MINIREVIEW

Amyloid oligomers: formation and toxicity of Aβ oligomers

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Introduction

Alzheimer’s disease (AD) is an age-related, progressive degenerative disorder that is characterized by synapse and neuron loss in the brain and the accumulation of protein-containing deposits (referred to as ‘senile plaques’) and neurofibrillary tangles. Insoluble amyloid β-peptide (Aβ) fibrillar aggregates found in extracellular plaques have long been thought to cause the neurodegenerative cascades of AD. However, accumulating evidence suggests that prefibrillar soluble Aβ oligomers induce AD-related synaptic dysfunction. The size of Aβ oligomers is distributed over a wide molecular weight range (from < 10 kDa to > 100 kDa), with structural polymorphism in Aβ oligomers of similar sizes. Recent studies have demonstrated that Aβ can accumulate in living cells, as well as in extracellular spaces. This review summarizes current research on Aβ oligomers, focusing on their structures and toxicity mechanism. We also discuss possible formation mechanisms of intracellular and extracellular Aβ oligomers.

Keywords
Alzheimer’s disease; amyloid β; formation and toxicity mechanism; intracellular and extracellular oligomers; soluble amyloid oligomers

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(Received 4 September 2009, revised 11 December 2009, accepted 6 January 2010)

doi:10.1111/j.1742-4658.2010.07568.x

Abbreviations
AD, Alzheimer’s disease; ADDL, Aβ-derived diffusible ligand; APP, amyloid precursor protein; Aβ, amyloid-β peptide; ER, endoplasmic reticulum; FCS, fluorescence correlation spectroscopy; HD, Huntington’s disease; LTP, long-term potentiation; MW, molecular weight; NGF, nerve growth factor; NMDAR, N-methyl-d-aspartate (NMDA)-type glutamate receptor; PD, Parkinson’s disease; polyQ, polyglutamine; PrP⁰, cellular prion protein.

Alzheimer’s disease (AD) is an age-related, progressive degenerative disorder that is characterized by synapse and neuron loss in the brain and the accumulation of protein-containing deposits (referred to as ‘senile plaques’) and neurofibrillary tangles. Insoluble amyloid β-peptide (Aβ) fibrillar aggregates found in extracellular plaques have long been thought to cause the neurodegenerative cascades of AD. However, accumulating evidence suggests that prefibrillar soluble Aβ oligomers induce AD-related synaptic dysfunction. The size of Aβ oligomers is distributed over a wide molecular weight range (from < 10 kDa to > 100 kDa), with structural polymorphism in Aβ oligomers of similar sizes. Recent studies have demonstrated that Aβ can accumulate in living cells, as well as in extracellular spaces. This review summarizes current research on Aβ oligomers, focusing on their structures and toxicity mechanism. We also discuss possible formation mechanisms of intracellular and extracellular Aβ oligomers.
inhibit many critical neuronal activities, including long-term potentiation (LTP), a classic model for synaptic plasticity and memory loss in vivo and in culture [12–15]. These studies strongly support the idea that soluble Aβ oligomers are the causative agents of AD; however, the biological and structural characteristics of Aβ oligomers and their formation mechanism remain unclear.

**Structure and size of soluble Aβ oligomers**

Many types of natural and synthetic Aβ oligomers of different sizes and shapes have been reported, which accounts for their biological and structural diversity and for the complexity of AD pathology (reviewed in [4,5,9–11]). SDS-stable dimers and trimers have been found in the soluble fractions of human brain and amyloid plaque extracts, which suggests that these low-MW Aβ oligomers could be the fundamental building blocks of larger oligomers or insoluble amyloid fibrils [16–18]. Aβ oligomers of similar sizes have also been secreted from cultured cells and have been shown to inhibit LTP in vitro [14]. The high toxicity of low-MW Aβ oligomers is also supported by in vitro studies showing that Aβ dimers are threefold more toxic than monomers, and that Aβ tetramers are 13-fold more toxic [19].

