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Redox Reactions of Copper Complexes Formed with Different β -amyloid Peptides and Their Neuropathological Relevance[†]

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Abstract

The binding stoichiometry between Cu(II) and the full-length β -amyloid A β (1–42) and the oxidation state of copper in the resultant complex were determined by electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) and cyclic voltammetry. The same approach was extended to the copper complexes of A β (1–16) and A β (1–28). A stoichiometric ratio of 1:1 was directly observed and the oxidation state of copper was deduced to be 2+ for all the complexes and residues tyrosine-10 and methionine-35 are not oxidized in the A β (1–42)-Cu(II) complex. The stoichiometric ratio remains the same in the presence of more than 10 fold excess of Cu(II). Redox potentials of the sole tyrosine residue and the Cu(II) center were determined to be ca. 0.75 V and 0.08 V vs. Ag/AgCl (or 0.95 V and 0.28 V vs. normal hydrogen electrode (NHE)), respectively. More importantly, for the first time, A β -Cu(I) complex has been generated electrochemically and was found to catalyze the reduction of oxygen to produce hydrogen peroxide. The voltammetric behaviors of the three A β segments suggest that diffusion of oxygen to the metal center can be affected by the length and hydrophobicity of the A β peptide. The determination and assignment of the redox potentials clarify some misconceptions in the redox reactions involving A β and provide new insight into the possible roles of redox metal ions in the Alzheimer's disease (AD) pathogenesis. In cellular environments, the reduction potential of the A β -Cu(II) complex is sufficiently low to react with antioxidants (e.g., ascorbic acid) and cellular redox buffers (e.g., glutathione), and the A β -Cu(I) complex produced could subsequently reduce oxygen to form hydrogen peroxide via a catalytic cycle. Using voltammetry, the A β -Cu(II) complex formed in solution was found to be readily reduced by ascorbic acid. Hydrogen peroxide produced, in addition to its role in damaging DNA, protein, and lipid molecules, can also be involved in the further consumption of antioxidants, causing their depletion in neurons and eventually damaging the neuronal defense system. Another possibility is that A β -Cu(II) could react with species involved in the cascade of electron transfer events of mitochondria and might potentially side-track the electron transfer processes in the respiratory chain, leading to mitochondrial dysfunction.

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Supporting Information Available: ESI-FTICR mass spectra of A β (1–28) and A β (1–42), an linear-ion trap mass spectrum of the A β (1–42)-Cu(II) complex, a voltammogram of Cu(II) in a thoroughly deaerated solution, voltammograms of ascorbic acid in the presence of A β (1–42)-Cu(II), and a table showing the relation between the A β (1–42)-Cu(II) complex and the molar ratio of Cu(II) and A β (1–42) are included in the Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder underscored by the appearance of senile plaques in disease-inflicted brains. The major components in senile plaques are peptides containing 39–43 amino acid residues (amyloid- β or A β peptides) generated from proteolytic cleavage of the amyloid precursor protein (APP) by β - and γ -secretases (1, 2). Such findings (2, 3) led to the hypothesis that deposition of A β fibrils and other aggregates is responsible for neuronal cell loss (4). However, how β -amyloid associates with the AD pathogenesis remains unclear.

Other characteristics in AD-affected brains include the enhanced level of oxidative stress manifested by extensive oxidation of proteins (5–7) and DNA (8–10), unusually high levels of metals (e.g., copper, zinc and iron) present in the senile plaques, and a decline of polyunsaturated fatty acids (11, 12) coupled with increased lipid peroxidation (13–15). On the basis of the amyloid hypothesis and the existence of oxidative stress, Butterfield and coworkers (15) proposed a model to account for neurodegeneration in AD, *viz.*, β -amyloid peptide-initiated oxidative stress and neurotoxicity. The aggregated amyloid peptide, perhaps in concert with complexed redox metal ions, produces free radicals and triggers a cascade of events, which include, but are not limited to, protein oxidation, lipid peroxidation, and cellular dysfunction. All of these detrimental processes result in death of neurons (15).

Together with these pathological characteristics, intracellular lesions, including impairment of mitochondrial energy metabolism, have also been purported to be a cause of the AD development (9, 10, 16, 17). In addition, impaired energy metabolism and altered cytochrome *c* oxidase activity are among the earliest detectable defects in AD (18–21). Recently, the linkage between mitochondrial dysfunction and A β peptides has been suggested by Deshpande *et al.* (22) who showed that, after treatment of neuronal cells with A β oligomers, mitochondrial redox potential dropped precipitously. A marked decrease in ATP level and drastic increases in caspase activation and lactate dehydrogenase (LDH) release were also observed prior to the occurrence of massive cell death (22). It also has been reported that synthetic A β inhibited activity of the human cytochrome *c* oxidase and the inhibitory effect is dependent on the copper content (23, 24). Only in the presence of copper does A β significantly inhibit the activity of human cytochrome *c* oxidase in mitochondria. These results suggest that copper plays a significant role in the neurotoxicity of A β .

The brain utilizes metal ions for many biochemical reactions, and cortical neurons release exchangeable copper and zinc ions during depolarization and neurotransmission (25, 26). Whether metal ions are involved in the pathogenesis of AD and what roles they play in the involvement of oxidative stress and AD development are not known. Yet, the fact that senile plaques in the neocortical region of the brains of AD patients contain up to millimolar concentrations of Zn²⁺, Cu²⁺, and Fe³⁺ (27) suggests that metal ions probably play a pivotal role in the generation of reactive oxygen species (ROS). A recent study by Raman spectroscopy of senile plaques extracted from postmortem samples demonstrated that copper and zinc ions are bound via histidine imidazole rings (28). More importantly, it was found that extensive methionine oxidation in A β has occurred in intact plaques (28). For metals to exert important influences on the AD pathogenesis, it is likely that a series of redox reactions facilitated by the metal-containing A β species have occurred, leading to the production of ROS and/or the interruption of electron transfer (ET) chain in the respiratory processes. Along this line, Cu(II) coordination with A β has been extensively investigated (29–34). In support of the important role of copper-containing A β species in ROS production, studies have shown that *in vitro* incubation of an A β /Cu(II) mixture with electron donors under aerobic condition produced hydrogen peroxide (35–37).

A number of techniques have been employed for examining the various aspects of the interactions between β -amyloid and copper ions (e.g. the structure of and binding sites in the complex) (34, 35, 38, 39). Although conflicting results have been reported on certain aspects (for example, the metal binding stoichiometry and affinity) (29, 34, 38, 39), it is generally accepted that metal ions are bound to the hydrophilic portion of $A\beta$ species (residues 1–16). Moreover, the involvement of histidine residues at positions 6, 13, and 14 has been ascertained by many studies (30, 34, 38). Given the wide existence of ET processes in cells in general and neuronal cells in particular, the introduction of exogenous redox-active species may alter the ET reactions or pathways in mitochondria. The accurate determination of redox potentials of $A\beta$ and its metal complexes will certainly help unravel their roles in oxidative stress, metal homeostasis and detoxification, and $A\beta$ aggregation/fibrillation. Surprisingly, other than a single experiment comparing the voltammetric behaviors of $A\beta(1-42)$ and its copper complex (35), a systematic effort has not been made to measure accurately the redox potentials of metal complexes of the full-length and different segments of $A\beta$ and to relate them to redox reactions in cellular milieu. Moreover, there exist inconsistencies in the interpretations of the redox reactions of the $A\beta$ -metal complexes (e.g., whether $Cu(II)$ can be reduced by $A\beta$ and, if the reduction does occur, which constituents in $A\beta$ cause the reduction) (27, 35, 37, 40). Compounded by the complexity in $A\beta$ structural elucidation and the possible involvement of $A\beta$ in many cellular processes, evidence regarding the effect of redox-active metal ions on ROS generation and the possible oxidations of the methionine residue near the C terminus (Met-35) and the tyrosine moiety in the hydrophilic domain (Tyr-10) remain either indirect or largely elusive.

Electrochemical methods can allow for the accurate and direct determination of potentials of redox-active biomolecules and provide insight about their ET reactions (41). By judiciously choosing the electrode materials and electrolyte system, one can achieve facile ET rates at the electrode/solution interface and reliably determine the redox potentials. In this study, we employed cyclic voltammetry (CV) and mass spectrometry (MS), to investigate the redox properties of several $A\beta$ variants and their copper complexes. We present strong evidence about the inability of $A\beta$, in the absence of a cofactor (i.e., a reductant), to reduce $Cu(II)$ and report on an experiment in which electrogenerated $A\beta$ - $Cu(I)$ complex can indeed facilitate the catalytic reduction of dissolved oxygen to hydrogen peroxide. Based on the redox potential of $A\beta$ - $Cu(II)$ and comparing it to those of selected cellular reductants, the implications of the $A\beta$ involvement in the production of ROS and the ET chain of mitochondria and their relations with AD pathogenesis are discussed.

MATERIALS AND METHODS

Materials

Lyophilized $A\beta(1-16)$, $A\beta(1-28)$, and $A\beta(1-42)$ (DAEFRHDSGYEVHHQK¹⁶LVFFAEDVGSNK²⁸GAIIGLMVGGVVIA⁴²) samples were purchased from American Peptide Co. Inc. (Sunnyvale, CA). $A\beta(1-28)$ and $A\beta(1-42)$ samples, generously provided by Prof. C. Glabe (University of California at Irvine, CA), were also used in this study. No difference in terms of experimental results between the two sources of $A\beta$ samples was found. Other chemicals were of analytical grade (Sigma-Aldrich). All the aqueous solutions were prepared using Millipore water (18 M Ω cm). Throughout the work, 1 mM $CuCl_2$ dissolved in 1 mM H_2SO_4 was used as the $Cu(II)$ stock solution. $A\beta(1-16)$ were prepared freshly by dissolving lyophilized powder samples in Millipore water or 5 mM NaOH. No apparent difference was observed for electrochemistry and MS results between samples dissolved in water and those dissolved in NaOH. To ensure no substantial aggregation occurs and to rid the solution of any aggregates, $A\beta(1-28)$ and $A\beta(1-42)$ samples were routinely prepared using a similar protocol developed by Teplow and coworkers (42) and Zorowski and coworkers (43). Briefly, $A\beta$ stock solutions (0.5

mM) were prepared daily by dissolving the lyophilized A β in 5 mM NaOH. This was followed by sonication for 1 min. The as-prepared solutions were centrifuged at 13,000 rpm for 30 min and the supernatants were pipetted out for further dilutions. During the relatively short voltammetric and MS measurements, A β (1–16) and A β (1–28) were not found by atomic force microscopy to aggregate considerably, whereas small amounts of oligomers of A β (1–42) were observed. By decreasing the A β (1–28) concentration and adding 10% dimethylsulfoxide (DMSO) into the A β (1–42) solution, aggregation in both cases was avoided or significantly retarded.

