Alzheimer’s disease (AD) is a neurodegenerative disorder associated with the accumulation of amyloid beta (Aβ) peptides and the formation of partially reduced oxygen species (PROS). Redox active transition metal bound Aβ active sites catalyze the pathological features of AD in the brain. Heme binding to these Aβ peptides has opened up a new direction in the field of AD.
1.1. AD

Alzheimer’s (AD), the most common form of dementia in the elderly, is a neurodegenerative and terminal disease. This old age disease is a serious threat with gradual increase of life expectancy. Brain disorder and slow loss of memory are key pathological features of AD. Confusion, mood swing and long term memory loss are prominent symptoms in the advanced stages of the disease\(^1\)\(^-\)\(^3\). Gradually the victim loses major bodily functions which ultimately lead to death. AD is a disorder with complex etiology and any specific or particular therapeutic approach is not the answer for its prevention or cure. Physically AD is characterized by massive loss of neurons and synaptic breakdown in the brains. Extracellular amyloid plaques and intracellular neurofibrillary tangles are hallmark features of AD\(^4\). Amyloid β (Aβ) peptide is the major constituent of the tangles and plaques\(^5\). Therefore, elucidation of the chemistry associated with Aβ peptides has drawn the attention of researchers worldwide.

1.2. AMYLOID β PEPTIDES AND ITS RELEVANCE IN AD

Different approaches have been taken to elucidate the chemistry behind AD. In the early 90s, the amyloid cascade theory\(^6\)\(^,\)\(^7\) had been proposed based on Aβ deposition in the brain which results in the formation of plaques and tangle. Aβ is a 39-43 amino acid residue containing peptide\(^8\), which is derived from a 770 residue containing post-translationally modified transmembrane protein called Amyloid Precursor Protein (APP)\(^9\)\(^-\)\(^11\). Three different proteolytic enzymes α-, β- and γ-secretases cleave APP at different sites to produce various Aβ chain lengths\(^12\). β-secretase cleaves APP between residues M671 and D672 in the amyloidogenic pathway to generate the extracellular N-terminus of Aβ. γ-secretase cleaves APP between residues A713 and T714 in the transmembrane domain yielding Aβ(1-42) peptide. On the other hand, the combined action of γ-secretase and α-secretase cleave between residues K687 and L688 in a non amyloidogenic pathway producing Aβ(17-42) peptide (Figure 1.1). Aβ(1-40) and Aβ(1-42) are the two most abundant fractions of Aβ. These Aβ peptides are composed of a hydrophilic fragment (first 16 amino acids from the N terminus) and a hydrophobic part (comprising of 17-42 amino acids)\(^13\). The hydrophilic portion of the Aβ peptide contains three histidine residues (at 6\(^{th}\), 13\(^{th}\) and 14\(^{th}\) position) and a tyrosine residue (at 10\(^{th}\) position), which are well known for their metal coordinating properties in various metalloenzymes\(^14\)\(^-\)\(^16\), while the hydrophobic amino acids (17-40) of Aβ peptide are responsible for aggregation and fibrillation\(^17\).
1.3. METALS AND AD

Another important factor in the neurodegeneration of AD is the involvement of transition metals\textsuperscript{18-21}. Zn, Cu and Fe dyshomeostasis and increased levels of these metals in the brain are key cytopathologies of AD\textsuperscript{21,22}. These transition metals (Zn, Cu and Fe to a lesser extent) are responsible for the aggregation of the Aβ peptides\textsuperscript{23,24}, as they are found at a much higher concentration in the neocortex of the brain, the region that is affected by AD\textsuperscript{25,26}. Treatment of the Aβ aggregates from post-mortem AD affected tissues with metal chelators leads to generation of soluble Aβ demonstrating the role of these transition metals in Aβ aggregation\textsuperscript{27,28}.

All the metal binding sites are present in the N-terminal hydrophilic region of the Aβ peptides (i.e. within the 1\textsuperscript{st} 16 amino acids)\textsuperscript{29}. Zn\textsuperscript{2+} ions have a significant contribution in the amyloid deposition in the transgenic mice (note, normally rodents do not show AD), which is a strong evidence of its plaque forming properties\textsuperscript{30,31}. Zn possibly exists in a four to six coordination environment in the Zn-Aβ complexes. NMR studies of the Zn-Aβ complexes propose a three histidine (at positions 6, 13 and 14) and a carboxylate (Glu11) ligation to the Zn ion\textsuperscript{32,33}, Asp1 at the N-terminus and water are likely to be the other ligands\textsuperscript{34}. Tyr10 and Arg5 are not involved in Zn binding\textsuperscript{35}.

