# Inhibitory Effect of a Quercetin Metabolite, Quercetin 3-*O*-*β*-D-Glucuronide, on Lipid Peroxidation in Liposomal Membranes

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To study the antioxidant activity of quercetin 3-*O*-*β*-D-glucuronide (Q3GA), which is one of the quercetin metabolites in the blood after intake of quercetin-rich food, the inhibitory effect of Q3GA on lipid peroxidation was estimated using phosphatidylcholine large unilamellar vesicles (PC LUV) as a biomembrane model. Iron ion, an aqueous peroxyl radical generator, a peroxynitrite generator, or lipoxygenase was used as the inducer of lipid peroxidation. In all cases, Q3GA inhibited lipid peroxidation significantly, although its inhibitory effect was lower than that of quercetin aglycon. The ultrafiltration of PC LUV containing Q3GA revealed that Q3GA has low but significant affinity with the membranes of phospholipid bilayers. It is therefore likely that Q3GA acts as an efficient antioxidant in membranous lipid peroxidation through its localization in the phospholipid bilayer. This conjugated quercetin metabolite seems to retain the ability to protect cellular and subcellular membranes from peroxidative attack by reactive oxygen species and peroxidative enzymes.

**Keywords:** *Quercetin 3-O-β-D-glucuronide; flavonoids; large unilamellar vesicles; biomembrane; lipid peroxidation*

## INTRODUCTION

It has become apparent that reactive oxygen species (ROS) generated in the human body cause lipid peroxidation, leading to the development of degenerative diseases including atherosclerosis. The antioxidant activity of dietary flavonoids has attracted much attention from the standpoint of their possible role in the prevention of atherosclerosis (*1*−*3*). Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a typical flavonoid, is widespread in plant foods such as vegetables and fruits. This flavonol-type flavonoid is known to inhibit lipid peroxidation by scavenging ROS and chelating transient metal ions responsible for the generation of ROS (*4*−*7*). Epidemiological evidence has suggested that the intake of dietary flavonoids including quercetin is beneficial to the prevention of atherosclerosis (*8*−*12*).

On the other hand, numerous studies using rodents have shown that quercetin is present as several quercetin metabolites in the blood plasma after oral administration of quercetin aglycone (*13*−*16*). In plant foods, quercetin is commonly found in the form of glycosides. Manach et al. (*17*) and our research group (*18*) showed that neither quercetin aglycon nor quercetin glycosides were detected in human plasma and that only conjugated quercetin metabolites, including glucuronides and sulfates, accumulated in the plasma after the intake of foods rich in quercetin glycosides. Furthermore, we recently identified quercetin 3-*O*-*β*-D-glucuronide (Q3GA) as an antioxidative metabolite present in rat plasma after oral administration of quercetin aglycon (*19*). Thus, dietary quercetin seems to exert a protective effect against lipid peroxidation occurring in vivo. However, little is known about the antioxidant activity of individual quercetin metabolites in biological systems such as cellular and subcellular membranes.

In this study, we measured the inhibitory effect of Q3GA and its related compounds, quercetin aglycon, quercetin 3-*O*-*β*-D-glucoside (Q3G), and luteolin (3′,4′,5,7-tetrahydroxyflavone), on membranous lipid peroxidation using phosphatidylcholine large unilamellar vesicles (PC LUV) as a biomembrane model. Q3GA and Q3G are derivatives of quercetin in which a glucuronide or glucoside substituent, respectively, is bound to the C-3 position of the diphenylpropane structure of quercetin (Figure 1). The effect of glucuronidation on the antioxidant activity of quercetin was therefore evaluated by comparison of the effectiveness of Q3GA with those of Q3G and quercetin aglycon.

## MATERIALS AND METHODS

**Materials.** Egg yolk phosphatidylcholine (PC), quercetin, and *dl*-α-tocopherol were obtained from Sigma Chemical Co. (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) and L-ascorbic acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Q3G and luteolin were obtained from Extrasynthese (Genay, France). 3-Morpholinosydnoniminnine-HCl (SIN-1) was purchased from Affinity Bioreagents Inc. (Golden, CO). Rabbit reticulocyte 15-lipoxygenase (15-LOX) was from Oxford Biomedical Research Inc. (Oxford, MI). Q3GA was chemically synthesized and purified as described elsewhere (*19*). Other reagents were of analytical grade and were used without further purification.

