Free Radical Research, Volume 38 Number 8 (August 2004), pp. 869–876

The Inhibitory Effect of Luteolin-7-*O*-glucoside on the Formation of Pentyl and 7-Carboxyheptyl Radicals from 13-Hydroperoxy-9,11-octadecadienoic Acid in the Presence of Iron(II) Ions

HIDEO IWAHASHI*, KAYO AKATA, ATSUKO SUNAGA, YOSHIKO TONE, NORIKO YAMADA and KEIKO IIJIMA

Department of Chemistry, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8509, Japan

Accepted by Professor E. Niki

(Received 14 August 2003; In revised form 12 April 2004)

A flavone glucoside, luteolin-7-*O*-glucoside (luteolin-7-G) inhibited the formation of pentyl and 7-carboxyheptyl radicals in the reaction of 13-hydroperoxy-9,11-octadecadienoic (13-HPODE) acid with iron(II) ions. The inhibitory effect of luteolin-7-G was diminished in the presence of EDTA. These results indicated that the inhibitory effects of luteolin-7-G occur partly through the chelation of iron ions. Measurement of visible spectra also showed that luteolin-7-G chelates iron ions. On the other hand, luteolin-7-G did not inhibit the reaction under anaerobic conditions, suggesting that oxygen molecules participate in the inhibition. Oxygen consumption measurements showed that the luteolin-7-G/iron ion complexes react with oxygen molecules in competition with 13-HPODE acid, and free iron ions exclusively react with 13-HPODE acid. The reaction of luteolin-7-G/iron ion complexes with oxygen molecules possibly diminishes the formation of pentyl and 7-carboxyheptyl radicals.

Keywords: Lipid peroxidation; Luteolin; Antioxidant; Radicals; HPLC-ESR-MS

INTRODUCTION

Flavonoids are expected to be promising potential compounds for combating free radicals pathologies such as ischaemia, anaemia, arthritis, asbestosis, etc. Flavonoids occur widely in the plant kingdom, and are especially common in leaves, flowering tissues and pollens. They are also abundant in woody parts such as stems and barks. Humans consume substantial amounts of flavonoids in fruits, vegetables, herbs and beverages.[1–3] The average diet contains approximately 1 g/day of mixed flavonoids.[4] Thus, it is of interest to examine the influence of flavonoids on human health in view of their widespread occurrence in food products and the relatively large quantities consumed by virtually the entire human population.

Flavonoids are known to act as strong scavengers of superoxide radicals ($O_2^{\cdot -}$),[5,6] hydroxyl radicals[7] and peroxyl radicals.[8] Singlet oxygen ($^1O_2$) is quenched by flavonoids.[9] Flavonoids are also able to scavenge NO radicals[10] and peroxynitrite.[11] Several papers showed that flavonoids inhibit lipid peroxidation in iron-dependent lipid peroxidation systems.[12–16] However, details of the mechanism of the inhibitory effect of flavonoids on the lipid peroxidation in iron-dependent lipid peroxidation systems have not been clarified. We focus on the inhibitory effects of a flavone glucoside, luteolin-7-*O*-glucoside (luteolin-7-G) (Fig. 1) on the following reaction:

$$LOOH + Fe^{2+} \rightarrow OH^- + LO^{\cdot} + Fe^{3+} \qquad (1)$$

where LOOH and $LO^{\cdot}$ represent 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE) (or 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid) and 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radicals [or 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyloxyl radicals], respectively (Scheme 1).[17]

*Corresponding author. Tel.: +81-73-441-0772. Fax: +81-73-441-0772. E-mail: chem1@wakayama-med.ac.jp

