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Mechanistic Studies of Catechins as Antioxidants against Radical Oxidation

Kazunari Kondo,*[,1] Masaaki Kurihara,† Naoki Miyata,† Takashi Suzuki* and Masatake Toyoda*
**Division of Foods and †Division of Organic Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan*

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The antioxidative mechanisms of catechins were studied by investigating products generated at the first stages by 2,2′-azobis(2-aminopropane)hydrochloride (AAPH)-induced radical oxidation, without any isolation, using LC/MS, spectrophotometry, and PM3 semiempirical molecular orbital (MO) calculations. Catechins were quite effective in scavenging peroxyl radicals in a liposomal system and in an aqueous system except for (−)-epigallocatechin (EGC). EGC was the least effective among four catechins tested. From the results of LC/MS and spectroscopic studies, (−)-epicatechin (EC) would be gently converted to an anthocyanin-like compound. According to the mechanisms, the compound produced from EC by radical oxidation can also function as an antioxidant. As a result, EC has a longer inhibition period (t_{inh} = 9360 s). On the other hand, EGC decreased shortly after oxidation (t_{inh} = 3420 s) and was transformed to a quinone-like compound. The addition of superoxide dismutase (SOD) reduced the chemiluminescence from EGC during oxidation. Active oxygen including superoxide anion radicals (O_2^-) may be produced in the case of EGC, but not in the case of EC. However, EGC has a more rapid scavenging effect on peroxyl radicals (k_{inh}/k_p = 232) than EC (k_{inh}/k_p = 41). The calculated C-H bond dissociation enthalpies (BDEs) for catechins at the C-2 position were unexpectedly low (65 kcal/mol) compared to O-H BDEs at phenolic sites (70 kcal/mol), suggesting that hydrogen at the C-2 position may be abstracted by free radicals. The authors propose the tentative antioxidative mechanisms of catechins depending on the experimental results and theoretical calculations.

Key Words: **catechins; LC/MS; MO calculation; superoxide anion radicals.**

Catechins are a group of polyphenolic compounds abundantly contained in green tea. The main polyphenolic components in green tea are (−)-epicatechin (EC)[2], (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG). Catechins are considered to exert protective effects against cancer and inflammatory and cardiovascular diseases (1–4). These protective effects have been mainly attributed to their antioxidative activities by scavenging free radicals. Chinese and Japanese people who are often in the habit of drinking tea have a very low incidence of coronary heart disease. Epidemiological studies have demonstrated that the consumption of catechins and other flavonoids is effective in lowering the risk of coronary heart disease (1, 2, 5, 6). This suggests that polyphenolic compounds like catechins may play an important role in scavenging free radicals such as hydroxyl radicals, peroxyl radicals, superoxide anion radicals, and nitric oxide in living systems. A large number of researchers have reported that catechins effectively suppress the formation of lipid peroxidation in biological tissues and subcellular fractions such as microsome and low-density lipoprotein (LDL) (7–11).

Recently, Unno *et al.* (12, 13) reported the absorption of (−)-epigallocatechin gallate after oral administration to rats and the ingestion of green tea powder in humans. They demonstrated that the concentration of EGCG in human plasma reached the highest level during the first 2 h and the maximal concentration was less than 142 ng/ml (0.3 μM). Recently, Nakagawa *et al.* (14) have also reported that the concentration of

[1] To whom correspondence should be addressed. Fax: 81-3-3707-6950. E-mail: kondo@nihs.go.jp.

[2] Abbreviations used: EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate; LDL, low-density lipoprotein; MO, molecular orbital; PC, phosphatidylcholine; AAPH, 2,2′-azobis(2-aminopropane)hydrochloride; DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid; ESI, electrospray ionization; BDE, bond dissociation enthalpies.

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EGCG in human plasma reaches about 4 μM after the ingestion of tea extract (including 535 mg of EGCG). We have been very interested in the fact that the human plasma concentration of catechins after the ingestion is at a level as low as 0.3–4.0 μM, and that people who often eat diets containing flavonoids have a low incidence of cancer and vascular diseases. This suggests that the biological activities and mechanisms of catechins may not be quite that simple. In addition, little is known about the antioxidative mechanisms of catechins, despite the fact that much attention has been paid to the inhibitory effect of catechins on lipid peroxidation.

There are several reports using ESR on mechanistic studies of catechins against radical oxidation (15–17). Recently, interesting results were reported by other groups. Gardner *et al.* (18) have reported the antioxidant potentials of tea catechins in aqueous and organic media by ESR study. Scavenging effects of catechins and their derivatives were reported by Nanjo *et al.* (19), indicating an interesting result that 3-, 5-, and 7-hydroxyl groups were not so important. However, these reports did not give any positive evidence about the reaction mechanisms. It is very important to clarify the antioxidative mechanisms of catechins to understand the potential of catechins in biological fluids.

We tried to elucidate the antioxidative mechanisms of catechins by investigating the products produced at the first stages of radical oxidation. When the reaction process was monitored with an UV detector, however, it was very difficult to clarify the antioxidative mechanisms of catechins because a large number of products were formed by radical oxidation and they were quite inseparable. For further detailed investigation of the mechanisms, unique techniques would be required. LC/MS is a potentially powerful tool for these purposes because of its high selectivity.

