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Inhibition of UVA and UVB Radiation-Induced Lipid Oxidation by Quercetin

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The flavonol quercetin is believed to provide protection against ultraviolet (UV)-induced damage to plants. As part of our investigations into the potential for quercetin to protect skin against UV-induced damage, we have measured the ability of quercetin to inhibit UV-induced lipid peroxidation in vitro in liposomes. Quercetin, which absorbs UV radiation at 255 and 365 nm, was determined to be a stronger inhibitor of lipid oxidation induced by UVB (3.7 radicals scavenged per molecule) than by lipid oxidation induced by UVA (1.9 radicals scavenged per molecule). The values for inhibition of UVB-induced lipid oxidation by quercetin are comparable to those when 2,2′-azobis(2-amidinopropane) was used as an oxidizing system (four radicals scavenged per molecule). The protective effect of quercetin appears to be mainly the result of scavenging of UV-generated radical species, although this may be decreased slightly in the UVA as a result of its absorption at 365 nm.

KEYWORDS: Quercetin; flavonol; antioxidant; sunscreen; ultraviolet radiation

INTRODUCTION

Ultraviolet (UV) radiation is known to have deleterious effects on cells, including damage to DNA, either directly (*1*) or via reactive oxygen species (*2*, *3*). In addition, UV radiation can affect cellular signaling (*4*), resulting in effects such as photoaging, immunosuppression (*5*), and damage to lipid membranes via reactive oxygen species-mediated processes (*6*).

Reactive oxygen species can be formed through absorption of UVA or UVB radiation by chromophores in the skin. These chromophores include NADH/NADPH, tryptophan, riboflavin (*7*), advanced glycation end products on cellular and extracellular proteins (*8*), unmodified extracellular matrix proteins (*9*), bilirubin, and various quinones (*10*). Absorption of UV radiation by the skin leads to excited state chromophores, which can transfer their excitation energy through an intermediate in a type I photoreaction or directly to molecular triplet oxygen in a type II photoreaction (*11*), resulting in the formation of reactive oxygen species such as superoxide ion ($O_2^{\bullet -}$) or singlet oxygen ($^1O_2$) (*7*).

Plant biosynthesis of the flavonol quercetin (**Figure 1**) and its glycosides appears to be photoprotective (*12*), as UV radiation causes increased quercetin biosynthesis in a number of plant species including *Vicia faba* (fava beans) (*12*) and *Brassica napus* (canola) (*13*). Quercetin could also serve as a skin photoprotectant in animal systems, as topically applied quercetin inhibited a UV radiation-induced increase in myeloperoxidase activity and reduction of glutathione levels in a HRS/J mouse model (*14*).

The precise mechanism of quercetin's protective effect, however, remains unclear. One possible mechanism for quercetin's photoprotective properties is the scavenging of reactive oxygen species, preventing oxidative damage to DNA and lipid peroxidation. Quercetin has been found to reduce cell death due to oxidative stress caused by buthionine sulfoximine in a concentration-dependent manner (*15*) and to scavenge free radicals produced by H_2O_2 (*16*), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) (*17*), and 2,2′-azobis (2,4-dimethylvaleronitrile) (AMVN) (*18*). Another possible photoprotective mechanism would be direct absorption of UV radiation [λ_{max} (CH_3OH) = 365 nm (UVA) and λ_{max} (CH_3OH) = 255 nm (UVC)], thus preventing UV radiation-induced formation of reactive oxygen species and direct DNA damage. The energy from this absorption could be dissipated as heat, light (*19*), or through decomposition of quercetin (*20*).

At present, it is unclear if the photoprotective effect of quercetin in plants is due to radical scavenging, UV radiation absorption, or a combination of these mechanisms (*13*, *21*−*23*). The purpose of this study is to determine the contribution from antioxidant properties and UV absorption of quercetin to UV radiation-induced lipid oxidation. To accomplish this goal, the relative ability of quercetin to prevent lipid oxidation in a model in vitro liposome system initiated by UVA, UVB, or AAPH-induced lipid oxidation was determined, and the photostability of quercetin to UVA and UVB radiation over the time of the lipid oxidation experiments was determined.

