Alkylperoxyl Radical-Scavenging Activity of Various Flavonoids and Other Phenolic Compounds: Implications for the Anti-Tumor-Promoter Effect of Vegetables

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We recently reported that alkylperoxyl radical (ROO•) enhanced carcinogenesis in rats treated with carcinogen (Sawa et al. *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7*, 1007−1012), and the tumor promoting action of ROO• could be reduced by addition of hot-water extracts of vegetables (Maeda et al. *Jpn. J. Cancer Res.* **1992**, *83*, 923−928). Here we described the ROO•-scavenging activity of flavonoids and nonflavonoid phenolics and their role in anti-tumor-promoter effects. A model molecular species, ROO•, was generated from *tert*-butyl hydroperoxide (*t*-BuOOH) and heme iron, and the scavenging of *t*-BuOO• was determined by (a) bioassay based on the bactericidal action of ROO•, (b) luminol-enhanced chemiluminescence, and (c) electron spin resonance. Of 17 authentic plant phenolics tested, 9 compounds (including rutin, chlorogenic acid, vanillin, vanillic acid, neohesperidin, gallic acid, shikimic acid, rhamnetin, and kaempferol) showed remarkably high ROO•-scavenging activity. Some of them were detected and quantified in hot-water extracts of mung bean sprouts, used as the model vegetable, and their contents increased after germination, which paralleled very well to the ROO•-scavenging capacity of the vegetable extracts. Thus, a diet rich in these radical scavengers would reduce the cancer-promoting action of ROO•. Consequently, the carcinogenic potentials of oxygen-related radicals may be suppressed.

Keywords: *Alkylperoxyl radical; flavonoids; phenolic compounds; anti-tumor-promoter; radical scavenger*

INTRODUCTION

Free radical species including superoxide anion ($O_2^{\bullet -}$) (Aruoma et al., 1989; Takeuchi et al., 1994), hydroxyl radical (•OH) (Floyd et al., 1986, 1988), and nitric oxide (•NO) (Wink et al., 1991; Nguyen et al., 1992) as well as their reaction products such as peroxynitrite ($ONOO^-$) (Inoue and Kawanishi, 1995; Yermilov et al., 1995; Spencer et al., 1996; Szabo and Ohshima, 1997) are known to cause many types of DNA damage, which is a major event in carcinogenesis (Guyton and Kensler, 1993). For instance, the reaction between •OH and DNA generates a large amount of 8-hydroxyguanine (8-OHG) (Floyd et al., 1988), which causes a G to T transversion (Kamiya et al., 1992), especially at hot spots, the most vulnerable sites of a tumor suppressor gene such as *p53*. We recently found that, in addition to these radicals, alkylperoxyl radicals (ROO•), generated from alkyl hydroperoxides (ROOH) and heme iron (Akaike et al., 1992), cleaved DNA quite effectively (Sawa et al., 1998). The importance of ROO• is due to a long half-life, i.e., ROO• or its adduct with heme group is stable for more than 30 min in biological systems (Akaike et al., 1992). This long half-life makes ROO• increasingly accessible to distant sites as compared with short-lived radicals such as $O_2^{\bullet -}$ and •OH. Furthermore, a diet containing heme plus a large amount of lipids, which would generate ROO•, indeed enhanced colon carcinogenesis in rats treated with *N*-nitroso-*N*-methylurea (Sawa et al., 1998). These findings suggest the cancer-promoting or carcinogenic potential of ROO•. It is therefore expected that scavengers of ROO• may play an important role in cancer prevention.

We recently found that water-soluble extracts of various vegetables possessed potent ROO•-scavenging capacity (Maeda et al., 1992). Those vegetable extracts also had a strong anti-tumor-promoter effect in the transformation assay using B-lymphocytes that carried Epstein−Barr virus (Raji cells). Raji cells were effectively transformed by the addition of phorbolmyristate acetate (PMA) (Maeda et al., 1992) to express the transformation antigen, the so-called EA (early) antigen. These results suggest that hot-water extracts of many vegetables contain a large amount of ROO• scavengers, and thus it would be rational to expect that components in vegetables are important dietary factors for reducing carcinogenic or cancer-promoting actions of ROO•. However, details of the components exhibiting ROO•-scavenging activity remain to be elucidated.

