Genes and Environment, Vol. 28, No. 2 pp. 41–47 (2006)

Review

A Planar Catechin Analogue as a New Type of Synthetic Antioxidant

Kiyoshi Fukuhara[1]

National Institute of Health Sciences, Tokyo, Japan

(Received March 3, 2006; Accepted April 21, 2006)

The protective role of antioxidants against free-radical associated diseases has been widely studied, leading to the development of new types of antioxidants to remove reactive oxygen species such as $O_2^{\bullet -}$ and $^{\bullet}OH$. We synthesized a new type of synthetic antioxidant in which the catechol (B ring) and chroman moieties (AC ring) within the (+)-catechin (CA) structure were constrained to be planar. As compared with CA, planar catechin (PCA) showed strong radical scavenging activities towards both galvinoxyl and cumylperoxyl radicals. Reduced prooxidant activity was also observed, consistent with the dianion form of PCA being weaker at generating $O_2^{\bullet -}$ than the dianion form of CA. PCA completely inhibited DNA-strand scission induced by the Fenton reaction, whereas CA exhibited not only antioxidant properties but also prooxidant properties consistent with enhanced DNA strand cleavage. As compared with hydrophilic CA, the lipophilicity of PCA due to its planarity may aid in penetration of these antioxidant molecules past the cell membrane. Further development of planar PCA may be a favorable approach towards new clinically useful antioxidants for the treatment of free-radical associated diseases.

Key words: antioxidant, (+)-catechin, planarcatechin, reactive oxygen species

Introduction

Oxidative damage to biomolecules such as DNA, carbohydrate, proteins and polyunsaturated fatty acids is thought to play a significant role in mutagenesis, cancer, aging, and other human pathologies. Therefore, considerable attention has been focused on the development of antioxidants to prevent or treat diseases associated with oxidative stress (1,2). Flavonoids such as (+)-catechin (CA) and quercetin are plant phenolic pigment products that act as natural antioxidants (3). Quercetin protects against oxidant injury and cell death (4) by scavenging free radicals (5,6), thereby preventing lipid peroxidation (7) and terminating chain-radical reactions (8). However, there have only been a few reports on the use of CA for the treatment of free radical-associated disease, even though the mechanism of oxygen radical scavenging has been well studied (9,10). The ability to scavenge free radicals must be improved and adequate lipophilicity is required to penetrate the cell membrane before it is suitable for clinical use. The superior antioxidant ability of quercetin results from the formation of a stable radical, due to the C2-C3 double bond and the resulting planar geometry which delocalizes the radical throughout the entire molecule (11). Since the B ring in CA is known to be perpendicular to the A ring (12), the radical-scavenging ability of CA might be improved by constraining the geometry of CA to be planar.

Among natural antioxidants, CA is commercially available as well as quercetin, resveratrol and α-tocopherol and is suited for starting material for synthetic antioxidant. In this review, I describe the synthesis and characterization of the antioxidant properties of planar catechin analogue (PCA) with respect to the chroman and catechol moieties of (+)-CA, by taking advantage of the formation of a bridge between the 3-OH group on ring C and C6' on ring B.

Synthesis

PCA was synthesized *via* the oxo-Pictet-Spengler reaction using CA and acetone with a silyl Lewis acid such as TMSOTf, TESOTf, or TBSOTf (13), as shown in Fig. 1. Typically, CA and 1.2 equivalents of acetone in THF were treated with 1.2 equivalents of TMSOTf at -5°C to form the PCA in 3 hours. The planarity of PCA was confirmed by single-crystal X-ray crystallography (14). X-ray analysis also showed that the stereochemistry of 3-H on ring C was maintained throughout the reaction without any acid-catalyzed racemization. Although the PCA synthesized by the reaction has two methyl groups as side chains, it is possible to synthesize planar catechin analogues so that the lipophilicity is controlled by varying the length of the alkyl side chains in place of methyl groups, by using

[1]Correspndence to: Kiyoshi Fukuhara, Division of Organic Chemistry, National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya, Tokyo 158-8501, Japan. Tel: +81-3-3700-1141, Fax: +81-3-3707-6950, E-mail: fukuhara@nihs.go.jp