We herein provide the effects and the antioxidative mechanisms of catechins using LC/MS, spectrophotometric analyses, and PM3 semiempirical molecular orbital (MO) calculations. The structures of compounds formed by radical oxidation were analyzed without any isolation.

MATERIALS AND METHODS

Soybean phosphatidylcholine (PC) was obtained from Avanti Polar-Lipid, Inc. (Alabaster AL). EC, EGC, ECG, and EGCG were from Kurita Industry Company (Tokyo, Japan). 2,2′-Azobis(2-aminopropane)hydrochloride (AAPH) was from Wako Pure Chemicals (Osaka, Japan). Diethylenetriamine-*N*, *N*, *N*′, *N*″, *N*″-pentaacetic acid (DTPA) was purchased from Dojindo Chemical Company (Kumamoto, Japan). All other chemicals were of analytical grade. All chemicals were used without further purification.

Preparation of unilamellar liposome and lipid peroxidation. Large unilamellar vesicles containing catechins (5 μM) were prepared by the extrusion method (20). Briefly, soybean PC and catechins were suspended in 0.7 mL of 10 mM Tris–HCl buffer, pH 7.4, containing 0.5 mM DTPA and vortexed for 1 min followed by ultrasonication for 30 s (21–23). The multilamellar suspension was transferred into a Liposofast Apparatus (Avestin, Co., Ottawa, Canada) and passed through a polycarbonate membrane (pore size 100 nm) 21 times. After the resulting unilamellar liposomes were preincubated for 5 min at 37°C, lipid peroxidation was initiated by the addition of AAPH (2 mM).

Determination of PC-hydroperoxides (PCOOHs). PCOOHs were determined by normal-phase HPLC according to the method of Miyazawa *et al.* (24). The column was Finepak SIL NH_2-5 (4.6 $\times$ 250 mm, 5 mm particle size; Japan Spectroscopic Co., Tokyo, Japan). The mixture of hexane, 2-propanol, methanol, and water (5:7:2:1, v:v:v:v) was used as an eluent, and PCOOHs were detected at 234 nm, using a molar absorption coefficient of 27,000.

Mass spectrometric analyses. An LCQ mass spectrometer (Thermoquest, Manchester, UK) equipped with an electrospray ionization (ESI) source and coupled to a Hitachi L-7000 series (Tokyo, Japan) HPLC (ESI-LC/MS) was used in a positive ionization mode for mass spectral confirmation of the reaction intermediates of catechins. Mass analyses were performed in the range of 10 mass units at the center of the molecular ion, called a "Zoom scan," to investigate the structure of the reaction intermediates. The column was TSK-GEL ODS-80Ts (2.0 $\times$ 150 mm; Toso, Tokyo, Japan). A mixture of methanol and water containing 1% acetic acid (2:8) was used as an eluent, and the flow rate was 0.2 ml/min. MS/MS analyses were achieved using helium as a collision gas at a pressure of 0.1 Pa (10^{-3} Torr).

The reaction conditions were the same as those for PC as described above. Fifty micromoles of catechins and 20 mM of AAPH were used in the LC/MS analyses. After oxidation was initiated by AAPH, aliquots of the reaction mixture (10 μl) were withdrawn and then injected onto the HPLC column, and the structures of the reaction intermediates were analyzed by LC/MS.

Spectrophotometric analyses. Spectrophotometric data of peaks B and C were obtained using a Toso HPLC system (Tokyo, Japan) with a photodiode array detector (MCPD-3600, Otsuka electronics Co., Japan). The column was TSK-GEL ODS-80Ts (4.6 $\times$ 150 mm). Aliquots of the reaction mixture (10 μl) were withdrawn and injected onto the column.

Semiempirical MO calculation. The program SPARTAN (v3.1) was used for all MO calculations described in this paper. First, the structures of catechins and catechin radicals were optimized by a conformation search (MacroModel v5.5; MM2*) and then the bond dissociation enthalpies of their structures (BDEs) were calculated using the semiempirical PM3 method (25).

RESULTS

Inhibitory Effect of Catechins on Liposomal Phospholipid Peroxidation

The structures of catechins tested are listed in Fig. 1. EC and ECG are compounds with a catechol structure in the B ring, which have hydroxyl groups at the 3′- and 4′-positions. EGC and EGCG are compounds with a pyrogallol structure, which have hydroxyl groups at the 3′-, 4′-, and 5′-positions. Figure 2 shows the inhibitory effect of catechins on lipid peroxidation in PC liposomes initiated by AAPH. Catechins were very effective in suppressing the formation of PCOOH. EGC was the least effective among four catechins used here, as shown in Fig. 2. EGC rapidly increased PCOOH after an initial inhibition period.

