JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9413–9417 9413
DOI:10.1021/jf101661w

Simple and Novel Screening Assay of Natural Antioxidants for Cu(II) Ion/Adrenaline-Mediated Oxidation of N-Terminal Amyloid β by Liquid Chromatography/Mass Spectrometry

Koichi Inoue,*,† Madoka Kaneko,† Tomoaki Hino,†,‡ and Hisao Oka†,‡

†Department of Physical and Analytical Chemistry, School of Pharmacy and ‡Graduate School of Human Ecology, Human Ecology Major, Kinjo Gakuin University, 2-1723 Omori, Moriyama-ku, Nagoya, Aichi 463-8521, Japan

In the present study, a novel assay for screening inhibitors of Cu(II) ion/adrenaline-mediated oxidative modification of N-terminal amyloid β (Aβ) peptides was developed using liquid chromatography with mass spectrometry (LC/MS). The physiological condition of Cu(II) ion/adrenaline in buffer (pH 7.4) at 37 °C for 90 min revealed a specific modification of N-terminal Aβ peptides, such as $A\beta_{1-6}$, $A\beta_{1-40}$, and $A\beta_{1-42}$, using trypsin digestion and LC/MS detection of the modified Aβ peptide. When this oxidative modification of the shorter N-terminal $A\beta_{1-6}$ was subjected to LC/MS, single charged ions from native peptide ($[M + H]^+$, *m*/*z* 774) were observed at *m*/*z* 729 and 685, corresponding to a decrease in mass of 45 and 89 Da, respectively, as compared with the original peptide. To determine the effect of specific antioxidants, a screening assay to find inhibitors of Cu(II) ion/adrenaline-mediated oxidation was developed based on the response ratio of *m*/*z* 685 to 774. LC/MS detection of the modified peptides allowed us to identify antioxidants that inhibit oxidative modification of $A\beta_{1-6}$ model peptide. The oxidative modification of $A\beta_{1-6}$ was inhibited by curcumin but not an isoflavone or catechin mixture or saponin or capsaicin, revealing a clear difference between antioxidants that inhibit oxidative modification and other antioxidants. This novel assay may allow for the identification of antioxidants that protect against oxidative modification of Aβ and other proteins related to oxidative stress by adrenaline and Cu(II) ions under normal physiologic conditions.

KEYWORDS: Amyloid-β; oxidation; Cu(II) ion; adrenaline; liquid chromatography/mass spectrometry; curcumin

INTRODUCTION

To investigate the nature and extent of damage to biological molecules by reactive oxygen species (ROS) and how this damage may be involved in degenerative diseases, many researchers have begun to investigate indirect and/or direct detection of ROS and the damage induced by ROS to individual matrix components in human models (*1*). Hydrogen peroxide (H_2O_2) also affects numerous intracellular signaling pathways, such as nonphagocytic cells expressing gp91 Phox protein and its homologues (*2*). Thus, the ROS redox reaction has been implicated in cellular signaling regulating normal processes and in the progression of diseases related to aging, including cardiovascular disease, cancer, immune system decline, brain dysfunction, and cataracts (*3*). A causal relationship with ROS without H_2O_2 would be established in the inevitable progression of oxidative damage to various molecules in a state of oxidative stress. On the basis of the general principle of life stress, oxidative stress is a factor of great importance with regard to three symptoms of aging, psychological state, and foods such as antioxidants. We focused on oxidative stress induced by ROS with regard to both aging and the psychological state and developed a novel assay to screen for specific antioxidants.

*To whom correspondence should be addressed. Tel/Fax: +81-52-798-0982. E-mail: kinoue@kinjo-u.ac.jp.

Alzheimer's disease is the most frequent neurodegenerative disorder, and diverse lines of evidence suggest that amyloid-β (Aβ) peptides have a causal role in its pathogenesis. Thus, it is a very important study that Aβ may be part of a mechanism controlling a positive regulator presynaptically and an aggregation regarding the neuronal circuit activity. Recently, the detection of specific oxidized Aβ peptides containing histidine (His) was reported using a metal-catalyzed oxidation (MCO) reaction of Cu(II) ion/ascorbic acid and liquid chromatography with mass spectrometry (LC/MS) as a potential biomarker of Alzheimer's disease (*4*). Moreover, the decarboxylation and deamination of the N-terminal aspartic acid (Asp) to pyruvate were detected in Aβ peptides in an oxidative reaction using time-of-flight (TOF)-MS (*5*). On the basis of these results, we considered that a model peptide containing N-terminal Asp and His residues would be useful for evaluating oxidative stress and studying the modification of biomolecules using Cu(II) ion/ascorbic acid (*6*). In a previous study, few or no modifications of a model peptide ($A\beta_{1-6}$) were observed in reactions with H_2O_2 and Cu(II) ion/other catecholamines (dopamine, noradrenaline, or acetylcholine), with the exception of adrenaline (*6*). These results suggest that oxidative modification of peptides did not occur due to damage inflicted by H_2O_2 but rather occurred due to the coexistence of endogenous adrenaline and Cu(II) ion. The reduced metabolism that occurs with aging is

