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Interaction of NO with Cu and Heme-Bound Aβ Peptides Associated with Alzheimer's Disease

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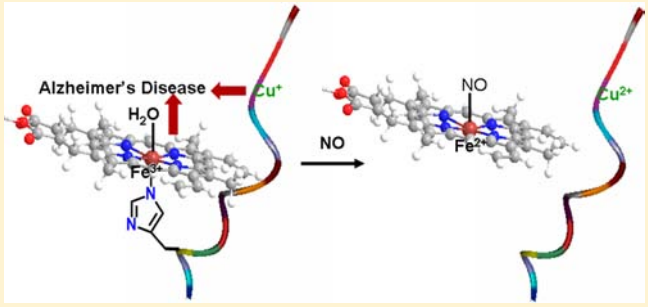
Supporting Information

ABSTRACT: Reduced Cu and heme has been invoked to be involved in Alzheimer's disease (AD). Recently the Aβ peptides have been demonstrated to bind heme and Cu simultaneously, and this complex produces significantly more toxic partially reduced oxygen species (PROS) than the Cu or heme-bound Aβ peptides. Here a combination of absorption, EPR, and resonance Raman spectroscopy along with kinetic assays are used to investigate the interaction of nitric oxide (NO) with the physiologically relevant form of Cu and heme-bound Aβ peptides, since a down-regulation of nitric oxide synthase activity is observed in patients suffering from AD. The data indicate that NO oxidizes the Cu(I) sites, making them less toxic toward PROS generation and releases heme from the Aβ peptides ameliorating the effects of heme binding to Aβ peptides associated with AD. This process involves a tyrosine-mediated electron transfer between the Cu and heme sites. These results provide a mechanistic pathway for the possible protective role of NO in AD.



1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder causing senile dementia.[1] This terminal disease is closely associated with a massive loss of neurons and synaptic breakdown in the brain. The most common biomarker of AD is the accumulation of neurotoxic amyloid beta (Aβ) peptides in the brain.[2,3] Transition metals like Cu and Fe bind to Aβ peptides[4−6] and in their reduced forms react with O_2 generating toxic partially reduced oxygen species (PROS),[7,8] leading to oxidative stress and oligomerization of Aβ peptides. Recently, it has been shown that heme binds to Aβ peptides.[9−12] This complexation diminishes the bioavailability of regulatory heme, leading to deficiency of heme required for normal biological processes.[13] Heme deficiency gives rise to specific symptoms, which are incidentally key pathological features of AD,[13] implicating a direct role of heme in this disease. The heme−Aβ complexes behave as peroxidases and can oxidize neurotransmitters like serotonin and 3,4-dihydroxyphenylalanine (DOPA) in the presence of H_2O_2, which might account for the abnormal neurotransmission observed in AD patients.[14,15] The active site of this complex has been characterized by spectroscopic techniques. His^{13} and His^{14} of Aβ peptide have been identified as the heme-coordinating residues.[12] Arg^5 residue present at the distal pocket H-bonds with the exchangeable water-derived ligand present at the distal position and donates a proton, thus driving the O−O bond heterolysis and making the heme−Aβ complexes function as peroxidases.[12]

The Aβ peptides have recently been shown to simultaneously bind both heme and Cu. The heme and Cu sites exhibit unique spectroscopic and electrochemical features that remain unperturbed in the presence of each other.[16,17] The PROS generated by the Cu(II)−Fe(III)heme−Aβ complexes is maximum relative to the Fe(III)heme−Aβ and Cu(II)−Aβ complexes, making them most toxic for AD.[16]

Nitric oxide (NO) is one of the most important signaling molecules present in the human body and plays complex roles in many biological processes.[18] It is synthesized from *L*-arginine by the enzyme nitric oxide synthase (NOS).[19,20] Recently, NO has been associated with AD since there is a decreased level of NO in patients suffering from AD.[21] The soluble guanylyl cyclase (sGC) enzyme binds NO and relays the NO signal.[22,23] This activated sGC elevates intracellular levels of a second messenger molecule, cyclic guanosine monophosphate (cGMP).[22] This is a key signal transduction system in the central nervous system (CNS) that maintains plasticity in the hippocampus and cerebral cortex of the brain.[24,25] This immensely important process for the formation of new memory is hampered in the AD brain possibly due to a decrease in the NO concentration. Thus, decreased levels of NO observed in the AD brain may contribute to memory impairment and neuronal cell death. Presently, it has been accepted that aggregated Aβ inhibits the NO signaling pathway and suppresses the protective effects of endogenous NO in the brain.[26] It has also been observed that the NO produced by neuronal NOS (nNOS) and endothelial NOS (eNOS) plays a