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760410001722033

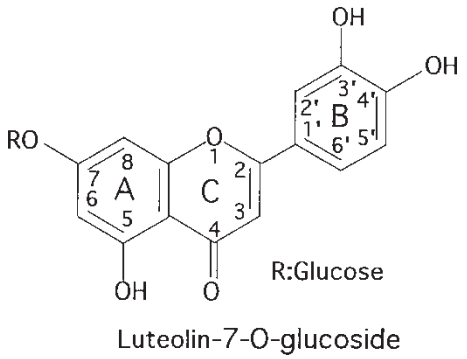
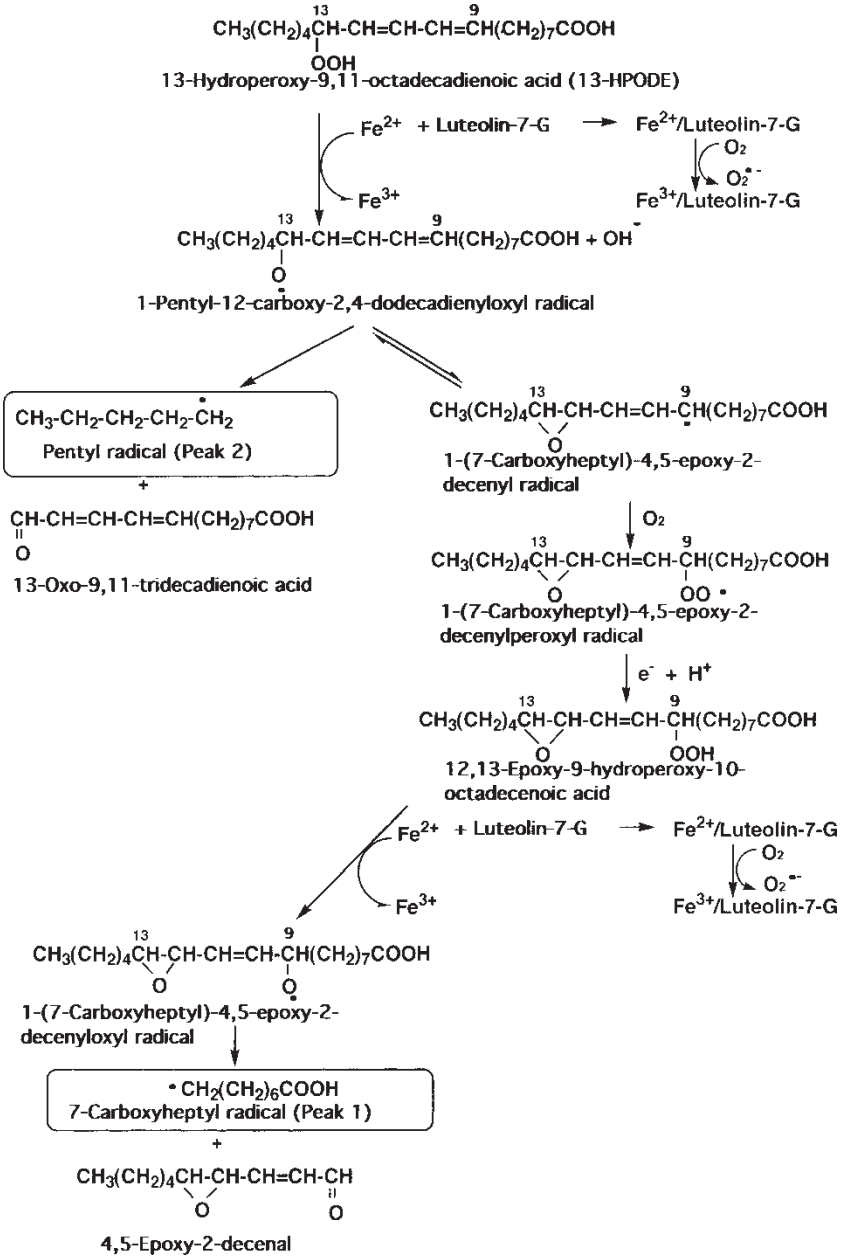


FIGURE 1 Structure of luteolin-7-*O*-glucoside. *R* is glucose.

MATERIALS AND METHODS

Materials

Luteolin-7-G was from Funakoshi Technical Services (Tokyo, Japan). α-(4-Pyridyl 1-oxide)-*N*-*tert*-butylnitrone (4-POBN) was purchased from Tokyo Kasei Kogyo, Ltd. (Tokyo, Japan). Ethylenediaminetetraacetic acid (EDTA) disodium salt and luteolin were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Soybean lipoxygenase (EC 1.13.11.12) Type V and linoleic acid were from Sigma Chemical Co. (St. Louis, MO, USA). Ammonium iron(II)



SCHEME 1 A possible mechanism for the inhibitory effect of luteolin-7-G on the formation of the 7-carboxyheptyl and pentyl radicals.

sulphate was obtained from Kishida Chemical Co. (Osaka, Japan). Glucose was purchased from Nakalai Tesque, Inc. (Kyoto, Japan). All other chemicals used were commercial products of the highest grade available.

Preparation of 13-HPODE Acid

The reaction mixture contained, in total volume of 25 ml, 1.5 mg/ml linoleic acid, 440 units/ml soybean lipoxygenase and 0.2 M boric acid (pH 9.0). The reaction was performed at 25°C under air for 1 h. The reaction mixture (25 ml) was acidified to pH 2.0 and extracted with 20 ml diethyl ether. The extracts were evaporated under reduced pressure and applied to the normal-phase HPLC.[19] The normal-phase HPLC used consisted of a model 7125 Rheodyne injector (Reodyne, Cotati, CA), a model Hitachi 655A-11 pump with a model L-5000 LC controller (Hitachi Ltd., Ibaragi, Japan) and a model SPD-M10AVP diode array detector (Shimadzu Co., Kyoto, Japan) with a model CLASS-LC10 LC workstation (Shimadzu Co.). The SPD-M10AVP diode array detector was operated from 200 to 350 nm in the HPLC system. The HPLC was performed on Zorbax SIL column (25.0 cm × 9.4 mm) (Du Pont Company, Wilmington, DE) with *n*-hexane-diethyl ether-acetic acid mixture (v/v, 1000:150:1) at 20°C. The flow rate was 3 ml/min. When the HPLC profile was monitored at 234 nm, four prominent peaks (P46, P56, P60 and P64) were observed at retention times of 46, 56, 60 and 64 min, respectively. Based on the paper by Teng and Smith,[19] we assigned the four peaks as follows: P46, 13-hydroperoxy-(9Z,11E)-octadeca-9,11-dienoic acid; P56, 13-hydroperoxy-(9E,11E)-octadeca-9,11-dienoic acid; P60, 9-hydroperoxy-(10E,12Z)-octadeca-10,12-dienoic acid; P64, 9-hydroperoxy-(10E,12E)-octadeca-10,12-dienoic acid. We collected the P46 and used it for the reaction. The concentration of 13-HPODE was determined from its absorbance at 234 nm ($\varepsilon = 25,600/\mathrm{cm/M}$).[20]