Recently, Lesne et al. [13] demonstrated that the level of SDS-stable Aβ nonamers and dodecamers (referred to as Aβ*56) correlated with memory deficits in an APP transgenic Tg2576 mice model. Purified dodecamers also induced a significant fall-off in the spatial memory performance of wild-type rats. These results suggest that nonamers and dodecamers are associated with deleterious effects on cognition. However, it is unlikely that these oligomers alone cause brain dysfunction. For example, young Tg2576 mice showed decreased dendritic spine density in the dentate gyrus, impaired LTP and impaired contextual fear conditioning, all at an age before the first dodecamer was detected [13,20].

Another recent report also showed that the Aβ*56 levels are not correlated with memory deficits in a certain transgenic mice model [21]. These results suggest that Aβ*56 is not the only key determinant of memory impairment. These oligomers could be classified as low-MW (< 50 kDa-) oligomers. However, natural Aβ oligomers with a wide-ranging MW distribution (from < 10 kDa to > 100 kDa) have been found in the AD brain [22], suggesting that Aβ oligomers of various sizes are associated with the disease.

There are also many reports of toxic oligomers from synthetic Aβ. Synthetic Aβ forms fibrillar aggregates that have properties similar to those found in AD plaques in the brain. In vitro studies using synthetic Aβ are useful to complement efforts to determine the disease mechanism. Snyder et al. [23] detected the formation of soluble Aβ assemblies, rather than fibrils, using an analytical ultracentrifugation technique, and Lambert et al. [12] reported the formation of small Aβ globular oligomers (5 nm in diameter) in Hams-F12 medium, which were referred to as Aβ-derived diffusible ligands (ADDLs). Importantly, ADDLs strongly bound to the dendritic arbors of cultured neurons, caused neuronal cell death and blocked LTP. The finding of ADDL in soluble brain extracts from the human AD brain using ADDL-specific antibody supports the idea that the existence of ADDLs in the human AD brain causes disease [24].

The formation of annular Aβ oligomers, with an outer diameter of 8–12 nm and an inner diameter of 2.0–2.5 nm (150–250 kDa), has also been reported [25,26]. As these annular Aβ oligomers could be preferentially formed from mutant Aβ (such as those carrying the Arctic mutation), and because the amyloid ‘pore’ resembles bacterial cytolytic β-barrel pore-forming toxins, it has been suggested that these doughnut-like oligomers could be responsible for the Aβ-associated cytotoxicity [26]. The largest globular assemblies are amylospheroïds [27], which are highly neurotoxic, off-pathway, spheroidal structures with diameters of 10–15 nm.

Although Aβ oligomer structures at atomic resolution are unclear, studies using conformation-dependent antibodies suggest that structural variants could exist among even morphologically similar Aβ oligomers. A difference in antibody-binding properties indicates a difference in epitope exposure. For example, Glabe et al. used two antibodies – A11 and OC – which are specific for oligomers and fibrils, respectively. They proposed two distinct types of oligomers: prefibrillar oligomers that are A11-positive and OC-negative, and fibrillar oligomers that are A11-negative and OC-positive [10]. As prefibrillar oligomers are not recognized by the fibril-specific antibodies, and are considered to be transient intermediates in the fibril-formation process, a conformational change is necessary for them to become fibrils. It should be noted that the oligomer-specific A11 antibody also recognizes soluble oligomers from various proteins, such as those from α-synuclein, islet amyloid polypeptide, polyglutamine (PolyQ), lysozyme, human insulin and prion peptide (106–126) [28]. These findings suggest that various proteins may form prefibrillar oligomers that share a common structure regardless of their amino acid sequence [8,28]. However, because the fibrillar oligomers are recognized by the fibril-specific antibody, but not by A11, they at
least, it is plausible that the fibrillar oligomer might represent fibril nuclei to which the monomers can attach before elongation [10]. Aβ oligomers formed at a low pH, but not those formed at a neutral pH, are recognized by the 6E10 antibody [29]. These results strongly suggest the existence of a structural polymorphism of Aβ oligomers.

