JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Oxidative Deamination of Benzylamine and Lysine Residue in Bovine Serum Albumin by Green Tea, Black Tea, and Coffee

MITSUGU AKAGAWA,*,† TOMOKO SHIGEMITSU,‡ AND KYOZO SUYAMA‡

Department of Biological Chemistry, Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-chyo, Sakai, Osaka 599-8531, Japan, and Division of Bioscience and Biotechnology for Future Bioindustries, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi Aoba-ku, Sendai 981-8555, Japan

Oxidative deamination by various polyphenolic compounds is presumed to be due to the oxidative conversion of polyphenols to the corresponding quinones through autoxidation. Here we examined the oxidative deamination by the polyphenol-rich beverages green tea, black tea, and coffee at a physiological pH and temperature. Green tea, black tea, and coffee extracts oxidatively deaminated benzylamine and the lysine residues of bovine serum albumin to benzaldehyde and α-aminoadipic δ-semialdehyde residues, respectively, in sodium phosphate buffer (pH 7.4) at 37 °C in both the presence and absence of Cu^{2+}, indicating the occurrence of an amine (lysyl) oxidase-like reaction. We also examined the effects of pH and metal ions on the reaction. The possible biological effects of drinking polyphenol-rich beverages on human are also discussed.

KEYWORDS: Polyphenol; oxidative deamination; amine oxidase; lysyl oxidase; caffeic acid; catechin; green tea; black tea; coffee

INTRODUCTION

Polyphenols are a large group of antioxidants naturally present in fruits, vegetables, and beverages such as wine, coffee, and tea. Orally administered polyphenols are widely distributed in mammalian tissues (*1*−*4*). Recent epidemiological studies have shown an inverse association between the intake of some beverages rich in polyphenols and the risk of cancer and cardiovascular diseases (*1*, *2*, *5*−*8*). These protective effects have been believed to be due to the antioxidant activity of the polyphenols (*9*−*11*), since reactive oxygen metabolites are considered important factors for cancer development and oxidation of low-density lipoproteins (LDL). Generally, plant polyphenols can act as free radical scavengers quenching hydroxyl radical (*9*), superoxide anion (*12*), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (*13*), etc.

Catechin administration has been reported to elevate lysyl oxidase activity in chick aorta, increase collagen stability, and protect against lathyrism induced by β-aminopropionitrile (BAPN), which is a specific inhibitor of the enzyme (*14*−*16*). Lysyl oxidase (EC 1.4.3.13), a kind of copper-containing amine oxidase, catalyzes the oxidative deamination of ε-amino groups of lysine residues to form α-aminoadipic δ-semialdehyde (AAS) residues, which are precursors of cross-links and are required for proper cross-linking of elastin and collagen (*17*). Furthermore, we have recently demonstrated that polyphenols in the presence of Cu^{2+} oxidatively deaminate benzylamine and the lysine residue of bovine serum albumin (BSA) under a physiological pH and temperature, indicating an amine (lysyl) oxidase-like activity (*18*). In general, *o*-diphenolic compounds have been known to be converted to the corresponding *o*-quinones in the presence of transition metal ions and O_2, concomitant with the H_2O_2 evolution, via so-called autoxidation (*19*, *20*). Moreover, various quinone compounds are known to undergo the oxidative deamination reaction (*21*, *22*). Actually, lysyl oxidase contains the amino-*o*-quinone cofactor lysine tyrosylquinone (LTQ) at the active site (*23*, *24*). On the basis of these facts, the proposed mechanism of the oxidative deamination by polyphenols is quinone-mediated oxidation as shown in **Figure 1**.

In the present study, we examined the oxidative deamination by the polyphenol-rich beverages green tea, black tea, and coffee and their polyphenolic components. Green tea, black tea, and coffee are the most widely consumed beverages in the world and will likely be the major source of polyphenols. Polyphenols in green tea are predominantly members of three subclasses: flavanols, flavones, and flavonols (*4*, *25*). Four major flavanols or catechins (flava-3-ols), (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate, constitute about a third of the dry weight of green tea. Whereas green tea undergoes little oxidation, the preparation of black tea, which constitutes about 80% of the tea production in the world, is primarily an oxidation step of tea leaves catalyzed by polyphenol oxidase. As a result, many oxidation products of

* To whom correspondence should be addressed: tel +81-72-254-9460; fax +81-72-254-9921; e-mail akagawa@biochem.osakafu-u.ac.jp.
† Osaka Prefecture University.
‡ Tohoku University.