ESR Measurements

The ESR spectra were obtained using a model JES-FR30 Free Radical Monitor (JEOL Ltd., Tokyo, Japan). Samples were aspirated into a Teflon tube centred in a microwave cavity. Operating conditions of the ESR spectrometer were: power, 4 mW; modulation width, 0.1 mT; centre of magnetic field, 337.000 mT; sweep time, 4 min; sweep width, 10 mT; time constant, 0.3 s. Magnetic fields were calculated by the splitting of MnO ($\Delta H_{3-4} = 8.69$ mT).

HPLC-ESR and HPLC-ESR-MS Analyses

HPLC-ESR and HPLC-ESR-MS analyses were performed as described in the previous paper.[18]

HPLC-UV Analyses

The HPLC used in the HPLC-UV consisted of a model 7125 injector (Reodyne) with a 5 ml sample loop, a model 655A-11 pump with a model L-5000 LC controller (Hitachi Ltd.), and a Model SPD-M10AVP diode array detector with a Model CLASS-LC10LC workstation (Shimazu, Kyoto, Japan). A semi-preparative column ($30 \times 10\,\mathrm{mm}^2$ in diameter) packed with TSKgel ODS-120T (5 mm particle size) (Tosoh, Tokyo, Japan) was used. The column was kept at 20°C throughout the analyses. For the HPLC-UV analyses, two solvents were used: solvent A, 50 mM acetic acid; solvent B, 50 mM acetic acid/acetonitrile (20:80, v/v). A combination of isocratic and linear gradient was used for the analyses of 13-oxo-9,11-tridecadienoic acid: 0–5 min, 100% A (isocratic) at flow rate 2.0 ml/min; 5–50 min, 100–0% A (linear gradient) at flow rate 2.0 ml/min; 50–60 min, 100% B (isocratic) at flow rate 2.0 ml/min.

Control Reaction Mixture of 13-HPODE with Iron(II) Ions

The control reaction mixture of 13-HPODE with iron(II) ions contained 140 μM 13-HPODE, 67 μM $Fe(NH_4)_2(SO_4)_2$, 0.1 M α-(4-POBN), and 37 mM phosphate-buffered solution (pH 7.4). 4-POBN is a spin-trapping reagent. The reaction was started by adding $Fe(NH_4)_2(SO_4)_2$. The reaction was performed for 2 min at 25°C. Anaerobic conditions were obtained with 3VP-C rotary pump (Hitachi Co., Tokyo, Japan) in Thunberg tubes.

Visible Absorption Spectra

Visible absorption spectra were measured using a model UV-160A ultraviolet-visible spectrophotometer (Shimadzu Co.). The measurements were performed at 20°C.

Oxygen Consumption

Oxygen consumption studies were performed with a Clark-type electrode (Yellow Spring Instrument Co., Yellow Spring, OH). All of the components for the control reaction mixture of 13-HPODE with $Fe(NH_4)_2(SO_4)_2$ were put into the sample chamber except for $Fe(NH_4)_2(SO_4)_2$. After a delay of 2 min for equilibrium, the reaction was started by the addition of $Fe(NH_4)_2(SO_4)_2$ at 20°C. For the purpose of calculation, the reaction mixture was assumed to contain the same amount of dissolved oxygen as pure water (281 μM at 20°C).

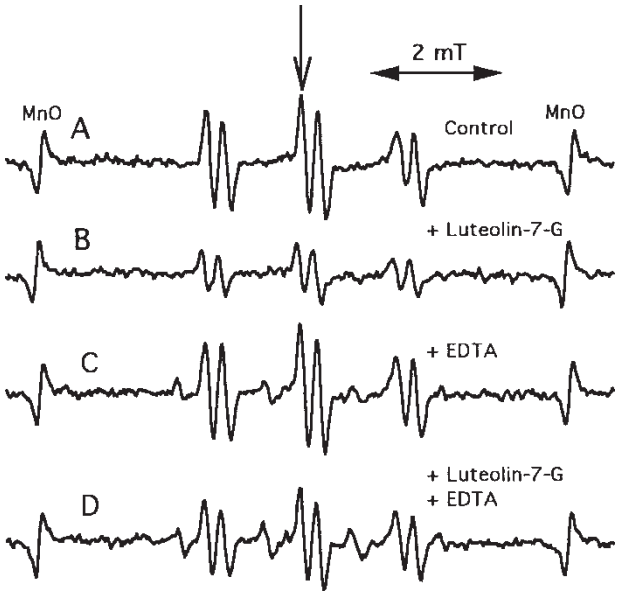


FIGURE 2 ESR spectra of the reaction of 13-HPODE with iron(II) ions in the presence of luteolin-7-G. ESR and reaction conditions were as described in the "Materials and methods". (A) Control reaction mixture; (B) control reaction mixture with luteolin-7-G (180 μM); (C) control reaction mixture with EDTA (330 μM); (D) control reaction mixture with luteolin-7-G (180 μM) and EDTA (330 μM).