Electrochemical measurements

All the electrochemical experiments were performed on a CHI 832 electrochemical workstation (CH Instruments, Austin, Texas) using a home-made plastic electrochemical cell. A glassy carbon disk electrode and a platinum wire were used as the working and counter electrodes, respectively. The reference electrode was Ag/AgCl and all the potential values are reported with respect to this electrode unless otherwise stated. Prior to each experiment, the glassy carbon electrode was polished with diamond pastes of 15 and 3 μ m and alumina pastes of 1 and 0.3 μ m in diameter (Buehler, Lake Bluff, IL). The electrolyte solution was a 10 mM phosphate buffer (pH, 7.4) containing 0.1 M Na₂SO₄. Although it has been noted that different buffer solutions may affect A β /Cu(II) binding and the subsequent H₂O₂ generation, we did not test other buffers because H₂O₂ had been detected in phosphate buffer containing A β and Cu(II).⁽³⁷⁾ Thus, the voltammetric studies in such an electrolyte solution are more relevant to the elucidation of the redox reactions of A β -Cu(II) and H₂O₂ generation. For A β (1–16) and A β (1–28), aliquots of A β from stock solutions were diluted with the phosphate buffer to desired concentrations. For voltammetric studies in the presence of Cu(II), these A β solutions were spiked with the Cu(II) stock solution to different A β /Cu(II) molar ratios. For voltammetric studies of A β (1–42), the same procedures were employed except that the 10 mM phosphate/0.1 M Na₂SO₄ (pH 7.4) solution containing 10% dimethylsulfoxide was chosen to retard the rapid aggregation of A β (1–42).

Detection of hydrogen peroxide

Based on the voltammetric data (*cf.* Results Section), the electrode potential was held at 0.07 V to reduce the A β /Cu(II) complex. Possible H₂O₂ generation was monitored using the Fluoro H₂O₂ detection kit (Cell Technology Inc., Mountain View, CA). In the presence of H₂O₂, 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) is oxidized to a fluorescent product, resorufin. This reaction is rapidly catalyzed by peroxidase in a homogeneous solution. Briefly, 50 μ L of the sample solution was added to 50 μ L aliquot of the reaction cocktail, which contained 100 μ L of 10 mM ADHP, 200 μ L of 10 U/mL horseradish peroxidase, and 4.7 mL of reaction buffer. The mixture was then incubated at room temperature in dark for 10 min. Subsequently the fluorescence intensity of resorufin was measured at an excitation wavelength of 550 nm with a Cary Eclipse Spectrofluorometer (Varian, Inc, Palo Alto, CA). By comparing the fluorescence intensity of resorufin of the sample solution to that of the control, H₂O₂ can be detected.

Electrospray ionization-Fourier transform ion cyclotron mass spectrometry (ESI-FTICR-MS)

The ESI-FTICR-MS experiments were conducted on an IonSpec FT-ICR mass spectrometer equipped with a 4.7-Tesla superconducting magnet (IonSpec Inc., Lake Forest, CA) and an LTQ linear-ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) operated in the high-resolution, “ultra zoom scan” mode. For the MS measurements, A β was first dissolved in a water/methanol solution (50/50 volume ratio) to yield a 50- μ M A β stock solution. Aliquots were then diluted with the water/methanol solution to a final concentration of 5 μ M. The A β solution and A β solution spiked with CuCl₂ at different

molar ratios were introduced to and analyzed by MS. The typical mass resolving power for the FTICR-MS is approximately 200,000.

RESULTS

Copper binds A β at 1:1 molar ratio and the oxidation state of copper is +2

The coordination chemistry between Cu(II) and A β has been investigated by various techniques, such as circular dichroism (CD) spectroscopy (34, 35), electron spin resonance (ESR) (34, 35, 38) and nuclear magnetic resonance (NMR) (34, 39). While all the studies indicate that Cu(II) can be incorporated mainly in the hydrophilic domain (residues 1 through 16), contradictory results have been reported regarding the coordination stoichiometry, the ligands involved, and the oxidation state of copper. A few papers suggested that A β -Cu(II) binding ratio is 1:2 (29, 34), whereas other papers showed that the ratio should be 1:1 (38, 39). MS can provide evidence for the number of copper ions bound per A β molecule. ESI-MS is particularly advantageous in that the soft ionization by ESI generates multiply charged species of proteins or peptides while keeping the molecular ions intact. Formation of multiply charged species also decreases the mass-to-charge ratios, shifting molecular ions of large biomolecules to the mass range readily accessible by most mass analyzers (44). Furthermore, when the MS employed has a high resolving power, the analysis of isotopic peaks affords an opportunity to deduce the oxidation state of the ligated metal ion. To our knowledge, the use of ESI coupled with high-resolution MS to study A β -metal ion binding has not been reported.

The predominate peaks in the ESI-FTICR mass spectra indicate that A β and its copper complex are of the 3+, 4+, and 5+ charge states, with the 4+ charge state being the most abundant. Figure 1 shows the m/z range covering the peaks corresponding to the 4+ charge states of free A β (1–28) and its copper complex. Clustered around m/z 816 and m/z 832 are the various isotopic peaks of the quadruply charged A β (1–28) and A β (1–28)-copper ion complex, respectively. An ESI-FTICR mass spectrum of A β (1–28) in the absence of Cu²⁺ exhibited peaks at around m/z 816 (See Figure S1 in the Supporting Information). The difference in the mass between the monoisotopic peaks of free A β (1–28) and A β (1–28)-Cu complex is $4 \times (831.387 - 816.142) = 60.980$ Da. Since the monoisotopic A β (1–28)-Cu(II) complex has two less protons, the actual mass difference is $60.980 + 2 = 62.980$, which is close to the nominal mass of a copper ion. Therefore, the ESI-MS result supports that the binding stoichiometry between A β (1–28) and Cu is 1:1. Further increasing the molar ratio between Cu(II) and A β (1–28) (up to 10) in the solution only changed the relative intensities of the free A β (1–28) and A β (1–28)-Cu(II) peaks, but did not create other peaks of different binding stoichiometries (e.g., 1:2 or 2:1).

The oxidation number of the copper ion in the complex can be determined from the measured m/z values of the 4+ ion of the complex. In this respect, if Cu(I) is involved in the formation of the complex, the complex will need to be associated with three protons to yield a 4+ ion; on the other hand, if Cu(II) is present in the complex, it would only require two protons to give a quadruply charged ion. The oxidation number of copper ion present in the complex can, therefore, be determined by comparing the experimentally measured m/z values of the isotope clusters for the quadruply charged complex with the calculated m/z values for the complexes that are associated with either Cu(I) or Cu(II) (see Table 1). Clearly, the deviations between the measured and calculated m/z values for the Cu(II)-complex are 15–23 ppm, which are markedly smaller than the 280–290 ppm deviations found for the differences between the measured and calculated m/z values for the Cu(I)-complex. These results, therefore, strongly support that the charge state of copper ion in the A β (1–28)-copper complex is 2+. Similar analysis of the isotope cluster peaks for the 3+ ion leads to the same conclusion (Table 1).

It has been proposed that Cu(II), in the presence of dissolved oxygen, could alter the redox state of A β (1–42), possibly by oxidizing Met-35 into its sulfoxide or sulfone analogs (30, 35, 36). Thus, since A β (1–28) does not comprise the methionine residue, it is conceivable that Cu(II) remains unchanged upon binding to A β (1–28). However, there has been a lack of direct spectroscopic evidence for chemical modifications of methionine even in A β (1–42) (except for samples produced under extreme conditions such as laser photolysis (45) or from autopsy (28)). It has been further contended that Met-35 oxidation could change the Cu(II) center to Cu(I), which remains coordinated by A β (1–42) (28, 35). If the methionine residue were chemically modified or the copper ion oxidation state were changed, the mass spectra of A β (1–42) treated with Cu(II) would contain peaks showing the addition of oxygen or different m/z values for the isotope clusters (*vide supra*).

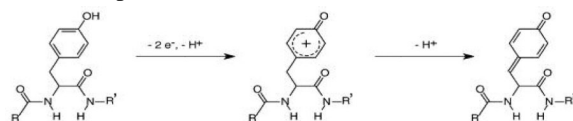
To verify whether the ligated Cu(II) could oxidize methionine, we collected mass spectra by spraying a solution containing A β (1–42) and Cu(II). Figure 2 is a representative mass spectrum in the mass range encompassing the 4+ charge states of A β (1–42) and A β (1–42)-copper ion complex. In Figure 2, peaks corresponding to both free A β (1–42) and A β (1–42)-Cu(II) complex were observed, but any peaks associated with oxidized A β (1–42) were absent. This observation strongly suggests that, under the present experimental conditions, A β (1–42) itself cannot be oxidized by Cu(II). This observation is further supported by our voltammetric data (*vide infra*). A detailed analysis of the positions of the peaks corresponding to the 4+ and 5+ charge states again indicates that Cu(II) was not reduced (Table 2), consistent with the fact that the methionine residue was not chemically modified. Our results are also in good agreement with the report from the Zagorski group who conducted NMR measurements of A β (1–40) in the presence of Cu(II) and demonstrated that Met-35 oxidation and Cu(II) reduction did not occur (39). We conducted two additional experiments to show that A β (1–42)-Cu(II) complex is indeed formed by mixing Cu(II) with A β (1–42). First, an FT-ICR mass spectrum collected from spraying a solution containing A β (1–42) did not exhibit peaks around m/z 1145 (Figure S2), indicating that A β (1–42)-Cu(II) does not exist in the absence of Cu(II). The second experiment involves the confirmation of the A β (1–42)-Cu(II) complex peak with ESI-MS measurement on a linear-ion trap mass spectrometer. As can be seen in Figure S3 and Table S1 in the Supporting Information section, the peaks around 1145 are even more pronounced. Due to the greater mass resolving power of FTICR-MS and to be consistent in comparing the spectra among various species, we focused mainly on the FTICR-MS results.

