Inhibition of Malonaldehyde Formation from Lipids by an Isoflavonoid Isolated from Young Green Barley Leaves

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The inhibitory effect of 2″-O-glycosylisovitexin (2″-O-GIV), isolated from young green barley leaves, on malonaldehyde (MA) formation from lipids was determined by gas chromatography. Two μmol of 2″-O-GIV inhibited MA formation from 0.2 mmol of squalene by almost 100% upon ultraviolet (UV) irradiation. Only 1 μmol of 2″-O-GIV inhibited 90% of MA formation from 0.2 mmol of ethyl linoleate upon UV irradiation. The effective quantities of 2″-O-GIV were much lower than those of either butylated hydroxytoluene or α-tocopherol. When ethyl linoleate, ethyl linolenate and ethyl arachidonate were oxidized by Fenton's reagent in the presence of 2″-O-GIV or α-tocopherol, both compounds showed similar activity toward MA formation. Both compounds gave maximum inhibition at doses of 0.1–0.3 μmol for ethyl linoleate, 0.1–0.5 μmol for ethyl linolenate and 0.1–0.5 μmol for ethyl arachidonate.

KEY WORDS: Antioxidant, isoflavonoid, lipid peroxidation, malonaldehyde formation.

Lipid peroxidation is purportedly associated with carcinogenesis, mutagenesis, aging and atherosclerosis. However, its mode of toxic action is not yet clearly understood. The formation of low-molecular weight reactive aldehydes, such as malonaldehyde (MA), may possibly play an important role in the toxic effects caused by lipid peroxidation (1–3). Because these aldehydes (including MA, formaldehyde and acrolein) possess a strong electrophilic nature, they react with nucleophiles, such as guanine and guanine nucleosides (4), to form adducts that may cause further biological complications.

Some lipid peroxides have reportedly caused several types of biological damage in experimental animals, for example, oxidative hemolysis of red blood cells (5), necrosis (6) and alterations in the fluidity of plasma membranes (7). Lipid peroxidation *in vivo* may be initiated by the intake of oxidized fats and subsequently cause damage. For example, consumption of oxidized fat by experimental animals was reportedly accompanied by weight loss, alteration in the size of organs and changes in triglyceride levels (8).

Various antioxidants have commonly been applied to inhibit lipid peroxidations. Recently, some chemicals that occur naturally in plants have begun to receive much attention as safe antioxidants, as they have been eaten by people and animals for years (9,10).

In the present study, the antioxidant activity of 2″-O-glycosylisovitexin (2″-O-GIV), isolated from young green barley leaves, was investigated by using lipid peroxidation systems.

MATERIALS AND METHODS

Materials. Squalene, ethyl linoleate, ethyl linolenate, ethyl arachidonate, butylated hydroxytoluene (BHT), Trizma hydrochloride and Trizma base were purchased from Sigma Chemical Co. (St. Louis, MO). α-Tocopherol, sodium dodecyl sulfate (SDS), hydrogen peroxide and 2-methylpyrazine were obtained from Aldrich Chemical Co. (Milwaukee, WI). N-Methylhydrazine was bought from Fluka Chemical Co. (Ronkonkoma, NY). Ferrous chloride was obtained from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). 2″-O-GIV was isolated from young green barley leaves (*Hordeum vulgare* L. var. nudum Hook) by using column chromatography with Amberlite XAD-2 nonionic polymeric absorbent (Aldrich Chemical Co.), according to a method reported previously (11).

Ultraviolet (UV) irradiation of squalene and ethyl linoleate with or without 2″-O-GIV, α-tocopherol or BHT. Squalene (0.2 mmol) or ethyl linoleate (0.2 mmol) was dissolved in 5 mL absolute ethanol in a 20-mL pyrex test tube with 2″-O-GIV (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 μmol), α-tocopherol (0.5, 1.0, 2.0, 4.0, 6.0, 8.0 or 10.0 μmol) or BHT (1.0, 2.0, 4.0, 8.0, 12.0, 16.0 or 10.0 μmol). The sample tubes were then irradiated for 6 h with a Rayonet RPR-100 chamber reactor (Southern New England Ultraviolet Co., Homden, CT) equipped with eight UV lamps ($\lambda = 300$ nm). One sample tube was wrapped with aluminum foil throughout the experiment to serve as a control.

