DOI:10.1021/jf102871g

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Transformation of Rutin to Antiproliferative Quercetin-3-glucoside by *Aspergillus niger*

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The flavonol quercetin in plants and foods occurs predominantly in the form of glycoside whose sugar moiety affects the bioavailability and the mechanism of its biological activities. The antiproliferative activities of quercetin derivatives such as quercetin aglycone, quercetin-3-β-D-glucoside (Q3G), and rutin were compared using six different cancer cell lines including colon, breast, hepatocellular, and lung cancer. The IC_{50} value of Q3G ranged between 15 and 25 μM in HT-29, HCT 116, MCF-7, HepG2, and A549 cells. In these five cell lines, Q3G showed the most potent growth inhibition, whereas rutin showed the least potency. Transformation of rutin to Q3G was conducted by controlling α-L-rhamnosidase and β-D-glucosidase activities from crude enzyme extract of *Aspergillus niger*. Carbon sources during culture and transformation conditions such as pH, temperature, and heat-stability were optimized. After 4 h biotransformation, 99% of rutin was transformed to Q3G and no quercetin was detected. This study presented an efficient biotransformation for the conversion of rutin to Q3G which was newly shown to have more potent antiproliferative effect than quercetin and rutin.

KEYWORDS: Quercetin-3-β-D-glucoside; biotransformation; antiproliferation; cancer; rutin; *Aspergillus niger*

INTRODUCTION

Flavonoids belong to naturally occurring polyphenolic compounds and exist ubiquitously in plants and foods including vegetables, fruits, medicinal herbs, and beverages such as tea and red wine. Among more than 4000 flavonoid compounds, most of the flavonoids are found as glycosides with different sugar moieties (*1*). Conjugation with various sugar molecules stabilizes the aglycones, increases their water solubility, and affects the bioavailability and bioactivity (*2*, *3*). Flavonoid glycosides are converted and modified to their deglycosylated, hydroxylated, methylated, sulfated, or glucuronidated forms during absorption and metabolism (*4*). These variations and modifications are related to the diversities in their bioavailabilities and physiological functions.

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a diglycoside of quercetin which is the predominant flavonol type of flavonoid in foods. Rutin is abundant in various plants such as buckwheat and apple as well as many plant-based beverages (*5*). It has been reported that rutin has several pharmacological functions such as antioxidative, cytoprotective, antiplatelet, antithrombic, vasoprotective, and cardioprotective activities (*6*−*11*). Quercetin, the aglycone form of rutin, is also an important dietary flavonoid with antioxidative, antiinflammatory, and antiproliferative properties (*12*). Even though rutin and quercetin share several biological activities, the antiproliferative effects of rutin and quercetin on cancer cell lines were significantly different. Quercetin exerted antiproliferative and cytotoxic activities on cancer cell lines of diverse lineage including colon cancer cell lines (*13*). On the other hand, rutin showed neither antiproliferative nor antitumorigenic effects in in vitro (*14*) and in vivo studies. When administered with 0−3% quercetin or rutin to azoxymethane (AOM)-induced F344 rats, the incidence of aberrant crypt foci (ACF) was markedly inhibited by quercetin but not by rutin (*15*, *16*). Quercetin-3-β-D-glucoside (Q3G, isoquercitrin) is not as abundant as quercetin-4′-β-D-glucoside (Q4′G) or quercetin-3,4′-diglucoside (Q3,4′G) in foods. The antiproliferative effect of Q3G on various cancer cell lines has not yet been investigated in comparison to the effects of rutin and quercetin.

Biotransformation of many effective compounds by a specific type of glycosyl hydrolase has many benefits. Bioavailabilities and biological properties of various saponins and flavonoid glycosides were modified after the hydrolysis of specific glycosyl groups or the conversion of glycones to aglycones (*17*−*19*). Biotransformation of rutin to Q3G and its increased bioactivity have not been reported. In this study, we optimized the production of Q3G using crude enzyme extract from a food-grade microorganism, *Aspergillus niger* (*A. niger*), and showed that Q3G exerted a more potent antiproliferative effect than quercetin and rutin on various cancer cell lines including colon, breast, hepatocellular, and lung cancer cells.

MATERIALS AND METHODS

Chemicals and Reagents. Rutin, Q3G, quercetin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO) unless specifically mentioned. All the materials needed for cell culture including media, serum, and related reagents were GIBCO products from

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 Published on Web 10/01/2010