MATERIALS AND METHODS

Materials. Quercetin, butylated hydroxytoluene (BHT), and Tris-HCl were purchased from Sigma (St. Louis, MO), 2,2′-azobis(2-amidinopropane) (AAPH) was from Monomer-Polymer & Dajac Laboratories, Inc. (Feasterville, PA), and 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All solvents were high-performance liquid chromatography (HPLC) grade. Water was purified using a Millipore Super Q water system with

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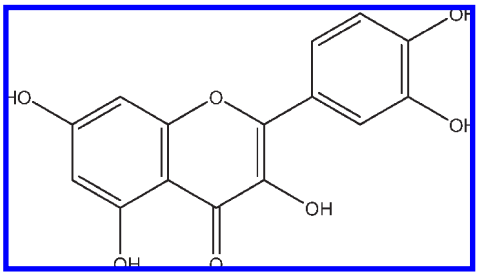

Figure 1. Structure of quercetin.

one carbon cartridge followed by two ion exchange cartridges (Bedford, MA). UV spectra were recorded using quartz cuvettes (VWR Canada) on an Agilent 8453 UV/vis photodiode array spectrophotometer with ChemStation software (Agilent Technologies Canada Inc., Mississauga, Canada).

Liposome Preparation. To prepare liposomes for determination of the antioxidant capacity of BHT in DLPC liposomes, 500 μL of DLPC in chloroform (1 mg/mL) and 15 μL of 500 μM BHT in acetonitrile were placed in a 5 mL culture tube and evaporated to dryness under a stream of N_2. The resulting residue was redissolved in 600 μL of 10 mM Tris-HCl buffer (pH 7.0) preheated to 50 °C and vortex mixed for 1 min. The liposome−BHT mixture was passed through a Hamilton extruder 17 times and transferred to a 2.5 mL screw top vial to which was added 800 μL of 10 mM Tris-HCl buffer (pH 7.0) preheated to 50 °C. The final concentration of DLPC was 6.4 μM.

AAPH-Induced Lipid Peroxidation. Oxidation was initiated with 200 μL of 0.75 M AAPH in water, and the tube was covered and placed in a heating block at 50 °C. The final concentrations of BHT and AAPH were 5.0 μM and 100 mM, respectively. Thirty microliter aliquots of sample were withdrawn and placed in 970 μL of cold methanol and placed on ice for later analysis every 30 s from $t = 0$ min to $t = 10$ min and then every 60 s from $t = 10$ min to $t = 25$ min. The absorbance of samples was read at 240 nm, and absorbance readings were plotted against time. A wavelength of 240 nm was used instead of the typical 232 nm as conjugated dienes showed optimal absorbance at this wavelength in our system (*24*). The lag time in the onset of oxidation of DLPC (and exhaustion of antioxidant capacity) was determined from the inflection point of the resultant graph. Determination of the lag time of quercetin-inhibited DLPC liposome oxidation by AAPH was performed in the same manner as for BHT except using 30 μL of 250 μM quercetin in methanol for a final concentration of 5.0 μM quercetin and 100 mM AAPH. Results presented are the summary of six replicates.

UV Radiation-Induced Lipid Peroxidation. Antioxidant stoichiometry of quercetin against UVB and UVA oxidation was determined as above for AAPH-induced oxidation of DLPC liposomes with the modification that AAPH was not added to the reaction mixture and oxidation was initiated by exposure to two FS20T12/UVB lamps (National Biological Corp., Beachwood, OH) filtered to remove UVC with an intensity of 1303 μW cm^{-2} at 310 nm as measured with a UVP UVX-31 sensor or two F20T12/BL/HO UVA lamps (National Biological Corp.) filtered to remove UVC with an intensity of 740 μW cm^{-2} at 365 nm as measured with a UVP UVX-36 sensor. Results presented are the summary of six replicates.

Calculation of Stoichiometric Ratio. The antioxidant stoichiometry of quercetin was calculated using eq 1 (*24*).

$$n = R_i\tau/[\mathrm{ArOH}] \quad (1)$$

where n is the stoichiometric ratio, R_i (μM min^{-1}) is the rate of chain initiation, [ArOH] (μM) is the concentration of antioxidant, and τ (min) is the lag time in the onset of oxidation with antioxidant present. Using a value of $n = 2$ (*24*) for BHT, R_i was calculated for the system using the BHT lag time, and an n value for quercetin was calculated using the quercetin lag time.

