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Apomyoglobin Sequesters Heme from Heme Bound Aβ Peptides

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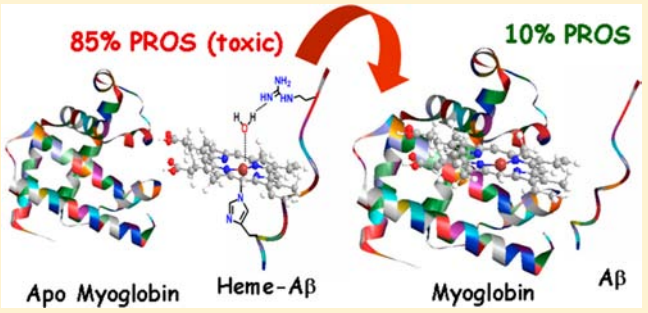
Supporting Information

ABSTRACT: A combination of absorption, electron paramagnetic resonance (EPR), and resonance Raman (rR) spectroscopy has been used to study the interaction of heme-Aβ and apomyoglobin (apoMb). The absorption spectrum of oxidized heme bound Aβ, characterized by a split Soret band at 364 and 394 nm, shifts to 408 nm on incubation with apoMb, characteristic of Myoglobin (Mb). The ν_4, ν_3, and ν_2 bands in the rR spectrum of heme-Aβ are observed at 1376, 1495, and 1570 cm^{-1}, which shift to 1371, 1482, and 1563 cm^{-1}, respectively on incubating with apoMb, implying formation of Mb. Similarly, heme transfer from reduced heme-Aβ to apoMb resulting in the formation of deoxyMb was also observed. Thus, spectroscopic data show that apoMb can sequester heme from heme-Aβ complexes both in oxidized and in reduced forms. Heme uptake by apoMb from native heme-Aβ(1−40) and Aβ(1−16) in both oxidized and reduced forms follow a biphasic reaction kinetics likely representing heme transfer from two dominating conformers of heme-Aβ in solution. The rate constants for the two steps involved in heme uptake by apoMb from heme-Aβ(1−40) are 11.5×10^4 M^{-1} s^{-1} and 7.5×10^3 M^{-1} s^{-1} while from heme-Aβ(1−16) are 6.0×10^4 M^{-1} s^{-1} and 7.5×10^3 M^{-1} s^{-1}. The rate constants for heme uptake by apoMb from reduced heme-Aβ(1−40) are 3.7×10^4 M^{-1} s^{-1} and 6.8×10^3 M^{-1} s^{-1} while for reduced heme-Aβ(1−16) are 2.0×10^4 M^{-1} s^{-1} and 6.0×10^3 M^{-1} s^{-1}. The heme uptake from heme-Aβ by apoMb leads to a dramatic reduction of PROS generation by the reduced heme-Aβ complexes.



1. INTRODUCTION

Alzheimer's disease (AD) is a terminal neuronal disorder that causes senile dementia. Memory loss and brain disorder are the characteristic features observed in AD.[1] Synaptic breakdown and loss of neurons in the hippocampus cortex are closely related with the memory loss observed in AD.[1] Aggregation of amyloid β (Aβ) peptides as neurofibrillary tangles[2−4] and formation of reactive oxygen species (ROS)[5,6] are the key biomarkers of this disease. Aβ peptides originate from large trans membrane Amyloid Precursor Protein (APP)[7,8] by proteolytic actions of different proteolytic enzymes.[9] Aβ peptides are normally found in biological fluids containing 39−42 amino acid residues.[8] Aβ peptides show cytotoxicity in both aggregated and soluble oligomeric forms.[10,11] Transition metals (Zn^{2+}, Cu^{2+}, and Fe^{3+}) present in AD brain[12−14] take part in the aggregation of Aβ.[15,16] Redox active transition metal ions (Fe^{3+} and Cu^{2+}) assist in ROS formation.[5,6] These transition metals in their reduced state (Fe^{2+}, Cu^{+}) spontaneously generate freely permeable, neurotoxic partially reduced oxygen species (PROS), $HO_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$, and so forth.[17,18] These highly reactive hydroxyl radicals generate lipid peroxidation adducts and nucleic acid adducts that are characteristics of AD pathology.[19−21] According to current studies heme (an Fe based cofactor) might also be involved in generating significant pathological features of AD.[22]

