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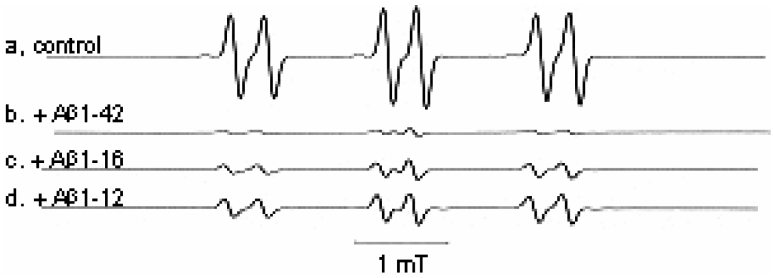

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Three Histidine Residues of Amyloid-β Peptide Control the Redox Activity of Copper and Iron

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ABSTRACT: Zinc, iron and copper are concentrated in senile plaques of Alzheimer disease. Copper and iron catalyze the Fenton−Haber−Weiss reaction, which likely contributes to oxidative stress in neuronal cells. In this study, we found that ascorbate oxidase activity and the intensity of ascorbate radicals measured using ESR spectroscopy, generated by free Cu(II), was decreased in the presence of amyloid-β (Aβ), the major component of senile plaques. Specifically, the ascorbate oxidase activity was strongly inhibited (85% decrease) in the presence of Aβ1−16 or Aβ1−42, whereas it was only slightly inhibited in the presence of Aβ1−12 or Aβ25−35 (<20% inhibition). Ascorbate-dependent hydroxyl radical generation by free Cu(II) decreased in the presence of Aβ in the identical order of Aβ1−42, Aβ1−16 > Aβ1−12 and was abolished in the presence of 2-fold molar excess glycylhystidyllysine (GHK). Ascorbate oxidase activity and ascorbate-dependent hydroxyl radical generation by free Fe(III) were inhibited by Aβ1−42, Aβ1−16, and Aβ1−12. Although Cu(II)−Aβ shows a significant SOD-like activity, the rate constant for the reaction of superoxide with Cu(II)−Aβ was much slower than that with SOD. Overall, our results suggest that His6, His13, and His14 residues of Aβ1−42 control the redox activity of transition metals present in senile plaques.

The hallmark lesion of Alzheimer disease (AD)[1] is the senile plaque, which mainly consists of amyloid-β (Aβ), a peptide of 40−42 amino acids in length. Aβ, whether extracellular as senile plaques or intracellular as oligomeric Aβ, is thought to subsequently cause the formation of neurofibrillary tangles and neuronal death. Although many hypotheses have been postulated concerning the pathogenesis of AD, the exact mechanism remains to be solved (*1*, *2*). Along with Aβ, one of the earliest events in AD is oxidative damage to vulnerable neurons (*3*−*6*), marked by 8-OHG (*7*), hydroxynonenal (*8*), nitrotyrosine (*9*), and myeloperoxidase.

Recent progress has shown that Aβ contributes to the initiation and progression of AD. Yankner et al. indicate that the trophic and toxic responses of neurons to Aβ1−40 depend on the stage of neuronal differentiation and the concentration of peptides (*10*). Toxicity of Aβ to neuronal cells is ascribed to the nature of Aβ (*11*, *12*). Aβ1−42 forms aggregates spontaneously through the formation of dimer and oligomers. Recent findings indicate that a dimer of Aβ1−42 interacts with the terminal oxidase of mitochondria, which causes the dysfunction of mitochondria leading to the generation of oxygen radicals (*13*). It has been demonstrated that Aβ1−42 binds equimolar copper with very high affinity (*14*) and the resulting Cu(I)−Aβ produces H_2O_2 through the reaction of Cu(I)−Aβ complex with O_2 (*15*−*17*). The resulting H_2O_2 contributes to the neurotoxicity of the peptides in primary neuronal cultures. The source of the reducing equivalents for the conversion of Cu(II)−Aβ to Cu(I)−Aβ is still uncertain.