10.1021/jf050843f CCC: $30.25
Published on Web 09/09/2005

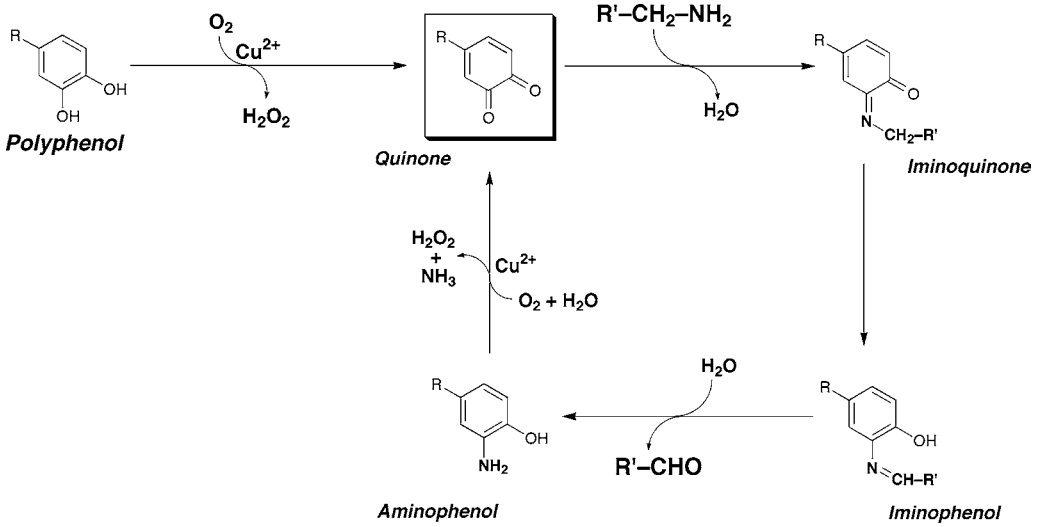

Figure 1. Proposed mechanism of amine oxidase-like activity of polyphenols.

polyphenols, in particular catechins, are present in black tea (*4*, *25*). The dimeric forms of these products have been identified as theaflavins, theaflagallins, theasinensins, and theacitrins. Thearubigens have been also characterized as high molecular weight, heterogeneous polyphenolic condensation products in black tea. The main polyphenol in coffee is chlorogenic acid. Catechol derivatives such as chlorogenic acid and caffeic acid are present at about 250 mg per cup of coffee (*4*, *26*).

In the present study, we incubated green tea, black tea, and coffee extracts with benzylamine or BSA under a physiological condition (pH 7.4, 37 °C) and determined the oxidative deamination by measuring the formation of both benzaldehyde and AAS by high-performance liquid chromatography (HPLC). We also examined the effects of pH and metal ions on the reaction. In addition, the possible biological effects of drinking polyphenol-rich beverages on human are discussed.

MATERIALS AND METHODS

Materials. Acetonitrile was of HPLC grade from Kanto Chemicals, Tokyo, Japan. (+)-Catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate were purchased from Funakoshi, Tokyo, Japan. Folin and Ciocalteu's phenol reagent was from Sigma−Aldrich, St. Louis, MO. All other chemicals were of analytical grade from Nacalai Tesque, Kyoto, Japan.

Oxidative Deamination of Benzylamine by Various Polyphenols. Each polyphenol (250 μM) was incubated with or without 250 μM $CuSO_4$ in 50 mM sodium phosphate buffer containing 5.0 mM benzylamine (pH 7.4) at 37 °C for 24 h with shaking in the dark. After incubation, the reaction was terminated by the addition of acetic acid (500 μL). We confirmed that this procedure stopped the reaction and completely converted the Schiff bases of benzaldehyde and benzylamine to the corresponding constituents. The samples (20-μL aliquots) were chromatographed with acetonitrile/0.02% aqueous phosphoric acid (4:6 v/v). The HPLC analysis was performed on a reversed-phase HPLC column (Cosmosil $5C_{18}$-AR-II, 150 × 4.6, Nacalai Tesque) with detection at 245 nm. The column oven was maintained at 40 °C. Benzaldehyde was eluted at 5.2 min at a flow rate of 1.0 mL/min.

Preparation of Black Tea, Green Tea, and Coffee Extracts. The extracts of black tea, green tea, and coffee were prepared by extracting 100 mg of black tea leaves (blend Darjeeling tea, R. Twining, London, U.K.), green tea leaves (blend Senchya, Ujien, Sendai, Japan), and ground coffee beans (Mocha blend, M. M. C., Yokohama, Japan) with 10 mL of distilled deionized water heated at 90 °C for 10 min. Resulting beverages were then filtered and cooled at room temperature.

Total Polyphenol Assay. Total polyphenol contents in green tea, black tea, and coffee extracts were analyzed by Folin assay (*27*). For the analysis, 10 mM (+)-catechin was used as the standard. An aliquot of standard, blank, or extract (300 μL) was added to 3.0 mL of Folin-Ciocarteu's reagent, previously diluted 1:10 with distilled deionized water. The mixture was then incubated at 25 °C for 20 min in the dark, followed by colorimetric measurement at 750 nm.