Flavonoids are abundant in the plant kingdom and have potent antioxidant and radical scavenging activities (Bors et al., 1990; Rice-Evans et al., 1996; Cao et al., 1997). Other phenolic compounds including vanillin and phenolic acids such as protocatechuic acid were also found to possess potent radical-scavenging activity (Zhou and Zheng, 1991). In this study, we examined the ROO•-scavenging activity of various flavonoids, phenolic

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acids, and simple phenols, which are referred as plant phenolics hereafter; the effects of these phenolic compounds on the ROO•-scavenging capacity of vegetables, and hence an anti-cancer-promoter effect, were demonstrated.

MATERIALS AND METHODS

Materials. Various authentic plant phenolics were a gift of late professor N. Ishikura of Kumamoto University. *tert*-Butyl hydroperoxide (*t*-BuOOH), (met)hemoglobin, and hematin were from Sigma Chemical Co., St. Louis, MO. Diethylenetriaminepentaacetic acid (DTPA) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) were from Dojindo Laboratories, Kumamoto, Japan. The culture medium (mannitol broth) used for the bioassay based on the bactericidal action of ROO• was prepared by adding D-mannitol (10 g) and phenol red (35 mg) to nutrient broth (18 g of powder) (Eiken Chemical Co., Ltd., Tokyo, Japan) per 1.0 L of pure water. The pH was adjusted to 7.4, followed by sterilization (121 °C, 20 min) (Akaike et al., 1995). Other reagents used were of reagent grade and were used without further purification.

Bioassay of ROO•-Scavenging Potential of Authentic Flavonoids and Other Phenolic Compounds. The ROO•-scavenging potential of authentic plant phenolics was determined by the bioassay method we reported previously, which is based on the bactericidal effect of ROO• but not RO• (alkoxyl) or alkyl (R•) radicals, which do not kill bacteria. *t*-BuOOH was used as a model of alkyl hydroperoxide (ROOH) in this study. Most of plant phenolics exhibited bactericidal or bacteristatic effect by themselves. Therefore, assay samples (plant phenolics) were diluted to the concentration at which no bactericidal or bacteristatic effect was apparent. *t*-BuOO• was generated from *t*-BuOOH via a heme iron-catalyzed reaction, in which ROO• is generated according to

$$\text{porphyrin } (Fe^{III}) + ROOH \rightarrow \text{porphyrin } (Fe^{IV}{=}O)^{+\bullet} + ROH \quad (1)$$

$$\text{porphyrin } (Fe^{IV}{=}O)^{+\bullet} + ROOH \rightarrow \text{porphyrin } (Fe^{IV}{=}O)^{+} + ROO^{\bullet} \quad (2)$$

where porphyrin $(Fe^{IV}{=}O)^{\bullet+}$ is the iron(IV)-oxoporphyrin radical. *Staphylococcus aureus* strain 209P, Gram-positive bacteria, was used in one series of experiments. The reaction mixture (a total of 0.9 mL) was composed of the following: 0.1 mL of *S. aureus* at 10^7 cfu/mL, 0.1 mL of hemoglobin at 1 mg/mL, 0.1 mL of the solution containing a test compound, and 0.6 mL of PBS [phosphate-buffered saline: 8.1 mM Na_2HPO_4 + 1.5 mM KH_2PO_4 + 2.7 mM KCl + 137 mM NaCl (pH 7.4)]. The reaction was initiated by adding 0.1 mL of 0.2 M *t*-BuOOH to the reaction mixture mentioned above at 37 °C, and it was allowed to stand for 30 min. After incubation in the ROO•-generating system, the bacterial suspension was serially diluted on a 96-well plastic multiplate in mannitol broth containing phenol red (as the pH indicator) followed by overnight incubation at 37 °C. Scavenging of ROO• by test compounds permits bacterial growth, accompanying production of acidic metabolites in the culture medium and color change of the medium. This color change allows us to identify the bacterial growth by macroscopic observation. The end point of dilution of the test samples was defined as the complete killing of bacteria with no color change.