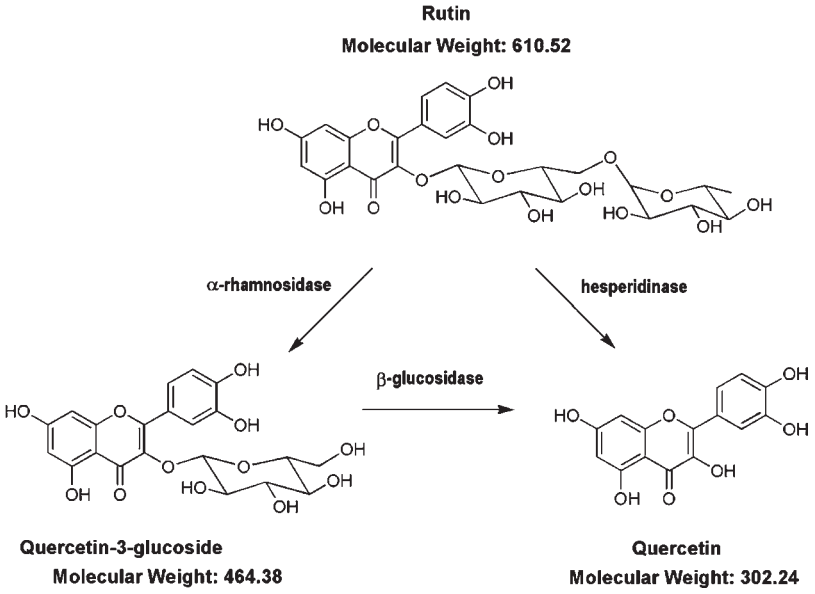

Figure 1. Structures of quercetin, quercetin-3-β-D glucoside, and rutin.

Invitrogen Life Technologies (Carlsbad, CA). HPLC-grade acetonitrile, methanol, and water were obtained from Fisher Scientific (Pittsburgh, PA).

Cell Culture. Human colorectal adenocarcinoma HT-29 (KCLB 30038), colorectal carcinoma HCT 116 (KCLB 10247), breast adenocarcinoma MCF-7 (KCLB 30022), and lung carcinoma A549 (KCLB 10185) cell lines were purchased from the Korean Cell Line Bank (Seoul, Korea). Human colorectal adenocarcinoma Caco-2 (ATCC, HTB-37) and liver hepatocelluar carcinoma HepG2 (ATCC, HB-8065) cells were obtained from American Type Culture Collection (Manassas, VA). All the cell lines were maintained and subcultured according to the distributor's instructions. Briefly, HT-29, Caco-2, and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) antibiotic-antimycotic (AA) solution. The MCF-7 cell line was cultured in DMEM supplemented with 0.01 mg/mL bovine insulin. HCT 116 and A549 cells were incubated in RPMI-1640 media containing 10% (v/v) FBS and 1% (v/v) AA solution at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Cell Proliferation Assay. For the assessment of cell proliferation and cytotoxicity, cells were seeded in 96-well plates at 5000−6000 cells/well with complete medium and allowed to adhere for 24 h. Cells were incubated for 72 h in the presence of various concentrations of quercetin, Q3G, or rutin. They were dissolved in dimethyl sulfoxide (DMSO), and the concentration of DMSO was not greater than 0.5% (v/v) in all experiments. Viable cell numbers were estimated by the MTT assay as described previously (*20*). At the end of incubation, 10% (v/v) MTT stock solution (5 mg/mL) was added to each well, followed by incubation at 37 °C for 2 h. The converted formazan product was dissolved in DMSO with gentle shaking, and the absorbance was measured at 540 nm using a microplate reader (Bio-Rad Laboratories, Philadelphia, PA).

Preparation of Crude Enzyme Extract of *Aspergillus niger*. *A. niger* van Tieghem KCTC 6906 was purchased from Korean Collection for Type Cultures (Daejeon, Korea) and cultured in potato dextrose agar (Difco, Detroit, MI) under aerobic conditions at 30 °C for 7 days. Crude microbial enzyme extract of *A. niger* was prepared according to the method described previously with a minor modification (*18*). The spores were harvested from the subcultured potato dextrose agar plate by scraping and suspending in the spore suspension solution (0.9% NaCl solution with 0.005% Tween 80). The collected spores were inoculated at 10^6 spores/mL into the basal medium with various sugars. The basal media contained $NaNO_3$ (0.5 g/L), K_2HPO_4 (1.0 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $FeSO_4 \cdot 7H_2O$ (0.01 g/L), and 0.5% (w/v) casamino acid with pH adjusted to 7.0. The culture broth was incubated at 30 °C for 8 days under aerobic conditions with shaking. The mycelia were removed by filtration using glass microfiber filter (GF/A, Whatman, Kent, U.K.), and the filtrate was concentrated using a centrifugal filter (Amicon Ultra, 30,000 MWCO, Millipore Korea, Seoul, Korea) at 4000*g* for 30 min at 4 °C. The concentrated filtrate was exchanged with the same volume of piperazine-HCl buffer (0.02 M, pH 6.0) using Centricon (Millipore, Seoul, Korea) to remove the small molecules and adjust the pH. This buffer-changed protein extract was used as microbial crude enzyme extract (CE) for the transformation of rutin, and the enzyme activity was calculated as previously described (*21*).