**Preparation of Large Unilamellar Vesicles.** The suspension of large unilamellar vesicles (LUV) was prepared according to the method described previously with slight modification (*4*). Briefly, chloroform solutions of egg yolk PC, which was purified to remove contaminating peroxides by

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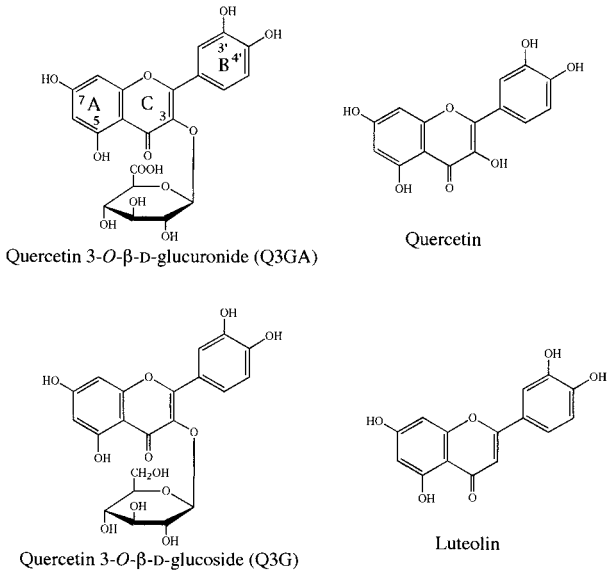


**Figure 1.** Structures of Q3GA and related compounds used in this study.

column chromatography before the experiments (*20*), and cholesterol were mixed in a test tube. The solvent was removed with a stream of nitrogen followed by evaporation under vacuum. The residue was dispersed in Tris-HCl buffer (10 mM, pH 7.4), which contained 0.5 mM diethylenetriaminepentaacetic acid (DTPA) to prevent the prooxidant effect of contaminating metal ions on the reaction. In the experiment with iron(III) nitrate and ascorbic acid, DTPA was not included in the buffer. The suspension was mixed using a Vortex mixer for 1 min followed by ultrasonic irradiation in an Astrason ultrasonifier for 30 s. LUV were obtained by the extrusion method using a LiposoFast apparatus (Avestin Co., Ottawa, ON, Canada). The suspension was passed through a polycarbonate membrane (pore size = 100 nm) 21 times. The resulting LUV suspension was diluted with the same volume of Tris-HCl buffer. The preparation was made just before the experiments.

**Lipid Peroxidation of LUV by Iron Ion and Ascorbic Acid.** Ethanol solutions of Q3GA or its related compounds at various concentrations (10 $\mu$L) were added to the LUV suspension (180 $\mu$L). After preincubation of the mixture in the dark at 37 °C for 5 min, oxidation was initiated by the addition of 10 $\mu$L of a solution of iron(III) nitrate enneahydrate [$Fe(NO)_3 \cdot 9H_2O$] and ascorbic acid (final concentrations = 0.4 and 4 mM, respectively) (*21*). Incubation was carried out in the dark at 37 °C with continuous shaking for 2.5 h. The amounts of thiobarbituric acid reactive substances (TBARS) were quantified by using the method of Uchiyama and Mihara (*22*).

**Lipid Peroxidation of LUV by AAPH or SIN-1.** Ethanol solutions of Q3GA and its related compounds at various concentrations (10 $\mu$L) were added to the LUV suspension (180 $\mu$L). After preincubation of the mixture in the dark at 37 °C for 5 min, oxidation was initiated by the addition of 10 $\mu$L of a solution of AAPH (final concentration = 20 mM) (*4*) or SIN-1 (final concentration = 2 mM). Incubation was carried out in the dark at 37 °C with continuous shaking for 3 h (AAPH) or 6 h (SIN-1). At the end of the incubation, the lipid fraction was extracted from the reaction mixture according to the method of Bligh and Dyer (*23*). Aliquots of the extracted fractions were subjected to HPLC analysis for the measurement of phosphatidylcholine hydroperoxide (PC−OOH) (*24*).