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protective role against Aβ-induced neuronal cell death, cerebrovascular dysfunction, and cerebral amyloid angiopathy.[27] The clinical trial of NO donors and cGMP analogues as therapeutics has been found to suppress cell death, prevent inflammatory responses in brain cells, and reverse learning and memory impairment through protein kinase G (PKG) activation.[28] NO produced by inducible NOS (iNOS) causes neurotoxicity forming reactive nitrogen species. Elevated iNOS level and decreased nNOS level in aged rats also show similar dual character as observed in human AD.[29] Thus, it appears that normal and sufficient bioavailability of NO is essential for inhibiting the risk of AD.

Here, we report that NO can bind to heme and Cu-bound Aβ complexes. The reduction potentials of the heme and Cu sites in the Aβ bound complexes are −0.17 V and 0.28−0.26 V versus NHE, respectively. Thus, reducing agents like vitamin C (E^o = −0.066 V vs NHE)[30] can selectively reduce the Cu site of the Cu(II)−Fe(III)heme−Aβ complex.[11,16] Interestingly, in a physiologically relevant mixed valent Cu(I)−Fe(III)heme−Aβ complex, NO can bind to the heme center and drive the Tyr10 residue-mediated electron transfer from the Cu to the heme center. This results in the formation of an oxidized Cu(II) site and Fe(II)(heme)−Aβ−NO complex. The oxidized Cu site generated in the process is less toxic, since it is less prone toward generation of oxidative stress. The resultant Fe(II)-(heme)−NO complex dissociates from the Aβ peptide. Thus, NO also provides a mechanism for removal of heme from the Aβ peptides.

2. EXPERIMENTAL METHODS

All reagents were of the highest grade commercially available and were used without further purification. Amyloid beta peptide, Aβ(1−16) (Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys) and its Try10Gly mutant were purchased from GL Biochem Ltd. (Shanghai) with >95% purity. Hemin, copper sulfate, and HEPES buffer were purchased from Sigma. Sodium nitrite, sodium dithionite, ascorbic acid, and sulfuric acid were purchased from Merck. D_2O was purchased from Cambridge Isotope Laboratory.

Aβ stock solution of 0.5 mM strength was prepared by dissolving in pH 7 Hepes buffer. A 5 mM hemin solution was prepared in 1 M NaOH solution. A copper sulfate solution was made of 10 mM strength in nanopure water. Ascorbic acid and sodium dithionite solutions of 20 mM strength were made by dissolving them in degassed buffer under anaerobic condition. The Fe(III)heme−Aβ complex was prepared by incubation of 1 equivalent of Aβ with 0.8 equivalent of heme for ∼6 h, and the Cu(II)−Aβ complex was prepared by incubating 0.8 equivalent of $CuSO_4$ with 1 equivalent of Aβ for ∼1 h. For the Cu(II)−Fe(III)heme−Aβ samples, 0.8 equivalent of heme was incubated with 1 equivalent of Aβ for ∼6 h followed by incubation with 0.8 equivalent of $CuSO_4$ for ∼1 h. The control sample was prepared by adding heme to the Cu(II)−Aβ complex (heme added to Cu−Aβ and Cu added to Fe(III)heme−Aβ produces the same Cu(II)−Fe(III)heme−Aβ complex), allowing no incubation time for the heme cofactor to bind with Aβ. All the EPR samples were either prepared at or calibrated to pH 7 by addition of H_2SO_4. To obtain Cu(I)Fe(III)heme−Aβ, the oxidized Cu(II)Fe(III)heme−Aβ was reduced with ascorbic acid under anaerobic conditions at pH 7 in a glovebox. Reducing the Cu(II) center in Cu(II)−Fe(III)heme−Aβ does not affect the Fe(III)heme center as evident from the absorption and EPR data (Figure S1 of the Supporting Information). Scrubbed NO gas (generated by adding saturated $NaNO_2$ solution to 20 M H_2SO_4 and purified by passing the generated gas through thoroughly degassed 4 M KOH and water), saturated buffer solutions (1.94 mM)[31] were prepared by purging NO through an anaerobic buffer for about 10 min.

