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A Soluble Flavonoid-glycoside, αG-Rutin, Is Absorbed as Glycosides in the Isolated Gastric and Intestinal Mucosa

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We investigated the absorption and metabolism of the highly soluble quercetin glycoside αG-rutin, a glucose adduct of insoluble rutin, using the isolated mucosa of the rat stomach and intestines equipped with the Ussing chamber. αG-rutin and rutin appeared in the serosal sides of the gastric body and all the intestinal mucosa after the addition of αG-rutin (1 mM) to the mucosal fluid. The degree of αG-rutin appearance was much lower in the gastric fundus than in the other parts. Quercetin was not found in the mucosal fluid of any mucosal specimen. The concentrations (μM) of αG-rutin and rutin in the serosal fluid as a result of transport from the mucosal side increased time-dependently and linearly with mucosal αG-rutin concentration (1, 10 or 100 mM). The highest transport was shown in the ileal mucosa. These results indicate that αG-rutin is partly hydrolyzed to rutin through the intestine and absorbed as such.

Polyphenols are widely distributed in the plant world, especially in fruits and vegetables, as secondary metabolites.[1] These compounds act as antioxidants both in foods and in the body.[2] Flavonoids, a kind of polyphenol, are ingested as natural food pigments or supplements, however, the absorption and metabolism of flavonoids, and especially of their glycosides, are not yet fully understood. The bioavailability of these compounds depends on intestinal absorption, which is defined by their chemical composition. Quercetin is a well-known natural flavonoid found in onions, green tea, and sophora.[3–5] It is present mainly as β-glycosides in plant foods. The nature of glycosylation probably influences the efficiency of quercetin absorption.[6] Quercetin-3-*O*-glucoside does not appear in systemic blood after perfusion of this quercetin-glycoside to the rat small intestine. It is suggested that hydrolysis by mucosal lactase phlorizin hydrolase (LPH) is a major step in the intestinal absorption of quercetin-glucoside.[7] Another report suggests that a glucose transporter (SGLT1) may facilitate the transport of quercetin-3-*O*-glucoside in the isolated mucosa of the rat jejunum and proximal colon,[8] but there is very little information about the intestinal absorption of other flavonoid glycosides.

Recently, a new quercetin glycoside, αG-rutin, has been developed and made available (Fig. 1). This glycoside is a highly water-soluble quercetin derivative, in which one D-glucose is attached to rutin (quercetin-3-*O*-glucosyl-rhamnose). Previous *in vivo* experiments have shown that rutin is neither readily absorbed nor metabolized in the small intestine.[6,9,10] It is hardly soluble in water or other solvents, which may be one reason for its low absorbability in the small intestine.[10–12] Rutin absorption might be increased by improvement of water-solubility. Nonetheless, the intestinal absorption of αG-rutin has not yet been evaluated.

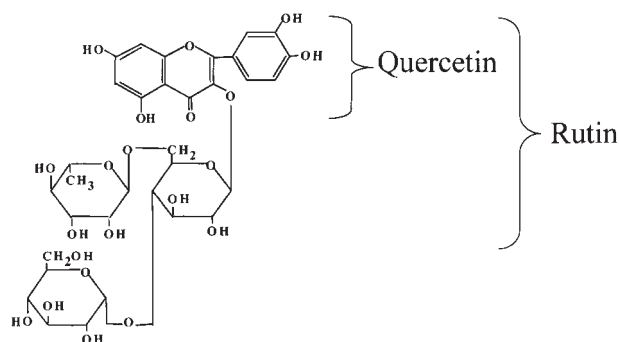The aim of the present study is to investigate the absorption and metabolism of the quercetin glycoside

Fig. 1. Structure of αG-Rutin.

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(αG-rutin) in the rat stomach and intestines. We examined its absorption and metabolism by the isolated mucosa of the stomach, small intestine, cecum, and colon with an Ussing chamber. αG-rutin and its metabolites were quantified by LC/MS analysis.