Studies revealed that heme metabolism is altered in AD patients' brains. Heme deficiency,[23] unregulated Fe homeostasis, increased levels of ferrochelatase, higher levels of heme oxygenase (HO),[24] increased level of heme degradation products[25] and diminished activity of mitochondrial complex IV[26−28] are common symptoms of AD that are associated with dysregulated heme metabolism. Recent studies reveal that regulatory heme binds Aβ[29,30] which might account for heme deficiency observed in AD patients.[24] The heme-Aβ complexes show peroxidase activity[29,30] in their oxidized form. These can effectively catalyze the oxidation of neurotransmitters such as serotonin, 3,4-dihydroxyphenylalanine, and 4-hydroxyphenylpyruvic acid by H_2O_2.[29,31] They also react with molecular O_2 in their reduced forms and generate toxic PROS.[32,33] While these scientific findings do not directly implicate heme as a causative agent in AD, they definitely poise the question. The active site of this heme-Aβ complex has been characterized by spectroscopic techniques.[30] One of the histidine residues present at the 13th and 14th position of Aβ possibly coordinate to heme.[30] There exists an exchangeable water derived ligand (pK_a − 6.8) at the distal pocket of this complex.[30,34] This water derived ligand is hydrogen bonded with the arginine residue (contains a positively charged guanidine group) present at the 5th position of the peptide sequence of the Aβ peptide.[30]

Myoglobin (Mb) is a heme based O_2 carrier protein originating in red muscles[35] that acts as a multifunctional

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protein in vertebrates.[36] Major bodily function of Mb is to transport O_2[35,36] similar to another globin protein hemoglobin (Hb). It transports O_2 in the blood from the sarcolemma to the mitochondria of vertebrate heart and red muscle. Mb also influences diffusion of O_2,[37] oxidative phosphorylation,[37] protection against oxidative damage,[38] enhancement of NO concentration gradients,[39,40] and inactivation of enzymes.[41] It contains a heme prosthetic group and a globular protein consisting of 153−154 amino acids.[42] The polypeptide backbone of Mb forms 8 α-helixes around the heme group. Histidine residue at the 93rd position coordinates to the Fe center. Another histidine group present at the 64th position is located at the distal pocket of Mb.[36] A recent study identifies a third globin type enzyme called neuroglobin (Ngb), mainly expressed in the brain. Ngb acts as an O_2 carrier.[43] It mimics the function of Mb in supplying O_2 to the mitochondrial respiratory chain.[44] These globin enzymes are essential for maintaining minimum O_2 levels and normal biological processes. Up-regulated globin enzymes in hypoxic conditions suggest a possible role of these enzymes in O_2 balance.[45,46] Ngb in human brain also plays protective roles in hypoxic conditions by enhancing O_2 supply to neurons. Hypoxic conditions also induce up-regulation of APP mRNA and Aβ.[47]

Hb isolated from brain tissues[48] catalyzes lipid peroxidation and causes neuronal cell death.[49,50] A recent report develops a positive correlation between Hb expression levels and cognitive impairment in aging rats.[51] A recent study aimed to identify Aβ-binding proteins that causes aggregation of Aβ has discovered Hb as a major Aβ binding protein.[52] Hb was also found to colocalize with Aβ in the soluble extracts and plaques of AD brain.[53,54] It has further been demonstrated that there exists a heme dependent interaction between Hb and Aβ that reduces the cytotoxicity of heme.[52] Not only Hb but Mb and cytochrome *c* (Cyt*c*) are also found to interact with Aβ.[52] In spite of several such reports in the past few years the exact nature of these interactions between Aβ and these heme containing proteins remain vastly unexplored. In parallel it is of potential interest to observe the interaction of the apoforms of these heme binding enzymes with heme-Aβ.

In this study we investigate the effect of apomyoglobin (apoMb) on heme-Aβ complexes. We show that heme bound human Aβ(1−40) and Aβ(1−16) peptides can transfer heme to apoMb by absorption, electron paramagnetic resonance (EPR), resonance Raman (rR) spectroscopic techniques, and gel electrophoresis. Kinetic analysis has been performed to determine the rate of heme uptake by apMb form heme-Aβ.

