Alzheimer's Disease

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Metallo-ROS in Alzheimer's Disease: Oxidation of Neurotransmitters by Cu^{II}-β-Amyloid and Neuropathology of the Disease**

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The generation of reactive oxygen species (ROS), including superoxide, peroxide, and free radicals, is associated with normal redox metabolic pathways as side-tracks that can be regulated through the action of superoxide dismutase, catalase, and some reducing agents under homeostasis.[1,2] However, long-term effects of such oxidative chemical imbalance in normal and disease states can be expected. ROS are often considered the culprits responsible for the devastating effects of oxidative stress,[3] which concern cancer, aging, heart diseases, arthritis, diabetes, and the etiology of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease (AD).[4] AD affects primarily the elderly and causes considerable distress for the patients and emotional suffering of their families and close friends. One mechanism proposed for the neurodegeneration in AD focuses on amyloid-β peptides (Aβ) and their metal complexes through formation of plaques and fibrils and generation of the ROS H_2O_2 and free radicals.[5–9]

Aggregation of Aβ of 40 or 42 amino acids (DAEFR HDSGY^{10} EVHHQ KLVFF^{20} AEDVG SNKGA^{30} IIGLM VGGVV^{40} IA) in the brain is the hallmark in AD neuropathology induced by metal binding[10–12] and is usually found as metal-containing plaques and insoluble fibrils. Similar pathological effects are also found in transgenic mouse models with human Aβ.[13,14] Moreover, soluble fragments of Aβ can be generated in vivo by insulin-degrading enzymes as well as α- and β-secretases.[15,16] Nevertheless, the cause or effect connection of the metallo-Aβ plaques with AD is still under debate.[17–19] Despite immense work in Aβ research, the potential risk of metal-centered oxidative catalyses by metallo-Aβ has been overlooked.[20] The Cu^{II} complexes of metal-binding domains of Aβ (CuAβ) have recently been demonstrated to exhibit metal-centered oxidative catalysis, consistent with type-3 copper oxidases.[21,22] To verify the biorelevance of this metal-centered catalysis, we have determined the oxidation of several catecholamine and indoleamine neurotransmitters catalyzed by $CuAβ_{1–40}$ and two metal-binding N-terminal fragments $CuAβ_{1–16}$ and $CuAβ_{1–20}$ under various biomimetic conditions. The studies described herein are expected to provide a chemical basis for a better understanding of the etiology of AD.

Dopamine is directly linked to the neurodegenerative Parkinson's disease.[23] As symptoms of Parkinson's disease are also recognized in AD patients,[24] disturbance of dopamine metabolism may be closely associated with AD. Dopamine is effectively oxidized aerobically to dopaquinone by $CuAβ_{1–40}$ at pH 7.0. The rate reaches saturation at high dopamine concentrations (Figure 1; Table 1), consistent with enzyme-like pre-equilibrium kinetics. There is an apparent cooperativity in the catalysis that is not usually expected in simple monomeric systems. This may be due to the tendency of the peptide to coagulate and/or the formation of a dinuclear center during catalysis. Fitting the results to the Hill equation[25] gives kinetic constants $k_{cat} = 7.48 \times 10^{-4}\ s^{-1}$ and $k_{cat}/K_m = 2.77\ M^{-1}\ s^{-1}$ and a Hill coefficient $\theta = 1.48$. This

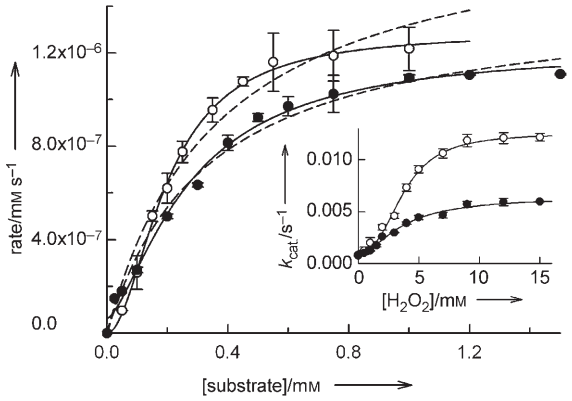

Figure 1. Oxidation of catechol (○) and dopamine (●) by 1.47 μM $CuAβ_{1–40}$ in 100 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES) buffer solution at pH 7.0 and 25 °C. Dashed traces are fittings to simple pre-equilibrium kinetics, whereas the solid traces are the fittings to the Hill equation. The inset shows the effect of H_2O_2 on k_{cat}, which indicates the presence of cooperativity.

