Protein Misfolding and Amyloid Formation in Alzheimer's Disease

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Abstract

The information necessary for proteins to correctly fold into biologically active three dimensional (3D) structures is present in the amino acid sequence. The ways by which proteins fold still remain one of the unexplained mysteries in the field of protein biochemistry. Investigating the impact and consequences of protein misfolding can help decipher the molecular causes behind the complex amyloid diseases such as Alzheimer's disease (AD) and Parkinson's disease. Various participating molecular entities like amyloid beta (Aβ), tau protein, and non-beta sheets are facilitating the pathogenesis of Alzheimer's disease. Understanding their structure as well as their mechanism of action is useful to decode the therapeutic treatment for these complex diseases.

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Protein misfolding • Amyloid formation • Amyloid beta structure • Amyloid fibrils • Alzheimer's disease • Neurodegenerative disorders

7.1 Introduction

One of the mystifying paradigms in biochemistry is to understand the dilemma of protein folding. In recent years, with the discovery of numerous diseases as protein folding disorders and with the sudden increase in the genomic information and the requirement for proficient methods to predict protein structure, protein folding developed into a fundamental subject in biological sciences (Brito et al. 2004). Protein folding diseases can be classified into two sets: (1) in first, extreme amounts of incorrectly folded proteins accumulate as unfolded proteins. This group represents amyloidosis diseases, such as Alzheimer's disease (2) in this group, a little fault in the genetic outline directs towards partial folding of a protein, thereby affecting its function.

Protein folding is accepted as a crucial issue in the twenty-first century. To correctly predict protein-folding patterns is tricky owing to the complex structure of proteins (Chen et al. 2012). In order to carry out their biological functions, all proteins should fold into specific three-dimensional structures. However, the genetic information for the protein gives merely the linear chain of amino acid residues (the primary structure) in the polypeptide backbone. The dilemma of protein folding can be broken down into three different nevertheless correlated issues: (1) kinetic process or pathway responsible for the native and biologically active conformation of the proteins; (2) the physical basis accountable for the stability of folded conformations; (3) determination of one particular folding process by the amino acid sequence, and resulting three-dimensional structure (Creighton 1990).

7.2 Protein Misfolding

Folding and unfolding are the crucial ways of producing and eliminating explicit forms of cellular activity. Also, processes such as translocation across membranes, trafficking, secretion, the immune response and regulation of the cell cycle are directly dependent on folding and unfolding proceedings (Radford and Dobson 1999). There are different levels of structures into which the protein chains can fold, however, a small number of fundamental confined folds, or secondary structures (for example; helices and beta sheets), are prevalent. Folding *in vivo* is a co-translational event with few an occasions, which means that it commences way ahead before the completion of protein synthesis with the growing chain still attached to the ribosome (Hardesty and Kramer 2001). For some proteins, on the other hand, the major part of their folding undergoes in the cytoplasm following their release from the ribosome. However, there are others that fold in specialized organelles, for example mitochondria or the endoplasmic reticulum (ER), after trafficking and translocation all the way through membranes (Bukau and Horwich 1998).

Specific proteins particularly molecular chaperones are, consequently, essential to aid proteins in folding and to avoid aggregation of folding intermediates. Failure to fold precisely, or to stay accurately folded, will as a result confer increase in the malfunctioning of living systems and consequently to disease (Horwich 2002). Several of these diseases, for instance, cystic fibrosis (Thomas et al. 1995) and a few types of cancer (Bullock and Fersht 2001), result from incorrect folding of proteins and not being capable to put into effect their appropriate function. Many of these disorders are familial since the possibility of misfolding is frequently larger in mutational variants. In cases of proteins that have an elevated inclination to misfold, escape the entire protective machinery and form unmanageable aggregates inside the cells or in extracellular space. A growing amount of disorders, together with Alzheimer's and Parkinson's diseases, the spongiform encephalopathies and type II diabetes, are directly related with the deposition of such aggre-

gates in tissues, such as brain, heart and spleen (Horwich 2002; Dobson 2003; Aguzzi and Calella 2009).

Protein folding diseases can be classified into two sets:

1. Firstly, as unfolded proteins, which are the result of abnormal accumulation of incorrectly folded proteins. The proteins in this group majorly are prevalent in causing amyloidosis diseases, such as Alzheimer's disease.
2. Secondly, due to minor faults in the genetic outline that directs towards partial folding of a protein, thereby affecting its function.

One of the simplest is to unfold the protein in a high concentration of chemical denaturant, such as guanidium chloride, and then dilute the solution rapidly, such that the denaturant concentration falls below that at which the native state is thermodynamically unstable. Another strategy that has been developed is to use a battery of complementary stopped-flow and quenched-flow techniques, each of which is capable of monitoring a specific aspect of the formation of native-like structure (Dobson et al. 1994). In addition, novel methods to initiate refolding reactions are being introduced (Ballew et al. 1996). These methods include the use of temperature jumps under conditions where cold denaturation takes place and an increase in temperature leads to refolding. In some cases, the rapid change of oxidation state of a metalloprotein can trigger the onset of the folding reaction (Pascher et al. 1996). With such approaches, folding events on the micro- and sub-micro-second time scale is becoming accessible (Dobson et al. 1998).