Redox potentials of A β -Cu(II) complexes

Since the proposed copper binding sites reside in the 16-amino acid N-terminal segment of A β (1–42), we first examined the redox behavior of A β (1–16) with or without Cu(II). Figure 3 is an overlay of voltammograms of A β (1–16) in a Cu(II)-free solution (thick solid curve), free Cu(II) (dashed curve), and A β (1–16) in the presence of an equimolar amount of Cu(II) (thin solid curve). To examine the effect of oxygen on the redox process involving A β (1–42), we also bubbled O₂ into the mixture of Cu(II) and A β (1–16) and then recorded the voltammogram (dash-dot-dash curve). Notice that the voltammogram of A β (1–16) (thick solid curve) is rather different than that of Cu(II) (dashed curve), which produced an irreversible reduction peak starting from around 0.0 V. This peak can be attributed to the catalytic reduction of oxygen by electrogenerated Cu₂O or CuOH layer (46). Due to the presence of trace amount of oxygen in solution, the follow-up catalytic oxidation of Cu₂O or CuOH prevents Cu₂O or CuOH from being further reduced to Cu(0). Therefore no copper stripping peak was observed. By thoroughly purging the solution with N₂, a copper stripping peak was observed in the CV (Figure S4). The comparison of the voltammogram of A β (1–16)-Cu(II) (thick solid curve in Figure 3) to the Cu(II) reduction voltammogram shows that the reduction current of A β (1–16)-Cu(II) complex is smaller, even though the concentration

of the former is twice as high as that of the latter. This suggests that Cu(II) is complexed, since the A β (1–16)-Cu(II) is expected to have a smaller diffusion coefficient. For the mixture of A β (1–16) and Cu(II), a pair of quasi-reversible waves (thin solid curve) was observed with an oxidation peak at 0.17 V and a reduction peak at ca. 0.0 V. The peak currents decrease inversely with the A β (1–16):Cu(II) molar ratio (Table S2). Apparently, catalytic reduction of O₂ by electrogenerated Cu₂O or CuOH occurs at a different potential (< -0.1 V) than the A β (1–16)-Cu(II) complex. We thus assign this pair of waves to the redox reactions between the A β (1–16)-Cu(II) and A β (1–16)-Cu(I) complexes and report the reduction potential of A β (1–16)-Cu(II) to be 0.085 V vs. Ag/AgCl. We noticed that this potential value is similar to that of histidine-rich peptide-Cu(II) complexes (46–48). The fact that the reduction peak is higher than the oxidation peak suggests that a catalytic follow-up reduction (49) takes place after A β (1–16)-Cu(II) is reduced. Another noteworthy point is that the oxidation peak became more pronounced as the potential scan rate is increased (data not shown). Furthermore, in the dash-dot-dash curve of Figure 3 the reduction peak was found to increase at the expense of the oxidation peak, suggesting the catalytic nature of the reaction and the involvement of O₂.

The A β (1–16) voltammogram exhibited a small and broad oxidation peak at the anodic side of Figure 3 (ca. 0.78 V). This oxidation peak is irreversible and disappears after the first cycle, and was observable even when Cu(II) was present (Note: the thin solid and dash-dot-dash curves in Figure 3 were acquired during the second cycle). Thus the peak must originate from the oxidation of a segment of the peptide and is irrelevant to the Cu(II) redox reaction. The peak potential is the same as that of A β (1–42) reported by and attributed to the Tyr-10 residue by Vestergaard et. al. (50). That the voltammogram of tyrosine solution alone (inset in Fig. 3) also showed an oxidation peak at a similar potential confirms our assignment. A tyrosine residue could undergo a two-electron, two-proton oxidation reaction (51), as depicted below:



In view that the hydrophobic segment in the A β -Cu(II) complex may affect the A β redox behavior, we conducted voltammetric experiments on A β (1–28) in the absence and presence of Cu(II). Superimposed in Figure 4 are the voltammograms of A β (1–28) alone (dashed curve) and A β (1–28) in the presence of an equimolar amount of Cu(II) (solid curve). The remarkable resemblance in the peak shapes between A β (1–16)-Cu(II) and A β (1–28)-Cu(II) indicates that the more hydrophobic segment in A β (1–28) does not affect the redox potentials of both the Cu(II) center and the Tyr-10 residue. In addition, A β (1–28) (dashed curve) has similar redox behavior as A β (1–16). The main difference is that A β (1–28) is more prone to aggregation and causes a more significant adsorption onto the electrode surface. To alleviate this effect, we decreased the concentration of A β (1–16) for the acquisition of the voltammograms in Figure 4. Again, analogous to A β (1–16), A β (1–28) displayed an oxidation peak at ca. 0.78 V, which can be ascribed to the Tyr-10 oxidation.

To clearly illustrate the effect of O₂ on the magnitude of the A β -Cu(II) reduction peak, we subtracted the voltammograms of A β (1–28)/Cu(II) in a N₂-purged solution from those of the same solution saturated with air (Figure 5). The plateaus at slow scan rates (e.g., 0.02 V/s) developed into peaks at higher scan rates. These features are again typical of an electrocatalytic reduction(49) and are also consistent with our findings related to A β (1–16) (*vide supra*). For oxygen to be catalytically reduced, oxygen molecules need to diffuse to and become associated with the newly electrogenerated A β (1–28)-Cu(I) center. We should note that, without background subtraction, the dependence of the steady-state current on O₂

purged into the solution is not as obvious. This suggests that the O₂ reduction is a diffusion-controlled process. Thus, O₂, which is hydrophobic, appears to have interacted more strongly with the hydrophobic segment inherent in A β (1–28) and consequently its movement towards the A β -Cu(II) center is retarded (52).

We then extended the same approach to the study of A β (1–42) and its complex with Cu(II). Since A β (1–42) has the strongest propensity to form aggregates, we added a small amount of dimethylsulfoxide (see Experimental) into the solution to impede the A β aggregation and to improve the reproducibility of the experiment. Several points can be extracted from the voltammograms in Figure 6. First, in the cathodic range (solid curve), the redox behavior of the A β (1–42)-Cu(II) is highly comparable to those of the A β (1–16) and A β (1–28) counterparts. Accordingly, we assigned the peak at \sim 0.02 V to the reduction of A β (1–42)-Cu(II) to A β (1–42)-Cu(I) and conclude that the redox potential of the Cu(II) center remains largely unaffected by the length of the hydrophobic segment of A β . Although the reduction current is much greater than the oxidation current during scan reversal, purging the solution with oxygen and nitrogen led to little change in the peak heights. This is in contrast to the cases of the shorter A β variants (*cf.* Figures 3 and 5). The insensitivity of reduction or oxidation current of the A β (1–42)-Cu(II) complex to the oxygen concentration could be caused by the slow coordination of oxygen molecules with the A β (1–42)-Cu(I) center due to the greater steric hindrance imposed by the longer length and the greater hydrophobicity of the A β (1–42) strand.

In the anodic range of the CV, a pair of small peaks, with the reduction peak potential at ca. 0.48 V and the oxidation peak potential at ca. 0.6 V, were observed. Again, these peaks are independent of Cu(II), since the peaks in solid and dashed curves in Figure 6 are almost congruent in this range. These peaks were assigned to the redox reaction of the Cu(II) center to its Cu(I) counterpart by Huang et al. (35). However, given its close proximity to the oxidation peak of Tyr-10 (*cf.* inset of Figure 3 and the reported value (50)) and its independence of Cu(II) in the solution, we feel that the assignment to the Tyr-10 redox reaction is more plausible. The greater reversibility of tyrosine oxidation in A β (1–42) with respect to those of A β (1–16) and A β (1–28) may be partially contributed by the adsorbate nature of A β (1–42). Moreover, it is well known that adsorption of redox species onto the electrode tends to yield redox peaks whose positions are different than those originated from the soluble species (49).

A β -Cu(I) complex catalyzes the reduction of oxygen to hydrogen peroxide

As mentioned above, the reduced form of the A β -Cu(II) complex can catalyze oxygen reduction, producing H₂O₂ as one of the possible products (30, 35, 37). To directly link the redox state of the Cu-containing A β complex to the H₂O₂ production, we carried out spectrofluorometric detection of H₂O₂ from A β (1–16)/Cu(II), A β (1–28)/Cu(II), and A β (1–42)/Cu(II) solutions that had been subject to controlled potential electrolyses.

After electrolyses of these solutions at 0.07 V for a period of time, the solutions were analyzed by a hydrogen peroxide detection kit. We chose 0.07 V because it is at the plateaus of the electrocatalytic reduction peaks of all the A β variants (thin solid curve in Figure 3 and solid curves in Figures 4 and 6). At this potential the complication of possible catalytic reduction of oxygen by unbound copper ion can also be excluded (*i.e.*, the potential is more positive than the onset potential of free Cu(II) reduction; *cf.* the dashed curve in Figure 3). As shown in Fig. 7, for all three A β variants, the fluorescence peak heights increase with the electrolysis time. Moreover, the resorufin fluorescence peaks are significantly greater than those in the buffer and Cu(II) solutions. Notice that the fluorescence intensity from the electrolyzed Cu(II) solution was not different than that from the buffer solution, again suggesting that the catalytic reaction involving Cu(II) and O₂ occurs at a different potential.

A β -Cu(II) complex solutions without being electrolyzed also exhibited similar responses to those of the buffer solution. All these observations indicate that it is the reduced A β -Cu(II) complex that is responsible for the H₂O₂ production.

A point particularly worth noting is that the fluorescence intensity increases inversely with the length of the A β variants. This explains why the catalytic reduction current becomes less sensitive to the concentration of dissolved oxygen as the A β strand becomes longer. Such a consistency reinforces our earlier contention that the movement of oxygen to the electrogenerated Cu(I) center is dependent on both the length and hydrophobicity of the A β strand.

DISCUSSION

As mentioned in the Introduction, it is generally believed that the Cu(II) binding domain in A β is within the segment comprising residues 1–14 (30, 34, 38, 53), with His-6, His-13, and His-14 having the strongest metal binding affinities. Our ESI-FTICR-MS study has demonstrated that the binding ratio between Cu(II) and A β (1–28) or A β (1–42) is 1:1, which is consistent with results by Karr *et al.* (38) and Garzon-Rodriguez *et al.* (54). We did not find other binding ratios as reported by others (29, 34). The ESI-FTICR-MS results are also in good agreement with our voltammetric data, which showed that the reduction current of A β (1–16)-Cu(II) complex leveled off once the molar ratio between Cu(II) and A β (1–16) had exceeded 1:1.

