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Aβ aggregation and possible implications in Alzheimer's disease pathogenesis

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Abstract

Amyloid β protein (Aβ) has been associated with Alzheimer's disease (AD) because it is a major component of the extracellular plaque found in AD brains. Increased Aβ levels correlate with the cognitive decline observed in AD. Sporadic AD cases are thought to be chiefly associated with lack of Aβ clearance from the brain, unlike familial AD which shows increased Aβ production. Aβ aggregation leading to deposition is an essential event in AD. However, the factors involved in Aβ aggregation and accumulation in sporadic AD have not been completely characterized. This review summarizes studies that have examined the factors that affect Aβ aggregation and toxicity. By necessity these are studies that are performed with recombinant-derived or chemically synthesized Aβ. The studies therefore are not done in animals but in cell culture, which includes neuronal cells, other mammalian cells and, in some cases, non-mammalian cells that also appear susceptible to Aβ toxicity. An understanding of Aβ oligomerization may lead to better strategies to prevent AD.

Keywords: Alzheimer's disease • Abeta • oligomerization/aggregation • peptide toxicity

Introduction

Alzheimer's disease (AD) belongs to a large cohort of diseases characterized by amyloidoses. Extracellular senile plaques, the foremost pathophysiological hallmark of AD are composed of a dense core of amyloid fibrils associated with degenerating neurites, astrocytes and astrocytic processes [1]. Amyloid β protein (Aβ) is the main protein component of senile plaques [2]. Extracellular Aβ is generated by proteolytic processing of amyloid precursor protein (APP) by β-secretase followed by γ-secretase at the cell surface. In the AD brain, there appears to be an apparent failure in regulating the production and clearance of Aβ, leading to increased levels of Aβ and consequently aggregation and neurotoxicity (Fig. 1). Studies using mouse models, cell culture, synthetic Aβ and biophysical methods have shown a strong correlation between increased levels of Aβ leading to acute dementia in AD [3–10]. Due to Aβ's ability to bind several different molecules and attain multiple physical states [11], our understanding of the key neurotoxic mechanism(s) causing cognitive decline in AD is incomplete. It is also increasingly believed that cognitive decline in AD is a result of multiple toxicity mechanisms of different Aβ forms.

The onset of AD supports an age-dependent dichotomous model including familial early onset cases (10% of all AD cases) and late onset cases. Increased production of Aβ is a feature of

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early onset AD and can be caused by mutations observed in APP and γ-secretase complex [12]. Three fully penetrant (autosomal dominant) genetic mutations (presenilin 1, presenilin 2 [components of γ-secretase complex] and APP) have been described for early onset AD. These mutations either alter APP metabolism or the nature of secreted Aβ. Thus the mechanism of amyloid plaque formation is primarily driven by increased local concentration of Aβ or due to the intrinsic aggregating property of the mutant Aβ form.

For late onset, which comprises 90% of AD cases, apolipoprotein E is the only genetic risk factor observed with moderate penetrance. However, senile extracellular amyloid plaques are observed in the majority of the late onset AD cases indicating that the mechanism of neurodegeneration could be similar. Although, late onset AD cases do not show signs of increased production of Aβ, it has been suggested that reduced degradation of Aβ by neprilysin and insulin-degrading enzyme [13, 14] and reduced perivascular drainage [15] may describe the elevated levels of Aβ in the AD brain. Several molecular mechanisms for clearance of Aβ have been demonstrated, including microglial clearance [16] *via* the macrophage scavenger receptor [17], receptor for advanced glycation end products [18], low-density lipoprotein receptor-related protein internalization and degradation of Aβ complexes with apolipoprotein E and α2-macroglobulin [19, 20].

Amyloid structure

Amyloidoses are associated with the misfolding of a native protein into a cytotoxic form, which occurs in parallel with, or as an alternative to physiological folding. This is followed by deposition in tissues in bundles of β-sheet fibrillar protein. The fibrillar form of proteins is a structure dominated by hydrogen bonding between the amino and the carbonyl groups of the main chain, rather than by specific interactions of the side chains observed in globular proteins [21]. Amyloid proteins also exhibit the ability to form multiple conformations [22]. According to the 'folding energy landscape theory', protein folding follows a funnel-like pathway in which the conformational intermediates progressively merge into a final species with minimum free energy and maximum stability [23]. However, in amyloid formation, at a minimum energy similar to that of the native protein state, the polypeptide acquires an alternative and relatively stable 'misfolded state' which is prone to aggregation [24]. The native structures and amino acid sequences of the proteins associated with amyloid diseases have been found to vary considerably; however, amyloid fibrils isolated from different sources share a common ultrastructure [25].