7.3 Amyloid Formation of Proteins

Protein misfolding leads to malfunctioning of cellular machinery, hence consequently resulting in a wide spectrum of diseases. One of these events being amyloid, where protein fragments or whole protein unfolds from their soluble forms to insoluble fibrils. Most of the times, these fibrils appear in the form of β-sheet, hence the term β-amyloid. These fibrils accumulate in different organs and lead to corresponding different diseases.

The basic amyloid structure contrasts strongly with the extremely original globular structures of most natural proteins. In these latter structures, the interactions linked with the exceedingly definite packing of the side chains appear to make ineffective the main-chain preferences (Dobson 1999). The polypeptide main chain and the hydrophobic side chains of a globular protein are basically concealed inside the folded structure. The switch into amyloid fibrils will be feasible only when they are in an exposed state, such as once the protein is partially unfolded (at low pH) or fragmented (by proteolysis). *In vitro* experiments show that their formation is then usually characterized by a lag phase, followed by a phase of quick growth (Caughey and Lansbury 2003). This behavior is characteristic of nucleated processes for instance crystallization; the lag phase can be removed by adding up of preformed aggregates to new solutions, a method well-known as seeding. An appealing proposal is that seeding by chemically tailored forms of proteins, resulting from deamidation or oxidative stress, might in various cases be a vital aspect in activating the aggregation process and the inception of disease (Nilsson et al. 2002). The aggregates that are formed initially are expected to be comparatively disorganized structures that expose to the outer environment a diversity of parts of the protein that are in general buried in the globular state (Bucciantini et al. 2002; Dobson 2003).

Amyloid fibrils represent just one of the kinds of aggregates which are produced by proteins, even though a considerable attribute of this particular group is that its extremely ordered hydrogen-bonded structure is expected to bestow its exceptional kinetic steadiness. Therefore, once formed, these aggregates can persevere for extensive times, permitting a progressive increase of deposits in tissue, and in fact facilitating seeding of the successive translation of additional amounts of the same protein into amyloid fibrils.

All amyloid deposits demonstrate definite optical behavior (such as birefringence) on binding to specific dyes such as Congo red. The latter discloses that the ordered core arrangement is composed of β-sheets whose strands run perpendicular to the fibril axis (Sunde and Blake 1997). The most persuasive confirmation for the last proclamation is that fibrils can be produced *in vitro* by numerous other peptides and proteins, together with distinguished molecules for instance, myoglobin, as well as by homopolymers such as polythreonine or polylysine (Dobson 2001, 2003; Yoon and Welsh 2004; Xu et al. 2010).

The central structure of the fibrils appears to be steadied mainly by interactions, predominantly hydrogen bonds, concerning the polypeptide main chain. Since the main chain is universal to all polypeptides, this observation describes why fibrils produced from polypeptides of extremely dissimilar sequence look like to be so similar (Dobson 1999). In a few cases, merely a handful of the residues of a particular protein might be implicated in this structure, with the rest of the chain being coupled in some other way with the fibrillar assembly; in others approximately the entire polypeptide chain seems to be occupied. Regardless of these complications, in the past few years, various experimental methods have come into view that allows the recognition and the thorough molecular investigation of misfolded oligomers (Bemporad and Chiti 2012).

In addition, the comparative aggregation rates for an extensive collection of peptides and proteins correlates with the physicochemical characteristics of the molecules for example charge, secondary-structure propensities and hydrophobicity (Chiti et al. 2003). Further, in numerous occasions, surface charges of the proteins have been established to function as "structural gatekeepers," that shun unnecessary interactions by negative design, such as, in the control of protein aggregation and binding (Kurnik et al. 2012).

Once a protein turns into toxic, a widespread conformational transformation takes place and it attains a motif, for instance the β-sheet. It may be noted that the β-sheet conformation is present in several functional native proteins like immunoglobulins; however, the conversion from α-helix to β-sheet is a feature of amyloid deposits (Kirkitadze et al. 2001). The uncharacteristic conformational change from α-helix to β-sheet exposes hydrophobic amino acids and encourages protein aggregation. The toxic arrangement is frequently capable of interacting with other native proteins and catalyzes their conversion into the toxic condition. Because of this capacity, they are known as infective conformations. The freshly made toxic proteins replicate the rotation in a self-sustaining loop, augmenting the toxicity and consequently leading to a disastrous result that finally destroys the cell or harms its function (Reynaud 2010).

Proteins carry out their normal functions accurately when the chains of amino acids, from which they are built, fold perfectly. Misfolded proteins can be toxic to the cells and amass into insoluble aggregates with other proteins. Ataxin-1 is extremely prone to misfolding owing to inherited flaws in the gene that lead to neurodegenerative diseases, as the glutamine amino acid is recurring in the protein chain of ataxin-1. Higher the number of glutamine residues, the greater toxic is the protein (La Spada and Taylor 2010). There are about 21 proteins that generally interact with ataxin-1 and manipulates its folding or misfolding such as GTP-binding nuclear protein Ran and adapter molecule Crk protein. Studies suggest 12 of these proteins increase the toxicity of ataxin-1 for the nerve cells, while 9 of the identified proteins reduce its toxicity (Petrakis et al. 2012). This structure endorses aggregation, for the reason that the proteins that interact with ataxin-1 and possess this domain increase the toxicity of mutated ataxin-1 (Tsuda et al. 2005).