RESULTS

ESR Measurements

An ESR spectrum ($a^N = 1.58$ mT and $a^H\beta = 0.26$ mT) was observed in the control reaction mixture of 13-HPODE with iron(II) ions (Fig. 2A). The ESR spectrum was hardly observed in the control reaction mixture without iron(II) ions (or 13-HPODE) (data not shown). On addition of 180 μM luteolin-7-G to the control reaction mixture, the ESR peak height decreased to 45% (±7%, $n = 3$) of the control reaction mixture (Fig. 2B), suggesting that luteolin-7-G inhibits the formation of the 13-HPODE-derived radicals. To establish whether or not chelation of iron ions is essential to the inhibitory effects, 330 μM EDTA was added to the control reaction mixture (Fig. 2C). On addition of the EDTA to the control reaction mixture, the ESR peak heights increased to 120% (±10%, $n = 3$) of the control reaction mixture. Addition of the luteolin-7-G into the control reaction mixture with the EDTA resulted in less effect on the ESR peak height [75% (±8%, $n = 3$) of the control mixture with the EDTA] (Fig. 2D). These results indicated that luteolin-7-G inhibits the formation of the 13-HPODE-derived radicals partly through the chelation of iron ions in the reaction mixture.

To know which moiety of luteolin-7-G is essential for the inhibitory effect, inhibitory effects of luteolin (or glucose) was examined. On addition of 180 μM luteolin to the control reaction mixture, the ESR peak heights decreased to 42% (±3%, $n = 3$) of the control reaction mixture. On the other hand, on addition of 180 μM glucose to the control reaction mixture, the ESR peak heights remained unchanged [97% (±10%, $n = 3$) of the control mixture]. The above results show that luteolin moiety is essential for the inhibitory effect.

UV/Visible Absorption Spectra of the Solutions Containing Luteolin-7-G and Iron(II) Ions

In order to discern whether or not iron(II) ions interact with luteolin-7-G, UV/visible absorption spectra of the solutions containing luteolin-7-G and iron(II) ions were measured (Fig. 3). The solution,

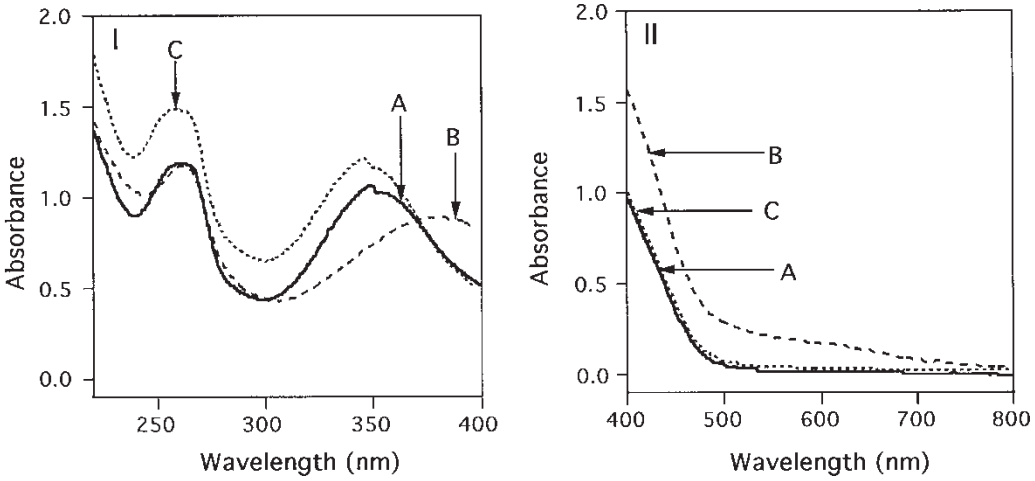


FIGURE 3 UV/visible absorption spectra of the solutions containing iron(II) ions and luteolin-7-G. UV/visible absorption spectra were observed for the solutions of iron(II) ions and luteolin-7-G in the following ranges: (I) 220–400 nm, (II) 400–800 nm. (I-A) 90 μM luteolin-7-G in 37 mM phosphate-buffered solution (pH 7.4); (I-B) a mixture of 90 μM luteolin-7-G and 33 μM $Fe(NH_4)_2(SO_4)_2$ in 37 mM phosphate-buffered solution (pH 7.4); (I-C) a mixture of 90 μM luteolin-7-G, 33 μM $Fe(NH_4)_2(SO_4)_2$ and 165 μM EDTA in 37 mM phosphate-buffered solution (pH 7.4); (II-A) 180 μM luteolin-7-G in 37 mM phosphate-buffered solution (pH 7.4); (II-B) a mixture of 180 μM luteolin-7-G and 67 μM $Fe(NH_4)_2(SO_4)_2$ in 37 mM phosphate-buffered (pH 7.4); (II-C) a mixture of 180 μM luteolin-7-G, 67 μM $Fe(NH_4)_2(SO_4)_2$ and 330 μM EDTA in 37 mM phosphate-buffered solution (pH 7.4).