Oxidation of ethyl linoleate, ethyl linolenate and ethyl arachidonate with Fenton's reagent (Fe^{2+}/H_2O_2) in the presence of 2″-O-GIV or α-tocopherol. An aqueous solution (5 mL) containing 10 μL of ethyl linoleate, ethyl linolenate or ethyl arachidonate and 0.25 mmol Trizma buffer (pH, 7.4), 1 mmol of ferrous chloride, 0.5 mmol of hydrogen peroxide, 0.75 mmol potassium chloride and 0.2% of surfactant SDS was stirred with 2″-O-GIV (0.03, 0.06, 0.125, 0.25, 0.50, 1.00, 2.00, 3.00 or 4.00 μmol for ethyl linoleate; 0.05, 0.10, 0.20, 0.40, 0.80, 1.00, 1.20, 1.60, 2.00, 3.00 or 4.00 μmol for ethyl linolenate; and 0.012, 0.025, 0.05, 0.10, 0.125, 0.50, 1.00, 2.00, 3.00 or 4.00 μmol for ethyl arachidonate) or α-tocopherol (0.03, 0.06, 0.125, 0.25, 0.50, 1.00, 2.00 or 3.00 μmol for ethyl linoleate; 0.02, 0.03, 0.04, 0.06, 0.10, 0.125, 0.20, 0.25, 0.40, 0.50, 1.00, 2.00, 3.00 or 4.00 μmol for ethyl linolenate; and 0.03, 0.06, 0.125, 0.25, 0.50, 1.00, 2.00, 3.00 or 4.00 μmol for ethyl arachidonate) for 16 h at 37°C in a 20-mL test tube. The oxidation of samples was stopped by adding 50 μL of 4% BHT ethanol solution (12). The sample tubes were covered with aluminum foil during incubation to avoid any influence of light on the lipid peroxidation.

Measurement of antioxidative activity. Antioxidative activity was determined by analyzing MA formed from squalene or ethyl linoleate upon UV irradiation or oxidation with Fenton's reagent. The gas chromatographic method for MA analysis reported previously (13,14) was used. The MA was reacted with N-methylhydrazine, and the resulting derivative, 1-methylpyrazole, was analyzed with 2-methylpyrazine as an internal standard by a gas chromatograph (Hewlett-Packard 5880, Avondale, PA) equipped with a fused-silica capillary column and a nitrogen-phosphorus detector.

RESULTS AND DISCUSSION

Figures 1 and 2 show the inhibitory activity of 2″-O-GIV, α-tocopherol and BHT toward MA formation from photo-

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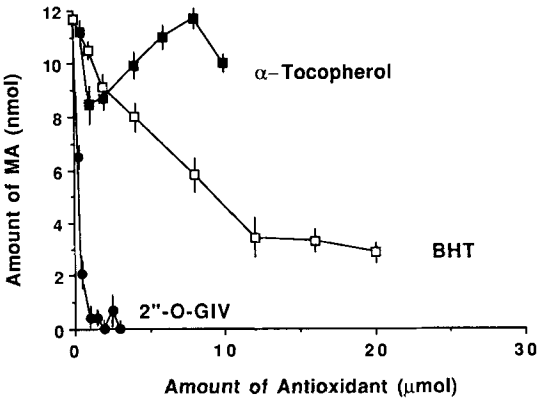

FIG. 1. Effect of 2″-O-glycosylisovitexin (2″-O-GIV), α-tocopherol or butylated hydroxytoluene (BHT) on formation of malonaldehyde (MA) from squalene upon ultraviolet irradiation.

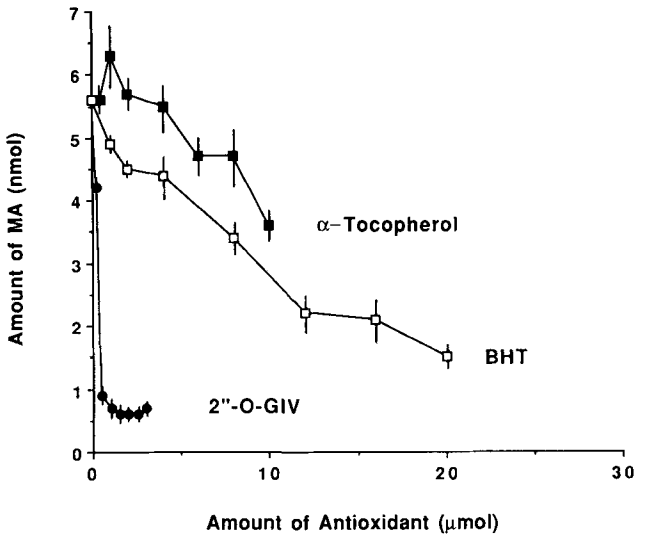

FIG. 2. Effect of 2″-O-glycosylisovitexin, α-tocopherol or butylated hydroxytoluene on formation of malonaldehyde from ethyl linoleate upon ultraviolet irradiation. Abbreviations as in Figure 1.