The capacity to form fibrillar amyloid structures is not exclusive to a specific group of proteins but is generic to all polypeptide chains [26]. α-helical proteins forming amyloid fibrils under appropriate *in vitro* conditions [27] and amyloid aggregates of non-pathogenic proteins cause toxicity to neuronal cells [28] implying that amyloid formation does not exclusively depend on the intrinsic nature of the protein.

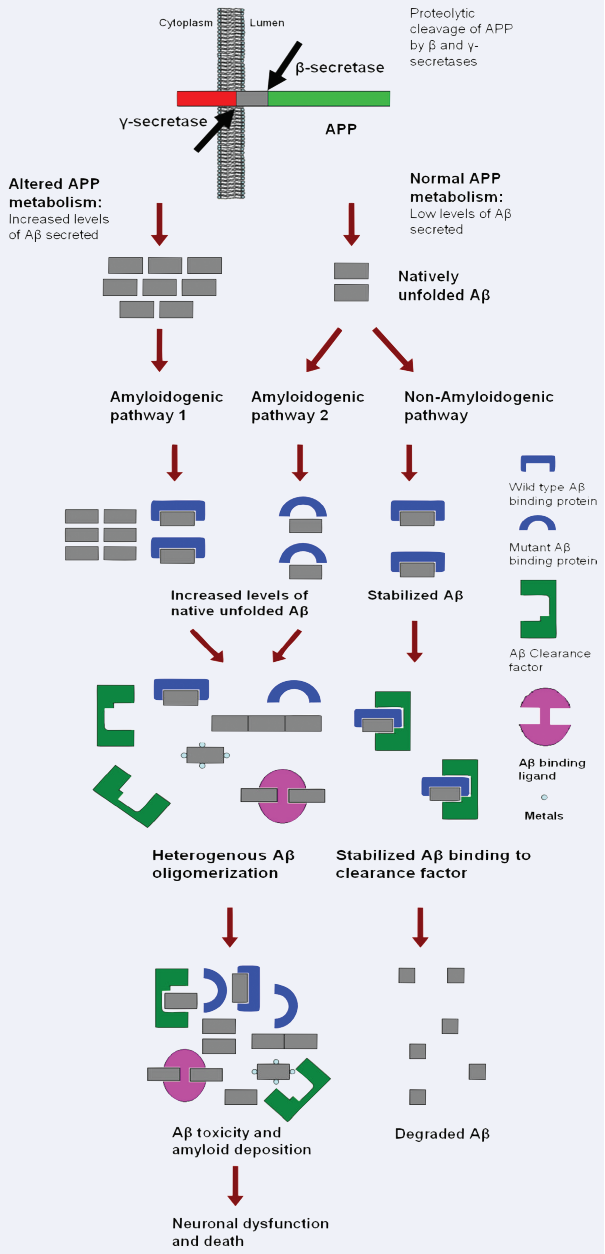

Fig. 1 Possible events leading to Aβ accumulation and neurotoxicity.

Mechanism of amyloid aggregation

Fibril formation is considered to be an aggregation pathway originating from a high entropic barrier and a thermodynamically unfavourable event [29]. The aggregation of Aβ is initiated by a conformational change from random coil or α-helix into a β-strand, quite similar to prion diseases. Hydrophobic interactions

Table 1 Pathogenic protein misfolding

Causes/factors	Misfolded protein/disease	Reference
Nature of the protein: (ageing, Hydrophobicity)	**Ageing:** transthyretin protein in senile systematic amyloidoses **Hydrophobicity:** Aβ, Prion proteins,	[35] [36] [37]
Concentration dependence	Aβ in Alzheimer's disease (AD)	[38]
Mutations in amino acid sequences in the protein	Hereditary amyloidosis APP in AD	[39] [4] see Table 2
Mutations in associating proteins	β2-Microglobulin mutations	[40]
Chemical modifications of the protein	Protonation of Aβ Oxidative modifications of Aβ	[2] [41] [42]
Protein folding machinery (Chaperones, heat shock proteins)	Aβ in AD Alpha synuclein in Parkinson disease	[43] [44]
Altered proteolysis or turnover of precursor protein	Mutations in Amyloid precursor protein in AD Presenilin mutations in AD	[4,5] [45,46]
Decreased clearance	Aβ in AD Alpha synuclein in Parkinson disease	[47] [48]
Time of incubation	Aβ in AD	[49]
Temperature and ionic strength	Aβ in AD	[50]
Local interacting factors (other proteins, metals, osmolytes)	Aβ and Metals Osmolytes and prions ApoE and Aβ	[51], [52], [53] [47,54,55]

are eventually maximized by β-sheet conformation [29, 30]. Aβ aggregation and fibril formation are nucleation dependent and the kinetics of fibril formation are determined by nucleation and fibril elongation rate [31]. Although, the formation of nuclei is thermodynamically unfavourable, the addition of monomeric molecules to the existing nuclei is favourable, and occurs by perpendicular hydrogen bonding to the axis of the amyloid nucleus [11].