Materials and Methods

Chemicals. αG-rutin was provided by Toyo Sugar Refining (Tokyo, Japan). Quercetin and rutin were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents and chemicals commercially available were of extra-pure grade.

Animals and diets. Male Wistar/ST rats (Japan SLC, Sizuoka, Japan) weighing 230–250 g were housed in individual cages in a temperature-controlled room at 22 °C throughout the experimental period. The rats were fed a 25% casein-sucrose diet consisting of 60.25% sucrose, 25% casein, 5% cellulose, 3.5% mineral mixture (AIN 93G), 1% vitamin mixture (AIN 93G), 5% corn oil, and 0.25% choline chloride for a week just before use.

These studies were approved by the Hokkaido University Animal Committee. The animals were maintained in accordance with Hokkaido University guidelines for the care and use of laboratory animals.

Absorption and metabolism by isolated gastro-intestinal mucosa. The stomach, small intestine (from the ligament of Treitz to the ileocecal junction), cecum, and colon were removed from six rats under anesthesia with pentobarbital (40 mg/kg body weight, Nembutal, Abbott, North Chicago, Illinois, U.S.A.). The outside and inside surfaces of the isolated segments were washed with ice-cold saline (154 mM NaCl). The excised small intestine was flushed with saline (20 ml). The stomach (whole sac), jejunum (15 cm distal from the Trietz ligament), ileum (15 cm proximal from the ileocecal junction), cecum (whole sac), and colon (middle and distal parts, each one third of the whole colon) segments were cut open along the mesenteric border to be a flat sheet and rinsed with an ice-cold balanced salt solution buffered by HEPES (HBS): 125 mM NaCl, 4 mM KCl, 10 mM D-glucose, 30 mM HEPES, and 1.25 mM CaCl$_2$, gassed with 100% O$_2$, pH 7.4. The stomach was divided into two parts, the upper stomach (gastric fundus) and the lower stomach (gastric body). The serosa and muscle layers of the segments were removed, and preparations consisting of the mucosal and submucosal tissues were mounted onto Ussing chambers (diffusion chamber system, Corning Costar, Cambridge, U.K.) which exposed a circular area of the epithelium (0.64 cm^2) to the medium. The serosal and mucosal sides of the specimens were bathed in 1 ml of HBS continuously gassed by 100% O$_2$. After a 30-min stabilization period, the serosal medium was replaced with fresh HBS, and the mucosal medium was replaced with 1, 10, or 100 mM

αG-rutin in HBS. After incubation for 30 or 60 min at 37 °C, the serosal solution was collected.

The integrity and viability of the tissue was checked by transepithelial electrical resistance (TEER, Millicell-ERS, Millipore, Billerica, Massachusetts, U.S.A.). The TEER value of the epithelial preparation in the gastric fundus, gastric body, jejunum, ileum, cecum, and colon was measured before and after the 30-min incubation period with 100 mM αG-rutin added to the mucosal chamber. The TEER value was indicated in terms of Ω cm^{-2}.

LC/MS analysis. The serosal fluid samples (100 μl) were added to 100 μl of MeOH, heated at 100 °C for 1 min, and centrifuged for 3 min at 9,000 × g. The supernatant was applied to oasis HLB cartridges (Waters, Milford, Massachusetts, U.S.A.), and the eluent was dried and dissolved in 100 μl of 50% MeOH solution (sample solution).