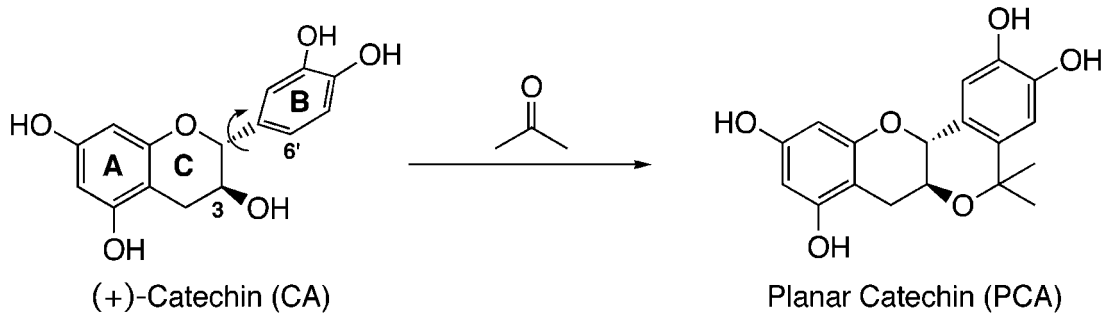

Fig. 1. Synthesis of PCA.

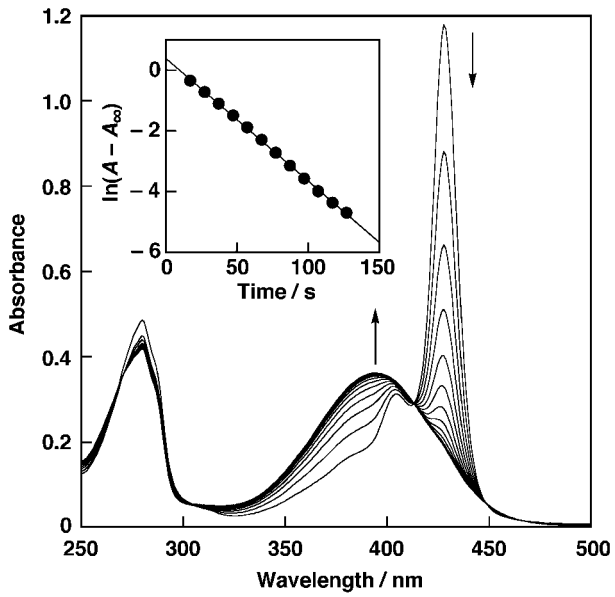

Fig. 2. Spectral change in the reaction of CA (1.5×10^{-4} M) with G$^{\bullet}$ (2.4×10^{-6} M) in deaerated MeCN at 298 K (Interval: 10 s). (Inset) First-order plot based on the change in absorption at 428 nm.

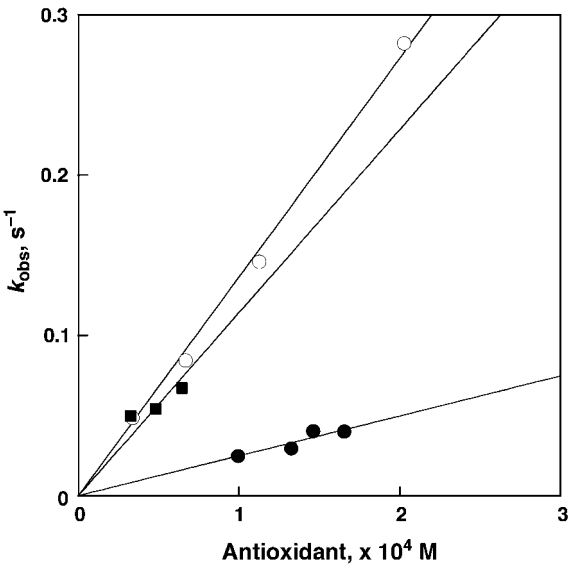

Fig. 3. Plot of the pseudo-first-order rate constant (k_{obs}) vs. the concentrations of CA (●), quercetin (■), and PCA (○) for hydrogen atom transfer from antioxidants to G$^{\bullet}$ (2.4×10^{-6} M) in deaerated MeCN at 298 K.

ketones with alkyl side chains in this reaction.