There have been several other attempts to examine Aβ oligomer structures to elucidate the mechanism of formation of Aβ oligomers. Studies using atomic force microscopy and scanning tunneling microscopy showed that the structures of dimers, tetramers and other low-MW Aβ oligomers were consistent with the model of the Aβ monomers as β-hairpins [30,31]. These low-MW Aβ oligomers are relatively compact, being 1–3 nm in height and 5–10 nm in width/length, and could be the fundamental building blocks of larger oligomers and protofibrils.

Bernstein et al. [18] developed a new method, called electrospray-ionization ion-mobility mass spectrometry, to obtain oligomer size distributions and the qualitative structure of each oligomer. Electrospray ionization allows a fixed population of different Aβ oligomer states in solution to be isolated from one another, and their size and shape could be determined using ion-mobility spectrometry. By analyzing the cross-sectional area of each oligomer obtained by ion mobility, the structure of the Aβ42 tetramer is theoretically assumed to take an open ‘V’ form, which is neither linear nor square. A planar hexagon form was assumed for the Aβ40 hexamer. It is interesting to note that a stacked hexamer paranuclei structure, rather than side-by-side planar hexagons, was suggested for the Aβ42 dodecamer, (Aβ*56). These authors also showed that oligomer size distribution was very different between Aβ42 and Aβ40, consistent with previous studies, indicating that their oligomerization pathways are different [25]. Although the oligomer structure in the gas phase may not be completely identical to that in solution, the information obtained using this novel technique probably reflects the characteristics of Aβ oligomers, at least in part, and may be useful for understanding the physical aspects of Aβ oligomers.

Further attempts to characterize Aβ oligomers at a single molecule level have been performed. Dukes et al. [32] and Ding et al. [33] recently reported oligomer size determination with single molecule spectroscopy using fluorescently labeled Aβ. By directly counting the photobleaching steps in the fluorescence of each oligomer on a cover-glass surface, the number of monomer molecules in individual oligomers could be determined, enabling the determination of more precise oligomer size distributions. For example, an Aβ40 sample incubated at a neutral pH was shown to be a mixture of monomers, dimers, trimers and tetramers, and the presence of zinc ion in the sample buffer increased the number of tetramers [33]. Although application of this method is limited to small oligomers, the single molecule approach overcomes the limitations of resolution and sample heterogeneity.

Analyses of the size of the Aβ oligomer in solution at the single molecule level have also been performed using fluorescence correlation spectroscopy (FCS), which detects the fluorescence of dye-labeled molecules in a very small confocal volume excited by a sharply focused laser beam [34]; FCS enables estimation of the size distribution of an oligomeric species in solution over a wide range of sizes (from monomers to large soluble particles) with a good time resolution (~1 min). From the fluorescence intensity fluctuations, one can calculate the number of molecules in the confocal volume and their diffusion times (corresponding to size). For example, the oligomer size distribution of the incubated Aβ40 sample showed a peak ranging from 50 to 120 nm, indicating the formation of large oligomers [34]. It should be noted that a low concentration (nm) of dye-labeled protein is required for single molecule detection using FCS.

Orte et al. [35] used a two-color single-molecule fluorescence technique (‘two-color coincidence detection’) to characterize oligomer formation of the SH3 domain of phosphatidylinositol 3-kinase, which is known to form small granular toxic aggregates. In this technique, fluorescence bursts from single oligomer particles made from protein monomers, each labeled with one of two fluorescent dyes that emit light at different wavelengths, are observed using optics similar to that of FCS. The coincident detection of both emitted wavelengths with dual excitation indicates the presence of oligomers consisting of more than one molecule. The size and population of oligomers can be determined from the fluorescence intensity and the frequency of such coincident bursts, respectively. Oligomer stability at low concentrations can be examined from changes in the monomer in solution, which can be evaluated from the frequency of noncoincident monomer bursts. Experimental data suggest that the stability of the SH3 domain of the phosphatidylinositol 3-kinase oligomer changes from unstable oligomer to stable oligomers that show no monomer dissociation [35]. It would be interesting to apply this method to examine the time-course of the stability of Aβ oligomers.
Although these *in vitro* studies provide insight into how Aβ monomers assemble into oligomeric complexes, further characterizations, by such as visualization of Aβ oligomer at the molecular level in living cells and animal models, may be required to elucidate the mechanism of formation of Aβ oligomers.

