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Neuroprotective Function of Non-Proteolytic Amyloid-β Chaperones in Alzheimer's Disease

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Abstract

This chapter attempts to explore protective role of chaperone proteins in the neurodegenerative diseases caused by amyloidosis. These chaperones prevent amyloid pathology either directly, through chemical interactions with amyloidogenic species to mediate their refolding, solubilization and degradation, or indirectly, by scavenging reactive oxygen species produced as by-products of amyloid aggregation. Here we focus on structural and morphological changes during aggregation of amyloids which have been identified using Nuclear magnetic resonance spectroscopy, X-ray crystallography, Electron microscopy, Atomic force microscopy and other biophysical techniques as well as interactions between chaperone proteins and amyloid moieties. Non-proteolytic chaperones mediate amyloid clearance and metabolism through conformational changes due to proximity binding. In this chapter, we delineate these interactions as well as the molecular mechanism of chaperones used to sequester ROS products of amyloidosis with focus on amyloid-β peptides associated with the Alzheimer's disease.

Keywords: amyloid-β, structural biology, aggregation, chaperone, neuroprotection, Alzheimer's disease

1. Introduction

Abnormal deposition of amyloids or "Amyloidosis" is hallmark of several chronic cerebrovascular diseases including neurodegeneration culminating into dementia. Efforts to develop targeted drugs against amyloids have been hindered since there is no universal mechanism that leads to protein misfolding or aggregation, and the aggregates usually do not correspond directly to clinical symptoms of the diseases. A clearer understanding of molecular interactions of amyloids can drive the ongoing therapeutic efforts to prevent aggregation of nascent amyloids into pathological species and to design timely interventions. In addition to aging, precursor mutations in genes and proteins, gene multiplication, expansion of amyloidogenic sequences, and xenobiotics such as air pollutants are risk factors usually associated with amyloidosis disorders [1]. There are different amyloidogenic species causing a variety of neuropathic diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Poly-glutamine disorders like Huntington disease (HD), Prion diseases including Creutzfeldt-Jakob disease, Lewy body disease, Amyotrophic Lateral Sclerosis (ALS) as well as metabolic diseases such as type II diabetes (T2D) and corneal dystrophy to name a few [2]. AD is a pandemic form of

dementia caused due to improper aggregation of amyloidogenic proteins-amyloid-β (Aβ), which is a cleavage product of amyloid precursor protein (APP) and tau, stabilizes microtubules in neurons [3]. Prognosis is also closely associated with aggregation of α-Synuclein (αS) protein into Lewy bodies usually concentrated in presynaptic terminals [4]. Human islet amyloid polypeptide (IAPP), also known as amylin, is secreted along with insulin from pancreatic β-islet cells. IAPP rich amyloid plaques, facilitated by insulin resistance, are hallmarks of T2D [5]. β-sheeted infectious isoform of cellular prion protein (PrP) causes transmissible spongiform encephalopathy (TSE), broadly known as prion disease [2].

In this chapter, we review various structures and conformations attained by Aβ peptides during the process of aggregation. We begin with introducing different morphologies and conformations attained by amyloids during this process. We outline mechanisms of amyloid pathology, either directly mediated by aggregates or indirectly through generation of oxidative stress. We have briefly alluded to chaperone functions of Heat shock protein (Hsp) family and their interactions to different amyloid structures as well as intracellular protease mechanisms. It is important to note that such intracellular proteases are almost entirely inefficient in dealing with large insoluble plaques. Here we emphasize on additional endogenous proteins which show potential as chaperones, albeit through different mechanisms. Towards the end, we discuss the role of oxidative stress in accelerating AD pathology and the effect of Aβ interactions with metal species. Mechanisms to alleviate oxidative stress and their possible protective role in AD have been discussed.

2. Structural and biophysical basis of amyloid aggregation

Membrane glycoprotein APP is cleaved by β-secretase and γ-secretase enzymes generating 36–49 residue long peptides, among which amyloidogenic Aβ(1–40) and Aβ(1–42) are well-known culprits in AD [6]. Unstructured monomeric amyloids polymerize to form fibrillar structures with characteristic cross-β morphology formed by in-register β-sheets which align parallel to the fibrillar axis whereas perpendicularly extended side-chains pack closely to form tight steric zippers [2]. Nuclear magnetic resonance (NMR) spectroscopy studies on amyloid monomers or fibrillar structures to identify characteristic dynamic structural features. X-ray crystallographic studies have helped in identifying structural motifs through fiber diffraction and mass-per-length studies through EM provide information regarding specific symmetries identified in filaments. NMR models of Aβ monomeric peptides show predominantly α-helices with propensity to convert into β-sheets [6]. The α-helical conformation is also observed among on-pathway transient intermediates, possibly mediated by interactions of hydrophilic N-terminal residues [7]. These intermediates then give way to β-sheet conformation in higher oligomeric species which eventually transition into mature fibrils. *In vitro* aggregation of Aβ monomers into fibrils can be carefully curated to obtained different fibril morphology. In solid state nuclear magnetic resonance (ssNMR) spectra, cross-peaks originating from hydrophobic core and C-terminal regions within amyloid fibrils, prepared without seeding indicate presence of polymorphs. Formation of homogenous fibrils from Aβ(1–42) seeds precludes aggregation of Aβ(1–40) [8].