Oxidative Deamination of Benzylamine by Green Tea, Black Tea, and Coffee. An aliquot (500 μL) of the freshly prepared extract of green tea, black tea, or coffee was put in a micro test tube, and 500 μL of 100 mM sodium phosphate buffer containing 10 mM benzylamine (pH 7.4) was added with or without 500 μM of each tested metal ion ($CuSO_4$, $VOSO_4$, $MnCl_2$, $AgNO_3$, $CoCl_2$, $FeCl_3$, $NiCl_2$, $ZnCl_2$, $CrCl_3$, $MgCl_2$, and $CaCl_2$). In some experiments, 500 μM ethylenediaminetetraacetic acid (EDTA) was added to the dilution buffer. The resulting mixtures were maintained between pH 7.3 and 7.5. The reaction mixtures were incubated at 37 °C for the indicated periods up to 72 h with shaking in the dark. After incubation, the reaction was terminated by the addition of acetic acid (500 μL). We confirmed that this procedure stopped the reaction and completely converted the Schiff bases of benzaldehyde and benzylamine to the corresponding constituents. Then, the production of benzaldehyde was measured by HPLC as previously described.

Effect of pH on Oxidative Deamination of Benzylamine. An aliquot (500 μL) of the freshly prepared extract of green tea, black tea, or coffee was put in a micro test tube, and 500 μL of either 100 mM sodium phosphate buffer (pH 6, 7, 8, and 9) or sodium acetate buffer (pH 5) containing 10 mM benzylamine was added with $CuSO_4$ (500 μM). After the measurement of pH, the reaction mixtures were incubated for 12 h at 37 °C with shaking in the dark. After incubation, the production of benzaldehyde was measured by HPLC as previously described.

Oxidative Deamination of Lysine Residue of BSA by Green Tea, Black Tea, and Coffee. An aliquot (1.0 mL) of the freshly prepared extract of green tea, black tea, or coffee was put in a Pyrex test tube with a Teflon-lined screw cap, and 1.0 mL of 100 mM sodium phosphate buffer containing 20.0 mg/mL BSA (pH 7.4) was added with or without $CuSO_4$ (500 μM). The mixtures were maintained between pH 7.3 and 7.5. The reaction mixtures were incubated at 37 °C for the indicated periods up to 48 h with shaking in the dark. After incubation, the mixture was treated with 2 mL of cold 10% (w/v) trichloroacetic acid (TCA) in an ice bath. After 5 min, the mixture was centrifuged at 2000*g* for 30 min, and the resulting pellet of precipitated protein was separated. The pellet was washed with 2 mL of cold 5% (w/v) TCA. Then the resulting protein was hydrolyzed for HPLC analysis as described below.

AAS was derivatized to a bisphenol derivative, 1-amino-1-carboxy-5,5-bis(*p*-hydroxyphenyl)pentane (ACPP), and determined with some modifications of a previously reported method (*18*, *28*) as follows. Briefly, the protein was hydrolyzed for 48 h at 110 °C with 4 mL of 6 M HCl containing 3% (v/v) phenol. The hydrolysate was dried under vacuum followed by a reconstitution in 500 μL of distilled water. After

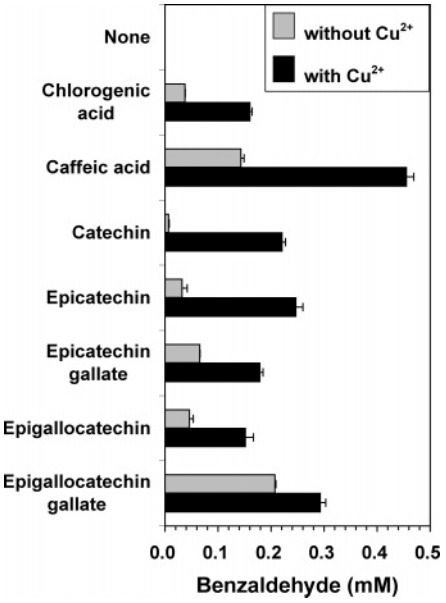

Figure 2. Oxidative deamination of benzylamine by polyphenols. Each polyphenol (250 μM) was incubated with or without 250 μM $CuSO_4$ in 50 mM sodium phosphate buffer containing 5.0 mM benzylamine (pH 7.4) at 37 °C for 24 h. After the reaction was terminated, the production of benzaldehyde was measured by HPLC. The values are shown as means ± SD ($n = 3$).

filtration with a syringe filter, a 20-μL aliquot was injected into an HPLC apparatus with a C-18 reversed-phase column (Superspher RP-18 ODS column, 150 × 4 mm, Merck, Darmstadt, Germany). The solvent was 100 mM sodium phosphate buffer/acetonitrile (5:1 v/v) containing 20 mM sodium dodecyl sulfate at pH 3.0. The flow rate was 1.0 mL/min, and the column oven was maintained at 40 °C.