**Possible mechanism of soluble oligomer formation and toxicity**

The mechanism of formation of soluble Aβ oligomer *in vivo* remains unclear. Glabe *et al.* [10] proposed that multiple Aβ oligomer conformations were produced via different pathways, indicating the complexity of the oligomer formation mechanism. The mechanisms of formation may also differ for extracellular and intracellular oligomers. In this section, we discuss possible formation mechanisms of extracellular and intracellular Aβ oligomers, and also discuss how these Aβ oligomers can cause cell death or neuronal impairment (Figs 1 and 2).

**Extracellular soluble Aβ oligomer formation and its toxicity**

A recent study by Yamamoto *et al.* [36] showed the formation of toxic Aβ oligomers in the presence of GM1 ganglioside. This Aβ oligomer was spherical, with a diameter of 10–20 nm and a molecular mass of 200–300 kDa, and therefore much larger than ADDL. Furthermore, Aβ monomers produced extracellularly can interact with GM1, and an Aβ complex with GM1 has been found in AD brain [37]. These observations support the idea that extracellular soluble Aβ oligomers could be formed by GM1. The Aβ oligomer–GM1 complex is not recognized using a seed-specific mAb, suggesting that the GM1-induced Aβ oligomer is formed via a pathway distinct from that of fibril

![Fig. 1. Formation and toxicity mechanisms of extracellular Aβ oligomers. Aβ is released extracellularly as a product of proteolytically cleaved, plasma membrane-localized amyloid precursor protein (APP). Extracellular Aβ oligomers can be formed in the presence of GM1 ganglioside on the cell membrane. GM1 induces Aβ oligomer-induced neuronal cell death mediated by nerve growth factor (NGF) receptors. Toxic non-fibrillar Aβ is also produced in the presence of αB-crystallin and ApoJ. A cellular prion protein (PrP<sup>C</sup>) acts as an Aβ oligomer receptor with nanomolar affinity, and mediates synaptic dysfunction. Furthermore, the membrane pore is formed by Aβ oligomers. The pores allow abnormal flow of ions, such as Ca<sup>2+</sup>, which causes cellular dysfunction. Binding of Aβ oligomers to the NMDA-type glutamate receptor (NMDAR) also causes abnormal calcium homeostasis, leading to increased oxidative stress and synapse loss. Binding of Aβ oligomers to the Frizzled (Fz) receptor can inhibit Wnt signaling, leading to cell dysfunctions such as tau phosphorylation and neurofibrillary tangles. Moreover, Aβ oligomer can induce insulin receptor loss from the neuronal surface and impaired kinase activity related to long-term potentiation.](image-url)
Furthermore, nonfibrillar Aβ can be produced in the presence of B-crystallin [38] and clusterin (also known as Apo J) [39], suggesting that extracellular Aβ oligomers could be formed by various bio-components such as proteins and gangliosides (Fig. 1). The GM1-induced Aβ oligomer induces neuronal cell death mediated by nerve growth factor (NGF) receptors, suggesting that binding of the Aβ oligomer to the NGF receptor is important for the toxicity mechanism [36] (Fig. 1). Potent alternation of NGF-mediated signaling by ADDL supports this concept [40]. Moreover, previous studies suggested that apoptotic cell death occurs through the interaction of Aβ with low-affinity NGF receptor [pan neurotrophin receptor (p75NTR)] and the activation of downstream signaling molecules, such as c-Jun N-terminal kinase (reviewed in ref. [41]). However, it has also been demonstrated that p75NTR promotes neuronal survival and differentiation, indicating that p75NTR might have diverse functions in both cell death and cell survival [42]. Consistent with this notion, there are also conflicting reports showing that p75NTR is protective against Aβ toxicity [43,44]. These results imply that the NGF-mediated toxicity mechanism is complicated.