HPLC. HPLC analysis was carried out at room temperature on a Waters 2695 Alliance system using a Waters 2996 photodiode array detector set at 365 nm. Aliquots of 50 μL were injected onto a 250 mm × 4.6 mm Allsphere ODS-2 column, 5 μm particle size (Alltech, Calgary, AB). Data were processed using Empower software (Waters, Milford, MA). Elution was carried out in gradient mode using two components: A = 0.1% formic acid in water and B = 0.1% formic acid in methanol. The gradient was as follows: 0−10 min, linear gradient from 90 to 40% A; 10−25 min, isocratic 40% A; 25−28 min, linear gradient from 40 to 90% A; and 28−30 min, isocratic 90% A. The flow rate was 1.2 mL/min. Irradiated quercetin samples ($n = 3$) were volume corrected, and HPLC peak height and area were compared to unirradiated quercetin to determine loss.

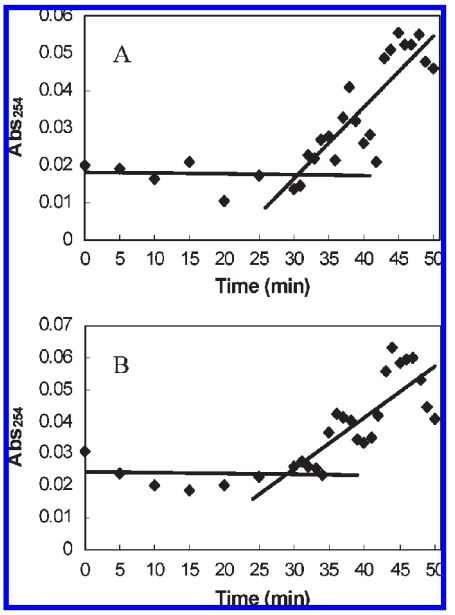

Figure 2. UVA (740 μW cm^{-2}, 365 nm)-induced oxidation of DLPC liposomes (6.4 μM) in 10 mM Tris-HCl buffer (pH 7.0) measured by formation of conjugated dienes absorbing at 240 nm. (**A**) Five micromolar BHT. (**B**) Five micromolar quercetin.

Statistics. Significant differences in stoichiometric ratios were assessed with one-way analysis of variance with Tukey's test for pairwise multiple comparisons using Prism 4 (GraphPad software, San Diego, CA). The level of significance was set at $P < 0.05$.

RESULTS

Stoichiometry—Quercetin AAPH Standardized vs BHT. The stoichiometric ratio for quercetin (5.0 μM) against AAPH-induced oxidation of DLPC liposomes was determined to be 4.26 ± 0.31 (standardized to 5.0 μM BHT, $n = 2$, and R_i = 3.07 μM min^{-1}).

Stoichiometry—Quercetin UVA and UVB. The ability of quercetin to inhibit UV radiation-induced oxidation of DLPC liposomes was determined by exposing liposomes to either UVA (365 nm) or UVB (310 nm) radiation. The reaction stoichiometry was standardized to 5.0 μM BHT ($n = 2$) resulting in an R_i (UVA) = 0.24 μM min^{-1} and an R_i (UVB) = 1.33 μM min^{-1}. **Figure 2** shows a representative example of the lag times (min), which are indicative of BHT- (**Figure 2A**) and quercetin- (**Figure 2B**) mediated inhibition of UVA-induced oxidation of DLPC liposomes. **Figure 3** shows a representative example of the lag times (min) for BHT- (**Figure 3A**) and quercetin- (**Figure 3B**) mediated inhibition UVB-induced oxidation of DLPC liposomes. The stoichiometric ratios for inhibition of UV-induced DLPC oxidation with quercetin as determined using the lag times and eq 1 were 1.93 ± 0.21 (UVA) and 3.73 ± 0.35 (UVB), where the values were significantly different ($P < 0.05$).

Stability of Quercetin to UVA and UVB Radiation. Quercetin was irradiated with UVA or UVB radiation in 10 mM Tris-HCl buffer (pH 7.0) for 60 min (at least two lag times) in the absence of DLPC liposomes, and HPLC-UV analysis (**Figure 4**) of the reaction mixture showed that quercetin was stable to our UV

conditions over the length of the experiment with less than 1% loss of quercetin.

