Chapter 9

Oligomerization of Proteins and Neurodegenerative Diseases

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Additional information is available at the end of the chapter

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1. Introduction

Oligomerization of amino acids by binding their peptide bonds (-CONH-) forms proteins (or peptides), which are the major components of our bodies. Although the primary sequence (the linear sequence of amino acids) of the protein mainly determines its characteristics, its secondary structures (the conformations) are also critical determinants of their shapes and functions. The conformation (random coil, α-helix, and ß-sheet) is restricted by the circumstances nearby proteins. The hydrogen bond between the amino acids in the peptide chain forms the α-helix structure. Meanwhile, the ß-sheets (ß-plated sheets) consist of ß-strands which are laterally connected peptide bonds with hydrogen bonds.

Recent neurochemical evidence indicates that the oligomerization of proteins and the formation of ß-sheet structures are linked with several neurodegenerative diseases such as Alzheimer's disease (AD), prion diseases, triplet repeat diseases, dementia with Lewy bodies (DLB). The disease-related proteins, such as ß-amyloid protein (AßP) in AD, prion protein in prion diseases, polyglutamine in triplet repeat disease, α-synuclein in DLB, are identical in each disease (Table 1). However, all of these amyloidogenic proteins share common characteristics in the formation of amyloid with ß-sheet structures, and in the exhibition of cytotoxicity. Therefore, a new concept termed "conformational disease" was proposed, suggesting that protein conformation is an important determinant of its toxicity, and consequently, the development of the related disease [1].

These conformational diseases are included in amyloidosis. At 1853, Virchow found the abnormal accumulates in tissues and named "amyloid", since they exhibited similar characteristics with *amylum*. At 1968, amyloid was determined to be the oligomers of proteins with ß-sheet structures. The accumulation of amyloid causes various diseases (amyloidosis) including familial amyloid polyneuropathy (FAP), amyloid-light chain amyloidosis, dialysis

Disease	The primary sequence of amyloidogenic protein or its fragment peptide	Metal	ß-sheet formation	Cyto-toxicity	Pore-formation
Alzheimer's disease	*AßP(1-42) and AßP(1-42)truncated C-terminal* *DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG VVIA*	Al, Zn, Cu, Fe	+	+	+
Prion disease	Prion protein: *PrP106-126* MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGG SRYPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPH GGGWGQPHGGGWGQPHGGGWGQGGGTHSQWN KPSKP*KTNMKHMAGAAAAGAVVGG*LGGYVLGSAMS RPIIHFGSDYEDRYYRENMHRYPNQVYYRPMDEYSNQ NNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVV EQMCITQYERESQAYYQRGSSMVLFSSPPVILLISFLIFL IVG	Zn, Cu, M, Fe	+	+	+
Dementia with Lewy bodies (DLB)	α-synuclein; *NAC* (a *fragment of α-synuclein*) MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEG VLYVGSKTKEGVVHGVTTVAEKTKEQVSNVGGAVVTG VTAVAHKTVEGAGNFAAATGLVKKDQKNESGFGPEG TMENSENMPVNPNNETYEMPPEEEYQDYDPEA	Cu, Fe, Al	+	+	+
Triplet- repeat disease	*Polyglutamine* MATLEKLMKAFESLKSF*QQQQQQQQQQQQQQQQQQ QQQQQQ*PPPPPPPPPPQLPQPPPQAQPLLPQPQPPPP PPPPPPGP	Fe	+	+	+
Diabetes mellitus	*Human amylin* KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	Cu, Al	+	+	+

* The sequence of fragment peptide of each amyloidogenic protein (PrP106-126, NAC, polyglutamine) is indicated in italic form.

Table 1. Characteristics of amyloidogenic proteins and the related peptides

amyloidosis, *etc* [2]. All of theses diseases share common properties about the deposition of amyloids in various tissues or organs with protease-resistant, insoluble fibril-like structures (amyloid fibrils), and stained by congo-red, ß-sheet specific dye. However, the component of amyloid is different in each disease. For example, the major component of amyloid in FAP patients is transthyretin, and ß2-microglobulin deposits in patients with dialysis amyloidosis. There are no effective treatments for amyloidosis.

