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Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma

Kyoji Yoshino, Masazumi Suzuki, Kiyotaka Sasaki, Toshio Miyase, and Mitsuaki Sano

Laboratory of Health Science, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

*The oxidative dimerization of (−)-epigallocatechin gallate (EGCG), the major catechin of tea leaves (*Camellia sinensis *L.), in authentic intestinal juice (pH 8.5) and mouse plasma (pH 7.8) was investigated. EGCG was unstable in the alkaline solutions over pH 7.4. The content of EGCG was decreased to 19.4% and 60.7% at 5 minutes in the intestinal juice and plasma, respectively. Three products—P-1 (theasinensin A), P-2 (a new dimerized product reported in a previous paper), and P-3 (theasinensin D, a rotational isomer of P-1)—were detected in these fluids. The sum of the molar contents of the three products formed after 5 minutes of incubation at 37°C corresponded to 35.1% and 21.9% of the degraded molar content of EGCG, respectively. These dimerization products of EGCG would be formed by the dehydrogenation and decarboxylation of EGCG under oxidative conditions in alkaline solutions. The formation of P-2 was greater than that of P-1 and of P-3 at 30 minutes of incubation in the intestinal juice and mouse plasma. Fe^{2+}-chelating activities of the three products were much higher than that of EGCG, and superoxide anion radical-scavenging activity of P-2 was also significantly higher than that of EGCG. The absorbance of P-2 administered to male ddY mice was studied. The content of P-2 in mouse plasma was less than that of administration of EGCG, but P-2 was absorbed quickly within 30 minutes and metabolized slowly. These dimerization products of EGCG are expected to contribute to in vivo* antioxidative activities enhanced by tea drinking. (J. Nutr. Biochem. 10:223–229, 1999)

Keywords: tea; (−)-epigallocatechin gallate; theasinensin; dimerization; antioxidant; alkaline fluids; intestinal juice; mouse plasma

Introduction

Catechins in green tea leaves (*Camellia sinensis* L.) are known to exhibit strong antioxidative activity in the plasma, liver, and kidney of rats and mice[1–3] and strong anticarcinogenic activity in rats in vivo.[4,5] To investigate the mechanisms of these effects in animal bodies, several studies have been conducted to determine their absorption, distribution, and metabolic fate.[6–9] In these reports, (−)-epigallocatechin gallate (EGCG) or other tea catechins administered to rats were detected in the plasma and organs such as the liver and kidney. It was demonstrated that the catechins are conjugated with sulfate and glucuronate by phenol sulfotransferases (EC 2.8.2.1) and UDP-glucuronyltransferase (EC 2.4.1.17) and are *O*-methylated by catechol *O*-methyltransferase (EC 2.1.1.6) or phenol *O*-methyltransferase (EC 2.1.1.25) in the animals.[10–12] Recently, we have reported that two products (P-1 and P-2, as shown in *Figure 1*) were formed easily from EGCG in rat plasma and bile.[12] P-1 was identified as theasinensin A, which is a component of fermented teas such as oolong and black tea,[13,14] and P-2 as a new compound.[12]

Plant flavonoids including tea catechins are known to be

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Address correspondence to Dr. Mitsuaki Sano, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka, Shizuoka 422-8526, Japan.
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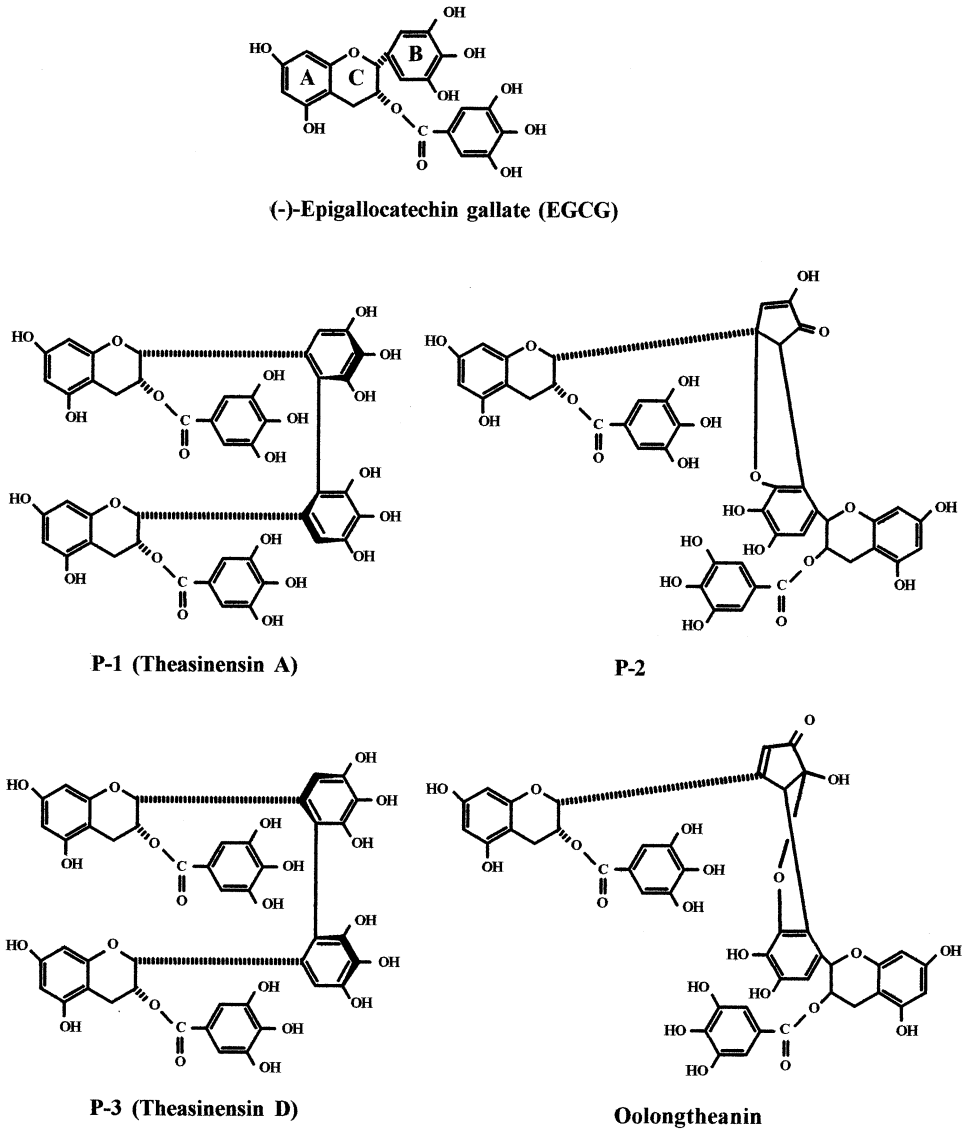

