Synthesis of Oligosaccharides of Genistein and Quercetin as Potential Anti-inflammatory Agents

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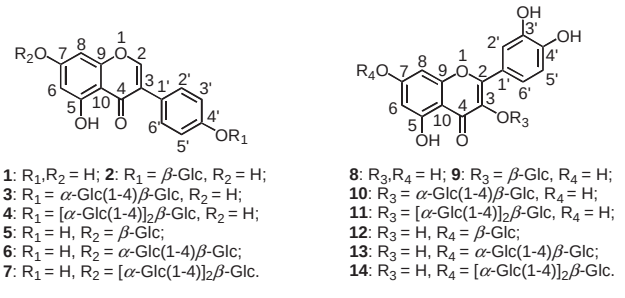
Genistein was glucosylated to its 7-*O*-β-D-glucoside by cultured cells of *Ipomoea batatas*, while cultured *Marchantia polymorpha* cells produced 4′-*O*-β-D-glucoside and 7-*O*-β-D-glucoside of genistein. On the other hand, quercetin was converted into its 3-*O*-β-D-glucoside by *I. batatas*, and *M. polymorpha* gave 3-*O*-β-D-glucoside and 7-*O*-β-D-glucoside of quercetin. These four glucosides were further glycosylated to the corresponding β-maltosides and β-maltotriosides by cyclodextrin glucanotransferase. Genistein 7-*O*-β-maltooligosaccharides, quercetin 3-*O*-β-glucoside, and quercetin 7-*O*-β-maltooligosaccharides showed potent inhibitory effects on histamine release from rat peritoneal mast cells.

Soy isoflavonoid, genistein, and onion flavonoid, quercetin, have been recognized as useful natural antioxidants, and have been reported to show antiallergic and anti-inflammatory activities such as inhibitory effects on histamine release from mast cells.[1] In spite of such physiological activities, their use as drugs and food ingredients is limited because of their water-insolubility and low absorbability after oral administration. Glycosylation using plant cultured cells and glycosyltransferases is useful for preparing water-soluble and stable glycosides from water-insoluble and unstable organic compounds.[2] Furthermore, it has been reported that glycosylation of lipophilic flavonoid, quercetin, improved its absorbability after oral administration.[3] From the viewpoint of pharmacological development of isoflavonoid and flavonoid, their glycoconjugation is of importance. We report, herein, the biocatalytic glycosylation of genistein and quercetin by cultured plant cells and cyclodextrin glucanotransferase (CGTase) to β-maltooligosides, and the inhibitory effects of the β-maltooligosides of genistein and quercetin on the histamine release from rat peritoneal mast cells.

Two plant cell cultures, i.e., *Ipomoea batatas* and *Marchantia polymorpha*, were tested for the glycosylation of genistein (**1**) and quercetin (**8**) (Figure 1).[4] Genistein (**1**) was glucosylated to genistein 7-*O*-β-D-glucoside (**5**) by cultured *I. batatas* cells in 61% yield. On the other hand, genistein 4′-*O*-β-D-glucoside (**2**, 9%) and genistein 7-*O*-β-D-glucoside (**5**, 30%) were isolated from cultured *M. polymorpha* cells treated with **1**. Although *I. batatas* cells had high potential to produce genistein 7-*O*-β-D-glucoside (**5**), no glycosylation occurred at the 4′-position of genistein with *I. batatas*.



1: R_1,R_2 = H; **2**: R_1 = β-Glc, R_2 = H;
3: R_1 = α-Glc(1-4)β-Glc, R_2 = H;
4: R_1 = [α-Glc(1-4)]$_2$$\beta$-Glc, R_2 = H;
5: R_1 = H, R_2 = β-Glc;
6: R_1 = H, R_2 = α-Glc(1-4)β-Glc;
7: R_1 = H, R_2 = [α-Glc(1-4)]$_2$$\beta$-Glc.

8: R_3,R_4 = H; **9**: R_3 = β-Glc, R_4 = H;
10: R_3 = α-Glc(1-4)β-Glc, R_4 = H;
11: R_3 = [α-Glc(1-4)]$_2$$\beta$-Glc, R_4 = H;
12: R_3 = H, R_4 = β-Glc;
13: R_3 = H, R_4 = α-Glc(1-4)β-Glc;
14: R_3 = H, R_4 = [α-Glc(1-4)]$_2$$\beta$-Glc.