In this chapter, we review the implication of protein oligomerization in the pathogenesis of these neurodegenerative diseases. Considering that the amyloidogenic proteins are commonly present in our brain, factors which influence oligomerization play crucial roles in their pathogenesis. As such factors, we focus on trace elements such as Al, Zn, Cu, and Fe. Metals have a property of firmly binding to metal-binding residues of proteins, such as tyrosine (Tyr)

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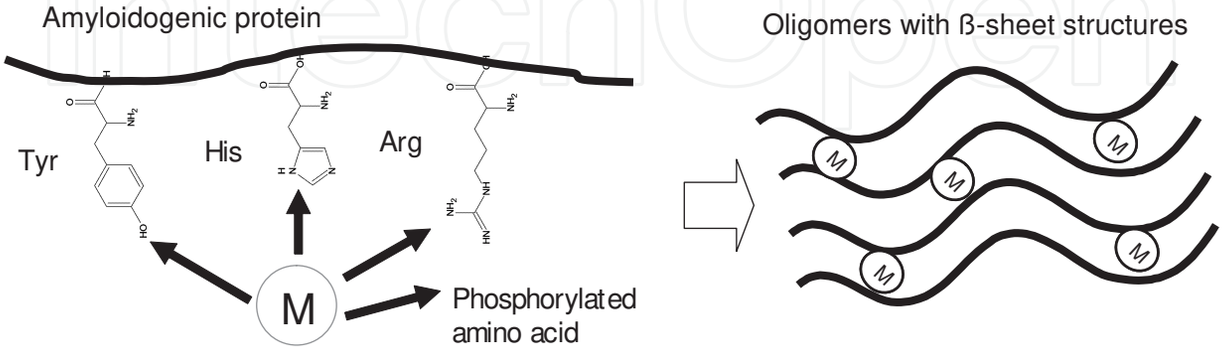
or histidine (His) or phosphorylated amino acids, and cause cross-linking of the proteins (Fig. 1). Furthermore, all of these amyloidogenic proteins were reported to have the ability to bind metals as shown in Table 1. Our and other numerous studies reported that oligomers cause neurodegeneration by induction of Ca^{2+} dyshomeostasis through the formation of amyloid channels on neuronal membranes [3,4]. The beneficial characteristics of carnosine (ß alanyl histidine) as a drug for the treatment for these neurodegenerative diseases are also discussed.



M stands for metal.

Figure 1. Trace elements acts cross-linkers of amyloidogenic proteins.

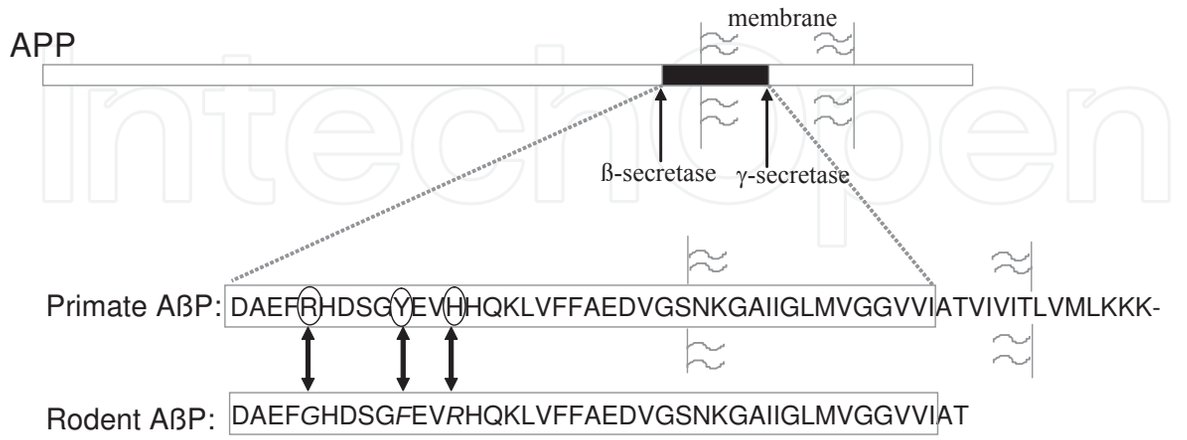
2. Alzheimer's disease and oligomerization of AßP

2.1. Amyloid cascade hypothesis

Alzheimer's disease (AD) is a severe type of senile dementia, affecting a large portion of elderly people worldwide. It is characterized by profound memory loss and inability to form new memories. The pathological hallmarks of AD are the presence of numerous extracellular deposits (senile plaques) and intraneuronal neurofibrillary tangles (NFTs). The degeneration of synapses and neurons in the hippocampus or cerebral cortex is also observed. The major components of NFTs are phosphorylated tau proteins, and that of senile plaques are ß-amyloid proteins (AßPs) [5]. Although the precise cause of AD remains elusive, numerous biochemical, cell biological, and genetic studies have supported the idea termed "amyloid cascade hypothesis" that the AßP accumulation and the consequent neurodegeneration play a central role in AD [6]. Moreover, recent studies on the identified AßP species have indicated that the oligomerization of AßP and the conformational changes are critical in the neurodegeneration process [7].