Figure 1 Chemical structures of (−)-epigallocatechin gallate and its dimerization products.

easily degraded in alkaline solutions.[15,16] In general, the plasma and bile fluids in mammals are mildly alkaline; for example, it is known that the pH values of the plasma and bile in human are 7.4 to 7.5 and 7.1 to 8.5, respectively. We have speculated that P-1 and P-2 were produced from EGCG in mildly alkaline biological fluids. Human intestinal and pancreatic juices also are known to be mildly alkaline, with pH values of 8.3 and 7.0 to 8.5, respectively. The formation of these products in the intestinal tract as well as the plasma and bile would be expected.

In the present study, we investigated the stabilities of tea catechins under various pH conditions and the antioxidative activities of the products from EGCG in authentic intestinal juice and mouse plasma. Furthermore, we also examined absorption of P-2 administered orally to ddY mice because P-2 had strong iron-chelating activity and superoxide anion radical-scavenging activity.

Materials and methods

Materials

(−)-Epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and EGCG, four major tea polyphenols, were purchased from Kurita Co. (Tokyo, Japan). P-1, P-2, and P-3 were isolated and purified from a reaction mixture consisting of EGCG in rat plasma in accordance with a method described previously.[12]

Stabilization of tea catechins at various pH

One milliliter of EC, ECG, EGC, or EGCG solution (1.0 mg/mL 10% ethanol) was added to 5 mL of 0.07 M phosphate buffer (pH 6.4), 0.04 M phosphate buffer (pH 7.4), 1.5% sodium carbonate (pH 11.2), authentic gastric juice (0.24% hydrochloric acid-0.2% sodium chloride solution, pH 1.8), or authentic intestinal juice (1.5% sodium hydrogen carbonate solution, pH 8.5). The reaction mixture was incubated at 37°C for 5 minutes. No digestive enzymes such as pepsin in gastric juice and pancreatin in intestinal

juice were added to the authentic fluids used in this study because the effects of proteins on the degradation of catechins were neglected. The contents of catechins and degradative products were determined by a high performance liquid chromatograph (HPLC) equipped with an ultraviolet detector at 280 nm.[17] Briefly, the HPLC column used was Wakosil $5C_{18}$ (4.6 × 150 mm; Wako Pure Chemical Ind., Ltd., Tokyo, Japan). The mobile phase was acetonitrile/N,N-dimethylformamide/0.1% phosphoric acid (6:2:40) and ran isocratically at a flow rate of 1.0 mL/min at 25°C.

Determination of total phenols

EGCG (0.2 mg) was incubated at 37°C for 0, 5, 15, and 30 minutes in 1 mL of authentic intestinal juice or heparinized plasma from male ddY 6-week-old mice (Japan SLC, Inc., Shizuoka, Japan). The pH of the plasma was 7.8. After the incubation, the reaction mixtures were neutralized to pH 7.0 with 0.1 N hydrochloric acid and the total volumes were adjusted to 3.0 mL with water. The contents of total phenols in the reaction mixtures were analyzed by the method of Folin and Denis.[18] Briefly, the reaction mixture consisting of 0.125 mL of sample solution, 0.375 mL water, and 0.5 mL Folin reagent (2.5% sodium tungstate and 0.5% phosphomolybdate in 1.25% phosphoric acid) was allowed to stand for 3 minutes at room temperature. The mixture was then mixed with 0.5 mL of 10% sodium carbonate and allowed to stand for 90 minutes at room temperature. The blue color of the solution was evaluated by a spectrophotometer at 700 nm. EGCG was used as a reference and the values of total phenols were expressed as EGCG equivalent.