Table 1: Kinetic parameters for dopamine oxidation by CuAβ.[a]

CuAβ	k_{cat} [$\times 10^{-3}\ s^{-1}$]	K_m [mM]	k_{cat}/K_m [$M^{-1}\ s^{-1}$]	$k_{rel.}$
1–40	0.748	0.27	2.77	85
1–20	11.6	0.90	12.9	1320
1–16	28.0	0.31	90.3	3180
1–40[b]	5.61	0.27	21	312
1–20[b]	99.0	0.52	190	5530
1–16[b]	230	0.68	339	12700

[a] In 100 mM HEPES buffer solution at pH 7.0 and 25 °C. [b] In the presence of 20 mM H_2O_2.

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reaction shows an 85-fold rate enhancement relative to the auto-oxidation of dopamine. The shorter $CuA\beta_{1-20}$ and $CuA\beta_{1-16}$ do not exhibit the apparent cooperativity and show 16- and 37-fold, respectively, higher activities than $CuA\beta_{1-40}$ toward dopamine oxidation in terms of k_{cat}. Catechol oxidation shows the same catalytic trend (Figure 1 and Table S1 in the Supporting Information) with $CuA\beta_{1-40}$ showing cooperativity ($\theta = 2.7$) and $CuA\beta_{1-16}$ exhibiting the highest activity. The results herein indicate that the soluble CuAβ fragments may be more severe in causing oxidative stress in the brain of AD patients than the coagulation-prone $CuA\beta_{1-40}$. Recent studies also suggested pathological significance of soluble forms of Aβ.[26]

Collectively termed catecholamines, dopamine, epinephrine, norepinephrine, and Dopa are catechol-containing neurotransmitters (while serotonin and its precursor 5-hydroxytryptophan are indole-containing neurotransmitters) that are involved in cognitive, behavioral, physical, physiological, and psychological functions.[27] Oxidation of these molecules may cause severe alteration in bioactivity, eventually leading to neuronal death.[28] Metabolic malfunctions of neurotransmitters are known phenomena in the physiology of AD[29,30] and have also been suggested to be related to the neuropathology of this disease.[29–32] However, a chemical mechanism for the neurotransmitter malfunction in AD is still unknown. These catecholamine neurotransmitters are found to be effectively oxidized aerobically to their respective *o*-quinone products by $CuA\beta_{1-20}$ (see Figure S1 and Table S1 in the Supporting Information). Herein, $CuA\beta_{1-20}$ is able to significantly accelerate the aerobic oxidation rate of these neurotransmitters by 333–2420 times in terms of k_{cat} relative to the auto-oxidation rate constant k_o.

Compared with that of catechol, the auto-oxidation rates of these neurotransmitters are nearly 10 times faster with respect to k_o (see Table S1 in the Supporting Information). However, their oxidation rates by CuAβ are approximately 130- to 980-fold slower than that of catechol, reflecting their higher resistance to oxidation by CuAβ by approximately 10^3- to 10^4-fold relative to catechol oxidation. The relatively higher stability against oxidative damage means that these molecules are better suited for the purpose of neurotransmission. Dopa shows a lower reactivity than other catecholamines, which is likely due to the inductive effect of the carboxy group on the side chain. Chirality of these neurotransmitters does not appear to play a role in this oxidation chemistry (see Table S1 in the Supporting Information).

The ROS H_2O_2 has been commonly suggested to be a culprit causing the oxidative stress in AD.[4,6] However, this ROS alone, at a concentration of 50.0 mM, does not significantly affect the oxidations of neurotransmitters (see Table S1 in the Supporting Information). Conversely, oxidations of these neurotransmitters by CuAβ in the presence of greater than 15 mM (> 0.051 %)[33] H_2O_2 under the same conditions exhibits significant rate enhancement (k_{rel}; see Table S1 and Figure S2 in the Supporting Information); for example, a rate enhancement of 312-fold relative to the auto-oxidation rate constant k_o for dopamine oxidation by $CuA\beta_{1-40}$ with $k_{cat} = 0.0056\ s^{-1}$ and $k_{cat}/K_m = 21\ M^{-1}s^{-1}$ was obtained. The oxidation of dopamine is dependent on the concentration of H_2O_2 and reaches a plateau at $[H_2O_2] > 15$ mM with $\theta = 3.57$ (Figure 1, inset), which indicates that the ROS H_2O_2 can bind to CuAβ to afford a metallo-ROS in a cooperative manner. Oxidation of catechol without the side chain by Cu-$A\beta_{1-40}$ exhibits a similar kinetic pattern (Figure 1 and Table S1 in the Supporting Information), confirming the cooperative nature of this oxidative catalysis.