αS binds to neuronal membranes in a highly α-helical state as opposed to the unfolded monomeric conformation [7]. The antipathic N-terminal sequence has tendency to form α-helical conformation, central region from aa 61–95 is highly hydrophobic and amyloidogenic, and the C-terminal domain provides flexibility to the protein without attaining any specific structure [7]. Prefibrillar αS shows

conformational plasticity and the soluble monomers combine to form unstable dimeric molecules which further aggregate into higher oligomers and fibrils. IAPP structure in detergent micelles shows a helical conformation kinked around residue His18 at neutral pH and an extended α-helix in acidic conditions; thus the ability to attain specific conformation also depends on its chemical environment [5].

2.1 Morphological differences in amyloid aggregates

Disordered or misfolded monomers can convert into a range of amyloid species, e.g., spherical oligomers, amorphous aggregates, annular oligomers, protofibrils, inclusion bodies or insoluble fibrils, based on the pathological pathways (**Table 1**). Dynamic intermediate oligomers form transient structures facilitating on-pathway conversion from misfolded monomers to fibrils. The spherical and annular aggregates get generated off-pathway and contribute highly to amyloid mediated neurotoxicity [2, 9]. The Lewy bodies formed by αS are example of intracellular amyloid inclusions [10]. Formation of a specific aggregated form is usually controlled by process of nucleation. In primary nucleation, seeds are formed by spontaneous aggregation of monomers followed by fibril elongation, whereas fragments from mature fibrils recruit monomers to facilitate the polymerization in secondary nucleation process. However, this process is specific to monomeric species; seeding with Aβ(1–42) fibrils does not contribute to aggregation of Aβ(1–40) [8]. Comparison between kinetic profiles of seeded and non-seeded Aβ(1–40) suggest that aggregation follows the seeded fibrils template [8]. Monomeric Aβ(1–40) samples form striated ribbons under constant agitation, and twisted fibrils under undisturbed conditions [11]. NMR can also be used to study kinetics of oligomer interactions by utilizing the constant exchange of detectable monomers with other invisible oligomeric species, through saturation transfer difference (STD) experiments [12]. Amyloid fibrils show protease resistance and are insoluble in detergents. However, their affinity to small lipophilic molecules is amenable for detection through biophysical assays using Congo red and thioflavin dyes [13]. These fluorescent dyes can be used to quantify β-sheet rich amyloid fibrils under laboratory conditions where fluorescent intensity linearly correlates with fibril formation [14]. Morphology of amyloid fibrils can be studied using transmission electron microscopy (TEM) by observing diffraction patterns. Whether fibrils form a ribbon-like or a striated pattern, can be calculated by mass-per-length constraints [15]. Different amyloid structures can be categorized

Amyloid structure	Structure/symmetry	Toxicity	Relevance	Refs.
Monomer	Disordered, random coil	Non-toxic	On pathway*	[20]
Small oligomers	Rich in β-sheet (spherical, annular, ADDLs)	Toxic	On pathway	[21, 22]
Amyloid inclusions	Amorphous aggregates	Non-toxic	Off pathway	[2, 23]
Protofilaments	Homogenous, up to 200 nm long	Toxic	On pathway	[24]
Fibrils	Cross-β	Non-toxic	On pathway	[25]
Plaque	Amorphous meshworks, fibril bundles, amyloid stars	Non-toxic	—	[26]

On-pathway structures are intermediates involved in aggregation of amyloids into fibrils.

Table 1.
Summary of amyloid structures observed in AD.

based on symmetry and periodicity with these methods [16]. Aβ40 fibrils structure have either 2-fold (2A) or 3-fold (3Q) rotational symmetry with a twisted morphology with periodicity of 120 ± 20 nm, and roughly 8 nm width [2, 11]. IAPP fibrils also lead to formation of striated and twisted ribbons [11].