Other reports on neuronal receptor-mediated toxicity mechanisms (reviewed in ref. [9]) have shown that ADDL binding to an N-methyl-d-aspartate (NMDA)-type glutamate receptor (NMDAR) causes abnormal calcium homeostasis, leading to increased oxidative stress and synapse loss [45,46]. ADDL can also induce the loss of insulin receptors from the neuronal surface [47,48] and impair LTP-associated kinase activity [49]. However, such insulin receptor impairment is inhibited by extracellular insulin, suggesting that insulin plays an important role in oligomer-induced cell death. Magdesian et al. [50] showed that Aβ oligomers binding to the Frizzled (Fz) receptor, an acceptor of Wnt protein, inhibited Wnt signaling, leading to cellular dysfunction. Wnt signaling, which promotes progenitor cell proliferation and directs cells into a neuronal

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**Fig. 2.** Formation and toxicity mechanisms of intracellular Aβ oligomers. Aβ can be localized intracellularly by the uptake of extracellular Aβ or by the cleavage of APP in endosomes generated from the ER or the Golgi apparatus. Extracellular Aβ is internalized through various receptors and transporters, such as formyl peptide receptor-like protein 1 (FPRL1) or scavenger receptor for advanced glycation end-products (RAGE). These receptor–Aβ complexes are internalized into early endosomes. Most Aβ in the endosome is degraded by the endosome/lysosome system. However, Aβ in the lysosome can leak into the cytosol by destabilization of the lysosome membrane. Although cytosolic Aβ can be degraded by the proteasomal degradation system, inhibition of the proteasome function by Aβ oligomers causes cell death. Suppression of protein aggregation by interactions with various cellular proteins, such as prefoldin (PFD) or other molecular chaperones, may cause the formation of Aβ oligomers.
although misfolded prion protein (PrPSc) is thought to mediate synaptic dysfunction. Interaction between PrPC and a co-receptor, such as glycogen synthase kinase-3b (GSK-3b) and increased beta-catenin levels. Inhibition of Wnt signaling by Aβ oligomers causes tau phosphorylation and neurofibrillary tangles, which suggests a Wnt-beta-catenin toxicity pathway [50].

A recent report by Nimmrich et al. [51] showed that Aβ oligomers can also impair presynaptic P/Q-type calcium currents, which are related to neurotransmission and synaptic plasticity in the brain, at both glutamatergic and gamma-amino butyric acid (GABA)-ergic synapses. This impairment is specific for Aβ oligomers, but not for Aβ monomer or fibrils. Although the detailed mechanism of this impairment remains unclear, the interaction of Aβ oligomers with synaptic proteins or channels may cause modification of the P/Q current. By contrast, another study showed that the cell membrane could be destabilized by the Aβ oligomer [52]. The membrane pores formed by the Aβ oligomer would allow the abnormal flow of ions, such as Ca2+, suggesting another plausible mechanism for Aβ oligomer toxicity [53,54]. Recent observations by Lauren et al. [55] indicate that cellular prion protein (PrPc) can act as an Aβ oligomer receptor with a nanomolar affinity, mediating synaptic dysfunction. Although misfolded prion protein (PrPSc) is thought to cause prion disease, the interaction between the Aβ oligomer and the prion does not require the infectious PrPSc conformation. This interaction may disrupt the interaction between PrPc and a co-receptor, such as NMDAR, impairing the neuron signal-transduction pathways. This discovery by Lauren et al. also suggests that AD is linked with other neurodegenerative diseases.

Recently, interactions between Aβ and alpha-synuclein in vivo and in vitro have recently been observed [56,57]. Alpha-synuclein is an aggregation-prone protein that causes Parkinson’s disease (PD), and interactions between alpha-synuclein and Aβ therefore indicate that AD and PD could be related. Aβ also promotes alpha-synuclein aggregation and toxicity. These results suggest that the AD and PD pathologies could overlap. Interestingly, interactions between Aβ and alpha-synuclein induce the formation of hybrid pore-like oligomers [58]. Aβ-treated cells expressing alpha-synuclein display increased current amplitudes and calcium influx, consistent with the formation of cation channels. It is therefore assumed that the hybrid pore-like oligomers may alter neuronal activity and cause neurodegeneration. These observations support the idea that there are various Aβ oligomer-formation pathways, and that cell death might occur via multiple pathways (Fig. 1).