Determination of EGCG and the degradation products

One milliliter of EGCG solution (1.0 mg/mL 10% ethanol) was added to 5 mL of the authentic intestinal juice or mouse plasma and the mixture was incubated at 37°C for 0 to 30 minutes. After the incubation, the reaction mixture was neutralized to pH 7.0 and the total volume was adjusted to 7.0 mL with water. Changes in the contents of EGCG and the degradative products were determined by HPLC.[17]

Antioxidative activities of EGCG and the dimerization products

Antioxidative activities of EGCG and the products formed from EGCG under alkaline conditions were evaluated by (1) Fe^{2+}-chelating activity[19] or (2) superoxide anion radical (O_2^-)-scavenging activity.[20]

For the first assay (formation of the complexes with Fe^{2+}), 0.25 mL of 6.96 mM ferrous sulfate, 0.25 mL of 14.2 mM potassium sodium tartrate, 0.5 mL of sample solutions, and 0.5 mL of water were added to 1.5 mL of 25 mM phosphate buffer (pH 7.5). Immediately, the blue color of the complexes formed with Fe^{2+} was evaluated by a spectrophotometer at 540 nm. The activities were expressed as the absorbance ratios of sample-Fe^{2+} complexes when the absorbance of EGCG-Fe^{2+} complex is 100. For the second assay (O_2^--scavenging activity), 0.5 mL of 15 μM phenazine methosulfate, 0.5 mL of 200 μM nitro blue tetrazolium, 0.5 mL of sample solution, and 0.5 mL of 750 μM nicotinamide adenine dinucleotide, a starter for the production of O_2^-, were added to 0.5 mL of 20 mM phosphate buffer (pH 7.4). The mixture was incubated at 25°C. Formation of nitro blue tetrazolium diformazan was determined by a spectrophotometer at 560 nm with the passage of time. The increasing absorption rate of the reaction mixture with samples was compared with that without samples, and the inhibition ratio was calculated by using the reaction rate. O_2^--Scavenging activity of the samples was expressed as the inhibition ratio for the reaction mediated by this radial.

The concentrations of the samples used were 37.0 μM for the former assay and 22.0 μM for the latter one.

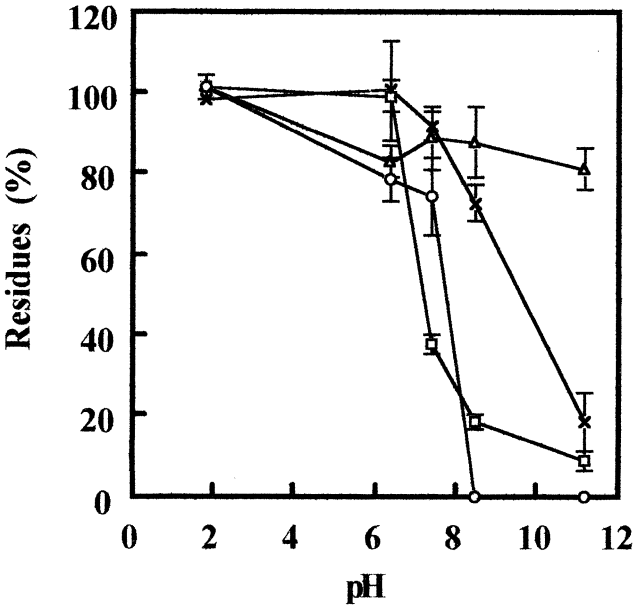

Figure 2 Stabilization of tea catechins at various pH values. -○--(−)-epigallocatechin. -△--(−)-epicatechin. □--(−)epigallocatechin gallate. -X--(−)-epicatechin gallate. Mean ± SD (N = 3).

Administration of EGCG and P-2 to male ddY mice

Male ddY mice (6 weeks old; Japan SLC, Inc.) were fasted for 6 hours before they were used. EGCG or P-2 was administered per os to four mice at a dose of 100 mg/kg body weight. Mice were euthanized under anesthesia with diethyl ether at 0, 30, 60, 120, and 300 minutes after the administration. The blood was obtained by heart puncture using a heparinized syringe; 50 μL of the plasma was added with 150 μL of 40 mM phosphate buffer (pH 6.8) and 20 μL of ascorbate-EDTA solution [40 mM phosphate buffer (pH 3.6) including 20% ascorbate and 0.1% EDTA]. The mixture was extracted with 2.5 mL of ethyl acetate and 2.0 mL of the ethyl acetate layer was dried under the flow of nitrogen gas. The residue was resolved in the solvent for HPLC analysis. The contents of EGCG and P-2 were determined with a HPLC system equipped with an electrochemical detector.[21] Briefly, the HPLC column was Shodex C18-5A (4.6 × 150 mm; Showa Denko, Tokyo, Japan). The mobile phase was acetonitril/100 mM phosphoric acid containing 0.1 mM Na_2EDTA (15:85) and ran isocratically at a flow rate of 0.5 mL/min. The eluent was monitored electrochemically (applied potential, +600 mV versus Ag/AgCl).