A number of in vivo studies have shown that metal ions such as copper, iron, and zinc can cause oxidative damage to neuronal cells (*18*−*20*). These observations are significant in view of the fact that senile plaques in AD contain elevated concentrations of copper, iron, and zinc (*19*). Surprisingly, although Cu(II)−Aβ is more toxic to cultured neurons than Aβ alone, these complexes are less toxic than copper alone (*21*). Consistent with this, Aβ has been reported to prevent the copper-induced lipid peroxidation of low-density lipoproteins (*22*), and iron-induced cell death is also reduced in the presence of Aβ1−42 (*23*). On the other hand, specific interaction of Aβ1−42 with oxidatively damaged membranes

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[1] Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer disease; SOD, superoxide dismutase; HRP, horseradish peroxidase; GHK, glycylhystidyllysine; ESR, electron spin resonance; PBN, *N*-*tert*-butyl-α-phenylnitrone; DMSO, dimethyl sulfoxide; MCLA, 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3-one ; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

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is suggested to cause Aβ1−42 to form fibrils (*24*) and lead to neurotoxicity. Given these conflicting ideas, it is perhaps not surprising that it is unclear whether Aβ1−42 is a prooxidant or an antioxidant (*25*, *26*).

In this report, we measured redox activity of Cu(II) bound to Aβ (Aβ1−12, 1−16, 1−28, 1−40, 1−42, 25−35) compared with free Cu(II). Using ascorbate oxidation as a sensitive indicator of redox activity, we found a marked reduction of ascorbate oxidation by Cu(II) or Fe(III) in the presence of Aβ1−40 or Aβ1−42.

MATERIALS AND METHODS

Materials. Aβ1−16, Aβ1−28, Aβ25−35, Aβ1−40, and Aβ1−42 were purchased from the Peptide Institute Inc. (Minoh-shi, Osaka). Aβ1−12 was obtained from Biosource (Camarillo, CA). All peptides were analyzed for purity using MALDI-MS before use. Xanthine oxidase from buttermilk, SOD from bovine erythrocytes, horseradish peroxidase (HRP), glycylhystidyllysine (GHK), and *N-tert*-butyl-α-phenylnitrone (PBN) were purchased from Sigma-Aldrich (St. Louis, MO). 2-Methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3-one hydrochloride (MCLA) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Tokyo Kasei Kogyo Co. and Nacalai Tesque, respectively. Other reagents used were of analytical grade. Reactions were carried out at 25 °C.

Effects of Aβ on the Ascorbate Oxidation by Copper or Iron. Oxidation of ascorbate was followed optically at 265 nm with a Shimadzu MPS 2000 spectrometer (Kyoto). Reaction mixture contained 100 μM ascorbic acid, 20 μM $CuCl_2$ or $Fe(NO_3)_3$, and 50 μM Aβ in 20 mM HEPES buffer (pH 7.0). Oxidation of ascorbate was started by the addition of ascorbate to a solution containing $CuCl_2$ or $Fe(NO_3)_3$ and Aβ.

Effects of Aβ on the Formation of Ascorbate Radicals by Copper or Iron. The formation of ascorbate radicals by copper or iron was observed with a JEOL JES-TE300 ESR spectrometer (Tokyo) with 100 kHz modulation. Reaction mixtures in the flat cell contained 1 mM ascorbic acid, 20 μM $CuCl_2$ or $Fe(NO_3)_3$, and 50 μM Aβ in 10 mM HEPES buffer (pH 7.0). Reactions were started by the addition of ascorbic acid. Relative intensity of ascorbate radicals in the steady state has compared with the intensity of the peak of doublet at higher magnetic field (Figure 3). Instrumental conditions were as follows: gain, 250; power, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.1 s; scan rate, 5 mT min^{-1}.

Determination of H_2O_2 Concentrations. A solution containing 100 μM ascorbic acid and 20 μM $CuCl_2$ in the absence or presence of 50 μM Aβ in 10 mM HEPES (pH 7.0) was incubated for 1 h and then subjected to the ABTS/HRP system. After incubation, the solution was mixed with ABTS (100 μM) and 40 nM HRP. The concentration of H_2O_2 was calculated from the increase of absorbance at 414 nm using molar absorption coefficient $\epsilon = 36\,000$ cm^{-1} at 414 nm (*27*).

Effect of Copper upon the Intrinsic Fluorescence of Aβ. Cu^{2+}-dependent quenching of the fluorescence assigned to Tyr residue of Aβ and the restoration by the addition of chelators has been reported (*28*). The fluorescence spectra (excitation at 280 nm) of 10 μM Tyr or Aβ were obtained in the absence or presence of varied concentrations of $CuCl_2$ with an RF-5300PC spectrofluorometer. Each point represents the mean ± SD of three experiments.