Cu plays an ambiguous role in amyloidogenesis. There are conflicting reports that Cu can either accelerate or slow down the process of amyloidogenesis\textsuperscript{36}. Cu also plays a major role in the generation of reactive oxygen species (ROS) (Figure 1.2) via Fenton type reaction\textsuperscript{37,38}. The Cu-Aβ complex has features of a type-2 Cu center with a 3N1O (three nitrogen based ligands and one oxygen based ligand) coordination environment\textsuperscript{39,40}. The 3N coordination of Cu can either be from three histidine residues\textsuperscript{41}, or two histidine residues and the N-terminus acting as the third ligand\textsuperscript{42}.  

**FIGURE 1.1.** Aβ(1-42 and 17-42) formation from APP by the proteolytic enzymes. Numbers in purple indicate the APP amino acid sequence. Amino acid sequence in green represents the hydrophilic part (1-16) and orange represents the hydrophobic part (17-42) of the Aβ peptide.
The O ligation can be contributed by Asp1, Glu3, Asp7, Tyr10, Glu11 or it could be from aqueous buffer. There exist reports of carbonyl oxygen of the peptide linkage acting as the O donor ligand. Some recent studies have reported the presence of two different Cu complexes of Aβ at physiological pH, namely component I and component II. The relative ratios of these two components are pH sensitive, component I being the dominant species at lower pH (pH ~ 7) while component II is the predominant species at higher pH (pH ~ 9).

Though Fe has not been associated for its amyloidogenic properties, it can have a marked influence in generating oxidative stress like with Cu. Fe and Cu generate toxic ROS, causing oxidative stress, which is a characteristic pathological feature of AD. This is in fact believed to precede amylo deposition, thus causing the early signs of AD. Transition metal ions get reduced in the presence of endogeneous reducing agents (ascorbate, α-tocopherol, or glutathione). These reduced metal centers (Fe²⁺, Cu⁺) can spontaneously react with molecular O₂ to generate freely permeable, neurotoxic partially reduced oxygen species (PROS), e.g. HO₂⁻, H₂O₂, HO etc. The toxic hydroxyl radicals generated can cause lipid peroxidation adducts and nucleic acid adducts which are characteristics of AD pathology.

Recent studies show an interesting fact that heme, a Fe containing metabolite binds with Aβ to form a heme based metalloenzyme-like active site. This active site exhibits peroxidase activity and can oxidize neurotransmitters in the brain. This heme binding to Aβ and its relevance to AD has opened up a new dimension in AD research.

**FIGURE 1.2.** A schematic presentation of transition metal induced ROS generation. M = transition metals like Fe and Cu.
1.4. HEME AND ITS ROLE IN AD.

Heme is a Fe containing prosthetic group originating in the mitochondria\textsuperscript{58}. It is the building block of many enzymes. These enzymes can function either as an electron carrier or as a catalyst for redox reactions\textsuperscript{59}. There are four types of heme in the eukaryotes: protoheme, heme-\textit{a}, \textit{b} and \textit{c}. Protoheme is the pool of free heme produced in the mitochondrial matrix\textsuperscript{60}. Protoheme is composed of an organic framework, protoporphyrin IX and a Fe metal centre. Succenyl-CoA condenses with glycine\textsuperscript{61}, to produce protoporphyrin IX in the mitochondrial matrix. The enzyme ferrochelatase, in the mitochondrial inner membrane inserts Fe in protoporphyrin IX to produce protoheme\textsuperscript{62}. Heme-\textit{b} and heme-\textit{c} are structurally similar to protoheme, but are covalently attached to specific proteins. Heme-\textit{a} biosynthesis from protoheme requires two modifications, farnesylation and formylation (Figure 1.3)\textsuperscript{63}. Mitochondrial complex IV is the only heme-\textit{a} containing system\textsuperscript{64}. Interestingly, heme-\textit{a} and mitochondrial complex IV declines in AD\textsuperscript{65}.