Biotransformation of Rutin to Q3G by Optimized Enzyme Extract of *A. niger*. Various biotransformation conditions with respect to pH, temperature, organic solvent, and duration of heat treatment were assessed. The α-L-rhamnosidase and β-D-glucosidase activities in each experiment were assayed by the degradation of respective substrates to produce free *p*-nitrophenols (pNPs). *p*-Nitrophenyl-α-L-rhamnopyranoside (pNP-Rha) and *p*-nitrophenyl-β-D-glucopyranoside (pNP-Glc) were used as substrates, and the concentration of released pNPs was measured by reading the absorbance at 405 nm using a microplate reader. One unit of enzyme activity corresponded to the amount of enzyme which liberated 1 μmol of pNP per min at 45 °C in 0.02 M piperazine-HCl buffer (pH 6.0).

For the biotransformation of rutin to Q3G, 50 μL of CE was resuspended in 375 μL of piperazine buffer and heat-treated at 70 °C for 30 min. Prepared CE (10% (v/v)), 10 mM rutin solution in methanol (15% (v/v)), and piperazine-HCl buffer (75% (v/v)) were mixed and incubated at 60 °C for 1 h. The reaction was terminated by boiling for 10 min and freeze-dried before analysis.

Analysis of Transformed Products by HPLC and LC/MS. The lyophilized samples were dissolved in methanol, filtered through 0.45 μm syringe filter (Pall, Ann Arbor, MI), and used for HPLC analysis. HPLC was conducted with a Dionex P680 instrument (Dionex Corporation, Sunnyvale, CA) equipped with an ASI-100 auto sampler (Dionex) and a UVD 170 UV−vis detector (Dionex). A Sunfire C_{18} column (150 mm × 4.6 mm, 3.5 μm particle size) from Waters (Milford, MA) and TCC-100 thermostatted column compartment (Dionex) were used, and the column was maintained at 30 °C during the separation. The mobile phase consisted of solvent A (0.1% (v/v) trifluoroacetic acid (TFA) in water, pH 2.5) and solvent B (acetonitrile) with the following gradient: 0−20 min, linear gradient from 20 to 40% B; 20−30 min, 40% B. The injection volume of standards and samples was 20 μL, and the flow rate was 0.5 mL/min. Transformed rutin was identified by comparison of the retention time (RT) and UV−vis absorbance profile to Q3G standard obtained from Sigma-Aldrich. Quercetin, rutin, and Q3G were quantified at 350 nm by using linear calibration curves of standards. LC/MS analysis in the ESI negative mode of transformed samples was also performed using a Hewlett-Packard Series 1100 HPLC instrument coupled to a quadrupole-time of flight mass spectrometer (Q-TOF MS) system (Micromass, Manchester, U.K.). The column and solvent conditions were same as those of HPLC analysis.