The results conclude that metallo-ROS is much more reactive than free ROS alone as far as H_2O_2 is concerned. Once again, the shorter $CuA\beta_{1-16(20)}$ fragments exhibit higher activity than $CuA\beta_{1-40}$ toward dopamine oxidation in the presence of H_2O_2 (Table 1). This observation suggests pathological significance of soluble Aβ fragments in AD. Herein, $CuA\beta_{1-20}$ is able to significantly accelerate oxidation rates of catechol and catecholamine neurotransmitters by 1350 to 2.24×10^5 times in terms of k_{cat} relative to k_o (see Table S1 in the Supporting Information). It is worth noting that the k_{cat}/K_m value of $1690\ M^{-1}s^{-1}$ for catechol oxidation by $CuA\beta_{1-16}$ approaches enzyme-like catalytic efficiency, which is 5.3 % of the activity of the catechol oxidase ($32\,000\ M^{-1}s^{-1}$) from gypsywort (*Lycopus europaeus*).[34]

As both H_2O_2 and the neurotransmitters can bind to the metal active center, the data are analyzed with a two-substrate random-binding mechanism according to the Hanes equation[25] (see Figure S2 in the Supporting Information) to afford the apparent and intrinsic dissociation constants K_S and K_{Si}, respectively (0.34 and 0.23 for catechol and 0.52 and 0.22 for dopamine, respectively). The ratios of K_S/K_{Si} are greater than one, indicating that the neurotransmitters and H_2O_2 affect the binding of each other in this bisubstrate reaction mechanism.[25,35]

$A\beta_{1-40}$ and fragments are found in various cellular environments, including soluble forms in the cytosol and insoluble forms as membrane-bound plaques. Herein, the detergent sodium dodecyl sulfate (SDS) is used to approximate the amphiphilic nature of the cell membrane. Structures of short Aβ fragments in the presence of SDS differ considerably from that in the absence of this micelle-forming surfactant.[36] The activities of CuAβs in the presence of micelles may offer insight into the nature of the structure and activity of Aβ. The rate constant k_{cat} for dopamine oxidation by $CuA\beta_{1-20}$ is largely affected by the soluble form of SDS (4.5 times) and noticeably influenced (80 %) by micelles with a critical micelle concentration (CMC) of around 8 mM[37] (Figure 2a). This pattern fits well to a binding mode with two different binding sites,[38] whereas that of $CuA\beta_{1-40}$ is only slightly influenced by soluble SDS (2-fold) and greatly affected by micellar SDS (8-fold). The rate constant for the shortest $CuA\beta_{1-16}$ is only slightly enhanced (85 %) at a saturating amount of SDS and is 14 % enhanced by micelles. Under our experimental conditions, SDS micelles do not influence the self-oxidation rates of neurotransmitters. The results indicate that the plaque-forming $CuA\beta_{1-40}$ exhibits more-significant oxidation chemistry when it is "solubilized" and incorporated into a hydrophobic environment, whereas the soluble $CuA\beta_{1-16(20)}$ are more powerful oxidation catalysts than $CuA\beta_{1-40}$ in aqueous environments (see Table S1 in the Supporting Information). The results seem to also corroborate the "opposing-activities" proposal for neuroprotection

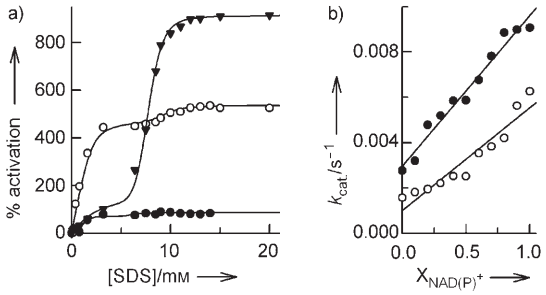

Figure 2. a) Effect of SDS on the oxidative activity of $CuA\beta_{1-16}$ (●), $CuA\beta_{1-20}$ (○), and $CuA\beta_{1-40}$ (▼) in 100.0 mM HEPES buffer solution at pH 7.0 and 25 °C. The data show the influence on the activity by both the monomeric and the micellar forms of SDS with a CMC of 8 mM. b) Influence of $NAD(P)^+$ (○) and NAD^+ (●) on the oxidative activity of $CuA\beta_{1-20}$ toward dopamine oxidation with a fixed total concentration C_{tot} of $[NAD(P)^+] + [NAD(P)H] = 10.0$ mM. $X = NAD(P)^+/C_{tot}$.

through proteolysis and aggregation,[39] wherein the highly reactive $A\beta_{1-16(20)}$ fragments are supposed to be eliminated by the former process, whereas the activity of $A\beta_{1-40}$ is much decreased by the latter process. The local concentrations of metallo-Aβ plaques can reach the mM range,[4] which can cause significant oxidative damages of proximal areas on the brain surface. Consequently, the devastating metallo-ROS chemistry owing to $CuA\beta_{1-40}$ and fragments in the brain of AD patients can have a widespread effect in different cellular environments, particularly in amphiphilic surroundings as demonstrated herein.