ESR Measurements. The effect of plant phenolics on the generation of *t*-BuOO• from *t*-BuOOH plus hemoglobin was investigated by electron spin resonance (ESR) spectroscopy using the spin trap agent, DMPO. Concentrations of samples in 0.1 M sodium phosphate buffer (pH 7.4) were 10 mM *t*-BuOOH, 0.1 mg/mL hemoglobin, 45 mM DMPO, and 0.5 mM DTPA with or without certain plant phenolics. ESR spectra were obtained with a JES-RE1X spectrometer (JEOL, Tokyo, Japan) using quartz flat cells (inner size 60 × 10 × 0.31 mm; effective sample volume of 160 μL) at room temperature under the following conditions: modulation frequently, 100 kHz; modulation amplitude, 0.079 mT; scanning field, 335.0 ± 5 mT; receiver gain, 2000; response time, 0.3 s; sweep time, 2 min; microwave power, 20 mV; and microwave frequency, 9.421 GHz. All spectra were recorded at 2 min after incubation at room temperature. ESR spectra obtained were assigned by using hyperfine splitting constants of the radical adducts of DMPO reported previously (Akaike et al., 1992).

Luminol-Enhanced Chemiluminescence Assay: Determination of ROO•-Scavenging Capacity of Vegetable Extracts. ROO•-scavenging capacity of mung bean sprout extracts was determined by measuring the inhibitory potential of the test compounds against the luminol-activated chemiluminescence of ROO•. The assay mixture contained 250 μL of PBS, 50 μL of 10 mM DTPA, and 50 μL of 0.1 M *t*-BuOOH; 50 μL of the test sample dissolved in dimethyl sulfoxide (DMSO) and 50 μL of 0.1 mM luminol were added and mixed well. The chemiluminescence assay was started by adding 50 μL of hemoglobin (1 mg/mL). The rate, peak intensity, and peak area of chemiluminescence were measured at 37 °C by using a chemiluminescence multichannel analyzer (Berthold Model LB4505 AT, Wildbad, Germany). The concentration of test compounds that gave 50% inhibition of chemiluminescence by *t*-BuOO• was expressed as an α-tocopherol or quercetin equivalent concentration.

Isolation and Identification of Flavonoids from Mung Bean Sprouts. Flavonoids from mung bean sprouts (Japanese moyashi) were isolated according to the literature (Mato and Ishikura, 1993). Mung beans were germinated at 25 °C on water-soaked cotton in Petri dishes, and the beans or the sprouts were illuminated at 10 klx for 18 h/day under a white fluorescent lamp. At various days after germination, bean sprouts were harvested and subjected to extraction and isolation of various flavonoids. One method of extraction was with hot water; the minced (size about 0.5−1 cm) bean sprouts (2.0 g) were boiled in water for 5 min and then homogenized with 20 mL of 70% methanol by using a mortar and pestle without contact with any metals. Another method of extraction was with cold water at or below 15 °C, again with a mortar and pestle, similar to the hot-water extraction. The homogenate was centrifuged at 10000*g* for 10 min. The supernatant was collected and concentrated in vacuo in a rotary evaporator. The concentrates thus obtained were dissolved with 1 mL of 70% methanol and applied to a high-performance liquid chromatography (HPLC) column equipped with a C18 reversed-phase column (4 × 250 mm, LiChrospher 100 RP-18, Merck, Darmstadt, Germany). A Tri Rotar-V HPLC system (JASCO, Tokyo, Japan) was used with an isocratic solvent, methanol/water/phosphoric acid (30/70/0.2, v/v/v), at a flow rate of 0.5 mL/min monitoring at 360 nm.

RESULTS

ROO•-Scavenging Activity of Authentic Flavonoids and Other Phenolic Compounds. When quercetin above 82.1 μM (final concentration) was added to the *t*-BuOO•-generating system in the presence of 20 mM *t*-BuOOH and 0.1 mg/mL hemoglobin, the growth of bacteria resumed, which shows that the bactericidal action of ROO• was neutralized. Thus, the minimal effective dose of quercetin was estimated to be 82.1 μM in this experimental setting. Figure 1 summarizes the ROO•-scavenging activity of various plant phenolics, with potency expressed as quercetin equivalent activity. Among the plant phenolics examined, rutin showed the highest ROO•-scavenging activity based on a molar basis. It is noteworthy that many plant phenolics other than rutin were also found to have significantly higher ROO•-scavenging activity than α-tocopherol (Figure 1). These phenolics included chlorogenic acid, vanillin, vanillic acid, neohesperidin, gallic acid, shikimic acid, rhamnetin, and kaempferol, all of which exhibited activity comparable to that of rutin (Figure 1). They

