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Active Site Environment of Heme-Bound Amyloid β Peptide Associated with Alzheimer's Disease

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Abstract: Recent reports show that there is a large increase in heme in the temporal brain of Alzheimer's disease (AD) patients, as heme, biosynthesized in brain cells, binds to amyloid β (Aβ), forming heme−Aβ complexes. This leads to the development of symptoms that are characteristic pathological features of AD, e.g., abnormal iron homeostasis, decay of iron regulatory proteins, dysfunction in mitochondrial complex IV, oxidative stress, etc. However, the active site resulting from heme binding to Aβ is not well characterized. For example, the coordinating residue, relevant second-sphere residues, and spin state of the Fe center are not known. In this study we have used wild-type and mutated Aβ peptides and investigated their interaction with naturally occurring heme. Our results show that, out of several possible binding sites, His^{13} and His^{14} residues can both bind heme under physiological conditions, resulting in an axial high-spin active site with a trans axial water-derived ligand. Peroxidase assays of these heme−peptide complexes along with pH perturbations indicate that Arg^5 is a key second-sphere residue that H-bonds to the trans axial ligand and is responsible for the peroxidase activity of the heme−Aβ complexes. The His^{13} and Arg^5 residues identified in this study are both absent in rodents, which do not show AD, implicating the significance of these residues as well as heme in the pathology of AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder causing senile dementia with no definite known cause.[1] With the increase of general life expectancy, AD has become a severe problem for the elderly. The major symptoms of AD are long- and short-term memory loss, confusion, mood swing, and decline of sense and thinking ability.[2−4] These symptoms generally appear at a later stage; thus, diagnosis in the early stage is difficult. AD is characterized by loss of neurons and breakdown of synaptic function in the hippocampus cortex, and this part of the neuronal cortex plays an important role in the formation of new memories.[1]

The two markers of AD are the aggregation of amyloid β (Aβ) peptides[1,5,6] and the generation of reactive oxygen species.[7−9] Aβ is derived by proteolysis of membrane-bound amyloid precursor protein. The long hydrophobic tail of Aβ (Gly29−Val40 or Gly29−Ala42) increases its hydrophobicity and reduces its solubility. Both Aβ(1−40) and Aβ(1−42) are neurotoxic in aggregated and soluble oligomeric forms which induce oxidative stress by the spontaneous generation of partially reduced oxygen species (PROS), e.g., H_2O_2 and other free radicals.[10,11] During this process of PROS generation, Aβ gets oxidized, forming proteolysis-resistant soluble dimers cross-linked via tyrosine or histidine residues of the Aβ peptides.[12] The H_2O_2 generated is freely permeable across cell membranes and in the presence of reduced transition-metal ions (Fe^{2+}, Cu^{+}) can generate neurotoxic hydroxyl radicals.[13] In fact, brains of patients with AD contain higher levels of Fe, Cu, and Zn.[14] It has been suggested from chemical analysis of several brain samples of AD patients that the generation of H_2O_2 through the reduction of O_2 by transition metals (e.g., Cu and Fe) precedes the formation of the amyloid aggregates and hence could be responsible for the early oxidative damages observed in AD.[15,16] This is further supported by recent developments where Cu is shown to bind Aβ peptides and form well-defined active sites.[17−19] The role and mechanism of action of the

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transition-metal-catalyzed chemistry associated with AD is thus being actively investigated.

Recent studies show that AD patients exhibit a remarkable 250% increase in heme *b* in their temporal brain.[20] It has been proposed that such abnormal heme concentration in the brain of AD patients is because heme, biosynthesized in brain cells, binds to Aβ, forming heme−Aβ complexes. This complexation depletes heme, required for biological processes, leading to heme deficiency in cells.[21] This ultimately results in symptoms such as increases in iron uptake, bilirubin, and heme oxygenase concentration,[22,23] abnormal iron homeostasis, decay of iron regulatory proteins, dysfunction in mitochondrial complex IV, oxidative stress, etc.[20,24] These symptoms are all characteristic pathological features of AD. Further it has been shown that the human Aβ(1−42)−heme complex shows peroxidase activity and can catalyze the oxidation of neurotransmitters such as serotonin, 3,4-dihydroxyphenylalanine, and 4-hydroxyphenylpyruvic acid by H_2O_2. This could be the probable reason for the abnormal neurotransmission observed in AD patients.[25,26]

The involvement of heme in AD pathology opens up a new dimension in AD research and may hold a key towards development of a cure. However, so far no information is available about the nature and properties of these heme−Aβ active sites. In this study we use absorption and electron paramagnetic resonance (EPR) spectroscopic techniques to identify the amino acid residues of the human Aβ peptide that bind to heme, using native human Aβ(1−40) peptide, isolated peptide fragments Aβ(1−16), Aβ(17−40), and Aβ(10−20), and various site-directed mutants. Peroxidase activity assays provide insight into the nature of the active site environment of these peptide−heme complexes, including the key second-sphere residues involved. We evaluate the effect of pH on these complexes to elucidate the nature of the exchangeable sixth ligand of the heme-bound Aβ peptide complexes.