Results

Figure 2 shows the stabilization of four major catechins in green tea leaves at various pHs. All of these compounds were very stable in the acidic solution (pH 1.8–6.4). When the pH of the solutions was above 7.4, EGC and EGCG were unstable. ECG was degraded under strong alkaline conditions (pH 11.2), which were thought to be an abnormal pH in animal bodies. At the pH of the intestinal juice (pH 8.5), EGC and EGCG were degraded markedly. EC was very stable at all pHs used in this study (pH 1.8–11.2).

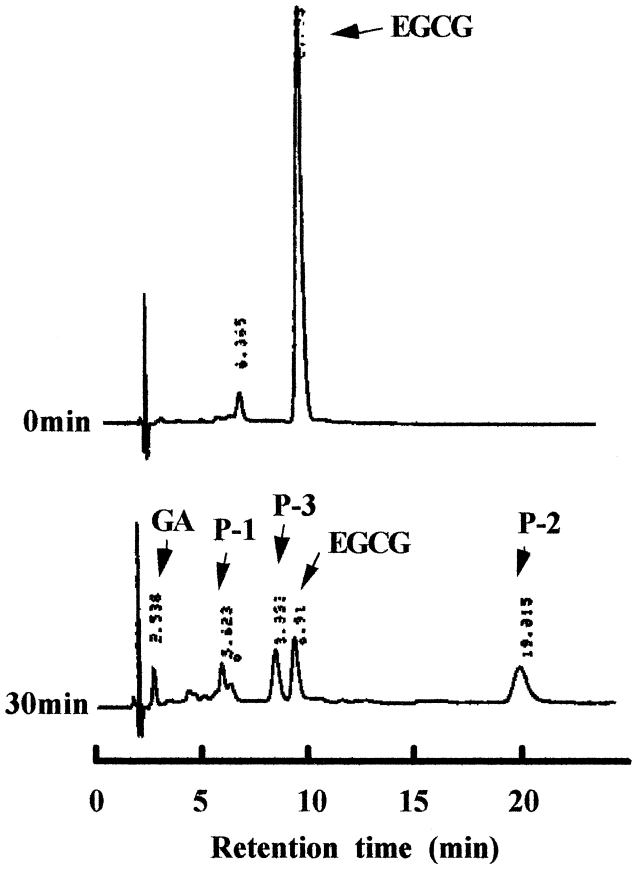

Figure 3 Chromatograms of (−)-epigallocatechin gallate (EGCG) and its dimerization products in authentic intestinal juice (pH 8.5). GA–gallic acid.

Figure 3 shows chromatograms of the products formed from EGCG in the intestinal juice. At 30 minutes of reaction time, a lowering of the peak of EGCG and the appearance of other peaks were observed. Retention times on the chromatograms were 8.8 minutes for EGCG and 2.5, 5.6, 8.0, and 19.0 minutes for the others. Small broad peaks also appeared between 4 and 5 minutes of retention time. The peaks at 2.5, 5.6, and 19.0 minutes of retention time agreed with those of the standard gallic acid (GA), P-1, and P-2. The peak eluted at 8.0 minutes was identified as theasinensin D, a rotational isomer of P-1, on the basis of spectroscopic evidence such as ultraviolet light, nuclear magnetic resonance, fast atom bombardment mass (FAB-MS), secondary ion mass spectrometry (SI-MS), and circular dichroism (CD). Its chemical structure is shown in *Figure 1*. The peaks at 4 to 5 minutes were from unknown compounds.

Figure 4 shows the changes in the contents of EGCG, the degradative products, and total phenols in the intestinal juice (pH 8.5) and/or mouse plasma. In the treatment using the intestinal juice, EGCG was degraded to 15.0% of the value at the start of treatment at 30 minutes of reaction time. The contents of P-2 and P-3 increased gradually. The content of P-1 was the highest at 5 minutes and decreased thereafter. Among these three products, P-2 was formed in the largest amount. In the treatment of mouse plasma, EGCG was slowly degraded as compared with the result for the intestinal juice, and the content at 30 minutes of reaction time was 21.8% of that at the start of the reaction. The contents of P-1 and P-3 increased gradually until 15 minutes and decreased thereafter. The content of P-2 increased slowly. In mouse plasma, the content of P-1 was lower and those of P-2 and P-3 were inversely higher in comparison with values for the intestinal juice.

The contents of total phenols in the intestinal juice and mouse plasma were degraded to 93.5% and 86.5%, respectively, of initial levels at 30 minutes of reaction time. In *Table 1*, the antioxidative activities of the dimerization products from EGCG in the mild alkaline fluids were compared with those of EGCG. Fe^{2+}-chelating activities of the three products (P-1, P-2, and P-3) were approximately two times that of EGCG. O_2^--Scavenging activities of P-2 were significantly higher than those of EGCG but the activities of other dimerization products were almost the same as that of EGCG.

Figure 5 shows the absorption of EGCG and P-2 in ddY male mice. When EGCG was orally administered to the mice, the highest concentration of EGCG in the plasma (2.5 μg/mL) was observed at 60 minutes. After 60 minutes, the concentration of EGCG in mouse plasma gradually decreased, and at 120 minutes was only 0.11 μg/mL. When P-2 was administered to mice, the highest concentration of P-2 in the plasma (1.1 μg/mL) was obtained at 30 minutes. After 30 minutes the concentration of P-2 in mouse plasma decreased slowly and at 120 minutes, it was still 0.38 mg/mL. Though the highest concentration of P-2 was 40.2% of that of EGCG in mouse plasma, P-2 was absorbed rapidly and metabolized slowly in mouse body compared with EGCG.