The $NAD(P)^+$/NAD(P)H ratios (NAD = nicotinamide adenine dinucleotide (phosphate)) vary according to changes in metabolism and is species/tissue dependent,[40] and thus are expected to affect the redox property of CuAβ in vivo. Changes in the homeostatic levels in terms of $NAD(P)^+$/NAD(P)H may reflect the neurochemical status under oxidative stress.[42] Metabolic changes have been noted to be associated with several age-related diseases, including neurodegenerative diseases.[41] It has been previously reported that these ratios are managed according to spatial and temporal constraints in the brain when under oxidative stress.[42] The oxidation activity of $CuA\beta_{1-20}$ is lowered by NAD(P)H. As the ratios of $NAD(P)^+$/NAD(P)H decrease, the activity in terms of k_{cat} toward dopamine oxidation decreases by 2.4 and 1.9 times, respectively (Figure 2b). Based on the proposed mechanism for catechol oxidation by Cu-$A\beta_{1-20}$,[21] the inhibitory effect of NAD(P)H is due to a shift in equilibrium toward H_2O_2 generation under reducing conditions.[21,40] The more pronounced inhibition caused by NADPH as compared with NADH might be attributed to the phosphate group in NADPH. Indeed, phosphate has been observed to be a competitive inhibitor toward the oxidation of dopamine, with $K_i = 4.7$ mM (see Figure S3 in the Supporting Information). The ratio of free NAD^+/NADH has been under debate, nevertheless, it has recently been suggested[43] to be around 600, which is consistent with the value based on potentiometric measurement.[44] The relatively smaller availability of the free form of NADH suggests that this biological reducing agent may not significantly influence the metal-centered oxidative catalysis of CuAβ under physiological conditions.

To reveal the mechanism of the full-length $A\beta_{1-40}$, the slow substrate 4,5-dichlorocatechol (DCC) was titrated into a solution of $CuA\beta_{1-40}$. The observation of a charge-transfer transition at 438 nm (Figure S4) is indicative of DCC binding to the Cu^{II} center (with an affinity constant of $6.4 \times 10^5\ M^{-1}$, see Figure S4 in the Supporting Information) analogous to its binding to the soluble $CuA\beta_{1-20}$.[22] Cooperativity is apparent in DCC binding (see Figure S5 in the Supporting Information), probably owing to the coagulating nature of $A\beta_{1-40}$. The stoichiometry of DCC:$CuA\beta_{1-40} = 1{:}2$ verifies the dinuclear nature of the catalysis. The activation profile during Cu^{II} titration to $A\beta_{1-40}$ is sigmoidal with a Hill coefficient of $\theta = 2.68$ (see Figure S5 in the Supporting Information), which further supports a dinuclear catalysis. Cooperativity is not usually expected in mononuclear catalysis wherein a linear correlation of the activity with the concentration of Cu^{II} is expected until one equivalent is reached.

The oxidation of catecholamine neurotransmitters by $CuA\beta_{1-20}$ and the stoichiometry for DCC binding are consistent with the action of dinuclear catechol oxidase.[21] The mechanism of this enzyme thus serves as a working model for the metal-centered oxidation of catecholamines by CuAβ. Under aerobic conditions, the catechol moiety binds to a di-Cu^{II} center (Figure 3, path A) and is oxidized through a two-electron transfer to afford di-Cu^{I} and the *o*-quinone product (path B). Di-Cu^+ then binds O_2 to form the metallo-ROS μ-peroxo-Cu_2 center (paths C and D), which may bind (path E) and oxidize (path F) another substrate. In the presence of an electron donor, such as NAD(P)H (path H), the oxidation of neurotransmitters is inhibited. Herein, H_2O_2 is formed to a certain extent that then enables production of other types of ROS and may exacerbate the oxidative destruction by going through the peroxide shunt (path G) upon forming the highly reactive metallo-ROS μ-peroxo-di-Cu^{II} intermediate. The metal-bound H_2O_2 as a metallo-ROS in equilibrium with three isoelectronic species[45] (path D) has been demonstrated

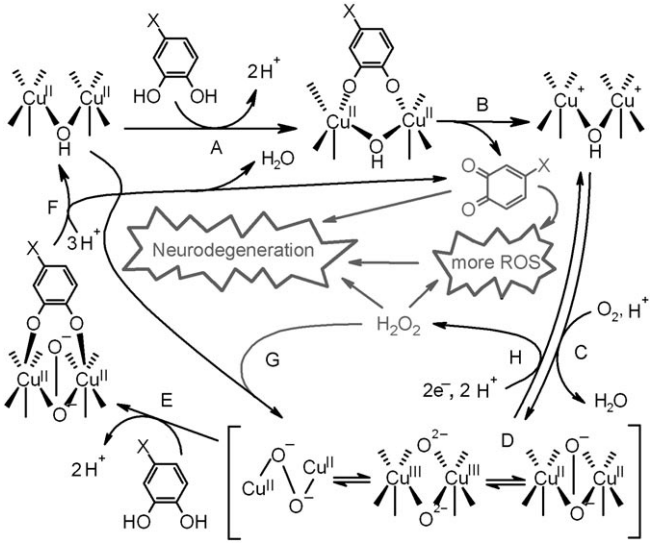

Figure 3. Mechanism for the oxidation of catecholamine neurotransmitters and the cause of neurodegeneration by CuAβ. The metal-bound H_2O_2 as a "metallo-ROS" is shown in path D.