Published on Web 08/13/2010

mostly due to an increased concentration of metal ions in the human body (*7*). Moreover, in a state of extended hyperexcitability, adrenaline rushes through the human body, causing oxidative stress to impair normal physiological functions by the coexistence of adrenaline and Cu(II) ion. On the basis of these observations, it has been hypothesized that natural antioxidants derived from foods and natural extracts may be beneficial to prevent and/or delay the occurrence of age-related cognitive deficits (*8*−*10*). Thus, the discovery of specific antioxidants from natural products is reevaluated to identify potential therapeutic agents for the treatment of age-related diseases based on the prevention of antioxidant activity.

The previous screening assay for identifying specific inhibitors using only $A\beta_{1-6}$ and ascorbic acid is not applicable under physiologic conditions (*6*). Moreover, $A\beta_{1-40}$ and $A\beta_{1-42}$ would be not shown for the specific antioxidant assay of modified peptides. In this study, our experimental design was modified to a simple physiologically relevant oxidation that consisted of Cu(II) ion/endogenous adrenaline-mediated ROS generation and modification of N-terminal $A\beta$ peptides, such as $A\beta_{1-6}$, $A\beta_{1-40}$, and $A\beta_{1-42}$. Moreover, this simple LC/MS detection of a native and modified model peptide was used to identify specific and natural inhibitors of Cu(II) ion/adrenaline-mediated oxidative modification of a peptide.

MATERIALS AND METHODS

Reagents. Synthetic $A\beta_{1-6}$ (peptide purity, 98.40%; molecular weight, 773) was obtained from Sigma-Aldrich (St. Louis, MO). $A\beta_{1-42}$ (human, 1−42) and $A\beta_{1-40}$ (human, 1−40) proteins were obtained from Peptide Institute, Inc. (Osaka, Japan). Trypsin (mass spectrometry grade, 16900 U/mg) was obtained from Promega Co. (Madison, WI). Cu(II) sulfate anhydrous ($CuSO_4$), ammonium hydrogencarbonate (NH_4HCO_3), H_2O_2, high-performance liquid chromatography-grade water, methanol, acetonitrile, ethanol, trifluoroacetic acid (TFA), 1 M Tris-HCl buffer (pH 7.5), dopamine hydrochloride, DL-adrenaline, acetylcholine chloride, isoflavone (aglycon) mixture from soybean, catechin mixture from green tea, saponin from soybean, and capsaicin were obtained from Wako Chemical Co. (Osaka, Japan). Curcumin was obtained from San-Ei Gen FFI Co. (Osaka, Japan). Noradrenaline was obtained from Sigma-Aldrich. Purified water was obtained using a Milli-Q Simplicity UV system (Millipore, Bedford, MA).

Analytical Equipment and Conditions. LC/MS was performed using an LCMS-2010EV system (Shimadzu Co., Kyoto, Japan) that was coupled to a quadrupole mass spectrometer fitted with an electrospray ionization source. LC separation was performed using a TSK-GEL ODS 100 V column (2.0 mm × 150 mm, 3 μm; Tosoh Co., Tokyo, Japan).

The mobile phase was comprised of 0.1% aqueous TFA (solvent A) and 0.1% TFA in methanol (solvent B). An LC linear gradient was used as follows: 10% solvent B at 0 min, 50% solvent B at 20 min, 95% solvent B at 20.1 min, 95% solvent B at 25.0 min, and 10% solvent B at 25.1 min with a flow rate of 0.2 mL/min. The injection volume was 10 μL. The column temperature was 40 °C. The mass spectrometer was operated with an electrospray source in positive ionization and single ion monitoring (SIM) modes for the analytical state. The electrospray ionization source conditions were a nebulizer gas rate of 0.18 L/min, CDL temperature of 230 °C, block temperature of 200 °C, probe voltage of +3.5 kV, interface temperature of 250 °C, and 1 s event time, respectively, and were obtained from a nitrogen source (N_2 Supplier model 24S, Anest Iwata Co., Yokohama, Japan). The chromatogram was acquired in the TIC mode in fragment table. This LC/MS system was operated using LC-MS software version 3.41-324.

Peptide Solutions. $A\beta$ peptides such as $A\beta_{1-6}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ were dissolved in their original vial with water and acetonitrile (50/50, v/v) by sonication for 30 s to give 1, 0.1, and 0.1 mmol/L solutions. The solutions were stored at −20 °C until use. These solutions were useful for solubility and stability studies and LC separation of these peptides for 6 months.