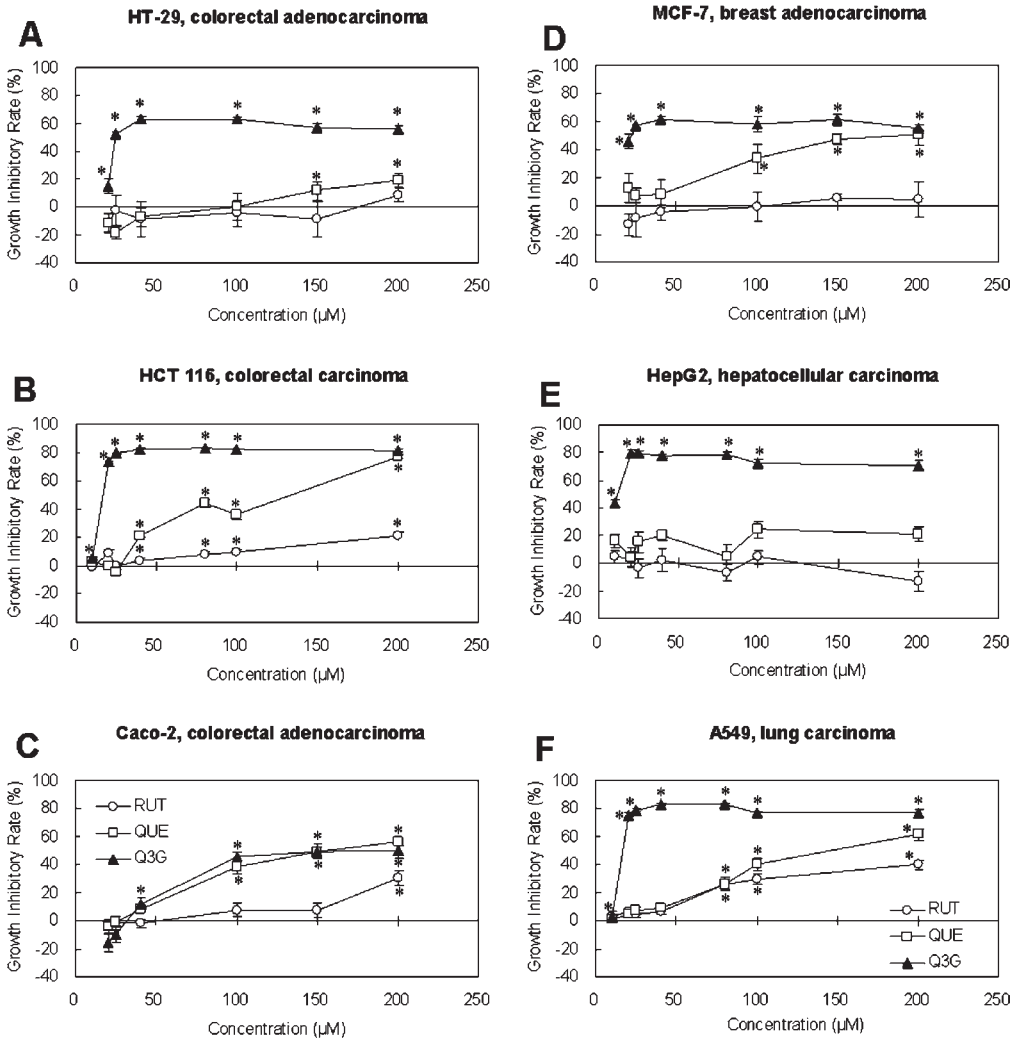

Figure 2. Antiproliferative effects of rutin (RUT, ○), quercetin (QUE, □), and quercetin-3-β-D glucoside (Q3G, ▲) on various cancer cell lines. HT-29 (**A**), HCT 116 (**B**), Caco-2 (**C**), MCF-7 (**D**), HepG2 (**E**), and A549 (**F**) cells were treated with indicated amounts of each compound from 10 to 200 μM for 72 h. Cell proliferation was estimated by the absorbance (Abs) at 540 nm after MTT assay. Growth inhibitory rate (%) = (Abs of control − Abs after treatment)/Abs of control ×100. DMSO (≤0.5%) was used as a vehicle control. (∗) Significantly different with DMSO control group ($p < 0.05$) when compared by Duncan's multiple range tests. Values are expressed as mean with error bars (SEM) ($n = 6$).

Statistical Analysis. Results are presented as mean values with their standard errors. One-way analysis of variance (ANOVA) followed by Duncan multiple comparison tests were used to test statistically significant differences between treatment groups using the SPSS statistical package (SPSS Inc., Chicago, IL).

RESULTS

Antiproliferative Effects of Quercetin and Its Glycosides on Various Cancer Cell Lines. To investigate the antiproliferative effects of quercetin, Q3G, and rutin (**Figure 1**) on cancer cells, six different cancer cell lines including colon, breast, hepatocellular, and lung cancer cells were treated with them at various concentrations (**Figure 2**). In five cell lines except Caco-2 cells, Q3G showed notably higher growth inhibitory effect than with quercetin or rutin. Concentrations of Q3G that caused 50% inhibition of cell proliferation (IC_{50}) ranged between 15 and 25 μM in these cell lines. In Caco-2 cells, the potency of Q3G to inhibit cell growth was similar to that of quercetin. The IC_{50} value of quercetin was greater than 80 μM in every cell line. HCT 116, HepG2, and A549 cell lines were more susceptible to Q3G than other cell lines. Quercetin showed less than 20% of growth inhibitory rate in HT-29 and HepG2 cell lines at concentrations up to 200 μM. In all the cancer cell lines, rutin showed the least potency in the inhibition of cell proliferation.

Induction of α-L-Rhamnosidase Activity Using Different Monosaccharide Carbon Sources. *A. niger* was inoculated and incubated in different compositions of carbon sources such as glucose (0.5% (w/v)), galactose (0.5% (w/v)), mannose (0.5% (w/v)), and rhamnose (0.1, 0.5, 1, and 2% (w/v)). During the culture period of 10 days, the activities of α-L-rhamnosidase and β-D-glucosidase from mycelia-free culture supernatant were measured every day. Rhamnose-containing media showed the greatest level of α-L-rhamnosidase activity (**Figure 3**). One percent (w/v) rhamnose was the most effective; however, lower (0.1% (w/v)) or higher (2% (w/v)) concentrations of rhamnose decreased the enzyme induction. When added with glucose (0.5% (w/v)), β-D-glucosidase activity was produced at the greatest level compared to other carbon sources.

Effects of pH, Temperature, and Addition of Methanol on Enzyme Activities of Crude Enzyme Extract (CE). For the efficient biotransformation of rutin to antiproliferative Q3G using enzyme extract, the inhibition of β-D-glucosidase activity was important as well as the induction of α-L-rhamnosidase activity. The effects of pH and temperature on *A. niger* crude enzyme extract from