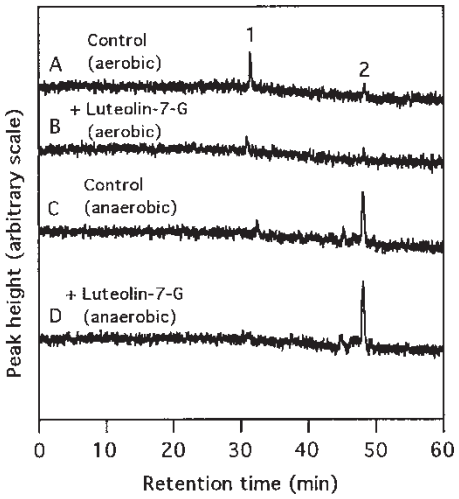


FIGURE 4 The HPLC-ESR analysis of the control reaction mixture of 13-HPODE with iron(II) ions. Reaction mixtures (0.9 ml) were applied to the HPLC-ESR. (A) Control reaction mixture under aerobic conditions, (B) control reaction mixture with 180 μM luteolin-7-G under aerobic conditions, (C) control reaction mixture under anaerobic conditions and (D) control reaction mixture with 180 μM luteolin-7-G under anaerobic conditions.

which contained both luteolin-7-G and $Fe(NH_4)_2(SO_4)_2$ in phosphate-buffered solution (pH 7.4), showed an absorption spectrum with λ_{maxs} of 260, 382 and around 600 nm (Fig. 3, I-B and II-B). The solution containing only luteolin-7-G in phosphate-buffered solution (pH 7.4) showed an absorption spectrum with λ_{maxs} of 260 and 350 nm (Fig. 3, I-A and II-A). When 330 μM EDTA, which is a strong chelating agent of iron(II) ions, was added to the solution containing both iron(II) ions and luteolin-7-G, an absorption spectrum reverted to the one of luteolin-7-G alone (Fig. 3, I-C and II-C). These results suggested that the luteolin-7-G/iron ion complexes form in the solution.

HPLC-ESR and HPLC-ESR-MS Analyses

In order to probe the effect of luteolin-7-G on the formation of respective 13-HPODE-derived radicals, the HPLC-ESR analyses were performed. On the HPLC-ESR elution profile of the control reaction mixture, two peaks were observed at the retention times of 31.0 ± 0.3 min ($n = 3$) (peak 1) and 48.2 ± 0.1 min ($n = 3$) (peak 2), respectively (Fig. 4A). In order to identify peaks 1 and 2, the HPLC-ESR-MS analyses were performed (Fig. 5). The HPLC-ESR-MS analysis of peak 1 compound gave ions at m/z 251 and 338 (Fig. 5A). The ion m/z 338 corresponds to the protonated molecular ions of the 4-POBN/7-carboxyheptyl radical adducts, $[M + H]^+$. A fragment ion at m/z 251 corresponds to the loss of $[(CH_3)_3C(O)N]$ from the protonated molecular ion. The HPLC-ESR-MS analysis of peak 2 gave ions at m/z 179 and 266 (Fig. 5B). The ion m/z 266 corresponds to the protonated molecular ion of the 4-POBN/pentyl radical adducts, $[M + H]^+$. A fragment ion at m/z 179 corresponds to the loss of $[(CH_3)_3C(O)N]$ from the protonated molecular ion. Thus, the two peaks are assigned as follows: peak 1, 4-POBN/7-carboxyheptyl radical adducts; peak 2, 4-POBN/pentyl radical adducts.