**Lipid Peroxidation of LUV by 15-LOX.** Ethanol solutions of Q3GA and its related compounds at definite concentration (5 $\mu$L) were added to the LUV suspension (90 $\mu$L). In this case the suspension was prepared with HEPES buffer (50 mM, pH 7.4). After preincubation of the mixture in the dark at 20 °C for 5 min, oxidation was initiated by the addition of 10 $\mu$L of a solution of 15-LOX (100 units) (*25*). Incubation was carried out in the dark at 20 °C with continuous shaking for 5 h. After the incubation was completed, the lipid fraction was extracted and subjected to HPLC analysis for the measurement of PC−OOH (*24*).

**Measurement of Partition Coefficient in Octanol/Water.** A methanol solution of Q3GA was placed in a test tube, and the solvent was removed with a stream of nitrogen. The residue was dispersed in Tris-HCl buffer (100 $\mu$L, 50 mM, pH 7.4) and octanol (100 $\mu$L) (*26*). The solution was mixed using a Vortex mixer for 1 min followed by centrifugation at 12000 rpm for 5 min at 4 °C. The concentrations of Q3GA in the upper and lower phases were measured by reversed phase HPLC as described below.

**Ultrafiltration of LUV.** Ethanol solutions of Q3GA and its related compounds (20 $\mu$L, final concentration = 20 $\mu$M) were added to the LUV suspension (180 $\mu$L). After the mixture was incubated in the dark at 37 °C for 10 min, it was filtered through an ultrafiltration membrane (UFC3TGC00, pore size = 10 nm) by centrifugation at 12000 rpm for 40 min (*27*). The filtrate solution was diluted with ethanol and injected into an HPLC column to measure the concentration of Q3GA and its related compounds.

**Measurement of Quercetin, Q3GA, Q3G, Ascorbic Acid, and α-Tocopherol.** Quercetin, Q3GA, and Q3G were measured by reversed phase HPLC with electrochemical detection at the oxidation potential of +800 mV using a TOA ICA-5212 amperometric detector (TOA Electronics Ltd., Tokyo, Japan) with a TSKgel ODS-80Ts column (Tosoh, Tokyo, Japan). The mobile phase consisted of methanol/water/acetic acid (49:49:2, v/v/v, for quercetin; 37:61:2, v/v/v, for Q3GA and Q3G). All eluting solvents contained 50 mM lithium acetate as supporting electrolyte and were used at a flow rate of 1.0 mL/min. Ascorbic acid was measured by reversed phase HPLC with electrochemical detection at the oxidation potential of +500 mV using a Shimadzu L-ECD-6A detector (Shimadzu, Kyoto, Japan) with a TSKgel ODS-80Ts column (Tosoh, Japan). Trifluoroacetic acid solution (pH 2.2) was used as the mobile phase, and the flow rate was set at 1.0 mL/min. α-Tocopherol was measured by HPLC using a Shimadzu RF-10AXL fluorescence detector (Shimadzu) and a TSKgel ODS-80Ts column (Tosoh) with methanol/water (93:7, v/v) as the mobile phase and a flow rate of 1.0 mL/min. The eluate was monitored by fluorescence detection with excitation at 295 nm and emission at 335 nm.

**Data Analysis.** Statistical analysis was evaluated by one-way analysis of variance followed by the Bonferroni/Dunn post hoc multiple-comparison test to determine the statistical significance of differences between means. A value of $p < 0.05$ was judged as statistically significant.

## RESULTS

**Effect of Q3GA and Related Compounds on Iron Ion-Induced Lipid Peroxidation of PC LUV.** Iron ion-induced lipid peroxidation of PC LUV was evaluated by measuring the amounts of TBARS produced, and the inhibitory effects of Q3GA and related compounds (at 20 $\mu$M) were estimated (Figure 2A). Quercetin aglycon exerted the highest inhibitory effect on this reaction system. Q3GA, Q3G, and luteolin also showed significant inhibition, although their inhibitory effects were weaker than that of quercetin aglycon. Their effectiveness increased in the order Q3GA < Q3G < luteolin < quercetin aglycon. The inhibitory effect of Q3GA increased with the elevation of its concentration between 20 and 100 $\mu$M (Figure 2B). These findings confirmed that Q3GA possessed a weak but significant inhibitory effect on iron ion-induced membranous phospholipid peroxidation.