2.2 Structural properties of amyloid aggregates

Basic structural architecture of amyloid fibrils consists of a characteristic 4.7 Å repeat through the cross-β structure [2]. Still, there exist some variations in morphology of different Aβ fibrils; the C-terminus in Aβ(1–40) is hidden within the core, while the corresponding residues are exposed on the surface of Aβ(1–42) [10]. Solid state NMR studies of amyloid fibril structures employ 2D dipolar-assisted rotation resonance (DARR) experiments and frequency selective rotational-echo double resonance (REDOR) experiments along with distance measurements between ^{13}C-^{13}C and ^{13}C-^{15}N nuclei [8]. Studies of Aβ(1–42) fibrils using ssNMR suggested unidirectional protofibril growth with two molecules coming together to form parallel intermolecular β-sheets. The ssNMR structure of homogeneous Aβ(1–42) fibrils (PDB ID: 2MXU) exhibits triple-β-strands encompassing residues 12–18, 24–33 and 36–40 respectively, connected by coil-and-turns at residues 19–23 and 34–42 [8]. The other structure of Aβ(1–42) fibrils with oxidized Met35 (PDB:2BEG), suggests structural inhomogeneity in N-terminal residues from 1 to 17 and presence of two β-strands in residues 18–26 and 31–42, respectively [17]. The AD related Aβ(1–42) polymorph shows double-horseshoe like cross-β structure where N terminus of monomeric Aβ(1–42) has L shape and the C terminus takes an S-shape. The structure comprises five in-register parallel strands with each fibril layer comprising of two molecules with hydrophobic side chains buried maximally [16]. In addition to hydrophobic core, parallel β-sheets also show polar zipper interactions through intermolecular hydrogen bonds. A recent 4.0 Å resolution ssNMR-cryoEM hybrid structure (PDB ID: 5OQV) of intertwined Aβ(1–42) protofilaments showed an approximate 21 screw helical symmetry with 4.67 Å rise [18]. This implicated step-wise shift between the subunits is similar to tau dimers. Three hydrophobic clusters involving Ala, Val, Phe, Leu, Ile, and Met residues expand along the fibril axis and contribute to overall stability of fibrils [18]. Tertiary interactions in Aβ(1–42) fibrils are different compared to Aβ(1–40) fibrils owing to differences in side-chain packaging in hydrophobic core of protofilaments [17]. The N-terminus of Aβ(1–40) peptides is disordered and highly prone to proteolysis whereas the ordered region from tyrosine residue onwards acquires a double-layered structure with a "β-arch" motif where two β-strands are separated by a short loop [19]. This motif is stabilized by formation of a salt bridge between Asp23 and Lys28 across the bend. Interactions between Lys28 and Ala42 observed in these fibrils differ to those found in Aβ(1–40) [8]. Similarly, salt bridges between Asp1 and Lys28, Asp7 and Arg5, Glu11 and His6 and His13, and Asn-Gln ladders further contribute to stability of the Aβ(1–42) fibril structure [16, 18]. Point mutations introduced in basic amino acid sequences lead to varied fibril architecture compared to the wild-type Aβ40 fibrils [2].

NMR structure of tau monomers shows detectable propensity to β-sheet, poly-proline helices and transient α-helical conformations. Aggregation of the R3 fragment consisting of 26 amino acid residues is strongly associated with formation of fibrils in the presence of polyanions as well as during self-assembly of pristine tau [7]. Phosphorylation of serine and threonine residues stabilize the α-helical conformation. αS fibrils acquire an overall assembly that mimics a Greek key as seen in atomic resolution structure of purified protein [7].

2.3 Role of amyloidosis in AD pathology

Amyloidosis in cerebrovascular system is mediated through aggregation of wild type proteins as the consequence of multivalent interactions in intrinsically disordered proteins or regions of proteins, mutations in amyloidogenic precursors, expansion of repeats in the amyloidogenic sequences, actions of proteases or chemical modifications on the precursor sequences, overexpression of the precursor, liquid–liquid phase separation, actions of small metabolites or age-related cell death [27–31]. The number of human proteins capable of causing amyloidosis has exceeded 50 and are associated with many neurological disorders based of variations in the disease precursors [2]. Different oligomeric and morphologically distinct species affect cells and tissues in different manner. Amyloid oligomers can be cytotoxic with the long-term potentiation disrupting membranes to cause ion permeability and homeostasis imbalance [2]. Fully matured fibrils contribute to the disease by disturbing lipid membranes and thus interfere with the general membrane bound cellular organelles, or by forming physical barriers which disrupt inter-cellular communications [2]. Structural inhomogeneity was observed in fibrils from the different clinical variants of amyloids in AD. Differences in morphology of Aβ aggregates can change the phenotype observed in different forms of AD. While a single Aβ structure is found in brain cortex in the cases of typical prolonged duration AD, different polymorphs are found in the cases of rapidly progressive form of AD [32]. There are six isoforms of tau protein in brain and CNS, longest one containing a stretch of 441 amino acids, with high tendency for phosphorylation and self-assembly. Tau filaments cause different tauopathies, including neurofibrillary tangles in AD [7]. Neurofibrillary tangles and neuropil threads are predominantly tau aggregates localized in neuronal cell bodies and processes, respectively, whereas the mature Aβ plaques are present extracellularly [33].