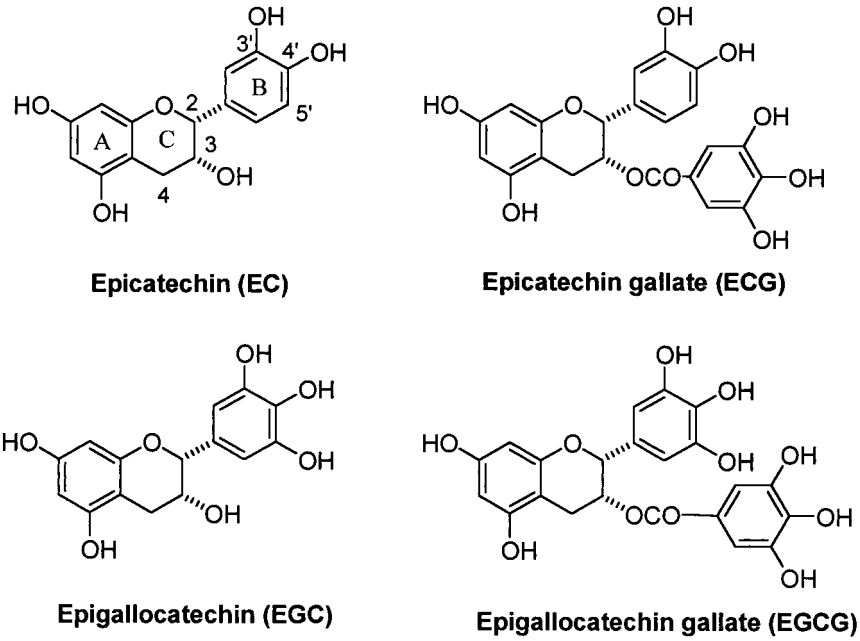

FIG. 1. Structures of four catechins.

Kinetic Studies on Liposomal Phospholipid Peroxidation

Kinetic studies were carried out to evaluate the potential of catechins as antioxidants in soybean PC liposomes according to the method of Ioku *et al.* (22). The inhibition period (t_{inh}) is the period in which the formation of PCOOH was inhibited by catechin, and the slope during this time is expressed as the inhibition rate (R_{inh}). The slope after the inhibition period is described as the propagation rate (R_p). R_p is the propagation rate in the absence of catechins. These parameters are obtained from the results in Fig. 2. The peroxyl radical-scavenging activities of catechins were determined in the PC liposomal system using AAPH as a radical initiator.

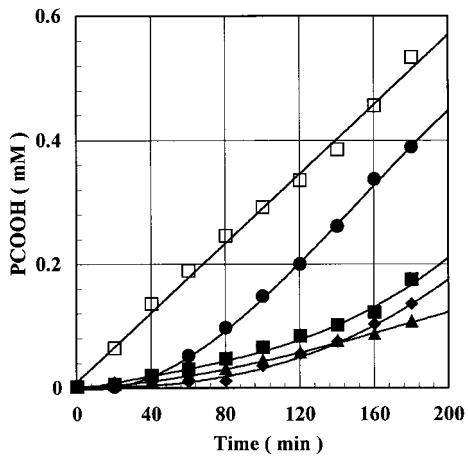

FIG. 2. Inhibitory effect of catechins on the peroxidation of soybean PC liposomes. The reaction system consisted of soybean PC (5 mM) and catechins (5 μM) in 10 mM Tris–HCl buffer (pH 7.4) containing 0.5 mM DTPA. Control (□), EC (■), EGC (●), ECG (▲), EGCG (◆).

Table I shows the results of calculations for catechins at the concentration of 5 μM. The inhibition period (t_{inh}) of EGC and EGCG was shorter than that of EC. In the case of ECG, a clear inhibition period was not observed because of the linear formation of PCOOH during the experiment. The ratio of the rate constant for inhibition (k_{inh}) to that for chain propagation (k_p) was determined by the equation

$$k_{inh}/k_p = [LH]/R_{inh} \cdot t_{inh},$$

where [LH] is the concentration of PC. The values of k_{inh}/k_p for EGC [232] and EGCG [628] were higher than that of EC [41]. On the other hand, the inhibition period (t_{inh}) of EC [t_{inh} = 9360 s] was longer than those of EGC [3420 s] and EGCG [4980 s]. The inhibitory effect of EC on peroxyl radicals lasted longer than that of EGC and EGCG, although the rate constant ratio of EC was lower than those of EGC and EGCG.

Reaction Intermediates Generated from EC and EGC by AAPH-Induced Radical Oxidation

EC and EGC were used as model compounds in this experiment. Reaction intermediates from EC and EGC were investigated using ESI-LC/MS (Zoom scan mode).

TABLE I

Inhibitory Effect of Catechins on the Peroxidation of Soybean PC Liposomes

	t_{inh} (s)[a]	R_{inh} ($M \cdot s^{-1}$)[a]	R_p'/R_p[b]	k_{inh}/k_p[c]	inhibition (%)[d]	n[e]
EC	9360	13.3×10^{-9}	0.95	41	68	3.1
EGC	3420	6.3×10^{-9}	1.06	232	26	1.1
ECG	—	—	0.21	—	79	—
EGCG	4980	1.6×10^{-9}	0.43	628	75	1.7

[a] t_{inh} and R_{inh}, the inhibition period and the inhibition rates in the presence of catechins, respectively.
[b] R_p and R_p', the radical propagation rates in the absence and presence of catechins, respectively.
[c] k_{inh}/k_p, the ratio of the rate constant for inhibition to that for chain propagation.
[d] Inhibition ratio (%) when control is 0%.
[e] n, stoichiometric number of radicals trapped by antioxidant.