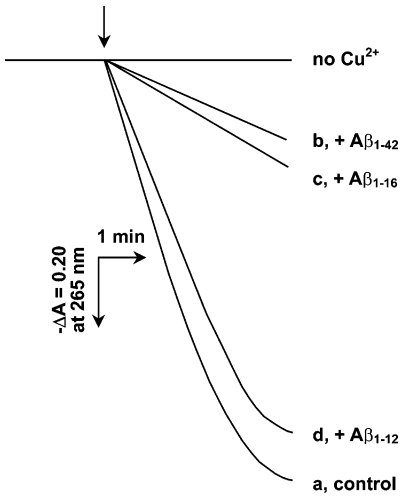

FIGURE 1: Ascorbate oxidation by copper in the absence or presence of Aβ. Concentrations were 100 μM ascorbic acid, 20 μM $CuCl_2$, and 50 μM Aβ in 20 mM HEPES buffer (pH 7.0). Reactions were carried out in the absence (a, control) or presence of Aβ1−42 (b), Aβ1−16 (c), and Aβ1−12 (d).

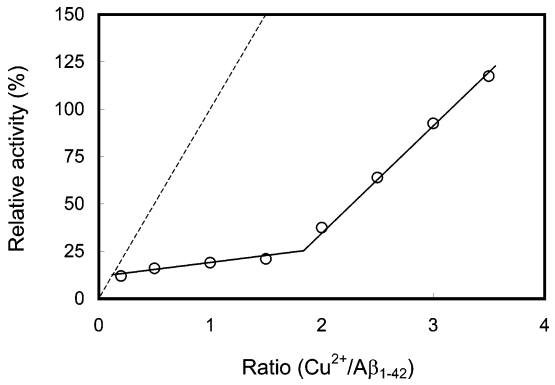

FIGURE 2: Effect of copper to Aβ1−42 ratio upon the ascorbate oxidation of copper. Concentrations were 100 μM ascorbic acid, 21 μM Aβ1−42, and various concentrations of $CuCl_2$ in 20 mM HEPES buffer (pH 7.0). Reactions were started by the addition of ascorbic acid to a solution that contained copper and Aβ1−42 that had been incubated for 10 min; 100% denotes the ascorbate oxidase activity in the absence of Aβ1−42 (dotted line).

Effects of Aβ on the Formation of Hydroxyl Radicals by Copper or Iron in the Presence of Ascorbic Acid and Hydrogen Peroxide. A reaction mixture comprised of 1 mM ascorbic acid, 1 mM H_2O_2, 20 μM $CuCl_2$ or $Fe(NO_3)_3$, and 50 μM Aβ in 25 mM phosphate buffer (pH 7.0) was freshly prepared. Hydroxyl radicals produced in the solution were detected with an ESR double-trapping method using 0.1 M PBN and 1 M DMSO (*29*). Relative intensity of the spin-adduct was compared with the intensity of the peak of the sextet at the highest magnetic field. Instrumental conditions were as follows: gain, 10; power, 5.0 mW; modulation amplitude, 0.1 mT; time constant, 0.3 s; scan rate, 2.5 mT min^{-1}.

Estimation of Rate Constant for the Reaction of Cu(II)−Aβ with Superoxide. Reaction mixtures containing 100 μM xanthine, 40 nM xanthine oxidase, 4.9 μM MCLA, 21 μM Aβ, and various concentrations of $CuCl_2$ in 20 mM HEPES