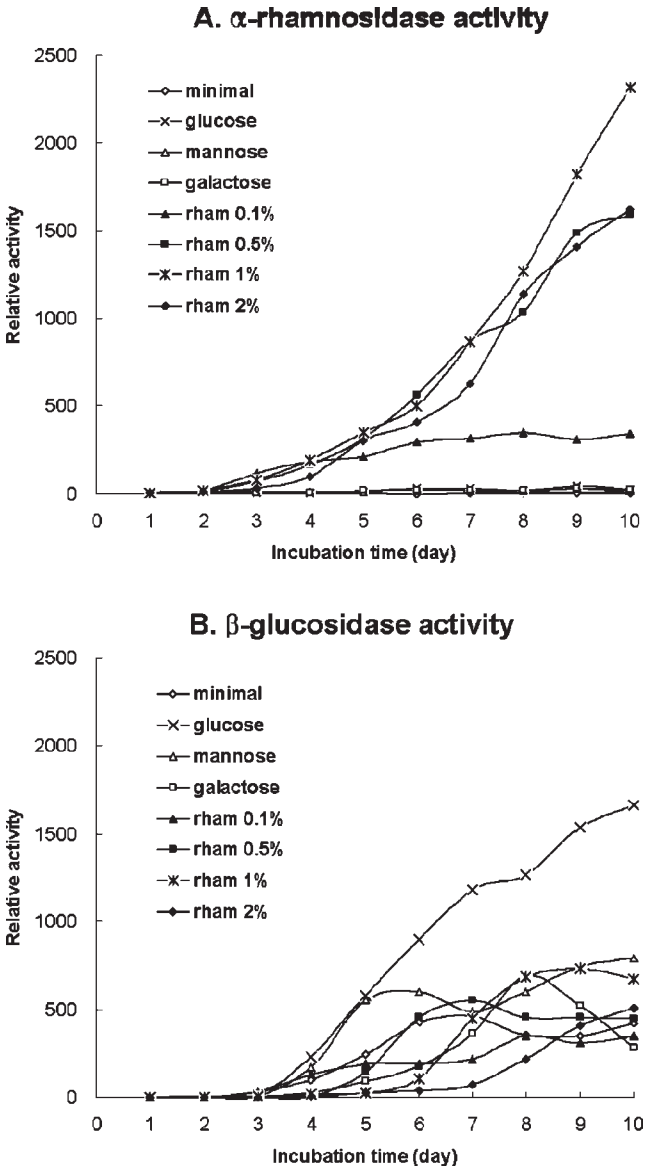

Figure 3. Induction of α-L-rhamnosidase (**A**) and β-D-glucosidase (**B**) activities in *A. niger* culture with different compositions of carbon sources for 10 days. Relative activity was measured and calculated using released pNP concentrations from respective substrates. (Minimal, basal media; glucose, basal media added 0.5% (w/v) glucose; mannose, basal media added 0.5% (w/v) mannose; galactose, basal media added 0.5% (w/v) galactose; rham *x*%, basal media added respective % (w/v) of rhamnose).

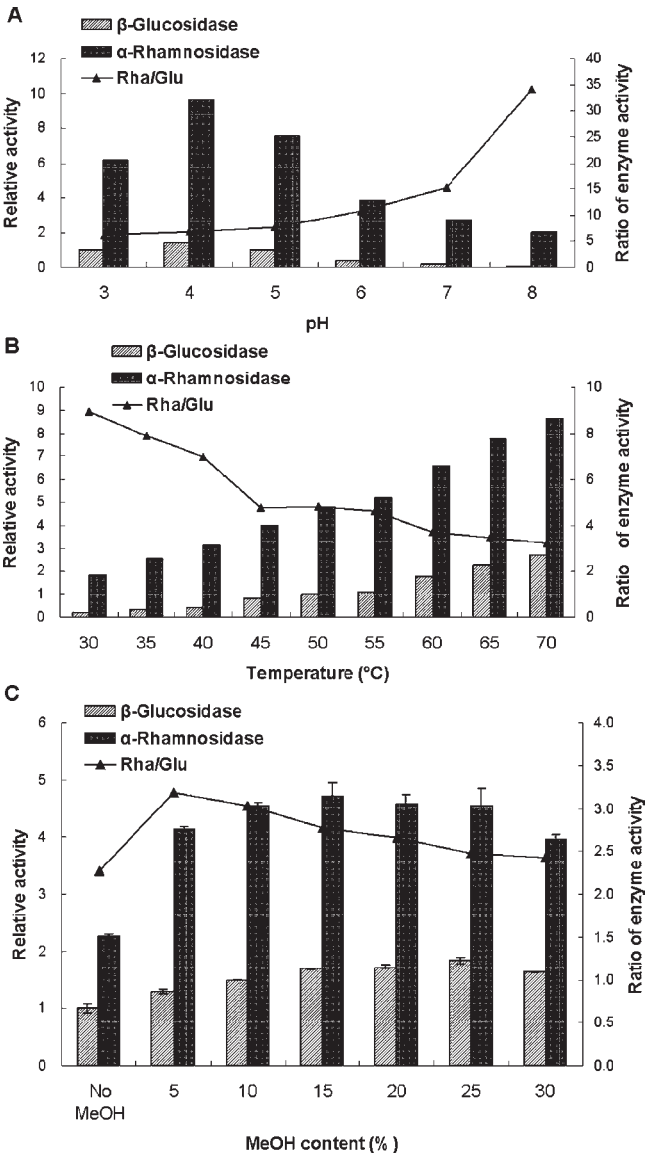

Figure 4. Effects of pH (**A**), temperature (**B**), and concentration of methanol (**C**) on α-L-rhamnosidase and β-D-glucosidase activities in CE. The ratio of two enzyme activities is represented as Rha/Glu (▲).

