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Alzheimer's Disease: A Heme–Aβ Perspective

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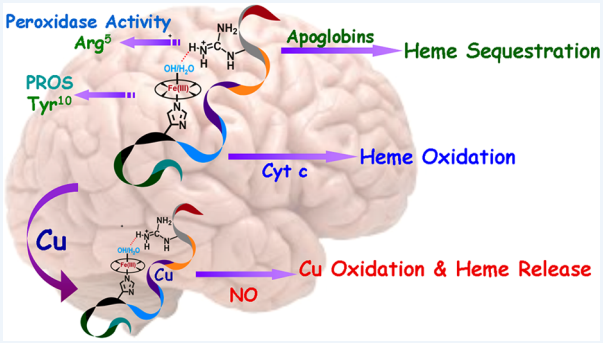
CONSPECTUS: Redox active iron is utilized in biology for various electron transfer and catalytic reactions essential for life, yet this same chemistry mediates the formation of partially reduced oxygen species (PROS). Oxidative stress derived from the iron accumulated in the amyloid plaques originating from amyloid β (Aβ) peptides and neurofibrillary tangles derived from hyperphosphorylated tau proteins has been implicated in the pathogenesis of Alzheimer's disease (AD). Altered heme homeostasis leading to dysregulation of expression of heme proteins and heme deposits in the amyloid plaques are characteristic of the AD brain. However, the pathogenic significance of heme in neurodegeneration in AD has been unappreciated due to the lack of detailed understanding of the chemistry of the interaction of heme and Aβ peptides. As a result, the biochemistry and biophysics of heme complexes of Aβ peptides (heme–Aβ) remained largely unexplored.



In this Account, we discuss the active site environment of heme bound Aβ complexes, which involves three amino acid residues unique in mammalian Aβ (Arg5, Tyr10, and His13) and missing in Aβ from rodents, which do not get affected by AD. The histidine residue binds heme, while the arginine and the tyrosine act as key second sphere residues of the heme–Aβ active site that play a crucial role in its reactivity. Generation of PROS, enhanced peroxidase activity, and oxidation of neurotransmitters such as serotonin (5-HT) are all found to be catalyzed by heme–Aβ in *in vitro* assays, and these reactivities can potentially be linked to the observed neuropathologies in AD brain. Association of Cu with heme–Aβ leads to the formation of heme–Cu–Aβ. The heme–Cu–Aβ complex produces a greater amount of PROS than reduced heme–Aβ or Cu–Aβ alone. Nitric oxide (NO), a signaling molecule, is found to ameliorate the detrimental effects of heme–Aβ and Cu bound heme–Aβ complexes by detaching heme from the heme–Aβ complex and releasing it into the environment solution. Heme–Aβ complexes show fast electron transfer with oxidized cytochrome c and rapid heme transfer with apomyoglobin and aponeuroglobin. NO, cytochrome c, and apoglobins can all lead to reduction in PROS generated by reduced heme–Aβ. Synthetic analogues of heme, offering a hydrophobic distal environment, have been used to trap oxygen bound intermediates, which provides insight into the mechanism of PROS generation by reduced heme–Aβ. Artificial constructs of Aβ on nonbiological platforms are used not only to stabilize metastable and physiologically relevant large and small amyloid aggregates but also to monitor the interaction of various drug candidates with heme and Cu bound Aβ aggregates, representing a tractable avenue for testing therapeutic agents targeting metals and cofactors in AD.

1. INTRODUCTION

Protein aggregation and amyloid formation are common features of several diseases like Alzheimer's disease (AD), Parkinson disease (PD), and type 2 diabetes mellitus (T2Dm), which are major potential threats for human health and have become subjects of rapidly increasing research activities.[1,2] AD is the most common neurodegenerative disorder clinically characterized by progressive dementia.[3] The established pathological feature of AD is neuritic senile plaques (SP), which predominantly contain amyloid-β (Aβ), and tau-containing neurofibrillary tangles (NFT) mainly in the frontal cortex and hippocampus region of the brain.[4] Aβ peptides are produced by the cleavage of trans-membrane amyloid precursor protein (APP) from the C-terminal region by β- and γ-secretases.[5−7] The amyloid cascade hypothesis and the toxicity of the oligomeric species of Aβ peptide provide the central pathogenic role of Aβ in AD etiopathology.[2,3] Recent failures of clinical trials in which drugs that prevent Aβ production and antibodies that target Aβ have been ineffective in preventing cognitive decline in AD patients, indicate that Aβ is not the sufficient and necessary risk factor for the progression of AD and therapeutics targeting Aβ alone are not sufficient for symptomatic patients.[8,9]

A significant abundance of redox active metals such as Cu and Fe in the plaques and the dyshomeostasis of these metals in the AD brain have invoked their role in the development of AD.[10,11] Fe accumulation occurs in both the plaques and tangles in the cortex and hippocampus, consistent with disease severity and cognitive impairment observed in AD brain.[12] Apart from this, altered heme homeostasis,[13] mitochondrial complex IV dysfunction,[14] and upregulation of both heme