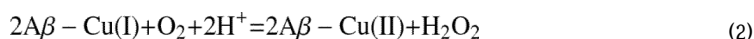
The reduction potentials of A β -Cu(II) deduced from Figures 3, 4, and 6 allowed us to gain insight about the factors or redox species that could be involved in the reduction of the A β -Cu(II) complex in the cellular milieu. Thus, for example, to reduce A β (1–42)-Cu(II), a species must have a reduction potential that is more negative (cathodic) than 0.08 V *vs.* Ag/AgCl or 0.28 V *vs.* normal hydrogen electrode, NHE (*cf.* Figure 6 and Table 3). Since the Tyr-10 redox peak appeared at ~0.78 V, we conclude that Tyr-10 cannot reduce A β (1–42)-Cu(II) *in vitro*. Several reports have suggested that Tyr-10 participates in the metal ion coordination. A Raman analysis of samples from deceased AD patients' brains indicated that the tyrosine residue in A β (1–42) was oxidized (28). Thus, it is clear that the A β -Cu(II) complex is not the sole species responsible for the Tyr-10 oxidation, and other cellular species or processes must be involved. Another amino acid residue that is susceptible to oxidation and has been implicated in the reduction of A β -Cu(II) and generation of ROS such as H₂O₂ is Met-35 (30, 45, 55). However, from the ESI-FTICR-MS data, we did not find ions corresponding to oxidized or chemically modified (e.g., oxygenated adducts) A β (1–42) and/or A β (1–42)-Cu(II). Previously the peak potential for the Met-35 oxidation to its radical cation has been reported to exceed 1.3 V *vs.* Ag/AgCl in aqueous solution (56). Such a value is almost 1.2 V more *positive* than the A β -Cu(II) reduction potential, suggesting that Met-35 would not be a viable reductant under the *in-vitro* condition. Moreover, in the natively unstructured A β (1–40) or A β (1–42) monomer, there is a wide separation between the metal center (close to the N terminus) and Met-35 (in the vicinity of the C terminus), which is unfavorable for facile ET. Therefore, based on the MS and voltammetric data, we also ruled out the possibility that Met-35 had undergone oxidation reactions with Cu(II) in solution and/or Cu(II) in the complex without involving other cellular species. This conclusion is in agreement with what was found by Hou and Zagorski (39).

In solution, A β binds Cu(II), and under an externally applied potential, the resultant A β -Cu(II) complex can be reduced to A β -Cu(I), once the cathodic scan has passed ~0.080 V:



Notice that the reduction potential for A β -Cu(II) complex is quite different than that reported by Huang et al.(35). As the redox potentials of copper complexed by histidine-containing peptides are in the range between -0.1 and 0.1 V (46–48, 57, 58), we believe that the redox potential around 0.1 V is more reasonable.

In the presence of dissolved oxygen, the electroreduced A β -Cu(I) center could react with O₂ in solution, producing H₂O₂:



The above reaction is responsible for the O₂-dependent reduction peaks exhibited in Figures 3–6. As the potential for the A β -Cu(II)/A β -Cu(I) (0.28 V vs. NHE) is lower than that for O₂/H₂O₂ (0.295 V vs. NHE, Table 3), reaction (2) is thermodynamically allowed. A much higher potential for A β -Cu(II)/A β -Cu(I), as that reported in Ref (35) (0.55 V vs. Ag/AgCl), however, would cause Reaction (2) to proceed in the reverse direction (i.e., H₂O₂ oxidation). Thus, our measured potential value explains the catalytic reduction of O₂ to H₂O₂ better. We should point out that the present study cannot rule out the possibility that some O₂ might be reduced to H₂O in a similar fashion to that catalyzed by Cu(II)-containing enzymes such as laccase.(59) However, the A β -Cu(II) complexes are not considered to be “enzymes”, and the direct four-electron reduction of O₂ to H₂O typically involves multiple copper centers.(59) Furthermore, based on the H₂O₂ detected and other published data (37), the reactions outlined above should constitute the major mechanism. To further confirm the proposed mechanism, we simulated the kinetics of ET reaction and the follow-up catalytic oxygen reduction. The simulated voltammogram, overlaid with the experimentally measured one, is provided in the Supporting Information, together with the parameters used for the simulation. The heterogeneous ET rate constant for A β (1–16)-Cu(II) at the electrode was estimated to be about $5 \times 10^{-4} \text{ s}^{-1}$ and the follow-up catalytic reaction rate constant was deduced to be about $1000 \text{ M}^{-1} \text{ s}^{-1}$. The reasonable agreement between the experimental and simulated voltammograms suggests that the proposed mechanism is quite likely. Moreover, the rather high reaction rate constant deduced for equation 2 indicates that there is a strong tendency for the reduced A β -Cu(II) complex to catalytically reduce O₂ to H₂O₂. The rapid depletion of ascorbic acid (Figure S6), initiated by the A β -Cu(II) complex, indicates the fast turnover rate (*vide infra*).

Based on our spectrofluorometric measurements (Figure 7), it is clear that a prerequisite for H₂O₂ generation is the conversion of A β -Cu(II) to A β -Cu(I). The difference in the dependence of the catalytic peak currents on the O₂ content in solution among the three A β -Cu(II) complexes implies that in AD patients, the amount of H₂O₂ produced in Reaction (2) is likely to be dependent on the A β sequence and length. It is generally known that A β (1–42) has a much stronger tendency to aggregate than A β (1–40), and the A β (1–42) content in senile plaques of AD patients is disproportionately high (60). Given the relatively high affinity of A β species towards metal ions, it has been shown that A β and its aggregates, upon complexation with redox-active metal ions such as Cu(II) and Fe(III), can generate H₂O₂ (35, 37). The level of H₂O₂ can be sufficiently high to cause cell death (37). Based on the aforementioned dependence of H₂O₂ amount on the strand length of A β species, we postulate that there might exist an alternative process responsible for the slow onset of AD symptom. If Reactions (1) and (2) are at work, the amount of H₂O₂ generated must be dependent on all the reactants (i.e., A β , Cu(II), and O₂). Since in senile plaques the content of A β and concentration of Cu(II) are high and the function of brains requires a constant supply of large amounts of O₂ (61), one would expect that H₂O₂ concentration be substantially high. In addition to metabolic degradation of species that can potentially generate ROS and scavenging of ROS (including H₂O₂) by antioxidants, we think that the

slow diffusion of O₂ to the metal center in the Cu(II)-Aβ complex should have dramatically hindered the H₂O₂ generation. Regarding the dependence of H₂O₂ generation on the Aβ sequence, it is interesting to note that mice do not develop AD (62) and its Aβ is mutated at two of the metal binding sites, *viz.*, His-13 and Tyr-10 (replaced by Arg and Phe, respectively). The decreased binding affinity of such a mutated Aβ towards Cu(II) leads to a dramatically lower H₂O₂ production (37). In the case of familial AD, the Aβ is point-mutated, which causes early onset AD. Interestingly, none of the mutations occurs in the metal binding region. These facts strongly suggest the important roles of metal binding and H₂O₂ production in the pathogenesis of AD.

During the CV scan reversal, Aβ-Cu(I) molecules that have not been completely oxidized by O₂ in solution (*cf.* thin solid curve in Figure 3 and thick solid curves in Figures 4 and 6), will be reoxidized:



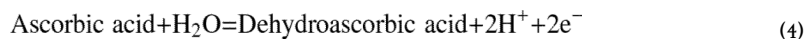
The concentration of Aβ-Cu(I) depends on the catalytic turnover rate, which is in turn governed by the accessibility of the Aβ-Cu(I) complex to oxygen and the oxygen concentration in solution. A declined oxygen level in solution results in the availability of more Aβ-Cu(I), which leads to a greater oxidation current during the reverse potential scan (*cf.* Figure 3). The function of metalloproteins in a biological process involving O₂ requires the binding to and activation of diatomic O₂ molecules by the metal center(s). Recent studies have captured the O₂-containing intermediates (63, 64). Similarly, the catalytic reduction of O₂ by Aβ-Cu(I) also entails the binding of O₂ to the copper center. It is conceivable that a longer Aβ segment will increase the steric hindrance for O₂ binding to the copper center and decrease the catalytic turnover rate. This is in accord with our experimental results. Given the slow and gradual development of AD, our contention appears to be plausible.

As stated in previous sections, a prerequisite for the catalytic production of H₂O₂ is the reduction of Aβ-Cu(II) to Aβ-Cu(I) and the subsequent binding of O₂. This process would not occur *in vitro* without a suitable electron donor. However, Aβ-Cu(II) complex could be reduced *in vivo* by redox reactions involving easily oxidizable cellular species (*i.e.*, antioxidants). There exists a wide range of intracellular and extracellular electroactive species and antioxidants, some of which, together with their reduction potentials, are listed in Table 3.

Species whose reduction potentials are more positive than the Aβ-Cu(II) reduction potential (0.280 V) are incapable of reducing Aβ-Cu(II). These include some important redox-active neurotransmitters such as dopamine, epinephrine, and norepinephrine. This is interesting, since, to our knowledge, there is no evidence directly linking the appearance of Aβ aggregates or senile plaques to the deficiency of these neurotransmitters. It is a well known fact that another neurodegenerative disease, Parkinson's disease, is directly related to dopamine deficiency and loss of dopaminergic neurons. The species listed in Table 3 that could (*i.e.* potentials more negative) reduce the Aβ-Cu(II) complex can be classified into three categories: extracellular species (*e.g.*, ascorbic acid), intracellular redox buffers (*e.g.*, glutathione), and membrane-bound redox species (especially those associated with mitochondria that governs cellular respiratory processes and bioenergetics).

Outside the neuronal cells, reduction of Aβ-Cu(II) to Aβ-Cu(I) by ascorbic acid and vitamin B12 is thermodynamically favored (Table 3). The reduced Aβ-Cu(I) can then catalyze the reduction of oxygen to form hydrogen peroxide. The as-formed hydrogen peroxide can either react further with antioxidants such as ascorbic acid and vitamin B12 or attack cell membrane and other organelles in brain. This certainly will deplete the antioxidants and

damage the cellular defense system, making the cells vulnerable. To verify that the A β -Cu(II) reduction by ascorbic acid is kinetically facile, we acquired CVs of ascorbic acid in the absence and presence of A β -Cu(II). As shown in Figure S6, without A β (1–16) and Cu(II) in the solution, an irreversible oxidation peak of ascorbic acid at *ca.* 0.42 V was observed. The irreversible reduction is due to the ring-closure reaction following the ET reaction, as shown in equation (4):



When A β -Cu(II) is present, the addition of an equimolar amount of ascorbic acid did not show the characteristic irreversible ascorbic acid oxidation peak. Only after the addition a few more molar equivalents did the oxidation peak appear in the first potential scan (Figure S6). Interestingly, the peak quickly disappeared in the second scan. When the solution was thoroughly purged, the ascorbic acid oxidation peak was observable regardless of the presence of A β -Cu(II). These observations thus demonstrate that ascorbic acid can rapidly reduce A β -Cu(II) to A β -Cu(I) and the resultant A β -Cu(I) catalyzes the oxygen reduction. The overall catalytic redox reaction cycle converts ascorbic acid to dehydroascorbic acid. The rapid depletion of ascorbic acid is also consistent with the high chemical reaction rate constant in the aforementioned kinetic simulation.