herein to be a more potent oxidative agent to cause neurodegeneration than H_2O_2 alone.

The plaque-forming $CuA\beta_{1-40}$ also shows significant metal-centered oxidative activity toward the relatively more inert phenol (see Figure S6a and Table S1 in the Supporting Information), showing $k_{cat} = 0.439$ and $1.43\ s^{-1}$ (with $\theta = 1.6$) and $k_{cat}/K_m = 0.351$ and $1.14\ M^{-1}s^{-1}$ in the absence and presence of 50.0 mM H_2O_2, respectively. The activity reaches saturation at high H_2O_2 concentrations (see Figure S6b in the Supporting Information), reflecting binding of H_2O_2 to the metal to afford metallo-ROS. Phenol hydroxylation and oxidation exhibits the same trend of reactivity with the shortest $CuA\beta_{1-16}$ showing the highest activity (see Table S1 in the Supporting Information). Herein, oxidation of phenol is dramatically enhanced by 6.64×10^6 and 1.41×10^5 times by $CuA\beta_{1-16}$ with and without 50.0 mM H_2O_2, respectively.

Serotonin can be hydroxylated and oxidized into its quinone form by CuAβ with significant rate enhancements ranging from $(324–3.03 \times 10^4)$-fold and $(8.41 \times 10^3–2.69 \times 10^5)$-fold relative to k_o without and with more than 50 mM H_2O_2, respectively (Figure 4 and Table S1 in the Supporting Information). As in the case of catecholamine oxidation, the oxidation of serotonin by $CuA\beta_{1-40}$ is slower than that by $CuA\beta_{1-(16,20)}$. The serotonin precursor 5-hydroxy-Trp can also be effectively hydroxylated and oxidized (see Table S1 in the Supporting Information). The apparent and intrinsic dissociation constants K_S and K_{Si} for phenol are determined to be 1.23 and 0.54 mM, respectively, from the Hanes plots, indicating that H_2O_2 binds to the metal center and decreases the binding of serotonin.[25,35] The hydroxylation/oxidation reactions seem to follow the tyrosinase mechanism.[46] However, Cu^{II}-Aβ is still highly active in the hydroxylation reaction even without H_2O_2, whereas the di-Cu^{II} Met form of tyrosinase is not. The large oxidation enhancements of the two indoleamines suggest that their oxidation by CuAβ, possibly taking place in the brain of AD patients, may alter serotonin-mediated physiological functions, including sleep disorders, mood changes, and anxiety often associated with AD patients.[47]

Cell-culture experiments reveal that catecholamines can exacerbate the oxidative stress caused by Aβ,[48] wherein the metal-centered oxidative catalysis has been overlooked.[20] Oxidation of catecholamines is known to generate neurotoxic quinone products[49] that are involved in protein modification (e.g., covalent modification of dopamine transporter[50]) and polymerizing tau protein into fibrils.[51] Age-related deficiencies of both dopamine and norepinephrine have been implicated in the vulnerability of noradrenergic neurons in the hippocampus,[52] which suffers significant damage in AD. Moreover, loss of noradrenergic neurons is linked to degradation of the locus ceruleus, which is rich in dopaminergic neurons that show severe lesions in AD.[53] Hence, a possible mechanism for reduction in neurotransmitter-regulated alertness response, delay-period activity, sleep cycle, mood stabilization, short-term memory, cognition, attention and problem-solving capability, satisfaction feeling, and coordination of physical movement experienced by AD patients[29–32] may be due to excessive oxidation of neurotransmitters, hinting at a possible neuropathological role of metallo-ROS associated with CuAβ.

In conclusion, the plaque-forming $CuA\beta_{1-40}$ and fragments thereof have been demonstrated to exhibit significant activities toward the oxidation of neurotransmitters with or without H_2O_2, which presumably can be further enhanced by interacting with membranes. The results suggest that an imbalance of neurotransmitter metabolism can be created near $A\beta_{1-40}$ plaques in AD. The results also suggest that small fragments of Aβ, owing to their soluble nature, can significantly disturb neurotransmission in a more systematic manner in the brain of AD patients and thus may play an important role in neuropathology of this devastating disease.