2.4 Toxicity of amyloid aggregates

Aβ(1–42) is more potent fibrillogenic Aβ variant in human brain, yet its concentration accounts for only about 5–10% of Aβ(1–40) concentration produced [33]. Accumulation of Aβ(1–42) in brain and cerebrovascular system due to its aggregation propensity, and by extension, the relative ratio of Aβ(1–42): Aβ(1–40) is a biomarker for AD [33]. Aβ(1–40) fibrils are less toxic compared to Aβ(1–42) fibrils which show differential toxicity and Aβ(1–43) fibrils are most cytotoxic [10]. Soluble Aβ in the form of low-n oligomers, Aβ-derived diffusible ligands and protofibrils are among major toxic contributors towards AD pathology [34]. Interactions of oligomeric species with other molecular complexes, metal ions and cellular membranes also impact the extent of their toxic effects in brain. N-terminus truncated Aβ peptides (Aβ(n–42), where n can vary from 2 to 11) are highly toxic and are usually present in Aβ deposits in AD brain [7]. Peptides cleaved at positions 3 or 11 are prone to pyroglutamylation and prominent components of Aβ deposits, likely owing to their resistance to proteolytic degradation [35]. αS is a major component of Lewy bodies and neurites causing neurotoxicity through dopaminergic mechanism and these aggregates are key identifiers for PD and Lewy body dementia [7]. Detection of αS aggregates early in peripheral nervous system can serve as early biomarkers before motor disabilities develop in PD patients [36]. Horizontal transfer of amyloid aggregates and oligomers between cells also contributes to propagation of disease pathology [37]. IAPP oligomers can impair insulin secretion in pancreatic cells and cause cell death and cellular uncoupling [7].

3. β-Amyloid chaperones

The main function of molecular chaperones is to facilitate their protein targets acquire proper functional conformation or fold into correct oligomeric assemblies [38]. Several proteins have been shown to bind misfolded amyloids and inhibit amyloid aggregation or promote refolding of polymorphs. They usually play other physiological roles, yet show tendency to prevent amyloidosis under specific stimuli. We will discuss some of these non-proteolytic chaperones in more detail below. Additionally, albumin binding to polymeric Aβ, α1-antitrypsin, immuno-globulins A and G are endogenous human cerebrospinal fluid (CSF) proteins with capability to control amyloid formation, although the inhibitory activity is lesser by two orders of magnitude compared to plasma [39].

3.1 Heat shock proteins

Heat shock proteins (Hsp) are the most commonly known chaperones in human body, divided into five classes Hsp70, Hsp90, Hsp60, Hsp40, and small Hsps, differing in protein size. Chaperones in Hsp60 and Hsp70 family mediate folding, Hsp33 family hold partially folded proteins till other chaperones or degradation system can act, and Hsp104 promotes solubilization of aggregated proteins [38]. Mutations in αA- or αB-crystallin and other small heat shock proteins have been linked to increased risk of certain amyloidosis conditions [40]. Hsp70 and Hsp90 are capable of utilizing ATP molecules to perform energy costly unfolding of stable misfolded aggregates and then convert them into properly folded conformations. Similarly other members like Hsp110 can act as disaggregases to forcibly solubilize preformed fibrillar aggregates [41]. Hsp70 recognizes KFERQ motif in proteins to mediate degradation by transporting them to lysosomal compartments inside the cell, a typical example of chaperone mediated autophagy [41]. Disaggregation machinery in humans mainly involves proteins from Hsp family namely, Hsp110, Hsp105, Hsp100, and Hsp70/40 co-chaperone cognates [41]. Proteins in Hsp40 co-chaperone family, mainly DnaJB6 and B8, can decrease the aggregation of polyglutamine peptides in HD in addition to modulating Hsp70 activity [41]. Binding of misfolded proteins and interaction with ATPase domain of Hsp70 are achieved via the highly conserved J domain. Anti-aggregation activity of Hsps, especially Hsp70, is at least partially dependent on presence of ATP [42]. ADP-bound forms of Hsp70 is generated as a result of the ATP hydrolysis by its co-chaperones. This form has high affinity for the hydrophobic residues in misfolded peptides and holds the unfolded ensemble till it spontaneously achieved proper conformational populations. Small Hsps (12–42 KDa) do not require ATP since they usually function as holdases and assist other proteins in Hsp chaperone complex [41]. Hsp104 is part of proteostasis network which regulates prion assembly in yeast, Hsp110 is its human counterpart which facilitates chaperone function of Hsp70 and Hsp40 [43]. Hsp90 and its co-chaperones interact with tau and a close control on their interaction can cause the oligomers to turn into benign species instead of toxic aggregates [44]. Free Hsp90 binds and releases substrates in its monomeric extended form, and attains a dimeric ATP-bound closed conformation mediate by co-chaperones p23/Sba1, Hsp70/90 organizing protein (HOP), Cdc37 and other proteins [41, 45]. Hsp60 chaperonins are mainly located in mitochondria and form heptameric, double ring complexes which provide isolated environment for protein folding with co-chaperonins Hsp10 forming the lid of this cavity. Unlike Hsp60, the cytosolic chaperonins in neurons such as TCP-1 Ring Complex (TRiC or CCT), do not require co-chaperones for their function and form a double

ring complex with each ring consisting of eight subunits. Hsp60 can interact with mutant αS in PD brain [41]. B-chain of monomeric or dimeric insulin can bind to IAPP monomers, thus preventing their aggregation [7]. Presence of chaperone proteins such as Hsp27 (HSPB1), αB-crystallin (HSPB5) and Hsp70 (HSPA1A) can decrease cell toxicity, possibly by binding Aβ oligomers and converting them to larger less toxic aggregates, however it not clear whether they bring about any change in secondary or tertiary structure [44]. HSPA6 is induced in neurons post heat shock, unlike other members of this family HSPA1A and HSP8, thus has a unique probable role as a human neuronal chaperone [46]. Hsp70 and Hsp90 have differential roles in case of inflammation and macrophage recruitment. Hsp90 can also stabilize neurotoxic proteins and should be carefully controlled to achieve required therapeutic outcomes.