Discussion

We reported previously that EGCG was degraded rapidly in rat plasma and bile.[12] It is well known that animal blood and bile are mildly alkaline, so the degradation of EGCG in these biological fluids was thought to have occurred under the same pH conditions. In that study, the pH of the plasma and bile from 5-week-old mice was 7.8 and 8.2, respectively. In fact, EGCG was degraded rapidly in the authentic intestinal juice (pH 8.5), which did not include digestive enzymes as shown in *Figures 2* and *4*. Enzymes from digestive juice were excluded from the study because they affect the ability of tea catechins to bind proteins.[22] The results shown in *Figure 2* are in agreement with those of Zhu et al.[15]

It is suggested that EGC and EGCG administered to animals would be stable in the stomach but partly degraded in the intestinal tract before their absorption, and, furthermore, that these catechins would be degraded to metabolites in blood, bile, and some organs after absorption. The rapid degradation of EGC and EGCG in these biological fluids is disadvantageous in terms of the bioavailability and pharmacologic effects of tea drinking. First, the levels of EGC and EGCG in green tea leaves were much higher than those of the other catechins.[17] Second, their biological activities such as antioxidative function were rather strong.[23,24] Third, tea catechins administered to animals were transferred rapidly into the plasma and bile.[12] In this study, however,

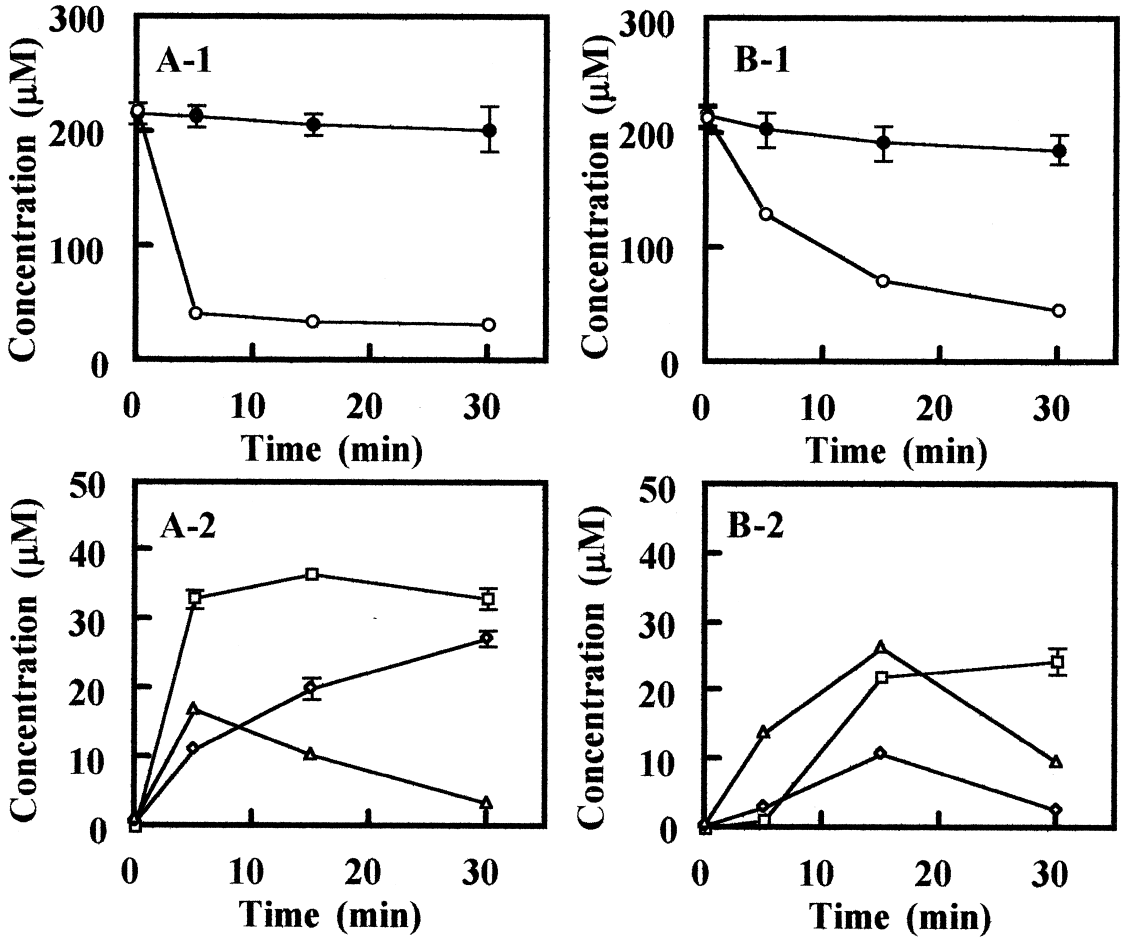

Figure 4 Changes in the contents of (−)-epigallocatechin gallate (EGCG) and its degradation products in authentic intestinal juice (pH 8.5) and mouse plasma. (*A-1, A-2*) In authentic intestinal juice (pH 8.5). (*B-1, B-2*) In mouse plasma (pH 7.8). -○--EGCG. -△--P-1. -□--P-2. -◇--P-3. -●--total phenols. Mean ± SD (N = 3).