Antioxidant Properties

The radical-scavenging activities of CA and PCA as well as that of quercetin were compared using the galvinoxyl radical (G$^{\bullet}$) as an oxyl radical species (14). Upon addition of CA to a deaerated MeCN solution of G$^{\bullet}$, the absorption band at 428 nm due to G$^{\bullet}$ disappeared immediately as shown in Fig. 2. This indicated that hydrogen abstraction from one of the OH groups on the B ring of CA by G$^{\bullet}$ had taken place to give the (+)-catechin radical (CA$^{\bullet}$) and hydrogenated G$^{\bullet}$ (GH). The decay of the absorbance at 428 nm due to G$^{\bullet}$ obeyed pseudo-first-order kinetics when the concentration of CA was maintained at more than 10-fold excess of the G$^{\bullet}$ concentration (inset of Fig. 2). The dependence of the observed pseudo-first-order rate constant (k_{obs}) on the concentration of CA is shown in Fig. 3, which demonstrates a linear correlation between k_{obs} and the concentration of CA. From the linear plot of k_{obs} vs. the CA concentration in Fig. 3, we determined that the second-order rate constant (k) for hydrogen abstraction of CA by G$^{\bullet}$ was 2.34×10^2 M^{-1} s^{-1}. The k values for PCA and quercetin were determined in the same manner to be 1.12×10^3 M^{-1} s^{-1} and 1.08×10^3 M^{-1} s^{-1}, respectively. Thus, the k values for PCA and quercetin are about 5-fold larger than that for CA. These results suggest that molecular planarity is essential for the radical-scavenging ability of antioxidants.

The hydroxyl radical is the most reactive oxygen-derived free radical and is responsible for aging and free radical-mediated injury. Therefore, the effects of CA, PCA, and quercetin on hydroxyl radical-mediated DNA breakage were investigated (14). DNA-strand scission in supercoiled pBR322DNA was induced by a hydroxyl

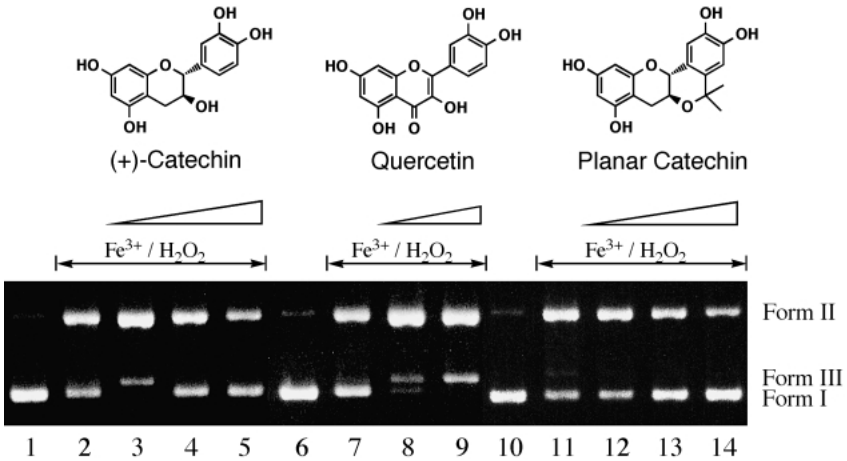

Fig. 4. Effects of CA, quercetin, and PCA on DNA breakage induced by Fe^{3+}/H_2O_2. Assays were performed in 50 mM sodium cacodylate buffer, pH 7.2, containing 45 μM bp of pBR322DNA, for 1 h at 37°C. Lanes 1, 6, and 10; DNA alone, lanes 2, 7, and 11; 10 mM H_2O_2 and 10 μM $FeCl_3$, lanes 3–5, 8 and 9, and 12–14; 10 mM H_2O_2 and 10 μM $FeCl_3$ in the presence of 0.25, 1.25, and 2.5 mM CA (lanes 3–5), 0.25 and 1.25 mM quercetin (lane 8 and 9), and 0.25, 1.25 and 2.5 mM PCA (lane 12–14).