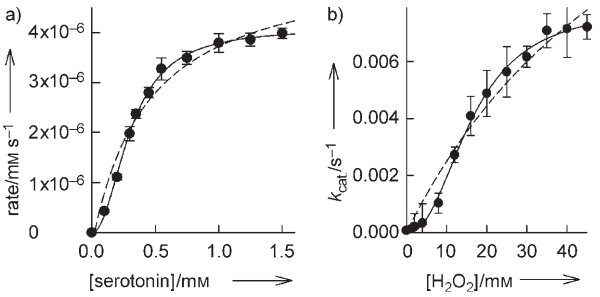

Figure 4. a) Aerobic oxidation of serotonin by 1.47 μM $CuA\beta_{1-40}$ in 100.0 mM HEPES buffer solution at pH 7.0 and 25 °C. Dashed traces are fittings to pre-equilibrium kinetics, whereas the solid traces are fittings to the Hill equation, which indicates the presence of cooperativity. b) Oxidation of serotonin by $CuA\beta_{1-40}$ in the presence of H_2O_2 as in (a).

Experimental Section

$Ab_{1-16(20)}$ were synthesized at the Peptide Center of the University of South Florida and confirmed with a Bruker MALDI-TOF mass spectrometer. $A\beta_{1-40}$ was purchased from Biopeptide Co., LLC (San Diego, CA). The Cu^{II} complex of $A\beta_{1-40}$ was prepared according to literature procedures[54] and the concentration determined with a standard (bicinchoninic acid) BCA assay. The CuAβ stock solutions were quickly divided up into aliquots to prevent concentration deviations caused by aggregation.

Kinetic measurements were performed and analyzed as previously described[21,22] ($\varepsilon_{product} = 32\,500\ M^{-1}cm^{-1}$ for phenol and catechol[55] and 28900, 27200, and $27\,500\ M^{-1}cm^{-1}$ for Dopa, dopamine, and epinephrine/norepinephrine, respectively,[56] and $35\,200\ M^{-1}cm^{-1}$ for serotonin and 5-hydroxytryptophan). The data were analyzed with the bisubstrate Hanes plot for experiments in the presence of H_2O_2 to yield the apparent K_S and intrinsic K_{Si} dissociation constants for the neurotransmitters and H_2O_2.[25]

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[1] K. Schuessel, S. Leutner, N. J. Cairns, W. E. Mueller, A. Eckert, *J. Neural Transm.* **2004**, *111*, 1167–1182.
[2] J. Apelt, M. Bigl, P. Wunderlich, R. Schliebs, *Int. J. Dev. Neurosci.* **2004**, *22*, 475–484.

[3] a) A. J. Núñez-Sellés, *J. Braz. Chem. Soc.* **2005**, *16*, 699 – 710; b) M. Valko, M. Izakovic, M. Mazur, C. J. Rhodes, J. Telser, *Mol. Cell. Biochem.* **2004**, *266*, 37 – 56; c) A. C. Maritim, R. A. Sanders, J. B. Watkins, *J. Biochem. Mol. Toxicol.* **2003**, *17*, 24 – 38; d) Review: Oxidative stress and disease: A. Spector, *J. Ocul. Pharmacol. Ther.* **2000**, *16*, 193 – 201.

[4] a) A. I. Bush, *Trends Neurosci.* **2003**, *26*, 207 – 214; b) D. A. Butterfield, *Free Radical Res.* **2002**, *36*, 1307 – 1313.

[5] M. J. Del Rio, C. Velez-Pardo, *Curr. Med. Chem. Cent. Nerv. Syst. Agents* **2004**, *4*, 279 – 285.

[6] C. Opazo, X. Huang, R. A. Cherny, R. D. Moir, A. E. Roher, A. R. White, R. Cappai, C. L. Masters, R. E. Tanzi, N. C. Inestrosa, A. I. Bush, *J. Biol. Chem.* **2002**, *277*, 40302 – 40308.

[7] R. Sultana, S. Newman, H. Mohmmad-Abdul, J. N. Keller, D. A. Butterfield, *Free Radical Res.* **2004**, *38*, 449 – 458.

[8] H. Engelberg, *Dementia Geriatr. Cognit. Disord.* **2004**, *18*, 278 – 298.

[9] D. A. Butterfield, *Curr. Med. Chem.* **2003**, *10*, 2651 – 2659.

[10] A. I. Bush, W. H. Pettingell, G. Multhaup, M. Paradis, J. Vonsattel, J. F. Gusella, K. Beyreuther, C. L. Masters, R. E. Tanzi, *Science* **1994**, *265*, 1464 – 1467.

[11] T. Miura, K. Suzuki, N. Kohata, H. Takeuchi, *Biochemistry* **2000**, *39*, 7024 – 7031.