3.2 Intracellular degradation machinery

Molecular chaperones are capable of binding and folding intracellular soluble amyloid aggregates, however solubilization of amyloid plaques is out of scope for these proteins. While these chaperones may not be able to completely reverse neurodegenerative symptoms, they play crucial anti-apoptotic functions through protein folding and degradation of unfolded or misfolded proteins. There are fundamental mechanisms underlying a chaperone function, namely, unfolded protein response (UPR), protein compartmentalization, heat shock response, chaperone-mediated autophagy and lysosome system, ER associated degradation (ERAD) and ubiquitin-proteasome system (UPS) [46]. Extracellular chaperones mediate proper protein folding and refolding by providing isolated environment or through intermolecular interactions. If proper folding cannot be achieved, they may play a role in mediating intracellular proteasomal degradation or microglial digestion of unfolded protein fragments. Cytosolic chaperone systems like Hsp70 and Hsp40, comprising heat shock cognate 70 (Hsc70), and their interacting proteins like C-terminus of Hsc70-interacting protein (CHIP) can mediate targeting of misfolded proteins to proteasome machinery [38]. Intracellular pathways for degradation of misfolded proteins include the UPS, 26S proteasome and lysosome-mediated phagocytosis [9]. Hsp70 recruits misfolded proteins, especially αS, for degradation by directing them to proteasomes or autophagy-lysosomal pathway [44]. UPS comprises of a cascade of enzymes E1 (Ub activating enzyme), E2 (Ub conjugating enzyme) and E3 (Ub ligase) facilitating binding of ubiquitin (Ub) to target proteins. Ligase enzymes in this cascade can employ chaperone proteins such as Hsc70 for recognition of exposed hydrophobic regions misfolded peptides and together mediate proteolysis of misfolded intracellular proteins. E4 enzyme from UPS can recruit additional Ub molecules to the protein substrate which can be either directed for degradation or other protein interactions based on their topology [41]. 26S proteasome particles degrades polyUb-conjugated proteins with the help of its two 19S regulatory particles responsible for recognition and de-ubiquitination and a 20S core particle through which proteolysis of these substrates into short peptides is mediated [41]. ERAD withholds misfolded proteins from continuing with on-going cargo to golgi apparatus for further downstream processes. UPR is initiated by migration of transcription factors to the nucleus causing upregulation of ER chaperones-encoding gene expression in response to increasing in unfolded proteins, such as Aβ aggregates in neuronal ER, which require assistance of chaperones present in the ER [47, 48]. Reduction in UPS proteolytic activity is linked with disease pathology in AD, PD, ALS, HD as well as TSEs.

3.3 Non-proteolytic amyloid chaperones

Aging is a major risk factor for many neurodegenerative diseases. The UPS system is known to get deregulated with increase in neuronal age and therefore offers less resistance to pathological protein aggregates. Proteins such as Human serum albumin show competitive binding towards Aβ oligomers, with its different domains binding to many oligomeric molecules [12]. It binds almost 90% of plasma Aβ peptides potentially through dual binding mechanism involving Aβ(1-40) monomers and protofibrils likely utilizing residues involved in fibril formation.

Here we suggest non-proteolytic endogenous proteins which show potential as AD therapeutics directed against Aβ, namely, Lipocalin-type prostaglandin D synthase (L-PGDS, also known as β-trace), apolipoprotein E (ApoE), α2 macroglobulin (α2M), haptoglobin and clusterin as alternatives to intracellular Aβ degradation machinery. These proteins have been sporadically studied for their holdase or transporter activity, yet their Aβ chaperone function is still unexplored. L-PGDS is the second most abundant protein in human CSF after albumin [49]. It exhibits dual functions, as a lipophilic ligand transporter in cells and as isomerase in arachidonic acid pathway to convert prostaglandin H2 to prostaglandin D2 [50]. L-PGDS plays protective roles in different neurological diseases including genetic demyelinating disease, brain injuries and multiple sclerosis [51]. It plays protective role against cerebral ischemia as well [52]. It promotes recruitment of astrocytes and glial cells to the source of injury [53]. Early stress stimulus can upregulate L-PGDS suggesting its unknown, yet novel stress protection mechanism. L-PGDS deficient mice showed additional neuro-nal apoptosis strongly indicating its important protective function in neurons and surrounding oligodendrocytes [51]. L-PGDS binds to various Aβ peptides including fibrils and is colocalized in Aβ plaques [54]. Furthermore, Aβ is physi-ologically secreted to CSF under normal conditions where L-PGDS is abundant. L-PGDS ratio in CSF:serum is already identified as early biomarker for detection of potential damage to blood–brain barrier and quantifying its complex with transthyretin in CSF is also suggested to be a possible diagnostic marker [55]. L-PGDS colocalizes with amyloid plaques and mediates inhibition of aggregates through cysteine residue [54]. Based on previous studies and our own findings, we posit that secreted L-PGDS binds to monomeric and prefibrillar Aβ and inhibits amyloid aggregation in synergy with its ability to break down mature fibrils.