radical-generating system using hydrogen peroxide in the presence of Fe^{3+} (Fenton reaction). As shown in Fig. 4, CA at a high concentration (1.25 and 2.5 mM) suppressed DNA strand breakage, while at a low concentration (0.25 mM) it exhibited pro-oxidant properties, consistent with enhanced DNA cleavage in comparison to cleavage without antioxidant. Quercetin only showed pro-oxidant effects at 0.25 and 1.25 mM. In agreement with previously published results,[15] the measured pro-oxidant effects of CA and quercetin may be attributed to autoxidation of the antioxidant in the presence of transition metal, leading to the generation of primary radicals such as the hydroxyl radical. In contrast to the pro-oxidant effects of CA and quercetin, the addition of PCA protected DNA from Fenton reaction-mediated damage at all concentrations tested. PCA exhibited marked hydroxyl radical-scavenging ability, which exceeded that of CA. Since PCA is very lipophilic compared to CA (data not shown), the high radical-scavenging ability of PCA might be very useful for suppressing free-radical associated events, especially in the cell membrane.

Radical Scavenging Mechanism

Considering the radical scavenging mechanism of phenolic compounds, there are two possibilities in the mechanism of hydrogen transfer reactions: either a one-step hydrogen atom transfer or an electron transfer followed by proton transfer. We have recently reported that the hydrogen transfer from CA to a galvinoxyl or cumylperoxyl radical proceeds *via* electron transfer from CA to galvinoxyl or cumylperoxyl radical (15). This transfer is accelerated in the presence of metal ions such as Mg^{2+} or Sc^{3+}, followed by proton transfer. In such cases, the coordination of the metal ion to the one-electron reduced species of galvinoxyl or cumylperoxyl radical may stabilize the product, resulting in acceleration of the electron transfer process. On the other hand, the hydrogen transfer reaction of vitamin E proceeds *via* a one-step hydrogen atom transfer process, in this case there is no effect of metal ions on the hydrogen transfer rate from vitamin E analogues to the galvinoxyl radical (16–18). Therefore, the effect of a metal ion on the rate of hydrogen transfer from PCA to cumylperoxyl radical can distinguish between one-step hydrogen atom transfer and electron transfer mechanisms in the radical scavenging reaction of PCA. The kinetics of hydrogen transfer from CA and PCA to cumylperoxyl radical have been examined in propionitrile at low temperature by use of ESR (19). The rate of hydrogen transfer from PCA was significantly faster than that from CA. The rate was also accelerated in the presence of $Sc(OSO_2CF_3)_3$. Such an acceleration effect by a metal ion indicates that the hydrogen transfer reaction proceeds *via* metal ion-promoted electron transfer from PCA to oxyl radical (RO.) followed by proton transfer rather than *via* a one-step hydrogen atom transfer, as shown in Fig. 5. As the radical scavenging mechanisms of both CA and PCA are both one-electron transfers, their one-electron oxidation potentials (E_{ox}^0) were determined by the SHACV method. The E_{ox}^0 value of PCA (1.01 V vs. SCE) was significantly more negative than that of CA (1.18V vs. SCE), indicating that electrochemical oxidation of PCA was easier than that of CA. The electron transfer mechanism is probably a consequence of the electrochemical ease of one-electron oxidation.

Reduced Antioxidant Activity

The antioxidant effects of flavonoids undoubtedly

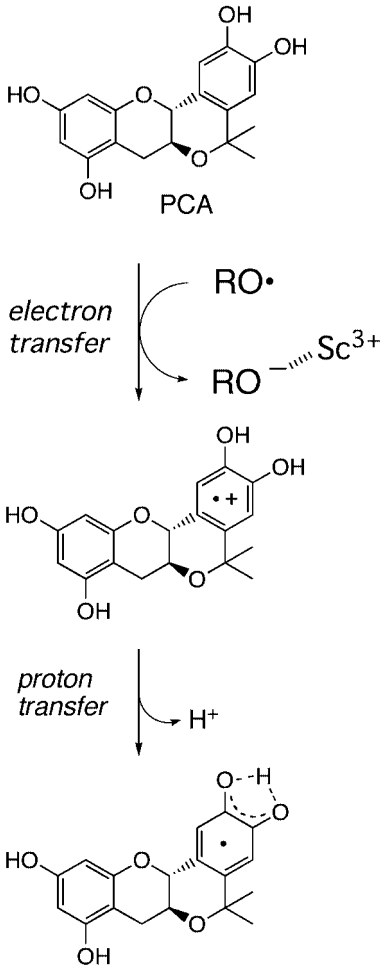

Fig. 5. Antioxidative mechanism of PCA to scavenge reactive oxygen species: Effect of metal ion.