**Effect of Q3GA and Related Compounds on Aqueous Peroxyl Radical-Induced Lipid Peroxidation of PC LUV.** We next examined the effect of Q3GA and related compounds at 20 $\mu$M on the formation

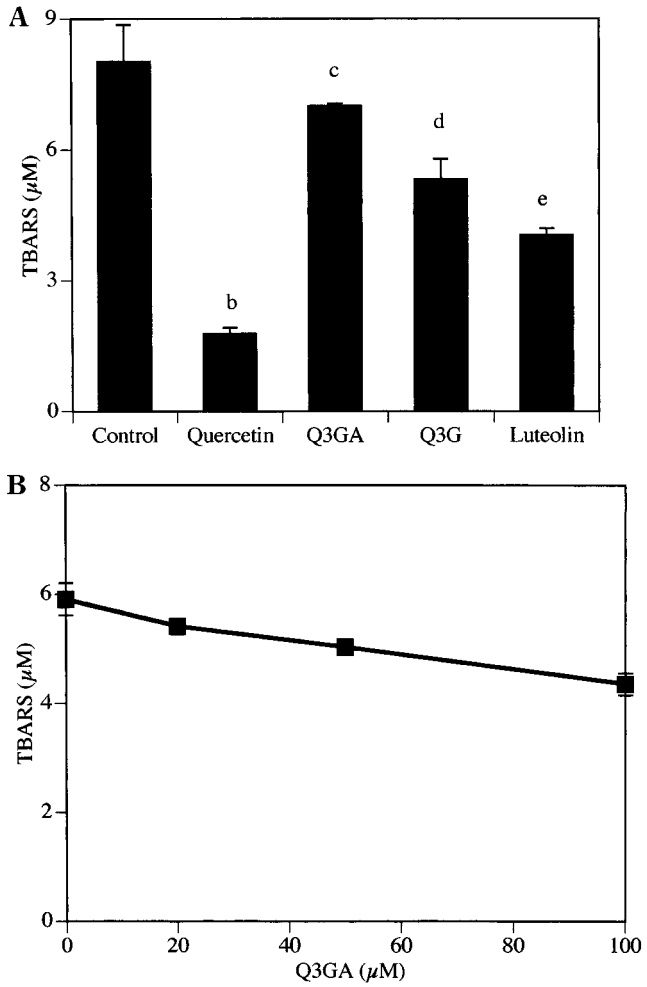


**Figure 2.** Effect of Q3GA and related compounds on iron ion-induced lipid peroxidation of PC LUV: (A) effect of Q3GA and related compounds at 20 $\mu$M; (B) effect of Q3GA at various concentrations (up to 100 $\mu$M). The reaction system consisted of PC (5 mM), cholesterol (2.5 mM), and Q3GA or related compounds (20 $\mu$M) in 10 mM Tris-HCl buffer (pH 7.4). Oxidation was started by the addition of iron(III) nitrate enneahydrate (0.4 mM) and ascorbic acid (4 mM) and allowed to proceed at 37 °C for 2.5 h. Results are the means ± SD of three independent experiments. Means with different letters are significantly different ($p < 0.05$).