Quercetin derivatives were identified and quantified with a Waters mass spectrometer-computer system through the positive ions at the electric spray ionization (ESI)-interface (ZQ 2000, Waters). The temperature of the capillary heater and the vaporization heater was maintained at 100 °C and 300 °C respectively. The flow rate of sheath gas (nitrogen) was 70 arb. LC/ESI-MS was carried out in the scan mode from m/z: 50 to 2,000 (M$^+$) and in the selected ion monitoring (SIM) mode m/z: 303 (M$^+$) for quercetin, m/z: 611 (M$^+$) for rutin, and m/z: 773 (M$^+$) for αG-rutin respectively. The HPLC system was fitted with a 5-μm C-18 Waters Puresil TM column (150 mm × 4.6 mm, Waters), and the temperature was maintained by a column oven set at 40 °C. Solvents A (water:methanol:trifluoroacetic acid, 70:30: 0.1, v/v/v) and B (methanol:trifluoroacetic acid, 100: 0.1, v/v), were run at a flow rate of 1 ml/min using a linear gradient up to 30% from 10% of solvent B until 20 min and back to 10% solvent B for the next 5 min, and then held in this condition for a further 5 min. Concentrations of flavonoids in the sample solution were estimated using the calibration curves of quercetin, rutin, and αG-rutin standard solution. UV chromatograms were also recorded at 360 nm.

Calculations. Statistical analyses were performed by one-way and two-way ANOVA. The differences among groups were analyzed by Duncan's multiple range test and were considered significant at $P < 0.05$.

Results

The LC/MS chromatograms of αG-rutin, quercetin, and rutin in the standard solution (A) and sample solution (B) are shown in Fig. 2. The retention times for αG-rutin and rutin were 7 min, and that of quercetin was 15 min. Intact αG-rutin and rutin were detected chromatographically by absorbance measurement at 360 nm in the serosal fluid 30 min after incubation, with the

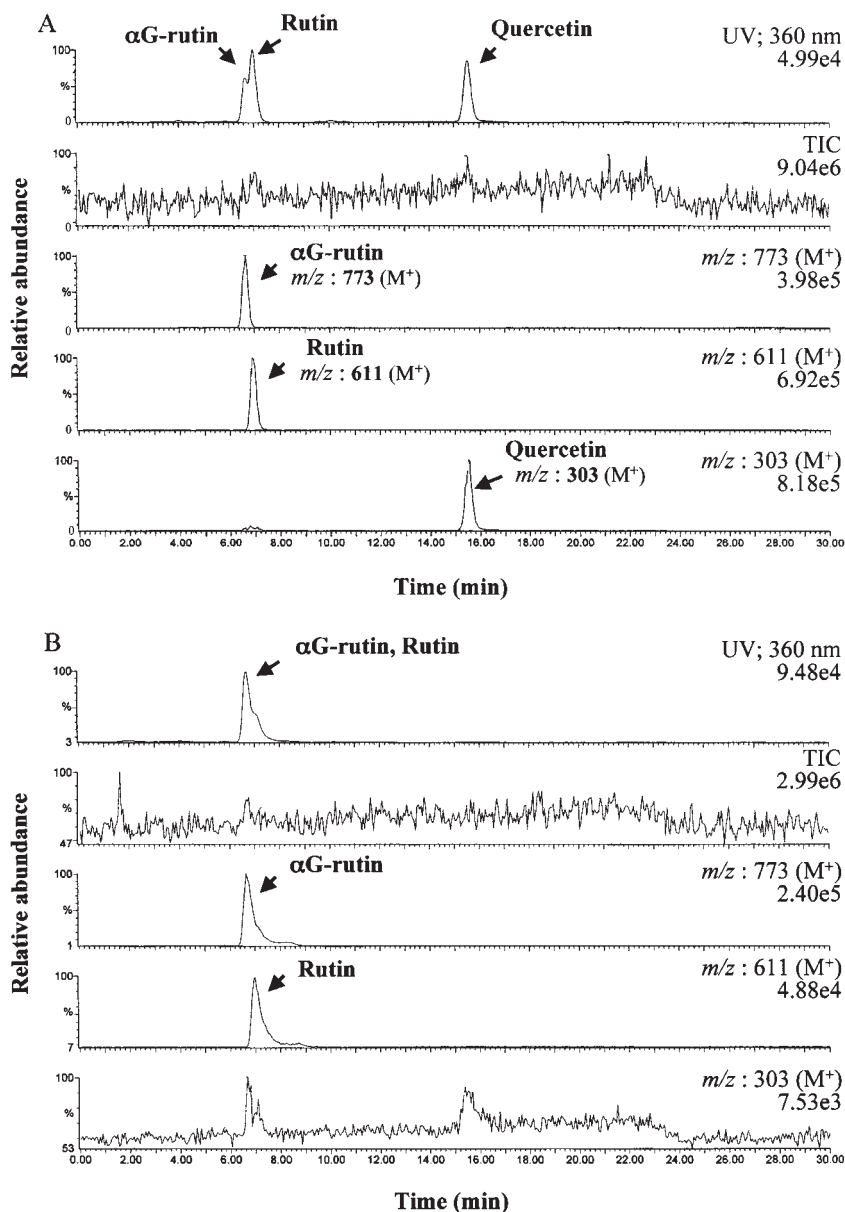**Fig. 2.** LC/MS Analysis of Standard of αG-Rutin, Rutin, and Quercetin (A), αG-Rutin and Rutin in the Serosal Fluid of the Ileum after Incubation for 30 min (B).