DISCUSSION

Recently, quercetin has come under study for its possible protective effects against UV radiation-induced skin damage (*14*). There are several mechanisms through which quercetin may provide protection against UV radiation, including absorbance of UV radiation (*14*), antioxidant activity (*15*), and interaction with signal transduction pathways (*4*). This study investigated the ability of quercetin in an in vitro system to inhibit lipid oxidation induced by UVA and UVB radiation. The antioxidant stoichiometry for quercetin was also determined using a thermal radical initiator (AAPH) for comparison. BHT ($n = 2$) was used to standardize the radical scavenging reaction for all systems, allowing for determination of the antioxidant stoichiometry of quercetin (*24*). BHT was stable to UVA/UVB radiation for the duration of the lipid oxidation experiments, and the calculated R_i values were consistent with control UVA/UVB radiation-mediated lipid oxidation (data not shown). UVA radiation produced a less rapid rate of lipid oxidation than did UVB radiation; however, both UVA and UVB lipid oxidation systems were standardized to BHT, so the stoichiometric ratio for inhibition of lipid oxidation by quercetin is relative to BHT for both systems.

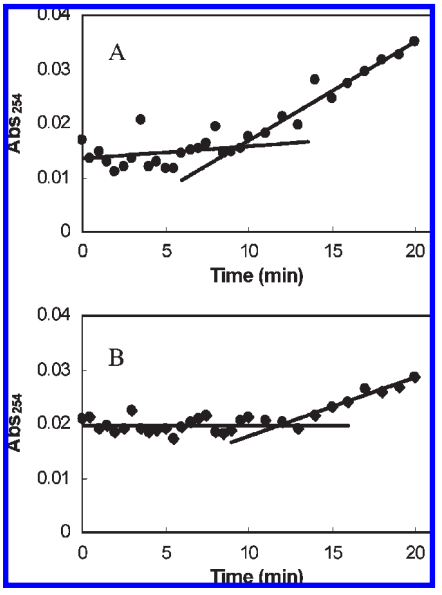

Figure 3. UVB (1303 μW cm^{-2}, 310 nm)-induced oxidation of DLPC liposomes (6.4 μM) in 10 mM Tris-HCl buffer (pH 7.0) measured by formation of conjugated dienes absorbing at 240 nm. (**A**) Five micromolar BHT. (**B**) Five micromolar quercetin.

A number of different systems have been used to quantify the antioxidant status of quercetin resulting in a range of antioxidant capacities reported. These include values of 4.7 (*25*) and 2.5 (*26*) mM Trolox for the Trolox equivalent antioxidant capacity (TEAC) ($ABTS^{+\bullet}$ as a radical source), 3.7 Fe^{3+} ions reduced per molecule for the ferric reducing antioxidant power assay (FRAP) (*26*), 3.5 radicals trapped per molecule for the AMVN-catalyzed oxidation of methyl linoleate (*18*), and 4.53−6.56 radicals trapped per molecule for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (*27*). Our stoichiometric ratio of 4.25 ± 0.27 radicals trapped per molecule for AAPH-mediated lipid oxidation appears consistent with the TEAC, FRAP, and AMVN studies (slightly higher than the FRAP and AMVN results) but is less than the DPPH study, although DPPH frequently overestimates antioxidant potency (*28*).

Numerous studies on the oxidation chemistry of quercetin have been carried out. Rice-Evans suggested that the antioxidant properties of quercetin were the result of a number of factors, although no attempt was made to explain the antioxidant mechanism of quercetin (*25*). This may not be unreasonable given the wide range of antioxidant values observed and that more than 20 quercetin-derived oxidation products have been identified in a wide variety of systems. Of these oxidation products, quercetin depside is the most frequently reported (*29*, *30*), the formation of which is the subject of much debate (*31*−*34*). Given these factors, a mechanistic discussion is beyond the scope of this study.

