are released into synaptic clefts during neuronal excitation. Zn^{2+} regulates Ca^{2+} influx through glutamate receptors (NMDA-R, Ca-A/K), modulates neuronal information and is implicated in the maintenance of synaptic plasticity and memory formation, similar to Ca^{2+}. Zn has important roles in neural communication. Cu^{2+} is also released into synaptic vesicles and regulates various receptors, similar to Zn^{2+}. APP is present in the presynaptic domain and PrP^C is localized to the postsynaptic domain. These proteins are closely associated with the synaptic cleft and have cytoprotective roles via the regulation of metal homeostasis and protection against free radicals. PrP^C binds to the AMPA-type glutamate receptors and regulates Zn^{2+} levels similar to ZIP Zn transporters. Additionally, the ZnT-1 Zn transporter is localized to postsynaptic membranes that express NMDA-type glutamate receptors and regulates Zn homeostasis. Moreover, PrP^C has SOD activity and also regulates Cu^{2+} levels, which influence APP processing. APP converts Cu^{2+} into Cu^{+} and regulates Cu at the synapse. PrP^C has ferrireductase activity, which converts Fe^{3+} to Fe^{2+}. APP binds to ferroportin and thereby regulates Fe^{2+} efflux. MT-3 and carnosine are released from glial cells, into synaptic clefts and are also implicated in the regulation of excess Zn. Abbreviations: ZnT-1, zinc transporter 1; AMPA-R, AMPA-type glutamate receptor; NMDA-R, NMDA-type glutamate receptor; MT-3, metallothionein 3.

PrP^C regulates the cellular uptake of Cu, Zn and Fe as discussed previously. PrP^C, an analogue of ZIP transporters, is localized to the postsynaptic membrane, binding with the AMPA-type glutamate receptor, which facilitates Zn influx. The ZnT-1 transporter, which enhances Zn efflux into the extracellular compartment, is also localized to the postsynaptic membrane and regulates the activity of the NMDA-type glutamate receptor [81, 82]. Thus, synaptic Zn levels are likely controlled by both ZnT-1 and PrP^C. In comparison, MT-3 that is secreted from neurons or glia may regulate Zn homeostasis at synapses. Uchida et al. found that neuronal growth inhibitory factor (GIF) that inhibits neurite extensions and prevents neuronal death was decreased in the brains of AD patients and determined that GIF is equivalent to MT-3 [83]. Therefore, MT-3 (GIF) is implicated in AD-associated neuronal death. Considering our results and other numerous findings, carnosine may be another regulator of metal homeostasis in the synapse, similar to MT-3; carnosine is synthesized in glial cells (astrocytes and oligodendrocytes) and secreted into synaptic clefts after the glutamate response.

PrP^C also regulates the level of Fe and the ratio between Fe^{3+} and Fe^{2+} as a ferrireductase. APP binds to a Fe transporter, ferroportin and regulates Fe efflux. Furthermore, PrP^C regulates Cu, which influences the expression of APP and the production of AβP. The roles of APP and PrP^C in the maintenance of metal homeostasis are essential for normal brain function; therefore, a disruption of this homeostasis may trigger the degeneration of synapses and ultimately lead to the pathogenesis observed in AD or prion diseases. Recent studies demonstrate that PrP^C plays critical roles in the cleavage of APP and the regulation of AβP levels [84]. PrP^C reportedly attenuates the oligomerization of AβP and the neurotoxicity [85]. Considering these results together, PrP^C is crucial for neuroprotection and the regulation of various neuronal processes because it modulates SOD, protects cells from free radicals and controls AβP neurotoxicity.

However, under pathological conditions (**Figure 4**), PrP^{Sc} enters into the brain via the ingestion of contaminated food, for example and then triggers the conformational conversion of PrP^C into PrP^{Sc}. Mn may act in these pathways. The loss of neuroprotective PrP^C induces the dyshomeostasis of trace elements and ultimately leads to apoptotic death of neurons. Excess Zn or Cu influences APP, induces conformational changes of AβP and enhances its neurotoxicity. Oligomerized PrP^{Sc}, as well as AβP, form pores in synaptic membrane, causing the disruption of Ca homeostasis, ultimately leading to apoptotic neuronal death. This working hypothesis offers insight into the mechanism of prion diseases.

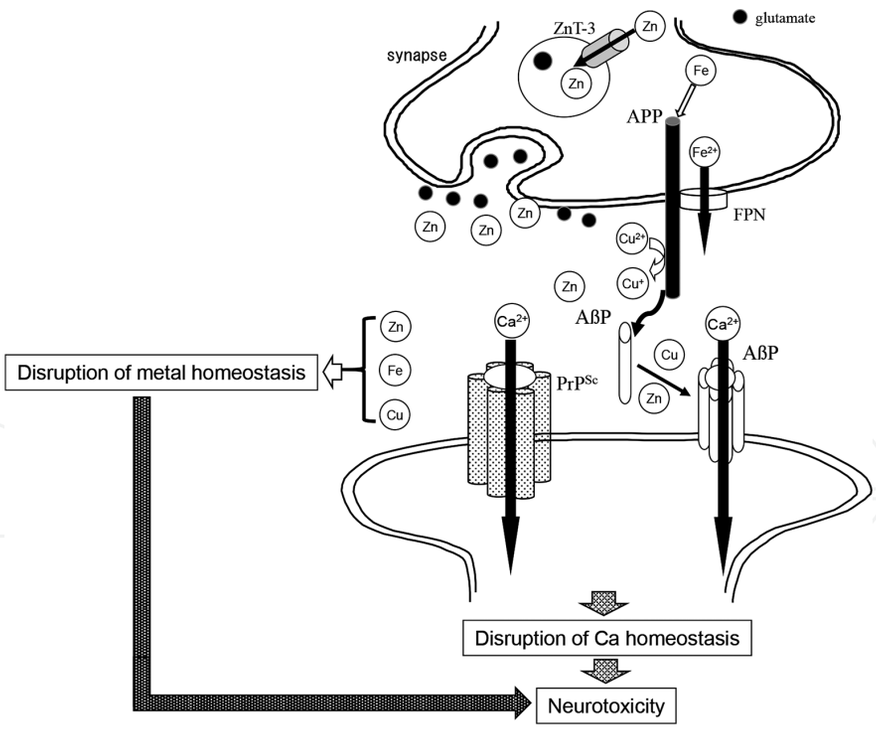


Figure 4. Hypothetical model of prion disease pathogenesis. In the pathological condition, PrP^{Sc} enters the brain and triggers the conversion of PrP^C, which depletes PrP^C and accumulates of PrP^{Sc}. The loss of the neuroprotective functions of PrP^C induces oxidative stress, enhance AβP neurotoxicity and ultimately lead to neuronal death. The accumulated PrP^{Sc} forms Ca^{2+}-permeable pores in the membrane and disrupted Ca^{2+} homeostasis. The imbalance of metals at the synapse triggers conformational changes in AβP, which enhances its neurotoxicity via the formation of Ca^{2+}-permeable pores. Subsequent Ca^{2+} dyshomeostasis leads to neuronal death.

In conclusion, our results shed light on the enigmatic roles of trace elements in the pathogenesis of prion diseases. However, further research is necessary particularly regarding the inhibitory mechanism of carnosine and the development of possible protective agents for prion diseases.

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