Other Solutions for a Screening Assay for Inhibitors of MCO. Various concentrations of Cu(II) ($CuSO_4$), adrenaline, dopamine, noradrenaline, and acetylcholine were prepared using pure water as the solvent. For the screening assay, an isoflavone (aglycon) mixture, catechin mixture, saponin, capsaicin, and curcumin were prepared using pure water or ethanol as the solvent (10, 50, and 500 ppm).

MCO with Cu(II) Ions/Adrenaline or Other Compounds. In the optimal condition, MCO reactions were performed at 37 °C with 0.1 mM $A\beta_{1-6}$, 0.01 mM $A\beta_{1-40}$, or $A\beta_{1-42}$ peptides, 0.01 mM Cu(II), and 0.5 mM adrenaline in 50 mM Tris-HCl/Tris buffer (pH 7.4) for 90 min. The MCO reactions were initiated by the addition of adrenaline. For the investigated conditions, MCO reactions were performed at 37 °C with 0.1 mM $A\beta_{1-6}$ peptide, 0.01 mM Cu(II), and other compounds (dopamine, noradrenaline, and acetylcholine) in 50 mM Tris-HCl/Tris buffer (pH 7.4) for 90 min. The MCO reactions were initiated by adding these compounds.

Trypsin Digestion. Oxidized $A\beta$ was obtained from the reaction with Cu(II)/adrenaline in Tris-HCl/Tris buffer (pH 7.4) for 90 min. Native $A\beta$ was obtained from the reaction without adding Cu(II)/adrenaline in Tris-HCl/Tris buffer. The $A\beta$ peptides were digested with trypsin in RapiGest SF (1.0 mg/50 mM NH_4HCO_3 solution, 1 mL). The enzyme solution was used in the ratio of 1/20 (v/v), and incubation was conducted at 37 °C for 2 h.

Solid Phase Extraction (SPE) To Stop the MCO. Reactions were terminated by removing the water-soluble substrates using SPE. An OASIS-HLB (1 mL, 30 mg, Waters Co., Milford, MA) cartridge was used. Before the reacted solutions were extracted, the SPE cartridge was conditioned by eluting 1.0 mL of methanol followed by 1.0 mL of 1% aqueous TFA. After the MCO reaction, the sample solution was diluted 2-fold with 1.0% aqueous TFA and eluted through an SPE cartridge. The cartridge was then washed with 1.0 mL of water. Methanol was added at a low flow rate to elute the peptides that were retained in the cartridges. The solutions were evaporated to dryness at 30 °C. The sample volumes were then adjusted to 1.0 mL of water/methanol (80/20, V/V) and measured by LC/MS.

Screening Assay for Inhibitors of Metal-Catalyzed Oxidative Modification. To evaluate the MCO with Cu(II)/adrenaline inhibitors, the optimal reaction was performed at 37 °C with 0.1 mM $A\beta_{1-6}$ model peptide, 0.01 mM Cu(II) ($CuSO_4$), test substances (1, 5, and 50 ppm), and 0.5 mM adrenaline in 50 mM Tris-HCl/Tris buffered to a pH of 7.4 for 90 min. The MCO reactions were initiated by the addition of adrenaline. The reaction was terminated by SPE and measured using LC/MS. In this experiment, the test natural antioxidants were isoflavone (aglycon) mixture, catechin mixture, saponin, capsaicin, and curcumin. Test substance solutions were obtained by resuspending the dry samples in water or ethanol. For control values, a control sample was prepared without test substances in water or ethanol and was used to determine the background MCO inhibition level. The antioxidant activity for MCO was expressed as the inhibition value and calculated using the following formula:

$$A\ (\text{control value}) = [(\text{SIM response of } m/z\ 685;\ \text{modified model peptides in control sample})/(\text{SIM response of } m/z\ 774;\ \text{native model peptide in control sample})]$$

$$B\ (\text{test value}) = [(\text{SIM response of } m/z\ 685;\ \text{modified model peptides in test sample})/(\text{SIM response of } m/z\ 774;\ \text{native model peptide in test sample})]$$

$$\text{antioxidant activity for MCO of peptide } (\%) = [(A-B)/A] \times 100$$

If the test substance had antioxidant activity toward this reaction, these values would be significantly increased. On the other hand, if the substance did not have antioxidant activity, these values would be only slightly altered, near-zero values.