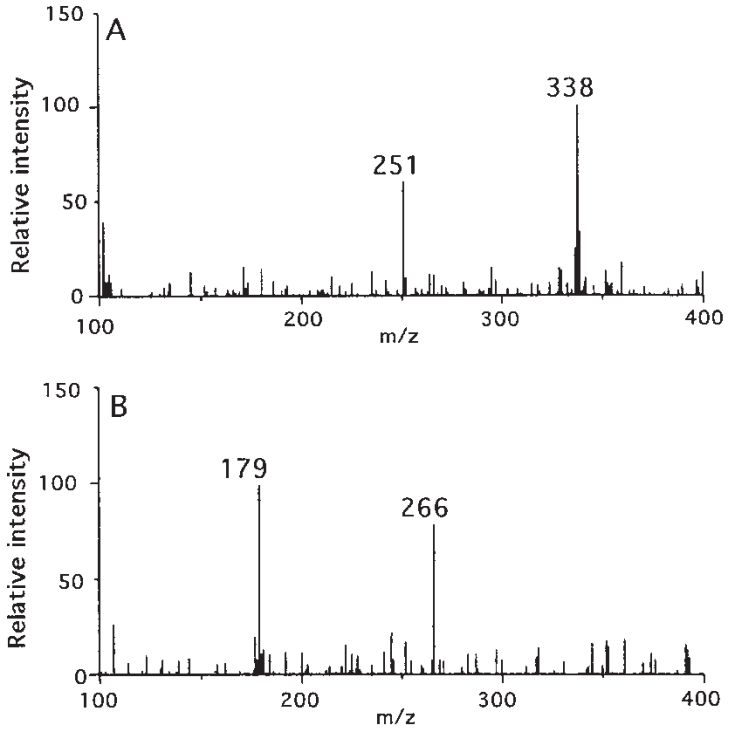


FIGURE 5 The HPLC-ESR-MS analysis of the control reaction mixture. (A) A mass spectrum of peak 1, (B) a mass spectrum of peak 2.

Under aerobic conditions, peak 1 was predominant (Fig. 4A). On the other hand, peak 2 became predominant under anaerobic conditions (Fig. 4C). Since oxygen molecules participate in the formation of 7-carboxyheptyl radicals from 13-HPODE (Scheme 1),[17] the formation of pentyl radicals becomes predominant in the reaction mixture under anaerobic conditions.

On addition of luteolin-7-G to the control reaction mixture under aerobic conditions, the peak height of peak 1 decreased to 44% ($\pm$ 13%, $n = 3$) of the control reaction mixture (Fig. 4B). On the other hand, on addition of luteolin-7-G to the control reaction mixture under anaerobic conditions, the peak height of 4-POBN/pentyl radical adducts (peak 2) remained unchanged [113% ($\pm$ 19%, $n = 3$) of the control reaction mixture] (Fig. 4D).

Detection of 13-Oxo-9,11,tridecadienoic Acid

13-Oxo-9,11-tridecadienoic acid and pentyl radicals form from 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radicals simultaneously (Scheme 1). In order to detect 13-oxo-9,11-tridecadienoic acid, HPLC-UV analyses were performed for the reaction mixture of 13-HPODE (140 μM) with Fe^{2+} (67 μM). A prominent peak was observed at the retention time of 50.2 min on the HPLC-UV analysis (280 nm) of the reaction under anaerobic conditions (Fig. 6B). An UV spectrum of the peak showed a λ_{max} of 279 nm. Mass spectrum measurement of the peak gave two prominent ions, m/z 225 and 207. The ion, m/z 225 corresponds to the protonated molecular ions of the 13-oxo-9,11-tridecadienoic acid, $[M + H]^+$. A fragment ion at m/z 207 corresponds to the loss of $[H_2O]$ from the protonated molecular ion.

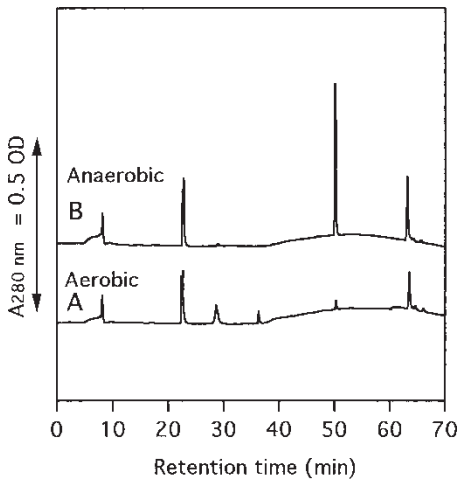


FIGURE 6 HPLC-UV analyses of the reaction mixture of 13-HPODE with ferrous ions. The reaction mixture contained 13-HPODE (140 μM) and Fe^{2+} (67 μM). The reaction was performed for 2 min at 25°C. The reactions were performed under aerobic condition (A) and anaerobic condition (B). Five hundred microlitres of the reaction mixture was applied to the HPLC-UV. UV detector was set at 280 nm.

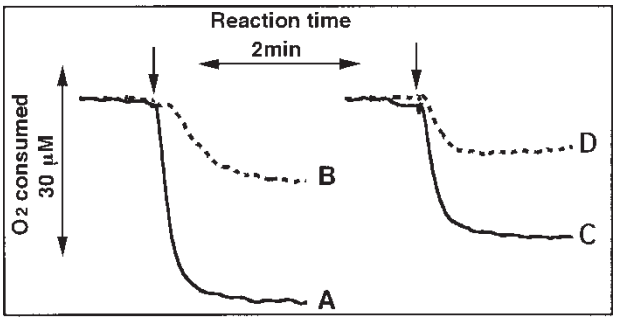


FIGURE 7 Oxygen consumptions in the reaction mixtures of 13-HPODE with $Fe(NH_4)_2(SO_4)_2$. The $Fe(NH_4)_2(SO_4)_2$ was added at the respective times indicated by arrows. (A) Control reaction mixture, (B) control reaction mixture without 13-HPODE, (C) control reaction mixture in the presence of 180 μM luteolin-7-G and (D) control reaction mixture without 13-HPODE in the presence of 180 μM luteolin-7-G.