A considerable number of environmental factors as well as some intrinsic properties of proteins can work in concert to cause amyloidogenesis (Fig. 1). Some of these factors, particularly those involved in AD, are listed in Table 1. These factors can influence the thermodynamic stability of the various accessible conformations of the protein potentially causing amyloidogenesis. Although, protein aggregation and amyloid formation have been thought to be cytotoxic, recent studies have identified novel biological functions for amyloidogenic protein fibrils in bacteria, fungi and even mammals [32]. Emerging evidence has indicated that rather than amyloidogenic aggregates, the oligomeric intermediates could be the toxic entities, which also have been observed in AD [33, 34]. Thus understanding the mechanism and factors causing Aβ aggregation and stability of the oligomeric intermediates have become more important.

Numerous causes/mechanism of Aβ aggregation have been observed previously. Although many studies have demonstrated the mechanism of Aβ aggregation, there are certain drawbacks in regard to the poor correlation of *in vivo* conditions to the controlled environment in an *in vitro* analysis. For example, Aβ assembly occurs in a very complex and dynamic environment; characterized by the presence of different proteins, membranes, metal ions etc., while *in vitro* experiments are done with extremely simplified conditions that may bias toward amyloid aggregation. Very little mechanistic/structural information is available regarding the exact conformational change and mechanism of Aβ aggregation caused by local environmental factors in AD brain.

Aβ: a natively unfolded protein?

The folding of proteins into their correct three-dimensional structure is critically important for their biological activity and normal functioning of the cell. With the human proteome reaching a size of more than 100,000 proteins, it is clearly evident that protein folding occurs in a crowded and sensitive environment highly prone to aggregation [26]. However, cellular systems have evolved to evade unfavourable protein aggregation. Negative selection has been observed to avoid alternating polar and hydrophobic residues that favour a β-sheet structure [56]. It is also suggested that residues showing increased vulnerability to aggregation are preferably located in different regions of the sequence from those that determine native protein folding, termed 'kinetic partitioning' [57]. Apart from amino acid sequence selection, biological systems have developed molecular chaperones and degradation mechanisms to control the rate of formation of unfavourable structures [58].

Table 2 Mutations affecting Aβ aggregation

Mutations	Aggregation/toxicity
Flemish (A21G) [59]	Short 2 h incubation shows elevated apoptosis, slower aggregation than wild-type [60]
Arctic (E22G) [61]	Increased rate of fibril formation [62]
Dutch (E22Q) [63]	Causes higher amount of apoptosis at physiological concentrations with 24 h incubation, faster aggregation than wild-type, increased rate of fibril formation [60, 62]
Italian (E22K) [64]	Aggregates rapidly [65]
Iowa (D23N) [66]	Aggregates rapidly [65]
Pyroglutamate-modified AβN3(pE) [67]	AβN3(pE)-40/42 peptides shows resistance to degradation by cultured astrocytes [68]

Therefore it becomes obvious that apart from intrinsic properties of protein to aggregate, failure of the protein folding machinery could also play a crucial role in amyloidogenesis [43]. Mutations in Aβ sequences (Table 2) have been identified in familial forms of AD which either increase the propensity to aggregate, or decrease the stability of the native state. However, no similar genetic evidence has been identified for the more prevalent sporadic AD, although widespread neuritic amyloid plaques are observed in majority of the sporadic AD cases [1]. This indicates that progressive Aβ aggregation in sporadic AD could be associated with Aβ binding and clearance factors [47].