α2M is also an extracellular glycoprotein showing potential chaperone proper-ties. It can prevent proteases from hydrolyzing proteins and mediates Aβ clearance through formation of α2M/protease complexes [56]. It protects cells from apoptosis through receptor interactions. α2M selectively binds proteins in non-native confor-mations and in process prevents their aggregation [56]. α2M decreases with age and its clearance mechanisms for Aβ get impaired [57]. A2M gene polymorphisms are associated with sporadic AD in some populations and α2M is also present in amyloid plaques [58]. ApoE isoforms E2, E3 and E4 can delay amyloid aggregation through differential activity as extracellular chaperones [59]. The suggested role of apoE4 as pathological chaperone is partly due to its role in delaying fibril formation from Aβ monomers leading to increase in toxic oligomeric species [60]. Isoform E3 and E2 show binding affinity to oligomeric Aβ as well, thus decreasing their neurotoxic effects [61]. Clusterin, or apolipoprotein J, is also extracellularly secreted and moonlights as a chaperone upon cellular stress stimulus [62]. Very similar to sHsps in its chaperone activity, clusterin preferentially interacts with off-pathway aggregates which are highly toxic and prone to precipitation [63]. Haptoglobin is very similar to α2M as an extracellular chaperone glycoprotein, though is activated in acidic

Chaperone	Function	Amyloid species	Chromosomal and cellular location	Brain expression levels (HPA) [66]	Refs.
Hsp70 (DnaK)	Holdase	Nascent amyloids and oligomers	6p21; intracellular	66.3 TPM	[42, 67, 68]
Hsp90	Holdase	Nascent amyloids and oligomers	14q32; intracellular	1021.5 TPM	[69, 70]
Hsp40 (DnaJ)	Holdase; disaggregase in ternary complex	Higher order aggregates	19p13; intracellular	110.5 TPM	[71]
αB crystallin (Hsp27)	Holdase	Amyloid fibrils	11q23; intracellular, membrane	1888.7 TPM	[72]
Hsp110	Disaggregase; holdase	Prefibrillar oligomers	13q12; intracellular	109.4 TPM	[73]
Hsp28 (sHsp)	Holdase	Unfolded or misfolded proteins	7q11; intracellular	98.7 TPM	[74]
Hsc70 (HSPA8)	Chaperone mediate autophagy	Nascent polypeptides	11q24; intracellular	1119.0 TPM	[75]
Hsp60	Chaperonin	Early oligomeric species	2q33; intracellular	141.6 TPM	[76, 77]
STUB1 (CHIP)	Ubiquitin-mediated protein degradation	Misfolded protein aggregated	16p13; intracellular	137.7 TPM	[78]
L-PGDS (β-trace)	Aggregation inhibitor	Monomers, fibrils	9q34; secreted	1224.7 TPM	[54]
Clusterin (ApoJ)	Stabilizer	Senile plaques/ diffusible aggregates	8p21; secreted	10875.4 TPM	[79]
α2-Macroglobulin	Inhibitor	Prefibrillar species	12p13; extracellular	172.0 TPM	[80]

Table 2.
Mode of function of endogenous amyloid chaperones.

environment and interacts with prefibrillar amyloids to prevent further aggregation [64]. These endogenous proteins and many others may play very important yet undiscovered role to maintain proteostasis in physiological environment.

Ig antibodies such as aducanumab, targeting specific oligomeric forms of Aβ have already reached clinical trials, showing promising therapeutic effects focusing on delaying cognitive decline. However recent withdrawals of some of these antibodies like bapineuzumab and solanezumab, have forced scientists to look for more robust options [65]. Enzymes such as insulin degrading enzymes, neprilysin, cathepsin B are capable of degrading amyloidogenic peptides. Enzymes belonging to peptidyl-prolyl cis/trans isomerase (PPIase) family, e.g., CypB, can convert

proline containing peptides from cis to trans conformational isomers thereby facilitating their folding process [47]. Some inherently amyloidogenic proteins such as PrP, hTTR and BRICHOS-domain containing proteins can paradoxically also inhibit aggregation of other amyloids. BRICHOS-domain containing protein ITM2B (Bri2) is a CNS membrane protein which shows chaperone functions for amyloids by binding to tyrosine and other charged residues [43]. Tetrameric form of Transthyretin (TTR) can also prevent aggregation of amyloid oligomers [44]. In recent years, some small molecules have also been put forward as possible inhibitors of amyloid aggregation—such as polyphenols, e.g., epigallocatechin gallate (EGCG), curcumin, resveratrol, etc. [7, 12]. Understanding inhibition mechanisms of non-proteolytic endogenous chaperone proteins and other molecules is very crucial to develop therapies targeting amyloid aggregates in AD (**Table 2**).