![Structure of protoheme and heme-a](image)

**FIGURE 1.3.** Structure of protoheme and heme-a.

It has been observed that heme deficiency in human brain cell lines causes depletion of monomeric APP to \(~50\%)\textsuperscript{66}. Abnormal forms of APP such as dimers and aggregates are formed in heme deficient human brain cells\textsuperscript{65}. This induced heme deficiency also selectively decreases the activity of mitochondrial complex IV, however, the other complexes I-III remain unperturbed\textsuperscript{67}. Other studies on heme metabolism in AD patients reveal that heme metabolism is altered in AD brains\textsuperscript{56}. Unregulated Fe accumulation\textsuperscript{68}, increased levels of ferrochelatase\textsuperscript{56}, higher levels of heme oxygenase (HO)\textsuperscript{68}, increased levels of heme degradation products\textsuperscript{69} and diminished activity of mitochondrial complex IV\textsuperscript{70}, are common symptoms of AD. Recent studies reveal that regulatory
heme binds Aβ and results in heme deficiency in AD patients. Both heme-\(b\) and heme-\(a\) bind Aβ to form heme-Aβ complexes\textsuperscript{57}. Assaying reports suggest a 250% increase of heme-\(b\) and 26% decrease of heme-\(a\) in AD patients’ brains compared to controls\textsuperscript{56}. Thus, there exists a possible connection between heme regulation pathway and AD.

Increased Aβ binds regulatory heme creating heme deficiency in AD pathology. Though the correlation between heme-\(a\) and heme-\(b\) and the reason behind decreased heme-\(a\)/heme-\(b\) ratio is not very clear, it has been proposed that poor heme-\(a\) maturation pathway as the key factor for decreased heme-\(a\) levels\textsuperscript{56}. An increased heme-\(b\) biogenesis in AD patients may be a compensatory action of decreased heme-\(a\) maturation\textsuperscript{56}. (depicted in Fig 1.4)

This increased heme concentration may generate oxidative stress and accelerate mitochondrial decay\textsuperscript{71}. Elevated protoheme also causes oxidative damage in human cells. To maintain the level of protoheme, its decaying process also gets accelerated. As a result higher levels of heme oxygensase\textsuperscript{69,72}, (it degrades heme in the endoplasmic reticulum and maintains heme balance) and elevated heme degradation products, (eg, bilirubin) are found in the brains of AD patients. On the contrary decreased level of mitochondrial heme-\(a\) affects the assembly of mitochondrial complex IV. Mitochondrial complex IV is a part of the electron transport chain (ETC) in the inner membrane of the mitochondria. ETC maintains the proton gradient across the mitochondrial membrane required for ATP synthesis\textsuperscript{73}. It also reduces mitochondrial O\(_2\) to H\(_2\)O in order to detoxify higher levels of molecular O\(_2\) present in the mitochondria\textsuperscript{74}. Thus, depleted heme-\(a\) availability leads to release of PROS by the mitochondria\textsuperscript{75,76}. Maturation of heme-\(a\) requires fernesylation and formylation of protoheme (Figure 1.3). Fernesyl pyrophosphate (FPP) is the source of fernesyl group incorporated in heme-\(a\). This FPP is not only the precursor of isoprenoids like heme-\(a\) but is also a building block of cholesterol. Hence, in order to have a higher biogenesis of heme-\(a\), tight regulation of cholesterol is essential\textsuperscript{77,78}. Higher biogenesis of cholesterol would eventually decrease the level of heme-\(a\). In reality neurons in AD brain contain higher levels of free cholesterol than the normal brain\textsuperscript{79}. This may be a possible reason behind the low maturation of heme-\(a\) in AD brain\textsuperscript{56}. 
FIGURE 1.4. Proposed model depicting excess Aβ induced heme deficiency (HD) and oxidative stress (OS) in AD brain. Heme-b after its biogenesis in the mitochondrial matrix is exported to the cytosol to join regulatory heme (step a), where it serves in diverse metabolic activities e.g. sterol synthesis, and also as a substrate for heme-a biosynthesis (step b). In AD brain, Aβ, derived from the APP (step c), increases (indicated by ↑). Both heme-b and heme-a can bind Aβ to produce heme-Aβ, (steps d and e), which acts as peroxidases and contributes to OS (step h) in AD. Excessive binding of heme with Aβ depletes the regulatory heme and heme-a (indicated by ↓) which causes depletion of mitochondrial complex IV (step f) leading to OS and up-regulation of heme-b (indicated by ↑) contributing to mitochondrial dysfunction. In the presence of free metals like Cu and Zn, Aβ forms aggregates (step g) which are pathological features of AD. This figure is adapted from reference 56.
1.5. REFERENCES