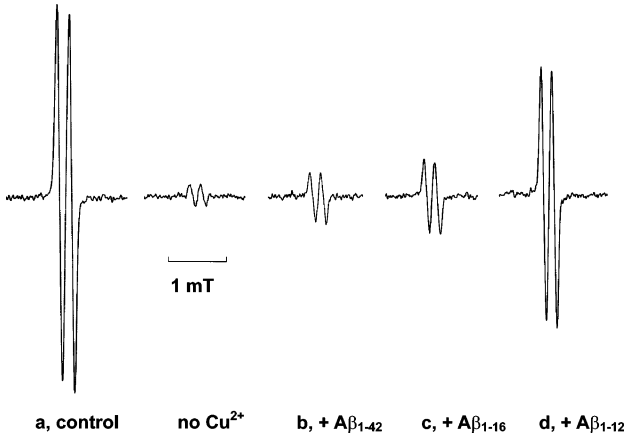

FIGURE 3: Effect of Aβ upon the formation of ascorbate radicals by copper. Concentrations were 1.0 mM ascorbic acid, 20 μM $CuCl_2$, and 50 μM Aβ in 20 mM HEPES buffer (pH 7.0). Reactions were carried out in the absence (a, control) or presence of Aβ1−42 (b), Aβ1−16 (c), or Aβ1−12 (d). ESR spectra were obtained at 2 min after reactions had been started. Instrumental conditions are described in Materials and Methods.

(pH 7.0) were freshly prepared. The velocity of superoxide generation by the xanthine oxidase system was 2.1 μM min^{-1} (*30*). Chemiluminescence of MCLA was observed with an Aloka BLR-301 luminescence reader. The rate constant for the reaction of Cu(II)−Aβ with superoxide is calculated kinetically based on the rate constant for the reaction of MCLA with superoxide (*31*).

RESULTS

Ascorbate oxidation was followed by the decrease of absorbance at 265 nm and initiated by the addition of copper (Figure 1). The 100 μM ascorbate was exhausted in 4 min by 20 μM Cu(II) under aerobic conditions. When the reaction was carried out both in the presence of copper and Aβ, the velocity of ascorbate oxidation decreased. When a molar ratio of Aβ to copper of 2.5 was used, the rank order of inhibitory effect (Aβ1−42, Aβ1−40, Aβ1−28, Aβ1−16 > Aβ25−35, Aβ1−12) was observed for ascorbate oxidation. Complete inhibition of ascorbate oxidation by copper was observed when a 2-fold molar excess of GHK over copper was added to the solution. Marked difference in the ascorbate oxidation was observed for Cu(II)−Aβ1−12 and Cu(II)−Aβ1−16. Ascorbate oxidation was performed using various molecular ratios of Cu(II) to Aβ1−42 (Figure 2). These results indicate that Aβ1−42 is able to inhibit the redox activity of 2 mol of Cu(II). Above 2 mol of Cu(II) to Aβ1−42, ascorbate oxidation increased with increasing concentration of Cu(II). The slope of the activity to Cu(II) concentration in the presence of Aβ1−42 was slightly decreased compared with that in the absence of Aβ1−42 (dotted line), which indicates that Aβ1−42 potentially interacts with more than 2 mol of Cu(II).

One-electron oxidation of ascorbate by free Cu(II) and Cu(II)−Aβ was confirmed by ESR spectroscopy. Properties of ascorbate radicals are well documented (*32*). Relation of the concentration of ascorbate radicals ($[Asc^{\bullet}]_s$) in the steady state and initial velocities (Vi) in the oxidation of ascorbate by free Cu(II) and Cu(II)−Aβ is expressed as follows. Ascorbate radicals decay only through dismutation reaction with a dismutation constant (Kd).

$$Vi = Kd[Asc^{\bullet}]_s^2$$

Steady-state concentration of ascorbate radicals decreased when the reactions were carried out in the presence of Aβ1−42 or Aβ1−16, whereas the intensity was reduced by 30% in the presence of Aβ1−12 (Figure 3). It has been shown that transition metals, such as copper or iron, catalyze the Fenton−Haber−Weiss reaction in the presence of reductants and oxygen (*33*). Reduced copper reacts with oxygen to form H_2O_2 through the intermediate, 2Cu(I) complex and O_2 complex formation. The resulting H_2O_2 reacts with the Cu(I) complex, generating hydroxyl radicals. Hydroxyl radicals rapidly react with DMSO to form DMSO radicals. In this reaction, hydroxyl radicals are detected as the PBN−CH_2-$SOCH_3$ adduct (Figure 4). The results are compatible with the observation that hydroxyl radical generation by Cu(II) was markedly inhibited by the coexistence of Cu(II) and Aβ1−42 or Aβ1−16 (Figures 1 and 3). With a 0.5 molecular ratio of copper to Aβ1−42, there is no free Cu(II) (*14*). Figure 4b indicates that Cu(II)−Aβ1−42 produces a significant amount of hydroxyl radicals in the presence of reductants.