Total ion chromatograms of the reaction mixtures produced from these two catechins are shown in Fig. 3. Two peaks were observed from EC at the retention times of 5.9 (peak A) and 8.2 (peak B) min 2 h after AAPH-induced radical oxidation was started. On the other hand, only one peak was produced from EGC (peak C, rt = 3.1 min) immediately after oxidation. The molecular ion peaks B and C were shown at m/z 289 and peak C at m/z 303 from the result of ESI-LC/MS (data not shown). The MS/MS spectra of peaks B and C are shown in Fig. 4. The patterns of the fragment ions for peaks B and C were quite different, although those of EC and EGC were completely the same. These results indicate that the structures in the B ring of peaks B and C are not the same. EC would gradually produce peak B after two hydrogen atoms were subtracted by peroxyl radicals, and EGC would form peak C immediately after abstraction of four hydrogen atoms described in Fig. 5. EGC was easily changed to peak C because of its susceptibility to oxidation. Peak A remains unknown.

Bond Dissociation Enthalpies for EC and EGC

To clarify the antioxidative mechanisms of catechins, BDEs were evaluated by PM3 semiempirical MO calculations. All MO calculations for catechins and catechin radicals were performed after the structural optimization. As shown in Fig. 6, C-H bond dissociation enthalpies at the C-2 position of EC and EGC were unexpectedly lower (64.8 and 64.5 kcal/mol, respectively) than BDEs for the phenolic O-H bond (70 kcal/mol), suggesting that the C-2 proton could easily be subtracted by free radicals.

Based on the results described above, the tentative mechanisms of catechins as antioxidants are shown in Fig. 5, which is also in agreement with the theoretical calculations.

DISCUSSION

The antioxidative effects and mechanisms of catechins were investigated using LC/MS, spectrophotometric analyses, and semiempirical MO calculations.

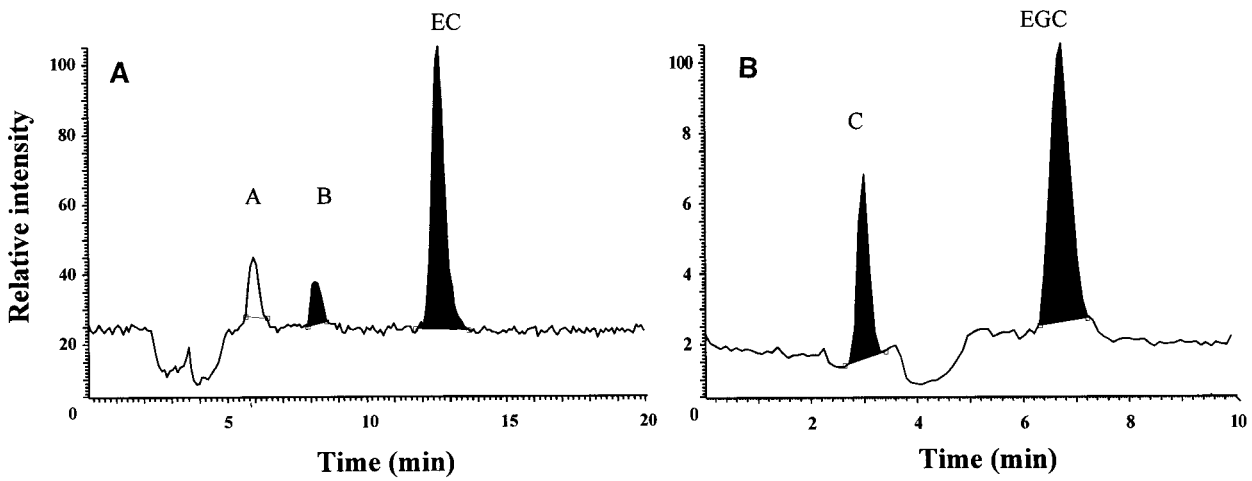

FIG. 3. Mass chromatograms of reaction mixtures (EC or EGC and AAPH). Oxidation was initiated by the addition of AAPH at 37°C. Reaction mixtures containing EC (A) or EGC (B) were withdrawn at intervals and then injected onto columns for LC/MS.