For NO complexes, one equivalent NO-saturated buffer solution (strength 1.94 mM) was added to Cu(I)Fe(III)heme−Aβ (100 μL, 0.5 mM) in an EPR tube, in the glovebox. For kinetics study, this sample was taken out of the glovebox within 1 min and frozen in liquid nitrogen to obtain the initial spectrum. For subsequent data points, the same sample was thawed in the glovebox and incubated; following which, it was frozen again in liquid nitrogen. A similar protocol was followed to prepare the deuterated samples. One equivalent of the NO-saturated solution was added to the Cu(I)Fe(III)heme−Aβ complex in a cuvette under anaerobic conditions to obtain the absorption data at various reaction times.

EPR samples were 0.5 mM in concentration and 100 μL in volume and were run at 77 K in a liquid nitrogen finger dewar. EPR spectra were obtained by a Jeol (JES FA200) spectrophotometer with the following parameters for the high-energy region: modulation width, 20 gauss; amplitude, 50; time const, 300 ms; power, 10 mW; frequency, 9.27 GHz. The parameters for the low-energy region included modulation width, 20 gauss; amplitude, 50; time const, 30 msec; power, 1 mW; frequency, 9.27 GHz. Absorption spectral data were obtained by a UV−vis diode array spectrophotometer (Agilent 8453). Resonance Raman spectroscopy 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).

3. RESULTS AND ANALYSIS

3.1. Absorption Spectroscopy. A Cu(II)−Fe(III)heme−Aβ complex of Aβ(1−16) was prepared by incubating 0.8 equivalent of Cu(II) with an equivalent of a Fe(III)heme−Aβ peptide complex. When the mixed valent Cu(I)−Fe(III)heme−Aβ complex was exposed to one equivalent of NO, it generated an absorption spectrum with the Soret band at ∼384 nm, within a couple of seconds (Figure 1, light green). Gradually,

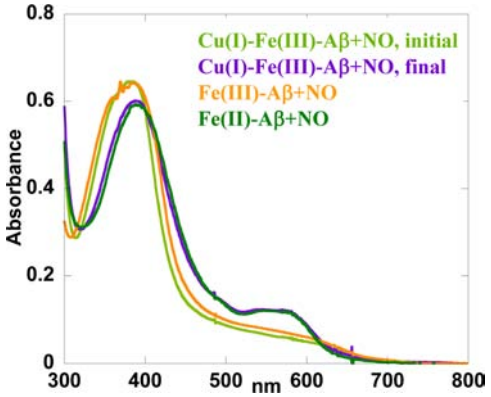

Figure 1. Absorption spectra of a Cu(I)−Fe(III)heme−Aβ complex with one equivalent NO after 10 s (light green), 60 s (purple) overlaid with Fe(III)heme−NO (orange), and Fe(II)heme−NO (dark green).

with time, the Soret band red-shifted to ∼394 nm and bands at 555 and 585 nm appeared in the Q-band region of the spectrum (Figure 1, purple). When one equivalent of NO was added to the Fe(III)(heme)−Aβ complex (no Cu), it formed a species having a Soret band at ∼384 nm (Figure 1, orange), similar to the spectrum generated on instantaneous NO exposure to the Cu(I)−Fe(III)heme−Aβ complex (Figure 1, light green). The absorption spectrum on addition of NO to the reduced Fe(II)heme−Aβ complex (no Cu), generated a spectrum having a Soret band at 394 nm and Q bands at 555 and 585 nm (Figure 1, dark green), very similar to that produced on prolonged NO exposure to the mixed valent Cu(I)−Fe(III)(heme)−Aβ complex (Figure 1, purple). Thus, the species formed on instantaneous exposure of NO to

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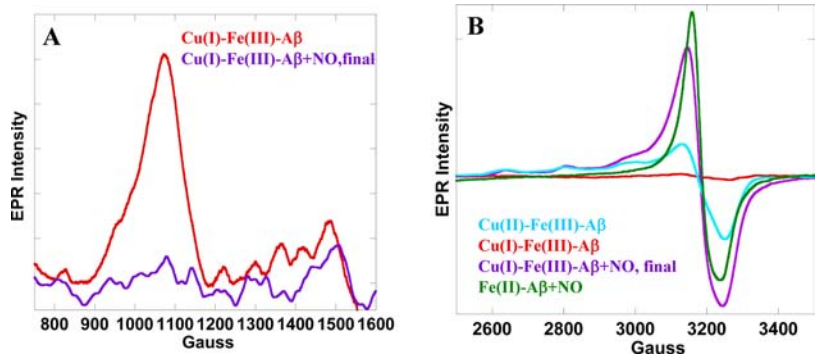

Figure 2. EPR spectra of Cu(I)−Fe(III)heme−Aβ (red), Cu(I)−Fe(III)heme−Aβ + NO (purple), Cu(II)−Fe(III)heme−Aβ (cyan), and Fe(II)heme−Aβ + NO (green) in the (A) low-field and (B) high-field regions.