2. MATERIALS AND METHODS

2.1. Materials. All reagents were of the highest grade commercially available and were used without further purification. Amyloid beta (Aβ) peptides (1−40) (sequence: Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val) and (1−16) (sequence: Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys) were purchased from GL Biochem (Shanghai) Ltd. with >95% purity. Hemin, Mb and the buffers were purchased from Sigma.

2.2. Sample Preparation. Aβ peptide stock solutions were made in 100 mM phosphate buffer at pH 7, and hemin solution was made in 1 M NaOH solution. Peptide stock solutions were 0.5 mM, and heme stock solution was 5 mM. Heme-Aβ complexes were prepared by incubating 1 equivalent of Aβ with 0.8 equivalent of heme solution for ∼2 h. ApoMb was extracted from Mb following reported protocol using ethyl methyl ketone. It was then dialyzed extensively. The apoprotein was then centrifuged to a concentrated solution.[55] The concentration of the Mb and apoMb solutions were determined using the molar extinction coefficient.[56] Heme-Aβ samples (both in oxidized and reduced form) were incubated with 1 equivalent of apoMb for ∼1 h. The kinetics of these reactions was monitored by absorption spectroscopy.

2.3. Absorption Spectroscopy. Absorption spectra of heme-Aβ (for both 1−40 and 1−16 sequences), Mb, and heme-Aβ-apoMb complex (1−40 and 1−16) were recorded in 1 mL buffer at pH 7. Final concentrations of the UV samples were 12.5 μM. All the spectral data were obtained by an UV−vis diode array spectrophotometer (Agilent 8453).

2.4. EPR Spectroscopy. EPR spectra were obtained by a Jeol (JES FA200) spectrophotometer. EPR samples were 0.4 mM in concentration with respect to heme (concentration of apoMb and Aβ were 0.5 mM) and were run at 77 K in liquid nitrogen finger dewar. EPR samples of heme-Aβ(1−40 and 1−16) were prepared by lowering the pH of a high pH sample (initial pH of the heme-Aβ samples were pH ∼11) to pH 7 with 0.5 M H_2SO_4. Mb and heme-Aβ-apoMb complexes were prepared at pH 7.

2.5. Resonance Raman Spectroscopy. Resonance Raman (rR) data were obtained using a Trivista 555 spectrograph (Princeton Instruments) and using 413.1 nm excitation from a Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). rR samples were 0.4 mM in concentration with respect to heme (concentration of apoMb and Aβ are 0.5 mM).

2.6. Gel Electrophoresis. In native gel electrophoresis method, 10% polyacrylamide gel was used as the resolving gel. The samples were prepared by mixing 20 μL of each sample (containing 20 μg samples) with equal volume of Laemmli buffer. Laemmli buffer was prepared following the standard protocol.[57] 4% stacking gel was prepared on top of the resolving gel, and the comb was inserted carefully. It was then allowed to stay for 1 h. After formation of the defined wells the gel was loaded in the electrophoresis setup. It was then filled with 1× tank buffer, and the samples were loaded in the wells. Electrophoresis run was monitored by the movement of bromophenol blue. Finally the gel was stained with Coomassie blue (G-250) stain to obtain the protein bands, and after complete staining, it was destained overnight with a solution of 25% methanol, 7% acetic acid, and water.

2.7. PROS Detection Assay. For PROS calculation, a xylenol orange assay was performed. A 3.9 mg portion of xylenol orange and 4.9 mg of Mohr's salt were taken in 5 mL of 250 mM H_2SO_4. A 200 μL portion of this solution was taken in 1.8 mL of nanopure water, and a calibration curve for the quantitative estimation of H_2O_2 was prepared using different concentrations of H_2O_2. The calibration curve was expressed as absorbance at a fixed wavelength of 560 nm vs concentration of H_2O_2 in μM units for a 2 mL volume. A blank was obtained with 1.8 mL of nanopure water and 200 μL of the xylenol orange solution. This served as the control. The heme-Aβ(1−40), Mb, and heme-Aβ-apoMb (1−40) complexes were reduced by dithionite (for all samples) under anaerobic conditions (observed by absorption), followed by their reoxidation by O_2 (this complete reoxidation of the reduced complexes by O_2 was confirmed by absorption spectroscopy). 200 μL of 0.025 mM reoxidized solutions were separately added to the cuvette containing the control. Absorbances of these solutions were recorded. The value of absorbance of the above solutions (after subtracting the control) at 560 nm when plotted on the calibration curve yielded the corresponding H_2O_2 concentrations.