There have been many mechanisms suggesting that A β penetrates cell membranes and enters cytoplasm (65–67). A β has been shown to exist inside (22, 68–70) and outside of neuronal cells in AD-affected brains. In PC12 cells and human fibroblasts A β oligomers were found to adsorb onto the cell surface and became internalized into the cytosol (71, 72). Given the relatively high binding affinity of A β to Cu(II) (29), Cu(II)-bound A β monomers and oligomers should be capable of penetrating the neuronal cell membrane. In addition, it is purported that APP from which A β is cleaved can function as a copper chaperone, regulating the intracellular copper concentration and homeostasis (73). Inside the neuronal cells, the glutathione redox couple (GSH/GSSG), present at millimolar concentrations, has a high redox buffering capacity. The GSH/GSSG couple not only maintains the function of mitochondria (74) but also regulates the apoptosis of cells (75–79). From Table 3, the reduction of A β -Cu(II) complex to A β -Cu(I) by glutathione is favored thermodynamically and H₂O₂ produced via Reaction (2) could consequently disrupt the potentiation of the GSH/GSSG couple. The possible decline in the cellular glutathione may not only induce abnormal apoptosis but also cause dysfunction of mitochondria. Another intracellular species whose reduction potential is lower than A β -Cu(II) complex is pyruvate, which serves as the fuel for mitochondria-mediated metabolic process. As shown by the potential values in Table 3, a redox reaction between A β -Cu(II) and pyruvate is possible. Similarly, given the presence of a high concentration of O₂ in brain and particularly in neuronal cells (61), the catalytic reduction of O₂ to H₂O₂ by A β -Cu(I) is likely to occur. The H₂O₂ would subsequently oxidize more pyruvate molecules and cause a depletion of the cellular pyruvate concentration. As a result, ATP production is decreased and mitochondria are starved energetically. This process may have some relevance to the observed decline in the production of ATP in AD patients (80–83). In line with our suggestion, therapeutic treatments of AD patients with pyruvate derivatives have shown promises in alleviating AD symptoms (84).

It has been well documented that mitochondrial insufficiencies contribute significantly to the pathophysiology of AD and mitochondrial dysfunction is also a hallmark of AD (80, 85–87). However, the intricate relationship between AD and mitochondrial dysfunction awaits further elucidation. A β has been found to exist inside mitochondria and deposited on mitochondria membranes in AD brains (81, 88). Recent studies demonstrated that, in the

presence of Cu(II), A β inhibits the activity of cytochrome *c* oxidase in mitochondria (22–24, 82). This provides a possible link between the dysfunction of mitochondria and A β -Cu(II). The function of mitochondria relies on the uninterrupted ET processes in the cascade of events at the membrane and in the intermembrane space. As shown in Table 3, the redox reactions of A β -Cu(II) with nicotinamide adenine dinucleotide (NADH), flavin mononucleotide (FMN_{H2}), flavin adenine dinucleotide (FADH₂), Coenzyme Q (CoQH₂), cytochrome *b*, cytochrome *c*₁ and cytochrome *c* in the ET chain are all thermodynamically allowed. If there is a reaction of A β -Cu(II) with any of the species, the normal electron flow will be side-tracked, leading dysfunction of mitochondria and triggering a mitochondrial death pathway. We should note that mitochondrial dysfunction has been shown to be associated with the AD development (80, 85, 86, 89).

Overall, A β -Cu(II) complex can react with redox-active molecules essential to neuronal cells and present in biological fluids. Thus, the accurate measurements of the redox potentials of various A β -Cu(II) species and the confirmation of H₂O₂ generation by A β -Cu(I) complex provides new insight into AD pathogenesis and offers some possible interpretations about certain AD symptoms and the roles of metal ions in AD neuropathology.

CONCLUSIONS

We have studied, for the first time, the interaction of A β with Cu(II) by high-resolution mass spectrometry. It reveals that Cu(II) coordinates with A β in a 1:1 ratio. Independent of the methionine residue, the oxidation state of the copper center in the complex is 2+. The presumed reduction reaction of Cu(II) center to Cu(I) cannot occur *in vitro*. The redox chemistry of the complexes of A β (1–16), A β (1–28), or A β (1–42) with Cu(II) was systematically investigated by cyclic voltammetry and the redox potential for the reduction of the copper center was determined to be 0.08 V (vs. Ag/AgCl). The A β -Cu(I) electrogenerated was found to catalyze the reduction of oxygen and produce hydrogen peroxide. The remarkable similarity among the voltammetric behaviors of these three complexes excludes the possibility that methionine-35 can be oxidized by the Cu(II) center. In addition, we found that the diffusion of oxygen to the Cu(II) center is dependent on the peptide length (steric hindrance) and hydrophobicity. To our knowledge, this is the first work showing that A β -Cu(I) can be controllably generated and studied in the presence and absence of O₂. The implication of the redox properties of the complex is discussed in the biological context. Based on the redox potentials of A β , A β -Cu(II) complexes, and common redox-active biomolecules, a number of redox reactions could occur. As a result, antioxidants may be depleted, which may destroy the normal protective system against oxidative stress. Using voltammetry and digital simulation, we show that the thermodynamically allowed reduction of Cu(II)-A β (1–42) by ascorbic acid, is kinetically facile. The redox reactions of the A β -Cu(II) complex with species in the ET chain of mitochondria are also thermodynamically favorable. Although whether these reactions occur *in vivo* remain to be investigated, the possibility raises an important aspect that such reactions could sidetrack the normal electron flow in the respiratory process of mitochondria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AD	Alzheimer's disease
Aβ	β -amyloid
ADHP	10-acetyl-3,7-dihydroxyphenoxazine
APP	Amyloid precursor protein
CV	Cyclic voltammetry
ESI-FTICR-MS	Electrospray ionization-Fourier transform ion cyclotron mass spectrometry
ET	Electron transfer
Met-35	Methionine at position 35
MS	Mass spectrometry
ROS	Reactive oxygen species
Tyr-10	Tyrosine at position 10

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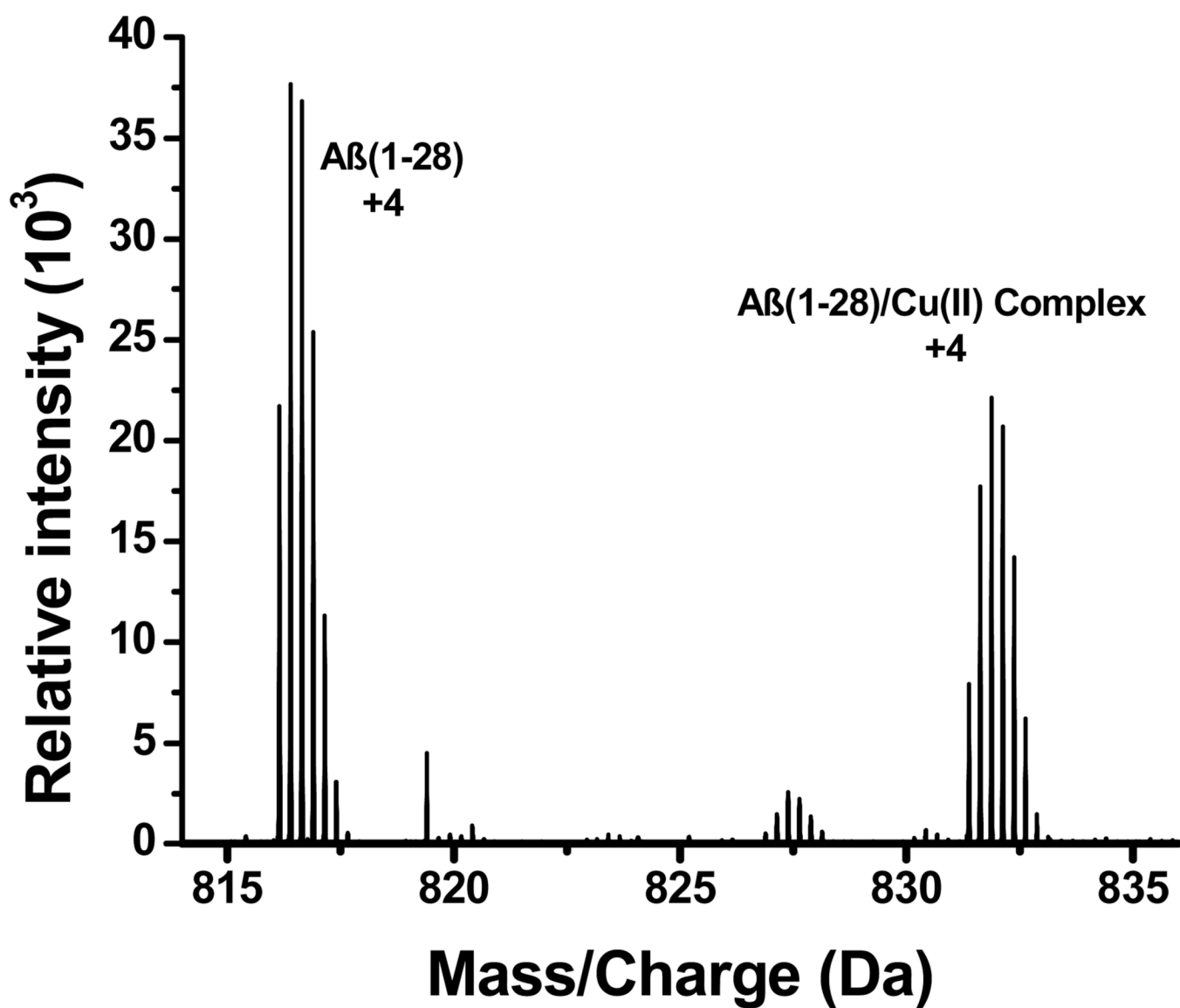


Figure 1.
Positive-ion ESI-MS of an Aβ(1–28)/Cu(II) mixture at 1:1 ratio.