Intracellular Aβ

Although Aβ was first identified as a component of extracellular amyloid plaques, ample evidence has demonstrated that Aβ is also generated intracellularly (reviewed in ref. [6]). Besides the plasma membrane, APP localizes to the trans-Golgi network, to the ER and to the endosomal, lysosomal and mitochondrial membranes. Aβ is produced by the sequential cleavage of APP by beta-secretase (also known as BACE) and gamma-secretase in endosomes as well as at the plasma membrane [59]. Aβ is also produced intracellularly within the ER and the trans-Golgi network system. Identification of the intracellular protein, endoplasmic reticulum associated binding protein (ERAB), which binds to Aβ, also strongly suggests the existence of intracellular Aβ [60].

In addition to Aβ being produced intracellularly, previously secreted Aβ that forms the extracellular Aβ pool can be taken up by cells and internalized into intracellular pools through various receptors and transporters, such as the nicotinic acetylcholine receptor, low-density lipoprotein receptor, formyl peptide receptor-like protein 1, NMDAR and the scavenger receptor for advanced glycation end-products [6] (Fig. 2). These receptor-associated Aβ complexes could be internalized into endosomes. Recent findings also support the idea that Aβ is present within the cytosolic compartment. Intracellular accumulation of Aβ in the multivesicular body is linked to cytosolic proteasome inhibition [61]. Furthermore, in vivo and in vitro proteasome inhibition also leads to higher Aβ levels [62,63]. As the proteasome is primarily located within the cytosol, these findings strongly suggest that Aβ is also located within the cytosolic compartment. Extracellular Aβ can enter the cytosolic compartment and inhibit the proteasome activity of cultured neuronal cells [62]. Clifford et al. [64] showed that fluorescently labeled Aβ which is injected into the tail of mice with a defective blood–brain barrier (which is common in AD patients) accumulates in the perinuclear cytosol of pyramidal neurons in the cerebral cortex. These observations strongly support the notion that neurons can take up extracellular Aβ in the cytosolic compartment.

The destabilization of intracellular membranes may also contribute to the presence of cytosolic Aβ. A high proportion of autophagosome-related vesicular structures, which would suggest impaired maturation of autophagosomes to lysosomes, has been found in the AD brain, but not in the normal brain [65]. Although most Aβ formed in endosomes is normally degraded within lysosomes, Aβ can accumulate in lysosomes in
the AD brain. Aβ within the lysosomal compartment destabilizes its membrane [66], which would also lead to the presence of Aβ in the cytosolic compartment.

**Intracellular soluble Aβ oligomer formation and its toxicity**

How intracellular Aβ monomers assemble and form soluble oligomers remains unclear. One possibility is that the uptake of extracellularly-produced Aβ oligomers occurs via endocytic pathways or various receptors and transporters, as described above (Fig. 2). Another possibility is that the interaction of Aβ with intracellular proteins results in oligomer formation. Recent observations by Yuyama et al. [67] showing GM1 accumulation in early endosomes, support the idea that intracellular GM1 could also induce Aβ oligomer formation. Recently, we found formation of toxic high-MW (50–250 kDa) soluble Aβ oligomers by the cytosolic molecular chaperone protein, prefoldin, *in vitro* [68]. In general, molecular chaperones stabilize and mediate the folding of unfolded proteins. Molecular chaperones play essential roles in many cellular processes, such as protein folding, targeting, transportation, degradation and signal transductions [69]. Prefoldin reportedly captures and delivers denatured protein to another cytosolic chaperone, chaperonin [70–73]. Our results also suggested that the interaction between prefoldin and Aβ oligomers prevents further aggregation and stabilizes the oligomer structure (Fig. 2).