2.8. Separation of the Samples. The complex (mixture of heme-Aβ and apoMb) was centrifuged using a 10 kD cutoff membrane to separates Aβ (∼4 kD) and Mb (∼17 kD). Spectroscopic techniques on the separated solutions were used to confirm the identity of the products.

3. RESULTS AND ANALYSIS

3.1. Absorption Spectroscopy. *3.1.1. Oxidized Heme-Aβ.* One equivalent heme has been experimentally demonstrated to covalently bind to Aβ(1−16) and Aβ(1−40)

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peptides. The absorption spectrum of heme bound Aβ(1−16) and Aβ(1−40) is characterized by a split Soret band at 364 and 394 nm and a broad Q-band at 606 nm (Figure 1). Mb has a

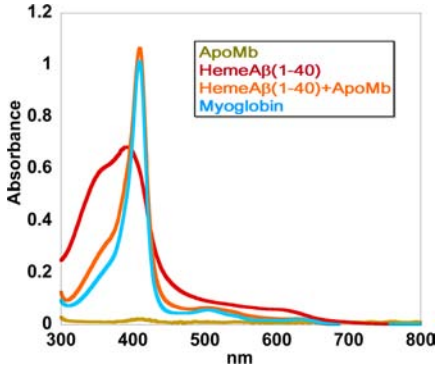

Figure 1. Absorption spectra of heme-Aβ (1−40), red; heme-Aβ (1−40) incubated with apoMb, orange; and Mb, cyan, in 100 mM phosphate buffer at pH 7.

sharp Soret band at 408 nm and Q bands at 500, 540, and 630 nm (Figure 1 and Supporting Information, Figure S1). ApoMb (without the heme cofactor) lacks any absorption bands in the visible region of the spectrum. When heme-Aβ and apoMb are incubated in 1:1 stoichiometric ratio, the resultant absorption spectrum resembles that of native Mb (Figure 1 and Supporting Information, Figure S1), implying that heme has transferred from heme-Aβ to apoMb. Further the 1:1 incubated mixture of heme-Aβ and apoMb has been separated by centrifugation, and the absorption spectrum of the Mb obtained after separation is identical to the native holoMb, confirming that heme has transferred from heme-Aβ resulting in formation of Mb (Supporting Information, Figure S2A).

3.1.2. Reduced Heme-Aβ. Reduction of heme-Aβ(1−16) and heme-Aβ(1−40) results in a decrease in intensity and shift of the Soret band to ∼388 nm with a shoulder at ∼423 nm relative to the oxidized form, and Q bands at 540 and 575 nm, (Figure 2 and Supporting Information, Figure S3). DeoxyMb

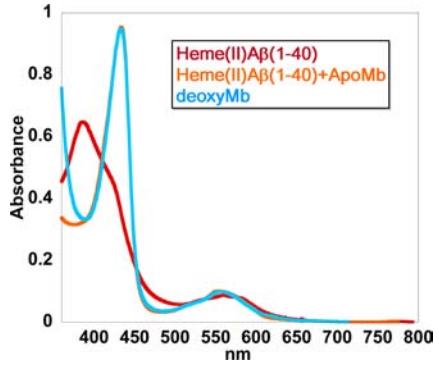

Figure 2. Absorption spectra of reduced heme-Aβ(1−40), red; reduced heme-Aβ(1−40) incubated with apoMb, orange; and deoxyMb, cyan, in 100 mM phosphate buffer at pH 7.

has a sharp Soret band at 435 nm and Q bands at 526 and 555 nm with a shoulder at 586 nm (Figure 2 and Supporting Information, Figure S3). When reduced heme-Aβ are incubated with apoMb, the experimentally observed spectrum is almost similar to that of deoxyMb (Figure 2 and Supporting Information, Figure S3), indicating that heme can also be transferred from reduced heme-Aβ to apoMb.