Experimental Methods

Materials. All reagents were of the highest grade commercially available and were used without further purification. Aβ peptides of different chain lengths (1−40, 1−16, 17−40, and 10−20) have been used for this study. All site-directed mutants are of the Aβ(1−16) peptide. The mutants used were His^6Gly, His^{13}Ala, His^{14}Ala, Tyr^{10}Gly, Arg^5Asn, double mutant His^{13}Gly, His^{14}Gly, and triple mutant His^6Gly, His^{13}Gly, His^{14}Gly. All peptides were purchased from Syn Bio Science Corp and GL Biochem (Shanghai) Ltd. with >95% purity. Hemin and the buffers were purchased from Sigma. Different buffers were used at different pH ranges: MES for pH 5−6.4, HEPES and phosphate for pH 7−8, and glycine for pH 9−10. All the peptide stock solutions were made in 100 mM phosphate buffer. Aβ(1−40), Aβ(17−40), and Aβ(10−20) peptide stock was made in 20 mM Hepes buffer, while hemin solution was made in 1 M NaOH solution. Peptide stock solutions were 0.5 mM, and the heme stock solution was 5 mM. Heme−Aβ complexes were prepared by incubating 1 equiv of both Aβ and heme solutions for ∼1 h. Absorption spectra were recorded by adding 20 μL of the heme−Aβ complex solution in 1 mL of buffer. All the spectral data were obtained by a UV−vis diode array spectrophotometer (Agilent 8453).

EPR spectra were obtained by a Jeol (JES FA200) spectrophotometer. EPR samples were 0.5 mM in concentration and were run at 77 K in a liquid nitrogen finger dewar. Low-pH EPR samples were prepared by lowering the pH of a high-pH sample with 0.5 M H_2SO_4.

Peroxidase Activity Measurement. 3,3′,5,5′-Tetramethylbenzidine (TMB) was used as the substrate for peroxidase activity measurement. A 10 mg portion of TMB was dissolved in 0.5 mL of glacial AcOH and 10 mL of AcOH/NaOAc buffer (1 M, pH 4.5). The solution was diluted to 25 mL with water. This was followed by addition of 100 μL of 30 volume H_2O_2. A 10 μL volume of 0.05 mM protein sample was added to the above solution. Kinetic traces were obtained by monitoring the increase of the 652 nm absorption band with time.[27]

Catalase Activity Measurement. Catalase activity was measured by following the rate of decay of H_2O_2 at 240 nm. A 1.5 mL volume of deionized water and 100 μL of 0.05 M H_2O_2 were mixed in a cuvette. A 20 μL volume of 0.5 mM protein was added to it. All spectra were recorded in the kinetic mode, with continuous stirring at 25 °C.[28]

Results and Analysis

Choice of Peptides. Human Aβ(1−40) was the chosen parent peptide for this study as it is the most common Aβ peptide found physiologically. Two fragments, the hydrophilic Aβ(1−16) and the lipophilic Aβ(17−40), have been separately considered to narrow the heme binding region (Scheme 1A). There are four residues in Aβ(1−40) that are known to bind heme biologically. They are the three histidine residues (His binds heme in hemoglobin, myoglobin, cytochrome *c* oxidase, etc.) His^6, His^{13}, and His^{14} and a tyrosine residue (Tyr binds heme in catalase), Tyr^{10}, which are all present in the Aβ(1−16) fragment (Scheme 1C). To identify the binding residue, site-directed mutants of Aβ(1−16) have been selected (Scheme 1D). Single mutants His^6Gly (i.e., Tyr^{10}, His^{13}, and His^{14} are the available coordinating residues), His^{13}Ala (i.e., His^6, Tyr^{10}, and His^{14} are the available coordinating residues), His^{14}Ala (i.e., His^6, Tyr^{10}, and His^{13} are the available coordinating residues),[27] and Tyr^{10}Gly (i.e., His^6, His^{13}, and His^{14} are the available coordinating residues) have been used to observe the effect of elimination of the possible binding ligands individually. Double mutant His^{13}Gly, His^{14}Gly (i.e., His^6 and Tyr^{10} are the available coordinating residues) shows the effect of His^6 and Tyr^{10} residues, and triple mutant His^6Gly, His^{13}Gly, His^{14}Gly (i.e., Tyr^{10} is the available coordinating residue) has been chosen to isolate the effect of the Tyr^{10} residue (Scheme 1D).

Absorption Spectroscopy. 1 equiv of heme covalently binds with 1 equiv of Aβ peptide.[26] The absorption spectra of 1 equiv of heme-incubated Aβ(1−40) peptide and the isolated peptide fragments Aβ(1−16) and Aβ(17−40) overlaid with heme are presented in Figure 1. The intensity decrease of the Soret band at ∼365 nm, the blue shift of the Q-band from ∼618 to ∼606 nm, and development of a new band at ∼693 nm relative to native heme indicate the formation of a heme−Aβ complex for Aβ(1−40) (Figure 1, red) and Aβ(1−16) (Figure 1, green). However, heme does not bind to Aβ(17−40) as indicated by