7.4 Protein Folding Diseases with Respect to Amyloid Formation

Accumulation of misfolded proteins can cause amyloid disease; the most prevalent one is Alzheimer's disease, while Parkinson's and Huntington's disease have related amyloid origins (Haass and Selkoe 2007). Every amyloid

disease involves primarily the aggregation of an explicit protein, even though a variety of other machinery together with supplementary proteins and carbohydrates are included in the deposits as soon as they are formed *in vivo*. Amyloid accumulation occurs in different parts of the body, leading to malfunctioning of bodily processes, which is termed as amyloid disease. The huge amounts of insoluble protein involved in various aggregation diseases can physically disturb particular organs and in doing so cause pathological behavior (Tan and Pepys 1994). Based on frequency of occurrence, amyloid diseases can be classified as sporadic or familial.

For neurodegenerative disorders, like Alzheimer's disease, the principal indications more or less definitely are a consequence from a 'toxic gain of function' related to aggregation (Taylor et al. 2002; Dobson 2003). Aggregation of the microtubule coupled protein tau is linked with numerous neurodegenerative disorders, together with Alzheimer's disease and front temporal dementia (Kfoury et al. 2012). In addition, the malfunction of proteins to fold accurately and efficiently is being linked with the failure of biological systems, and considerable varieties of diseases are currently identified to be related with the misfolding of proteins. A few of these diseases, for instance cystic fibrosis, result from mutations, which obstructs with the standard folding and secretion of particular proteins. Others, like Alzheimers and Creutzfeldt-Jakob diseases, are linked to the later switch of typical soluble proteins into insoluble amyloid plaques and fibrils (Prusiner 1992). Population of helical intermediates and their stabilization by means of interactions with membranes may be a significant cause by which the progression of aggregation directs to toxicity (Pappu and Nussinov 2009).

The danger of achieving any of these diseases amplifies considerably with age. The mechanistic justification for this relationship is that with age (or due to mutations), the subtle equilibrium of the synthesis, folding, and degradation of proteins is perturbed, following in the production and increase of misfolded proteins that form aggregates (Lindholm et al. 2006).

Amongst the environmental factors which raise the possibility of suffering degenerative diseases is contact to substances that have an effect on the mitochondria, resulting in enhancement of oxidative harm to proteins (Jenner and Olanow 1996). On the other hand, it is apparent that no single environmental factor is accountable. Besides, there are genetic factors as well. For instance, in the simplest forms of familial Parkinson's disease, mutations are related with dominant forms of the disease. In Alzheimer's disease, and for other fewer widespread neurodegenerative diseases, the genetics can be still extra difficult, because diverse mutations of the similar gene and combinations of these mutations may influence disease risk in a different way (Bertram and Tanzi 2004).

7.5 Alzeihmer's Disease as a Protein Folding Disease

7.5.1 Mechanism

The causal pathway of Alzheimer's disease (AD) is a complex mechanism comprising of different proteins. As per amyloid cascade hypothesis, AD is caused due to the formation of a peptide (protein) known as amyloid beta (or beta amyloid, Aβ), which clusters as senile plaques on the blood vessels and accumulation of amyloid fibrils on the outside surface of neurons of the brain leading to the destruction of neurons. This in turn inhibits the conventional impulses being transmitted by the affected neurons. Aβ has been shown to impair mitochondrial function in PC12 cells (Pereira et al. 1998). Aβ peptide is created by enzyme clipping of the normal neuron membrane protein known as amyloid precursor protein (APP) (Hartmann et al. 1997). Enzymes can clip APP in ways that do not result in Aβ formation. Moreover, there are two forms of amyloid beta peptide, one of which has 40 amino acids and another one that has 42 amino acids. The enzymes that cleave APP are known as secretases (Lathia et al. 2008). The two enzymes that initially compete to cleave APP are alpha-secretase (α-secretase) and beta-secretase (β-secretase,

BACE1) (Lathia et al. 2008). If alpha-secretase cleaves APP, there is no formation of Aβ. If APP is cleaved by beta-secretase it can then be further cleaved by gamma-secretase (γ-secretase) to form either a 40 amino acid amyloid peptide ($Aβ_{40}$) which is soluble and mostly innocuous—or a 42 amino acid peptide ($Aβ_{42}$) which clumps together to form insoluble amyloid plaques (Hartmann et al. 1997). Alpha-secretase cleavage occurs at the cell surface, whereas beta-secretase acts at the endoplasmic reticulum (Hartmann et al. 1997). Gamma-secretase produces $Aβ_{42}$ if cleavage occurs in the endoplasmic reticulum and $Aβ_{40}$ if the cleavage is in the trans-Golgi network (Hartmann et al. 1997).

7.5.2 Aβ Peptide Oligomerization

Aβ can exist as various aggregation states such as monomers, oligomers and eventually insoluble fibrils. The broad term 'oligomers' comprises diverse types of assembly for instance dimers, trimers, protofibrils, ADDLs (Aβ-derived diffusible ligands) and annular or pore-like oligomers. It was also recommended that oligomers may possibly be categorized into prefibrillar or fibrillar oligomers as they have dissimilar aggregation pathways (Cerf et al. 2009; Bitan et al. 2005).