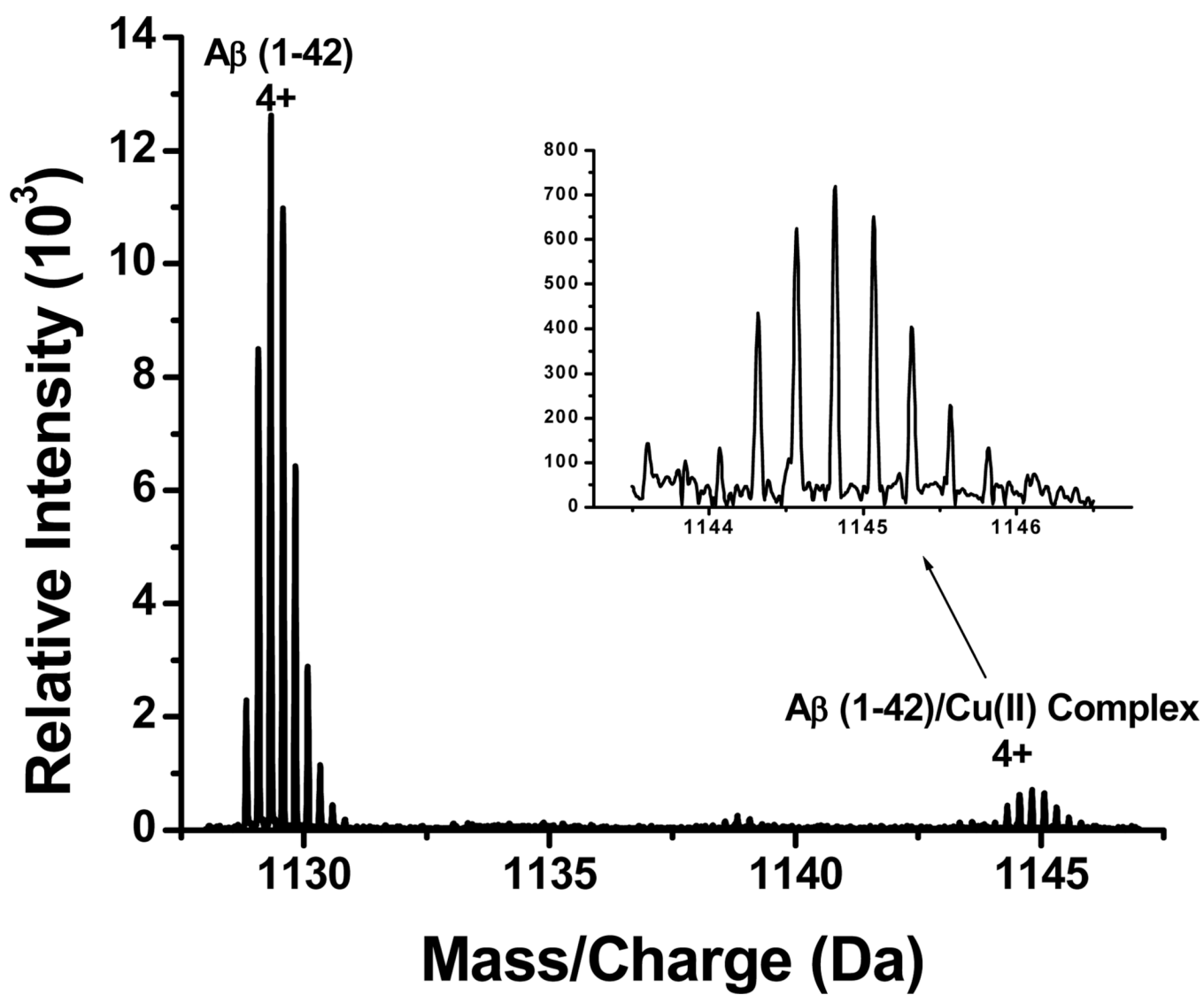


Figure 2. Positive-ion ESI-MS of an Aβ(1-42)/Cu(II) mixture at 1:1 ratio.

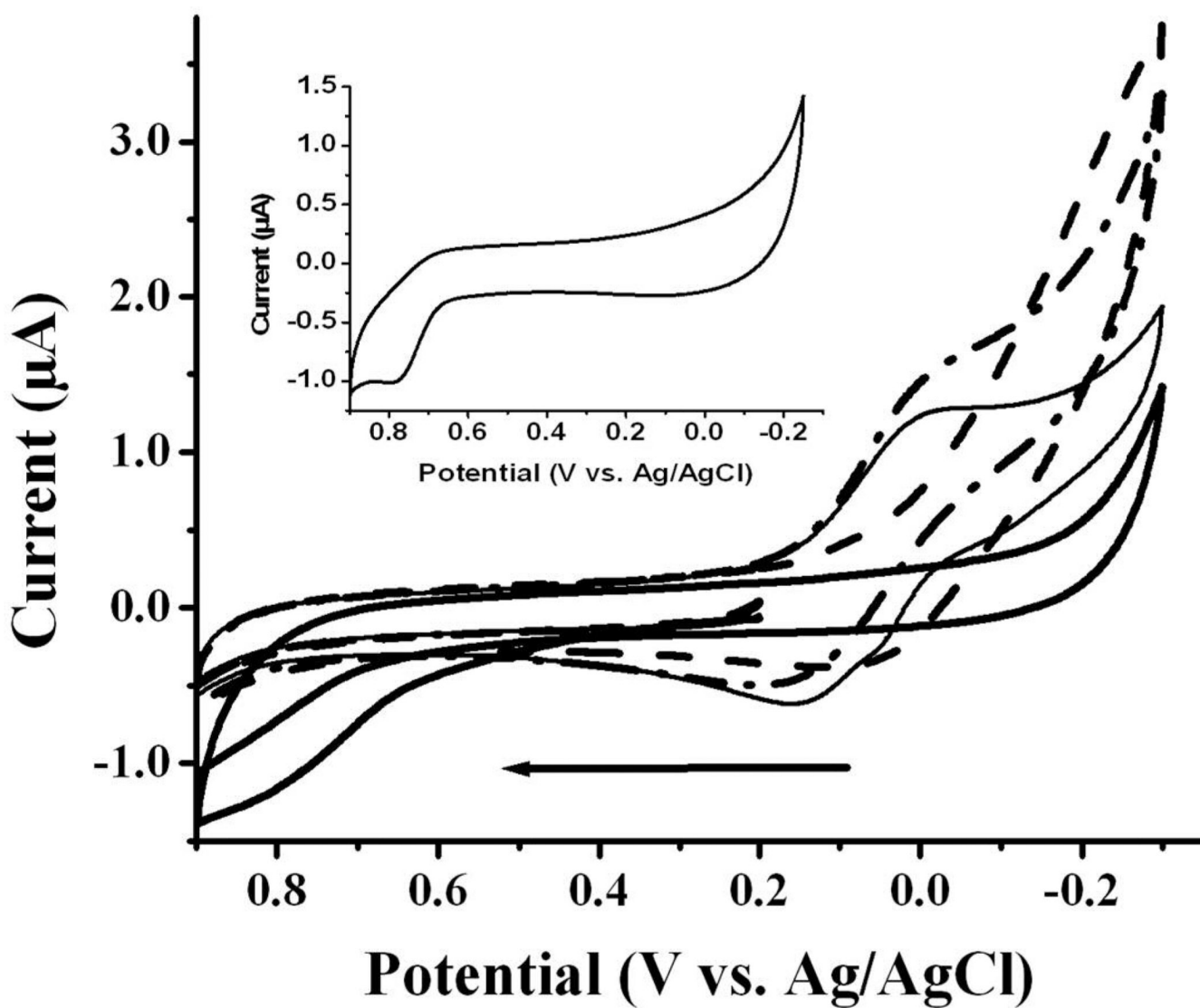


Figure 3.

The cyclic voltammograms of 200 μM Aβ (1–16) (thick solid curve), 100 μM Cu(II) (dashed curve), a mixture containing 200 μM Aβ (1–16) and 200 μM Cu(II) (thin solid curve) and an O₂-saturated Aβ (1–16)/Cu(II) mixture (dash-dot-dash). A voltammogram from a 50 μM tyrosine solution is shown in the inset. All solutions were prepared with a buffer containing 5 mM phosphate and 0.1 M Na₂SO₄ (pH 7.4) and data were obtained at a glassy carbon disk electrode with a diameter of 3 mm. The scan rate was 20 mV/s and the arrow indicates the initial scan direction.