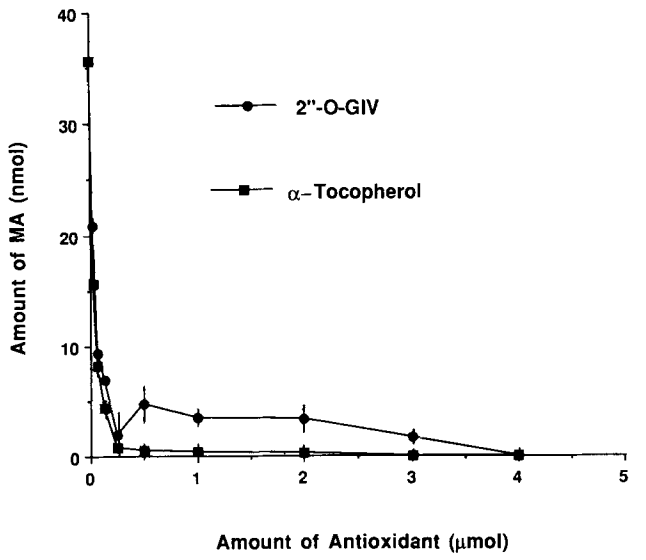

FIG. 3. Effect of 2″-O-glycosylisovitexin or α-tocopherol on malonaldehyde formation from ethyl linoleate oxidized by Fenton's reagent. Abbreviations as in Figure 1.

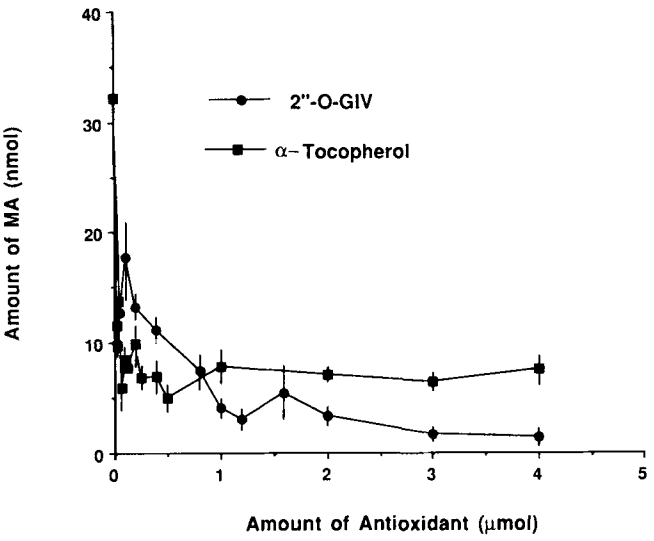

FIG. 4. Effect of 2″-O-glycosylisovitexin or α-tocopherol on malonaldehyde formation from ethyl linolenate oxidized by Fenton's reagent. Abbreviations as in Figure 1.

degradation of squalene and ethyl linoleate, respectively. The values are averaged from at least two replicate experiments. Great inhibition of MA formation was achieved by the addition of 2″-O-GIV. The addition of 2 μmol of 2″-O-GIV to 0.2 mmol of squalene inhibited the formation of MA by almost 100%. BHT also inhibited the formation of MA from squalene. However, it required 12 μmol of BHT to attain the minimum amount of 3.4 nmole MA from 0.2 mmol of squalene. 2″-O-GIV required only 1 μmol to inhibit 90% of MA formation from 0.2 mmol of ethyl linoleate, whereas the maximum inhibitions obtained by BHT (20 μmol) and α-tocopherol (10 μmol) were 60 and 20%, respectively.