we showed that strong antioxidants such as P-1, P-2, and P-3 were produced in the intestinal juice and plasma by the degradation of EGCG. In particular, the production of P-2, which has potent antioxidative activities, was greater than that of P-1 and of P-3 at 30 minutes of reaction time in these fluids. The phenomenon was in agreement with the results of experiments using rat plasma and bile.[12]

Table 1 Comparison of antioxidative activities of (−)-epigallocatechin gallate (EGCG) and the degradative products

	Fe^{2+}-chelating activity (absorbance ratio)*	O_2^--scavenging activity (inhibition %)†
EGCG	100 ± 1.70	47.3 ± 2.69
P-1	196 ± 3.57[a]	39.8 ± 1.56
P-2	234 ± 19.9[a]	70.1 ± 5.97[b]
P-3	202 ± 5.59[a]	51.6 ± 2.56

Values are the mean ± SD (N = 3).
*Absorbance ratio of the complexes with 37 μM samples and Fe^{2+} when the absorbance of EGCG-Fe^{2+} complex is 100.
†Inhibition ratio of 22 μM samples for the reaction mediated by O_2^-.
Significantly different from the activity of EGCG by Student's t-test: [a]$P < 0.001$; [b]$P < 0.01$.

P-1 (theasinensin A) is known to be a component of fermented tea leaves.[25] P-3 (theasinensin D) is an optical isomer formed by restricted rotation of theasinensin A and also exists in oolong tea leaves.[13] Hashimoto et al.[13,14] speculated that theasinensins would be produced by dehydrogenation and intermolecular coupling of two flavan pyrogallol rings (B rings) in EGCG via the formation of radicals in the fermentation process. The theasinensins could be further changed to oolongtheanins via further dehydrogenation and decarboxylation. We confirmed the formation of P-1 and P-3 from EGCG in some mild alkaline fluids such as mammalian plasma, bile, and the authentic intestinal juice in the present and a previous study.[12]

Yoshioka et al.[26] and Guo et al.[27] reported that the ability to donate hydrogen was elevated in the aromatic B ring of EGCG in an alkaline solution and the radicals formed easily. That is to say, the reaction that occurred on EGCG in alkaline solution would be an oxidation.[28] Therefore, it was suggested that P-1 and P-3 were produced by the oxidation via the formation of radicals under mild alkaline conditions as well as the reaction in fermented tea leaves. Yoshioka et al.[26] also reported that the ability to donate hydrogen of the gallyl moiety in the aromatic B ring in EGCG was higher

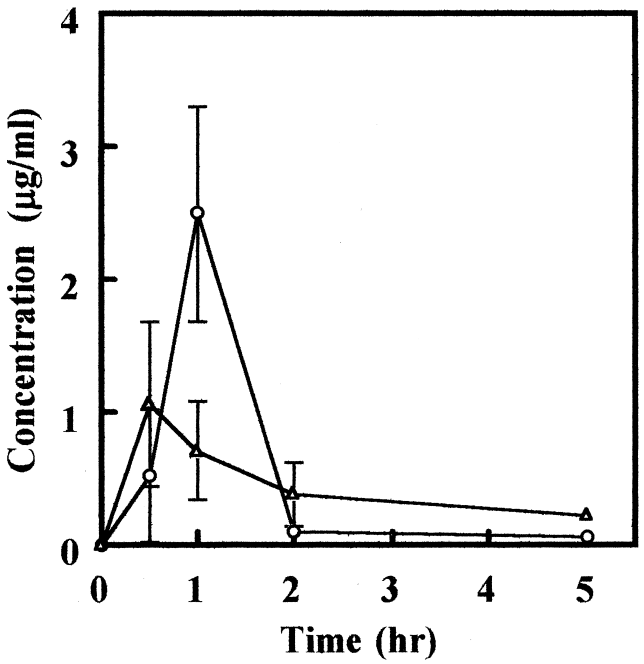

Figure 5 Concentrations of (−)-epigallocatechin gallate (EGCG) and P-2 in the plasma of ddY mice following oral administration of EGCG or P-2 (100 mg/kg). -○--EGCG (μg/mL). -△--P-2 (μg/mL). Values are presented as the mean ± SEM (N = 4).

than that of the galloyl moiety in ECG under alkaline conditions. Their evidence could explain the result of *Figure 2*—that is, EGCG and EGC were more unstable than EC and ECG in the authentic intestinal juice (pH 8.5). We previously thought that P-2 would be produced from P-1, because the production of P-2 was delayed in comparison with that of P-1 in rat plasma and bile.[12] The same phenomenon was observed in the present experiments using the authentic intestinal juice (pH 8.5) and mouse plasma.

Hashimoto et al.[13,14] speculated that theasinensins could be changed to oolongtheanins via dehydrogenation and decarboxylation. The chemical structure of P-2 was very similar to that of oolongtheanin, as shown in *Figure 1*. Therefore, it was very likely that P-2 would be produced from P-1 under mild alkaline conditions.