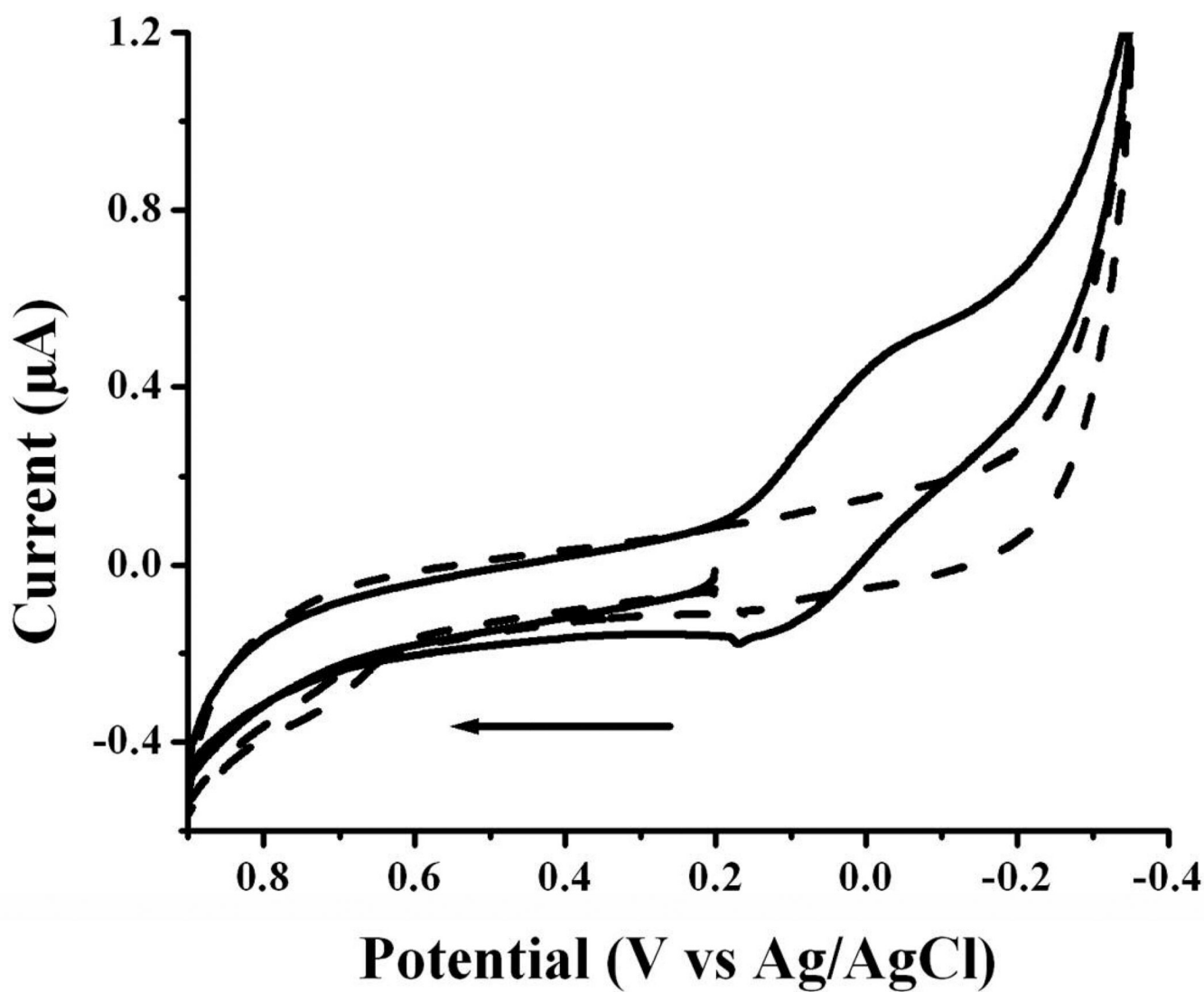


Figure 4. The cyclic voltammograms of 50 μM Aβ (1–28) (dashed curve) and a mixture of 50 μM Aβ (1–28)/50 μM Cu(II) mixture (solid curve) acquired at a glassy carbon disk electrode with a diameter of 3 mm at the scan rate of 20 mV/s. The arrow indicates the scan direction.

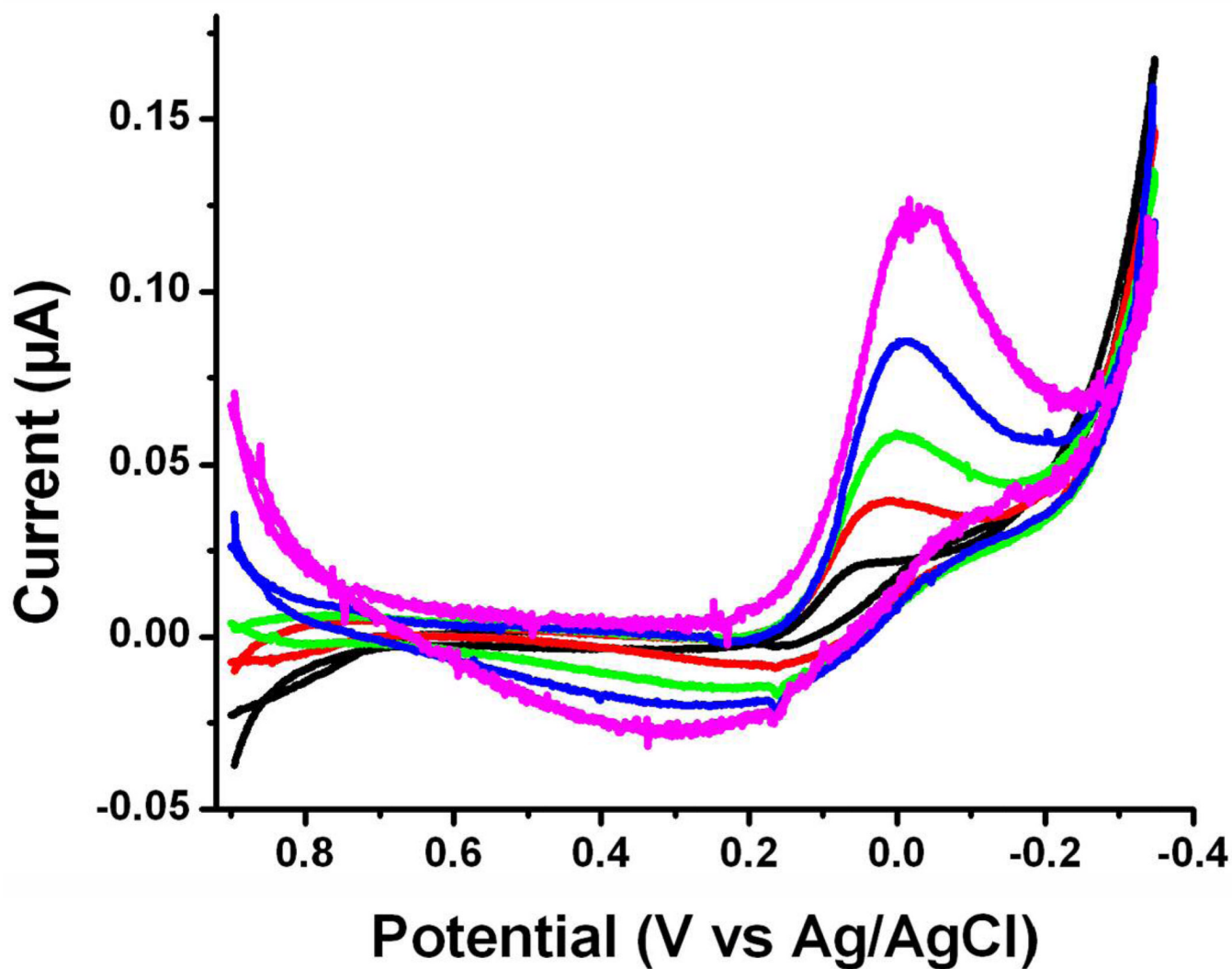


Figure 5. The cyclic voltammograms generated by subtracting voltammograms of a 1:1 mixture of A β (1–28)/Cu(II) in a N₂-purged solution from those of the same mixture in an air-saturated buffer solution at different scan rates, 0.01 V/s (black), 0.02 V/s (red), 0.04 V/s (green), 0.08 V/s (blue) and 0.16 V/s (purple). A glassy carbon disk electrode with a diameter of 3 mm was used as the working electrode.

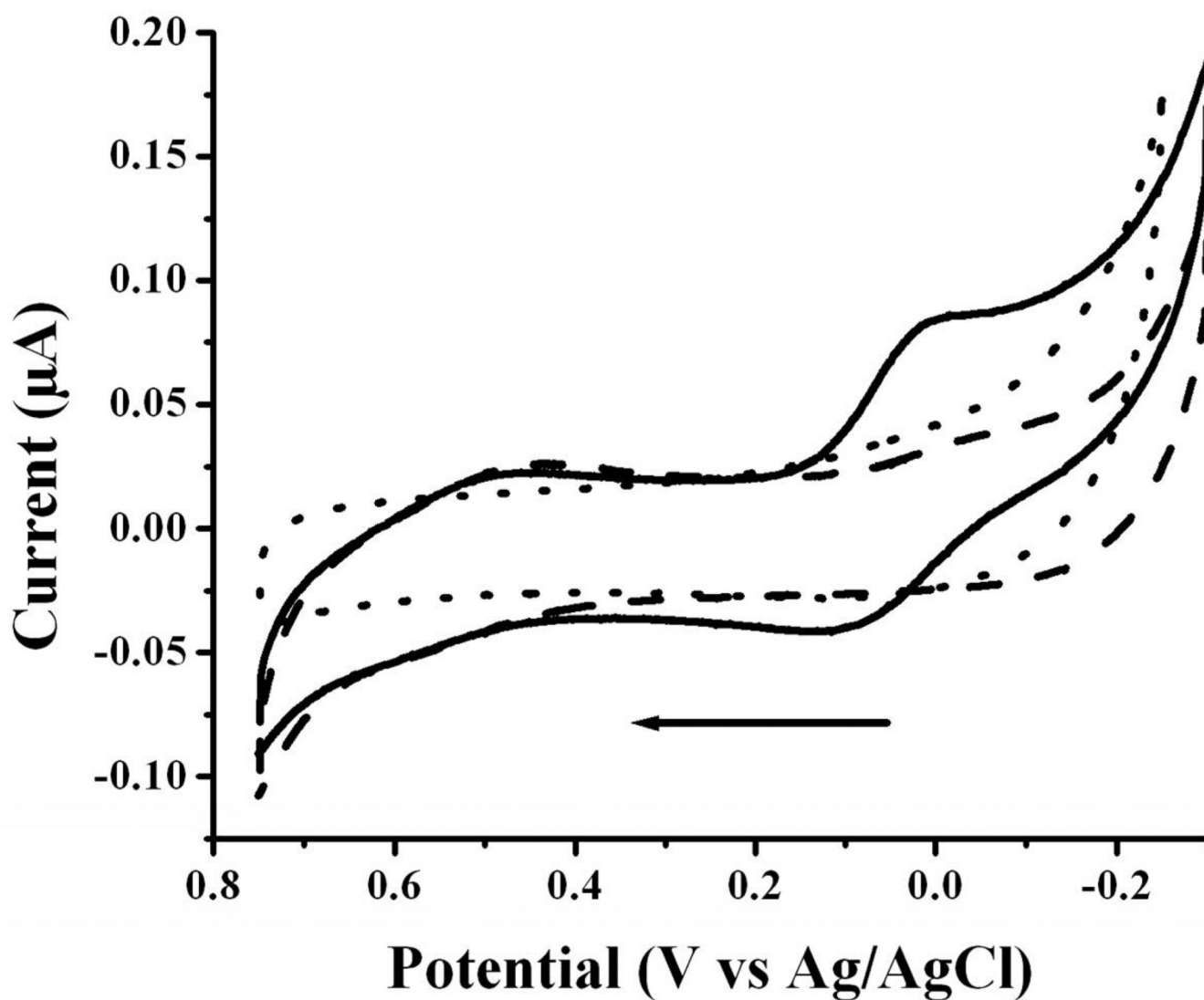


Figure 6. Cyclic voltammograms of 50 μM A β (1–42) (dashed curve) and a mixture of 50 μM A β (1–42) and 50 μM Cu(II) (solid curve) in a 5 mM phosphate buffer containing 0.1 M Na₂SO₄ and 10% dimethylsulfoxide or DMSO (pH 7.4). The background voltammogram was collected from the same electrolyte solution without A β (1–42) and Cu(II) (dotted curve). A glassy carbon disk electrode with a diameter of 3 mm was used as the working electrode. The scan rate was 20 mV/s.