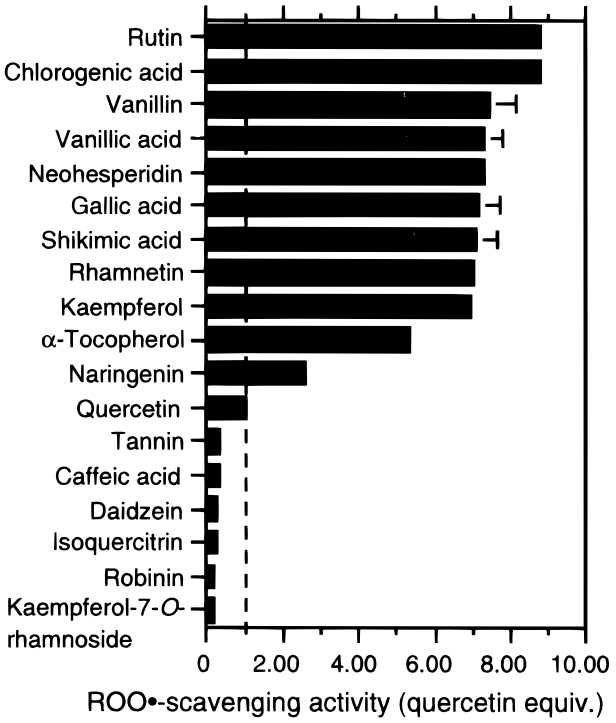

Figure 1. ROO•-scavenging activity of various flavonoids and phenolic compounds determined by bioassay. ROO•-scavenging activity based on a molar basis is expressed as a quercetin equivalent activity of means ± SD of three determinations.

were about 7−9-fold more potent than quercetin or 1.5-fold more potent than α-tocopherol. These results suggest that certain flavonoids and phenolic acids can scavenge ROO• effectively.

The scavenging of a model peroxyl radical, i.e., *t*-BuOO•, by plant phenolics was confirmed by ESR spectroscopy. Figure 2 shows the ESR spectra of DMPO adducts in the reaction systems containing *t*-BuOOH and hemoglobin with or without certain flavonoids. The reaction mixture without hemoglobin did not produce a spectrum of a DMPO−radical adduct (Figure 2A), which shows that hemoglobin (or heme) is required for the generation of *t*-BuOOH-derived radicals. The ESR spectrum in Figure 2B was found to contain a mixture of both DMPO−OOBu and DMPO−OBu: $a_N = 1.43$ mT, $a_H^\beta = 1.07$ mT, and $a_H^\gamma = 0.15$ mT for DMPO−OOBu; $a_N = 1.48$ mT and $a_H^\beta = 1.65$ mT for DMPO−OBu, consistent with the results reported previously (Davies, 1988; Akaike et al., 1992). The addition of either quercetin (Figure 2C) or kaempferol (Figure 2D) completely suppressed the generation of DMPO−OOBu adduct, suggesting that both flavonoids scavenge *t*-BuOO•. Similar results were also obtained for other nonflavonoid phenolics such as vanillic acid and protocatechuic acid (data not shown). These results suggest that reduction of bactericidal action of *t*-BuOO• by plant phenolics may be attributed to their *t*-BuOO•-scavenging action.

Flavonoids and Radical-Scavenging Capacity of Mung Bean Sprout Extracts. Mung bean sprouts were used as a model vegetable to study the role of flavonoids in the ROO•-scavenging capacity of hot-water extracts of vegetables. For this purpose, we first investigated the composition and the contents of flavonoids in the water extracts of mung bean sprouts. Both hot- and cold-water extracts prepared contained detectable amounts of six flavonoids, i.e., robinin, rutin, kaempferol, quercetin, isoquercitrin, and kaempferol-7-*O*-rhamnoside (Figure 3), which were identified by HPLC (Figure 4). This result was consistent with the report by Mato and Ishikura (1993). We further examined the changes in flavonoid contents in the extracts during the germination phase of mung beans. Their content of all flavonoids except isoquercitrin increased in extracts