RESULTS AND DISCUSSION

We have assumed that the polyphenols are oxidatively converted to the corresponding quinones and gain an amine oxidase-like activity as shown in **Figure 1** (*18*). In general, *o*-diphenolic compounds are converted to *o*-quinones in the presence of transition metal ions and O_2, concomitant with H_2O_2 production, via the so-called autoxidation (*19*, *20*). We examined oxidative deamination by the polyphenol-rich beverages green tea, black tea, and coffee and their polyphenolic components in the presence or absence of metal ions at a physiological pH and temperature. Oxidative deamination was determined by measuring the formation of benzaldehyde and AAS from benzylamine and the lysine residue of BSA by HPLC.

Oxidative Deamination of Benzylamine by Various Polyphenols. We examined the oxidative deamination using epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, and catechin, which are abundant in green tea and black tea, and chlorogenic acid and caffeic acid, which occur at high content in coffee. In both the presence and absence of 250 μM Cu^{2+}, a significant amount of benzaldehyde was produced by the incubation of benzylamine (5.0 mM) and each polyphenol (250 μM) in 50 mM phosphate buffer (pH 7.4) at 37 °C during 24 h as shown in **Figure 2**. The oxidation was apparently accelerated by the addition of Cu^{2+} in agreement with our previous studies (*18*), indicating that Cu^{2+} catalyzes the autoxidation of polyphenols.

Oxidative Deamination of Benzylamine by Green Tea, Black Tea, and Coffee. Figure 3 shows the formation of benzaldehyde during incubation of green tea, black tea, and coffee extracts. In both the presence and absence of Cu^{2+}, the formation of

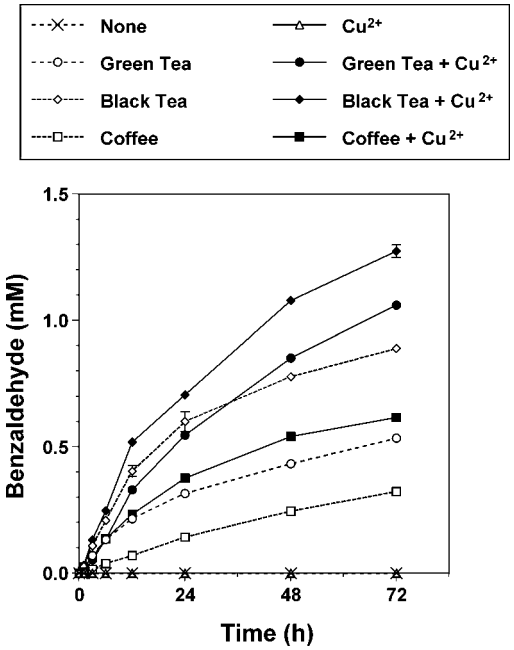

Figure 3. Time course of oxidative deamination of benzylamine by green tea, black tea, and coffee extracts. An aliquot (500 μL) of each extract (10 mg of solid/mL) was added to 500 μL of 100 mM sodium phosphate buffer containing 10 mM benzylamine (pH 7.4) with or without $CuSO_4$ (500 μM). Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. The mixtures were incubated at 37 °C for 0–72 h. After the reaction was terminated, the production of benzaldehyde was measured by HPLC. The values are shown as means ± SD ($n = 3$).

benzaldehyde with all extracts increased without reaching a plateau over the incubation time period. The presence of Cu^{2+} accelerated the generation of benzaldehyde with the extracts in a similar manner as with tested polyphenols. This observation supports the general character of the metal-dependent effects of the autoxidation (*19*, *20*). The final formation of benzaldehyde by green tea, black tea, and coffee after 72 h was 1.06, 1.27 and 0.61 mM, respectively. The black tea extracts had the greatest activity, followed by the green tea extract. Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. The oxidative deamination reaction is considered to be dependent on the contents and compositions of polyphenols in the extracts.

Because the oxidative deamination was apparently accelerated by the addition of Cu^{2+}, we investigated the effects of physiologically important metal ions on the reaction. The formation of benzaldehyde in the presence of various metal ions tested was highest in the case of Co^{2+} and VO^{2+}, followed by Cu^{2+}, whereas other metal ions did not significantly affect the reaction as summarized in **Figure 4**. In the absence of each extract, the formation of benzaldehyde was not observed in incubation of benzylamine with or without any metal ions.