LC/MS chromatogram monitoring relative absorption at 360 nm and selected ion monitoring (SIM) mode m/z: 303 (M$^+$) for quercetin, m/z: 611 (M$^+$) for rutin, and m/z: 773 (M$^+$) for αG-rutin.

mucosal fluid containing 1 mM of αG-rutin (Fig. 2B).

αG-rutin and rutin concentrations in the gastric fundus, gastric body, jejunum, ileum, cecum, and colon increased time-dependently from 30 min to 60 min. (Fig. 3). The highest concentrations of αG-rutin and rutin were found in the ileal serosal fluid at 60 min, and was 0.03% of total αG-rutin in the mucosal fluid.

αG-rutin and rutin in the serosal fluid of the mucosal specimens of the gastric fundus, gastric body, jejunum, ileum, cecum, and colon dose-dependently increased with increased αG-rutin (1, 10, or 100 mM) in the mucosal fluid (fluid, see Fig. 4). Increases in rutin in the serosal fluid were comparable to those of αG-rutin in the ileal mucosa up to 10 mM αG-rutin in the mucosal fluid,

but the rutin concentration was much lower than that of αG-rutin at 100 mM in both the mucosa. The concentrations of rutin in the serosal fluid of the cecum and colon were very low at all the concentrations of mucosal αG-rutin. The concentrations in the gastric fundus were much lower than in the other parts of the mucosa. The transport rates of αG-rutin and rutin into the serosal fluid with 100 mM αG-rutin in the mucosal fluid were 0.14 and 0.01 in the gastric fundus, 0.49 and 0.17 in the gastric body, 2.04 and 0.39 in the jejunum, 2.90 and 0.68 in the ileum, 3.83 and 0.20 in the cecum, and 0.95 and 0.01 nmol/min/cm^2 in the colon respectively. Quercetin and quercetin conjugates were not detected in the serosal fluid.
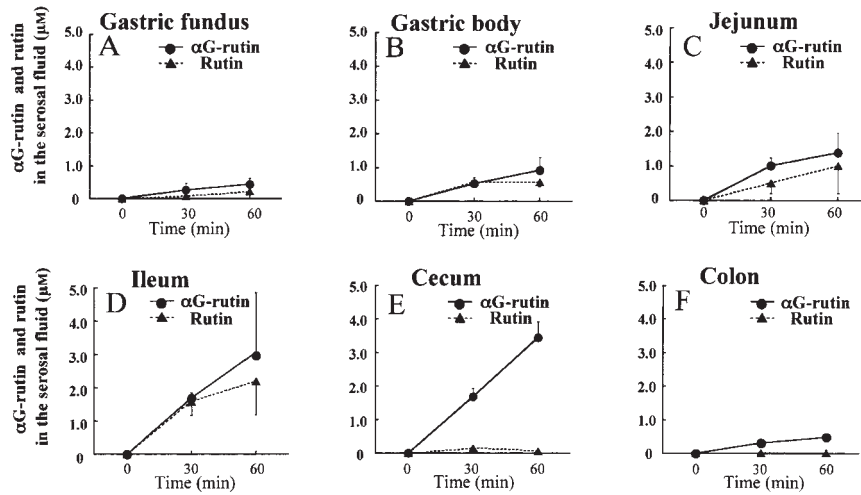
Fig. 3.　Concentrations of αG-Rutin and Rutin in the Isolated Stomach Fundus (A), Stomach Body (B), Jejunum (C), Ileum (D), Cecum (E), and Colon (F) of Rats after Incubation for 30 min or 60 min at 37 °C.