Proteins are required to possess fast folding kinetics and high stability to minimize the risk of protein aggregation in the cell [69]. But certain proteins may require higher-order structural disorders mediated by binding to ligands to fulfil their function [70, 71]. Such proteins have greater structural plasticity to favour ligand binding and could be classified under the emerging class of 'natively unfolded proteins' [72]. The presence of natively unfolded proteins in the cell is believed to provide a simple solution to having large intermolecular interfaces for diverse ligand binding and regulation, and smaller protein, genome and cell sizes [73]. There is an increasing belief that amyloidogenic proteins could be natively unfolded proteins [74]. Aβ is known to bind a large array of extracellular and cell-associated ligands (Table 3, Fig. 1). However, Aβ interaction with different molecules has not been clearly understood in relevance to its biological function or pathology. Although not experimentally proved, Aβ shows characteristics of a 'natively unfolded protein'. Under normal conditions, Aβ could be bound to a ligand essential for a normal function and occurrence of Aβ aggregation in sporadic AD may be a result of absence or structural disorder of its ligand. Thus it is suggested that the clinical manifestations observed in AD could be either the toxic property of the pre-fibrillar intermediate or loss of function of native Aβ.

Ambiguities in synthetic Aβ studies

The dynamic nature of Aβ in physiological conditions has been a major concern in determining the mechanism of Aβ toxicity. Since aggregating Aβ is neurotoxic, cell loss and Aβ deposition are considered to correlate with the severity of disease symptoms. However, several studies predict that events preceding neuronal cell death may provide a better explanation to the progressive decline in cognition in AD [103–105]. There are robust correlations between the levels of soluble Aβ and the extent of synaptic loss and severity of cognitive impairment [33, 34]. In these studies, the term soluble Aβ describes all isoforms of Aβ that remain in the supernatant following high speed centrifugation ($>$100,000 $\times$ *g*) of tissue extracts. Although these studies have not identified a specific assembly form(s) of soluble Aβ, it is clearly implied that non-fibrillar assemblies are the main cause of synaptic dysfunction leading to cognitive decline in AD. Identifying a particular Aβ species as the main cause of synaptic loss has posed serious difficulties owing to the heterogenic nature of Aβ. Monomeric Aβ has the ability to associate into higher-ordered aggregates depended on several interdependent factors. Thus, it is difficult to unequivocally attribute toxicity to a discrete species. Consequently, much confusion has resulted regarding the variable behaviour of different peptide stocks [106, 107]. Table 4 refers to the literature describing a range of Aβ isoforms. Not all descriptions are unique, but it is clear that many isoforms have been observed, ranging from monomers to oligomers of various sizes to protofibrils, fibrils and aggregates.

Apart from the propensity of Aβ42 to generate multiple conformations, it possesses oxidative [113], hydrolytic [114], and surfactant properties [115]. It is also clear that different Aβ assemblies can possess distinct toxicity mechanisms [116] in different cell lines, even in yeast [111].

Formation of amyloid plaques

Aβ deposits in the brain are usually referred to as senile plaques. Aβ plaques can also be observed in cognitively normal individuals [117–119]. The major variation between Aβ deposits in normal individuals and those found in AD patients is their distribution [118]. In

Table 3 Aβ interacting molecules

Cofactor	Reference
Metals:	
Copper	[2]
Zinc	[51]
Aluminium	[75]
Iron	[76]
CSF and plasma proteins:	
Albumin	[77]
Lipoprotein: ApoE	[55]
Insulin	[78]
Serum amyloid P	[79]
Other plasma proteins: IgG, IgA, IgM α1-Antitrypsin, Transferrin, α2-Macroglobulin, α1-Antichymotrypsin Antithrombin III, Transthyretin and Fibrinogen	[80]
Cell Surface Receptors:	
Transforming growth factor β receptor	[81]
Insulin receptor	[82]
NMDA receptor	[83]
p75 neurotrophin receptor	[84]
Receptor for advanced glycation End products (RAGE)	[85]
Formyl peptide receptor-like 1	[86]
Amyloid precursor protein	[87]
Scavenger receptors SR-A, SR-BI	[17]
α7nicotinic acetylcholine receptor (α7nAChR)	[88]
CD47, CD36, α6β1-integrin	[89]
Serpin-enzyme complex receptor (SEC-R)	[90]
Integrin β1	[91]
HSP	[92]
Intracellular proteins:	
Aβ binding alcohol dehydrogenase	[93]
Chaperone proteins	[94]
20S proteasome	[95]
Extracellular matrix proteins:	
Heparin sulphate Agrin Laminin, Collagen-like Alzheimer amyloid plaque component CLAC.	[96] [97] [98] [99]
Others:	
Membrane lipids	[100]
Chondroitin sulphate-derived Monosaccharides and Disaccharides	[101]
Cholesterol	[102]

Table 4 Different isoforms of Aβ

Aβ isoform
Soluble dimers [108], tetramers and oligomers [2]
Non-amyloidogenic amorphous aggregates [109]
Amyloidogenic fibrils [109]
Fibrillar aggregates [50]
Amyloid proto-fibrils [31]
Amyloid derived diffusible ligands [110]
Soluble non-fibrillar [111]
Hexamer, nonamer, dodecamer, Aβ*56 (56-kD soluble Aβ assembly) [112]

addition to Aβ, other proteins accumulate within senile plaques, including apolipoprotein E (apoE), α2-macroglobulin, interleukins, components of the complement system, α2-macroglobulin receptor, low-density lipoprotein receptor-related protein, collagenous Alzheimer amyloid component [120–124] and also dystrophic neurites, reactive astrocytes, and microglial cells [121, 125].