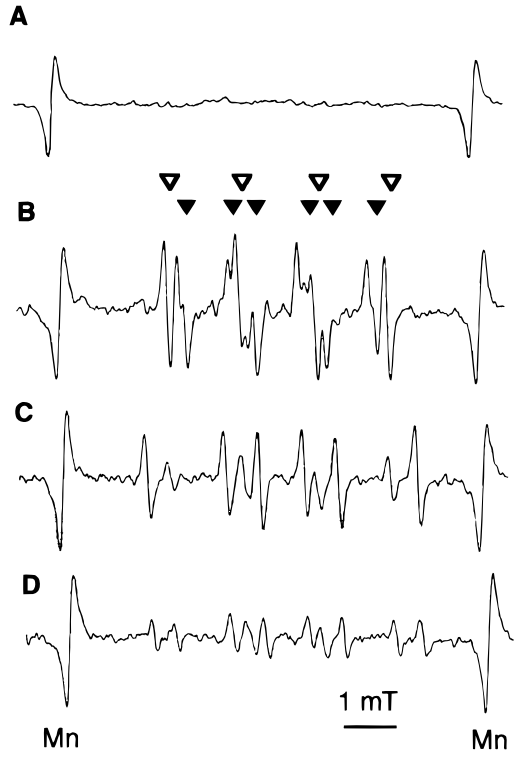

Figure 2. ESR spectra of DMPO spin adducts in systems containing *t*-BuOOH and hemoglobin with or without flavonoids. (A) Spectrum obtained for the reaction mixture of 10 mM *t*-BuOOH, 0.5 mM DTPA, and 45 mM DMPO in 0.1 M sodium phosphate buffer, pH 7.4. (B) Spectrum obtained for reaction system A plus 0.1 mg/mL hemoglobin. (C) Spectrum obtained for reaction system B plus 1 mM quercetin. (D) Spectrum obtained for reaction system B plus 1 mM kaempferol. All spectra were recorded at 2 min after incubation at room temperature. Spectra assigned: DMPO−OOBu (▼), DMPO−OBu (▽).

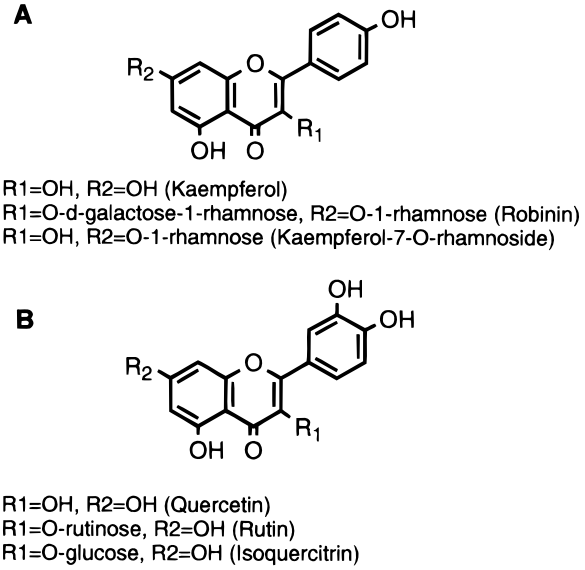

Figure 3. Chemical structures of flavonoids isolated from water extracts of mung bean sprouts. (A) Kaempferol glycosides. (B) Quercetin glycosides.

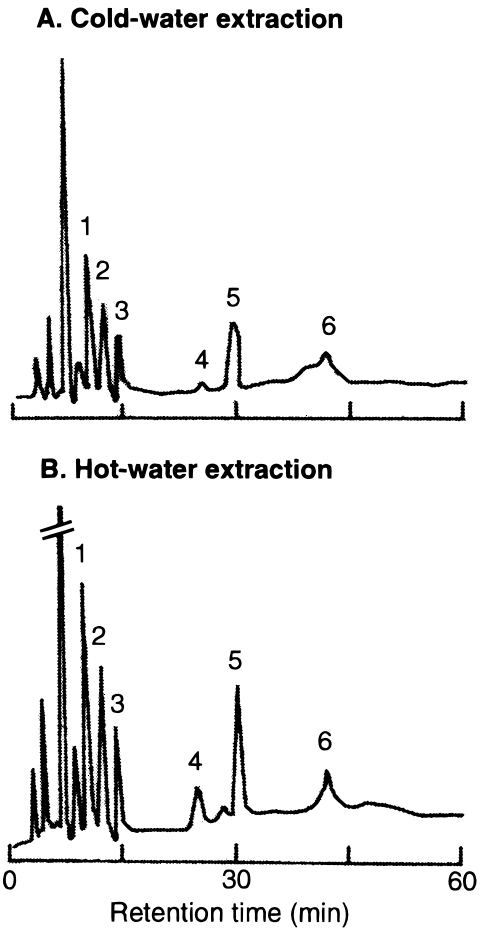

Figure 4. HPLC profiles of (A) cold-water and (B) hot-water extracts of the stems of mung bean sprouts at 8 days after germination. Peaks were identified by using authentic samples as follows: 1, quercetin; 2, kaempferol-7-*O*-rhamnoside; 3, robinin; 4, rutin; 5, isoquercitrin; 6, kaempferol. See text for details.