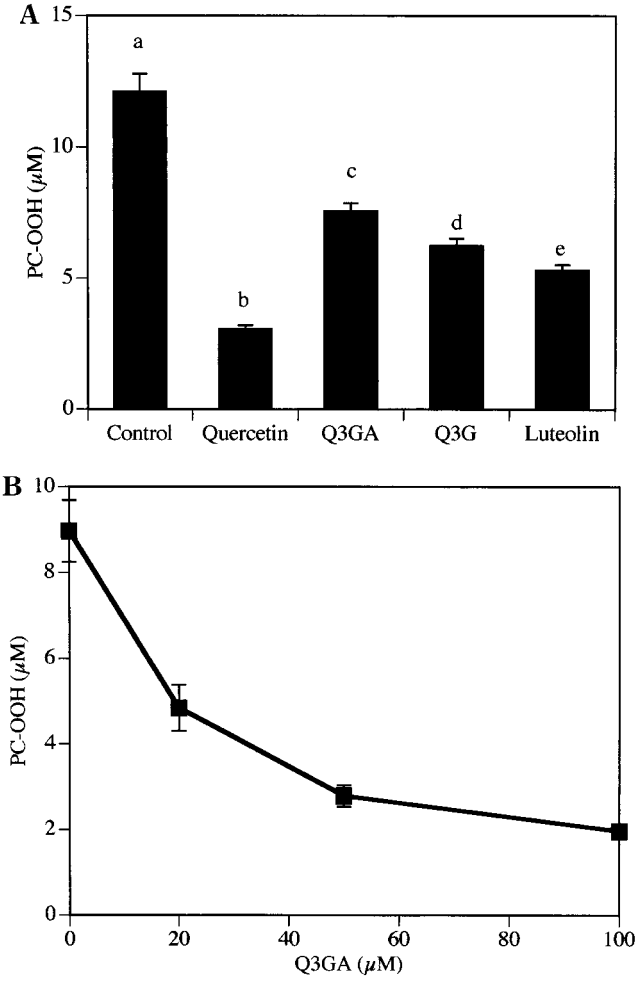


**Figure 3.** Effect of Q3GA and related compounds on AAPH-induced lipid peroxidation of PC LUV: (A) effect of Q3GA and related compounds at 20 $\mu$M; (B) effect of Q3GA at various concentrations (up to 100 $\mu$M). The reaction system consisted of PC (5 mM), cholesterol (2.5 mM), and Q3GA or related compounds in 10 mM Tris-HCl buffer (pH 7.4) containing DTPA (0.5 mM). Oxidation was started by the addition of AAPH (20 mM) and allowed to proceed at 37 °C for 3 h. Results are the means ± SD of three independent experiments. Means with different letters are significantly different ($p < 0.05$).

of PC−OOH from PC LUV that were exposed to AAPH (Figure 3A). Quercetin aglycon most effectively inhibited PC−OOH formation, whereas Q3GA, Q3G, and luteolin showed weaker effects than quercetin aglycon. Nevertheless, the amount of PC−OOH formation in the presence of Q3GA was significantly lower than that formed in the control reaction. The inhibitory effect of Q3GA was comparable to that of Q3G but weaker than that of quercetin aglycon. The effectiveness of the inhibition by Q3GA was increased with the elevation of its concentration in the range of 20−100 $\mu$M (Figure 3B). These findings confirmed that Q3GA has a significant inhibitory effect on aqueous peroxyl radical-induced lipid peroxidation of PC LUV.

**Effect of Q3GA and Related Compounds on SIN-1-Induced Lipid Peroxidation of PC LUV.** We also examined the effect of Q3GA and related compounds on SIN-1-induced lipid peroxidation of PC LUV (Figure 4). Quercetin aglycon, Q3GA, and Q3G significantly inhibited the formation of PC LUV, as compared with the control reaction. Their inhibitory effect increased in the order Q3GA < Q3G < quercetin aglycon. Q3GA exerted a marked inhibitory effect on SIN-1-induced lipid peroxidation of PC LUV, although its effect was weaker than those of Q3G and quercetin aglycon.

**Effect of Q3GA and Related Compounds on 15-LOX-Induced Lipid Peroxidation of PC LUV.** Lipid peroxidation of PC LUV in the presence of 15-LOX was evaluated by measuring the formation of PC−OOH. Figure 5 shows the effect of Q3GA and related compounds on this reaction system. All tested compounds significantly inhibited the formation of PC−OOH as compared with the control reaction. Their inhibitory effects increased in the order Q3GA < Q3G < quercetin aglycon. The findings showed that Q3GA exerted a substantial inhibitory effect on 15-LOX-induced lipid peroxidation of PC LUV, although its effect was weaker than that of quercetin aglycon.