contribute to their antimutagenic and chemopreventive activities. However, the fact that flavonoids themselves have antibacterial and bactericidal activities, as well as being mutagenic and pro-/co-carcinogenic, should be considered when contemplating their clinical use. Their harmful effects are thought to be due to their prooxidant activities. In the presence of Cu(II), CA induces oxidative DNA damage and fatty acid peroxidation (20), due to production of reactive oxygen species *via* electron transfer from CA to molecular oxygen mediated by Cu(II). However, this prooxidant effect is also observed for the dianion of CA produced by the reaction between CA and 2 equiv of tetramethylammonium methoxide (21,22). The one electron-transfer reaction from dianion to molecular oxygen proceeds to form $O_2^{\bullet -}$. The same reaction is also shown by the dianion (PCA^{2-}) of PCA, forming $O_2^{\bullet -}$ by electron-transfer oxidation from PCA^{2-} to O_2 as confirmed by a low-temperature ESR (23). A characteristic ESR $g_{//}$ value of 2.070 due to $O_2^{\bullet -}$, together with an ESR $g_{//}$ value of 2.050 for protonated $O_2^{\bullet -}$ ($HO_2^{\bullet}$) was observed for an O_2-saturated MeCN solution of PCA and 2 equiv of MeO^- at 77 K, as shown in Fig. 6a. During $O_2^{\bullet -}$ generation, the resultant radical anion from PCA underwent an intramolecular proton transfer to give an *o*-semiquinone radical anion form ($PCA^{\bullet -}$), with a characteristic ESR g value of 2.0048 at 298 K, as shown in Fig. 6b.

If one molecule of PCA^{2-} reacts with one molecule of O_2, the rate of electron transfer from PCA^{2-} to molecular oxygen should show first-order dependence. In fact, the increase in absorbance at 485 nm due to the radical anion of PCA obeyed pseudo-first-order kinetics under conditions where the O_2 concentration was maintained at more than 10-fold excess relative to the PCA^{2-} concentration. The pseudo-first-order rate constant (k_{obs}) increases linearly with increases in O_2 concentration. The slope of the linear plot of k_{obs} vs. [O_2] gave the second-order rate constant of the electron transfer (k_{et}) from PCA^{2-} to O_2: $2.8 \times 10^{-2}\,M^{-1}\,s^{-1}$. This k_{et} value is about half of that determined for CA ($5.8 \times 10^{-2}\,M^{-1}\,s^{-1}$), indicating that electron transfer from PCA^{2-} to O_2 proceeds slower than from CA. While PCA provides efficient protection against DNA strand breakage induced by Fenton reaction, the low k_{et} value implies that, in physiologically relevant systems, the ability of PCA to generate oxygen radicals responsible for its prooxidant activity might not be as high as that of CA. Among natural antioxidants, α-tocopherol and ascorbic acid are typical compounds which are useful for the treatment or prevention of diseases associated with oxidative stress. However, administrating a large amount of such antioxidants is unfavorable because of their prooxidant properties, similarly to CA or quercetin. Therefore, the use of PCA, rather than natural antioxidants such as CA, quercetin, α-tocopherol, ascorbic acid, etc., might be favorable for the treatment of diseases associated with oxidative stress due to suppression of oxidant injury as a side-effect arising from the antioxidant itself.

Biological Properties

In addition to antioxidant ability, catechin is known to have several biological activities, including anti-allergic effects, inhibition of α-glucosidase, antibacterial, antivirus, and antitumor activities, though none of these activities are particularly strong. Therefore, the inhibitory effects of PCA on α-glucosidase from *Saccharomyces cerevisiae* and *Bacillus stearothermophilus* were evaluated (13). Surprisingly, in contrast to the relatively weak inhibitory effect of catechin with an IC50 > 500 μM, PCA exhibited strong inhibitory effects of the respective α-glycosidases with $IC_{50} = 1.2\,\mu M$ (*S. cerevisiae*) and 0.7 μM (*B. stearothermophilus*). As α-glycosidase catalyzes the final step in the digestive process of carbohydrates, the strong inhibitory effect on α-glycosidase suggested that PCA may be used as a lead