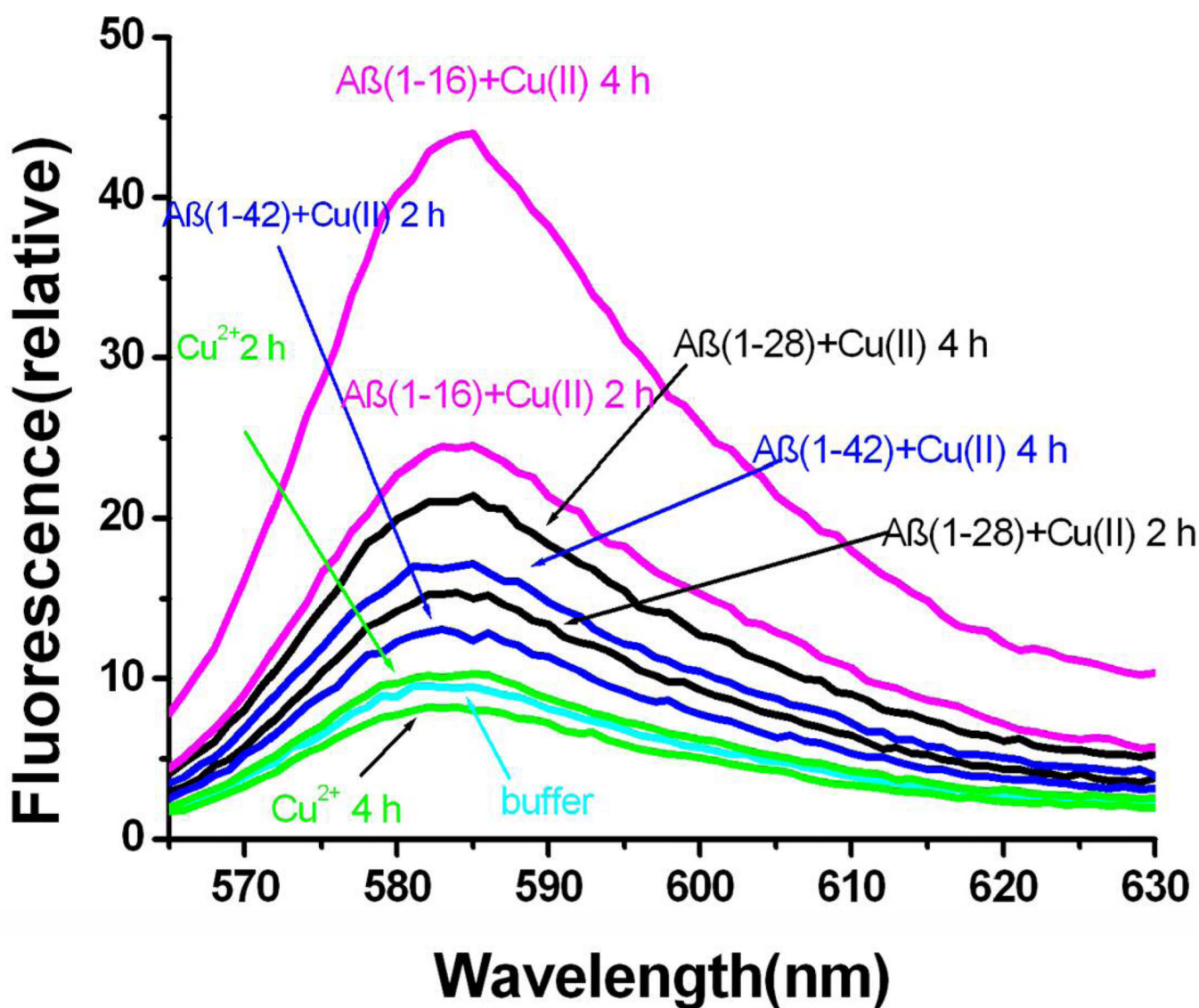


Figure 7.

The fluorescence spectra of resorufin for mixtures containing Cu(II) and different A β species upon electrolyses for different times: 100 μ M A β (1–16)/Cu(II) (pink curves), 100 μ M A β (1–28)/Cu(II) (black curves), 50 μ M A β (1–42)/Cu(II) (blue curves), 100 μ M Cu (II) solution (green curves) and buffer solution (light blue). In the electrolyses, a normal three-electrode system was used and a glassy carbon working electrode with an area of 0.071 cm² was held at 0.07 V. The solution volume for each was 300 μ L. The electrolysis times are indicated next to the respective curves.

Table 1
Extracting the copper oxidation state from mass peaks of the A β (1–28)-Cu(II) adduct

	A β -Cu(I)					A β -Cu(II)				
	Meas. <i>m/z</i>	Meas. Rel. Abund. (%)	Calcd. <i>m/z</i>	Calcd. Rel. Abund. (%)	Dev. (ppm)	Meas. <i>m/z</i>	Meas. Rel. Abund. (%)	Calcd. <i>m/z</i>	Calcd. Rel. Abund. (%)	Dev. (ppm)
3+ Ion	A	1108.145	15	1108.491	48	310	1108.155	48	9	
	A+1	1108.475	77	1108.825	85	320	1108.489	85	13	
	A+2	1108.806	100	1109.159	100	320	1108.823	100	15	
	A+3	1109.139	75	1109.493	88	320	1109.157	88	16	
	A+4	1109.470	30	1109.827	61	320	1109.491	61	19	
4+ Ion	A	831.387	36	831.620	48	280	831.368	48	23	
	A+1	831.634	80	831.871	85	280	831.619	85	18	
	A+2	831.883	100	832.121	100	290	831.869	100	17	
	A+3	832.134	93	832.372	88	290	832.120	88	17	
	A+4	832.382	64	832.622	61	290	832.370	61	15	
A+5	832.634	28	832.872	33	290	832.620	33	17		

Table 2

Extracting the copper oxidation state from the measured m/z values of the $A\beta(1-42)$ -Cu(II) adduct

$A\beta(1-42)$ -Cu	$A\beta$ -Cu(I)						$A\beta$ -Cu(II)					
	Meas. m/z	Meas. Rel. Abund. (%)	Calcd. m/z	Calcd. Rel. Abund. (%)	Dev. (ppm)	Calcd. m/z	Calcd. Rel. Abund. (%)	Dev. (ppm)	Calcd. m/z	Calcd. Rel. Abund. (%)	Dev. (ppm)	
4+ Ion	A	1144.068	19	1144.306	25	210	1144.054	25	12			
	A+1	1144.317	62	1144.556	62	210	1144.304	62	11			
	A+2	1144.568	87	1144.807	92	210	1144.555	92	11			
	A+3	1144.818	100	1145.057	100	210	1144.805	100	11			
	A+4	1145.069	91	1145.308	87	210	1145.056	87	12			
	A+5	1145.318	56	1145.558	62	210	1145.306	62	11			
	A+6	1145.569	31	1145.809	37	210	1145.557	37	11			
A+7	1145.819	19	1146.059	20	210	1145.807	20	10				
5+ Ion	A	915.457	32	915.646	25	210	915.444	25	14			
	A+1	915.656	54	915.847	62	210	915.645	62	12			
	A+2	915.856	83	916.047	92	210	915.856	92	12			
	A+3	915.055	100	916.247	100	210	916.046	100	10			
	A+4	916.256	94	916.448	87	210	916.246	87	11			
	A+5	916.456	66	916.648	62	210	916.446	62	11			
	A+6	916.656	40	916.848	37	210	916.647	37	10			
	A+7	916.859	23	916.049	20	210	916.847	20	12			
A+8	917.063	10	916.249	9	200	917.048	9	16				

Table 3Redox potentials of A β -Cu(II) complex and common redox species of biological relevance.

System	E ⁰ (V vs NHE)
Norepinephrine	0.384 ⁽¹⁾
Epinephrine	0.372 ⁽¹⁾
Dopamine	0.370 ⁽²⁾
O ₂ /H ₂ O ₂	0.295 ⁽⁴⁾
Cytochrome <i>a</i>	0.290 ⁽¹⁾
A β -Cu(II)	0.280
Cytochrome <i>c</i>	0.250 ⁽¹⁾
Hemoglobin	0.152 ⁽³⁾
CoQ/CoQH ₂ ^a	0.100 ⁽¹⁾
Ascorbic acid	0.051 ⁽³⁾
Cytochrome <i>b</i>	0.040 ⁽¹⁾
Fumarate/Succinate	0.031 ⁽⁴⁾
Myoglobin	0.005 ⁽¹⁾
Crotonyl-CoA/Butyryl-CoA	-0.015 ⁽⁴⁾
FMN/FMNH ₂ ^b	-0.120 ⁽¹⁾
Oxaloacetate/malate	-0.166 ⁽⁴⁾
Pyruvate/lactate	-0.185 ⁽⁴⁾
Glutathione	-0.228 ⁽¹⁾
Vitamin B12	-0.244 ⁽¹⁾
NAD ⁺ /NADH ^c	-0.320 ⁽¹⁾
FAD/FADH ₂ ^d	-0.327 ⁽¹⁾

^aCoenzyme Q (ubiquinone) oxidized/reduced forms^bFlavin mononucleotide oxidized/reduced forms^cNicotinamide adenine dinucleotide oxidized/reduced forms^dFlavin adenine dinucleotide oxidized/reduced forms⁽¹⁾G. Dryhurst, K. M. Kadish, F. Scheller, R. Renneberg, *Biological Chemistry*, Vol. 1, Academic Press, New York, London⁽²⁾D. C.-S. Tse, T. Kuwana, *Anal. Chem.*, 50 (1978), 1315.⁽³⁾B. E. Conway, *Electrochemical Data*, Greenwood Press Publisher. New York⁽⁴⁾A. L. Lehninger, D. L. Nelson, M. M. Cox, *Principles of Biochemistry*, Worth Publishers. New York