AßP is a small peptide of 39–43 amino acid long. It is derived from the proteolytic cleavage of a large precursor protein (amyloid precursor protein; APP). AßP is secreted by the cleavage of its N-terminal by ß-secretase (BACE), followed by the intra-membrane cleavage of its C-terminal by γ-secretase. Genetic studies of early-onset cases of familial AD indicated that APP mutations and AßP metabolism are associated with AD. It was also revealed that mutations

in the presenilin genes account for the majority of cases of early-onset familial AD. Presenilins have been revealed to be one of γ -secretases, and their mutations also influence the production of AßP and its neurotoxicity.



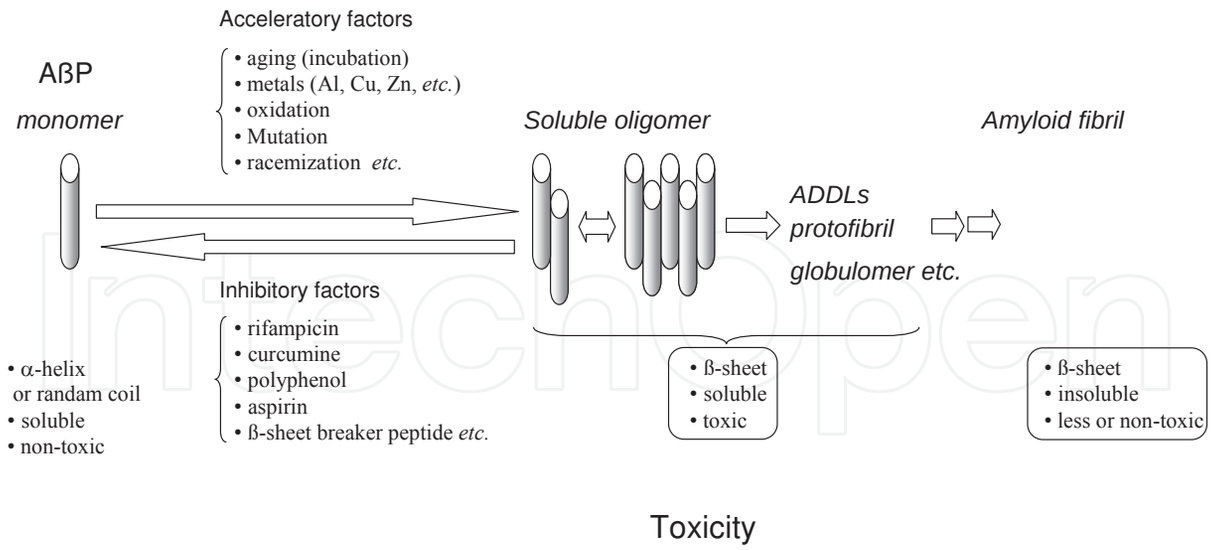
AßP is secreted from its precursor protein, APP by transmembrane cleavage. Sequences of primate AßP(1-42) and rodent AßP(1-42) are shown. The comparison between the sequence of primate (human or monkey) Aß(1-42) and rodent (rat or mouse) Aß(1-42) is depicted.

Figure 2. Structure of AßP

Yankner *et al.* reported that the first 40 amino acid residues of AßP (AßP(1–40)) caused the death of cultured rat hippocampal neurons or the neurodegeneration in the brains of experimental animals. Thereafter, it was agreed upon that the aggregation and the subsequent conformational change of AßP contribute to its neurotoxicity. AßP is a hydrophobic peptide with an intrinsic tendency to self-assemble to form insoluble oligomers with ß-pleated sheet structures. Pike *et al.* revealed that aged AßP(1–40) (aggregated under incubation at 37°C for several days) were considerably more toxic to cultured neurons as compared to freshly prepared AßP(1–40). Simmons *et al*. revealed ß-sheet contents of AßP observed by circular dichroism (CD) spectroscopy correlates with its neurotoxicity.

Furthermore, the longer peptide variant, AßP(1–42), has the characteristics of immediate polymerization compared to AßP(1–40). AßP(1–42) enhances the aggregation of AßP(1–40) and becomes a seed of the amyloid fibrils. AßP (1–42) is more abundant in the brains of AD patients as compared to those of age matched controls. The mutations of APP and those of presenilin genes induce the increased production of AßP (1–42) in the transfected cell lines.