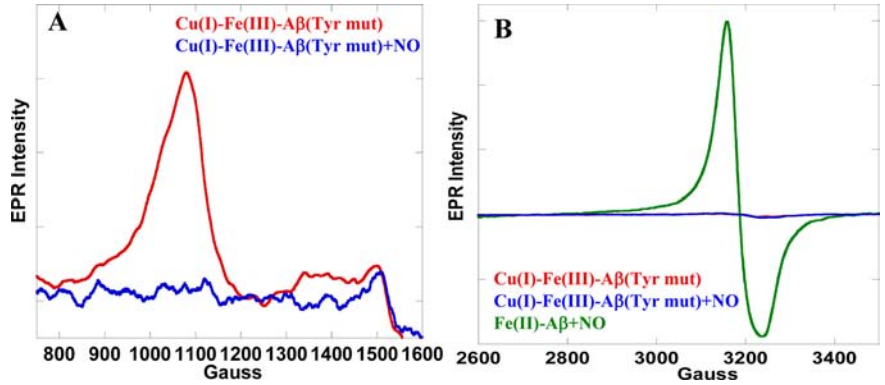

Figure 3. EPR spectra of Cu(I)−Fe(III)heme−Aβ of Tyr10 mutant at pH 7 (red), Cu(I)−Fe(III)heme−Aβ of Tyr10 mutant + NO, final (blue), and Fe(II)heme−Aβ of wild type + NO, final (green), in the (A) low-field and (B) high-field regions. The red spectrum is obscured by the blue spectrum in Figure 3 B.

Cu(I)−Fe(III)heme−Aβ is likely a Fe(III)heme−NO species, which then converts to a Fe(II)heme−NO species.

3.2. EPR Spectroscopy. The reaction of NO with the mixed valent Cu(I)−Fe(III)heme−Aβ complex was monitored by EPR spectroscopy as well. The starting Fe(III) center showed a high-spin S = 5/2 axial signal in the EPR spectrum, while the reduced Cu center was EPR silent (Figure 2, red). With the exposure of this to one equivalent of NO, the high-spin axial EPR signal diminished with the concomitant increase in the EPR signal intensity in the high-field, low-spin region of the spectrum (Figure 2, purple). An intense signal observed in the high-field g = 2 region of the EPR spectrum indicated formation of an S = 1/2 species (Figure 2B, purple). This signal is similar to that of the one electron-reduced Fe(II)heme−Aβ−NO complex (Figure 2B, green). Additionally, the presence of hyperfine features of an oxidized Cu species was observed with $A_{II} \sim 170 \times 10^{-4}$ cm^{-1}, $g_{II} \sim 2.239$, and $g_{\perp} \sim 2.046$ (Figure 2B, purple). These parameters are identical to the EPR parameters of the Cu(II) site of the Cu(II)−Fe(III)heme−Aβ complex (Figure 2B, cyan), indicating that the Cu(I) site of the Cu(I)−Fe(III)heme−Aβ complex gets oxidized by one electron to Cu(II) upon NO exposure as well.

Reaction of NO with Cu(I)−Fe(III)heme−Aβ complex of a Tyr10Gly mutant was evaluated. Interestingly, for this mutant complex, the high-spin S = 5/2 Fe(III)heme signal decreased upon addition of NO, implicating the formation of the same Fe(III)heme−NO species (Figure 3 A and Figure S2 of the Supporting Information) as the wild-type peptide. However, no low-spin signal appeared (Figure 3 B).