We investigated the formation of P-2 when P-1 or P-3 was added to the authentic intestinal juice and mouse plasma. In contrast to our prediction, no formation of P-2 was observed until 30 minutes of reaction time in any cases, although P-1 and P-3 were degraded easily under mild alkaline conditions. This result suggests that the precursors of P-2 would be different from those of P-1 and P-3. Further experiments are needed to clarify the mechanisms for the formation of these products from EGCG in mild alkaline solutions.

The degradation of EGCG in mouse plasma was slower than that in the authentic intestinal juice (pH 8.5), as shown in *Figure 4*. This phenomenon would be due to the following two facts. First, the pH of mouse plasma (pH 7.8) is lower than that of intestinal juice (pH 8.5). Second, mouse plasma includes a large amount of proteins. Authentic intestinal juice generally contains approximately 3 mg/mL of pancreatin, and the contents of the animal intestinal tract include more proteins, although the intestinal juice used in the experiment did not contain any enzymes. The addition of 2.8 mg/mL of pancreatin or bovine serum albumin to the reaction system shown in *Figure 4* suppressed the degradation of EGCG by 7% or 18%, respectively (data not shown). The presence of the proteins might inhibit the degradation of EGCG under mild alkaline conditions. The sum of the molar contents of P-1, P-2, and P-3 corresponded to 35.1% and 34.8% of the molar contents of degraded EGCG at 5 and 30 minutes, respectively.

Phenol groups in tea catechins are known to be very important for the antioxidative activity and anticarcinogenic function, in which the antioxidative activity is thought to be an essential factor.[29] The content of total phenols changed little, as shown in *Figure 4*. P-1, P-2, and P-3 maintain almost all the phenol groups originated from EGCG in these structures; thus it was expected that many phenolic structures would remain when EGCG was treated with mild alkaline solution.

Iron-chelating[30] and free radical-scavenging[31] abilities were presented to explain the antioxidative activity of plant polyphenols such as tea catechins. The two antioxidative systems play important roles in the oxidative stress that occurs in animals. These processes involve initiation and propagation of the oxidation, respectively. As to the antioxidative activities of P-1, P-2, and P-3, Fe^{2+}-chelating activities of P-1, P-2, and P-3 were much higher than that of EGCG, and O_2^--scavenging activities of P-1, P-2, and P-3 were also higher or approximately similar to that of EGCG.

P-1, P-2, and P-3 are dimerization products of EGCG, though the gallyl moiety that originated from the aromatic B ring of EGCG was destroyed in P-2. Therefore, it could be suggested that the galloyl moiety in these products plays a key role in the Fe^{2+}-chelating activities, and the galloyl and gallyl moieties in their O_2^--scavenging activities. This idea was supported by the result that Fe^{2+}-chelating activities of ECG were higher than those of EGC (data not shown).

P-2, a main antioxidative product formed from EGCG under mild alkaline conditions, was administered orally to male ddY mice, because some of the EGCG administered to animals might be changed to this compound in the intestinal tract. The concentration of P-2 detected in mouse plasma was less than that of EGCG but P-2 was absorbed immediately and degraded slowly compared with EGCG. In the case of human intake of tea, EGCG in the small intestinal tract could be degraded by oxidation under mild alkaline condition. It is expected that some of the dimerization products such as P-1, P-2, and P-3 are quickly taken up in the blood stream and exhibit strong antioxidative activities in organs and tissues. In particular, the potent iron-chelating activity is thought to play an important role in the prevention of oxidative stress. These results suggest that the dimerization products partly contribute to the various pharmacologic effects of EGCG observed in previous studies.

References

1 Yoshino, K., Tomita, I., Sano, M., Oguni, I., Hara, Y., and Nakano, M. (1994). Effects of long-term dietary supplement of tea polyphenols on lipid peroxide levels in rats. *Age* **17,** 79–85

2 Sano, M., Takahashi, Y., Yoshino, K., Shimoi, K., Nakamura, Y., Tomita, I., Oguni, I., and Konomoto, H. (1995). Effect of tea (*Camellia sinensis* L.) on lipid peroxidation in rat liver and kidney: A comparison of green and black tea feeding. *Biol. Pharm. Bull.* **18,** 1006–1008

3 Yoshino, K., Goto, T., Kuribayashi, H., Tamura, M., Sano, M., Murata, H., and Tomita, I. (1998). Antioxidative and hypolipidemic effects of dietary green tea polyphenols in ddY mice fed fish oil-rich diet. *J. Food Hyg. Soc. Japan* **39,** 192–198

4 Yamane, T., Takahashi, T., Kuwata, K., Oya, K., Inagake, M., Kitao, Y., Suganuma, M., and Fujiki, H. (1995). Inhibition of N-methyl-N′-nitrosoguanidine-induced carcinogenesis by (−)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res.* **55,** 2081–2084

5 Matsumoto, N., Kohri, T., Okushio, K., and Hara, Y. (1996). Inhibitory effects of tea catechins, black tea extract and oolong tea extract on hepatocarcinogenesis in rat. *Jpn. J. Cancer Res.* **87,** 1034–1038

6 Unno, T. and Takeo, T. (1995). Absorption of (−)-epigallocatechin gallate into the circulation system of rats. *Biosci. Biotech. Biochem.* **59,** 1558–1559