BHT is one of the most effective synthetic antioxidants currently known. However, use of synthetic chemicals, particularly in foods, has begun to be restricted because of possible hazard to humans. For example, it has been reported that a statistically significant incidence of lung tumors was observed in female B6C33F_1 mice fed a diet containing 0.3% BHT (15). Therefore, an intensive search has been made for safer antioxidants from natural resources. α-Tocopherol, which is naturally present in cottonseed, sunflowerseed and rice (16), has been the most widely used natural antioxidant for foods and pharmaceutical products. However, α-tocopherol did not satisfactorily inhibit formation of MA in the present study. α-Tocopherol and 2″-O-GIV were irradiated alone in an ethanol solution at 37°C for 16 h (Figs. 1 and 2). The UV-irradiated α-tocopherol sample showed many products in thin-layer chromatography, suggesting that α-tocopherol was broken down by UV irradiation and became inactive. On the other hand, 2″-O-GIV was not degraded by UV irradiation (data not shown).

Figures 3, 4 and 5 show the antioxidative activity of 2″-O-GIV and α-tocopherol toward ethyl linoleate, ethyl

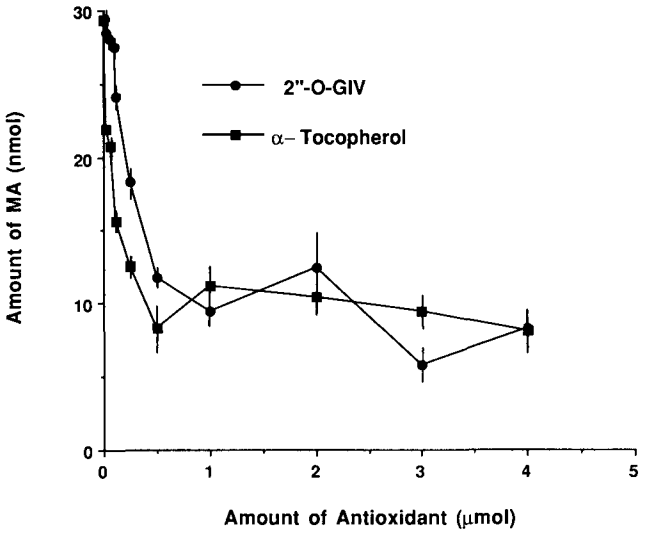

FIG. 5. Effect of 2″-O-glycosylisovitexin or α-tocopherol on malonaldehyde formation from ethyl arachidonate oxidized by Fenton's reagent. Figures as in Figure 1.

linolenate and ethyl arachidonate oxidized by Fenton's reagent, respectively. The values are averaged from at least two replicate experiments. 2″-O-GIV showed quite similar antioxidative activities to those of α-tocopherol. In the oxidation of ethyl linoleate (Fig. 3), the formation of MA was inhibited almost 100% in the presence of 4 μmol and 3 μmol of 2″-O-GIV and α-tocopherol, respectively. In the case of ethyl linolenate (Fig. 4), 2″-O-GIV showed dose response activity, and it inhibited nearly 95% of MA formation at the level of 4 μmol. α-Tocopherol required only 0.05 μmol to obtain maximum inhibition (85%), but its activity diminished slightly and leveled off with the presence of greater amounts of α-tocopherol. In the case of ethyl arachidonate (Fig. 5), the antioxidative activity of 2″-O-GIV and α-tocopherol was similar. Both compounds revealed maximum inhibition at doses between 0.5–1 μmol. These results indicate that the inhibitory activity of 2″-O-GIV toward MA formation from fatty acid ethyl esters oxidized by Fenton's reagent was similar to that of α-tocopherol.

It has been suggested that lipid peroxidation plays a key role in damaging biological processes resulting from excessive exposure to UV light (17). Moreover, aging of skin has been reportedly associated with peroxidation of skin lipids, such as squalene (18). It has been hypothesized that the toxicity associated with lipid peroxidation is mediated by the production of reactive carbonyl compounds, including MA (19). For example, MA is mutagenic in bacterial assays (2), and it has been shown to increase the incidence of skin cancers in experimental animals (3). As mentioned above, because lipid peroxidation has been known to cause many pathological complications, including aging, mutagenesis and carcinogenesis, use of an antioxidant for preventing occurrence of lipid peroxidation in biological systems should be investigated further.

The isoflavonoid investigated in the present study demonstrated significant antioxidative activity toward lipid peroxidation and, furthermore, can be obtained in large quantities from a natural source at low cost.

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