An effect of Cu(II) concentration upon Tyr fluorescence of Aβ was observed. The intensity of fluorescence decreased with increasing concentration of Cu(II) (Figure 5A), and the quenching of fluorescence was saturated at 2 mol of Cu(II) to Aβ. The quenched fluorescence with 0.3 mol of Cu(II) to Aβ1−12, Aβ1−16, and Aβ1−28 was completely abrogated by the addition of 3-fold molar excess of GHK to Aβ. On the other hand, the reduced intensity of Cu(II)−Aβ1−42 was unchanged by this 3-fold molar excess of GHK (Figure 5B). Bound copper to Aβ1−12, Aβ1−16, or Aβ1−28 was removed by 3-fold molar excess of GHK to Aβ.

Histidine residues are ligated to Cu(II) in Cu,Zn−SOD. Because the reduction potentials ($E_{m,7}$) for $O_2/O_2^{\bullet-}$ and $O_2^{\bullet-}/H_2O_2$ couple are −160 and 900 mV, respectively, the reduction potential of Cu(II) complexes, which show SOD-like activity, must lie between them (*34*). Huang et al. reported that the reduction potential for the Cu(II)−Aβ1−42/Cu(I)−Aβ1−42 couple is estimated to be +500 to +590 mV, a value much higher than that (−80 mV) for the Cu(II)/Cu(I) couple in phosphate (*16*). Rate constants for the reaction of superoxide with Cu(II) Aβ are shown in Figure 6 and summarized in Table 1. The rate constant for the reaction of superoxide with Cu(II)−Aβ1−12 is the highest, being approximately 10^4 times slower than that with SOD (*35*).

Since the concentration of iron in the senile plaques is higher than copper (*19*), ascorbate oxidation by Fe(III) was compared with that by Cu(II). At the same concentration of Fe(III) (20 μM), the velocity of ascorbate oxidation was 10% of that by Cu(II) (Table 2). Comparable to Cu(II), ascorbate oxidase activity and ascorbate-dependent hydroxyl radical generation by free Fe(III) were inhibited by Aβ1−42, Aβ1−16, and Aβ1−12 (Figure 7, parts A and B).

DISCUSSION

One-electron oxidation of ascorbate by free Cu(II) and Cu(II)−Aβ has been confirmed by the formation of ascorbate radicals in the steady state (Figure 3). Kinetics of the reaction of Cu(I) complex with oxygen or H_2O_2 have been reported

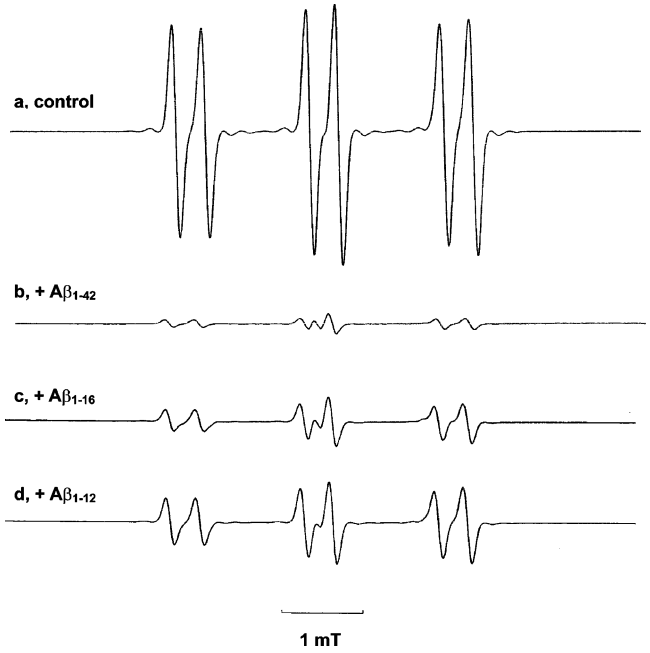

FIGURE 4: Effect of Aβ upon the formation of hydroxyl radicals by copper. Concentrations were 1.0 mM ascorbic acid, 1.0 mM H_2O_2, 20 μM $CuCl_2$, 0.10 M PBN, 1.0 M DMSO, and 50 μM Aβ in 25 mM phosphate buffer (pH 7.0). Reactions were carried out in the absence (a, control) or presence of Aβ1−42 (b), Aβ1−16 (c), and Aβ1−12 (d). Instrumental conditions are described in Materials and Methods.