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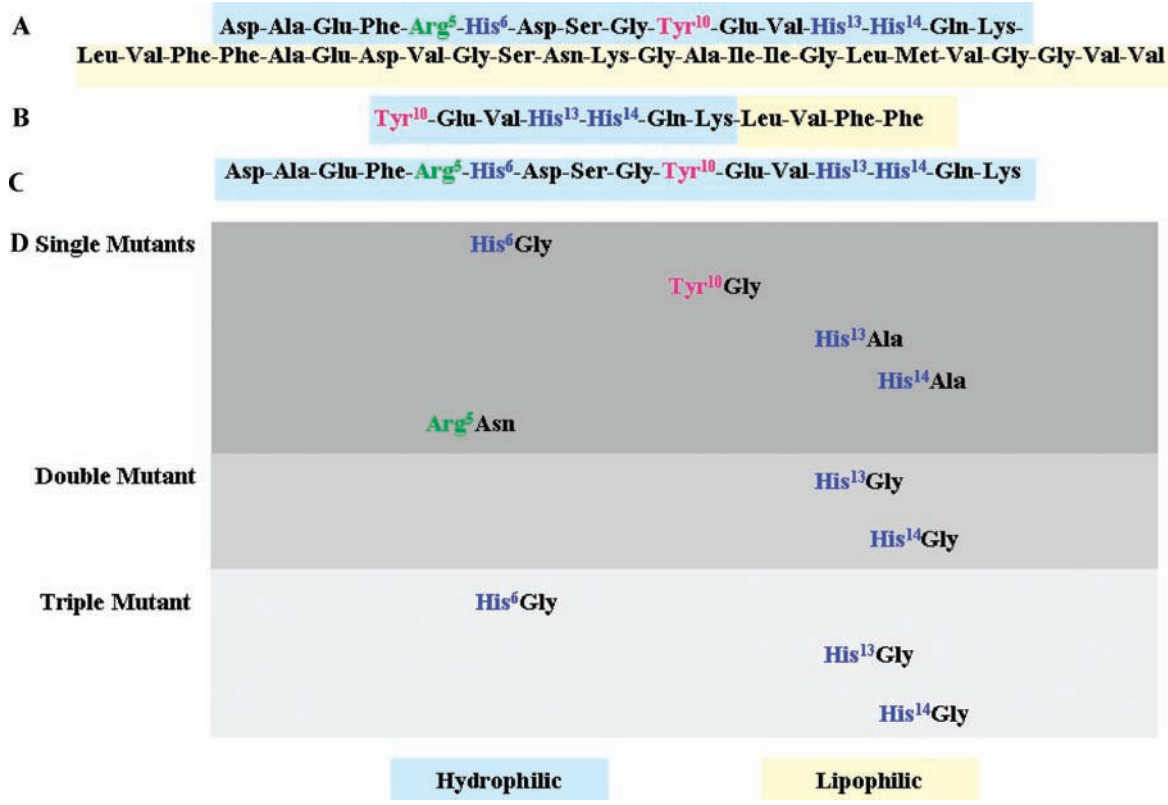
Scheme 1. Amino Acid Sequence of Human (A) Aβ(1−40) Showing the Hydrophilic Region in Blue and the Lipophilic Region in Yellow, (B) Aβ(10−20), (C) Aβ(1−16) Highlighting the Residues That Have Been Mutated in This Study, and (D) Mutants of the Aβ(1−16) Peptide



the absence of spectral changes relative to heme (Figure 1, blue) even after prolonged incubation. This is consistent with the absence of a heme-binding residue in this lipophilic peptide sequence. The similar nature of the spectra for both Aβ(1−40) and Aβ(1−16) indicates that the heme-binding residue lies within the 1−16 peptide sequence. Aβ(10−20) (Scheme 1B) binds heme and results in a spectrum similar to that of the Aβ(1−40) and Aβ(1−16) peptides (Figure 1, yellow). This indicates that the coordinating residue lies between Tyr^{10} and Lys^{16}.

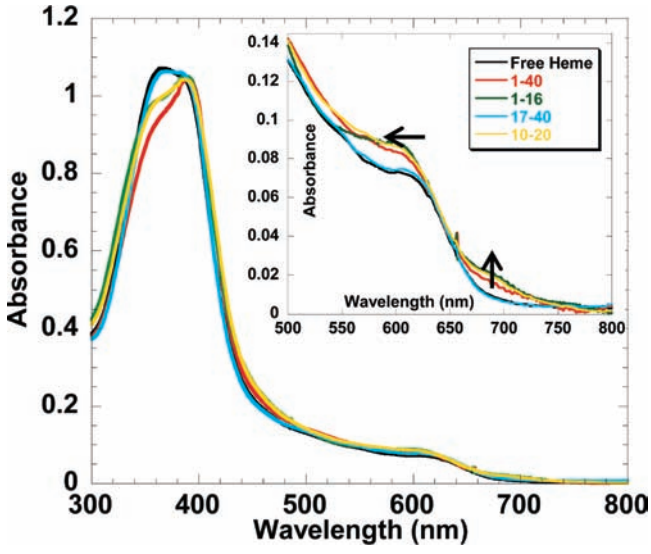


Figure 1. Absorption spectra of heme-incubated Aβ peptides at pH 7: (1−40), red; (1−16), green; (17−40), blue; (10−20), yellow; free heme, black. Inset: enlarged Q-band region showing changes as indicated by arrows relative to heme.