[12] R. E. Tanzi, L. Bertram, *Cell* **2005**, *120*, 545 – 555.

[13] I. H. Cheng, J. J. Palop, L. A. Esposito, N. Bien-Ly, F. Yan, L. Mucke, *Nat. Med.* **2004**, *10*, 1190 – 1192.

[14] K. Jin, V. Galvan, L. Xie, X. M. Ou, O. F. Gorostiza, D. E. Bredesen, D. A. Greenberg, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 13363 – 13367.

[15] G. Evin, A. Weidemann, *Peptides* **2002**, *23*, 1285 – 1297.

[16] G. Evin, A. Zhu, D. Holsinger, C. L. Masters, Q. Li, *J. Neurosci. Res.* **2003**, *74*, 386 – 392.

[17] A. Kontush, C. Berndt, W. Weber, V. Akopian, S. Arlt, S. Schippling, U. Beisiegel, *Free Radical Biol. Med.* **2001**, *30*, 119 – 128.

[18] Y. Luo, P. Butko, *Oxid. Stress Dis.* **2006**, *22*, 395 – 408.

[19] J. Hardy, *Curr. Alzheimer Res.* **2006**, *3*, 71 – 73.

[20] a) D. P. Smith, D. G. Smith, C. C. Curtain, J. F. Boas, J. R. Pilbrow, G. D. Ciccotosto, T.-L. Lau, D. J. Tew, K. Perez, J. D. Wade, A. I. Bush, S. C. Drew, F. Separovic, C. L. Masters, R. Cappai, K. J. Barnham, *J. Biol. Chem.* **2006**, *281*, 15145 – 15154; b) F. E. Ali, F. B. Separovic, C. J. Barrow, R. A. Cherny, F. Fraser, A. I. Bush, C. L. Masters, K. J. Barnham, *J. Pept. Sci.* **2005**, *11*, 353 – 360; c) P. I. Moreira, K. Honda, Q. Liu, M. S. Santos, C. R. Oliveira, G. Aliev, A. Nunomura, Z. Akihiko, X. Zhu, M. A. Smith, G. Perry, *J. Neur. Trans.* **2005**, *112*, 921 – 932.

[21] G. F. Z. da Silva, W. M. Tay, L.-J. Ming, *J. Biol. Chem.* **2005**, *280*, 16601 – 16609.

[22] G. F. Z. da Silva, L.-J. Ming, *Angew. Chem.* **2005**, *117*, 5637 – 5640; *Angew. Chem. Int. Ed.* **2005**, *44*, 5501 – 5504.

[23] D. B. Calne, J. W. Langston, *Lancet* **1983**, *322*, 1457 – 1459.

[24] a) S. M. Ditter, S. S. Mirra, *Neurology* **1987**, *37*, 754 – 760; b) E. Masliah, E. Rockenstein, I. Veinbergs, Y. Sagara, M. Mallory, M. Hashimoto, L. Mucke, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12245 – 12250.

[25] V. Leskovac, *Comprehensive Enzyme Kinetics*, Kluwer, Boston, **2002**.

[26] a) K. Heinitz, M. Beck, R. Schliebs, J. R. Perez-Polo, *J. Neurochem.* **2006**, *98*, 1930 – 1945; b) R. Kayed, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman, C. G. Glabe, *Science* **2003**, *300*, 486 – 489; c) F. Rabanal, J. M. Tusell, L. Sastre, M. R. Quintero, M. Cruz, D. Grillo, M. Pons, F. Albericio, J. Serratosa, E. Giralt, *J. Pept. Sci.* **2002**, *8*, 578 – 588.

[27] a) A. F. Arnsten, B. M. Li, *Biol. Psychiatry* **2005**, *57*, 1377 – 1384; b) S. F. Leibowitz, G. F. Weiss, G. Shor-Posner, *Clin. Neuropharmacol.* **1988**, *11*, S51 – S71.

[28] E. B. Larson, W. A. Kukull, R. L. Katzman, *Annu. Rev. Public Health* **1992**, *13*, 431 – 449.

[29] J. B. Friedman, D. N. Adler, K. L. Davis, *Biol. Psychiatry* **1999**, *46*, 1243 – 1252.

[30] H. Umegaki, N. Tamaya, T. Shinkai, A. Iguchi, *Exp. Gerontol.* **2000**, *35*, 1373 – 1382.

[31] R. J. Gruen, J. Ehrlich, R. Silva, J. W. Schweitzer, A. J. Friedhoff, *Psychiatry Res.* **2000**, *93*, 55 – 61.

[32] J. Li, M. Zhu, A. B. Manning-Bog, D. A. Di Monte, A. L. Fink, *FASEB J.* **2004**, *18*, 962 – 964.