3.2. EPR Spectroscopy. *3.2.1. Oxidized Heme-Aβ.* Heme-Aβ(1−16) and heme-Aβ(1−40) complexes show a broad high spin $S = 5/2$ signal with $g \sim 6.0$. When incubated with apoMb, the signal slightly shifts to high field and becomes sharper, similar to that of Mb (Figure 3 and Supporting Information,

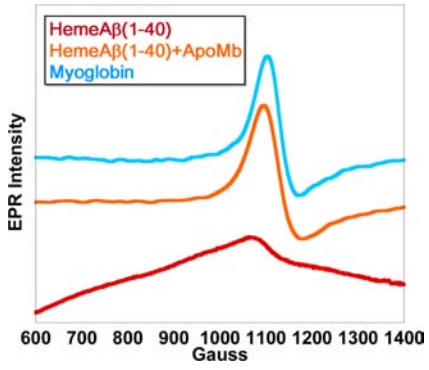

Figure 3. EPR spectra of heme-Aβ(1−40), red; heme-Aβ(1−40) incubated with apoMb, orange, and Mb, cyan, in 100 mM phosphate buffer at pH 7 in the low field region.

Figure S4), implying formation of Mb. This indicates transfer of heme from heme-Aβ to apoMb, consistent with the absorption data (Figure 1 and Supporting Information, Figure S1). This has further been confirmed by the EPR spectrum of the Mb obtained after centrifugation of the incubated mixture of heme-Aβ and apoMb, which is identical to native Mb (Supporting Information, Figure S2C).

3.3. Resonance Raman Spectroscopy. *3.3.1. Oxidized Heme-Aβ.* The oxidation state, coordination number, and spin state marker bands, ν_4, ν_3, and ν_2 respectively, observed at 1376, 1495, and 1570 cm^{-1} for heme-Aβ(1−40) (Figure 4) indicate

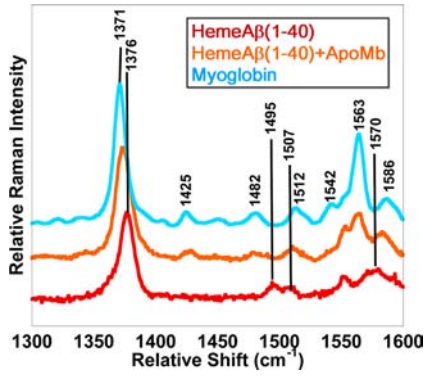

Figure 4. High frequency rR spectra of heme-Aβ(1−40), red; heme-Aβ(1−40) incubated with apoMb, orange, and Mb, cyan, in 100 mM phosphate buffer at pH 7.

the presence of a six coordinate high-spin Fe^{3+} active site[58,59] (small intensities at 1507 (ν_3) and 1579 (ν_2) cm^{-1} for heme-Aβ(1−40) indicates the presence of minor amount of a six coordinated low-spin Fe^{3+} species[60,61]). The marker bands of Mb appear at 1371 (ν_4), 1482 (ν_3), 1512 (ν_{38}), 1542 (ν_{11}), 1563 (ν_2), and 1586 (ν_{37}) cm^{-1} (Figure 4) indicating the presence of a six coordinate high-spin Fe^{3+} active site.[62,63] When heme-Aβ and apoMb are incubated in 1:1 stoichiometric ratio, the resultant rR spectrum confirms the formation of

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native Mb (Figure 4), implying that heme has transferred from heme-Aβ to apoMb. Similar results were obtained for heme-Aβ(1−16) (Supporting Information, Figure S5).

3.3.2. Reduced Heme-Aβ. The characteristic oxidation state marker band ν_4 for reduced heme-Aβ(1−40) appears at 1360 cm^{-1} indicating the presence of Fe^{2+} species. The coordination number and spin state marker bands, for heme-Aβ(1−40) show a mixture at 1558 and 1584 cm^{-1} (ν_2), 1471 and 1493 cm^{-1} (ν_3) which indicate the presence of both five coordinate high spin ferrous form as well as six coordinate low spin ferrous species[59,64] (Figure 5). The marker bands for deoxyMb appear

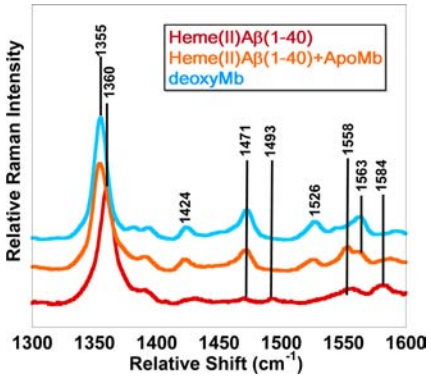

Figure 5. High frequency rR spectra of reduced heme-Aβ(1−40), red; reduced heme-Aβ (1−40) incubated with apoMb, orange, and deoxyMb, cyan, in 100 mM phosphate buffer at pH 7.