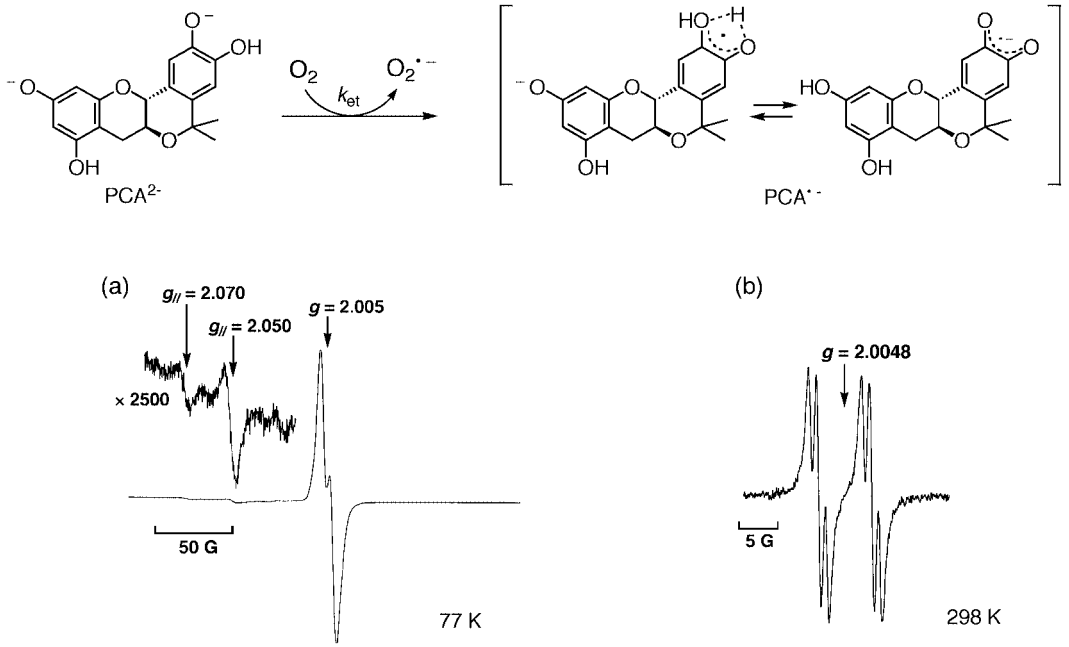

Fig. 6. ESR spectrum of $O_2^{\bullet -}$ (a) and $PCA^{\bullet -}$ (b) generated in the reaction of PCA^{2-} (3.4×10^{-3} M) with O_2 (1.3×10^{-2} M) in MeCN.

compound for the development of antidiabetic therapeutics, similar to acarbose and voglibose which are known to reduce postprandial hyperglycemia primarily by interfering with carbohydrate digesting enzymes and delaying glucose absorption.

Potent antiviral activity of PCA was also shown by significant inhibition of Newcastle Disease Virus infection of BHK cells (24). Considering the strong inhibitory effect on α-glucosidase, the antiviral activity of PCA may be attributed to inhibition of glucosidase during protein synthesis that is essential for virus proliferation (25).

Conclusion

The primary goal of this project was to develop a novel antioxidant, with potential for clinical usage and/or chemoprevention of diseases associated with reactive oxygen species (ROS). There are two kinds of strategy for the development of synthetic antioxidants: one is design of a new type of antioxidant, the structure of which is quite different from natural antioxidants, and the other is chemical modification of natural antioxidants to improve antioxidant capacities. A recent topic in synthetic antioxidants is the development of edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MUI-186). Edaravone has been reported to show potent free radical scavenging actions toward ROS such as $O_2^{\bullet -}$, H_2O_2, and HClO, which may be involved in the tissue destructive effects of reperfusion after ischemia (26). As a neuroprotective agent, edaravone has been clinically prescribed in Japan since 2001 to treat patients with cerebral ischemia. Flavonoids have been shown to exert prominent chemopreventive effects towards oxidative stress-derived injury. However, few studies on synthetic flavonoids with improved radical scavenging ability have been reported.