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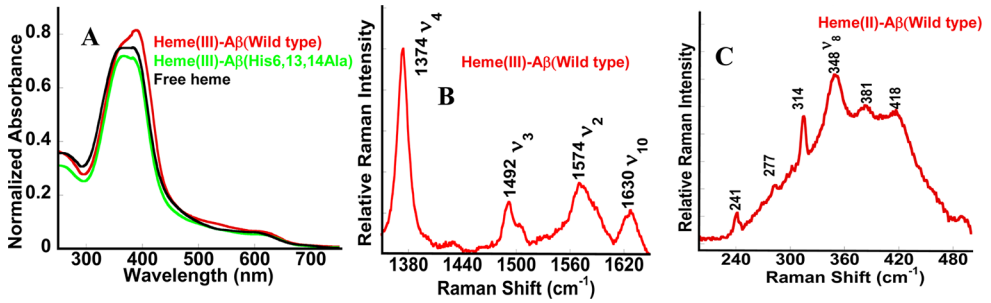


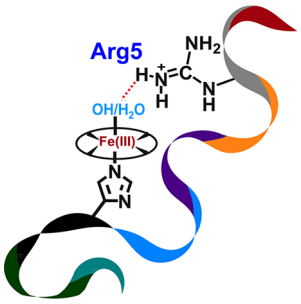
Figure 1. (A) Absorption spectra of heme−Aβ, free heme, and heme−Aβ(His6,13,14Ala) and rR spectra of (B) oxidized and (C) reduced heme−Aβ (in 100 mM, pH 7, phosphate buffer, at concentration 0.02 mM, excited at ∼413.1 nm, laser power ≈ 15 mW on the sample). Part A adapted with permission from ref 25. Copyright 2011 American Chemical Society.

oxygenase (HO)[15] and biliverdin reductase-A (BVR-A)[16] activity along with colocalization of heme protein (hemoglobin) in the plaques are observed in AD,[17] suggesting a significant role of heme in the disease progression. However, the pathophysiological role of impaired HO-1/BVR-A activity in AD is debatable, because not only increased levels of these enzymes but also their post-translation modifications must be taken into account in their antioxidant activity under oxidative and nitrosative stress.[16,18] Recent reports provide strong evidence regarding the covariance of Aβ plaques with the heme rich deposits found in the region of the brain consistent with the pathological profile of neurodegeneration in AD.[19] This Account elaborates the active site environment of heme bound Aβ and the interaction of heme−Aβ complexes with different biomolecules[20−24] and discusses the plausible pathological role of heme in the neurodegeneration in AD.

2. ACTIVE SITE OF HEME−Aβ

Heme binds 1 equivalent of Aβ to form a 1:1 heme−Aβ complex characterized by a broad Soret band at ∼398 nm along with charge transfer band at 632 nm in the absorption spectra[25] (Figure 1A). The high frequency resonance Raman (rR) spectrum displays major bands at 1374 (ν_4), 1492 (ν_3), 1574 (ν_2), and 1630 (ν_{10}) cm^{-1} (Figure 1B) signifying the predominant presence of a five-coordinate high-spin heme-Fe(III) center (the presence of a weak band at 1505 cm^{-1} indicates the presence of a minor low-spin species). The formation of the high-spin heme center is further confirmed by the EPR signal at $g \approx 6.0$ corresponding to $S = 5/2$ spin state.[25,26] The identical nature of spectral features of heme−Aβ(1−16) and heme−Aβ(1−40) suggests that the heme binding residue lies within the hydrophilic residue 1−16 region of the peptide. There are three histidine residues (His6, His13, and His14) and one tyrosine residue (Tyr10) present in Aβ(1−16), which are commonly known to coordinate to heme(Fe) in different biological systems. Spectroscopy on different site directed mutants indicate that Aβ peptide binds heme through one of the histidine residues (possibly His13).[25,26] Further the possible ν_{Fe-His} stretching mode for reduced heme−Aβ in the low frequency rR spectrum is observed at 241 cm^{-1} (Figure 1C), akin to those observed in peroxidases, provides direct evidence of the presence of the Fe−His coordination.[27] Heme−Aβ(1−16) exhibits a pK_a of ∼6.8 ± 0.3, whereas heme−Aβ(10−20) and heme−(Arg5Asn)Aβ(1−16) show a pK_a of ∼8.2 ± 0.3, which is similar to that of myoglobin (a histidine coordinated heme protein) that has a water-derived sixth ligand and lacks any strongly basic hydrogen bonding residue in the distal pocket.[28] Thus, the above pK_a values likely represent the protonation equilibrium of the axial water derived ligand of heme−Aβ. The decrease in pK_a in case of the native heme−Aβ(1−16) complex is consistent with the presence of H-bonding interaction between the water derived trans axial ligand and the distal Arg5 residue. This H-bonding with the positively charged protonated guanidine side group of Arg5 will stabilize a bound hydroxide more than the water, thus lowering its pK_a. Thus, the active site environment of heme−Aβ complex has a histidine ligated to heme at the proximal pocket along with a water derived sixth ligand present at the distal pocket, hydrogen bonded to the Arg5 residue (Scheme 1).