RESULTS AND DISCUSSION

Cu(II) Ion/Adrenaline-Mediated Oxidative Modification of N-Terminal $A\beta_{1-6}$ Peptide. A previous study indicated that $A\beta_{1-6}$ peptide containing His residues underwent spontaneous N-terminal Asp oxidative decarboxylation (decreased 45 Da) and alkoxyl radical pathway modification (decreased 89 Da) using MCO in Tris-HCl/Tris buffer (*6*). The m/z 774 $[M + H]^+$ on a SIM mode, and the optimal LC conditions were used to evaluate the $A\beta_{1-6}$ peptide before and/or after MCO with Cu(II) ion/ascorbic acid. After MCO of $A\beta_{1-6}$, two novel oxidized peptides were observed at m/z 729 and 685, corresponding to a decrease in mass of 45 and 89 Da, respectively, as compared with the $A\beta_{1-6}$ peptide. In

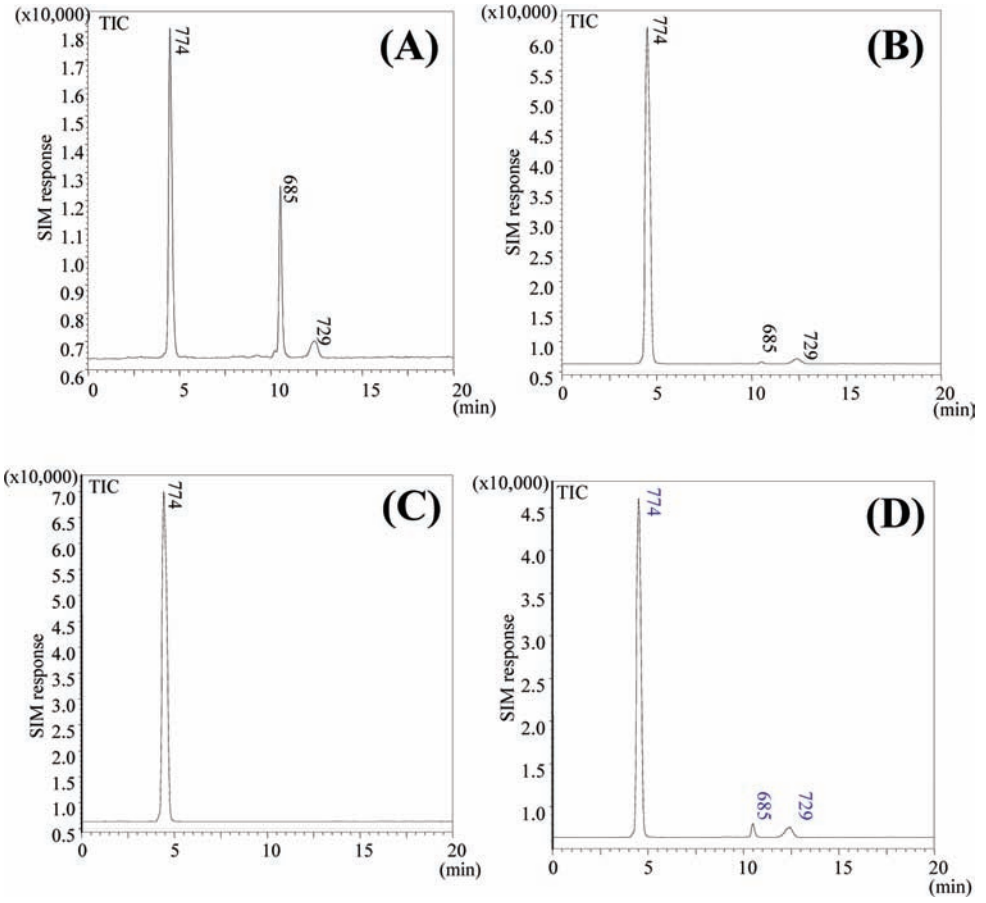

Figure 1. SIM chromatograms of native (MW 773) and modified model peptides with copper(II) ions and catecholamines. (**A**) Adrenaline, (**B**) norepinephrine, (**C**) acetylcholine, and (**D**) dopamine. The reaction was performed at 37 °C with 0.1 mM Aβ_{1-6}, 0.01 mM Cu(II), and 0.5 mM concentrations of each compound in 50 mM Tris-HCl/Tris buffer (pH 7.4) for 90 min.

addition, no modification was observed in the H_2O_2 reactions. This reaction using such a high concentration of ascorbic acid, however, is not a realistic or physiologic condition. Thus, alternate endogenous compounds were investigated in MCO with Cu(II) ion. The SIM chromatograms of native Aβ_{1-6} and oxidized peptides are shown in **Figure 1**. This experimental data showed a simple physiologically available oxidation that consisted of Cu(II) ion/adrenaline-mediated ROS generation and modification of the N-terminal Aβ_{1-6} peptide (**Figure 1A**). In contrast, the MCO reaction with noradrenaline, acetylcholine, and dopamine did not modify Aβ_{1-6} (**Figure 1B−D**). Therefore, the modified peptides were characterized by a specific MCO reaction of Aβ_{1-6} that contained both His and N-terminal Asp residues in Cu(II) ion/adrenaline in Tris-HCl/Tris buffer. Moreover, the optimal concentration of adrenaline was investigated in MCO with Cu(II) ion. Adrenaline concentrations were changed while maintaining the Cu(II) concentration at 0.01 mM for 90 min. The adrenaline had little effect at concentrations of less than 0.1 mM (**Figure 2**). Therefore, the optimal condition for this reaction was 0.01 mM Cu(II)/0.5 mM adrenaline in Tris-HCl buffer (pH 7.4) for 90 min, based on a previous report (*6*).