Recent approaches using size-exclusion chromatography, gel electrophoresis, and atomic force microscopy have demonstrated that there are several stable types of soluble oligomers: naturally occurring soluble oligomers (dimers or trimers), ADDLs (AßP-derived diffusible ligands), AßP globulomers, or protofibrils. Hartley separated aggregated AßP(1–40) into low–molecular-weight (mainly monomer), protofibrillar, and fibril fractions by size-exclusion chromatography, and found that the protofibrillar fraction caused marked changes in the electrical activity of cultured neurons and neurotoxicity. Walsh *et al.* found that the intracere-



AβP monomers exhibit random or a-helix structures. However, under aging conditions or in the presence of some acceleratory factors, Aß self-aggregates and forms several types of oligomers (SDS-soluble oligomers, ADDLS, globulomers, or protofibrils *etc.*) and finally forms insoluble aggregates termed amyloid fibrils. Oligomeric soluble Aßs are toxic, although the monomeric and fibril ones are rather nontoxic.

Figure 3. Oligomerization of AβP

bral administration of the conditioned medium with cultured cells transfected with the human APP gene inhibited long-term potentiation (LTP), which is a form of synaptic information storage well known as a paradigm of memory mechanisms. They also demonstrated that LTP was blocked by SDS-stable low-molecular-weight oligomers (dimers, trimers, or tetramers) but not AßP monomers or larger aggregates. The natural AßP oligomers (derived from the cerebrospinal fluid of AD patients) cause the loss of dendritic spines, synapses, and LTP blockage. Klein and the colleagues reported that AßP-derived diffusible ligands (ADDLs) obtained from sedimentation by clustering are highly toxic to cultured neurons. They also reported that ADDLs inhibited LTP and exhibited adverse effects on synaptic plasticity such as abnormal spine morphology, decreased spine density, and decreased synaptic proteins. Based on these and other numerous findings, it is widely accepted that AßP oligomers are synaptotoxic and neurotoxic, but not monomer or fibrils. These studies further strengthened and modified the amyloid cascade hypothesis, which suggest that AßP oligomers are neurotoxic and crucial for the pathogeneis of AD [8,9].

2.2. Metal-induced oligomerization of AßP

Considering that AßP is secreted from APP into the brain of young people or of normal subjects, factors which influence (accelerate or delay) the oligomerization may become important determinants of the pathogenesis of AD. Various factors, such as the concentration of peptides, the oxidations, mutations, and racemization of AßP, pH, composition of solvents, temperature, and trace elements, can influence the oligomerization processes. A considerable amount of asparagines (Asp) or serine (Ser) residues of AßP accumulated in senile plaques are

racemized. Tomiyama *et al.* reported that racemized D-Asp^{23}-AßP easily aggregates compared to the L-type. Meanwhile, several substances such as rifampicin, curcumin, and aspirin have been reported to inhibit AßP oligomerization *in vitr*o. Rifampicin, a drug used to treat Hansen's disease, may be an interesting inhibitor of oligomerization since patients with Hansen's disease have a low susceptibility to AD. Aspirin and other NSAIDs (non-steroidal anti-inflammatory drugs) inhibit the AßP oligomerization and simultaneously attenuate inflammation.

Among these factors, trace elements such as aluminum (Al), zinc (Zn), copper (Cu), iron (Fe) are of particular interest. The accumulation of AßP is rarely observed in the brains of rodents (rats or mice) as compared to humans or monkeys. As shown in Fig.2, the amino acid sequence of human and rodent AßP are similar, yet they differ by three amino acids. However, rodent AßP exhibits less tendency to oligomerization compared to human AßP [10]. Considering that these three amino acids (Arg^{5}, Tyr^{10}, and His^{13}) have the ability to bind metals and that trace metals have cross-linking ability, trace elements might play important roles in the accumulation of AßP in the human brain.

Exley *et al.* first demonstrated that Al induces a conformational change in AßP(1-40) by CD spectroscopy. Furthermore, exposure to Al causes the accumulation of AßP in cultured neurons or in brains of experimental animals or human. Pratico *et al.* found that Al-fed mice transfected with the human APP gene (Tg 2576) exhibited pathological changes similar to those of the AD brain, including a marked increase in the amount of AßP both in the secreted form and the accumulated form: an increased deposition of senile plaques was also observed [11]. The neuropathological case study of the accidental Al-exposure that occurred in 1988 at Camelford (Cornwall, U.K.) indicated that the exposure to Al, even if it is short-term, could cause the accumulation of AßP and exhibit severe amyloid angiopathy [12]. Since there have been studies indicating the link between Al in drinking water and the pathogenesis of AD, Al-induced oligomerization may directly implicated in AD pathogenesis [13].