at 1355 (ν_4), 1471 (ν_3), and 1563 (ν_2) cm^{-1} indicating the presence of five coordinate high-spin Fe^{2+} species[65] (Figure 5). When reduced heme-Aβ and apoMb are incubated in 1:1 stoichiometric ratio, the resultant rR spectrum confirms the formation of deoxyMb (Figure 5), implying that heme has been extracted from reduced heme-Aβ to apoMb. Similar results were obtained for heme-Aβ(1−16) (Supporting Information, Figure S6).

3.4. Kinetics. The rates of heme sequestration by apoMb from heme-Aβ(1−16) and heme-Aβ(1−40) were followed using absorption spectroscopy. The data show that both the reactions follow biphasic reaction kinetics (Figure 6). The rate constants for the two steps involved in heme uptake by apoMb from heme-Aβ(1−40) are 11.5×10^4 M^{-1} s^{-1} and 7.5×10^3 M^{-1} s^{-1} while from heme-Aβ(1−16) are 6.0×10^4 M^{-1} s^{-1} and 7.5×10^3 M^{-1} s^{-1}. Both these steps involve shifting of

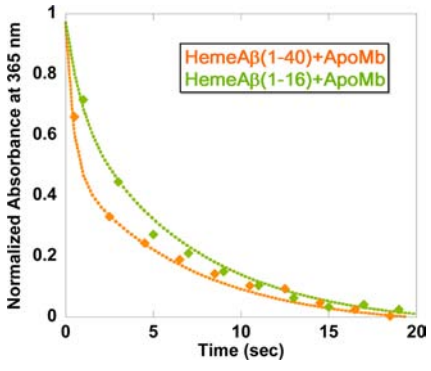

Figure 6. Kinetic traces for heme sequestration by apoMb followed by the increase in absorption intensity at 365 nm (A vs time plot) from heme-Aβ(1−40), orange, and heme-Aβ(1−16), green.

absorption intensity from 365 nm (characteristic of heme-Aβ) to 408 nm (characteristic of Mb). Thus both of these steps involve heme transfer from heme-Aβ by apoMb, as any other process, for example, dissociation of a peptide−protein (Aβ-Mb) complex, is unlikely to be associated with the observed changes in the absorption data. The two steps likely indicate heme transfer from different conformers of heme-Aβ in solution to apoMb at different rates. The higher rate constant (first step) for heme uptake by apoMb from heme-Aβ(1−40) relative to heme-Aβ(1−16) might indicate a weaker binding of heme to Aβ(1−40) than Aβ(1−16). Alternatively this may also represent stronger interaction of apoMb with Aβ(1−40), (which is relatively more hydrophobic than Aβ(1−16)), which may enable facile docking prior to heme transfer. Calculations using molar extinction coefficients of heme and Mb show that heme transfer from heme-Aβ(1−40) to Mb is ∼99.9%.

The rates of heme sequestration by apoMb from reduced heme-Aβ(1−40) and reduced heme-Aβ(1−16) were also monitored using absorption spectroscopy. The data show that both reactions follow biphasic reaction kinetics (Figure 7). The

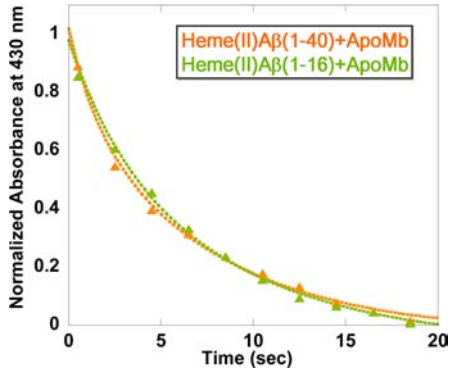

Figure 7. Kinetic traces for heme sequestration by apoMb monitored by the increase in absorption intensity at 430 nm (A vs time plot) from reduced heme-Aβ(1−40), orange, and reduced heme-Aβ(1−16), green.

rate constants for heme uptake by apoMb from reduced heme-Aβ(1−40) are 3.7×10^4 M^{-1} s^{-1} and 6.8×10^3 M^{-1} s^{-1} while for reduced heme-Aβ(1−16) are 2.0×10^4 M^{-1} s^{-1} and 6.0×10^3 M^{-1} s^{-1}. Heme transfer from heme-Aβ(1−40) to Mb is ∼94%.