about 2.5−10-fold with growth of mung bean sprouts (Figure 5A,B). Furthermore, hot-water extracts contained a 2−4-fold higher amount of these flavonoids than did cold-water extracts (Figure 5B).

As reported previously, hot-water extracts of many green vegetables showed a potent ROO•-scavenging capacity (Maeda et al., 1992). In the present study, the ROO•-scavenging capacity of mung bean sprout extracts was further investigated during the germination phase, from day 0 to day 10 (Figure 5C). As shown in Figure 5C, the ROO•-scavenging capacity of cold-water extracts increased with the growth of sprouts and reached a maximum at days 6−8 after germination. Hot-water extracts of the bean sprouts harvested on days 6−8, when it is time to eat them, contained about 4−5-fold higher ROO•-scavenging capacity as compared with the cold-water extracts. These changes in ROO•-scavenging capacity of the extracts were consistent with those in the flavonoid contents of the extracts just mentioned, suggesting that these flavonoids are synthesized during the germination phase. When the ROO•-scavenging capacity of mung bean sprout extracts was plotted against the theoretically calculated ROO•-scavenging capacity (i.e., amount × specific ROO•-scavenging activity of each flavonoid), both values showed a linear relationship (Figure 6, $r = 0.92$). This result strongly supports a contribution of these plant phenolics to the ROO•-scavenging activity of mung bean extracts.