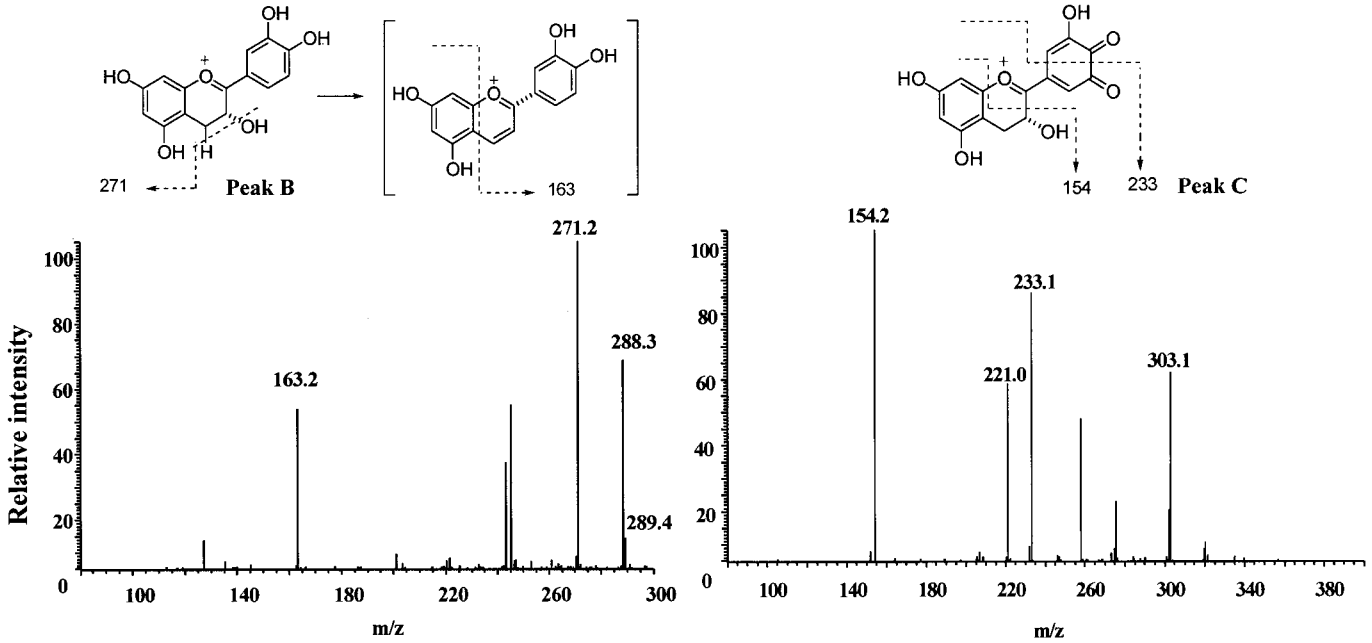

FIG. 4. MS/MS spectra obtained for peak B (left) and peak C (right). Reaction mixtures containing EC or EGC were injected onto the HPLC column, and MS/MS analyses of the ion at *m/z* 289 (peak B) and *m/z* 303 (peak C) were performed using LC/MS equipped with the ESI source operating in a positive ionization mode.

AAPH, which generates peroxyl radicals by the reaction with oxygen, was used as an initiator of lipid peroxidation in the liposomal system and of radical oxidation in water. In order to clarify the antioxidative mechanisms of catechins, in this work, the effect of transition metals was excluded by the addition of DTPA in both systems.

Catechins have multiple biological activities including anticarcinogenic, antiinflammatory effects (3, 4, 26–30). These protective effects are in large part attributed to the antioxidative activities. Catechins were effective in scavenging peroxyl radicals in the liposomal system. Bors *et al.* (31) have proposed that the three criteria for effective radical scavenging are (a) the *o*-dihydroxyl structure in the B ring, which confers higher stability to the radical form and participate in electron delocalization, (b) the 2,3-double bond in conjugation with a 4-oxo function in the C ring, which is responsible for electron delocalization from the B ring, and (c) the 3- and 5-OH groups with 4-oxo function in the A and C rings for maximum radical scavenging potential.

Thus, quercetin satisfies all three criteria mentioned above and seems to be a more effective antioxidant than catechins, which lack the determinants (b) and (c).

In the present work, the effect of catechins except for EGC against peroxyl radicals were almost the same as that of quercetin or greater in PC-liposomes and in water, although the electron-donating ability of catechins is lower than that of quercetin. Rice-Evans *et al.* (32) and Salah *et al.* (8) reported total antioxidative activity of catechins in aqueous medium using ABTS cation radical. They described that the compounds with the trihydroxyl structure in the B ring exerted the greatest antioxidative activity. The system they used, however, was based on the activity for a short period. Antioxidative action is not so simple. There are antioxidants that are effective only for a short time and that can act as prooxidants. In the liposomal and aqueous system we used, EGC and EGCG were the most effective catechins for a short period (within 20 min). Antioxidative activity is considered to be the ability to trap the chain-carrying peroxyl radicals by donation of the phenolic hydrogen atom in the A and B rings (33). The low reduction potential of flavonoids is responsible for their antioxidative effectiveness. Therefore, the effectiveness of catechins for peroxyl radical scavenging cannot be explained by the results described above.

LC/MS/MS analyses of the intermediates generated from EC and EGC (peaks B and C) suggest that EC ($m/z = 290$) could be gradually converted to compound 3 ($m/z = 289$) via compound 2 after abstraction of two hydrogen atoms at the phenolic and C-2 positions, and that EGC could be rapidly transferred to compound 8 via compounds 6 and 7 after abstraction of four hydrogen atoms (Fig. 5). Figure 5 illustrates the proposed mechanisms of catechins as antioxidants against radical oxidation. Unlike other flavonoids, such as quercetin, only catechins have a benzylic proton on the C ring,