3.5. Reactivity. Reduced heme-Aβ reacts with molecular O_2 reducing it to toxic PROS and in the process itself gets oxidized (Figure 8, red). On the other hand deoxyMb forms stable oxy adduct (oxyMb) on exposure to molecular O_2. OxyMb has a Soret band at 418 nm and Q bands at 545 and 584 nm (Figure 8, cyan). Reduced heme-Aβ(1−40) after incubation with apoMb forms a similar oxy complex when exposed to molecular O_2 (Figure 8, orange).

The rR spectra of the O_2 exposed reaction mixture (mixture of reduced heme-Aβ(1−40) and apoMb) and oxyMb are identical, with the Fe−O_2 stretch arising at 572 cm^{-1} (Figure 9). This further implies that heme from reduced heme-Aβ is sequestered by apoMb to form deoxyMb, which then subsequently forms oxyMb on exposure to O_2.

PROS detection assay shows that reduced heme-Aβ produces ∼85 ± 5% H_2O_2. It involves a two electron reduction of molecular O_2, one of which is obtained from reduced heme while the other is donated by the Tyr10 residue.[32] DeoxyMb

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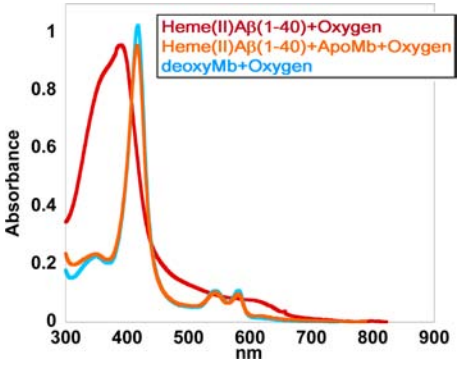

Figure 8. Absorption spectra of reduced heme-Aβ(1−40) + O_2, red; reduced heme-Aβ(1−40) incubated with apoMb + O_2, orange, and deoxyMb + O_2, cyan, in 100 mM phosphate buffer at pH 7.

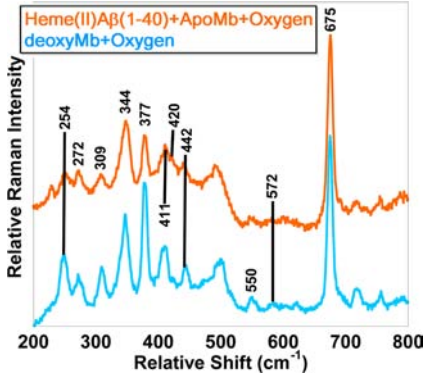

Figure 9. rR spectra of reduced heme-Aβ(1−40) incubated with apoMb + O_2, orange, and deoxyMb + O_2, cyan, in 100 mM phosphate buffer at pH 7.

and reduced heme-Aβ(1−40) incubated with apoMb produce negligible H_2O_2 (∼10 ± 5%) on reaction with O_2 (Figure 10). This clearly demonstrates that heme sequestration by apoMb can minimize the risk of oxidative stress in AD.

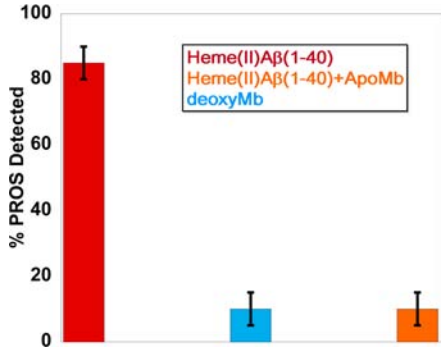

Figure 10. H_2O_2 detection assay of reduced heme-Aβ(1−40) + O_2, red; reduced heme-Aβ (1−40) incubated with apoMb + O_2, orange, and deoxyMb + O_2, cyan, in 100 mM phosphate buffer at pH 7.