Previously, we reported that the oxidative deamination reaction by phenolic compounds markedly inhibited by the addition of a chelating reagent (*18*). Polyphenols are known to form complexes with Cu^{2+} through their aromatic hydroxyl groups, and the postulated mechanism of the Cu^{2+}-catalyzed autoxidation of polyphenols is that polyphenols form a complex with Cu^{2+} in the first step (*19*). Therefore, we examined the effect of a chelating reagent, EDTA, on the reaction (**Figure 5**). In the absence of Cu^{2+}, the addition of EDTA hardly affected the generation of benzaldehyde by all extracts but inhibited it

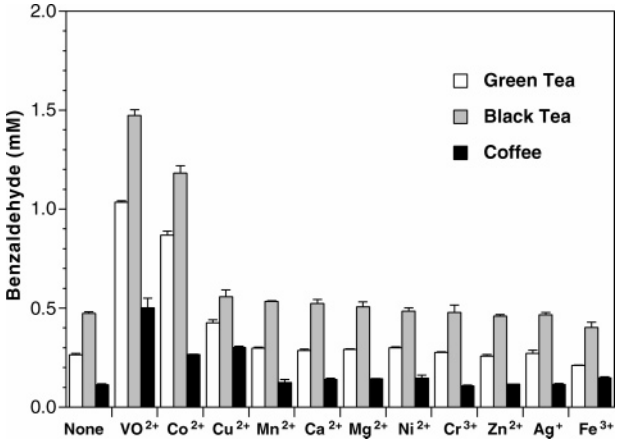

Figure 4. Effects of various metal ions on oxidative deamination of benzylamine by green tea, black tea, and coffee extracts. An aliquot (500 μL) of each extract (10 mg of solid/mL) was added to 500 μL of 100 mM sodium phosphate buffer containing 10 mM benzylamine (pH 7.4) with or without indicated metal ions (500 μM). Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. The mixtures were incubated at 37 °C for 12 h. After the reaction was terminated, the production of benzaldehyde was measured by HPLC. The values are shown as means ± SD ($n = 3$).

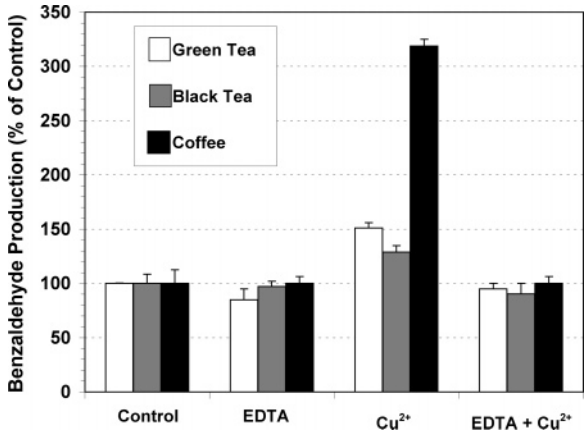

Figure 5. Effect of EDTA on oxidative deamination of benzylamine by green tea, black tea, and coffee extracts. An aliquot (500 μL) of each extract (10 mg of solid/mL) was added to 500 μL of 100 mM sodium phosphate buffer containing 10 mM benzylamine (pH 7.4) with or without indicated materials (500 μM). Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. The mixtures were incubated at 37 °C for 12 h. After the reaction was terminated, the production of benzaldehyde was measured by HPLC. The values are shown as means ± SD ($n = 3$).

in the presence of Cu^{2+} to control levels. The inhibition of the Cu^{2+}-catalyzed reaction by EDTA might also be ascribed to prevention of the formation of a complex between polyphenols and Cu^{2+}.

The autoxidation rate of polyphenols has been reported to be accelerated with increasing pH value (*19*). Furthermore, we previously reported that the amine oxidase-like activity of polyphenols is pH-dependent. Therefore, the effect of pH on the reaction with the green tea, black tea, and coffee extracts was also investigated. As shown in **Figure 6**, in agreement with our previous observation (*18*), the formation of benzaldehyde by all beverages was markedly elevated at higher pH. At pH 5, benzaldehyde production was not observed.

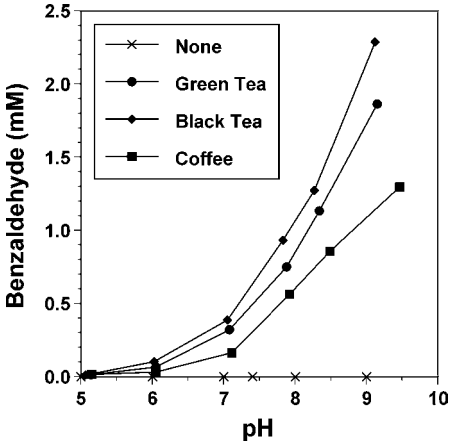

Figure 6. Effect of pH on oxidative deamination of benzylamine by green tea, black tea, and coffee extracts. An aliquot (500 μL) of each extract (10 mg of solid/mL) was added to 500 μL of 100 mM sodium phosphate buffer (pH 6–9) or 100 mM sodium acetate buffer (pH 5) containing 10 mM benzylamine with or without $CuSO_4$ (500 μM). Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. After the pH was adjusted, the reaction mixtures were incubated at 37 °C for 12 h. After the reaction was terminated, the production of benzaldehyde was measured by HPLC. The values are shown as means ± SD ($n = 3$).