(*36*, *37*). In this reaction, oxygen is directly converted to H_2O_2 without forming superoxide. The present result indicates that ascorbate oxidation by Cu(II)−Aβ proceeds in a similar manner.

Since H_2O_2 plays a role in the oxidative damage to neuronal cells, much attention has been paid to the generation of H_2O_2 by Cu(II)−Aβ complex in the presence of reductants. The formation of H_2O_2 has been reported in the reaction of ascorbate with free Cu(II) or Cu(II)−Aβ (*15*, *24*). Almost 60% of reducing equivalents of ascorbate are found as H_2O_2 formed in the reaction (*24*). After ascorbate is consumed, we have determined the concentration of remaining H_2O_2 using the HRP/ABTS system. Less than 25% reducing equivalents of ascorbate were found as H_2O_2 for coincubation of free Cu(II) and ascorbate or Cu(II)−Aβ1−12 and ascorbate. There is no significant amount of H_2O_2 observed for Cu(II)−Aβ1−16 or Cu(II)−Aβ1−42 system. Reaction of Cu(I) complex with H_2O_2 has been reported (*36*, *37*). The present results show that hydroxyl radicals were generated during the reaction of Cu(II)−Aβ with ascorbate (Figure 4) indicating that formed H_2O_2 was decomposed through the reaction with Cu(I) complex and through catalase reaction by Cu(II)−Aβ.

Although Aβ is reported to spontaneously produce radicals (*38*), this was subsequently shown to be an artifact due to the presence of contaminating oxidant (*39*, *40*). On the other hand, it is well-established that generation of H_2O_2 by AD-related peptides, including Aβ, may be related to the pathogenesis of AD (*7*, *12*, *15*).

Although, as described above, there has been a focus on the oxidative properties of Aβ, there is also compelling evidence that Aβ shows antioxidant activity (*22*, *23*, *41*). Hydroxyl radical generation by free Cu(II) is decreased in the presence of Aβ (Figure 4). There is no difference in

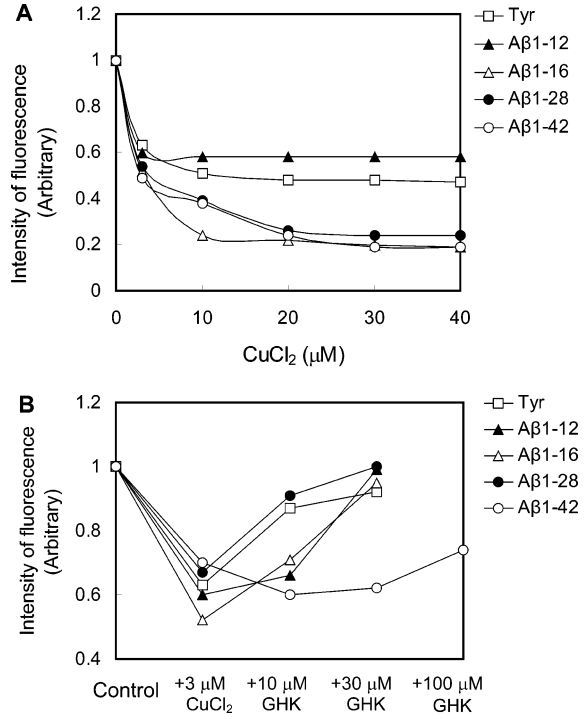

FIGURE 5: Effect of copper upon the fluorescence of Aβ. (A) Effect of copper concentrations upon the fluorescence of Aβ. Fluorescence spectra (excitation at 280 nm) of 10 μM Tyr (open rectangle) or Aβ1−12 (closed triangle), Aβ1−16 (open triangle), Aβ1−28 (closed circle), and Aβ1−42 (open circle) in 25 mM phosphate buffer (pH 7.0) were obtained in the absence or presence of various concentrations of $CuCl_2$. (B) Effect of GHK concentrations upon the fluorescence of Cu(II)−Aβ. Spectra were obtained by the addition of 10, 30, or 100 μM GHK to a solution (25 mM phosphate, pH 7.0) containing 3.0 μM $CuCl_2$ and 10 μM Tyr (open rectangle) or Aβ1−12 (closed triangle), Aβ1−16 (open triangle), Aβ1−28 (closed circle), and Aβ1−42 (open circle).