3.3. Resonance Raman Spectroscopy. Resonance Raman spectroscopy is a powerful technique that has been used extensively to determine the coordination number, spin state, and oxidation state of heme Fe.[32] Excitation into the Soret band of heme results in an enhancement of vibrations associated with heme, and the high-frequency region of the spectrum contains characteristic marker bands that are sensitive to the oxidation and spin state and coordination of heme Fe.[32−34] The oxidation state of the heme can be determined from the π-electron density marker, the ν_4 band, while the spin and coordination state can be determined from the markers, ν_3, ν_2, and ν_{10} bands, which are sensitive to the core size of the heme macrocycle.[35−37] The ν_4, ν_3, and ν_2 and ν_{10} bands for the prolonged NO exposed Cu(I)−Fe(III)(heme)−Aβ complex were observed at 1377, 1511, 1595, and 1652 cm^{-1}, respectively, in the resonance Raman spectrum (Figure 4, purple) of the complex. The Fe(II)heme−Aβ−NO (no Cu) complex of the wild type also has vibrations identical to that of the mixed valent NO complex (Figure 4, dark green). The resonance Raman spectrum of the Fe(II)−NO complex of free heme (no Aβ, Figure 4, black) shows the vibrations at energies identical to those of Cu(I)−Fe(III)heme−Aβ−NO and Fe(II)heme−Aβ−NO complexes. Since the Fe(II)−NO complex of free heme is five coordinate in nature, the data demonstrate that the above two complexes are also five-coordinate species. Note that the above experimentally obtained frequencies are also typical of a five-coordinate Fe(II)−NO species,[38,39] further implying that the nitrosyl complexes formed are five coordinate in nature. The N−O

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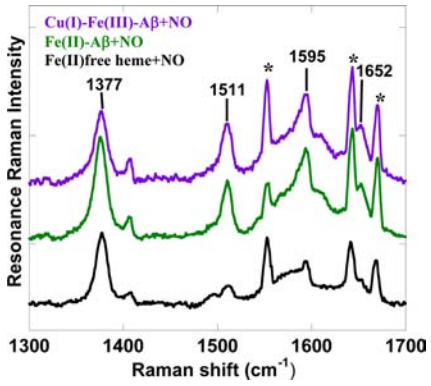

Figure 4. High frequency region of resonance Raman spectra of Cu(I)−Fe(III)heme−Aβ + NO (purple), Fe(II)heme−Aβ + NO (dark green), and Fe(II)free heme + NO (black). The data were obtained with an excitation wavelength of 413.1 nm (15 mW) at 77 K. (* indicates plasma lines from the laser).

stretch could not be identified because of overlapping heme ring modes.

3.4. Kinetics. The kinetics of the NO reaction of the Cu(I)−Fe(III)heme−Aβ complex of the wild-type Aβ peptide was followed using EPR spectroscopy. The data show that the rate of decrease of the high-spin Fe(III) signal is ∼3.5 times faster than the rate of formation of the Fe(II)heme−NO signal and the rate of oxidation of the Cu site, the later two processes having the same rates (Figure 5). This implies that the binding

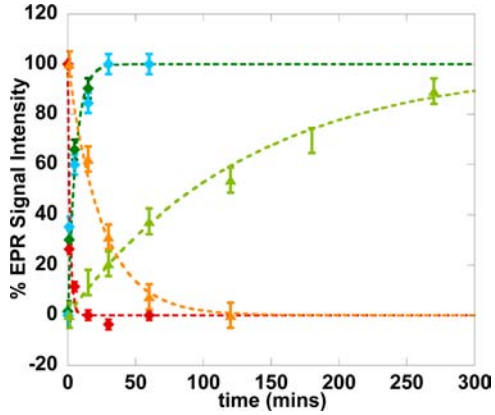

Figure 5. Kinetic traces of loss of high-spin Fe(III) EPR signal intensity in aqueous (red) and deuterated buffer (orange), increase in intensity of Cu hyperfine features in aqueous buffer (cyan), and increase in intensity of Fe(II)heme-NO signal intensity in aqueous (dark green) and deuterated buffer (light green), along with simulations of the rates in dotted lines.

of NO to the Fe(III)heme site forming an EPR silent Fe(III)heme−NO species (Figure S2 of the Supporting Information) is relatively fast. The rate of electron transfer from the reduced Cu site to the Fe(III)heme−NO site, forming the oxidized Cu site and the Fe(II)heme−NO species, is the rate determining step (rds).

The kinetics followed in deuterated buffer showed significant decrease in the rate of NO binding to the Fe(III)heme site, as well as the rate of electron transfer (Figure 5). The primary isotope effect for the first step (i.e., NO binding to the heme site) is ∼18 ± 5 and is derived from a proton-coupled displacement of hydroxide by NO.[40] The primary isotope effect of the second step involving electron transfer from the Cu to the ferric heme nitrosyl species is ∼25 ± 5.