Oxidative Deamination of Lysine Residue of BSA by Green Tea, Black Tea, and Coffee. Our previous study showed that the incubation of rutin, gallic acid, chlorogenic acid, and caffeic acid with BSA converts the lysine residues of BSA to AAS in the presence of Cu^{2+} (*18*). Therefore, the oxidative deamination of lysine residues by green tea, black tea, and coffee extracts was also monitored with reaction time by the formation of AAS. As shown in **Figure 7**, all extracts oxidatively deaminated the lysine residue of BSA to AAS in both the presence and absence of Cu^{2+}. Furthermore, a marked acceleration of the reaction was also observed in the presence of Cu^{2+}. The concentration of AAS increased with the incubation period (48 h). The black tea extract had the greatest activity against BSA, followed by the green tea extract, in a similar manner against benzylamine. The final formation of AAS with the green tea, black tea, and coffee extracts in the presence of Cu^{2+} after 48 h was 13.4 (0.93), 30.1 (2.08), and 6.0 (0.41) nmol/mg of BSA (mol/mol of BSA), respectively.

Biological Significance of Oxidative Deamination by Green Tea, Black Tea, and Coffee Extracts. We have assumed that polyphenols are oxidatively converted to the corresponding quinones and gain the amine oxidase-like activity at a physiological pH and temperature. In the present study, we examined the oxidative deamination activity of green tea, black tea, and coffee extracts. Green tea, black tea, and coffee extracts oxidatively deaminated benzylamine and the lysine residues of BSA to benzaldehyde and AAS residues, respectively, in phosphate buffer (pH 7.4) at 37 °C in both the presence and absence of Cu^{2+}.

Recent research has demonstrated that orally administered polyphenols are widely distributed in mammalian tissues and the maximum concentration of any individual polyphenols in plasma rarely exceeds 1 μM after the consumption of 10−100 mg of a single compound (*1*−5). However, the total polyphenol concentration in plasma seems to be higher due to the presence of metabolites formed in tissues or by the colonic microflora. Actually, polyphenols are more effectively absorbed in the small intestine.

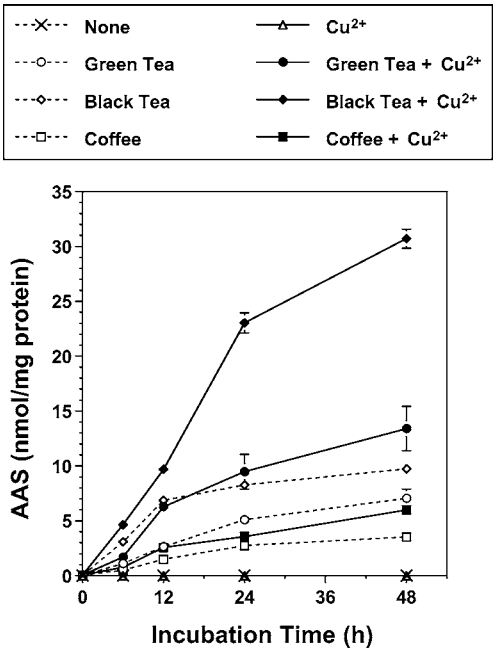

Figure 7. Time course of oxidative deamination of lysine residue in BSA by green tea, black tea, and coffee extracts. An aliquot (1.0 mL) of each extract (10 mg of solid/mL) was added to 1.0 mL of 100 mM sodium phosphate buffer containing 20 mg/mL BSA (pH 7.4) with or without $CuSO_4$ (500 μM). Contents of total polyphenols in green tea, black tea, and coffee extracts were 5.13, 5.31, and 1.75 mM of catechin equivalents, respectively. The mixtures were incubated at 37 °C for 0–48 h. After the reaction was terminated, AAS was measured by HPLC as described under Materials and Methods. The values are shown as means ± SD ($n = 3$).

Catechin administration has been reported to raise lysyl oxidase activity in chick aorta, increase collagen stability, and protect against lathyrism induced by BAPN, which is a specific inhibitor of the enzyme (*14*−*16*). Lysyl oxidase is the only essential enzyme for the generation of AAS, which forms various inter- and intramolecular cross-links via aldol condensation or Schiff base formation spontaneously (*29*−*31*), and gives high insolubility to elastin and collagen. The inhibition of cross-linking gives rise to serious pathologies such as lathyrism and several inborn diseases of collagen and elastin (*17*). The cross-links are very important to maintain the normal properties of connective tissues. This protective effect of catechin is consequently considered to be due to the lysyl oxidase-like activity of catechin. Therefore, it is conjectured that the usual drinking of polyphenol-rich beverages may cause modification in connective tissues. Interestingly, Young et al. (*32*) have recently reported that intake of flavonoid-containing juice increases AAS residues in plasma proteins. Our results suggest that this action is attributable to the lysyl oxidase-like activity of polyphenols.