When the reaction was performed under aerobic conditions, the HPLC-UV peak height decreased to 11% of the one observed for the reaction under anaerobic conditions (Fig. 6A). This result supports Scheme 1.

Oxygen Consumption

In order to investigate the role of oxygen molecules in the inhibition, oxygen consumption was measured (Fig. 7). When $Fe(NH_4)_2(SO_4)_2$ was added to the control reaction mixture, oxygen molecules were consumed to a great extent (Fig. 7A). The results are consistent with Scheme 1. On the other hand, addition of $Fe(NH_4)_2(SO_4)_2$ to the control reaction mixture with luteolin-7-G resulted in less oxygen consumption (Fig. 7C). These results indicated that luteolin-7-G inhibits oxygen consumption in the control reaction mixture of 13-HPODE with iron(II) ions.

When $Fe(NH_4)_2(SO_4)_2$ was added to the control reaction mixture without 13-HPODE in the presence of luteolin-7-G, a rapid oxygen consumption was observed (Fig. 7D). On the other hand, a slow oxygen consumption was observed in the control reaction mixture without 13-HPODE in the absence of luteolin-7-G (Fig. 7B).

DISCUSSION

Luteolin-7-G inhibited formation of the 7-carboxyheptyl radical in the control reaction mixture of 13-HPODE with iron(II) ions under aerobic conditions (Fig. 4B). UV/visible spectra of the solution containing iron(II) ions and luteolin-7-G clearly showed the formation of the luteolin-7-G/iron ion complexes (Fig. 3). Because luteolin-7-G did not inhibit the radical formation in the presence of EDTA (Fig. 2D), the formation of the luteolin-7-G/iron ion complexes are essential for the inhibitory effects of luteolin-7-G.

Luteolin-7-G possibly inhibits the reaction (1), i.e. the reaction between 13-HPODE acid and 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radicals [or between 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid and 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyloxyl radicals] because iron(II) ions participate in the reaction (Scheme 1). The catechol in luteolin-7-G appears to be essential for the chelation of iron ions. The catechol seems to chelate iron ions through the two hydroxyl groups. Indeed, *o*-benzenediol inhibits the formation of 13-HPODE-derived radicals.[17] Thus, the catechol in luteolin-7-G seems to be one of the important factors for the inhibition. On the other hand, Ferrali *et al.* showed that both the hydroxyl (in C_3) and the carbonyl (in C_4) group of the C ring (Fig. 1) are necessary to bind iron ions in the other flavonoids such as quercetin and kaempferol.[16]

On addition of iron(II) ions to the phosphate-buffered solution without luteolin-7-G, reaction (2) occurs with a slower oxygen consumption (Fig. 7B) compared with reactions (1.1)–(1.3) (Fig. 7A):

$$\mathrm{Fe^{2+}/phosphate\ ions} + \mathrm{O_2} \rightarrow \mathrm{Fe^{3+}/phosphate\ ions} + \mathrm{O_2^{\cdot -}} \quad (2)$$

$$\mathrm{LOOH} + \mathrm{Fe^{2+}} \rightarrow \mathrm{OH^-} + \mathrm{LO^{\cdot}} + \mathrm{Fe^{3+}} \quad (1.1)$$

$$\mathrm{LO^{\cdot}} \rightarrow \mathrm{epoxyL^{\cdot}} \quad (1.2)$$

$$\mathrm{epoxyL^{\cdot}} + \mathrm{O_2} \rightarrow \mathrm{epoxyLOO^{\cdot}} \quad (1.3)$$

where LOOH, $LO^{\cdot}$, $epoxyL^{\cdot}$ and $epoxyLOO^{\cdot}$ are 13-HPODE acid, 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radicals, 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyl radicals, 1-(7-carboxyheptyl)-4,5-epoxy-2-decenylperoxyl radicals, respectively. Thus, iron(II) ions exclusively react with LOOH in the reaction mixture containing both LOOH and O_2 in the absence of luteolin-7-G.

On the other hand, on addition of iron(II) ions to the phosphate-buffered solution with luteolin-7-G, reaction (3) occurs with a comparable oxygen consumption rate as reactions (1.1)–(1.3) (Fig. 6D):

$$\mathrm{Fe^{2+}/luteolin-7-G} + \mathrm{O_2} \rightarrow \mathrm{Fe^{3+}/luteolin-7-G} + \mathrm{O_2^{\cdot -}} \quad (6)$$

where Fe^{2+}/luteolin-7-G (or Fe^{3+}/luteolin-7-G) is a luteolin-7-G/Fe^{2+} (or a luteolin-7-G/Fe^{3+}) complex. Reactions (1.1)–(1.3) possibly proceeds in competition with reaction (3) in the reaction mixture containing both LOOH and O_2 in the presence of luteolin-7-G. Thus, formation of the 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical from 13-HPODE [or formation of the 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyloxyl radical from 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid] is possibly inhibited in the presence of luteolin-7-G under aerobic conditions. A possible mechanism of the inhibitory effect of luteolin-7-G on the formation of the 7-carboxyheptyl radical (or the pentyl radical) is shown in Scheme 1. Luteolin-7-G did not inhibit the pentyl radical formation under anaerobic conditions (Fig. 4D), supporting the above mechanism.