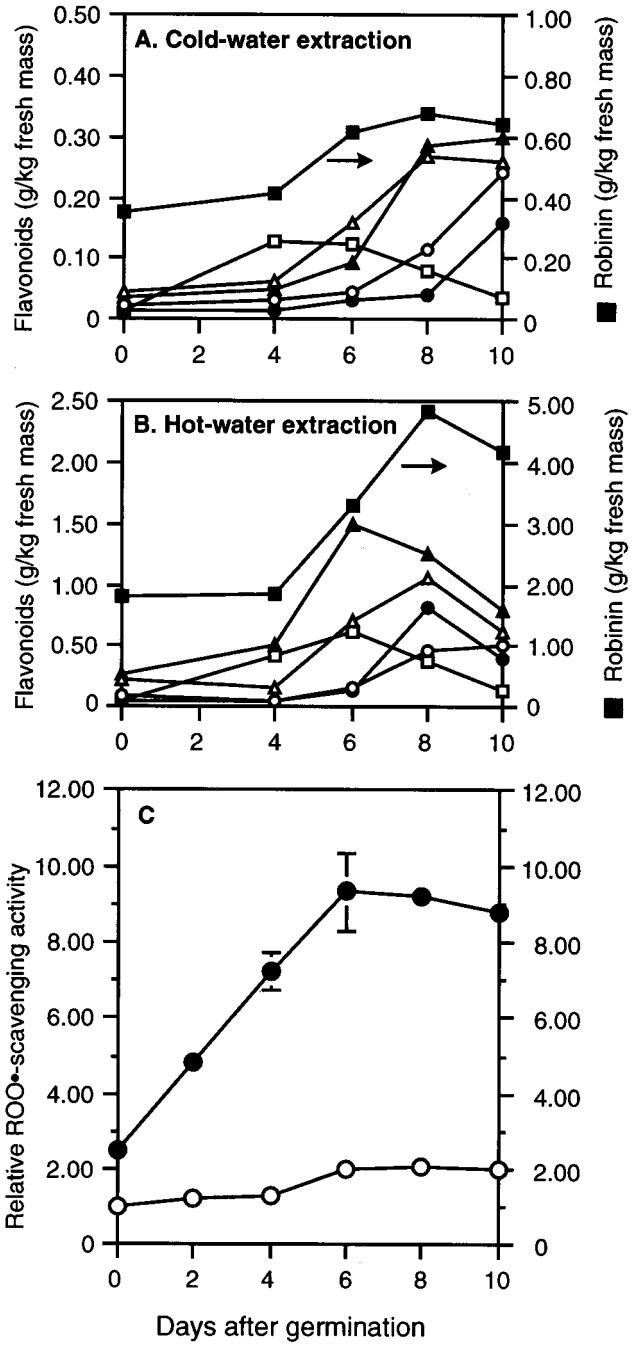

Figure 5. (A) Change in flavonoid content in cold-water extracts of mung bean sprouts after germination. (B) Change in flavonoid content in hot-water extracts of mung bean sprouts after germination. The amount of flavonoids was determined by reversed-phase HPLC with the flavonoids as shown in Figure 4. Flavonoids were rutin (▲), isoquercitrin (□), robinin (■), kaempferol (○), kaempferol-7-*O*-rhamnoside (●), and quercetin (△). (C) Relative ROO•-scavenging activity of cold-water (○) and hot-water extracts (●) of mung bean sprouts as determined by chemiluminescence. The relative ROO•-scavenging activity was obtained by calculating the ratios of each value to the value of the cold-water extract at day 0.

DISCUSSION

Some flavonoids and nonflavonoid phenolic compounds have been reported to show ROO•-scavenging activity (Torel et al., 1986; Bors et al., 1990; Laranjinha et al., 1994; Rice-Evans et al., 1996; Cao et al., 1997). The present study demonstrated that various flavonoids and other phenolic compounds are effective scavengers

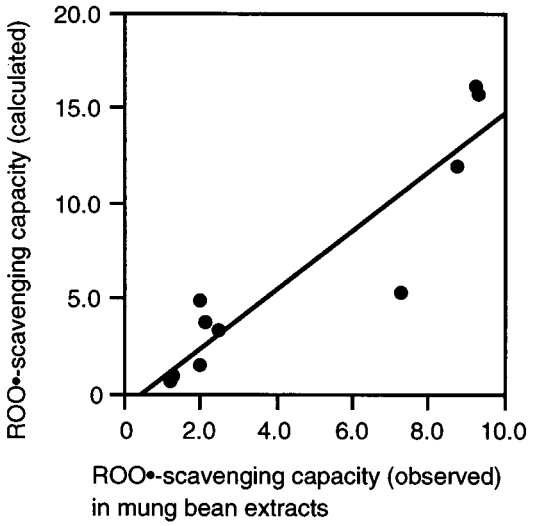

Figure 6. Correlation between ROO•-scavenging capacity of water extracts of mung bean sprouts determined by chemiluminescence and the capacity calculated from the contents and specific ROO•-scavenging activity of flavonoids in extracts of mung bean sprouts. Specific ROO•-scavenging activity was determined by bioassay as shown in Figure 1. The correlation factor was 0.92.

for ROO•. Specific radical-scavenging potency varied greatly among the flavonoids and phenolic compounds tested. For instance, rutin showed an ROO•-scavenging activity 33 times higher than that of isoquercitrin, which is structurally similar to rutin (Figure 1). The minimal effective dose of plant phenolics obtained from bioassay was quite reproducible, so we could compare the minimal effective dose of other antioxidants that had been determined previously (Akaike et al., 1995). Results showed that the activity of rutin and chlorogenic acid was remarkably greater than that of representative water-soluble antioxidants, e.g., 8 times greater than L-cysteine, 17 times greater than reduced glutathione, and 33 times greater than ascorbic acid based on a molar basis (Akaike et al., 1995).

The high potential of phenolic compounds to scavenge ROO• may be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl group as described in eq 3

$$ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet} \qquad (3)$$

This reaction gives the phenoxyl radical (ArO•), which subsequently undergoes a change to a resonance structure by redistributing unpaired electron on the aromatic ring. Thus, phenoxyl radicals exhibit much lower reactivity as compared with ROO•. An additional verification may be that ArO• is ESR silent even in the presence of DMPO (Figure 2). Consequently, ArO• would react further to form unreactive compounds, probably by radical−radical coupling.