[33] The concentration of the off-the-shelf H_2O_2 solution from drug stores in the U.S. is 3%.

[34] A. Rompel, H. Fischer, D. Meiwes, K. Buëldt-Karentzopoulos, A. Magrini, C. Eicken, C. Gerdemann, B. Krebs, *FEBS Lett.* **1999**, *445*, 103 – 110.

[35] J. R. Florini, C. S. Vestling, *Biochim. Biophys. Acta* **1957**, *25*, 575 – 578.

[36] C. Morgan, M. Colombres, M. T. Nunez, N. C. Inestrosa, *Prog. Neurobiol.* **2004**, *74*, 323 – 349.

[37] a) G. W. Buchko, A. Rozek, D. W. Hoyt, R. J. Cushley, M. A. Kennedy, *Biochim. Biophys. Acta* **1998**, *1392*, 101 – 108; b) P. C. Griffiths, J. A. Roe, B. L. Bales, A. R. Pitt, A. M. Howe, *Langmuir* **2000**, *16*, 8248 – 8254.

[38] M. N. Harris, J. D. Madura, L.-J. Ming, V. J. Harwood, *J. Biol. Chem.* **2001**, *276*, 19310 – 19317.

[39] E. Cohen, J. Bieschke, R. M. Perciavalle, J. W. Kelly, A. Dillin, *Science* **2006**, *313*, 1604 – 1610.

[40] S.-J. Lin, L. Guarente, *Curr. Opin. Cell Biol.* **2003**, *15*, 241 – 246.

[41] a) F. Garcia Soriano, L. Virag, P. Jagtap, E. Szabo, J. G. Mabley, L. Liaudet, A. Marton, D. G. Hoyt, K. G. Murthy, A. L. Salzman, G. J. Southan, C. Szabó, *Nat. Med.* **2001**, *7*, 108 – 113; b) Q. Zhang, D. W. Piston, R. H. Goodman, *Science* **2002**, *295*, 1895 – 1897.

[42] L. K. Klaidman, S. K. Mukrherjee, J. D. Adams, Jr., *Biochim. Biophys. Acta* **2001**, *1525*, 136 – 148.

[43] Q. Zhang, D. W. Piston, R. H. Goodman, *Science* **2002**, *295*, 1895 – 1897.

[44] H. Sies, *Metabolic Compartmentation*, Academic Press, London, **1982**.

[45] L. M. Mirica, X. Ottenwaelder, T. D. P. Stack, *Chem. Rev.* **2004**, *104*, 1013 – 1045.

[46] A. Sánchez-Ferrer, J. N. Rodriguez-López, F. García-Cánovas, F. García-Carmona, *Biochim. Biophys. Acta* **1995**, *1247*, 1 – 11.

[47] L. Volicer, M. Z. Wrona, W. Matson, G. Dryhurst, *Bioimaging Neurodegener.* **2005**, 85 – 93.

[48] W. Fu, H. Luo, S. Parthasarathy, M. P. Mattson, *Neurobiol. Dis.* **1998**, *5*, 229 – 243.

[49] a) D. G. Graham, *Mol. Pharmacol.* **1978**, *14*, 633—643; b) D. G. Graham, S. M. Tiffany, W. R. Bell, Jr., W. F. Gutknecht, *Mol. Pharmacol.* **1978**, *14*, 644 – 653; c) T. G. Hastings, *J. Neurochem.* **1995**, *64*, 919 – 924.

[50] S. B. Berman, M. J. Zigmond, T. G. Hastings, *J. Neurochem.* **1996**, *67*, 593 – 600.

[51] a) M. J. LaVoie, T. G. Hastings, *J. Neurosci.* **1999**, *19*, 1484 – 1491; b) I. Santa-Maria, F. Hernandez, M. A. Smith, G. Perry, J. Avila, F. J. Moreno, *Mol. Cell. Biochem.* **2005**, *278*, 203 – 212.

[52] A. J. Nazarali, G. P. Eynolds, *Cell. Mol. Neurobiol.* **1994**, *14*, 581 – 587.

[53] M. A. Miller, P. E. Klob, J. B. Levernz, E. R. Peskind, M. A. Raskind, *J. Neurochem.* **1999**, *73*, 2028 – 2036.

[54] X. Huang, C. S. Atwood, R. D. Moir, M. A. Hartshorn, J. P. Vonsattel, R. E. Tanzi, A. I. Bush, *J. Biol. Chem.* **1997**, *272*, 26464 – 26470.

[55] S. G. Srivatsan, P. Nigamb, M. S. Raob, S. Verma, *Appl. Catal. A* **2001**, *209*, 327 – 334.

[56] J. N. Rodríguez-López, J. Escribano, F. García-Cánovas, *Anal. Biochem.* **1994**, *216*, 205 – 212.