The mechanism described here is applicable only to the iron-dependent lipid peroxidation systems. Flavonoids inhibit the lipid peroxidation even in the iron-independent lipid peroxidation systems[8] where flavonoids seems to act as H-atom donors to the peroxy radical.

References

[1] Herrmann, K. (1976) "Flavonols and flavones in food plants: a review", *J. Food Technol.* **11**, 433–448.
[2] Hertog, M.G.L., Hollman, P.C.H. and Katan, M.B. (1992) "Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands", *J. Agric. Food Chem.* **40**, 2379–2383.
[3] Hertog, M.G.L., Hollman, P.C.H., Katan, M.B. and Kromhout, D. (1993) "Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands", *Nutr. Cancer* **20**, 21–29.
[4] Kuhnau, J. (1976) "The flavonoids. A class of semi-essential food components: their role in human nutrition", *World Rev. Nutr. Diet* **24**, 117–191.
[5] Baumann, J., Wurm, G. and Bruchhausen, F.V. (1980) "Hemmung der prostaglandinsynthetase durch flavonoide und phenolderivate im vergleich mit deren O_2^- radikalfangereigenschaften", *Arch. Pharm.* **313**, 330–336.
[6] Bors, W., Heller, W., Michel, C. and Saran, M. (1990) "Flavonoids as antioxidants: determination of radical-scavenging efficiencies", *Methods Enzymol.* **186**, 343–355.
[7] Husain, S.R., Cillard, J. and Cillard, P. (1987) "Hydroxyl radical scavenging activity of flavonoids", *Phytochemistry* **26**, 2489–2491.
[8] Torel, J., Cillard, J. and Cillard, P. (1986) "Antioxidant activity of flavonoids and reactivity with peroxy radical", *Phytochemistry* **25**, 383–385.
[9] Sorata, Y., Takahama, U. and Kimura, M. (1984) "Protective effect of quercetin and rutin on photosensitized lysis of human erythrocytes in the presence of hematoporphyrin", *Biochim. Biophys. Acta* **799**, 313–317.
[10] Krol, W., Czuba, Z.P., Threadgill, M.D., Cunningham, B.D.M. and Pietsz, G. (1995) "Inhibition of nitric oxide ($NO^{\cdot}$) production in murine macrophages by flavones", *Biochem. Pharmacol.* **50**, 1031–1035.
[11] Ohshima, H., Yoshie, Y., Auriol, S. and Gilibert, I. (1998) "Antioxidant and pro-oxidant actions of flavonoids: effects on DNA damage induced by nitric oxide, peroxynitrite and nitroxyl anion", *Free Radic. Biol. Med.* **25**, 1057–1065.
[12] Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A. and Potapovitch, A.I. (1989) "Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation", *Biochem. Pharmacol.* **38**, 1763–1769.
[13] De Whalley, C.V., Rankin, S.M., Hoult, J.R.S., Jessup, W. and Leake, D.S. (1990) "Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages", *Biochem. Pharmacol.* **39**, 1743–1750.
[14] Kozlov, A.B., Ostrachovitch, E.A. and Afanas'ev, I.B. (1994) "Mechanism of inhibitory effects of chelating drugs on lipid peroxidation in rat brain homogenates", *Biochem. Pharmacol.* **47**, 795–799.
[15] Yoshino, M. and Murakami, K. (1998) "Interaction of iron with polyphenolic compounds: application to antioxidant characterization", *Anal. Biochem.* **257**, 40–44.
[16] Ferrali, M., Signorini, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D. and Comporti, M. (1997) "Protection against

oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity", *FEBS Lett.* **416**, 123–129.
[17] Iwahashi, H. (2000) "Some polyphenols inhibit the formation of pentyl radical and octanoic acid radical in the reaction mixture of linoleic acid hydroperoxide with ferrous ions", *Biochem. J.* **346**, 265–273.
[18] Iwahashi, H., Nishizaki, K. and Takagi, I. (2002) "Cytochrome c catalyses the formation of pentyl radical and octanoic acid radical from linoleic acid hydroperoxide", *Biochem. J.* **361**, 57–66.
[19] Teng, J.I. and Smith, L.L. (1985) "High-performance liquid chromatography of linoleic acid hydroperoxides and their corresponding alcohol derivatives", *J. Chromatogr.* **350**, 445–451.
[20] Lindstrom, T.D. and Aust, S.D. (1984) "Studies on cytochrome P-450-dependent lipid hydroperoxide reduction", *Arch. Biochem. Biophys.* **233**, 80–87.