Aβ oligomers are tiny, soluble oligomers, which consist of five or six monomer components or ADDLs and protofibrils (Baumketner et al. 2006; Oda et al. 1995; Younkin 1995; Lambert et al. 1998; Wang et al. 1999; Klein et al. 2001). The deposition of Aβ is slow and prolonged and may possibly extend for over two decades (Villemagne et al. 2013). The process of amyloid plaque formation starts with an increased production of total Aβ and Aβ42/Aβ40 ratio, which gradually leads into oligomerization of $Aβ_{42}$ and ultimately forms $Aβ_{42}$ deposits that result in inflammatory responses, astrocytic activation, synaptic spine loss and neuritic dystrophy (Haass and Selkoe 2007). It has been also reported in the literature that the structure of Aβ rather than sequence plays the principle role in Aβ induced toxicity (Celej et al. 2012). β-Sheets have been reported as dominating structures in Aβ oligomers (Cerf et al. 2009). The studies have also reported spectral similarities between Aβ oligomers and pore-forming porins and suggested that the ability of Aβ oligomers to form a porin-like structure might be associated with their toxicity in AD. Currently, inhibiting aggregation of the Aβ peptide by rational design of small inhibitor molecules may be a daunting task because of the unavailability of the high-resolution structure of toxic Aβ aggregate. However, other approaches such as high-throughput screening successfully identified certain compounds exhibiting promising inhibitory effect on Aβ aggregation (McKoy et al. 2014).

Process of Aβ fibrillation involves a conformational shift, which ultimately leads to the formation of extended β-sheets (Kirkitadze et al. 2001). Involvement of an oligomeric α-helix containing intermediate was also proposed as a key step in Aβ fibrillogenesis (Kirkitadze et al. 2001). Aβ peptide 1–40 fibril polymorphs share a common parallel β-sheet organization and possess similar peptide conformations, but differ in overall symmetry and in other structural aspects. In one of the study on disease-associated mutant, D23N $Aβ_{1–40}$, researchers have reported stabilized parallel and antiparallel β-sheets within amyloid fibrils (Sawaya et al. 2007). The role of antiparallel β-sheet structures are suggested to fibrils that are formed by short peptides with one β-strand segment only.

Certain structural models for Aβ fibrils contain a β-hairpin with intramolecular backbone hydrogen bonding between β-strand segments on either side of a β-turn present between Val-24 and Asn27 (Lazo and Downing 1998), Gly-25 and Lys-28 (Balbach et al. 2002; Li et al. 1999; George and Howlett 1999), or Ile32 and Gly-37 (Tjernberg et al. 1999). A variety of other aggregates have also been identified, including Aβ protofibrils and soluble oligomers of various sizes. NMR (nuclear magnetic resonance) studies suggest (Bertini et al. 2011; Lansbury et al. 1995; Paravastu et al. 2008; Petkova et al. 2005, 2006) Aβ fibrils are highly polymorphic, with molecular structures that depend on aggregation conditions. Detailed structural models for fibrils formed in vitro have been developed from experimental data showing that fibril polymorphs can

differ in specific aspects of peptide conformation and inter-residue interactions as well as overall structural symmetry (Bertini et al. 2011; Lührs et al. 2005). Aβ aggregates, also formed *in vitro*, provide evidence for peptide conformations similar to those in fibrils but with reduced structural order and different supramolecular organizations. Molecular structures of Aβ aggregates that develop in human brain tissue have not been characterized in detail, but evidence exists that structural variations may be biomedically important: (1) structurally distinct fibrils can have different levels of toxicity in neuronal cell cultures (Petkova et al. 2005); (2) propagation of exogenous Aβ amyloid within transgenic mouse brains depends on the source of the exogenous Aβ-containing material (Meyer-Luehmann et al. 2006; Stöhr et al. 2012). Experiments on tissue from two Alzheimer's disease patients with distinct clinical histories showed a single predominant 40 residue Aβ ($A\beta_{40}$) fibril structure in each patient; however, the structures were different from one another. A molecular structural model developed for $A\beta_{40}$ fibrils from one patient reveals features that distinguish *in vivo* from *in vitro* produced fibrils. The data suggest that fibrils in the brain may spread from a single nucleation site, that structural variations may correlate with variations in AD and that structure-specific amyloid imaging agents may be an important future goal (Lu et al. 2013).

Numerous models of the Aβ fold in amyloid fibrils have been anticipated, occasionally merging structural constraints of diverse experimental techniques or from samples that form under dissimilar conditions (Fändrich et al. 2011). Nevertheless, different circumstances can generate fibrils with unlike peptide conformation. Generally fibril models consider a U-shaped peptide fold, which is termed a β-arc (or β-arch) (Fändrich et al. 2011). U-shaped peptide models are derived from molecular dynamics simulations, partly applying structural limitations from solid-state NMR, or other biophysical methods. At times, it has been recommended that these models fit cryo-EM reconstructions (Fändrich et al. 2011). On the other hand, reconstruction of an $A\beta_{1–40}$ fibril at elevated resolution (0.8 nm), in which the cross β-sheet structure is directly determined, does not conform to the previous U-shaped peptide models (Fändrich et al. 2011). The 0.8 nm reconstruction presents cross-sectional dimensions that are significantly outsized than those predicted by the U-shaped models, and hence, ought to cover the peptide in a different structural arrangement (Fändrich et al. 2011).