rhamnose-added culture were investigated by pNP assay. The optimal pH was 4 for both enzymes; however, the ratio of α-L-rhamnosidase activity to β-D-glucosidase activity (Rha/Glu) was increased as pH increased (**Figure 4A**). The activities of α-L-rhamnosidase and β-D-glucosidase were increased as temperature increased up to 70 °C. The addition of 5−15% (v/v) methanol showed greater increase in α-L-rhamnosidase activity than β-D-glucosidase activity (**Figure 4B** and **C**). Although the Rha/Glu ratio was the highest at 30 °C and decreased at higher temperatures, the relative α-L-rhamnosidase activity at 30 °C was about 25% compared to 70 °C.

Inactivation of β-D-Glucosidase by Heat Treatment. The heat-stabilities of α-L-rhamnosidase and β-D-glucosidase were investigated at 60, 65, and 70 °C for 1 h. β-D-Glucosidase activities were more rapidly decreased than those of α-L-rhamnosidase at 65 and 70 °C. By the heat treatment for 30 min at 70 °C, β-D-glucosidase activity reached zero, but α-L-rhamnosidase activity was decreased by only 50% compared to the nonheat treated control (**Figure 5**).

Analysis of the Biotransformed Rutin by HPLC and ESI-LC/MS. Before transformation, the pH of CE was adjusted to 6.0 with piperazine-HCl buffer containing 15% (v/v) methanol, followed by heat treatment at 70 °C for 30 min in order to inactivate β-D-glucosidase activity. Transformation of rutin by prepared CE was performed at 60 °C for 4 h. In 1 h, 86.4% of rutin was transformed to Q3G which was measured by HPLC analysis compared with standard compounds (**Figure 6A** and **B**). ESI mass spectra of transformed rutin and standard Q3G in negative ion mode showed an intense peak at m/z 463.7, which corresponded to the deprotonated ion $[M - H]^-$ of Q3G (**Figure 6C** and **D**). After 4 h of biotransformation, 99% of rutin was transformed to Q3G and no quercetin was detected (**Table 1**).

DISCUSSION

Overall 4000 flavonoids are ubiquitously distributed in plants as ester, ether, or glycosidic derivatives or mixtures. In mammals,

flavonoids occur only through dietary intake including fruits, vegetables, grains, nuts, tea, and wine. As a major flavonoid present in the human diet, 20−100 mg of quercetin is ingested daily by dietary intake (*22*). Quercetin in foods exists mainly as β-glycosides. The sugar moiety of the quercetin glycoside greatly affects the bioavailability of quercetin from foods (*3*, *23*−*25*). The in vivo bioactivities of quercetin are affected by the degree of its intestinal absorption from the diet (*12*).

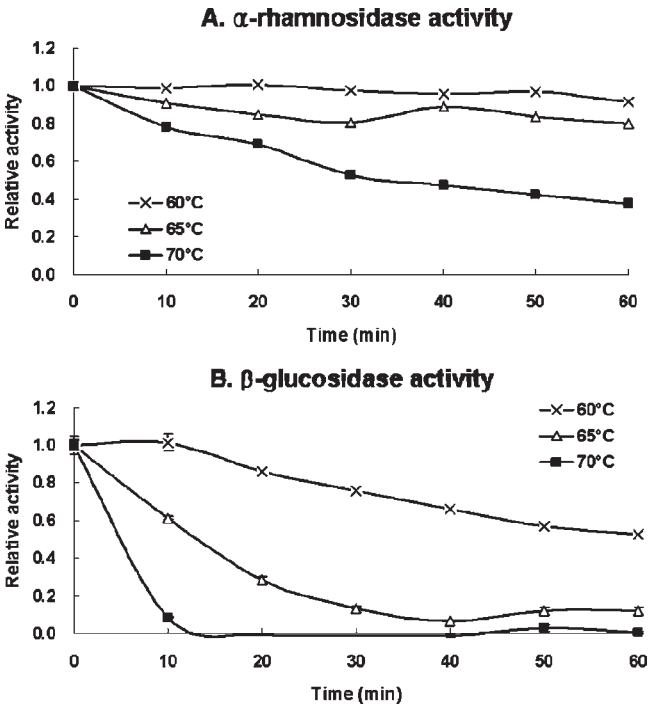

Figure 5. Time course heat inactivation of α-L-rhamnosidase and β-D-glucosidase in CE. Changes of enzyme activities at different temperatures (60, 65, and 70 °C) during 1 h were determined by pNP assay.