4. Oxidative stress

Oxidative stress in human body is mainly mediated by reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl free radical ($\bullet OH$), superoxide ($O_2^{\bullet-}$), peroxyl ($\bullet OOR$), hypochlorous acid (HOCl), nitric oxide (NO), peroxynitrite ($ONOO^-$) and other reactive nitrogen species. Increase in concentration of these reactive species can trigger downregulation of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase, thioredoxin and small molecules such as melatonin and coumarin [81]. Endogenous sources of ROS include components in mitochondrial electron transport chain and NADPH oxidase, which can trigger pathological responses leading to lipid peroxidation, DNA damage and cell death mediated by toxic products. Amyloid plaques have been shown to contain multi-fold concentrations of metals like iron (Fe), copper (Cu) and zinc (Zn) [81]. Intracellular Aβ can also trigger production of highly toxic molecules such as 4-hydroxyl-2,3-nonenal (HNE) and malandialdehyde through interactions with Fe or Cu which can catalyze Fenton reactions to generate more OH^- ions. Hydroxyl radicals can also be generated from other brain ROS through Haber-Weiss reaction, particularly within mitochondria which are most prone to such oxidative damage [82]. Such ROS are generated as by-products of amyloid aggregation and are involved with metallobiology of dementia. High concentrations of Fe in brain have drastic effects in cases of dementia leading to cognitive decline through interactions with brain amyloids [83]. Metal ions are crucial for many brain functions and Fe and Cu even possess active sites for binding antioxidant SOD. However, increase in concentrations of some of these metals can cause high levels of toxicity. Twenty percent of cases in familial ALS is affected by mutation in a single enzyme SOD1 functionally affected by Cu/Zn [83]. Metal ions have propensity to bind high affinity, well-protected and redox-shielded binding sites of proteins, and in higher concentrations they can bind to other putative active sites in proteins involved in pathology of various degenerative diseases [84]. While zinc compounds have been tested for their probable therapeutic role in overcoming cognitive degeneration, Zn^{2+} ion has also been implicated as a contributor to formation of amyloid plaques which act as metal sinks [84]. Such mitochondrial damage and mutations have been associated with age related late-onset, non-autosomal dominant AD pathology [85].

4.1 Amyloid-β and oxidative stress

Aβ binds and reduces Fe^{3+} and Cu^{2+} in presence of endogenous reducing agents to generate H_2O_2 further producing other partially ROS [86, 87]. Studies have revealed the role of metal ions such as Fe, Cu and Zn in inducing Aβ aggregation

and oligomerization [88–90]. Brain regions rich in Aβ(1–42) show increased oxidative stress, possibly mediated through redox interactions with the only methionine (Met35) present in peptide sequence [91]. Amyloids oligomers can also trigger ROS generation [2]. The three histidine residues-His6, His13 and His14, facilitate Aβ coordination with transition metal ions. These residues get protonated in acidic environment and may increasingly contribute to aggregation at low pH. Cu^{2+} interacts with Aβ and oxidizes sulfur of Met35 to activate formation of disulfide bonds leading to dimerization and other oligomeric formations. Soluble oxidized aggregates avoid clearance causing enrichment of brain regions with these agglomerates. Aβ peptides can successfully recruit ions of metals like Fe, Cu and Zn through sulfide group of Met35 and a chelating domain which involves Asp1 and all three histidines, in a bid to induce redox complexes capable of bringing about oxidative insults [7]. Dityrosine cross-linked Aβ dimers along with nitrotyrosine cross-linked proteins are also associated with oxidative stress [92]. Cell death from oxidative stress is a cumulative result of alteration in proteostasis, protein phosphorylation and glucose metabolism as downstream consequences of increase in Aβ42 concentration [91].

4.2 Pathophysiology of ROS

Excessive free radical production as a result of oxidative stress at cellular levels causes protein oxidation and lipid peroxidation [91]. Lipid peroxidation leads to break down of unsaturated fatty acids among other components of membrane phospholipids, leading to accumulation of isoprostanes, acrolein, thiobarbiturate-reactive substances, etc. [84, 92]. Glutamate receptors overstimulation can trigger downstream cell death cascades through increased calcium influx and generation of nitric oxide species [48]. 8-hydroxy-2′-deoxyguanosine (OHDG) is one of the oxidative markers for DNA, found in PD patients [36]. These products impair glucose transport and glutamate uptake, hence contributing to cell apoptosis. ROS cause imbalance in metal and ion homeostasis, for example Ca^{2+}, which can trigger imbalance in downstream signaling cascades. Oxidative damage can lead to hydroxylation of nucleic acids and carbonylation of proteins. Carbonyls are markers of protein oxidation and have been found concentrated in frontal brain regions of AD patients [92]. Free radicals generated as result of amyloid oligomerization or aggregation can directly mediate mitochondrial damage which triggers neuronal death through downstream pathways, one of them being cytochrome C reduction [48]. OS in PD cases may be a result of deregulation of dopamine-iron redox pathway, since αS can alter expression of enzymes indirectly regulating dopamine synthesis [84]. αS is also known to directly interact with metal ions causing protein aggregation. ALS is mainly characterized by loss of motor neurons, which combined with SOD mutations diminishing its free radical scavenging abilities can exacerbate the impacts to oxidative injury [84]. Oxidative markers localized in plaques and NFTs are toxic products such as 3-nitrotyrosine, HNE, pyrraline and pentosidine, while metal enriched protein carbonyls including ferritin, catalyst, Cu/Zn-SOD and Mn-SOD [93].