4. DISCUSSION

The absorption data indicate that apoMb can quantitatively uptake heme from oxidized heme-Aβ and produce native Mb (Figure 1 and Supporting Information, Figure S1). EPR spectrum of oxidized heme-Aβ shows a high spin $S = 5/2$ signal with $g \sim 6.0$. This signal eventually shifts to higher field and becomes sharper when incubated with apoMb, leading to the formation of Mb (Figure 3 and Supporting Information, Figure S4). The rR data obtained for heme-Aβ, Mb, and heme-Aβ incubated apoMb in the oxidized form also confirm heme transfer from heme-Aβ to apoMb with subsequent formation of Mb (Figure 4 and Supporting Information, Figure S5). This heme transfer from heme-Aβ to apoMb is also characterized by native gel electrophoresis (Supporting Information, Figure S7). Thus spectroscopic data suggest heme uptake by apoMb from toxic heme-Aβ complexes. Moreover, when the 1:1 incubated mixture of heme-Aβ and apoMb was separated, the resulting Mb solution had spectroscopic features identical to those of the native holoMb, confirming heme transfer from heme-Aβ to apoMb generating native Mb (Supporting Information, Figure S2A, S2B, S2C).

Kinetic analysis shows that the rate of heme uptake by heme-Aβ(1−40) is faster than heme-Aβ(1−16) (Figure 6). This might imply a stronger heme binding to Aβ(1−16) or a better interaction of apoMb with Aβ(1−40). Spectroscopic data indicate ∼100% heme transfer from oxidized heme-Aβ to apoMb. However, the heme transfer reaction is biphasic in nature and likely represents heme transfer from two dominating conformers of heme-Aβ in solution.

Absorption and rR data (Figure 2, 5, and Supporting Information, Figure S3 and S6) demonstrate that incubation of apoMb with reduced heme-Aβ complexes leads to heme sequestration by apoMb resulting in the formation of deoxyMb. There is 94% heme transfer from reduced heme-Aβ to apoMb. The rate of heme uptake by apoMb from reduced heme-Aβ also shows biphasic kinetics like the oxidized form.

The heme-Aβ complexes have been shown to behave as peroxidases and Arg[5] has been identified as the key residue required for making these complexes function as peroxidases.[30] The peroxidase activity of heme-Aβ complexes enables them to catalyze the oxidation of organic small molecules including neurotransmitters like serotonin, 3,4-dihydroxyphenylalanine, and 4-hydroxyphenylpyruvic acid in the presence of H_2O_2.[29] This could be the probable reason for the observed abnormal neurotransmission in AD patients. Heme-Aβ sites ($E^\circ = -0.42$ V, i.e., −0.17 V vs NHE) can be reduced by physiological reducing agents like glutathione ($E^0 = -0.25$ V vs NHE) or NADH ($E^\circ = -0.45$ V vs NHE). Reduced heme-Aβ is known to produce toxic PROS.[32] Thus, heme-Aβ complexes can exhibit harmful activities in both oxidized and reduced forms.[29,30,32] The present study shows that apoMb, which is normally not available in the cerebrospinal fluid, can extract the possible neurotoxic heme component from heme-Aβ in both the oxidized and the reduced forms, making them less toxic. Thus heme uptake from heme-Aβ by apoMb or the apo forms of structurally similar proteins like hemoglobin and neuroglobin might exhibit a protective role against the possible damages by heme-Aβ.

5. CONCLUSION

In summary, we have spectroscopically demonstrated that heme bound human Aβ(1−40) and Aβ(1−16) peptides can transfer heme to apoMb in both the oxidized and the reduced forms, resulting in the formation of Mb and deoxyMb, respectively. Kinetic analysis has been performed to determine the rate of heme uptake by apoMb form heme-Aβ in both oxidized and reduced forms. The rate of heme uptake from the native heme-Aβ(1−40) is faster than that of heme-Aβ(1−16), and the rate of heme uptake is much slower in reduced heme-Aβ relative to

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oxidized heme-Aβ complexes. These results might implicate that apoMb can play a protective role against heme-Aβ.

■ ASSOCIATED CONTENT

Supporting Information

Gel electrophoresis, absorption, EPR, rR data of heme-Aβ(1−16), and absorption, EPR, CD data of separated Mb after the incubation. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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