Cu(II) Ion/Adrenaline-Mediated Oxidative Modification of Aβ_{1-40} and Aβ_{1-42} Peptides. Under the optimal MCO conditions of oxidized Aβ_{1-6}, the reaction was fully saturated with 0.01 mM Cu(II)/0.5 mM adrenaline in Tris-HCl buffer (pH 7.4) for 90 min. The adrenaline induces an abnormal or defective state in the peptide by constantly increasing Cu(II) ion levels. This discovery suggests a possible link between a state of hyperexcitability and oxidative stress in diseases of aging. Furthermore, the effects of

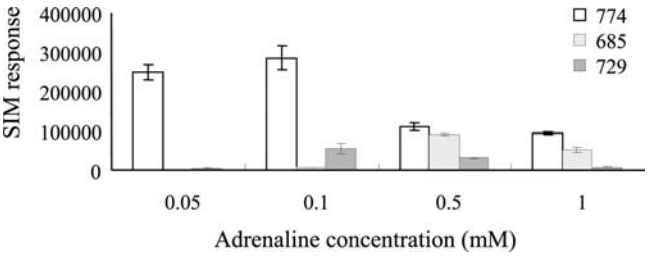

Figure 2. Investigation of the optimal concentration of adrenaline in MCO reaction. The reaction was performed at 37 °C with 0.1 mM Aβ_{1-6}, 0.01 mM Cu(II), and 0.05−1.0 mM adrenaline in 50 mM Tris-HCl/Tris buffer (pH 7.4) for 90 min.

this Cu(II) ion/adrenaline-mediated oxidative modification were unknown for the important Aβ peptides 1−40 and 1−42. Thus, we investigated whether Aβ peptides 1−40 and 1−42 were oxidized by the Cu(II) ion/adrenaline reaction. The resulting peptides were subjected to trypsin digestion, stopped by SPE, and analyzed by LC/MS. A tryptic fragment corresponding to N-terminal ^{1}DAEFR^5 was observed with a singly charged ion corresponding to $[M + H]^+$ m/z 637. The retention time (7.8 min) and mass spectrum were identical with those of an authentic peptide (^{1}DAEFR^5) obtained from trypsin digestion of all Aβ peptides that had not been subjected to MCO (**Figure 3A**,**C**,**E**). Typical SIM chromatograms of Aβ peptides from trypsin digestion are shown in **Figure 3A**,**C**,**E**. Aβ peptides 1−40 and 1−42 were oxidized using Cu(II) ion/adrenaline and subjected to