4.3 Protective mechanisms from ROS

Chaperones can bind ROS generated as by-product of amyloids and thus prevent triggering breakdown of homeostasis. α2-Macroglobulin can directly bind Aβ and potentially act as a chaperone in addition to its zinc-binding capabilities which can help mitigate redox activity of Aβ [94]. Zn^{2+} is redox-inert and may be helpful in mitigating metal mediated Aβ redox activity. ApoE can mediate Aβ clearance as a chaperone depending on specific isoform interactions; ε4 may potentially increase

Aβ pathology [95]. ApoE interaction with Aβ is modulated by metals [93]. There are two key requirements for metal interactions in dementia-either supplementation or chelation [83]. Metal chelators can help solubilization of Aβ plaques. Effects of chaperones on metal ions need to be carefully curated to maintain metal homeostasis in brain and other organs of nervous system. Several endogenous brain proteins show potential protective response against ROS in diseased brain, including β-trace protein, which is the second most abundant protein in CSF. Competitive binding of metal ions by chaperone proteins may be advantageous in decreasing generation of ROS by-products. Overexpression of metal-affinity proteins have been shown, as example of iron-binding ferritin. Antioxidant alpha-tocopherol has been reported to potentially slow AD progression in addition to action of metal chelators such as clioquinol and desferrioxamine [92]. L-PGDS scavenges ROS and in process protects against neuronal cell death with its ligand binding function intact [96]. It also reduces cytotoxicity mediated by oxidation of heme metabolites such as bilirubin [97]. We have found that L-PGDS can directly interact with Aβ-heme complex and lower its peroxidase activity (to be published). Other non-proteolytic chaperones such as α2M, clusterin and haptoglobin, also show neuroprotective potential in similar manner as L-PGDS and are good candidates for more comprehensive oxidative stress related studies.

5. Conclusions

Alzheimer's disease is a debilitating neurodegenerative condition and is projected to be a major risk factor for global population by the year 2050 [98]. One of the key theories, known as "amyloid cascade hypothesis" postulates that oligomerization of amyloid-β (Aβ) in brain is the key pathological event in AD [99]. Although complete explanation of causation in AD is yet to be established, it has been universally accepted that amyloidosis (perhaps provoked by environmental factors) plays a crucial role in AD progression [99–101]. Conformational alterations in Aβ which lead to its conversion from soluble peptide to insoluble aggregates are considered as a key mechanism in pathogenesis of neurodegenerative diseases such as AD [102]. Early amyloid aggregates can act as biomarkers in most dementia related maladies and associate observed clinical symptoms to underlying pathophysiological mechanisms. Till date, we do not have any therapeutic solution for aggregation of amyloids. Current gold-standard biomarkers in neurodegeneration are neuroimaging systems of degeneration and detectable clinical symptoms represent pathological changes causing irreversible damage to nervous system [33, 36]. Success of theranostic efforts will rely on rational drug design based on a proper understanding of molecular structures and mechanisms involved in aggregation. Aβ mediates AD pathology through direct inhibition of neuronal interactions and signaling cascades, and triggers oxidative stress in the process. Here we have discussed mechanisms of action of amyloid chaperones with focus on Aβ chaperones to better understand their modus operandi. Apart from the protein quality control machinery and housekeeping complexes responsible for maintaining general proteostasis, we have discussed importance of other endogenous chaperones which step up in time of crisis, often as response to stress stimuli. Proteins such as L-PGDS, clusterin, α2M have are involved in many physiological processes, with elusive function as amyloid chaperones. These proteins can provide alternative mechanisms to control amyloid aggregates in events of failure of intracellular Hsp complexes and proteasome machinery. Additionally, their affinity to redox active components may provide additional protective mechanisms against Aβ mediated oxidative stress.

Conflict of interest

Authors declare no conflict of interest.

Abbreviations

Aa	amino acid
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
Aβ	amyloid-β
CNS	central nervous system
CSF	cerebrospinal fluid
DARR	dipolar assisted rotation resonance
EM	electron microscopy
HD	Huntington disease
HNE	hydroxy-2,3-nonenal
Hsc	heat shock cognate
Hsp	heat shock protein
HSR	heat shock response
IAPP	islet amyloid polypeptide
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
OHDG	8-hydroxy-2′-deoxyguanosine
PD	Parkinson's disease
PDB	protein data bank
PrP	prion protein
REDOR	rotational echo double resonance
ROS	reactive oxygen species
SOD	superoxide dismutase
ssNMR	solid state nuclear magnetic resonance
T2D	type II diabetes
TEM	transmission electron microscopy
TSE	transmissible spongiform encephalopathy
Ub	ubiquitin
UPR	unfolded protein response
UPS	ubiquitin proteasome system
αS	α synuclein

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