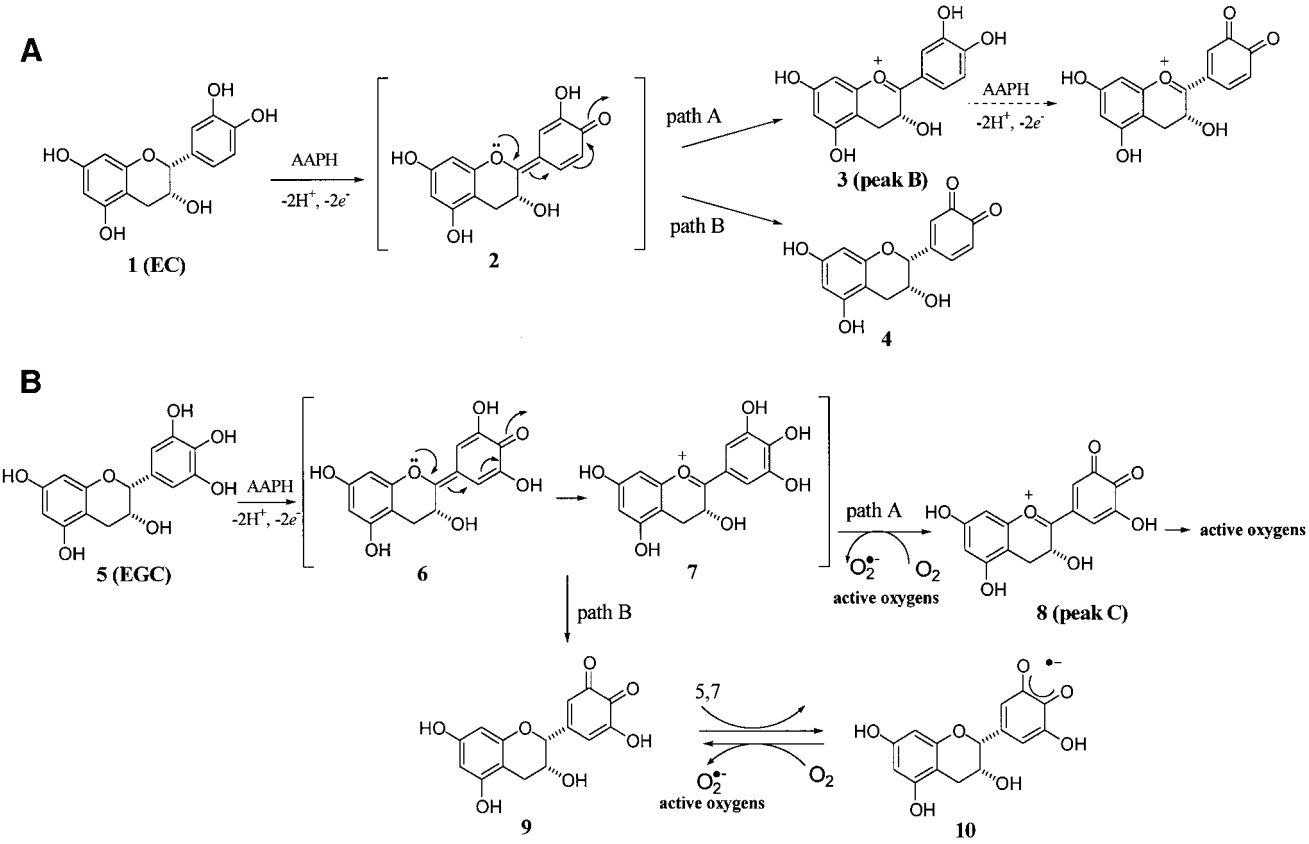

FIG. 5. Proposed antioxidative mechanisms of catechins in radical oxidation.

which could be easily abstracted by free radicals as with an allylic proton. Researchers have never discussed the antioxidative activity from this point of view. In general, antioxidative activity has been considered to be the potential to scavenge free radicals by donating a hydrogen atom of the phenolic O-H. Recently, Wright *et al.* (34) have reported the theoretical calculation of phenolic O-H BDEs of simple phenolic antioxidants such as phenol and substituted phenols. We have calculated here not only the phenolic O-H but also all of the C-H BDEs of catechins. PM3 semiempirical MO calculations have shown that BDEs at the C-2 position of EC and EGC are lower than expected (64.8 and 64.5 kcal/mol, respectively). These results indicate that EC would be gradually converted to compound 3 (path A) and perhaps compound 4 (path B) via compound 2, and that compound 3, an anthocyanin-like precursor, can also act as an antioxidant. This hypothesis was supported by the fact that the decrease in EC was extremely slow during radical oxidation,

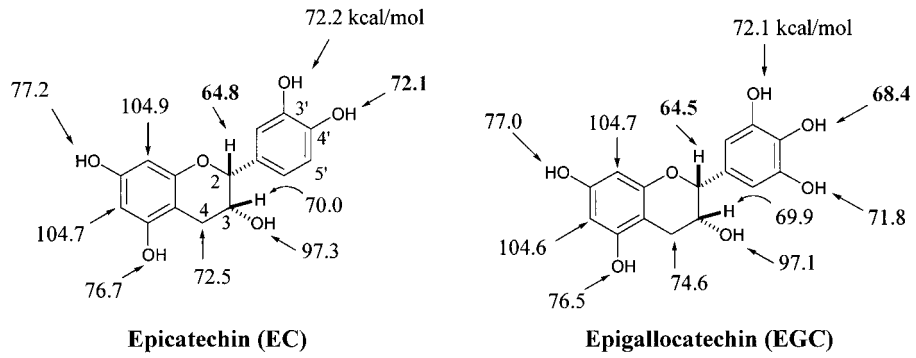

FIG. 6. Calculated bond dissociation enthalpies for EC and EGC. All values were obtained by PM3 semiempirical MO calculations.

whereas the decrease in EGC was very fast. The rate of EC for scavenging peroxyl radicals was six times slower than that of EGC (kinetic study). The decrease in EC was actually much slower than that of EGC. Consequently, it seems that EC showed a longer inhibition period [t_{inh} = 9360 s]. On the other hand, EGC would be changed into compounds 8 and 9 via compounds 6 and 7 (Fig. 5), and active oxygen such as superoxide anion radicals (O_2^-) might be produced during the transformation processes from compound 7 to compound 8 and perhaps from compound 10 to compound 9.