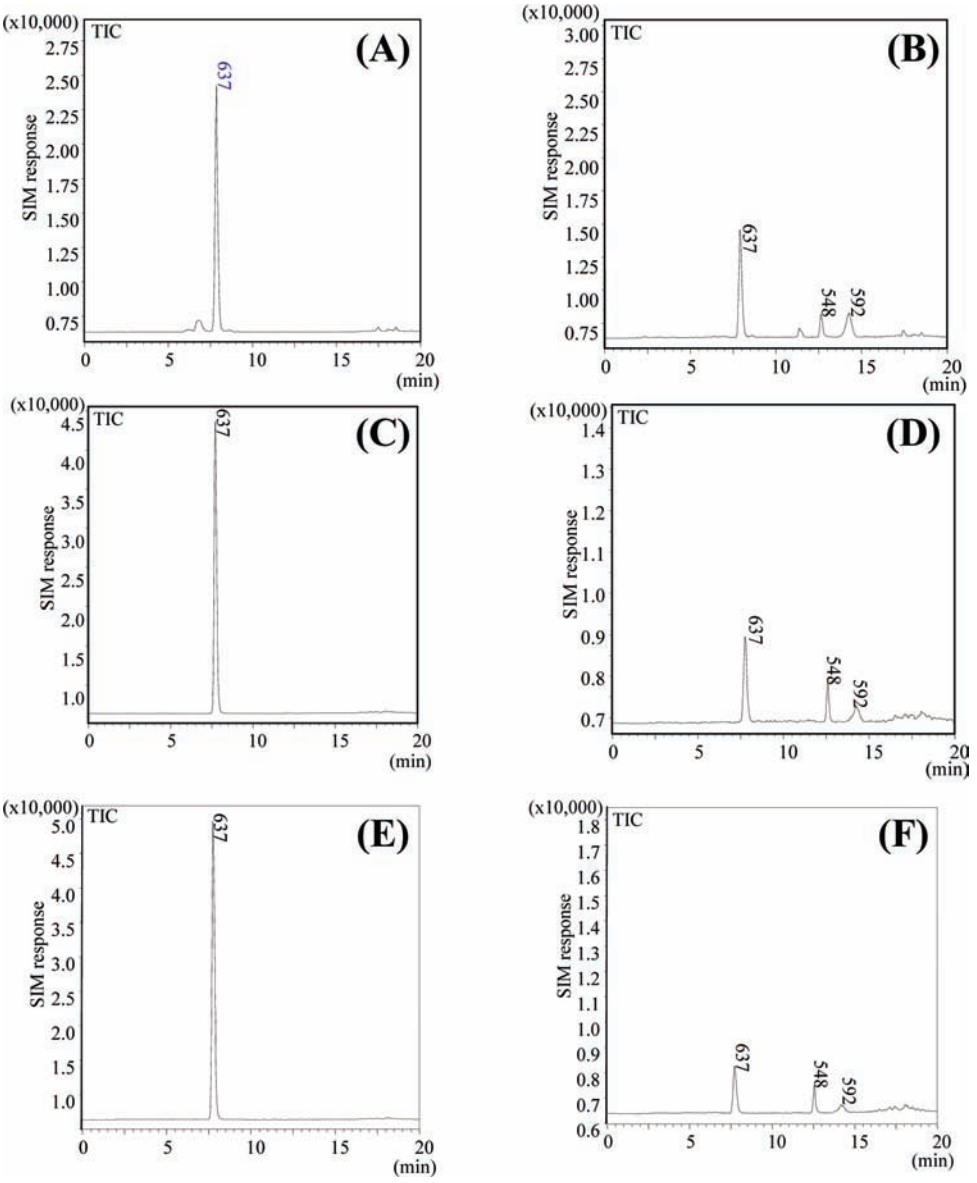

Figure 3. SIM chromatograms of tryptic peptides corresponding to native and oxidized [1]DAEFR[5] from Aβ type 1−6, 1−40, and 1−42. (**A**) Tryptic Aβ_{1-6} peptide, (**B**) tryptic oxidized Aβ_{1-6} peptide with Cu(II)/adrenaline, (**C**) tryptic Aβ_{1-40} peptide, (**D**) tryptic oxidized Aβ_{1-40} peptide with Cu(II)/adrenaline, (**E**) tryptic Aβ_{1-42} peptide, and (**F**) tryptic oxidized Aβ_{1-42} peptide with Cu(II)/adrenaline. Monitoring ions: *m*/*z* 637, 592, and 548 for native and oxidized [1]DAEFR[5].

trypsin digestion for the identification of specific oxidative modification of N-terminal Asp oxidative decarboxylation (decreased by 45 Da) and alkoxyl radical modification (decreased by 89 Da). Two tryptic peptides corresponding to oxidized [1]DAEFR[5] (decreased 89 Da and 45 Da) with retention times of 12.6 and 14.2 min, respectively, were observed by LC/MS with the SIM mode (**Figure 4B**,**D**,**F**). Thus, short (Aβ_{1-6}) and long (Aβ_{1-40} and Aβ_{1-42}) peptides could be oxidized by the Cu(II) ions/adrenaline reaction. It was recently reported that the brains of Alzheimer's disease patients display evidence of Cu(II) ion dyshomeostasis and increased oxidative stress (*11*) and that Cu(II) ions-Aβ complexes have been identified in Alzheimer's disease and catalytically oxidize cholesterol and lipid to generate H_2O_2 and lipid peroxides (*12*). A computational study indicated an MCO mechanism with Cu-Aβ peptide complexes (*13*). Thus, Cu(II) ions are important factors for the oxidative reaction in Alzheimer's disease. We also tested the efficacy of reducing agents on MCO-induced modification of Aβ peptides. Adrenaline is rapidly oxidized (*14*). This report is consistent with the concept that adrenaline plays a key role in reducing Cu(II) to Cu(I) to initiate a one-electron reduction of molecular oxygen. On the basis of these previous and current results, the first process is that Cu(II)-Aβ

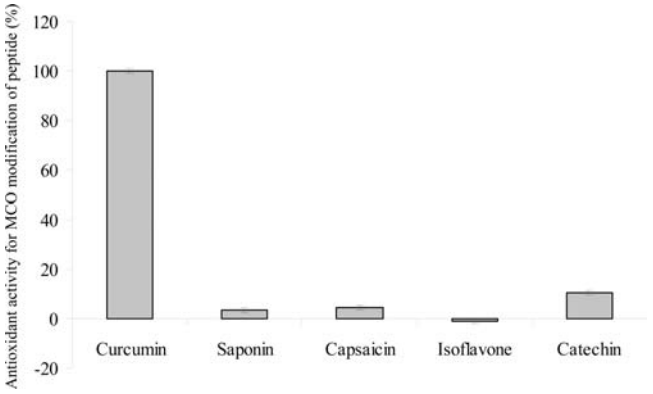

Figure 4. Antioxidant activity against MCO modification of the Aβ_{1-6} peptide using SIM response ratios of modified peptides (*m*/*z* 685) per native (*m*/*z* 774) peptide in control and test (50 ppm) reactions.