Scheme 1. Schematic Representation of the Active Site Environment of Heme−Aβ



Aβ peptides can physiologically bind Cu and heme simultaneously. Absorption, EPR, and CV data indicate that at low concentration (0.02 mM) Aβ peptides bind both heme and Cu simultaneously, where both sites remain electronically and electrochemically uncoupled from each other.[26,29]

3. REACTIVITY

3.1. Peroxidase

Peroxidases are the heme containing enzymes that catalyze the oxidation of various substrates in the presence of H_2O_2. Heme−Aβ catalyzes the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB), a classic substrate for peroxidases, in the presence of H_2O_2 (Figure 2).[25,30] Interestingly, TMB oxidation is inhibited by neurotransmitters like serotonin and 3,4-dihydroxyphenylalanine (DOPA), indicating that these neurotransmitters can also act as substrates for heme−Aβ complexes, thereby possibly accounting for the abnormal neurotransmission observed in AD patients.[30] Peroxidases are known to contain a highly conserved arginine residue in the distal pocket, which assists in the O−O bond heterolysis by protonation of the distal oxygen atom of a Fe(III)−OOH intermediate species

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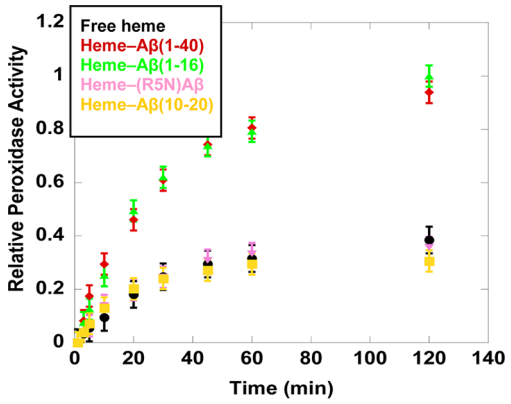


Figure 2. Kinetic traces for peroxidase activity of different heme−Aβ complexes. Adapted with permission from ref 25. Copyright 2011 American Chemical Society.

(also known as compound 0). This role of the arginine residue is also described as the "pull effect".[31] The protonation assisted O−O bond cleavage of compound 0 results in the formation of the reactive oxidant compound I; formally described as an Fe(IV)=O porphyrin π cation radical species.[31,32] The fact that Aβ(10−20) and (Arg5Asn)Aβ(1−16) fail to exhibit any enhanced peroxidase activity relative to free heme (Figure 2) despite binding heme suggests that the noncoordinating Arg5 residue is essential in making these heme−Aβ complexes function as peroxidases.

Serotonin (5-HT) is an essential neurotransmitter for cognitive functions and formation of new memories.[33] A deficit in 5-HT dependent neuronal activity is somewhat specific for AD.[33] Both the absorption spectra and HPLC results indicate that 5-HT is catalytically oxidized by heme−Aβ and H_2O_2 primarily to its dimer dihydroxybitryptamine (DHT) (Scheme 2).[23,30] The Arg5 residue facilitates the generation of the active catalyst (compound I) below neutral pH, while the active substrate formation requires the ionization of the phenolic group of 5-HT, which is feasible at alkaline pH. A combination of these two opposing effects results in the observed highest activity at neutral pH. The Tyr10 residue likely facilitates the O−O cleavage by translocating the proton required for generation of compound I. The 5-HT radical (B, Scheme 2) disproportionates to form DHT and an intermediate quinone imine that subsequently hydrolyzes and undergoes aerobic oxidation to form neurotoxic tryptamine-4,5-dione (Scheme 2). This generation of toxic species and their accumulation can have detrimental sequelae and provides a possible mechanistic explanation for the key cytopathologies observed in AD brain.[23,30]

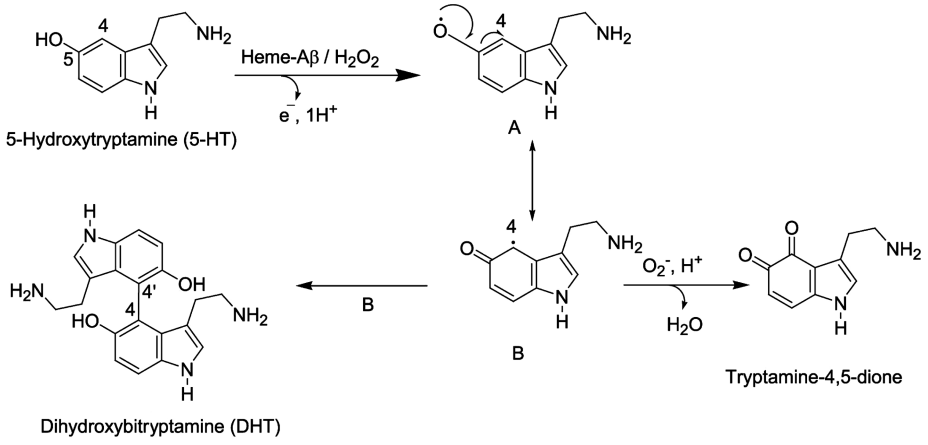
3.2. Oxygen Reactivity

Redox active transition metals like Fe(II) and Cu(I) are known to react with O_2 generating partially reduced oxygen species (PROS), like $O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$ etc. PROS are easily diffusible and neurotoxic species, giving rise to lipid peroxidation adducts and nucleic acid adducts, which are pathological features of AD. Ascorbate (vitamin C), α-tocopherol (vitamin E), NADH, or glutathione can act as endogenous reducing agents.[26,29]

One electron reduction of O_2 generates $O_2^{\bullet-}$, which after disproportionation should yield ∼50% H_2O_2 (Figure 3A), while two electron reduction of O_2 generates 100% H_2O_2 (Figure 3A). It is observed that reduced heme−Aβ, Cu−Aβ, and heme−Cu−Aβ generate ∼90%, ∼ 84%, and ∼130% H_2O_2, respectively, when exposed to O_2 (Figure 3B). These results indicate a two electron reduction of O_2 to H_2O_2 by heme−Aβ and Cu−Aβ and a three electron reduction by heme−Cu−Aβ. The reduced metal center in heme−Aβ and Cu−Aβ can provide only one electron (which would result in 50% PROS generation) whereas reduced heme−Cu−Aβ can provide two electrons (to produce 100% PROS) for O_2 reduction.[25] Thus, the additional e^- for O_2 reduction must be obtained from the Aβ peptide. It is observed that the generation of PROS decreases by ∼50% in the absence of the Tyr10 residue for all the complexes, demonstrating that Tyr10 is the source of the second electron during PROS formation (Figure 3B) as observed in several enzyme systems.[34] The transfer of an electron from Tyr10 to O_2 will subsequently lead to the formation of a tyrosyl radical that can cause dimerization of the peptides cross-linked via the tyrosine residue, which are characteristic features of AD.[35]