The present study confirmed the presence of flavonoids in hot-water extracts of mung bean sprouts, and the content of these flavonoids increased during the germination of mung beans. Commonly used mung bean sprouts in oriental food are harvested 7 days after germination, which coincides with the time of highest anti-ROO• activity. Interestingly, boiling in hot water for 5 min for extraction drastically increased the amount of flavonoids in the extracts, i.e., by 4−5 times (Figure 5). This may be due to the enhanced release of flavonoids via destruction of plant cell walls by boiling, and thus the components become more accessible to the solvent water. The finding of enhanced extraction by hot water but not by cold water is consistent with results for other leafy vegetables reported previously (Maeda et al., 1992). However, no remarkable qualitative alteration in HPLC profiles of the extracts was observed with or without boiling (Figure 4), which suggests that the boiling procedure did not generate new products. Thus, the enhanced extractability of flavonoids by boiling (for 5 min) is a plausible explanation for the high ROO•-scavenging capacity in hot-water extracts. Consequently, the cooking process may become important when considering the bioavailability of ROO• scavengers from whole vegetables. In addition, the high ROO•-scavenging potential of several green teas may be attributed, at least in part, to the effect of heat treatment of the tea leaves during tea manufacturing.

We want to emphasize the importance of the ROO•-species in the context of carcinogenesis because (a) ROO• has a much longer half-life (~30 min or longer) in biological systems (Akaike et al., 1992), whereas $O_2^{\bullet-}$ and hydroxyl radicals have half-lives of less than a millisecond order; (b) ROO• could cleave DNA effectively and hence cause mutation, thus initiating or promoting transformation of carcinogenesis (Sawa et al., 1998); (c) ROO• seems to exhibit a cancer-promoter effect, as we showed in the Epstein−Barr virus-transforming B-lymphocyte system (Maeda et al., 1992), that is consistent with another report that peroxide of diacylglycerol, which can also be converted to ROO• as described here, exhibits a tumor-promoter effect via protein kinase C activation (Yamamoto et al., 1997). Furthermore, ROO• was readily generated from dietary components such as oxidized edible oils and heme iron (rich in red meat and hence the heme group), implicating these radicals as risk factors for diets high in both fat and red meat. This cancer-accelerating effect of ROO• makes the scavenging activity of plant phenolics important in cancer prevention.

Studies of grape wines reported that in red wine the total phenolic content correlated well with relative antioxidant activity (for example, Frankel et al., 1993). However, Heinonen et al. (1998) reported that the total phenolic content of berry and fruit wines did not correlate with antioxidant activity in view of lipid hydroperoxide formation. In their study, however, the specific antioxidant activity of each phenolic compound was not examined. Also, they used a non-water-permissible oil-in-water system, an essentially nonaqueous system in which water-soluble phenolics may not be accessible to the oil phase. On the other hand, the present study indicated that the ROO•-scavenging potentials of the plant phenolics differed greatly from one another. For instance, rutin's ROO•-scavenging activity was 33 times higher than that of isoquercitrin, which is structurally similar to rutin (Figure 1) Therefore, quantification of the ROO•-scavenging activity of each phenolic would allow assessment of the total antioxidant potency of vegetables or plants.

Nardini et al. (1997) demonstrated that administration of caffeic acid to rats increased the level of α-tocopherol in both plasma and lipoprotein. This suggests an alternative action of phenolic compounds, i.e., phenolic compounds indirectly increase the ROO•-scavenging capacity in vivo by increasing the level of α-tocopherol.

In conclusion, it was found that certain flavonoids and phenolic acids are effective scavengers for alkylperoxyl

radicals. Thus, these phenolic compounds may play an important role in the effect of vegetable foods. Furthermore, they became increasingly available after boiling. Diets rich in these flavonoids and phenolics may therefore have a great potential for reducing alkylperoxyl radical content and thus radical-mediated pathogenesis such as carcinogenesis.

ABBREVIATIONS USED

ROO•, alkylperoxyl radical; *t*-BuOOH, *tert*-butyl hydroperoxide; $O_2^{\bullet -}$, superoxide anion; •OH, hydroxyl radical; PMA, phorbol myristate acetate; RO•, alkoxyl radical; R•, alkyl radical; ArO•, phenoxyl radical; ESR, electron spin resonance; NO, nitric oxide; $ONOO^-$, peroxynitrite; ROOH, alkyl hydroperoxide; PBS, phosphate-buffered saline; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DTPA, diethylenetriaminepentaacetic acid; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; 8-OHG, 8-hydroxyguanine.

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