Bush *et al.* demonstrated that Zn^{2+} and Cu^{2+}caused the oligomerization of AβP [14]. However, the metal-induced oligomerization of AßP and other amyloidogenic proteins are complex and controversial. The morphology of AßP oligomers treated with Al, Cu, Fe, and Zn were reported to be quite different. Zatta and his colleagues demonstrated that metals including Al, Cu, Fe, Zn differentially alter the oligomerization of AßP and its toxicity. We have shown that Al enhances the polymerization of AßP(1-40) and forms SDS-stable oligomers *in vitro* by immunoblotting and precipitation [15,16]. The oligomerized AßP(1-40) is heat- or SDS-stable but redissolves on adding deferoxamine, a chelator of Al. The oligomerization induced by Al is more marked than that induced by other metals, including Zn, Fe, Cu, and Cd. Furthermore, while Zn-aggregated AßPs are rarely observed on the surface of cultured neurons several days after its exposure, Al-aggregated AßPs bind tightly to the surface of cultured neurons and form fibrillar deposits. These results suggest that Al-induced AßP oligomers have a strong affinity to membrane surfaces and undergo minimal degradation by proteases compared to Zn-induced oligomers. Furthermore, AßP coupled with Al was reported to be highly toxic compared to normal AßP.

Considering the implications of metals in AD pathogenesis, chelation therapy for AD treatment is of great interest. Clioquinol (quinoform), a chelator of Cu^{2+} or Zn^{2+}, inhibits oligome-

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rization of AßP and attenuates the accumulation of amyloid in the brains of experimental animals. Clinical trials using its analogue PBT2 are under investigation. DFO, a chelator of Al and Fe, attenuates the decline of daily living skills in AD patients. Silicates, which couple with Al and reduce its toxicity, are also candidates for chelation therapy in AD [17].

2.2. Oligomerization-induced neurotoxicity of AßP

There is a considerable interest regarding the mechanisms by which AßP oligomers cause neurotoxicity. Exposure to AßP causes various adverse effects on neuronal survivals such as the production of reactive oxygen species, the induction of cytokines, the induction of endoplasmic reticulum (ER) stresses, and the abnormal increase of intracellular calcium levels ($[Ca^{2+}]i$), *etc* [18]. Although these effects may be interwoven, the disruption of Ca^{2+} homeostasis is regarded to be an important determinant considering it occurs upstream of the other effects [19,20]. Ca^{2+} ions are essential for the normal brain functions. They are involved with key enzymes such as kinases, phosphatases, and proteases. Therefore, its influx is severely controlled, and the intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) are strictly conserved by Ca^{2+} channels, *etc*. Ca^{2+} is also implicated in the phosphorylation of the tau protein or in APP sequestration. Increasing evidence indicates that presenilins are involved in capacitative Ca^{2+} entry or endoplasmic reticulum (ER) Ca^{2+} signaling, and that their mutations affect Ca^{2+}-regulated functions including AßP production [21].

There is considerable interest regarding the mechanism by which AßPs interact with neurons and disrupt Ca^{2+} homeostasis. In 1993, Arispe *et al.* first demonstrated that AßP(1–40) directly incorporates into artificial lipid bilayer membranes and forms cation-selective ion channels [22]. The channels termed "amyloid channels" were revealed to be giant multi-level pores and can allow a large amount of Ca^{2+} to pass through. Their activity was blocked by Zn^{2+} ions, which are abundantly present in the brain. Furthermore, soluble AßP oligomers but not amyloid fibrils were reported to increase the membrane permeability. Durell *et al.* proposed a 3-D structural model of the amyloid channels obtained from a computer simulation of the secondary structure of AßP(1–40) in membranes that showed 5- to 8-mers aggregating to form pore-like structures on the membranes. The multimeric (tetramer to hexamer) pore-like structures of AßPs on reconstituted membranes were observed using atomic force microscopy. Jang *et al.* established a model of amyloid channels on the membranes and observed that pentamer AßPs form pores, and their dimensions, shapes, and subunit organizations are in good agreement with AFM studies [23]. These results strongly support the hypothetical idea termed "amyloid channel hypothesis", which suggests that the direct incorporation of AßPs and the subsequent imbalances of Ca^{2+} and other ions through amyloid channels might be the primary event in AßP neurotoxicity. In this respect, AßP might share the mechanism of toxicity with a similar mechanism underlying the toxicity of various antimicrobial or antifungal peptides that also exhibit channel-forming activity and cell toxicity.