The formation of an Fe−O_2 intermediate (Figure 3C, blue) is the first step involved in the PROS generation pathway by the Fe(II) center, and this intermediate has been trapped and characterized in numerous heme proteins and their synthetic analogues.[36] The Fe−O_2 intermediate involved in the O_2 reduction pathway by heme−Aβ was trapped using picket

Scheme 2. Proposed reaction Pathway of Oxidation of Serotonin (5-HT) by Heme−Aβ and H_2O_2



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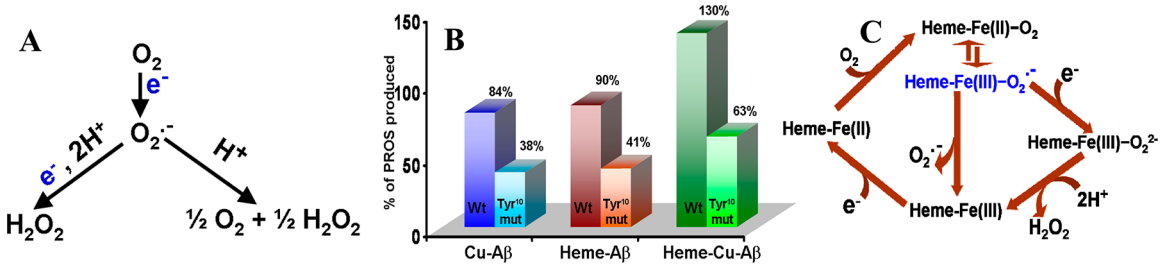

Figure 3. (A) Schematic representation of O_2 reduction by one and two electrons, (B) % PROS detected for wild-type and the Tyr10 mutant, and (C) schematic representation of PROS formation by heme−Aβ. Adapted with permission from ref 29. Copyright 2011 American Chemical Society.

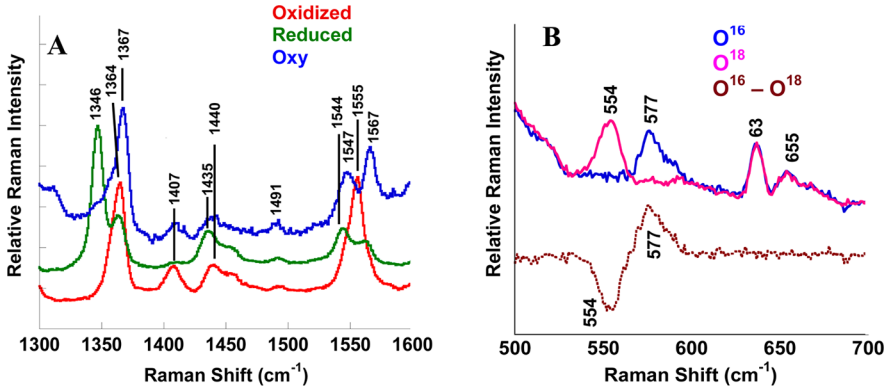

Figure 4. rR spectra of (A) pfp-Aβ, reduced pfp-Aβ, and Fe−$O^{16}{}_2$ and (B) Fe−O_2 of pfp-Aβ with O^{16}, O^{18}, and the difference spectrum in DMF solvent. Adapted with permission from ref 38. Copyright 2013 Royal Society of Chemistry.