**Partition Coefficient of Q3GA in Octanol/Water.** Q3GA was subjected to partitioning between octanol and water to estimate its lipophilicity. The partition coefficient of Q3GA, represented by the ratio of its concentration in the octanol phase to that in the water phase, was determined to be 0.008 ± 0.002. Compounds with such lower partition coefficients are considered to

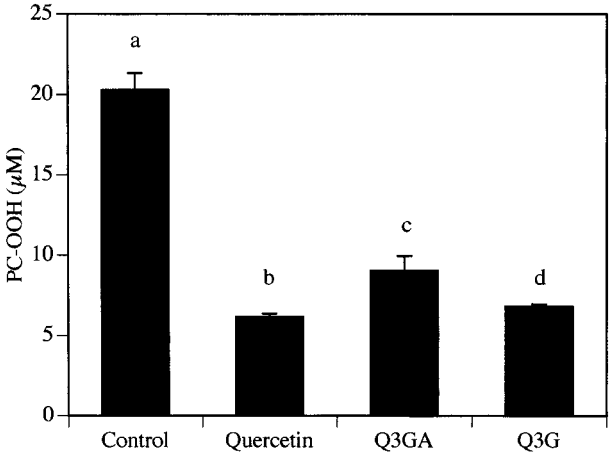


**Figure 4.** Effect of Q3GA and related compounds on SIN-1-induced lipid peroxidation of PC LUV. The reaction system consisted of PC (5 mM), cholesterol (2.5 mM), and Q3GA or related compounds (10 μM) in 10 mM Tris-HCl buffer (pH 7.4) containing DTPA (0.5 mM). Oxidation was started by the addition of SIN-1 (2 mM) and allowed to proceed at 37 °C for 6 h. Results are the means ± SD of three independent experiments. Means with different letters are significantly different ($p < 0.05$).

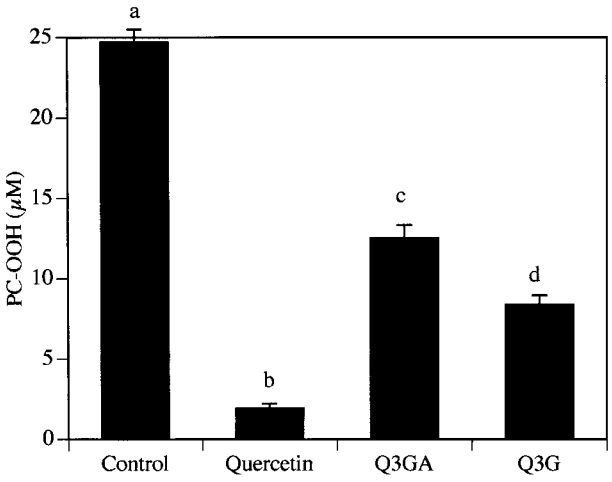


**Figure 5.** Effect of Q3GA and related compounds on 15-LOX-induced lipid peroxidation of PC LUV. The reaction system consisted of PC (5 mM), cholesterol (2.5 mM), and Q3GA or related compounds (20 μM) in 10 mM HEPES buffer (pH 7.4). Oxidation was started by the addition of 15-LOX (100 units) and allowed to proceed at 20 °C for 5 h. Results are the means ± SD of three independent experiments. Means with different letters are significantly different ($p < 0.05$).

be hydrophilic compounds. The partition coefficients for quercetin aglycon and Q3G have already been reported by our research group as 65.7 ± 3.1 and 5.0 ± 0.6, respectively (*26*). Therefore, Q3GA was the most highly hydrophilic among these three compounds.

**Ultrafiltration of PC LUV Containing Q3GA or Related Compounds.** PC LUV suspensions containing Q3GA or related compounds were ultrafiltrated through an ultrafiltration membrane with a pore size of 10 nm to measure the affinity of each compound toward phospholipid membranes. No phospholipid was passed through the membranes because the average size of the PC LUV particle (100 nm) was much larger than the pore size (10 nm). Table 1 shows the concentration of Q3GA and related compounds in the filtrates. After ultrafiltration, 50−100 μL of liposomal solution passed through the membrane and accumulated as filtrate

**Table 1. Concentration of Q3GA and Related Compounds in the Filtrate after Ultrafiltration of PC LUV Suspension[a]**

| compound | concn (μM) | compound | concn (μM) |
|---|---|---|---|
| quercetin | 0.97 ± 0.3[a] | α-tocopherol | 0.067 ± 0.06[c] |
| Q3GA | 12.9 ± 1.6[b] | ascorbic acid | 20.2 ± 0.06[d] |
| Q3G | 12.6 ± 1.4[b] | | |