complexes (specific sites: Asp^1 and His^6) are formed in aqueous buffer. Then, a specific MCO reaction of Aβ peptides occurs due to the presence of adrenaline and dissolved oxygen. This irreversible modification may occur in the progression of diseases related to aging regarding Cu(II) ions and adrenaline.

Screening Assay for Natural Antioxidants of Cu(II) Ion/Adrenaline-Mediated Oxidation of N-Terminal Aβ_{1-6} Peptide. Using LC/MS detection of the modified peptide, we also investigated the efficacy of a reducing agent (adrenaline) and Cu(II) ions on MCO-induced modification of Aβ peptides. Thus, the screening assay of natural antioxidants for Cu(II) ion/adrenaline-mediated oxidation was developed using LC/MS with Aβ_{1-6} peptide for physiologically realistic application. Aβ_{1-6} is a shorter peptide for MCO than the other Aβ peptides and does not require trypsin digestion for LC/MS detection.

To evaluate the substances that inhibit MCO, MCO was induced with the Aβ_{1-6}, Cu(II), test substances (1, 5, and 50 ppm), and adrenaline in Tris-HCl buffer (pH 7.4) for 90 min. The reaction was then terminated by SPE and measured by LC/MS. The antioxidant activity against MCO modification of the peptide was calculated based on the LC/MS-SIM response ratio of modified peptides (m/z 685) per native (m/z 774) Aβ_{1-6} peptide in control and test reactions. Natural antioxidants, isoflavone mixture, catechin mixture, saponin, capsaicin, and curcumin, were selected for preliminary test. In these results of screening assay, test antioxidants of isoflavone, catechin, saponin, and capsaicin did not inhibit MCO-induced Aβ modification with Cu(II) ion/adrenaline. Low concentrations (1 and 5 ppm) did not inhibit this reaction. On the other hand, curcumin at a concentration of 50 ppm (136 μM) more effectively inhibited this reaction than did the other compounds (**Figure 4**). Thus, on the basis of the findings obtained using this novel assay, there are major differences between curcumin and other known antioxidants. An interaction between Cu(II) and curcumin by resonance light scattering technique was recently reported (*15*). The interaction between Cu(II) ion and curcumin is important because the binding of curcumin to these metals is considered useful for the treatment of Alzheimer's disease (*16*). In addition, the keto−enol tautomerism of curcumin derivatives indicated that a novel target has Aβ-binding ability for both therapy and Aβ imaging in Alzheimer's disease (*17*). Our data lend support to this notion regarding the interaction between curcumin, Cu(II), and/or N-terminal Aβ site against Aβ oxidative modification.

In conclusion, this novel and simple screening assay of Cu(II) and adrenaline-mediated MCO inhibitors allowed for the discovery of interesting effects of natural antioxidant such as curcumin. A previous assay was used for only Aβ_{1-6} with Cu(II)/ascorbic acid for antioxidants of specific modification of peptide. In this assay, we used the Aβ_{1-40} and Aβ_{1-42} and Cu(II)/endogenous adrenaline-mediated ROS generation and modification of peptides. In addition, the modified Aβ_{1-42} has been important factor regarding to immunogenic, oligomergenic, fibrillogenic, neurotoxic, and associated with disease states (*18*). Thus, the use of this newly developed assay allows us to determine specific antioxidants that are effective against MCO of peptides related to degenerative diseases. Our approach overcomes the limitations of previous antioxidant assays and directly measures the specific MCO of Aβ peptides for the evaluation of new natural antioxidants.

ABBREVIATIONS USED

ROS, reactive oxygen species; Aβ, amyloid β; MCO, metal-catalyzed oxidation; LC/MS, liquid chromatography with mass spectrometry; His, histidine; Asp, aspartic acid; SPE, solid phase extraction; SIM, single ion monitoring.

LITERATURE CITED

(1) Rees, M. D.; Kennett, E. C.; Whitelock, J. M.; Davies, M. J. Oxidative damage to extracellular matrix and its role in human pathologies. *Free Radical Biol. Med.* **2008**, *44*, 1973–2001.

(2) Lambeth, J. D. NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* **2004**, *4*, 181–189.

(3) Rhee, S. G. H_2O_2, a necessary evil for cell signaling. *Science* **2006**, *312*, 1882–1883.