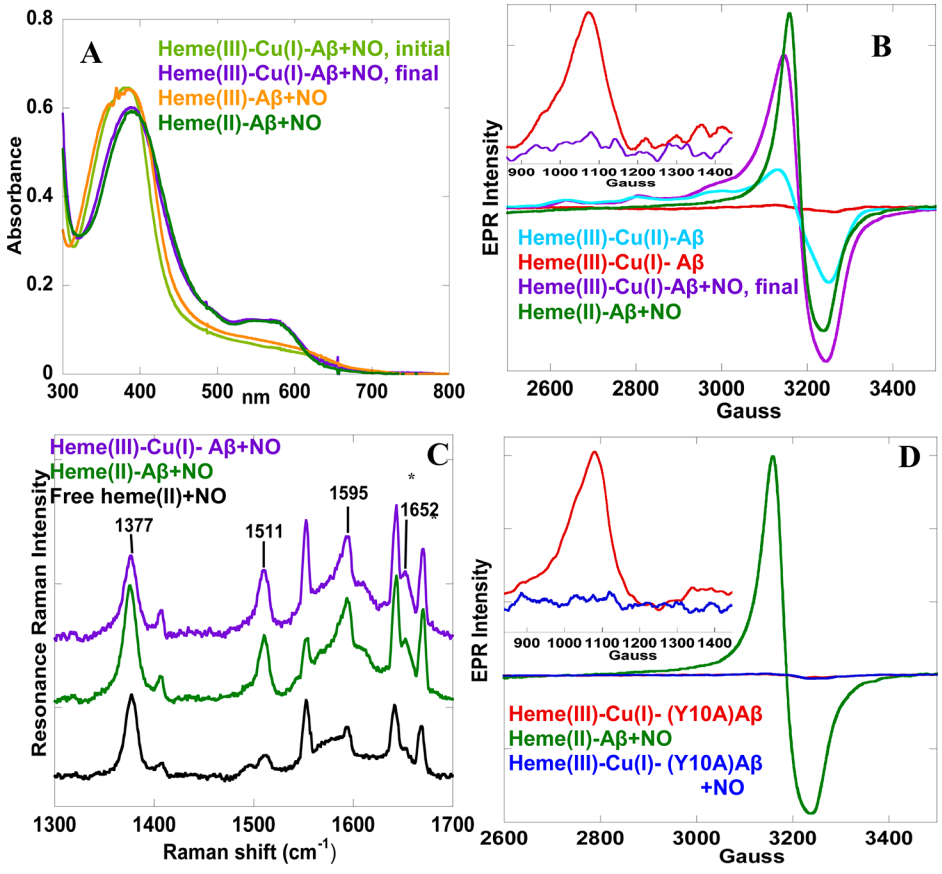

Figure 5. (A) Absorption, (B) EPR (inset, low field EPR), and (C) rR spectra of heme-Fe(III)−Cu(I)−Aβ(1−16) complex with 1 equiv of NO (initial and final), heme-Fe(III)−NO and heme-Fe(II)−NO and (D) EPR spectra of heme-Fe(III)−Cu(I)−(Tyr10Ala)Aβ(1−16), Cu(I)−heme-Fe(III)−(Tyr10Ala)Aβ(1−16) + NO, and heme-Fe(II)−NO. Adapted with permission from ref 21. Copyright 2013 American Chemical Society.

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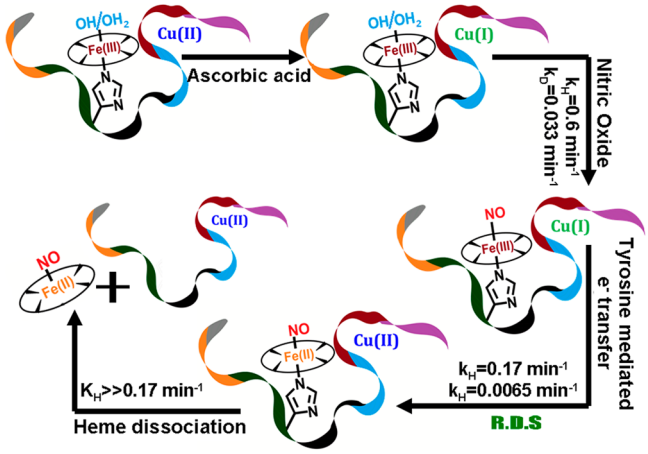
fence porphyrin (Pfp) (Fe(α4-TpivPP)Br),[37] a synthetic mimic to natural heme. On exposure of the reduced Pfp−Aβ complex to O_2 at −80 °C an Fe−O_2 intermediate was formed with ν_4 and ν_2 bands at 1367 and 1567 cm^{-1} in the rR spectrum, respectively, indicating the presence of a low-spin heme-Fe(III) center (Figure 4A). This low-spin Fe−O_2 adduct is EPR silent, as observed in the Fe−O_2 adducts of different heme proteins and synthetic porphyrins.[38] Additionally, in the low frequency region of the rR spectrum, the Fe−O stretching frequency is observed at 577 cm^{-1} (Figure 4B), which shifts to 554 cm^{-1} on O^{18} substitution, directly indicating that it is derived from O_2.[38] These values are consistent with Pfp−O_2 adducts with a trans imidazole ligand.[39] This Fe−O_2 species is the first intermediate involved in PROS generation by Aβ peptides that has been trapped and characterized in a synthetic porphyrin scaffold. The use of synthetic analogues of heme has enabled the successful modeling of this Fe−O_2 intermediate.[38]

3.3. Nitric Oxide

Nitric oxide (NO) is one of the key signaling molecules present in the human body and plays complex roles in many biological processes including neurotransmission, vascular homeostasis, and immune response.[40] A decrease in NO concentration hampers the signal transduction process of new memory formation in brain.[41,42] Decreased levels of NO have been observed in cultured neurons in the presence of Aβ.[43] It has been observed that NO plays a protective role against Aβ induced neuronal cell death, cerebrovascular dysfunction, and cerebral amyloid angiopathy.[44] Thus, decreased levels of NO may contribute to synaptic failure, which leads to memory impairment and neuronal cell death observed in AD.[45] However, excess levels of NO may generate harmful reactive nitrogen species, which cause cellular damage. Neuronal cells can minimize the NO and PROS induced stress in the presence of radical scavengers like bilirubin[46,47] (by the formation of N-nitro-bilirubin),[48] generated in the brain from biliverdin by biliverdin reductase-A.[40,49,50] Bilirubin exhibits neurotrophin like activity through regulation of NO release in pheochromocytoma (PC12) and rat primary cultures of cerebellar granule cells.[51]