The planar catechin analogue that we synthesized from CA was constrained to be planar compared with the structure of CA, by taking advantage of the formation of a bridge between the 3-OH group on ring C and C6' on ring B. As compared with CA, PCA showed strong radical scavenging activities towards both galvinoxyl radicals and cumylperoxyl radicals. The $O_2^{\bullet -}$ generating ability of the dianion form of PCA was much lower than that of CA, suggesting that PCA may be a promising novel antioxidant with reduced prooxidant activity. In fact, PCA inhibited DNA-strand scission induced by the Fenton reaction efficiently, whereas CA exhibited not only antioxidant properties in the same reaction but also prooxidant properties consistent with enhanced DNA strand cleavage. The prevention of polyphenols toward coronary diseases and cancer is due to antioxidant properties of polyphenols which should rely, at least in part, on their ability to inhibit lipid peroxidation in plasma low-density lipoproteins (LDL). The proper lipophilicity of PCA owing to its molecular planarity might be favorable for its antioxidant effect into LDL or cell membrane. If the hydrophobicity of PCA could be controlled so as to fine-tune its membrane binding and penetration into the phospholipid bilayer, PCA might be valuable in the development of a new type of clinically useful antiox-

idant. Therefore the synthesis of planar catechin analogues, the lipophicity of which is controlled by changing the length of the alkyl chains instead of methyl group in PCA and their antioxidant abilities are currently underway in our laboratories (13).

Acknowledgment: I thank Dr. I. Nakanishi at National Institute of Radiological Sciences and my colleagues at National Institute of Health Sciences for their enthusiasm in pursuing this field of research. I thank Dr. H. Okuda at National Institute of Health Sciences and Dr. N Miyata at Nagoya City University for helpful discussions. This work was supported partly by a Grant from the Ministry of Health, Labour and Welfare, by a Grant-in-Aid for Research of Health Sciences focusing on Drug Innovation (KH51058) from the Japan Health Sciences Foundation and by Grant-in-Aids for Scientific Research (B) (No. 17390033) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1 Terao J, Piskula M, Yao Q. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. Arch Biochem Biophys. 1994; 308: 278–84.
2 Elangovan V, Sekar N, Govindasamy S. Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene induced tumorigenesis. Cancer Lett. 1994; 87: 107–13.
3 Kühnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. Word Rev Nutr Diet. 1976; 24: 117–91.
4 Greenspan HC, Aruoma OI. Oxidative stress and apoptosis in HIV infection: a role for plant-derived metabolites with synergistic antioxidant activity. Immunol Today. 1995; 15: 209–13.
5 Bors W, Michel C, Saran M. Flavonoid antioxidants: rate constants for reactions with oxygen radicals. Methods Enzymol, 1994; 234: 420–9.
6 Jovanovic SV, Steenken S, Tosic M, Marjanvic B, Simic MG. Flavonoids as antioxidants. J Am Chem Soc. 1994; 116: 4846–51.
7 Decharneux T, Dubois F, Beauloye C, Wattiaux-De Coninck S, Wattiaux R. Effect of various flavonoids on lysosomes subjected to an oxidative or an osmotic stress. Biochem Pharmacol. 1992; 44: 1423–8.
8 Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry. 1986; 25: 383–5.
9 Dangles O, Fargeix G, Dufour C. Antioxidant properties of anthocyanins and tannins: a mechanistic investigation with catechin and the 3,4,7-trihydroxyflavylium ion. J Chem Soc Perkin Trans 2. 2000; 1653–63.
10 Valcic S, Burr JA, Timmermann BN, Liebler DC. Antioxidant chemistry of green tea catechins. New oxidation products of (−)-epigallocatechin gallate and (−)-epigallocatechin from their reactions with peroxyl radicals. Chem Res Toxicol. 2000; 13: 801–10.
11 Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996; 20: 933–56.
12 van Acker SABE, de Groot MJ, van den Berg DJ, Tromp MNJL. Doone-Op den Kelder G, van der Vijgh WJF, Bast A. A quantum chemical explanation of the antioxidant activity of flavonoids. Chem Res Toxicol. 1996; 9: 1305–12.
13 Hakamata W, Nakanishi I, Masuda Y, Shimizu T, Higuchi H, Nakamura Y, Oku T, Saito S, Urano S, Ozawa T, Ikota N, Miyata N, Okuda H, Fukuhara K. Planar catechin analogues with alkyl side chains, as a potent antioxidant and an α-glucosidase inhibitor. J Am Chem Soc. in press.
14 Fukuhara K, Nakanishi I, Kansui H, Sugiyama E, Kimura M, Shimada T, Urano S, Yamaguchi K, Miyata N. Enhanced radical-scavenging activity of a planar catechin analogue. J Am Chem Soc. 2002; 124: 5952–3.
15 Nakanishi I, Miyazaki K, Shimada T, Ohkubo K, Urano S, Ikota N, Ozawa T, Fukuzumi S, Fukuhara K. Effects of metal ions distinguishing between one-step hydrogen- and electron-transfer mechanisms for the radical-scavenging reaction of (+)-catechin. J Phys Chem A. 2002; 106: 11123–6.
16 Nakanishi I, Fukuhara K, Shimada T, Ohkubo K, Iizuka Y, Inami K, Mochizuki M, Urano S, Itoh S, Miyata N, Fukuzumi S. Effects of magnesium ion on kinetic stability and spin distribution of phenoxyl radical derived from a vitamin E analogues: mechanistis insight into antioxidative hydrogen transfer reaction of vitamin E. J Chem Soc Perkin Trans 2. 2002; 1520–4.
17 Nakanishi I, Matsumoto S, Ohkubo K, Fukuhara K, Okuda H, Inami K, Mochizuki M, Ozawa T, Itoh S, Fukuzumi S, Ikota N. EPR study on stable magnesium complexes of phenoxyl radicals derived from a vitamin E model and its deuterated derivatives. Bull Chem Soc Jpn. 2004; 77: 1741–4.
18 Nakanishi I, Kawashima T, Ohkubo K, Kanazawa H, Inami K, Mochizuki M, Fukuhara K, Okuda H, Ozawa T, Itoh S, Fukuzumi S, Ikota N. Electron-tansfer mechanism in radical-scavenging reactions by a vitamin E model in a protic medium. Org Biomol Chem. 2005; 3: 626–9.
19 Nakanishi I, Ohkubo K, Miyazaki K, Hakamata W, Urano S, Ozawa T, Okuda H, Fukuzumi S, Ikota N, Fukuhara K. A planar catechin analogue having a more negative oxidation potential than (+)-catechin as an electron-transfer antioxidant against a peroxyl Rradical. Chem Res Toxicol. 2004; 17: 26–31.
20 Hayakawa F, Kimura T, Maeda T, Fujita M, Sohmiya H, Ando T. DNA cleavage reaction and linoleic acid peroxidation induced by tea catechins in the presence of cupric ion. Biochem Biophys Acta. 1997; 1336: 123–31.
21 Nakanishi I, Fukuhara K, Ohkubo K, Shimada T, Kansui H, Kurihara M, Urano S, Fukuzumi S, Miyata N. Superoxide anion generation *via* electron-transfer oxidation of catechin dianion by molecular oxygen in an