(4) Inoue, K.; Garner, C.; Ackermann, B. L.; Oe, T.; Blair, I. A. Liquid chromatography/tandem mass spectrometry characterization of oxidized amyloid beta peptides as potential biomarkers of Alzheimer's disease. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 911–918.

(5) Kowalik-Jankowska, T.; Ruta, M.; Wiśniewska, K.; Łankiewicz, L.; Dyba, M. Products of Cu(II)-catalyzed oxidation in the presence of hydrogen peroxide of the 1−10, 1−16 fragments of human and mouse beta-amyloid peptide. *J. Inorg. Biochem.* **2004**, *98*, 940–950.

(6) Inoue, K.; Nakagawa, A.; Hino, T.; Oka, H. Screening assay for inhibitors of metal-catalyzed oxidative modification using liquid chromatography mass spectrometry with N-terminal amyloid-β model peptide. *Anal. Chem.* **2009**, *81*, 1819–1825.

(7) Bush, A. I. The metallobiology of Alzheimer's disease. *Trends Neurosci.* **2003**, *26*, 207–214.

(8) Rossi, L.; Mazzitelli, S.; Arciello, M.; Capo, C. R.; Rotilio, G. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. *Neurochem. Res.* **2008**, *33*, 2390–2400.

(9) Covas, M. I. Bioactive effects of olive oil phenolic compounds in humans: reduction of heart disease factors and oxidative damage. *Inflammopharmacology* **2008**, *16*, 216–218.

(10) Brambilla, D.; Mancuso, C.; Scuderi, M. R.; Bosco, P.; Cantarella, G.; Lempereur, L.; Di Benedetto, G; Pezzino, S.; Bernardini, R. The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: A point of view for an assessment of the risk/benefit profile. *Nutr. J.* **2008**, *7*, 29.

(11) Hung, Y. H.; Bush, A. I.; Cherny, R. A. Copper in the brain and Alzheimer's disease. *J. Biol. Inorg. Chem.* **2010**, *15*, 61–76.

(12) Hung, Y. H.; Robb, E. L.; Volitakis, I.; Ho, M.; Evin, G.; Li, Q. X.; Culvenor, J. G.; Masters, C. L.; Cherny, R. A.; Bush, A. I. Paradoxical condensation of copper with elevated beta-amyloid in lipid rafts under cellular copper deficiency conditions: implications for Alzheimer disease. *J. Biol. Chem.* **2009**, *284*, 21899–2907.

(13) Barman, A.; Taves, W.; Prabhakar, R. Insights into the mechanism of methionine oxidation catalyzed by metal (Cu(2+), Zn(2+), and Fe(3+)) - amyloid beta (Abeta) peptide complexes: A computational study. *J. Comput. Chem.* **2009**, *30*, 1405–1413.

(14) Costa, V. M.; Silva, R.; Ferreira, L. M.; Branco, P. S.; Carvalho, F.; Bastos, M. L.; Carvalho, R. A.; Carvalho, M.; Remião, F. Oxidation process of adrenaline in freshly isolated rat cardiomyocytes: formation of adrenochrome, quinoproteins, and GSH adduct. *Chem. Res. Toxicol.* **2007**, *20*, 1183–1191.

(15) Chen, Z.; Zhu, L.; Song, T.; Chen, J.; Guo, Z. A novel curcumin assay with the metal ion Cu (II) as a simple probe by resonance light scattering technique. *Spectrochim. Acta, Part A* **2009**, *72*, 518–522.

(16) Zhang, H. Y. One-compound-multiple-targets strategy to combat Alzheimer's disease. *FEBS Lett.* **2005**, *579*, 5260–5264.

(17) Yanagisawa, D.; Shirai, N.; Amatsubo, T.; Taguchi, H.; Hirao, K.; Urushitani, M.; Morikawa, S.; Inubushi, T.; Kato, M.; Kato, F.; Morino, K.; Kimura, H.; Nakano, I.; Yoshida, C.; Okada, T.; Sano, M.; Wada, Y.; Wada, K. N.; Yamamoto, A.; Tooyama, I. Relationship between the tautomeric structures of curcumin derivatives and their Abeta-binding activities in the context of therapies for Alzheimer's disease. *Biomaterials* **2010**, *31*, 4179–4185.

(18) Rossi, M.; Piovesan, P.; Ghirardi, O.; Mastroianni, D.; Bombardi, V.; Battistini, L.; Cencioni, M. T.; Capobianco, D.; Borsini, F.; Colombo, M.; Verdoliva, A. Immunogenic, antigenic, fibrillogenic and inflammatory properties of new simplified beta-amyloid peptides. *Mol. Immunol.* **2009**, *46*, 2524–2532.

Received for review May 5, 2010. Revised manuscript received August 2, 2010. Accepted August 4, 2010. This study was supported in part by Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (Grant No. 20790043).