When the physiologically relevant mixed-valent species heme-Fe(III)−Cu(I)−Aβ is exposed to NO, the changes in the absorption spectra (Figure 5A), along with the disappearance of the high-spin axial EPR signal (Figure 5B, inset), signify NO binding to the heme-Fe(III) center forming an EPR silent Fe(III)−NO center. A gradual appearance of a low-spin EPR signal with time, corresponding to $S = 1/2$ indicates the formation of a Fe(II)−NO species (Figure 5B). Additionally, the presence of hyperfine features characteristic of Cu(II) (Figure 5B) indicates the formation of oxidized Cu(II) (Scheme 3). The kinetic data suggest that the formation of the heme-Fe(III)−NO adduct is followed by a slower electron transfer from the reduced Cu center to the heme-Fe(III)−NO center resulting in the formation of Fe(II)−NO and concomitant oxidation of the Cu(I) center (Scheme 3). The NO adduct formation shows a KIE of ∼18 ± 5, whereas the electron transfer step exhibits a KIE of ∼25 ± 5, which may indicate a proton-coupled electron transfer (PCET) mechanism (Scheme 3). In the absence of the Tyr10 residue, no electron transfer from the Cu to the heme-Fe(III)−NO center is observed (Figure 5D). This signifies that Tyr10 is in close proximity to both centers to mediate an electron transfer, as observed in other long-range electron transfers in peptide

Scheme 3. Schematic Representation of Reaction of Heme-Fe(III)−Cu(I)−Aβ with NO[a]



Adapted with permission from ref 21. Copyright 2013 American Chemical Society [a]k_H and k_D were determined in H_2O and D_2O solvents, respectively.

systems.[52,53] The rR spectra (Figure 5C) indicates that the generated Fe(II)−NO species is five-coordinate in nature, implying that the electron transfer step is followed by dissociation of the Fe(II)−NO species from the peptide scaffold (Scheme 3). This dissociation of the Fe(II)−NO species from the peptide backbone is commonly observed in various NO bound Fe systems due to significant trans-effect exerted by NO.[54−56] While in a protein matrix the heme stays bound near the active site due to secondary interactions (e.g., hydrogen bonding with the propionate group), in a small peptide like Aβ, it diffuses into the solution.[21]

Heme and Cu binding to Aβ peptides has been invoked to have detrimental effects in AD.[29] The two otherwise electronically uncoupled paramagnetic centers undergo electron transfer upon NO binding generating Cu(II) from Cu(I), which should produce less oxidative stress in the body.[21] Moreover, once the heme-Fe(III)−NO species gets reduced by the Cu site, the ferrous nitrosyl species formed dissociates from the Aβ peptide. Therefore, NO helps in releasing heme from heme−Aβ, ameliorating the effects of heme binding to Aβ associated with AD. Hence NO might play a significant role in reducing the risks arising from redox-active heme and Cu bound Aβ peptides associated with AD.[21] A recent report suggests that the NO/cGMP (cyclic guanosine monophosphate) pathway may be an important therapeutic target for the treatment of neurodegeneration.[57]

3.4. Electron Transfer

Cytochrome c (Cyt c), a mitochondrial hemoprotein, is normally found in the intermembrane spaces of the mitochondria.[58] It acts as an essential electron donor of Cyt c oxidase in the respiratory chain in cells.[59] It can translocate from mitochondria to the cytosol and can also be released into body fluids from apoptotic or necrotic cells.[60] Recent studies have shown that the Aβ can interact with cytochromes.[61]

Addition of a stoichiometric amount of oxidized Cyt c (Soret at 410 nm) to reduced heme−Aβ (Soret at 383 nm) results in the appearance of a Soret band at 416 nm corresponding to reduced cytochrome c, with a shoulder at 364 nm corresponding to oxidized heme−Aβ. The formation of sharp Q bands at 520 and 550 nm characteristic of reduced

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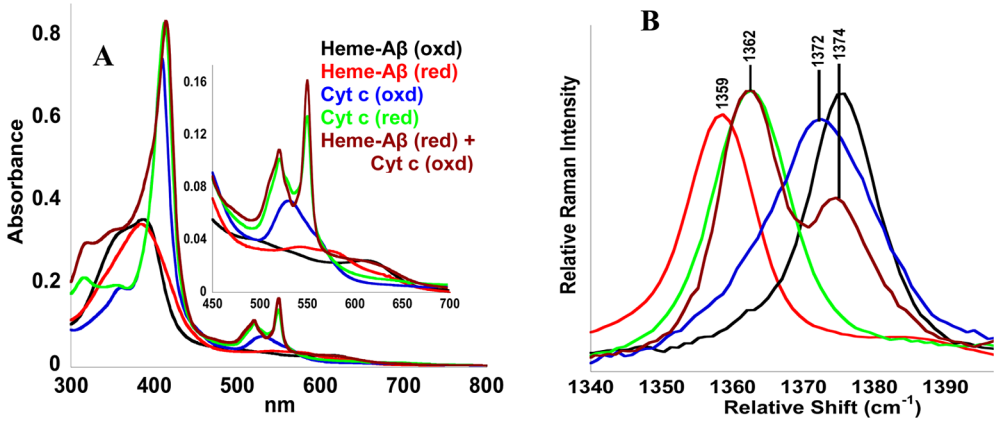

Figure 6. (A) Absorption and (B) rR spectra (excitation 413.1 nm, laser power ≈ 15 mW on the sample, ν_4 region) of oxidized and reduced heme−Aβ, Cyt c and the 1:1 mixture of reduced heme−Aβ and oxidized Cyt c in 100 mM, pH 7, hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. Adapted with permission from ref 20. Copyright 2013 Royal Society of Chemistry.