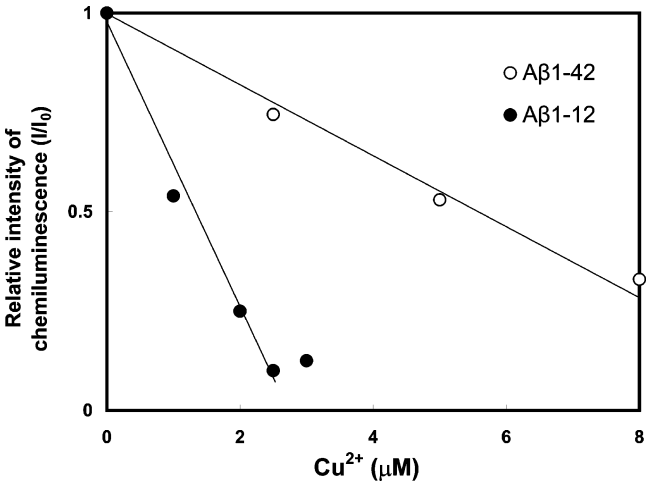

FIGURE 6: Reaction of superoxide with Cu(II)−Aβ. Concentrations were 100 μM xanthine, 40 nM xanthine oxidase, 4.9 μM MCLA, 21 μM Aβ1−42 (open circle) or Aβ1−12 (closed circle) and various concentrations of $CuCl_2$ in 20 mM HEPES buffer (pH 7.0). Intensity of chemiluminescence denotes the chemiluminescence of MCLA in the absence (I_0) or presence (I) of $CuCl_2$.

inhibitory effect of Aβ1−42 and Aβ1−40, and the effect of Aβ was in the order Aβ1−42, Aβ1−40, Aβ1−16 > Aβ1−12. The ligands of Cu(II) are located in the N-terminus and are postulated to be 3N1O. The Cu(II) binding site consists of three histidine residues (His6, His13, His14) and the carboxyl group of N-terminus (*28*, *42*). The results presented

Table 1: Rate Constant for the Reaction of Superoxide with Cu^{2+} Complex[a]

Cu^{2+} complex	rate constant (M^{-1} s^{-1})
GHK	1.1×10^4
$A\beta_{1-12}$	2.0×10^5
$A\beta_{1-16}$	1.1×10^5
$A\beta_{1-42}$	3.1×10^4

[a] Experimental conditions and calculation procedure for the rate constant are described in Materials and Methods.

Table 2: Effects of Aβ upon the Ascorbate Oxidation by Fe^{3+} [a]

Cu^{2+} complex	ascorbate oxidation (μM/min)	%
Cu^{2+}	39	975
Fe^{3+}	4.0	100
+ $A\beta_{1-42}$	0.4	10
+ $A\beta_{1-16}$	0.8	20
+ $A\beta_{1-12}$	3.6	90

[a] Experimental conditions are described in Materials and Methods. Rate of ascorbate oxidation is estimated from the initial decrease of absorbance at 265 nm.

here suggest that these three histidine residues strongly inhibit the redox activity of copper. It is important to note that Cu(II)−Aβ1−42 inhibits the activity of free Cu(II) by 90% and therefore is still able to generate small amounts of hydroxyl radicals in the presence of ascorbate (Figure 4). It has been noted that Aβ1−42 shows a specific interaction with membrane phospholipid (*24*), where Cu(II) bound to Aβ1−42 accelerates oxidative damage to lipid membrane. Site-specific hydroxyl radical generation by copper has been reported (*43*). When Cu(II)−Aβ1−42 is incubated in the presence of H_2O_2 or ascorbate, the Cu(II) binding site is selectively modified (*44*, *45*). When human Cu(II)−Aβ1−16 is incubated in the presence of H_2O_2, conversion of His to 2-oxo His and decarboxylation and deamination of terminal aspartic acid residue have been confirmed. The resulting modified Cu(II)−Aβ1−16 is suggested to be less reactive than native Cu(II)−Aβ1−16 (*45*). However, our results indicate that modified Cu(II)−Aβ1−42 may be more redox active than Cu(II)−Aβ1−42, because Cu(II) in modified Cu(II)−Aβ1−42 is more loosely bound than that in native Cu(II)−Aβ1−42.