7 Okushio, K., Matsumoto, N., Kohri, T., Suzuki, M., Nanjo, F., and Hara, Y. (1996). Absorption of tea catechins into rat portal vein. *Biol. Pharm. Bull.* **19,** 326–329

8 Chen, L., Lee, M.-J., Li, H., and Yang, C.S. (1997). Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab. Dispos.* **25,** 1045–1050

9 Nakagawa, K. and Miyazawa, T. (1997). Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. *J. Nutr. Sci. Vitaminol.* **43,** 679–684

10 Lee, M.-J., Wang, Z.-Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, A.D., and Yang, S.C. (1995). Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol.* **4,** 393–399

11 Piskula, M. and Terao, J. (1998). Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *J. Nutr.*, **128,** 1172–1178

12 Tomita, I., Sano, M., Sasaki, K., and Miyase, T. (1998). Tea catechin (EGCG) and its metabolites as bio-antioxidants. In *ACS Symposium Series No. 701*, pp. 209–216, Oxford University Press, New York, NY, USA

13 Hashimoto, F., Nonaka, G., and Nishioka, I. (1988). Tannins and related compounds. LXIX. Isolation and structure elucidation of B,B′-linked bisflavanoids, theasinensins D-G and oolongtheanin from oolong tea. (2). *Chem. Pharm. Bull.* **36,** 1676–1684

14 Hashimoto, F., Nonaka, G., and Nishioka, I. (1992). Tannins and related compounds. CXIV. Structures of novel fermentation products, theogallinin, theaflavonin and desgalloyl theaflavonin from black tea, and changes of tea leaf polyphenols during fermentation. *Chem. Pharm. Bull.* **40,** 1383–1389

15 Zhu, Q.Y., Zhang, A., Tsang, D., Huang, Y., and Chen, Z.-Y. (1997). Stability of green tea catechins. *J. Agric. Food Chem.* **45,** 4624–4628

16 Tamura, M. and Yoshino, K. (1997). Degradation of tea polyphenols in alkaline solutions and the antioxidative activities. *J. Ecotech. Res.* **3,** 263–266

17 Yoshino, K. and Hara, Y. (1992). Determination of catechins and theaflavins in tea infusions by high-performance liquid chromatography. *Numazu Coll. Tech. Res. Ann.* **27,** 87–91

18 Folin, O. and Denis, W. (1915). A colorimetric method for the determination of phenols (and phenol derivatives) in urine. *J. Biol. Chem.* **22,** 305–308

19 Iwasa, K. and Torii, H. (1962). A colorimetric determination of tea tannin with ferrous tartrate. *Tea Res. J.* **26,** 87–91

20 Nishikimi, M., Rao, N.A., and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* **46,** 849–854

21 Umegaki, K., Esashi, T., Tezuka, M., Ono, A., Sano, M., and Tomita, I. (1996). Determination of tea catechins in food by HPLC with an electrochemical detector. *J. Food Hyg. Soc. Japan* **37,** 77–82

22 Sazuka, M., Itoi, T., Suzuki, Y., Odani, S., Koide, T., and Isemura, M. (1996). Evidence for the interaction between (−)-epigallocatechin gallate and human plasma proteins fibronectin, fibrinogen, and histidine-rich glycoprotein. *Biosci. Biotech. Biochem.* **60,** 1317–1319

23 Matsuzaki, T. and Hara, Y. (1985). Antioxidative activity of tea leaf catechins. *J. Agr. Chem. Soc. Jpn.* **59,** 129–134

24 Yoshino, K., Hara, Y., Sano, M., and Tomita, I. (1994). Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by *tert*-butyl hydroperoxide. *Biol. Pharm. Bull.* **17,** 146–149

25 Hashimoto, F., Nonaka, G., and Nishioka, I. (1989). Tannins and related compounds. LXXVII. Novel chalcan-flavan dimers, assamicains A, B and C, and a new flavan-3-ol and proanthocyanidins from the fresh leaves of *Camellia sinensis* L. var. *assamica* KITAMURA. *Chem. Pharm. Bull.* **37,** 77–85

26 Yoshioka, H., Sugiura, K., Kawahara, R., Fujita, T., Makino, M., Kamiya, M., and Tsuyumu, S. (1991). Formation of radicals and chemiluminescence during the autoxidation of tea catechins. *Agric. Biol. Chem.* **55,** 2717–2723

27 Guo, Q., Zhao, B., Li, M., Shen, S., and Xin, W. (1996). Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochim. Biophys. Acta* **1304,** 210–222

28 Yoshioka, H., Rin, K., and Tsuyumu, S. (1990). Light emission and the formation of radicals after grinding tea leaves. *Agric. Biol. Chem.* **54,** 3105–3110

29 Xu, Y., Ho, C.-T., Amin, S.G., Han, C., and Chung, F.-L. (1992). Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res.* **52,** 3875–3879

30 Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., Cillard, P., and Cillard, J. (1993). Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diometin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol.* **45,** 13–19

31 Scott, B.C., Butler, J., Halliwell, B., and Aruoma, O.I. (1993). Evaluation of the antioxidant actions of ferulic acid and catechins. *Free Rad. Res. Comms.* **19,** 241–253