One molecule of Aβ(1−40) and Aβ(1−16) quantitatively binds one molecule of heme. The triple mutant His^{6}Gly, His^{13}Gly, His^{14}Gly when incubated with heme shows no spectral changes relative to heme, indicating that it does not bind heme (Figure 2, green). The fact that Tyr^{10} is the only available coordinating residue in this mutant peptide clearly indicates that Tyr^{10} is not a coordinating ligand for these Aβ peptides. This has been further probed by the Tyr^{10}Gly mutant, which binds heme like native Aβ(1−40), as reflected

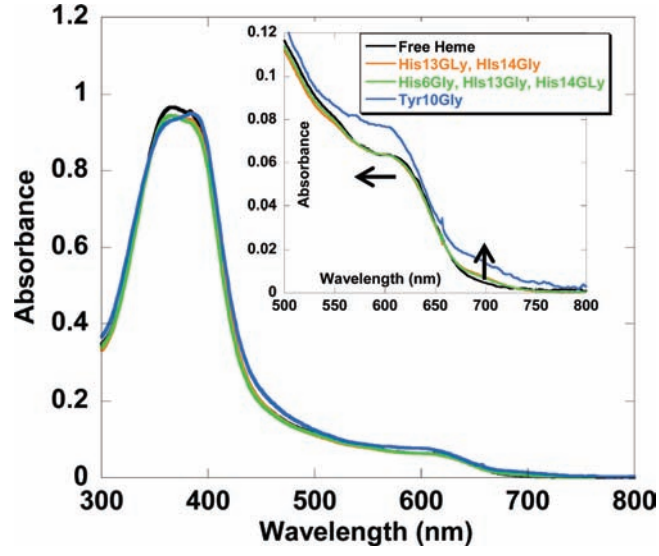


Figure 2. Absorption spectra of heme-incubated mutants of Aβ(1−16) peptide at pH 7: double mutant His^{13}Gly, His^{14}Gly, orange; triple mutant His^{6}Gly, His^{13}Gly, His^{14}Gly, green; Tyr^{10}Gly, blue; free heme, black. Inset: enlarged Q-band region showing changes as indicated by arrows relative to heme.

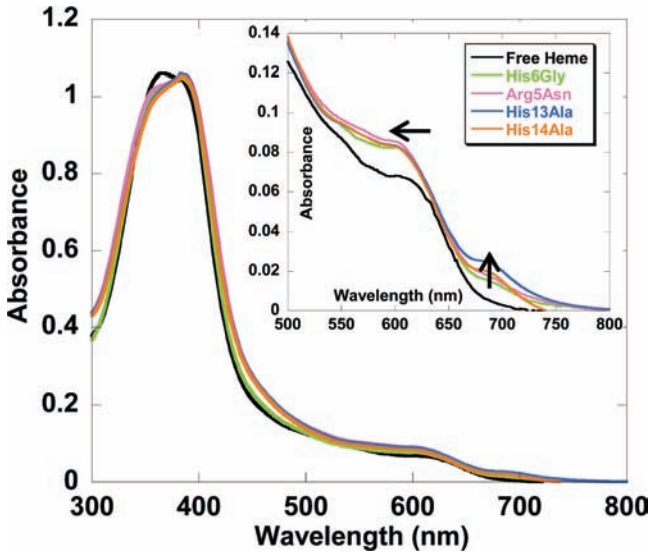


Figure 3. Absorption spectra of heme-incubated mutants of Aβ(1−16) peptide at pH 7: His^{6}Gly, green; Arg^{5}Asn, pink; His^{13}Ala, blue; His^{14}Ala, orange; free heme, black. Inset: enlarged Q-band region showing changes as indicated by arrows relative to heme.

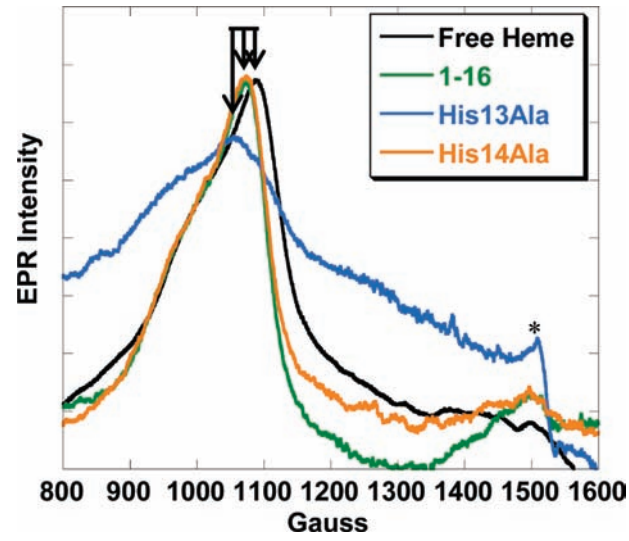


Figure 4. EPR spectra of heme−Aβ complexes at 77 K: (1−16), green; His^{13}Ala mutant of (1−16), blue; His^{14}Ala mutant of (1−16), orange; free heme, black. Arrows indicate the shift in the EPR signal. The asterisk indicates a negligible amount of rhombic high-spin signal observed at $g \approx 4.3$.