7.5.3 Role of Aβ Structure in Alzheimer's Disease

In general, amyloids are large elongated structures having varying lengths and ultrastructural forms that make NMR and X-ray crystallography difficult (Toyama and Weissman 2011). Prion Het-s from fungus *Podospora anserine*, peptide Aβ related to AD and X-ray structures of short amyloidogenic peptides from various amyloid systems are a few of the well-studied amyloids. The most important structural feature of amyloid fibers is primarily a β-sheet structure, either in parallel and antiparallel form (Toyama and Weissman 2011) (Fig. 7.1). In amyloid structures, β-sheets can come together into a tertiary fold in a variety of different ways such as β-sandwich (Prion Het-s) or β-solinoid (Aβ) (Toyama and Weissman 2011).

In general, two types of aggregates are found in the brains of AD patients (Toyama and Weissman 2011) viz., intracellular neurofibrillary tangles (aggregates composed of the hyperphosphorylated protein tau) and extracellular plaques (made up of aggregates of the Aβ peptide)

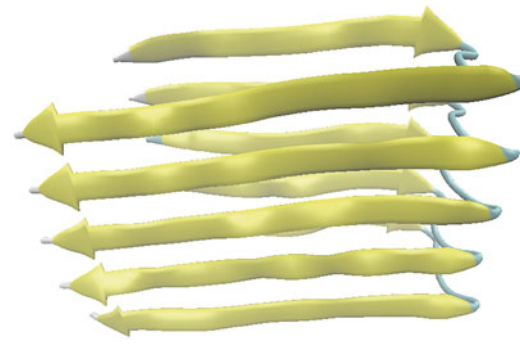

Fig. 7.1 3D structure of Alzheimer's Abeta (1–42) fibrils (PDB ID: 2BEG)

(Glenner and Wong 1984; Kosik et al. 1986). Aβ is a 39–42-residue peptide that originates from amyloid-β precursor protein (membrane protein) after proteolytic cleavage by β- and γ-secretases (Kang et al. 1987). X-ray fiber diffraction has previously revealed the presence of frequent cross-β diffraction pattern among extracellular plaques isolated from AD patients (Kirschner et al. 1986). Fourier Transform Infrared Spectroscopy (FTIR) and CD showed that the truncated Aβ peptide aggregates are mainly composed of β-sheet structures with β-sheets in an antiparallel arrangement (Lansbury et al. 1995). SSNMR has also played a significant role in understanding the high-resolution structures of Aβ fibers (Toyama and Weissman 2011). Early studies on truncated Aβ peptides ($A\beta_{34–42}$, $A\beta_{16–22}$, $A\beta_{11–25}$, and $A\beta_{14–23}$) also suggested antiparallel β-sheet structure (Lansbury et al. 1995; Balbach et al. 2000; Gordon et al. 2004; Bu et al. 2007). However, an in-register parallel sheet arrangement was observed for truncated $A\beta_{10–35}$ (Antzutkin et al. 2002). Interestingly, the SSNMR structures of full length $A\beta_{1–40}$ and $A\beta_{1–42}$ fibers revealed an in-register parallel β-sheet pattern (Antzutkin et al. 2000, 2002). From these studies it appeared that antiparallel β-sheet may perhaps be an artifact due to the truncation of hydrophobic and amphiphilic regions on Aβ (Toyama and Weissman 2011). Recently, a single predominant 40 residue Aβ ($A\beta_{40}$) fibril structure from two AD patients having different clinical histories and neuropathology was described by using simplified amyloid extraction and seeding methods (Lu et al. 2013).

The formation of amyloid fibrils can lead to a disease such as Alzheimer's disease, Diabetes type 2 and the spongiform encephalopathies (e.g., Mad cow disease), where each disease may be characterized by a particular protein or peptide that aggregates (Rambaran and Serpell 2008). In order to have a pathogenic consequence, these amyloid fibrils are deposited extracellularly in the tissues (Pepys 2001). However, all amyloid structures are not always cytotoxic as reported by the discovery of functional mammalian amyloid structure that functions in melanosome biogenesis (Fowler et al. 2006) that requires the formation of detergent-insoluble, lumenal Pmel17 fibers (Berson et al. 2003). Pmel17 is a transmembrane glycoprotein and its proteolytic processing yields a 28-kDa transmembrane fragment (Mβ) that degrades, and an 80-kDa lumenal fragment (Mα) which self-assembles into fibers that form the core of mature melanosomes (Marks and Seabra 2001; Berson et al. 2001, 2003). The recombinant Mα (rMα) accumulates more rapidly than any recognized polypeptide into amyloid fibers by at least four orders of magnitude quicker than the Aβ and α-synuclein peptides linked with Alzheimer and Parkinson disease (Fowler et al. 2006). Although some similarities have been observed between functional amyloidogenesis and pathogenic amyloid formation, they also exhibit remarkable differences. For instance, in case of gelsolin amyloid disease, proteolytic breakdown of mutant gelsolin during secretion by the proprotein convertase furin leads to slow, unregulated extracellular pathogenic gelsolin amyloid deposition (Chen et al. 2001). Similarly, Mα amyloid formation also begins by the activity of proprotein convertase but produces a functional amyloid structure (Fowler et al. 2006).