aprotic medium. Chem Lett. 2001; 1152–3.
22 Nakanishi I, Miyazaki K, Shimada T, Inami K, Mochizuki M, Urano S, Okuda H, Ozawa T, Fukuzumi S, Ikota N, Fukuhara K. Kinetic study on the electron-transfer oxidation of the phenolate anion of a vitamin E model by molecular oxygen generating superoxide anion in an aprotic medium. Org Biomol Chem. 2003; 1: 4085–8.
23 Fukuhara K, Nakanishi I, Shimada T, Miyazaki K, Hakamata W, Urano S, Ikota N, Ozawa T, Okuda H, Miyata N, Fukuzumi S. A planar catechin analogue as a promising antioxidant with reduced prooxidant activity. Chem Res Toxicol. 2003; 16: 81–6.
24 Hakamata W, Muroi M, Nishio T, Oku T, Takatsuki A, Osada H, Fukuhara K, Okuda H, Kurihara M. N-linked oligosaccharide processing enzymes as molecular targets for drug discovery. J Appl Glycosci. in press.
25 Mehta A, Zitzmann N, Rudd PM, Block TM, Dwek RA. α-Glucosidase inhibitors as potential broad based anti-viral agents. FEBS Letters. 1998; 430: 17–22.
26 Okatani Y, Watatsuki A, Enzan H, Miyahara Y. Edaravone protects against ischemia/reperfusion-induced oxidation damage to mitochondria in rat liver. Eur J Pharmacol. 2003; 50: 465–72.