from its absorption spectrum (Figure 2, blue), confirming that Tyr^{10} is not the residue binding heme. Thus, one of the three available histidine residues (His^6, His^{13} or His^{14}) coordinates heme. The double mutant His^{13}Gly, His^{14}Gly does not bind heme (Figure 2, orange), similar to the triple mutant. Thus His^6, available for binding heme, is not the coordinating residue either. This means that either His^{13} or His^{14} is the heme-binding ligand. This has also been established by the single residue mutant His^{6}Gly (with Tyr^{10}, His^{13}, and His^{14} as available coordinating residues) (Figure 3, green), which binds heme, implying that either His^{13} or His^{14} binds heme. To distinguish between His^{13} and His^{14} residues, the single mutants His^{13}Ala and His^{14}Ala were selected. The absorption spectra of both the above heme−Aβ mutants show spectral features characteristic of heme-bound forms (Figure 3, blue and orange). Thus, either His^{13} or His^{14} is capable of binding heme. Absorption spectroscopy is not sensitive enough to distinguish between His^{13} and His^{14} coordination.

EPR Spectroscopy. EPR spectroscopy is a powerful technique that is sensitive to small changes in the electronic structure of paramagnetic species. Figure 4 shows the 77 K, X-band EPR spectra of the heme−Aβ(1−16) complex overlaid with free heme. The EPR spectrum of the heme-bound peptide is shifted to lower field relative to that of heme, indicating the formation of heme-bound species. The spectrum of the heme−peptide complex shows an axial high-spin ($S = 5/2$) Fe signal with $g \approx 6.0$. No high-field, low-spin signals were observed at this temperature. These data also show the presence of rhombic splitting in the primarily axial $g \approx 6.0$ signal.[29] Thus, the Aβ peptide binds heme through histidine ligands (either His^{13} or His^{14}) and forms an axial high-spin Fe^{III} active site with rhombic distortion like those found in hemoglobin, myoglobin, and several peroxidases.[30−32]

EPR measurements were performed on the heme−Aβ complexes of the His^{13}Ala (i.e., heme bound to His^{14}) and His^{14}Ala (i.e., heme bound to His^{13}) mutants of Aβ(1−16) to spectroscopically differentiate between the two possible histidine ligands (His^{13} and His^{14}) that have been identified as the heme-binding ligands from absorption experiments (vide supra). The EPR spectrum of the His^{14}Ala mutant of Aβ(1−16) peptide shows similar features compared to that of the Aβ(1−16) peptide (Figure 4, orange and green, respectively). Thus, the His^{13} residue binds heme in the His^{14}Ala mutant, resulting in an active site which is spectroscopically similar to that of native heme−Aβ(1−16). However, the EPR spectrum of the His^{13}Ala mutant (Figure 4, blue) is different from those of the native heme−Aβ complexes and the His^{14}Ala mutant where the spectrum is more rhombic (i.e., the g_1 and g_2 components of $g_{perp} \approx 6.0$ signal are split more) relative to that of heme−Aβ(1−16) (Figure 4, green) and less intense.[33] The difference between the EPR data of His^{13}Ala and those of His^{14}Ala and native Aβ(1−16)−heme complexes indicates that His^{14} binds heme when His^{13} is unavailable. Thus, we conclude that, though His^{13} and His^{14} can bind heme, when both residues are present as in Aβ(1−16), His^{13} probably binds heme preferentially.

Peroxidase Activity. The peroxidase (histidine-coordinated heme enzyme) activities of the native heme−Aβ complexes and their mutants and free heme were tested by following the catalytic oxidation of the substrate TMB by H_2O_2. The heme complexes of Aβ(1−40) and Aβ(1−16) show similar peroxidase activity (Figure 5, red and green, respectively), further indicating similar active site environments of the heme-bound complexes in these peptides. This peroxidase activity is similar to reported values of heme complexes of

(29) Note that due to the low resolution of the data at 77 K we cannot accurately determine the *E*/*D* parameter. However, the data show changes that can be used for diagnostic purposes.

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(33) The reduced signal intensity of the His^{13} mutant could result from its increased rhombicity or small changes in the axial ZFS tensor, **D**, which could lead to greater population of the $g_{perp} = 6$ EPR excited state.

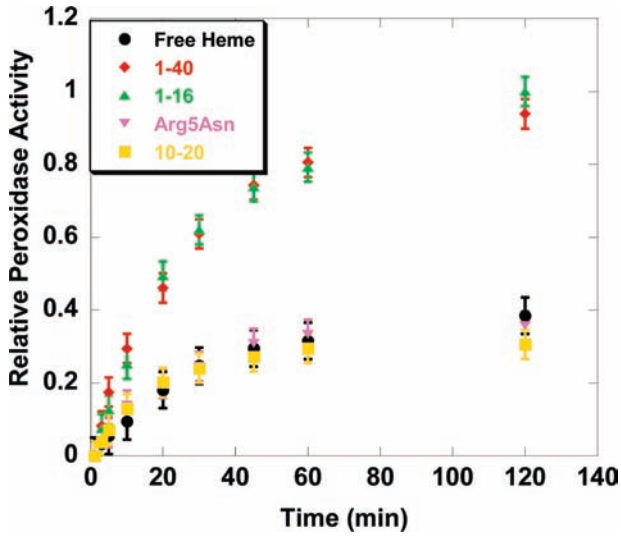

Figure 5. Kinetic traces for peroxidase activity, monitoring the increase of the 652 nm absorbance intensity, for different heme−Aβ complexes: (1−40), red; (1−16), green; (10−20), yellow; Arg^5Asn mutant of (1−16), pink; free heme, black.