To determine whether AßPs form channels on neuronal cell membranes as well as artificial lipid bilayers, we employed membrane patches from a neuroblastoma cell line (GT1-7 cells), which exhibit several neuronal characteristics such as the extension of neuritis and the expression of neuron-specific proteins or receptors [24]. After exposing the excised membrane

patches of GT1-7 cells in the bath solution to AßP(1–40), the current derived from the amyloid channels appeared. The amyloid channels formed on the GT1-7 cell membranes were cation-selective, multilevel, voltage-independent, long-lasting ones; the channel activity was inhibited by the addition of Zn^{2+}, and recovered by a zinc chelator, *o*-phenanthroline [25]. These features were considerably similar to those observed on artificial lipid bilayers. Meanwhile, AßP(40–1), a peptide bearing the reversed sequence of AßP(1–40), did not form any channels. Thus, we can conclude that AßPs are directly incorporated into neuronal membranes to form calcium-permeable pores. In order to test the amyloid channel hypothesis, we examined whether AßP altered the $[Ca^{2+}]_i$ levels in neurons by a high-resolution multi-site video imaging system with fura-2 as the cytosolic free calcium reporter fluorescent probe. This multisite fluorometry system enables the simultaneous long-term observation of temporal changes in $[Ca^{2+}]_i$ of more than 50 neurons. We could observe AßP-induced abnormal increase in $[Ca^{2+}]_i$ in GT1-7 cells [26-28] as well as in primary cultured rat hippocampal neurons [29]. Shortly after exposure to AßP (1–40), a marked increase in $[Ca^{2+}]_i$ occurred among many, but not all neurons. We also observed apoptotic death of cultured neurons after the exposure to AßPs and the consequent rise in the $[Ca^{2+}]_i$ levels.

Considering the results of our study together with those of the other studies, we propose the following hypothetical scheme of neurodegeneration induced by oligomerization of AßP (Fig. 4).

AßPs are normally secreted from APP into the cerebrospinal fluid and are usually degraded proteolytically by neprilysin within a short period. However, upregulation of the AßP secretion from APP, or an increased ratio of AßP(1–42) to AßP(1–40) may render AßPs liable to be retained in the brain. It has been demonstrated that APP or presenilin gene mutations promote this process. AßP possesses positive charges at neutral pH. Therefore, the net charge of the outer membrane surface may be a determinant when secreted AßPs bind to cellular membranes (Fig.4 (A)). The distribution of phospholipids on cellular membranes is usually asymmetrical and negatively charged phospholipids such as PS exist on the inner membrane surfaces. Disruption of the assymetrical distribution is the first hallmark of apoptotic cell death [30]. Therefore, the binding of AßP to neuronal membranes seldom occur in normal and young brains. This idea may explain why AD occurs in aged subjects meanwhile AßPs are secreted in the brains of young subjects. After incorporation into the membrane, the conformation of AßPs change and the accumulated AßPs aggregate on the membranes (Fig. 4(B)). The ratio of cholesterol to phospholipids in the membrane may alter membrane fluidity, thereby affecting the process from step (A) to (B). AßP oligomerization *in vitro* will also enhance the channel formation velocity. Considering that natural oligomers (dimers or trimers) are more toxic as compared to monomers or fibrils, it is provable that these oligomers might form tetrameric or hexameric pores and exhibit neurotoxicity. Micro-circumstances on the membranes, such as rafts, are suitable locations that facilitate this process. Finally, aggregated AßP oligomers form ion channels (Fig.4 (C)) leading to the various neurodegenerative processes. The processes required for channel formation (from steps (A) to (C)) may require a long life span and determine the rate of the entire process. Unlike endogenous Ca^{2+} channels, these AßP channels are not regulated by usual blockers. Thus, once formed on membranes, a continuous flow of

$[Ca^{2+}]_i$ is initiated. However, zinc ions (Zn^{2+}), which are secreted into synaptic clefts in a neuronal activity-dependent manner, inhibit AßP-induced Ca^{2+} entry, and thus have a protective function in AD.

Once AßP channels are formed on neuronal membranes, homeostasis of Ca^{2+} and other-ion will be disrupted. Disruption of Ca^{2+} homeostasis triggers several apoptotic pathways such as the activation of calpain, the induction of caspase, and promote numerous degenerative processes, including the production of reactive oxygen species (ROS) and the phosphorylation of tau, thereby accelerating neuronal death. Mutations of presenilins cause disturbances in the capacitive Ca^{2+} entry and may influence these pathways. Free radicals also induce membrane disruption, by which unregulated Ca^{2+} influx is further amplified. The disruption of Ca^{2+} homeostasis also influences the production and processing of APP. Thus, a vicious cycle of neurodegeneration is initiated. This hypothesis explains the long delay in AD development; AD occurs only in senile subjects despite the fact that Aßs are normally secreted also in younger or in normal subjects. Various environmental factors, such as foods or trace metals, as well as genetic factors will influence these processes and contribute to AD pathogenesis [31].