In addition to amyloid precursor protein, many other plasma proteins such as atrial natriuretic factor (ANF), wild-type transthyretin, β2-microglobulin, etc. have been identified that form amyloids (Fowler et al. 2006). Regardless of the noticeable differences in amino acid sequences and native structure of these amyloidogenic peptides, they all exhibit a widespread β-sheet conformation of their polypeptide backbone (Eanes and Glenner 1968; Sunde et al. 1997). Therefore, it is very much likely that this characteristic feature bestows the fibrillar, proteolytic resistant as well as insoluble characteristics to all types of amyloid (Fowler et al. 2006).

7.5.4 Conformational Changes in Aβ

Alteration in specific proteins or peptides from their native conformations and subsequent aggregation as insoluble fibrils has been proposed as causative agents in several neurodegenerative

disorders including AD (Celej et al. 2012). These conformational inequities in proteins explicate differences in various neural diseases (Guo et al. 2013). Growing evidence suggests abnormal accumulation of fibrillar Aβ in extraneuronal spaces and intraneuronal aggregates of abnormally modified tau proteins as a principal causation event in AD (Martha et al. 2012; Cerf et al. 2009). It is generally accepted that Aβ accumulation in brain is the primary event and tau protein formation could be one of the consequences of an imbalance between production and clearance of Aβ (Cerf et al. 2009). Up to 38–43 residues long Aβ released after the proteolytic cleavage of amyloid precursor protein have been reported as a primary component of amyloid plaques (Cerf et al. 2009)**.** The key components of these amyloid plaques are highly amyloidogenic, less abundant, early deposited $A\beta_{1–42}$ and $A\beta_{1–40}$ (Cerf et al. 2009).

There is evidence towards monomeric $A\beta_{42}$ peptides conformational states as building block of amyloid fibers (Baumketner et al. 2006). A conformational shift from α-helix to β-sheet of protein structure has been reported during the aggregation of amyloid fibrils associated with AD (Ding et al. 2003).

Although universal structure for Aβ fibrils does not exist, several antiparallel β-sheets structural models have been proposed by earlier studies (Chaney et al. 1998; George and Howlett 1999; Li et al. 1999; Tjernberg et al. 1999; Lazo and Downing 1998). Native Aβ peptide in AD is α-helix rich and polymorphic at molecular structural level (Tycko 2013; Ding et al. 2003).

Process of Aβ fibrillation involves a conformational shift which ultimately leads to the formation of extended β-sheets (Kirkitadze et al. 2001)**.** Involvement of an oligomeric α-helix containing intermediate was also proposed as a key step in Aβ fibrillogenesis (Kirkitadze et al. 2001). Aβ peptide 1–40 fibril polymorphs shares a common, parallel β-sheet organization and possess similar peptide conformations but differ in overall symmetry and in other structural aspects. In one of the study, on disease-associated mutant, D23N $A\beta_{1–40}$, researchers have reported stabilized parallel and antiparallel β-sheets within amyloid fibrils (Sawaya et al. 2007). The role of antiparallel β-sheet structures are suggested to fibrils that are formed by short peptides with one β-strand segment only.

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7.5.5 Misfolding in Neurofibril Tangles

The neurofilament is a linear 9–10 nm microfilament found in the neuronal cell body, the axon and the dendrites. It has an inadequately distinct lumen, short "side arms" project from it and it appears to be made up of globular subunits. Unlike neurotubules, the neurofilaments are steady and can be voluntarily secluded by subcellular fractionation. The neurofibrillary tangles (NFTs) are also found in the brain of normal aged humans (Iqbal et al. 1975). NFTs are aggregates of hyper phosphorylated tau protein that are generally identified as a main marker of Alzheimer's disease. Alzheimer's disease at the neuropathological stage is characterized by the build-up and aggregation of misfolded proteins: intracellular aggregates of tau in the NFTs and extracellular aggregates of Aβ deposits in forms of parenchymal amyloid plaques and congophilic amyloid angiopathy (CAA) (Hoozemans et al. 2005; Scholtzova et al. 2014).

The formation of amyloid plaques and NFTs are thought to contribute to the degradation of the neurons (nerve cells) in the brain and the subsequent symptoms of Alzheimer's disease (Strittmatter and Roses 1996). The conventional understanding is that tau attaches to microtubules and helps with their formation and stabilization. On the other hand, when tau is hyperphosphorylated, it is incapable to bind to microtubules and becomes unstable. The unbound tau clumps together in formations called NFTs (Spires-Jones

et al. 2009). These lesions, eventually, develop into filamentous NFTs, which hamper with several intracellular functions. Correlation among the quantitative aspects of Alzheimer's disease (neuron loss, neuritic plaque and neurofibrillary tangle load) and anger is normally observed in Alzheimer's patients. It was established that simply a raise in NFTs load was linked with the harshness of aggression and chronic aggression in Alzheimer's patients (Churchyard and Lees 1997).