4. DISCUSSION

The reduction potentials of the Cu and heme sites of Cu(II)−Fe(III)heme−Aβ complexes are 0.28−0.26 V and −0.17 V versus NHE,[11,16] respectively. Thus, the Cu site can selectively be reduced by abundant physiological reducing agents like vitamin C (E^o = −0.066 V vs NHE),[30] forming Cu(I)−Fe(III)heme−Aβ, the physiologically relevant form. Aβ is known to bind reduced Cu[41−43] and oxidized heme.[9−12] EPR and absorption data indicate that the Cu and heme sites of Cu(II)Fe(III)heme−Aβ and Cu(I)Fe(III)heme−Aβ complexes remain unperturbed in the presence of each other (Figures S1 and S3 of the Supporting Information).[16]

From the absorption data, we deduce that NO binds to the Fe(III)heme site of Cu(I)−Fe(III)heme−Aβ forming a Fe(III)heme−NO species (Figure 1, light green). This is then reduced to form a Fe(II)heme−NO complex (Figure 1, purple) upon prolonged exposure.[44] The EPR results indicate simultaneous formation of a Cu(II) site, identical to that of Cu(II)−Aβ and a Fe(II)heme−NO species on NO exposure to the Cu(I)−Fe(III)heme−Aβ complex (Figure 2B). The resonance Raman data confirm that the final product of the NO reaction with the mixed valent Cu(I)−Fe(III)heme−Aβ complex (Figure 4, purple) is a ferrous nitrosyl species, which is five coordinate in nature (Figure 4, black).[38,39] This implies that reduced Cu-bound Aβ directly transfers an electron to the Fe(III)heme−NO species, reducing it to the Fe(II)heme−NO form. The heme then dissociates from the Aβ peptide on NO binding to generate the final five coordinate Fe(II)heme−NO species. Note that the interaction of NO with Cu(I) is known to produce the Cu(I)−nitrosyl species.[45] However, the binding constant of NO with Cu(I) is much less than heme.[46] Since one equivalent of NO is added in this study, the Fe(III)heme−NO adduct formation will be thermodynamically more favorable. However, the possibility of formation of an intermediate Cu(I)−NO adduct, prior to the formation of Fe(III)heme−NO, cannot be eliminated.

A control sample was prepared where heme was added to the Cu-bound Aβ peptide, but no incubation time was allowed for heme binding. This complex thus has Cu bound to Aβ, but not heme (i.e., the heme is free in solution). Such a variation was chosen to evaluate the possibility of an intermolecular oxidation of the Cu site by a five-coordinate Fe(III)heme−NO species. When one equivalent of NO was added to the Fe(III)free heme−Cu(I)−Aβ peptide complex, it generated an absorption spectrum corresponding to a Fe(III)free heme−NO complex (Figure 6A). However, no subsequent formation of a Fe(II)heme−NO signal was observed. The same reaction was also monitored by EPR spectroscopy. Similar to the Cu(I)−Fe(III)heme−Aβ complex, the high-spin S = 5/2 axial EPR signal of Fe(III) was decreased upon NO addition (Figure 6B), however, with no development of any low-spin signal (Figure 6C). No Cu(II) hyperfine features were observed either. Thus, in the Fe(III)free heme−Cu(I)−Aβ complex, where free heme can potentially bind the NO, no electron transfer from the Cu(I) to the Fe(III)free heme−NO complex occurred. Thus, Fe(III)heme−NO needs to be bound to the Aβ peptide for electron transfer from the Cu site to the ferric nitrosyl site to occur (i.e., the electron transfer is intra molecular). Further, this also implies that formation of a five-coordinate Fe(II)heme−NO species, due to the heme dissociation from the Aβ peptide,