It is important to note that about 60% of Aβ in senile plaques is missing the N-terminal amino acids of Aβ1−42 (*46*). Aβ activates phagocytic cells to elicit inflammation (*47*), which liberates cytokines and reactive oxygen species. Modification of the N-terminal aspartic acid of Aβ1−16 in the presence of Cu(II) and H_2O_2 has been reported (*44*). The present results (Figure 6, Table 1) indicate that Cu(II)−Aβ scavenges superoxide and that resulting H_2O_2 modifies Aβ.

The copper- and iron-catalyzed hydroxyl radical generation, the Fenton−Haber−Weiss reaction, is described in detail elsewhere (*33*). Copper chelators present in tissues, such as histidine, GHK, and human serum albumin, strongly inhibit the reaction. On the other hand, iron-catalyzed hydroxyl radical generation may occur in vivo, since, except for iron-binding proteins, no such iron chelators are present. Our results (Figure 7 and Table 2) show that Fe(III)-catalyzed ascorbate oxidation and hydroxyl radical generation are inhibited in the presence of Aβ1−16 or Aβ1−42.

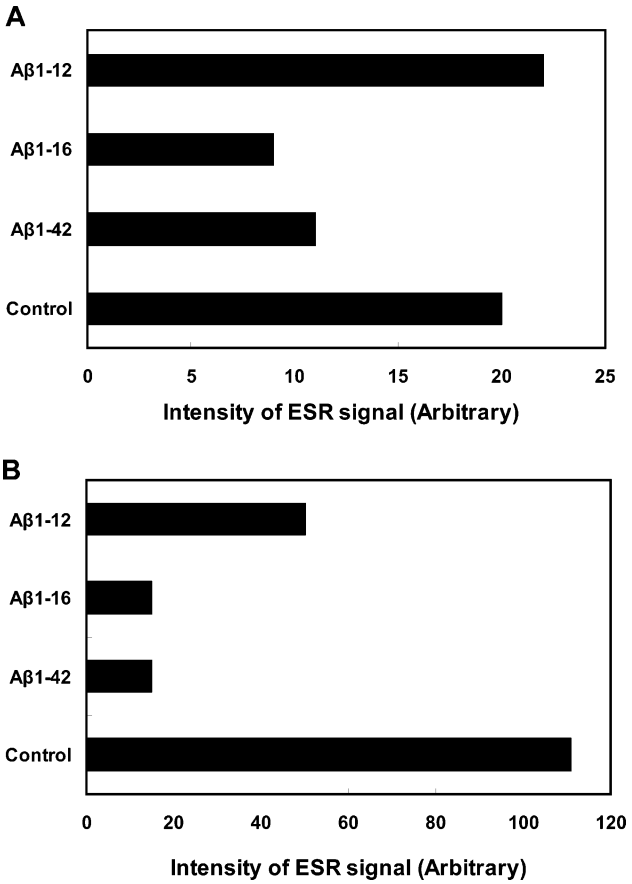

FIGURE 7: Effect of Aβ upon the formation of ascorbate radicals (A) and hydroxyl radicals (B) by iron in the absence or presence of Aβ. (A) Concentrations were 1.0 mM ascorbic acid, 20 μM $Fe(NO_3)_3$, and 50 μM Aβ. Reactions were carried out in 20 mM HEPES buffer (pH 7.0) in the absence (control) or presence of Aβ1−12, Aβ1−16, or Aβ1−42. Instrumental conditions are described in Materials and Methods. (B) Concentrations were 1.0 mM ascorbic acid, 1.0 mM H_2O_2, 20 μM $Fe(NO_3)_3$, 0.10 M PBN, 1.0 M DMSO, and 50 μM Aβ in 25 mM phosphate buffer (pH 7.0). Reactions were carried out in the absence (control) or presence of Aβ1−12, Aβ1−16, or Aβ1−42. Instrumental conditions are described in Materials and Methods. The results are means of three experiments.

These data indicate a marked antioxidant face of Aβ which is often considered a prooxidant protein. However, dependent upon the circumstances, such prooxidant or antioxidant aspects are a commonality among antioxidants but not prooxidants. Indeed, although not all prooxidants are antioxidants, all antioxidants are also prooxidants (*25*, *26*). Therefore, Aβ is likely an important antioxidant.

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