[a] Initial concentrations of Q3GA and related compounds in the LUV suspensions were 20 μM. Values are mean ± SD of three independent experiments. Means with different letters are significantly different.

solution. α-Tocopherol was found to possess a high affinity to PC LUV, as shown by the fact that the concentration of α-tocopherol in the filtrate was quite low. In contrast, ascorbic acid showed little affinity to PC LUV, as indicated by the fact that its concentration in the filtrate was little changed from its concentration in the suspension before filtration. The concentration of quercetin aglycon in the filtrate was intermediate between that of α-tocopherol and that of ascorbic acid. Comparison of their concentrations in the filtrates suggests that quercetin aglycon possesses considerable affinity to PC LUV, although it is lower than that of α-tocopherol. The higher concentrations of Q3GA and Q3G than of quercetin aglycon in the filtrate solution indicated their lower affinities to PC LUV. However, both Q3GA and Q3G showed significant affinity to PC LUV, because the concentrations of both Q3GA and Q3G in the filtrate were significantly lower than that of ascorbic acid. Therefore, it can be concluded that Q3GA possesses low but significant affinity to PC LUV.

## DISCUSSION

The aim of this study was to examine the antioxidant activity of Q3GA, which is one of the possible quercetin metabolites from dietary quercetin. We recently found that Q3GA accumulated in rat plasma after oral administration of quercetin aglycon (*19*). We also conducted a study of quercetin metabolites and their related derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein (LDL) and demonstrated that quercetin metabolites with conjugation at the 3-position in the C ring retain considerable antioxidant activity, as compared with those with conjugation at the 4′-position in the B ring (*28*). Here we estimated the inhibitory effect of Q3GA, possessing a conjugated glucuronide group at the 3-position in the C ring, on lipid peroxidation using PC LUV as a biomembrane model. Iron ion, an aqueous peroxyl radical generator (AAPH), a peroxynitrite generator (SIN-1), and lipoxygenase (15-LOX) were used as inducers of lipid peroxidation, because these reagents are widely used as initiators in in vitro models of membrane lipid peroxidation.

Several studies have indicated that the catechol structure (*o*-dihydroxyl structure) in the B ring is essential for the radical-scavenging activity of flavonol-type flavonoids and that a hydroxyl group at the 3-position in the C ring is important for maximizing the activity (*5*, *29*). It was therefore of great interest to determine the influence of glucuronidation at the 3-position on the antioxidant activity of quercetin. We measured the antioxidative activity of Q3G, Q3GA, and luteolin, in addition to that of quercetin aglycon, on iron ion- and AAPH-induced lipid peroxidation. The order of their inhibitory effects in each system increased as

follows: Q3GA < Q3G < luteolin < quercetin aglycon. These findings therefore confirmed that introduction of a glucuronide group to the hydroxyl group at the 3-position significantly decreases the antioxidative activity of quercetin. However, Q3GA suppressed iron ion-induced lipid peroxidation and AAPH-induced lipid peroxidation in a concentration-dependent manner (Figures 2B and 3B). These results imply that Q3GA retains substantial radical-scavenging activity and metal ion-chelating activity due to the presence of the catechol structure, which is responsible for metal ion chelating (*30*), as well as radical scavenging.

Peroxynitrite is known to be produced in the vascular system by the reaction of nitric oxide and superoxide generated by endothelial cells and macrophages (*31*). This reactive nitrogen species is thought to participate in the oxidative modification of LDL (*32*). Haenen et al. (*33*) proposed that the catechol structure in the B ring and a free hydroxyl group at the 3-position are also responsible for peroxynitrite scavenging, as they are for free radical scavenging. Our study showed that the inhibitory effects of Q3GA and related compounds on SIN-1-induced lipid peroxidation of PC LUV increased in the order Q3GA < Q3G < quercetin aglycon (Figure 4). SIN-1 is known to produce superoxide and nitric oxide simultaneously and thus act as a peroxynitrite generator (*34*). It is therefore likely that the peroxynitrite-scavenging activity of Q3GA is lower than that of quercetin aglycon because it lacks the free hydroxyl group at the 3-position in the C ring.