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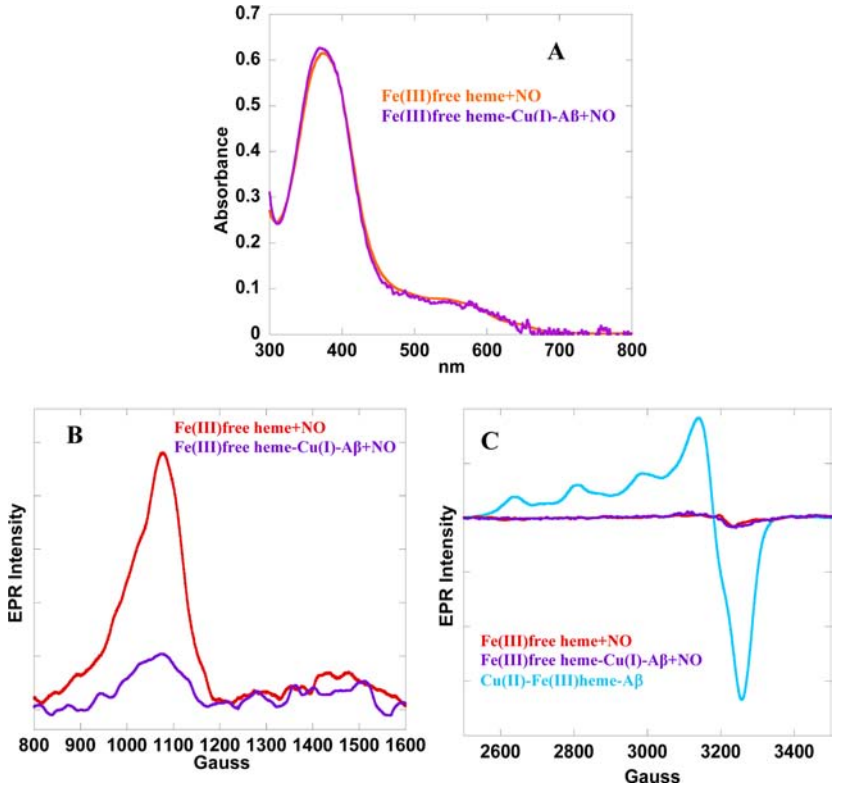


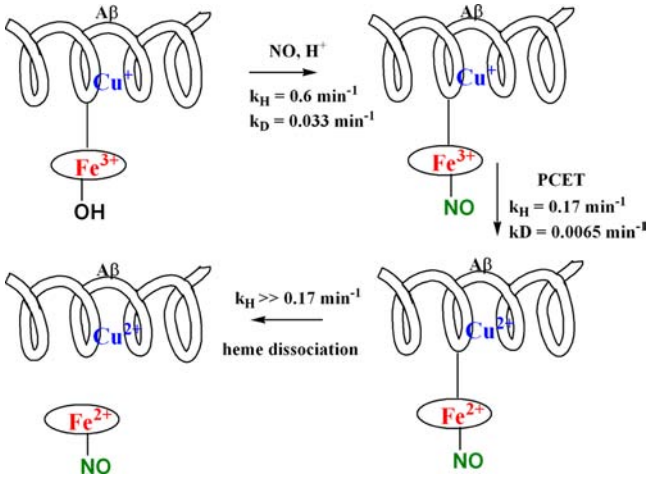
Figure 6. Absorption spectra of Fe(III)free heme + NO (orange) and Fe(III)free heme−Cu(I)−Aβ + NO (purple). (A) EPR spectra of Fe(III)free heme−Cu(I)−Aβ (red), Fe(III)free heme−Cu(I)−Aβ + NO, final (purple), and Cu(II)−Fe(III)heme−Aβ−NO (cyan) in (B) low-field and (C) high-field regions.

occurs only after the electron-transfer step, since no electron transfer is observed in this control sample containing Fe(III)free heme−Cu(I)−Aβ, where free heme (analogous to dissociated heme from Aβ) is five coordinate in nature. Thus, the six-coordinate Fe(III)heme−NO acts as an oxidizing agent for Cu.

Tyr10 is one of the amino acid residues absent in rodents which are not affected by AD.[15] The reaction of NO with the Cu(I)−Fe(III)heme−Aβ complex of the Tyr10Gly mutant indicated formation of a Fe(III)heme−NO complex; however, there was no subsequent formation of the reduced Fe(II)-heme−NO complex, nor did the Cu site get oxidized (Figure 3). It has been shown that Tyr10 does not bind either heme or Cu.[12,47,48] Thus, Tyr10 likely mediates the electron transfer from the Cu to the NO-bound Fe(III)heme site. Tyrosine is well-known to mediate long-range electron transfer in biology.[49,50] Note that given the close proximity of His13 (proposed to be involved in heme binding) and His14 (proposed to coordinate to Cu), and the fact that histidine-mediated electron-transfer pathways are well-known in metal active sites,[51,52] a His−His electron-transfer pathway between the Cu and heme sites may be invoked. However, current results, which show that a Tyr10 mutant abolishes electron transfer from the Cu to the heme site, and past studies (which show two electron oxidations of Cu(I) and Tyr10 or Fe(II)heme and Tyr10 by molecular O_2)[16] are more consistent with a Tyr10-mediated electron-transfer pathway.