Aβ(1−42) and is ∼3 times faster compared to that of free heme.[25] The triple mutant His^6Gly, His^{13}Gly, His^{14}Gly and double mutant His^{13}Gly, His^{14}Gly show peroxidase activity comparable to that of free heme (Figure S1A, Supporting Information). This is consistent with the fact that these mutants do not bind heme. The single mutants of the Aβ(1−16) peptide, His^6Gly, His^{13}Ala, and His^{14}Ala, bind heme and show activity comparable to that of the wild-type Aβ(1−16) and Aβ(1−40) peptides (Figure S1B). This implies that, though the active sites are not identical for His^{13}- and His^{14}-bound heme complexes, the peroxidase activity is not affected significantly by changing the coordinating histidine residues.

Interestingly, when peroxidase activity was tested for the heme−Aβ(10−20) complex (Figure 5, yellow), it did not show any enhanced activity compared to heme (Figure 5, black), in contrast to the other heme−Aβ complexes of peptides that bind heme. This is counterintuitive, because the Aβ(10−20) peptide contains both the coordinating histidine residues (His^{13}, His^{14}). This suggests that one or more noncoordinating residues in the 1−9 peptide region are essential for the heme−Aβ complexes to function as a peroxidase. It is well-known that peroxidase activity requires the presence of an acidic arginine residue in the distal side of heme which is protonated at physiological pH and provides the proton required for the heterolytic cleavage of the O−O bond of H_2O_2.[34,35] It is possible that the acidic Arg^5 residue might be in the distal pocket of heme and serve as the proton source required for peroxidase activity.

In fact, it was observed that the Arg^5Asn mutant of Aβ(1−16) binds heme (Figure 3, pink); however, the heme-bound complex when tested for peroxidase activity showed no enhanced activity compared to native heme, comparable to Aβ(10−20) (Figure 5, pink). This clearly demonstrated that Arg^5 is the key distal residue required for these heme−Aβ complexes to function as peroxidases.

Catalase Activity. The catalase (tyrosine-coordinated heme enzyme) activities of the heme−Aβ complexes have been tested, and it has been found that these heme complexes do not show any catalase activity with respect to free heme (Figure S2, Supporting Information). This further supports the conclusion drawn from spectroscopy that Tyr^{10} does not coordinate to heme to form catalase-type active sites.[36]

pH Perturbation of the Trans Axial Ligand. The observed peroxidase activity, which requires binding of H_2O_2 to the heme Fe trans to the axial His^{13} ligand, implies the presence of an exchangeable sixth ligand, trans to the axial histidine. The nature of this sixth ligand was investigated by pH perturbation.

The heme−Aβ(1−40) and heme−Aβ(1−16) complexes exhibit decreases in the Soret band (∼370 nm) and Q-band (∼635 nm) intensities and a blue shift of the Q-band on going from low to high pH with a pK_a of ∼6.8 ± 0.3 (Figures 6A,B and 7). The pH effect of heme−Aβ(10−20) shows similar spectral changes (Figure 6C), but the pK_a obtained was ∼8.2 ± 0.3 (Figure 7), which is similar to that observed for myoglobin (Figure S3, Supporting Information). This observed pH effect (pK_a ≈ 8.2) likely reflects the protonation equilibrium of a water-derived ligand (as in the case of myoglobin) at the distal side of the heme; i.e., there is a water bound at low pH and a hydroxide bound at high pH (Figure S4, Supporting Information).[37] It is worth mentioning that the pK_a of the metal-bound histidine ligand (His^{13}) is much lower than the observed pK_a values.[38] The higher pK_a observed for Aβ(10−20) is likely due to the absence of the Arg^5 residue. In fact, the Arg^5 mutant exhibits a pK_a of ∼8.2 ± 0.2 (Figures 6D and 7), similar to that of Aβ(10−20). This is consistent with the presence of H-bonding interaction between the water-derived trans axial ligand and the distal Arg^5 residue. This H-bonding with the positively charged protonated guanidine side group of Arg^5 will stabilize a bound hydroxide more than the water, thus lowering its pK_a. A similar lowering of the pK_a of a heme-bound axial water ligand by H-bonding to an arginine residue has also been observed in the Thr^{67}Arg mutant of myoglobin.[39]