Numerous interpretations of familial forms of Alzheimer's disease point towards that genetic factors resulting in alterations in β-amyloid are adequate to cause Alzheimer's disease, as well as tangles and other neurofibrillary changes (Hardy and Selkoe 2002). These observations offer strong confirmation that β-amyloid can generate or intensify neurofibrillary changes, at least following the early stages of amyloid deposition. Histologically, the neuronal cytoskeleton curls, factually, into structures known as NFTs. External to the cell, the Aβ peptide aggregates into clumps called oligomers, that gather and lead to the formation of amyloid plaques derived from the studies of a disorder identified as mild cognitive impairment (MCI) (a potential prodrome to dementia); the growth of noticeable entorhinal NFTs is measured to be the histologically correlate of MCI and, considered as the indication of early Alzheimer's disease (Gandy 2005).

7.5.6 Non-Beta Sheet Fibrils and Pathogenic Agents

General mechanism of aberrant protein aggregation has been reported by β-sheet conformation of Aβ fibrils. However, some studies also reported role of paired α- helical filaments (PHFs) conformation in the pathogenesis of AD (Sadqi et al. 2002). PHFs are abnormal twisted filaments, about 20 nm in width, and are composed of hyperphosphorylated tau protein (Ksiezak-Reding and Wall 2005). They are found in Alzheimer's disease, NFTs and other neurodegenerative disorders such as tauopathies (Braak and Braak 1991; Spillantini et al. 1997). PHFs have also been reported in brains of aging mammals (Cork et al. 1988; Schultz et al. 2000).

Moreover, tau-protein PHF's primary constituent has been also reported to contain high abundance of helix-breaking amino acids and is unstructured in solution (Sadqi et al. 2002). These internalized PHFs are capable of accelerating the formation of GFP-Tau-positive inclusions (aggresomes) in a pericentriolar location of the cells (Santa-Maria et al. 2012). It was also shown that PHFs from extracellular human AD can be internalized by cultured cells and may propagate a misfolded state to native soluble intracellular tau protein (Santa-Maria et al. 2012). PHFs enter the cells by an endocytic pathway known as fluid-phase endocytosis that involves engulfment by the cell membrane (Santa-Maria et al. 2012). However, additional studies are required to identify whether classical or rare endocytic pathways are responsible for internalization and cellular transport of PHFs (Hansen and Nichols 2009). Another mechanism for fibrillar polyglutamine aggregates (in Huntington disease) has been proposed, in which the aggregates were internalized by just crossing the membrane wall in various cell lines such as HEK 293 and N2a cells (Ren et al. 2009). The PHFs can be formed by the full-length human tau protein, as well as by the three or four repetitive tau segments (Yu et al. 2012).

Small oligomeric forms of α-synuclein have also been reported as a responsible factor of neuronal death (Conway et al. 2000). It has been reported that α-synuclein assemblies adopt a common cross-β structure with β-strands perpendicular to the fibril axis (Apetri et al. 2006). Both parallel and antiparallel β-sheet orientations have been reported in the amyloid fibrils (Apetri et al. 2006). In contrast, it was described that spheroidal α-synuclein oligomers are loaded with β-sheet structure and switch from monomer to oligomers involves a secondary structural shift from natively unfolded protein to primarily β-sheet (Volles et al. 2001; Apetri et al. 2006). Throughout the development of α-synuclein filaments, there is significant decrease in α-helical content and increases in β-sheet structure, whereas the

involvement of extended and PPII structure is negligible (Apetri et al. 2006). Higher toxicity of α-synuclein oligomers and their ability to destruct membranes has been reported both *in vitro* and *in vivo* models (Celej et al. 2012; Kim et al. 2009; van Rooijen et al. 2008; Kayed et al. 2004). Conformational organization of oligomeric α-synuclein contains β-sheet structural elements (Celej et al. 2012; van Rooijen et al. 2008). There is strong evidence which highlights multi-pathway aggregation of α-synuclein and must be considered for investigations concerned on molecular mechanisms of this protein fibrillation (Hong et al. 2011). Recently, it was shown that α-synuclein protein misfolding cyclic amplification (PMCA) technique may possibly be employed as a high throughput screening method for the discovery of new α-synuclein anti-aggregating compounds that may inhibit α-synuclein fibril formation (Herva et al. 2014). Some of the soluble Aβ oligomers are currently extensively accepted as main pathogenic structures in AD (Hefti et al. 2013).

Another insidious pathologic feature of AD called tauopathy has been also known to be promoted by oligomerization. The tau oligomers have been reported as a pathological structure associated with AD progression in mouse models and plays a key role in neurodegeneration and behavioral impairments (Davidowitz et al. 2008; Berger et al. 2007; Maeda et al. 2006). Higher toxicity of tau oligomers have been reported in scientific literatures compared with tau filaments and has been suggested as precursors of tau filaments (Davidowitz et al. 2008; Gómez-Ramos et al. 2006, 2008).