3. Prion diseases and other amyloidosis

The disease-related amyloidogenic proteins exhibit similarities in the formation of ß-pleated sheet structures, abnormal deposition as amyloid fibrils in the tissues, and introduction of apoptotic degeneration. Prion diseases, including human kuru, Creutzfeldt-Jakob disease, and bovine spongiform encephalopathy (BSE), are associated with the conversion of a normal prion protein (PrP^C) to an abnormal scrapie isoform (PrP^{SC}) [32]. The ß-sheet region of PrP^{SC} is suggested to play a crucial role in its transmissible degenerative processes. A peptide fragment of PrP corresponding to residues 106–126 (PrP106–126) has been reported to cause death in cultured hippocampal neurons. We investigated the oligomerization of PrP106-126 and its neurotoxicity on primary cultured rat hippocampal neurons [33]. As AßP, PrP106-126 formed amyloid-like fibrils with ß–sheet structures by observation with atomic force microspope and by thioflavin T staining during the aging process. The oligomerization and formation ß-sheet structure enhanced the neurotoxicity of PrP106-126. The co-existence of Zn or Cu inhibited ß-sheet formation of PrP106-126 and attenuated its neurotoxicity. Furthermore, the thickness of PrP106-126 fibrils was decreased in the presence of Zn or Cu.

Electrophysiological and morphological studies have revealed that PrP106-126 exhibits similarities in the formation of amyloid channels as well as AßP [34]. Lin *et al.* reported that PrP106–126 forms cation-permeable pores in artificial lipid bilayers. The activity of PrP channels was also blocked by Zn^{2+}. Kourie *et al.* investigated the detailed characteristics of channels formed by PrP106–126, concluding that it was directly incorporated into lipid bilayers and formed cation-selective, copper-sensitive ion channels. They also revealed that quinacrine, a potent therapeutic drug, possibly blocks amyloid channels induced by PrP106-126.

The oligomerization and fibrillation of α-synuclein has been implicated in the formation of abnormal inclusions, termed Lewy bodies, and the etiology of dementia with Lewy bodies

(DLB). Non-amyloid component (NAC), a fragment peptide of α-synuclein, accumulates in Alzheimer's senile plaques and causes apoptotic neuronal death. Lashuel *et al.* demonstrated by electron microscope observation that α-synuclein forms annular pore-like structures [35].

The elongation of a polyglutamine-coding CAG triplet repeat in the responsible genes is based on the pathogenesis of triplet-repeat disease such as Huntington's disease or Machado-Joseph disease. Hirakura *et al.* reported that polyglutamine formed ion channels in lipid bilayers.

Lal *et al*. investigated the oligomerization and conformational changes of AßP, synuclein, amylin, and other amyloidogenic proteins using gel electrophoresis and AFM imaging, and demonstrated that these amyloidogenic proteins form annular channel-like structures on bilayer membranes [36]. We have demonstrated that these amyloidogenic peptide also cause the elevations in $[Ca^{2+}]_i$ as well as AßP [3,31]. Considering these results together as shown in Table 1, it is suggested that the oligomerization of disease-related amyloidogenic proteins and the introduction of apoptotic degeneration by disruption of calcium homeostasis *via* unregulated amyloid channels may be the molecular basis of neurotoxicity of these diseases.

4. Conclusion

This hypothesis about the pathogenesis of conformational diseases may help in the development of drugs for these diseases. We focus carnosine (ß-alanyl histidine) as such a protective drug. Carnosine is a naturally occurring dipeptide and is commonly present in vertebrate tissues, particularly within the skeletal muscles and nervous tissues [37]. It is found at high concentrations in the muscles of animals or fish which exhibit high levels of exercise, such as horses, chickens, and whales. Thus, it is believed that carnosine plays important roles in the buffering capacities of muscle tissue and the administration of carnosine has been reported to induce hyperactivity in animals.

Secretion from synapses of AßP, and its direct incorporation into membranes and formation of oligomeric amyloid channels are depicted. Details are discussed in the text.

In the brain, a considerable amount of carnosine is localized in the neurons of the olfactory bulb. It is secreted into synaptic clefts along with the excitatory neurotransmitter glutamate during neuronal excitation. Carnosine reportedly has several beneficial effects including the antioxidant activity, the chelating ability to metal ions, the inhibition of the Maillard reaction. Furthermore, carnosine is reported to have anti-crosslinking properties. Attanasio *et al.* reported that carnosine inhibited the fibrillation of alpha-crystallin. It was also demonstrated that carnosine inhibited the oligomerization and subsequent neurotoxicity of AßP. Corona *et al.* showed that dietary supplementation of carnosine attenuated mitochondrial dysfunction and the accumulation of AßP in Alzheimer's model mice [38]. We also showed that carnosine attenuated the neuronal death induced by prion protein fragment peptide (PrP106-126) by changing its conformation [33]. Carnosine level is significantly reduced in the serum of AD patients. These results suggest possible beneficial effects of carnosine as a treatment for AD and prion diseases. We also demonstrated that carnosine attenuates Zn-induced neuronal