15-LOX has been also proposed to play a role in the oxidation of LDL by endothelial cells and macrophages during the early events of atherosclerosis (*35*). We previously measured the inhibitory effect of quercetin and quercetin glucosides on 15-LOX-induced lipid peroxidation of human LDL and concluded that the catechol structure in the B ring largely contributes to the activity of quercetin in the prevention of LDL lipid peroxidation (*36*). Our finding that Q3GA inhibited 15-LOX-induced PC LUV lipid peroxidation confirms that the catechol structure is also important for the prevention of 15-LOX-induced lipid peroxidation occurring in membranous phospholipids.

The above results obtained using different peroxidation inducers demonstrate that Q3GA exerts a considerable inhibitory effect on lipid peroxidation in phospholipid membranes induced by ROS and peroxidative enzymes. However, it should be noted that the effectiveness of Q3GA is significantly lower than that of quercetin aglycon in all cases. In general, the localization of antioxidants should be taken into account in order to understand the effectiveness of antioxidants in heterogeneous membrane systems. Nakayama et al. (*37*) suggested from centrifugation experiments of liposomal suspensions that quercetin possesses high affinity to phospholipid membranes. We assessed the affinity of Q3GA toward phospholipid membranes by ultrafiltration of LUV. The results shown in Table 1 indicate that Q3GA possesses much lower affinity toward phospholipid membranes as compared with quercetin aglycon. However, Q3GA showed significantly higher affinity to phospholipid membranes than ascorbic acid. The values of the partition coefficients show that the introduction of a glucuronide group to the 3-position in the C ring increases the hydrophilicity dramatically. Thus, it is reasonable that the affinity of Q3GA toward the phospholipid surface of PC LUV was much lower than that of quercetin aglycon. The lower antioxidant activity of Q3GA may have been due to the elevation of hydrophilicity, in addition to the loss of the free hydroxyl group. Nevertheless, Q3GA retains significant antioxidant activity because of its catechol structure and its affinity for phospholipid membranes.

Recently, Movileanu et al. (*38*) reported that quercetin aglycon could penetrate the lipid bilayer of a liposomal suspension depending on the acidity of the medium. Arora et al. (*39*) claimed that rutin, a quercetin glycoside, partitions into the hydrophobic core of phospholipid membranes. We suppose that the planar structure of quercetin, the diphenylpropane structure, may explain its high affinity for phospholipid membranes and that quercetin aglycon protects membrane lipids against peroxidation by trapping the chain-initiating radicals and metal ions in the interface of biomembranes (*40*). Conjugated metabolites of quercetin are likely to possess a capacity for interaction with phospholipid membranes because of their diphenylpropane structures, although the conjugation decreases the lipophilicity dramatically. The diphenylpropane structure of Q3GA may interact with the hydrophobic core of membranous phospholipids. Interestingly, quercetin aglycon was reported to be mutagenic and to act as a prooxidant (*41*−*43*); however, dietary quercetin is known to accumulate in blood plasma not as the aglycon but as glucuronide- and/or sulfate-conjugated metabolites (*17*, *18*). It was recently confirmed that several glucuronide conjugates of quercetin accumulate in human plasma after intake of quercetin-rich foods (*44*). It is likely that these metabolites contribute to the antioxidative defense against membranous phospholipid peroxidation occurring in vivo.

In conclusion, Q3GA, one of the conjugated metabolites of quercetin in the blood, inhibits membranous phospholipid peroxidation induced by ROS and peroxidative enzymes, although its effectiveness is lower than that of quercetin aglycon. Q3GA seems to act as an antioxidant in biomembrane systems via its catechol structure and affinity to phospholipid membranes.

## ABBREVIATIONS USED

ROS, reactive oxygen species; Q3GA, quercetin 3-*O*-*β*-D-glucuronide; Q3G, quercetin 3-*O*-*β*-D-glucoside; PC LUV, phosphatidylcholine large unilamellar vesicles; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; SIN-1, 3-morpholinosydnoniminnine-HCl; 15-LOX, 15-lipoxygenase; DTPA, diethylenetriaminepentaacetic acid; PC−OOH, phosphatidylcholine hydroperoxides; LDL, low-density lipoprotein.

## ACKNOWLEDGMENT

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