EGC was also the least effective catechin among four catechins used here in water when measured by the chemiluminescence method (data not shown), and a rapid increase of chemiluminescence was observed after a short period. The chemiluminescence was reduced by adding SOD (44% inhibition) in water. The lower effectiveness of EGC against peroxyl radicals may be due to formation of superoxide anion radicals (O_2^-). However, it can be difficult to discuss the low antioxidative activity of EGC in the liposomal system with DTPA only from the result of formation of superoxide anion radicals. The low antioxidative activity might be due to other active oxygen species such as hydroxyl radicals or EGC radicals. When measured by the chemiluminescence method without DTPA, in water, in Tris–HCl buffer, and in phosphate buffer, EGC was the least effective. Therefore, the low antioxidant activity of EGC is due to the formation of active oxygen species. Thus, it is thought that EGC was the least effective in scavenging peroxyl radicals in the liposomal system. This result was in agreement with that in LDL (7). We investigated the same experiment using EC and EGC in ethanol. However, peaks A, B, and C were not detected. This suggests that the antioxidative activity and mechanism are affected by the solvent system and perhaps the radical species. Gardner *et al.* (18) also reported that the activity of catechins in water was lower than that in ethanol, especially EGC and EGCG, which have the trihydroxyl structure in the B ring. This may be due to prooxidative action. The UV spectra of compounds 3 (peak B) and 8 (peak C) were obtained using a photodiode array detector with HPLC (Fig. 7). The spectrum of compound 3 was almost the same as that of EC except for a bathochromic shift, but compound 8 showed another absorption at 308 nm, indicating that compound 3 retains aromatic B ring whereas compound 8 is different from EGC in the B ring.

In conclusion, catechins proved to be powerful antioxidants against lipid peroxidation. EC is converted to a compound that can also act as an antioxidant, in addition to EC itself. On the other hand, EGC is transformed to an oxidized compound that cannot function as an antioxidant and produces active oxygen (O_2^-).

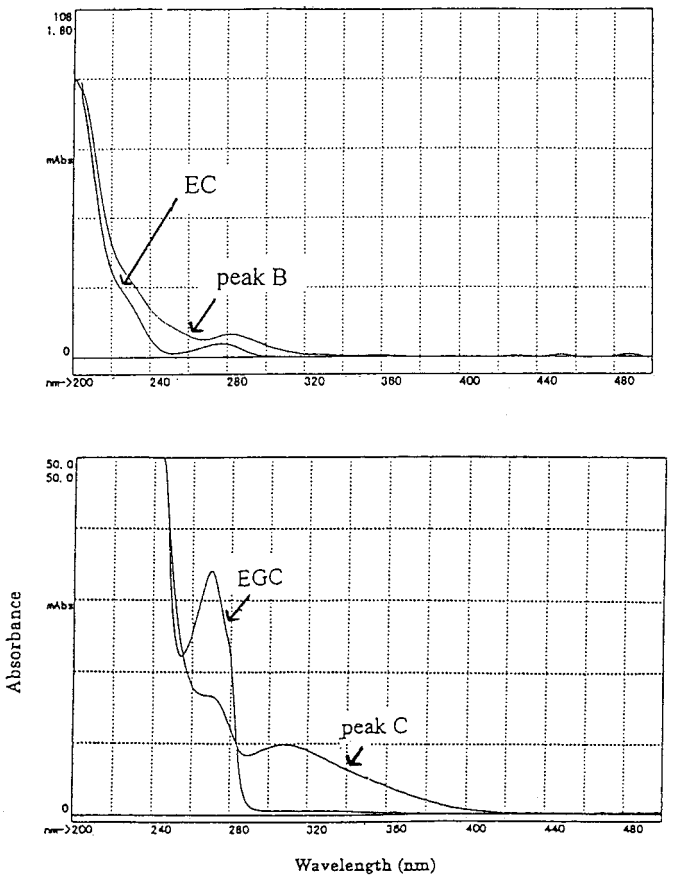

FIG. 7. Spectra of peak B (top) formed from EC and peak C (bottom) formed from EGC. Spectra were measured by HPLC with a photo diode array detector.

Therefore, EC has a much longer inhibition period for lipid peroxidation than EGC. EC is effective in scavenging peroxyl radicals for a longer period, whereas EGC is excellent antioxidants for scavenging peroxyl radicals more quickly.