Discussion

The results presented in this study indicate that a residue in the hydrophilic 1−16 region of human Aβ peptide binds heme (Scheme 1, Figure 1). EPR data indicate that this forms a high-spin Fe^{III} active site (Figure 4; Figure S5, Supporting Information). In the absence of all the possible coordinating histidine residues (triple mutant His^6Gly, His^{13}Gly, His^{14}Gly), heme does not bind to the Aβ peptides, implying that one of the three histidine residues (His^6, His^{13}, or His^{14}) binds heme (Figure 2). This further implies that the only available coordinating residue, Tyr^{10}, does not bind heme, which is also supported by the fact that a tyrosine-depleted mutant (Tyr^{10}Gly) binds heme (Figure 2). Also these complexes do not show any catalase activity (Figure S3, Supporting Information), which requires the coordination of the tyrosine residue. Mutating the His^{13} and His^{14} residues simultaneously

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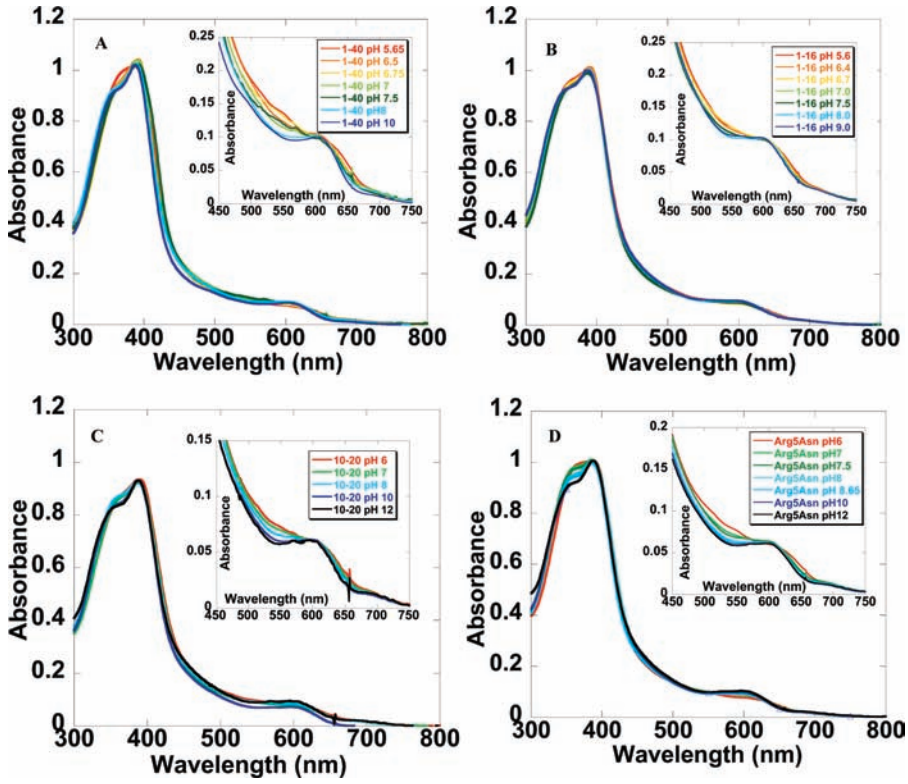

Figure 6. Absorption spectra of heme−Aβ complexes at different pH values: (A) Aβ(1−40), (B) Aβ(1−16), (C) Aβ(10−20), (D) Arg^{5}Asn mutant of Aβ(1−16). Inset: enlarged Q-band region.

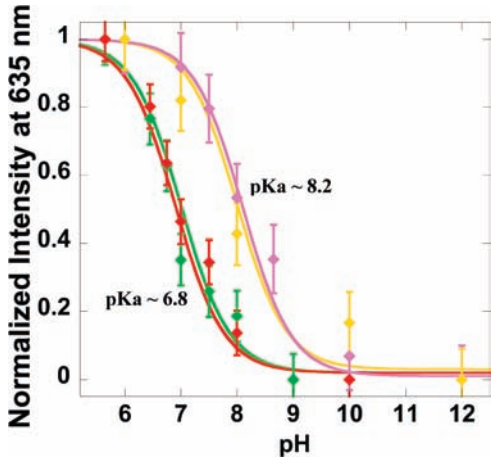

Figure 7. pK_a plots of heme−Aβ complexes with error bars: (1−40), red; (1−16), green; (10−20), yellow; Arg^{5}Asn mutant of (1−16) peptide, pink. All data points from Figure 6 have been used for each pK_a determination.

(double mutant His^{13}Gly, His^{14}Gly) prohibits heme binding, showing that His^6 does not bind heme (Figure 2). The His^{13}Ala mutant binds heme, and so does His^{14}Ala, as determined from absorption spectroscopy (Figure 3). This implies that either His^{13} or His^{14} can coordinate to heme. EPR spectra of the heme complexes of these two mutants (Figure 4) show that the His^{14}Ala (His^{13} being the only available coordinating residue) mutant exhibits an EPR spectrum very similar to that of the heme complex of the native Aβ(1−16), but the His^{13}Ala (His^{14} being the only other coordinating residue) mutant exhibits higher rhombicity than the heme complex of the native Aβ(1−16) peptide. The increased rhombicity can arise from differences in the orientation of the imidazolyl ring of these two hisitidine residues with respect to the g tensors or differences in the second-sphere environment of the active site.[40] While the exact origin of this effect is unclear, the data indicate that probably His^{13} binds heme in the native Aβ(1−16) peptide.