　　　HBS without αG-rutin was applied to the serosal bath and 1 mM αG-rutin-HBS was applied to the mucosal bath. Values are means ± SEM, n = 3.
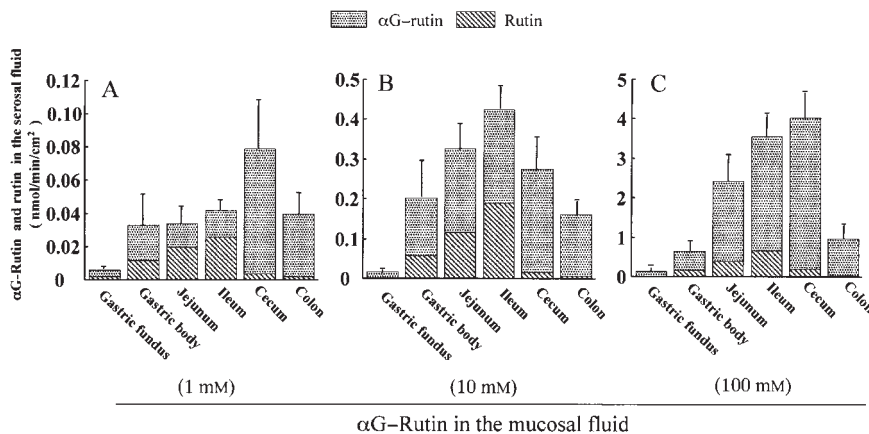

Fig. 4.　Dose-dependent Transportation of αG-Rutin in the Isolated Small Intestinal and Cecal Mucosa.

　　　HBS containing 1 (A), 10 (B), or 100 (C) mM αG-rutin was applied to the mucosal bath and incubated for 30 min at 37 °C. Values are means ± SEM, n = 7.

　　The TEER in the six intestinal portions before and after incubation for 30 min are shown in Table 1. The 30-min incubation with 100 mM αG-rutin did not affect the TEER for any portion of the intestine. The values in the mucosa of the gastric fundus were more than 10 times those of the other parts.

Discussion

　　In this study, we examined the absorption and metabolism of a D-glucose adduct of rutin, αG-rutin, using the isolated mucosa of the rat stomach and intestines. The serosa layers of the isolated mucosa were removed in the epithelial preparation used. Hence, as compared with an everted intestine sac, the ability to transport a substance could be measured more sensitively and precisely using an Ussing chamber. This procedure has been used for the transport of flavonoids in the gastrointestinal mucosa.[13] Hu *et al.*[14] demonstrated that intestinal tissue was viable for 3 h with an Ussing chamber. We also measured TEER and confirmed the viability of the above.