The predominant type of quercetin glycoside varies among the plant species. Onion and apple peels are commonly consumed foods that contain significant amounts of quercetin (*26*). While onion contains mainly Q4′G and Q3,4′G, apple mainly contains galactosides, rhamnosides, and arabinosides (*27*−*29*). Q4′G accounted for up to 45% of quercetin compounds in onions (*30*). Rutin is found in various foods such as buckwheat, asparagus, citrus fruits, black tea, apple peels, and oats (*31*). The bioavailability of quercetin from rutin was lower than that of Q4′G, and the absorption of rutin depended on the hydrolysis of rhamnosyl residue by bacterial α-L-rhamnosidases in gastrointestinal tract (*32*, *33*). Q3G is not an abundant flavonol glycoside in foods; however, the bioavailability of Q3G was 50−180% higher than that of quercetin in pigs, dogs, and rats (*34*−*36*). In contrast to the delayed absorption of rutin, Q3G is relatively rapidly absorbed mainly in the small intestine. The removal of rhamnosyl residue from rutin increased the bioavailability of quercetin up to 12-fold (*34*, *36*).

The sugar moiety affects the bioactivity as well as the bioavailability. In many in vitro and in vivo studies related to the bioactivities of quercetin and its glycosides, the effectiveness of quercetin and rutin was different. When administered with 0−3% quercetin or rutin to azoxymethane (AOM)-induced F344 rats, the incidence of aberrant crypt foci (ACF) was markedly inhibited by quercetin but not by rutin (*15*, *16*). Regarding cytotoxic effects of quercetin and rutin on colon cancer cells, only quercetin was effective in inhibiting HT-29 cell growth (*37*). Besides, quercetin with rhamnosyl moieties such as rutin and quercetin-3-rhamnoside (Q3R) did not show the antioxidant effect (*38*) and the inhibitory effect on glucose transport through intestinal glucose transporters (*39*). Shen et al. showed that quercetin was able to induce apoptosis in the human leukemia cell line, HL-60; however, neither rutin nor Q3R affected the viability of cells even at a dose of 80 μM (*14*). The addition of rutinose (rhamnoglucose) or rhamnose into quercetin attenuated the apoptosis-inducing activity of quercetin.

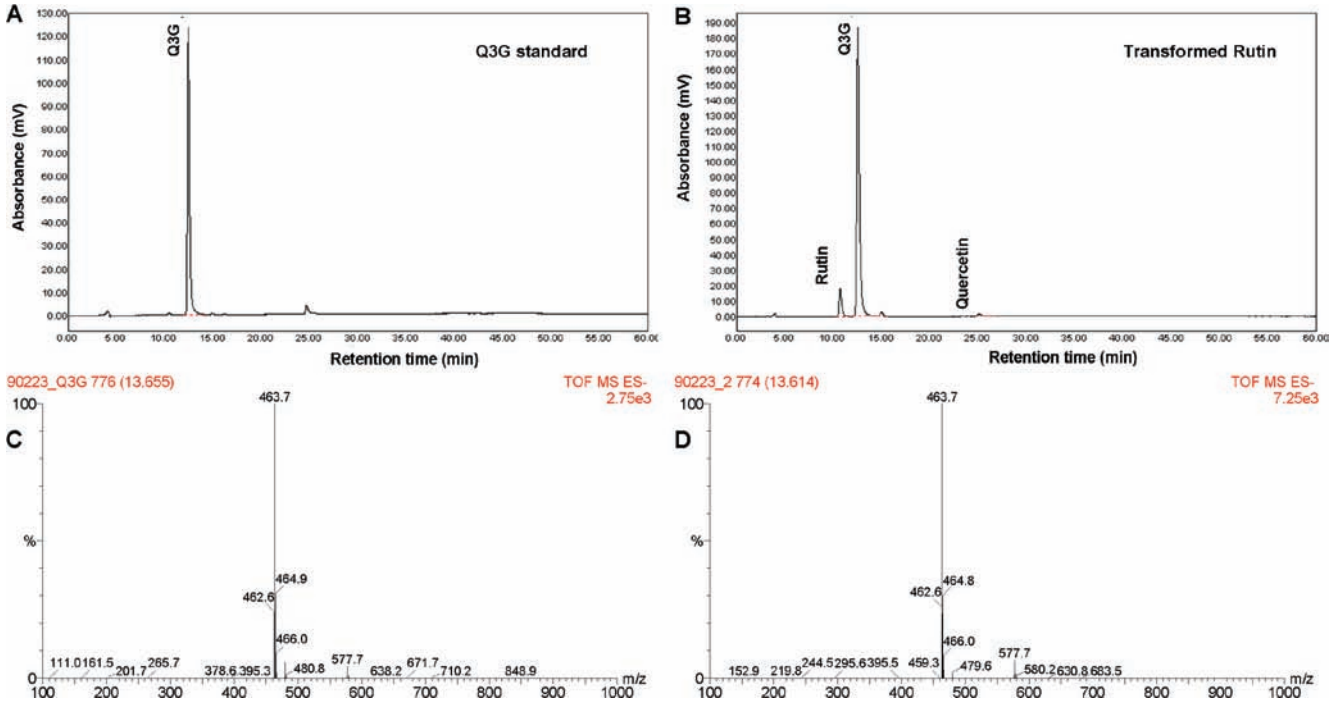

Figure 6. HPLC chromatograms (**A** and **B**) and LC/MS profiles in the ESI negative mode (**C** and **D**) of Q3G standard and biotransformed rutin. The *m*/*z* ratio of biotransformed rutin was 463.7 (*m*/*z*), the same as that of Q3G standard.