7.5.7 Role of Posttranslational Modifications in Alzheimer's Disease

The hyperphosphorylated varieties of microtubule associated protein (tau) have been proposed as another pathological hallmark of AD. Changes in the quantity or the structure of tau protein may possibly influence its function as a microtubules stabilizer (Kolarova et al. 2012). Several studies reported unusual posttranslational modifications like hyperphosphorylation, acetylation, glycation, nitration, truncation and others accountable for changed tau structure in AD (Kolarova et al. 2012; Mondragón-Rodríguez et al. 2009; Binder et al. 2005; Kuhla et al. 2007; Carrell and Gooptu 1998). Recently, it was anticipated that not just the amount of neurofibrillary tangles but also state of proteolysis at C-terminus (associated with conformational changes) defines AD progression (Kolarova et al. 2012). The studies on mutation and their adverse effects on the stability of the EPHB2 gene are inferred from the studies and its impact with its reactivity with NMDA receptor and the function in the AD (Tayubi et al. 2014).

7.5.8 Role of Metal Ions in Alzheimer's Disease

The bioavailable metal ions predominantly iron, zinc and copper play a crucial role in the pathogenesis of AD. The studies have provided a proof that normalizing metal ion represents a valid therapeutic target (Duce et al. 2011). Among accepted aggravating factors, metal ions like (Al, Zn, Cu and Fe) could precisely damage protein aggregation of Aβ; aggregation of Aβ by self-assembly into oligomers or amyloids is a central event in AD (Drago et al. 2008). Organization of metal ions, mainly copper and zinc, in vivo, which modulates the aggregation process in Aβ, and the impact of Cu and Zn on the aggregation of Aβ reveal some general trends (Faller et al. 2013). Some suggestion has been made about the Aβ precipitation, and toxicity in AD is caused by abnormal interactions with metal ions, especially Zn, Cu and Fe. However, Aβ might also participate in normal metal-ion homeostasis (Bush 2003). Mainly the concentrations of Cu and Zn affects the types of aggregation intermediates formed and the binding of metal ions changes both the structure and charge of Aβ in Alzheimer's disease (Faller et al. 2013). The decrease in overall charge at physiological pH increases overall

driving force for aggregation, but may favour more precipitation over fibrillation, whereas the induced structural changes seem more relevant for the amyloid formation (Faller et al. 2013).

7.6 Therapeutic Strategies for Alzheimer's Disease

Alzheimer's disease and Parkinson's disease are the most common forms of age-related neurodegenerative disorders (Trojanowski et al. 1998). There are still no effective treatments to prevent, halt or reverse Alzheimer's disease, but research advances over the past three decades could change this gloomy picture. Genetic studies demonstrate that the disease has multiple causes. Interdisciplinary approaches combining biochemistry, molecular and cell biology, and transgenic modelling have revealed some of its molecular mechanisms (Huang et al. 2013) Progress in chemistry, radiology and systems biology is beginning to provide useful biomarkers, and the emergence of personalized medicine is poised to transform the pharmaceutical development and clinical trials (Huang et al. 2013). Therapeutic therapy at the molecular level is being targeted at key proteins, which are in AD mechanism such as tau protein, Aβ protein and Apoe4 protein (Huang et al. 2013). Currently drugs target acetylcholinesterase and NMDA receptors are being used to curb the symptoms of AD. Recent studies on plant extracts exhibit promising results, which can be further exploited for finding candidate drugs for Alzheimer's disease. Phytochemicals from families like Araceae, Umbelliferae, etc., are being utilized for decoding pharmacological treatment for AD (Dastmalchi et al. 2007).

7.7 Conclusion and Future Directions

At present, protein folding has turned out to be a spotlight of interest in pharmaceutical investigation: it is feasible that novel approaches to the treatment of diseases such as Alzheimer's are to be found within its intricate pathways. In view of the fact that the number of AD patients is likely to amplify over the next 30 years, huge enhancement in healthcare expenses and care burden due to AD are expected as well. As a consequence, proficient and cost-effective treatment will be main waves for AD research. It is believed that Aβ oligomers could be the intermediates in developmental pathway of amyloid fibril rather than necessary factor in fibril formation. In the most recent years, it is becoming progressively more apparent that not the plaques but Aβ oligomers are the principle pathogenic agent in AD. There are strong evidences in scientific literature that oligomeric intermediates are comparatively more toxic and correlate well with Alzheimer's symptomology than final insoluble Aβ aggregates. Lesser diameter Aβ oligomer multitudes can diffuse into synaptic clefts much easier to induce neuronal and synaptic dysfunction compared with insoluble Aβ plaques.

Since last three decades, scientists have made significant development in understanding malformed brain function in Alzheimer's disease. While novel drugs take years to manufacture from conception to market—and since drugs that appear hopeful in early-stage studies may not work as anticipated in extensive trials—it is essential that Alzheimer's and associated dementias study continue to speed up. Among the majority of vigorously examined categories of possible amyloid inhibitors are natural phenols, an extensive group of plant molecules. Additional information and persuasive clinical trials are essential before these compounds are used for humans. However, these molecules undeniably show strong potential to be effective therapeutic agents against amyloid diseases. These natural molecules may serve dual purpose, viz.; they may be used as tools to study the amyloid aggregation pathway and as molecular scaffolds to widen our knowledge about additional dynamic and biologically obtainable drugs.

Nevertheless, it is expected in near future that numerous drug classes will demonstrate to be proficient and safe intended at undoing the neurodegeneration in AD with the assistance of biomarkers that will foresee expansion of the disease before progression of obvious dementia.

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