The reaction of NO with the Cu(I)−Fe(III)heme complex of the wild-type Aβ peptide, as determined from spectroscopy and kinetics, is summarized in Scheme 1. NO binds to the Fe(III) center of the heme−Aβ peptide (Aβ−Fe(III)heme−OH + NO + H^+ → Aβ−Fe(III)heme−NO + H_2O) with a

Scheme 1. Schematic Representation of Reaction of Cu(I)−Fe(III)heme−Aβ with NO[a]



[a]NO binds to Fe(III)heme−Aβ in the first step. In the second step, an electron is transferred from the Cu(I) to the Fe(III)heme−NO species. This is the rds and involves Tyr10. The next step is fast and involves dissociation of Fe(II)−NO from the Aβ peptide. The ligands of the heme and Cu sites have not been included.

primary isotope effect of ∼18 ± 5. Subsequently, the reduced Cu(I) site acts as a reducing agent, reducing the Aβ-bound ferric−NO species to the Aβ-bound ferrous−NO form. The rate of electron transfer from the reduced Cu site to the Fe(III)heme−NO site, forming the oxidized Cu site and the Fe(II)heme−NO species, is the rds. This electron transfer is mediated by the Tyr10 residue. This step has a KIE of ∼25 ± 5,

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which could be derived from a proton-coupled electron transfer (PCET) step involved in the oxidation of the Cu site (i.e., $Cu^{+}(H_2O) \rightarrow Cu^{2+}(OH^{-}) + H^{+} + e^{-}$.[53] PCET processes are known to have large deuterium kinetic isotope effects.[50,54,55] Since the absence of the tyrosine residue abolishes the electron transfer from the Cu to the heme site, we propose that it is involved in the electron-transfer pathway. In spite of trapping the Aβ-bound Fe(III)heme−NO species in EPR, its resonance Raman data could not be obtained, due to its well established photolability. The Fe(II)heme−NO complex formed then dissociates from the Aβ peptide, forming a five-coordinate species. Note that no six-coordinate Fe(II)heme−NO species was observed in the time-dependent EPR experiments, suggesting that the rate of dissociation of the Fe(II)heme−NO species from the Aβ peptide (final product is a five-coordinate Fe(II)heme−NO species) is possibly much faster than the rate of electron transfer from Cu to the heme site. The dissociation of the Fe(heme)−His(peptide backbone) bond on NO binding is well-known in several enzyme active sites.[56−58] 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.

5. CONCLUSION

Heme and Cu-binding to Aβ peptides has been invoked to have detrimental effects in AD.[16,17] Heme deficiency leads to abnormal iron homeostasis, increase in bilirubin and heme oxygenase concentration, decay of iron regulatory proteins, dysfunction in mitochondrial complex IV, and oxidative stress.[10,13,14] Cu-bound Aβ peptides will have Cu in the reduced form under physiological conditions. Cu(I) is prone to produce toxic PROS in the body, generating oxidative stress.[7] When heme and Cu are simultaneously bound to Aβ peptides, maximum PROS are produced.[16] NO binds the heme site of the physiologically relevant Cu(I)−Fe(III)heme−Aβ peptide complex. The two otherwise electronically uncoupled paramagnetic centers undergo electron transfer upon NO binding. This electron transfer from the Cu to the heme site is mediated by a tyrosine residue. In the process, Cu(II) gets generated from Cu(I), which is much less toxic for AD relative to the reduced form and will not produce oxidative stress in the body. Moreover, once the ferric(heme) nitrosyl species gets reduced by the Cu site, the ferrous nitrosyl species formed dissociates from the Aβ peptide. Thus, NO helps in releasing heme from the Aβ peptides and can ameliorate 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. In fact, NO has also been proposed to be involved in defending the heme−Cu active site of cytochrome *c* oxidase, the terminal enzyme in the respiratory chain, against antagonists like cyanide and carbon monoxide.[59,60] Interestingly, the physiological NO levels are decreased in patients affected with AD, which might imply that NO cannot implement its protective role in the body against heme and Cu. Maintaining an optimal NO level in the body might promote a significant reduction of risks associated with AD. Thus, not surprisingly, NO-releasing agents are already in clinical trials for AD.[28]

■ ASSOCIATED CONTENT

Supporting Information

Absorption and EPR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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