Table 1. Conversion Rate of Rutin to Q3G by Optimized CE for 4 h[a]

	transformation time (h)		
	1	2	4
rutin (mM)	0.11 ± 0.01	0.02 ± 0.01	0.01 ± 0.002
Q3G (mM)	1.29 ± 0.02	1.42 ± 0.035	1.49 ± 0.002
quercetin (mM)	0.1 ± 0.008	n.d.	n.d.
conversion rate (%)	86.4 ± 1.4	94.4 ± 2.3	99.4 ± 0.1

[a] Rutin was converted to Q3G by optimized CE with 99% yield for 4 h. Conversion rate (%) = (concentration of transformed Q3G (mM)/1.5 (mM) of rutin) × 100. n.d. = not detected.

Previously, the effect of Q3G on cancer cell proliferation has not been tested in comparison with its parent glycoside rutin or its aglycone quercetin. Q3G showed notable growth-inhibitory effects in colon, breast, hepatocellular, and lung cancer cells in the present study. The IC_{50} value of Q3G was between 15 and 25 μM, and that of quercetin was greater than 80 μM. Rutin showed the least antiproliferative effect on five epithelial cancer cells. The growth inhibitory effects of quercetin and rutin were consistent with the results from other studies (*37*, *40*).

The reason why Q3G showed more potent antiproliferative effect than quercetin might be related to the specific transport system such as glucose transport carrier SGLT1. It was reported that the conjugation of a glucose to a phenyl compound resulted in active absorption from the mucosal side to serosal side by the glucose transport system (*42*). Experimental evidence suggested that Q3G and Q4′G were transported by the sodium-dependent glucose transporter (SGLT1) and subsequently deglycosylated within the enterocyte by cytosolic β-glucosidase (*25*, *43*, *44*). Quercetin itself was not transported by SGLT1 or GLUT2 (*39*). The existence of a specific antiproliferatory signaling cascade after transport of Q3G to enterocytes remains to be further studied. In Caco-2 cells, the effect of Q3G and quercetin showed similar inhibitory patterns. This might result from the cell line specificity which expresses different kinds of transporters or glycoside hydrolases. Day et al. proposed that quercetin glycosides were mainly deglycosylated by lactase phlorizen hydrolase (LPH) before absorption by passive diffusion of the released aglycone (*45*). As shown in the assessment of the β-glucosidase activity including LPH in different colon cancer cell lines, Caco-2 cells had higher activity than HT-29 cells by 124% (*46*). Therefore, it might be suggested that Q3G was transformed to quercetin more rapidly in Caco-2 cells than in other cancer cell lines, which might explain similar effects between Q3G and quercetin.

In plant foods there are various flavonoid glycosides with rhamnosyl moieties such as Q3R, rutin, hesperidin (hesperetin-7-glucoside-6-β-1-rhamnoside), neohesperidin (hesperetin-7-glucoside-2-β-1-rhamnoside), narirutin (naringenin-7-glucoside-6-β-1-rhamnoside), and naringin (naringenin-7-glucoside-2-β-1-rhamnoside). As mentioned above, rhamnosyl residue had a great influence on the bioavailability and the bioactivity of flavonoid glucosides. It was also shown that the bitter taste of citrus was caused by naringin and the debittering of citrus juices could be accomplished by the removal of rhamnose (*47*). With an aim to improve industrial processes and absorption of the rhamnoside flavonoids in the small intestine, studies were recently carried out to selectively remove rhamnosyl moieties using purified fungal enzyme preparations and recombinant organisms (*48*−*51*). *Aspergillus aculeatus* NW240 was reported to produce two different α-L-rhamnosidases; however, it possessed potential pathogenicity (*52*). One of the *Lactobacillus plantarum* genes which encoded rhamnosidases was expressed in *Lactococcus lactis* as a potential candidate in food processing (*51*). However, transformation efficacy of rutin by the enzyme lysate from this recombinant microorganism was 13.2% after 24 h. In our results, the *A. niger* strain which can be used as a food-grade microorganism showed an outstanding transformation rate of rutin to Q3G of 99% in 4 h. *A. niger* has been used as a common enzyme source such as hesperidinase; however, the high β-D-glucosidase activity was an obstacle for the successful removal of rhamnose from rutin. By the optimization of the carbon source in the growth medium and the biotransformation conditions, we were able to produce the enzymic preparations with high α-L-rhamnosidase activity and no detectable β-D-glucosidase activity. Besides, the transformed Q3G was shown to be a more potent antiproliferative glucoside than its parent aglycone, quercetin. Considering the improved bioavailabilty of Q3G compared to rutin, the result of the present study is expected to provide a useful enzymatic biotransformation method with no additive purification or recombinant process for the production of antiproliferative Q3G from rutin.

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Received for review July 23, 2010. Revised manuscript received September 7, 2010. Accepted September 13, 2010. This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Science and Technology (MG08-0303-4-0), Republic of Korea.