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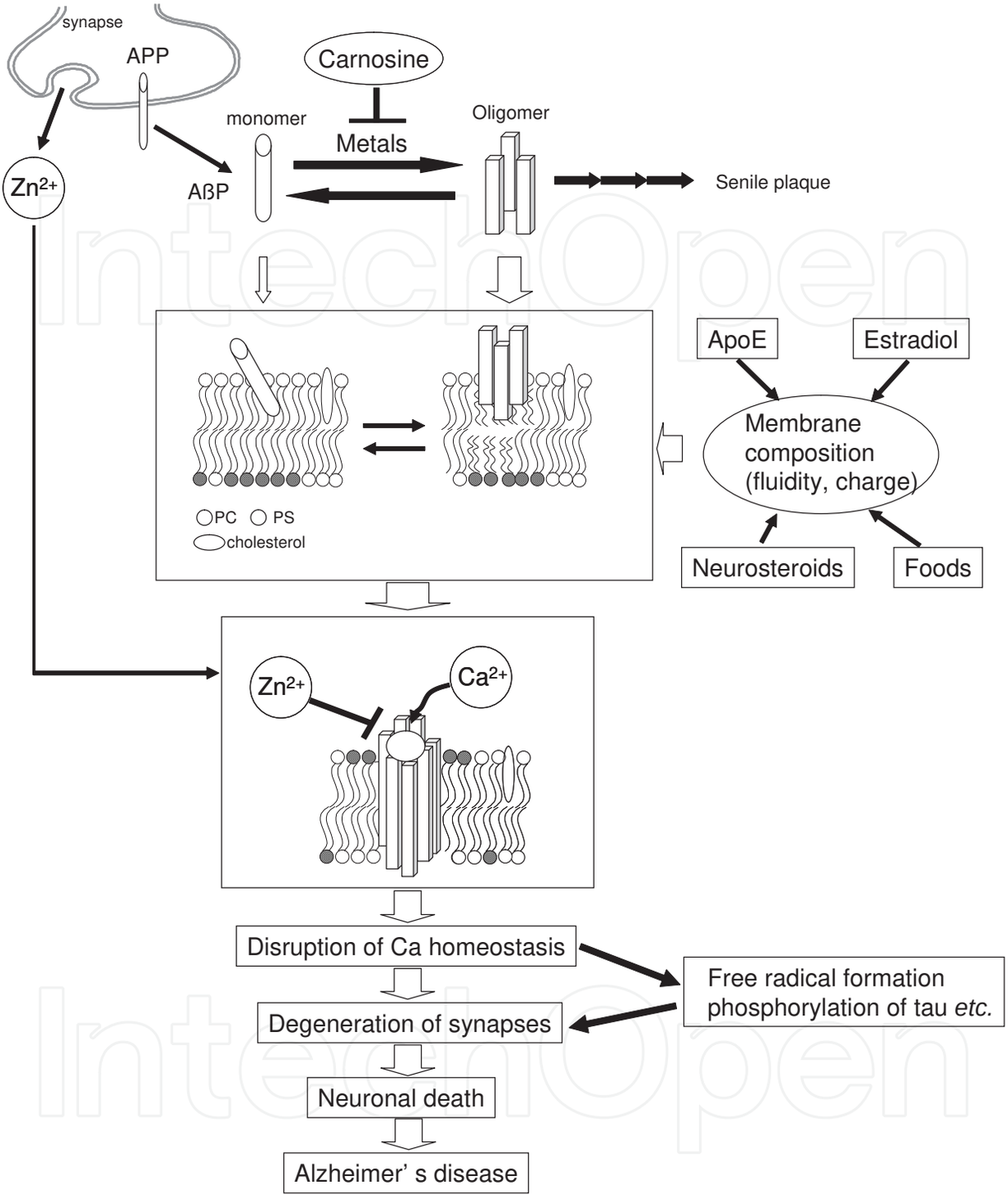

Figure 4. Amyloid channel hypothesis

death and becomes a candidate for drugs of vascular dementia [39,40]. All of these functions of carnosine (e.g., antioxidant, anti-glycating, anti-crosslinking, and scavenging toxic aldehydes) are related to the aging processes. The level of carnosine varies during development and is low in the aged animals. Therefore, it is highly possible that carnosine protects against

external toxins and acts as an endogenous protective substance against neuronal injury, senescence, and aging. We have applied patents for carnosine and related compounds as drugs for vascular type of senile dementia (Patent No. 5382633, Patent No. JP5294194).

In conclusion, further research into the role of protein oligomerization and Ca homeostasis via amyloid channels might lead to the development of new treatments for neurodegenerative diseases.

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