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REFERENCES

1. Renaud, S., and de Lorgeril, M. (1992) *Lancet* **339,** 1523–1526.
2. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., and Kromhout, D. (1993) *Lancet* **342,** 1007–1011.
3. Brown, J. P. A. (1980) *Mutat. Res.* **75,** 243–277.
4. Middeton, E., and Kondasmami, C. (1992) *Biochem. Pharmacol.* **43,** 1167–1179.
5. Whitehead, T. P., Robinson, D., Allaway, S., Syms, J., and Hale, A. (1995) *Clin. Chem.* **41,** 32–35.
6. Fuhrman, B., Lavy, A., and Aviram, M. (1995) *Am. J. Clin. Nutr.* **61,** 549–554.

7. Miura, S., Watanabe, J., Tomita, T., Sano, M., and Tomita, I. (1995) *Biol. Pharm. Bull.* **17,** 1567–1572.
8. Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P., and Rice-Evans, C. (1995) *Arch. Biochem. Biophys.* **322,** 339–346.
9. Desmarchelier, C., Barros, S., Repetto, M., Latorre, L. R., Kato, M., Coussio, J., and Ciccia, G. (1997) *Planta Med.* **63,** 561–563.
10. Meyer, A. S., Heinonen, M., and Frankel, E. N. (1998) *Food Chem.* **61,** 71–75.
11. Pearson, D. A., Frankel, E. N., Aeschbach, R., and German, J. B. (1998) *J. Agric. Food Chem.* **46,** 1445–1449.
12. Ueno, T., and Takeo, T. (1995) *Biosci. Biotech. Biochem.* **59,** 1558–1559.
13. Ueno, T., Kondo, K., Itakura, H., and Takeo, T. (1996) *Biosci. Biotech. Biochem.* **60,** 1558–1559.
14. Nakagawa, K., Okuda, S., and Miyazawa, T. (1997) *Biosci. Biotech. Biochem.* **61,** 1981–1985.
15. Yoshida, T., Mori, K., Hatano, T., Okumura, T., Uehara, I., Komagoe, K., Fujita, Y., and Okuda, T. (1989) *Chem. Pharm. Bull.* **37,** 1919–1921.
16. Yoshioka, H., Sugiura, K., Kawahara, R., Fujita, T., Makino, M., Kamiya, M., and Tsuyumu, S. (1991) *Agric. Biol. Chem.* **55,** 2717–2723.
17. Guo, Q., Zhao, B., Li, M., Shen, S., and Xin, W. (1996) *Biochim. Biophys. Acta* **1304,** 210–222.
18. Gardner, P. T., McPhail, D. B., and Duthie, G. G. (1998) *J. Sci. Food Agric.* **76,** 257–262.
19. Nanjo, F., Goto, K., Seto, R., Suzuki, M., Sakai, M., and Hara, Y. (1996) *Free Radical Biol. Med.* **21,** 895–902.
20. MacDonald, R. C., MacDonald, R. I., Menco, B. Ph. M., Takeshita, K., Subbarao, N. K., and Hu, L. (1991) *Biochim. Biophys. Acta* **1061,** 297–303.
21. Terao, J., Piskula, M., and Yao, Q. (1994) *Arch. Biochem. Biophys.* **308,** 278–284.
22. Ioku, K., Tsushida, T., Takei, Y., Nakatani, N., and Terao, J. (1995) *Biochim. Biophys. Acta* **1234,** 99–104.
23. Tesoriere, L., Bongiorno, A., Pintaudi, A. M., D'Anna, R., D'Arpa, D., and Livrea, M. A. (1996) *Arch. Biochem. Biophys.* **326,** 57–63.
24. Miyazawa, T., Suzuki, T., Fujimoto, K., and Yasuda, K. (1992) *J. Lipid Res.* **33,** 1051–1058.
25. Van Arnum, S. D., and Stepsus, N. (1997) *Tetrahedron Lett.* **38,** 305–308.
26. Havsteen, B. (1983) *Biochem. Pharmacol.* **32,** 1141–1148.
27. Akiyama, T., Ishida, J., Nalagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukami, Y. (1987) *J. Biol. Chem.* **262,** 5592–5595.
28. Robak, J., Shridi, F., Wolbis, M., and Krolikowska, M. (1988) *Polish J. Pharmacol. Pharm.* **40,** 451–460.
29. Sogawa, S., Nihro, Y., Ueda, H., Izumi, A., Miki, T., Matsumoto, H., and Satoh, T. (1993) *J. Med. Chem.* **36,** 3904–3909.
30. Chan, M. M., Fong, D., Ho, C. T., and Huang, H. I. (1997) *Biochem. Pharmacol.* **54,** 1281–1286.
31. Bors, W., Heller, W., Michel, and Saran, M. (1990) *in* Methods in Enzymology (Packer, L., and Glazer, A. N., Eds.), Vol. 186, pp. 343–355, Academic Press, San Diego.
32. Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996) *Free Radical Biol. Med.* **20,** 933–956.
33. Jovanovic, S. V., Hara, Y., Steen, S., and Simic, M. G. (1995) *J. Am. Chem. Soc.* **117,** 9881–9888.
34. Wright, J. S., Carpenter, D. J., McKay, D. J., and Ingold, K. U. (1997) *J. Am. Chem. Soc.* **119,** 4245–4252.