Apart from extracellular accumulation, Aβ is also known to form insoluble pools intracellularly (reviewed in [126]). Recent studies have confirmed the build up of intracellular Aβ in neuronal cells as an early event in AD pathogenesis [127, 128] which precedes formation of amyloid plaques in the brain [129]. Intracellular Aβ has been postulated to originate from the result of intracellularly localized APP proteolysis [130–132] or by receptor associated uptake of extracellularly secreted Aβ [133–137]. As the majority of Aβ is secreted, it is suggested that APP proteolysis predominantly occurs at the cell membrane or cleaved Aβ is rapidly secreted [126]. Previous studies also have shown that intraneuronal Aβ is observed only in transgenic models based on APP overexpression [138, 139] and not in wild-type. Thus it seems like that the origin of intracellular Aβ is largely by uptake from extracellular media. Intracellular Aβ is also implicated in synaptic dysfunction and associated cognitive decline [140]. However, the nature of intracellular Aβ assembly, mechanism of action and its relevance to AD pathology needs to be addressed.

Role of Aβ in AD pathogenesis

Memory impairment including the loss of the ability to form and retain new episodic memories is the hallmark of early stages of AD. Cognitive impairment is often attributed to synaptic dysfunction and neuronal cell loss particularly in the cells interconnecting the hippocampal formation with the associating structures crucial for memory [141, 142]. Depleted neurotransmitters [142], 25 to 35% decrease in the synapses [143, 144] and quantitative correlations of postmortem cytopathology with cognitive deficits indicate that

synaptic loss is more robustly correlated than the numbers of plaques or tangles, or extent of cortical gliosis [145].

Lesne *et al.* [112] study implicated a unique and novel Aβ isoform (Aβ*56: 56-kD soluble Aβ assembly) as the key neurotoxic Aβ isoform responsible for cognitive decline in APP overexpressing Tg2576 mice, based on its abundance, stability and occurrence during memory decline. However, a more recent study [108] has identified Aβ dimers from the soluble extract of AD cerebral cortex tissues. They also specifically attribute Aβ dimers to the loss of long-term potentiation, enhanced long-term depression, reduced dendritic spine density in normal rodent hippocampus and memory disruption of a learned behaviour in normal rats. Whether it is Aβ dimer or Aβ*56, the different toxic Aβ species identified might reflect differences in the toxicity assays and Aβ detection methods used. Even though the increase in soluble Aβ levels and aggregation in the brain has been consistently observed as the main indicator of cognitive decline, the localization of the Aβ accumulation in the brain has not been specified.

A recent study has elaborated the extraction of Aβ from different anatomical compartments (extracellular soluble, intracellular soluble, membrane associated and extracellular insoluble) [146]. It identified membrane-associated and intracellular Aβ in the temporal neocortex of AD patients to be more closely related to AD symptoms than other measured Aβ species. This study has addressed the very important aspect of Aβ localization and accumulation in AD pathogenesis.

It therefore becomes essential to identify and systematically categorize the factors responsible for Aβ's native form and pathological aggregation based on its relevance to physiological role or synaptic failure in AD.

Conclusion

Aβ plays the central role in neurodegeneration in AD. It is widely accepted that Aβ has a wide array of biological activities and affinities, which have not been definitively mapped to its native or pathological role in the brain. Although, there has been much progress in understanding the role of Aβ in AD, there are several important questions still unanswered including the physiological nature and function of APP/Aβ in the normal ageing brain, how Aβ contributes to the vascular defects observed in AD, what are the genetic risk factors associated with late onset AD, what causes vulnerability to Aβ toxicity, is AD caused by loss of native Aβ function or by its pathogenic form and are AD symptoms caused by the cumulative effect of different toxic Aβ forms or exclusive to a particular form. With Aβ emerging as one of the primary targets for immunotherapy and target based drug design for AD, it becomes essential to gain further insight into Aβ caused cognitive decline in AD.

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