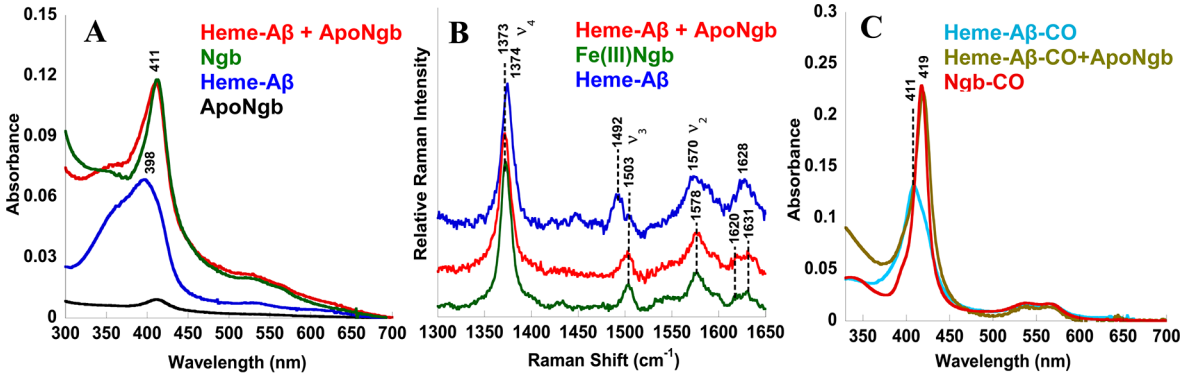

Figure 7. (A) Absorption and (B) rR spectra of heme-Fe(III)−Aβ(1−40), heme-Fe(III)−Aβ(1−40) + apoNgb, Fe(III)Ngb, and apoNgb and (C) absorption spectra of heme-Fe(II)−Aβ(1−40)−CO, heme-Fe(II)−Aβ(1−40)−CO + apoNgb and Ngb−CO in 100 mM, pH 7, phosphate buffer. Adapted with permission from ref 24. Copyright 2015 Springer.

cytochrome c and disappearance of the 539 and 574 nm bands (reduced heme−Aβ) (Figure 6A) signifies the reduction of Cyt c by heme−Aβ. Oxidized Cyt c and reduced heme−Aβ shows major ν_4 bands at 1372 and 1359 cm^{-1} respectively, while their resultant mixture shows ν_4 bands at 1362 and 1374 cm^{-1}, reflecting the formation of reduced Cyt c and oxidized heme−Aβ, respectively (Figure 6B).[20] Thus, the reaction of reduced heme−Aβ with oxidized Cyt c results in the formation of oxidized heme−Aβ and reduced Cyt c. This implies direct electron transfer from reduced heme−Aβ to Cyt c.

The electron transfer from heme-Fe(II)−Aβ to oxidized Cyt c follows second order kinetics (1st order wrt heme−Aβ and Cyt c) with a rate constant of ~1.3×10^5 M^{-1} s^{-1}.[20] A decrease in rate is observed with increase in the buffer concentration and addition of an electrolyte (KCl) suggesting that an increase in ionic strength of the medium decreases the rate of electron transfer. This suggests that docking of the positively charged Cyt c with the negatively charged Aβ is likely the rate-determining step (rds). An electron transfer from reduced heme−Aβ to oxidized Cyt c helps to reduce PROS generation and neurotoxicity of reduced heme−Aβ.[20]

3.5. Heme Transfer

Globins are well-known O_2 binding heme proteins found in bacteria, protists, algae, fungi, plants, and animals.[62] In vertebrates, two types of pentacoordinate globins are well-known, hemoglobin (Hb) in blood and myoglobin (Mb) in muscles. Among the hexacoordinate globins, cytoglobin (Cgb) and neuroglobin (Ngb) are found in mammals.[63,64] Ngb is found in the central and peripheral nervous system and in some endocrine tissues, whereas Cgb is found in almost all tissues.[65] Apoglobins are known to have high affinity toward heme and possess heme sequestering ability.[66]

Absorption and rR data (Figure 7A,B) indicate that when heme−Aβ and aponeuroglobin (apoNgb) are added in a 1:1 ratio, there appears a characteristic feature of a six-coordinate low-spin heme complex (Q bands at 535 and 560 nm in the absorption and ν_3 and ν_2 bands at 1503 and 1578 cm^{-1} in the rR spectra), which resembles those of holoNgb (Figure 7A,B).[24] This implies heme sequestration by apoNgb from heme−Aβ leading to the formation of Ngb. Similarly apoMb extracts heme from heme−Aβ resulting in the formation of Mb.[22] Further, apoglobins can also sequester heme from reduced heme−Aβ complex forming reduced hologlobins. Apart from sequestering heme from heme−Aβ, apoNgb can also sequester heme−CO from the heme−Aβ−CO complex to form Ngb−CO (Figure 7C).[24]

Because the heme sequestration from heme−Aβ by apoNgb or apoMb leads to the formation of Ngb and Mb, respectively, which are known to form the corresponding oxy complexes in the presence of molecular O_2, the extent of harmful PROS induced toxicity and damage is largely reduced.[22] Moreover, Ngb formed after heme transfer has a well documented role in neuroprotection.[67] The generated Ngb may increase O_2

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concentration in the neuronal cells and detoxify other reactive species since it can act as a radical scavenger.[68] So apoNgb and apoMb may play a physiologically protective role against the advancement of AD.[22,24]

4. AN ARTIFICIAL PLATFORM TO ASSAY O_2 REACTIVITY AND EFFECT OF SMALL MOLECULES

Aβ(1−16) appended with a cysteine residue (Aβ_{Cys}) at the C-terminus forms self-assembled monolayers (SAM) of Aβ peptides on Au electrodes.[69,70] AFM image of this SAM indicates the presence of fibrillar structures with a height distribution (8−12 nm) (Aβ_{WT}) (Figure 8) signifying the