Quercetin inhibited UVB radiation-induced lipid oxidation at the same level (3.73 ± 0.35) ($P > 0.05$) as observed in the AAPH system but was only half as strong an inhibitor of UVA radiation-induced lipid oxidation (1.93 ± 0.21) ($P < 0.05$). Previous studies by Chen (*23*) on the ability of quercetin to inhibit UVB (300 nm) radiation-induced lipid oxidation showed that for a 30 min incubation, 50 μM quercetin completely prevented the formation of thiobarbituric acid-reactive substances (TBARS), while other phenolics allowed some measurable level of TBARS. This result agrees with our observations that quercetin is a potent inhibitor of UVB-mediated lipid oxidation but gives no clear indication of the absolute scavenging ability of quercetin. In addition, Chen's study was limited to the UVB range, whereas we have observed a difference between the antioxidant capacity of quercetin in UVA and UVB systems.

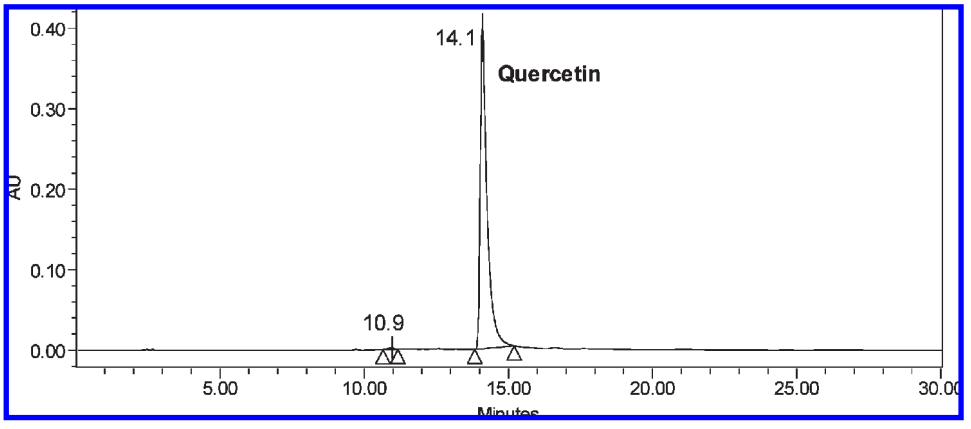

Figure 4. HPLC-UV (300 nm) chromatogram of quercetin (5 μM, 14.1 min) in 10 mM Tris-HCl buffer (pH 7.0) after 60 min of exposure to UVA radiation (740 μW cm^{-2}, 365 nm).

One possible explanation for the decreased antioxidant capacity in the UVA experiments is photodecomposition, since quercetin absorbs strongly in the UVA range (365 nm). Quercetin has been reported to be unstable to UV radiation, although over a longer time period (15 h) than in our experiments (*20*). We found no evidence for photodecomposition of quercetin over 60 min (**Figure 4**), which is greater than the length of either the UVA or the UVB lipid oxidation experiments.

A fluorescence study on UVA and UVB irradiation of low micromolar concentrations of quercetin-7-glucoside in aqueous solution by Smith (*35*) suggested that UV radiation energy was dissipated through the phototautomerization of an excited keto form to an excited state enol, which reverted to the enol ground state, finally tautomerizing to the keto form. At 30 μM quercetin-7-glucoside, a mixture of ground state keto−enol tautomers resulted, both of which produce fluorescence emissions through excited state species. Absorption of UVA radiation by quercetin at the excitation wavelength (365 nm) may lead to greater levels of the excited state species reported by Smith (*35*). These excited state species may possess decreased antioxidant properties and result in an overall decrease in the antioxidant stoichiometry of quercetin as was observed in our study. It is noteworthy, however, that a study of UVA radiation-induced oxidation in the skin of Sprague−Dawley rats found that quercetin protected against malondialdehyde formation and loss of glutathione peroxidase and glutathione reductase activity, suggesting that quercetin retains antioxidant properties in vivo that are observed in our in vitro studies (*36*).

In conclusion, we have demonstrated that quercetin can effectively inhibit UV radiation-induced lipid oxidation in vitro, quercetin is twice as effective at inhibiting lipid oxidation initiated by UVB vs UVA radiation, and quercetin is stable to UV radiation over the length of our experiments.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane); $ABTS^{+\bullet}$, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid); AMVN, 2,2′-azobis (2,4-dimethylvaleronitrile); BHT, butylated hydroxytoluene; DLPC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TBARS, thiobarbituric acid-reactive substances; TEAC, Trolox equivalent antioxidant capacity.

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