Molecular chaperones are potent suppressors of protein aggregation, leading to neurodegenerative disorders such as AD, PD and Huntington’s disease (HD) [74–76]. Various molecular chaperones are upregulated in patients and co-localize with aggregated proteins in plaques/inclusion bodies. These molecular chaperones prevent aggregation *in vivo* and *in vitro*; for example, the cytosolic chaperonin CCT can inhibit aggregation of the polyglutamine (polyQ) expansion protein, which causes HD *in vivo* and *in vitro* [77–79]. Reduced CCT levels also enhance the aggregation and toxicity of polyQ in neuronal cells, strongly supporting the idea that molecular chaperones can be a defense against the aggregation of misfolded protein. Importantly, however, our findings also suggest the possibility that the suppression of protein aggregation may cause the formation of toxic oligomeric species, which is consistent with previous results showing that toxic nonfibrillar Aβ was produced in the presence of zB-crystallin [38] and clusterin (also known as Apo J) [39]. These results suggest that intracellular Aβ oligomers could be produced by interaction with various cellular proteins, including molecular chaperone proteins (Fig. 2).

The toxicity mechanism of intracellular Aβ oligomers also remains unclear. Microinjection of Aβ or a cDNA-expressing cytosolic Aβ induces the cell death of primary neurons and the simultaneous formation of low-MW Aβ oligomers [80]. Furthermore, intracellular Aβ accumulation is closely correlated with apoptotic cell death via the P53-BAX pathway [81]. Recently, Mousnier et al. [82] reported a possible prefoldin-mediated proteasomal protein-degradation pathway. It is therefore plausible that Aβ oligomer–prefoldin complexes could bind to proteasome, causing proteasome dysfunction and subsequent cell death. This idea is supported by interaction studies between Aβ oligomers and proteasome, which showed that the proteasomal function was inhibited while interacting with Aβ [63]. Impairment of proteasomal function by the Aβ oligomer also leads to age-related pathological accumulation of Aβ and tau protein [63]. Recent research has shown that the dysfunction of autophagy, a lysosomal pathway for degrading organelles and proteins, is related to neurodegenerative diseases, including AD and PD [65,76]. These observations support the idea that the toxicity mechanism of intracellular oligomers may be different from that of extracellular oligomers (Fig. 2). However, more studies, particularly those focused especially on the proteolysis system in AD brains, are necessary to understand AD pathology in relation to intracellular soluble Aβ oligomers.

**Concluding remarks**

It has long been argued that insoluble Aβ fibrillar aggregates found in extracellular amyloid plaques initiate the neurodegenerative cascades of AD. However, recent emerging results indicate that prefibrillar soluble Aβ oligomers are the key intermediates in AD-related synaptic dysfunction. Various amyloidogenic proteins can form toxic soluble oligomers, suggesting that soluble oligomers are the general key factors in various diseases such as AD, PD, HD and other amyloidosis [5,28,83]. Although much research effort is being directed towards characterizing oligomer states, their conformations and formation mechanisms remain unclear. Recent evidence suggests that the size of Aβ oligomers is distributed in a wide MW range (from < 10 kDa to > 100 kDa), and that there is structural polymorphism of Aβ oligomers, even for those of a similar size. The biochemical properties of these oligomers in relation to disease pathology also seem to differ depending on their sizes and structures.
Aβ can form various distinct oligomeric states via various pathways. The formation and toxicity mechanisms of extracellular and intracellular Aβ oligomers can also be different from one another. Regardless of the complexity of the oligomer-formation mechanism, recent findings suggest that Aβ oligomers can be formed through interactions between Aβ monomers/oligomers and cellular proteins/biomolecules, such as molecular chaperones and lipids. Prevention of aggregation may cause the formation/stabilization of oligomer states.

Acknowledgements

The authors are grateful for financial support from the Japan Science Technology Agency (PRESTO Program, MS), RIKEN (Nano-scale Science and Technology Research, TZ) and the Japanese Society for the Promotion of Science (TZ). We wish to thank Drs Karin Sörjberg, Naofumi Terada (RIKEN) and Hiroshi Kubota (Akita University) for helpful comments.

References


glutamate receptor-dependent signaling pathway. 

J Neurosci 27, 2866–2875.


Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell* 93, 863–873.