Furthermore, polyphenols may undergo the oxidative deamination of various amines and induce physiological effects in vivo. Amine oxidases, which are a heterogeneous family of enzymes, catalyze the oxidative deamination of various monoamines, diamines, and polyamines produced endogenously or absorbed as dietary or xenobiotic substances. The physiological function in living organisms of amine oxidases is not completely established but is certainly related to biogenic amines metabolism and therefore involved in essential processes such as cell growth and differentiation. The catalytic products of oxidative deamination of amines (hydrogen peroxide and aldehyde) exert a cytotoxic effect and therefore are considered cell growth inhibitors (*33*). Actually, it has been reported that bovine serum amine oxidase can slowly release cytotoxic products and induce tumor cell death by apoptosis (*34*). Semicarbazide-sensitive amine oxidase (SSAO) catalyzes the conversion of methylamine to formaldehyde. This enzyme is located on the surface of the cytoplasmic membrane and in the cytosol of vascular endothelial cells, smooth muscle cells, and adipocytes. Thus, SSAO activity has been shown to be capable of regulating glucose transport in adipose cells (*35*). It has been independently discovered that the primary structure of vascular adhesion protein 1 (VAP-1) is identical to that of SSAO. VAP-1 regulates leukocyte migration and is related to inflammation. Moreover, vascular SSAO activity has been reported to be a source of vasoactive signaling molecules that generally relax blood vessels (*36*). Whereas the SSAO-derived formaldehyde has been shown to initiate protein cross-linking, leading to the alteration of protein structure (*37*). Thus, this process is thought to cause protein deposition associated with chronic pathological disorders. On the basis of our results, the drinking of polyphenol-rich beverages can be expected to induce the amine oxidase-like activity in vivo. However, currently, there is no evidence that the amine oxidase activity is increased by the intake of polyphenols. Further work will be needed to elucidate the amine oxidase-like activity of polyphenols and its relevance to physiological functions in vivo.

ABBREVIATIONS USED

AAS, α-aminoadipic δ-semialdehyde; ACPP, 1-amino-1-carboxy-5,5-bis(*p*-hydroxyphenyl)pentane; BAPN, β-aminopropionitrile; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; SSAO, semicarbazide-sensitive amine oxidase; TCA, trichloroacetic acid; VAP-1, vascular adhesion protein 1.

LITERATURE CITED

(1) Manach, C.; Regerat, F.; Texier, O.; Agullo, G.; Demigne, C.; Remesy, C. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutr. Res.* **1996**, *16*, 517−544.

(2) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(3) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr,* **2005**, *81*, 230S−242S.

(4) Morrice, P. C.; Wood, S. G.; Duthie, G. G. High-performance liquid chromatographic determination of quercetin and isorhamnetin in rat tissues using β-glucuronidase and acid hydrolysis. *J. Chromatogr. B* **2000**, *738*, 413−417.

(5) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.

(6) Manach, C.; Mazur, A.; Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* **2005**, *16*, 77−84.

(7) Arts, I. C.; Hollman, P. C. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* **2005**, *81*, 317S−325S.

(8) Vita, J. A. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. *Am. J. Clin. Nutr.* **2005**, *81*, 292S−297S.

(9) Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical Biol. Med.* **1994**, *16*, 845−850.

(10) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(11) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(12) Sichel, G.; Corsaro, C.; Scalia, M.; Di Bilio, A. J.; Bonomo, R. P. In vitro scavenger activity of some flavonoids and melanins against O_2^-. *Free Radical Biol. Med.* **1991**, *11*, 1−8.