　　We found that αG-rutin is transported through the gastric and intestinal mucosa in intact form. Transport of αG-rutin increased time-dependently in the mucosa of the whole alimentary tract, including the stomach. It has been reported in *in vivo* studies that rutin is scarcely absorbed as such in the stomach or small intestine, is but hydrolyzed by enterobacterial enzymes and absorbed from the colon.[12,15,16] In contrast, we found that αG-rutin is transported through the mucosa of the stomach and small intestine. These results suggest that αG-rutin is more efficiently absorbed after oral ingestion than rutin. Furthermore, we found that αG-rutin and rutin

Table 1. TEER in the Rat Intestinal Epithelium before and after Incubation for 30 min with αG-Rutin

	TEER ($\Omega\,cm^{-2}$)			
	Pre-incubation		Post-incubation (100 mM αG-rutin)	
Stomach fundus	2,668 ± 622.0	b	2,840 ± 683.0	b
Stomach body	163 ± 14.0	a	178 ± 14.0	a
Jejunum	160 ± 5.4	a	160 ± 10.0	a
Ileum	140 ± 5.5	a	148 ± 7.3	a
Cecum	170 ± 11.8	a	208 ± 22.0	a
Colon	205 ± 19.0	a	230 ± 18.0	a
ANOVA *P*-values				
Portions (P)		<0.001		
Pre- or post-incubation (I)		0.7823		
P × I		0.9995		

Values are means ± SEM, n = 6. Values in two columns not sharing a superscript letter are significantly different (*P* < 0.05) by Duncan's multiple-range test. TEER, transepithelial electrical resistance.

were absorbed in the gastric body but not in the gastric fundus comparably with the other intestines, suggesting that the gastric body might be involved in absorption in the stomach. We observed very high TEER in the mucosa of the gastric fundus. The keratinization of the mucosal surface of the gastric fundus may be involved in the high TEER and low absorptive activities in this segment. The mechanism for flavonoid glycoside absorption in the stomach has not been clarified, and should be in the future.

We found that comparable amounts of rutin and αG-rutin appeared in the serosal fluid in the jejunal and ileal mucosa despite the occurrence of αG-rutin in the cecum and colon. αG-rutin is stable for the analytical procedure used in this study. Its preparation contains less than 1% rutin (data not shown). We also confirmed that rutin was present in the mucosal fluid of the jejunum and ileum specimens (9.2% and 16.1% of αG-rutin respectively). These results suggest that rutin is produced mainly from αG-rutin on the mucosal side of the small intestine. It has been shown that β-glycosidase in the rat small intestine efficiently hydrolyzes quercetin-3-*O*-β-glucosides, whereas rutin is a poor substrate for this enzyme.[17,18] Lactose-phlorizin hydrolase (LPH) in the sheep small intestine can also hydrolyze some quercetin glucosides, but not rutin.[19] These previous reports agree with our present result that quercetin aglycone was not detected in the mucosal or serosal fluids. The transport pathway responsible for rutin ought to be clarified.

The mechanism by which intact αG-rutin passes through the gastric and intestinal mucosa is not yet known. It has been found that quercetin-3-*O*-glucoside is absorbed as glycoside into the mucosal cells by way of sodium-dependent glucose transport[8,20,21] but it is uncertain whether that αG-rutin, consisting of three sugar moieties, passes through the brush border membrane as it is. There was a linear increase in αG-rutin transport from the mucosal side to the serosal side in the three intestinal segments at concentrations up to 100 mM αG-rutin (Fig. 4). This unsaturable increase in transport

suggests that a simple diffusion is responsible for αG-rutin absorption. It is more likely that αG-rutin is transported via tight junctions of the intestinal mucosa by diffusion.[13] Morand *et al.*[6] showed that quercetin was conjugated with glucuronide in the intestinal cells. After ingestion of quercetin aglycone or quercetin-3-*O*-glucoside, they are absorbed and conjugated in the intestinal cells. Absorption of αG-rutin through the paracellular pathway might be associated with no detection of quercetin aglycone or its conjugate in the serosal fluid. The difference in metabolism between these two glycosides might be due to differences in the absorptive pathways.

We conclude that a considerable portion of αG-rutin was absorbed intact across the mucosa of the stomach and intestine and was in part hydrolyzed to rutin on the mucosal side of the small intestine.

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