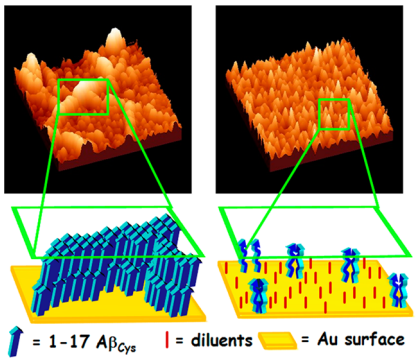

Figure 8. Probable arrangements of Aβ_{WT} (left) and A$\beta_{1:9}$ (right) on Au surface as observed in their AFM images. Adapted with permission from ref 69. Copyright 2014 Royal Society of Chemistry.

formation of large aggregates of Aβ.[70] Formation of a homogeneous distribution of small clusters is observed in the presence of a mixed SAM solution (A$\beta_{1:9}$) when Aβ_{Cys} and diluent 1-cysteine are used in 1:9 ratio (Figure 8).[69] Unlike *in vivo*, where the oligomers are transient species involved in fibril formation and hence are not stable entities, here these oligomers, bearing the same organization, are stable species offering an unprecedented opportunity for investigation of their reactivities and toxicities in detail. These peptide assemblies readily bind both heme and Cu.

Incubation of 8-hydroxyquinoline (HQ), analogous to clioquinol, with Cu−Aβ SAM removes the redox couple observed for the Cu(II)/Cu(I) system (Figure 9A) indicating removal of Cu from Cu−Aβ SAM. HQ takes higher concentration and more time to remove Cu from large Aβ aggregates (Aβ_{WT}), whereas it easily removes Cu from small aggregates (A$\beta_{1:9}$).[69,70] Thus, the chelation process is both thermodynamically and kinetically more inefficient for the large Cu−Aβ aggregates relative to the smaller aggregates.[69] In the presence of methylene blue (MB), a potential drug for AD, the strong O_2 reduction current by heme−Aβ disappears and a new redox couple assigned as MB absorbed over SAM surface is observed (Figure 9B).[70] Thus, MB can significantly reduce heme induced toxicity by inhibition of O_2 reduction and hence acts as an antioxidant (Figure 9B). It takes a higher concentration and longer time for the inhibition of O_2 reduction for large aggregates compared with small aggregates.[69] Thus, the chelation or inhibition of PROS production is mostly inefficient for large aggregates, and hence they are more toxic. The above data necessitates breaking of the larger fibrils to oligomers prior to any Cu/heme specific treatment.[69]

5. CONCLUSION

Co-localization of some heme proteins within the SP and deposits of heme in amyloid fibrils in AD brain instigated our group to investigate heme-bound Aβ complexes. The well-defined active site of heme binding involving the Arg5, Tyr10, and His13 residues unique to mammalian Aβ, ROS generation, and modest peroxidase activity exhibited by the resultant heme−Aβ complexes fits the pathology of an AD brain. The interaction of heme−Aβ with NO (heme release), Cyt c (electron transfer), and apoglobins like apoNgb or apoMb (through heme sequestration) is in line with observed pathologies like down-regulation of NO, hypometabolism, abnormal heme homeostasis, and overexpression of heme oxygenase, etc. These experimental findings suggest at least at a preliminary level that heme may be significantly involved in AD. We feel that further research into the likely involvement of heme and heme proteins in AD is warranted. Apart from the biophysical investigations of heme−Aβ complexes, the abiological model for aggregated amyloid on conducting surfaces developed is a powerful tool to investigate the reactivity of redox active metal/cofactor bound to fibrillar and oligomeric Aβ and to screen drugs designed to target these. These constructs are amenable to a large range of spectroscopy, microscopy, and electrochemical techniques allowing high resolution, ultrasensitive investigations of metal−Aβ complexes.

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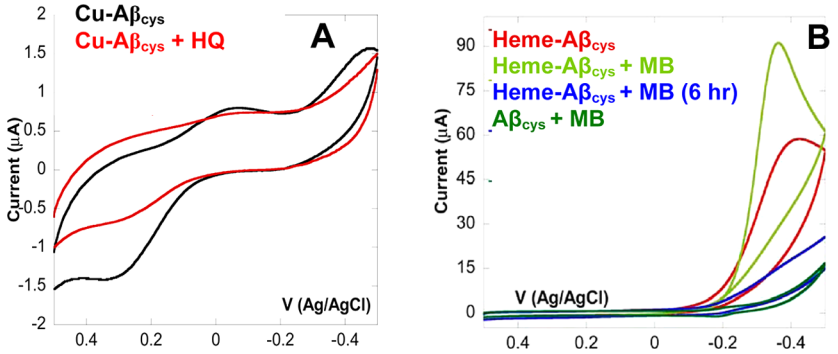

Figure 9. CV of (A) Cu−Aβ_{Cys} in absence (black) and presence of HQ (red), (B) heme−Aβ_{Cys} (red), heme−Aβ_{Cys} + MB after instantaneous addition (green), heme−Aβ_{Cys} + MB after 6 h (blue), and Aβ_{Cys}+MB (dark green) in air saturated, pH 7 buffer. Adapted with permission from ref 70. Copyright 2012 American Chemical Society.

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Acc. Chem. Res. 2015, 48, 2556−2564

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Notes

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