Scheme 2. Amino Acid Sequences of Human, Pig, Monkey, Rabbit, etc. (Top) and Rat, Mouse, and Chinese Hamster (Bottom) Aβ(1−16) Highlighting the Amino Acids That Differ between the Two Peptide Sequences

Asp-Ala-Glu-Phe-Arg^5-His-Asp-Ser-Gly-Tyr^{10}-Glu-Val-His^{13}-His-Gln-Lys

Asp-Ala-Glu-Phe-Gly^5-His-Asp-Ser-Gly-Phe^{10}-Glu-Val-Arg^{13}-His-Gln-Lys

The sixth ligand of the heme-bound Aβ complexes has an exchangeable water-derived ligand, which has a pK_a of ~6.8 for both Aβ(1−40) and Aβ(1−16) (Figures 6 and 7). However, Aβ(10−20) and the Arg^{5}Asn mutant of Aβ(1−16), both of which lack the Arg^5 residue, show similar spectral changes on perturbing the pH, but with a higher pK_a (~8.2). This lowering of pK_a in the presence of Arg^5 is consistent with the presence of H-bonding interaction between the heme-bound water ligand and the arginine residue, which stabilizes the hydroxide form more than the water form.

The peroxidase activities of the Aβ(1−40)−heme and Aβ(1−16)−heme complexes show enhanced activity relative to free heme, suggesting the formation of a histidine-bound peroxidase-like active site (Figure 5). However, Aβ(10−20), though it binds heme, fails to show any enhanced peroxidase activity unlike the other Aβ peptides. Peroxidases have a conserved arginine residue in the distal pocket of the active site,

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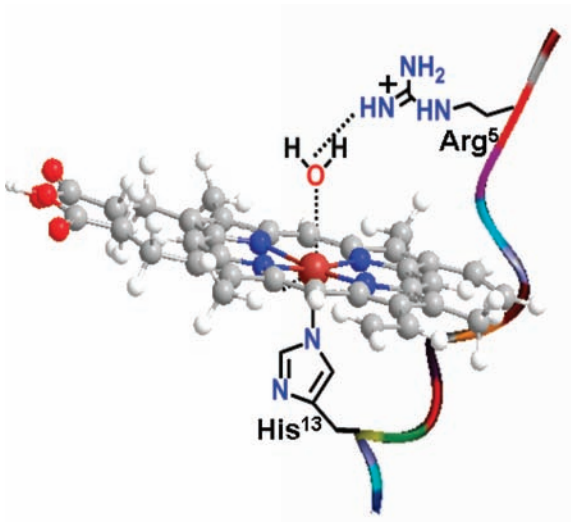

Figure 8. Schematic representation of the active site environment of heme−Aβ(1−16), which may be extrapolated for native Aβ(1−40).

which provides a proton for the O−O bond cleavage in the catalytic cycle. Aβ(10−20) lacks an arginine residue (Arg^5) which is present in both Aβ(1−40) and Aβ(1−16) and might be crucial for peroxidase activity. In fact, an Arg^5Asn mutant binds heme, but shows no peroxidase activity, confirming its role in making these heme−Aβ complexes function as peroxidases. It is important to note that rodent (i.e., rats, mouse, etc.) Aβ, which lacks this Arg^5 residue (Scheme 2), binds heme but shows no marked enhancement in peroxidase activity relative to free heme,[26] similar to Aβ(10−20) and Arg^5Asn peptides, consistent with the above conclusion. It is also important to note that His^{13} is absent in rodent Aβ; thus, His^{14} could be the probable coordinating ligand. The fact that the two key residues (Arg^5 and His^{13}) involved in the heme−Aβ active site of humans/pigs/monkeys are absent in rodent Aβ peptides is very interesting, as AD does not affect these rodents.[41] This further stresses the importance of heme binding to Aβ in the pathology of AD.

Summary

In summary, we have established that either His^{13} or His^{14} of Aβ peptide can bind heme at the proximal side. However, in the native human Aβ(1−16) peptides, His^{13} probably binds heme specifically (Figure 8). The open distal position of the heme−Aβ complex has an exchangeable water-derived ligand with a pK_a of 6.8. The acidic Arg^5 residue present at the distal side directly H-bonds with the axial water ligand, lowering its pK_a. It also induces a pull effect by providing a proton required to drive the O−O bond heterolysis, hence making these heme−Aβ complexes function as peroxidases. These findings shed light on the nature of interaction of heme with Aβ peptides, which is a key step toward unraveling the role played by heme in AD. The two Aβ residues critical for the active site of the heme−Aβ complex, His^{13} and Arg^5, are both absent in the Aβ of rodents, which do not show AD, further illustrating that heme may play a greater role in AD than previously thought.

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Supporting Information Available: Peroxidase activity of all the peptides and mutants, catalase activity kinetic traces and absorption spectra, pH effect (absorption and EPR spectra) and pK_a plot of myoglobin, EPR spectra of Aβ(1−16) at low and high pH, EPR spectra of the heme complex of the Arg^5Asn mutant of Aβ(1−16), extended absorption spectra of all heme−Aβ complexes, and high-field EPR spectra of heme−Aβ complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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