(13) Nanjo, F.; Goto, K.; Seto, R.; Suzuki, M.; Sakai, M.; Hara, Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biol. Med*. **1996**, *21*, 895−902.
(14) Cetta, G.; Gerzeli, G.; Quartieri, A.; Castellani, A. A. Protective effect of flavonoids on the collagen of lathyritic rats. *Experientia* **1971**, *27*, 1046−1048.
(15) Orloff, S.; Rao, V. H.; Bose, S. M. Effect of catechin flavonoids on the cross-linking of lathyritic collagen. *Indian J. Biochem. Biophys*. **1974**, *11*, 314−317.
(16) DiSilvestro, R. A.; Harris, E. D. Evaluation of (+)-catechin action on lysyl oxidase activity in aortic tissue. *Biochem. Pharmacol*. **1983**, *32*, 343−346.
(17) Smith-Mungo, L. I.; Kagan, H. M. Lysyl oxidase: properties, regulation and multiple functions in biology. *Matrix Biol*. **1998**, 16, 387−398.
(18) Akagawa, M.; Suyama, K. Amine oxidase-like activity of polyphenols. *Eur. J. Biochem.* **2001**, *268*, 1953−1963.
(19) Rinaldi, A. C.; Porcu, C. M.; Curreli, N.; Rescigno, A.; Finazzi-Agrò, A.; Pedersen, J. Z.; Rinaldi, A.; Sanjust, E. Autoxidation of 4-methylcatechol: a model for the study of the biosynthesis of copper amine oxidases quinonoid cofactor. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 559−567.
(20) Rinaldi, A. C.; Porcu, C. M.; Oliva, S.; Curreli, N.; Rescigno, A.; Sollai, F.; Rinaldi, A.; Finazzi-Agrò, A.; Sanjust, E. Biosynthesis of the topaquinone cofactor in copper amine oxidases evidence from model studies. *Eur. J. Biochem*. **1998**, *251*, 91−97.
(21) Shah, M. A.; Bergethon, P. R.; Boak, A. M.; Gallop, P. M.; Kagan, H. M. Oxidation of peptidyl lysine by copper complexes of pyrroloquinoline quinone and other quinones. A model for oxidative pathochemistry. *Biochim. Biophys. Acta* **1992**, *1159*, 311−318.
(22) Akagawa, M.; Suyama, K. Characterization of a model compound for the lysine tyrosylquinone cofactor of lysyl oxidase. *Biochem. Biophys. Res. Commun.* **2001**, *281*, 193−199.
(23) Wang, S. X.; Mure, M.; Medzihradszky, K. F.; Burlingame, A. L.; Brown, D. E.; Dooley, D. M.; Smith, A. J.; Kagan, H. M.; Klinman, J. P. A cross-linked cofactor in lysyl oxidase: redox function for amino acid side chains. *Science* **1996**, *273*, 1078−1084.
(24) Wang, S. X.; Nakamura, N.; Mure, M.; Klinman, J. P.; Sanders-Loehr, J. Characterization of the native lysine tyrosylquinone cofactor in lysyl oxidase by Raman spectroscopy. *J. Biol. Chem.* **1997**, *272*, 28841−28844.
(25) Beecher, G. R.; Warden, B. A.; H. Merken. Analysis of tea polyphenols. *Proc. Soc. Exp. Biol. Med*. **1999**, *220*, 225−228.
(26) Yen, W. J.; Wang, B. S.; Chang, L. W.; Duh, P. D. Antioxidant properties of roasted coffee residues. *J. Agric. Food Chem*. **2005**, *53*, 2658−2663.
(27) Vinson, J. A.; Proch, J.; Bose, P. Determination of quantity and quality of polyphenol antioxidants in foods and beverages. *Methods Enzymol*. **2001**, *335*, 103−114.
(28) Jahanmard, E.; Suyama, K. Bisphenol derivative of allysine for high-performance liquid chromatographic analysis of allysine residue of proteins. *J. Chromatogr. B* **2000**, *739*, 273−280.
(29) Nakamura, F.; Suyama, K. Analysis of aldosine, an amino acid derived from aldol cross-link of elastin and collagen by high-performance liquid chromatography. *Anal. Biochem*. **1994**, *223*, 21−25.
(30) Akagawa, M.; Yamazaki, K.; Suyama, K Cyclopentenosine, major trifunctional cross-linking amino acid isolated from acid hydrolysate of elastin. *Arch. Biochem. Biophys*. **1999**, *372*, 112−120.
(31) Akagawa, M.; Suyama, K. Mechanism of formation of elastin cross-links. *Connect. Tissue Res*. **2000**, *41*, 131−141.
(32) Young, J. F.; Nielsen, S. E.; Haraldsdóttir, J.; Daneshvar, B.; Lauridsen, S. T.; Knuthsen, P.; Crozier, A.; Sandström, B.; Dragsted, L. O. Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *Am. J. Clin. Nutr*. **1999**, *69*, 87−94.
(33) Pietrangeli, P.; Mondovi, B. Amine oxidases and tumors. *Neurotoxicology*. **2004**, *25*, 317−324.
(34) Averill-Bates, D. A.; Cherif, A.; Agostinelli, E.; Tanel, A.; Fortier, G. Antitumoral effect of native and immobilized bovine serum amine oxidase in a mouse melanoma model. *Biochem. Pharmacol*. **2005**, *69*, 1693−1704.
(35) Yu, P. H.; Wright, S.; Fan, E. H.; Lun, Z. R.; Gubisne-Harberle, D. Physiological and pathological implications of semicarbazide-sensitive amine oxidase. *Biochim. Biophys. Acta*. **2003**, *1647*, 193−199.
(36) Conklin, D. J.; Cowley, H. R.; Wiechmann, R. J.; Johnson, G. H.; Trent, M. B.; Boor, P. J. Vasoactive effects of methylamine in isolated human blood vessels: role of semicarbazide-sensitive amine oxidase, formaldehyde, and hydrogen peroxide. *Am. J. Physiol. Heart. Circ. Physiol*. **2004**, *286*, H667−H676.
(37) Gubisne-Haberle, D.; Hill, W.; Kazachkov, M.; Richardson, J. S.; Yu, P. H. Protein cross-linkage induced by formaldehyde derived from semicarbazide-sensitive amine oxidase-medicated deamination of methylamine. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 1125−1132.

Received for review April 14, 2005. Revised manuscript received July 24, 2005. Accepted August 11, 2005.

JF050843F