Figure 1. Structures of substrates **1** and **8**, and glycosylation products **2**–**7** and **9**–**14**.

Table 1. Water-solubility of genistein (**1**), quercetin (**8**), and their glycosides **2**–**7** and **9**–**14**

Compound	Water-solubility /μmol mL^{-1}	Fold[a,b]
1	1.1×10^{-3}	1
2	4.1×10^{-2}	37
3	0.7	6.4×10^{2}
4	1.9	1.7×10^{3}
5	3.2×10^{-2}	28
6	0.5	4.5×10^{2}
7	1.2	1.0×10^{3}
8	3.6×10^{-3}	1
9	1.5×10^{-1}	42
10	1.2	3.3×10^{2}
11	3.1	8.5×10^{2}
12	1.4×10^{-1}	38
13	1.0	2.7×10^{2}
14	2.5	6.9×10^{2}

[a]Fold of water-solubility of glycosides **2**–**7** is expressed relative to that of their aglycone **1**, normalized to 1. [b]Fold of water-solubility of glycosides **9**–**14** is expressed relative to that of their aglycone **8**, normalized to 1.

Genistein 4′-*O*-β-D-glucoside (**2**), which had been produced by *M. polymorpha*, was glycosylated to genistein 4′-*O*-β-maltooligosaccharides in the presence of soluble starch by CGTase from *Bacillus macerans*.[5] Two products were obtained and identified as genistein 4′-*O*-β-maltoside (**3**, 32%) and genistein 4′-*O*-β-maltotrioside (**4**, 12%). Genistein 7-*O*-β-D-glucoside (**5**), which had been produced by *I. batatas*, was converted into genistein 7-*O*-β-maltoside (**6**, 30%) and genistein 7-*O*-β-maltotrioside (**7**, 7%) by CGTase.

Quercetin (**8**) was subjected to the same sequential glycosylation system using plant cell culture and CGTase. Cultured *I. batatas* cells converted quercetin (**8**) into quercetin 3-*O*-β-D-glucoside (**9**, 55%), whereas *M. polymorpha* glucosylated **8** to quercetin 3-*O*-β-D-glucoside (**9**, 19%) and quercetin 7-*O*-β-D-

Table 2. Effects of genistein (**1**), quercetin (**8**), and their glycosides **2**–**7** and **9**–**14** on histamine release from rat peritoneal mast cells

Compound	Histamine release/%[a]
None	39
1	6
2	37
3	41
4	45
5	4
6	6
7	12
8	9
9	12
10	44
11	43
12	7
13	10
14	15

[a]Compound 48/80 (0.35 μg/mL)-induced histamine release from rat peritoneal mast cells after treatment with or without test sample.

glucoside (**12**, 6%). Quercetin 3-*O*-β-D-glucoside (**9**) produced by *I. batatas* was then glycosylated to two β-maltooligosaccharides, quercetin 3-*O*-β-maltoside (**10**, 40%) and quercetin 3-*O*-β-maltotrioside (**11**, 14%), by CGTase. Also, quercetin 7-*O*-β-D-glucoside (**12**), which had been produced by *M. polymorpha*, was converted into quercetin 7-*O*-β-maltoside (**13**, 36%) and quercetin 7-*O*-β-maltotrioside (**14**, 10%) by CGTase.

The water-solubility of genistein β-glycosides **2**–**7** and quercetin β-glycosides **9**–**14** was examined and compared with that of aglycones, genistein (**1**) and quercetin (**8**) (Table 1).[6] In the case of genistein 4′-*O*-β-D-maltotrioside (**4**), conjugation of genistein (**1**) to three glucose units enhanced the water-solubility to 1.9 μmol/mL, which was 1.7 thousand fold higher than that of **1** (1.1×10^{-3} μmol/mL). The water-solubility of quercetin 3-*O*-β-D-maltotrioside (**11**) was increased to 3.1 μmol/mL, which was 8.5×10^2 fold higher than that of quercetin (**8**) (3.6×10^{-3} μmol/mL).

Next, effects of genistein (**1**), quercetin (**8**), and their β-glycosides **2**–**7** and **9**–**14** on compound 48/80-induced histamine release from rat peritoneal mast cells were examined (Table 2).[7,8] Rat peritoneal mast cells released a high level of histamine (control, 39%) when stimulated with 0.35 μg/mL of compound 48/80. Genistein (**1**) and quercetin (**8**) effectively inhibited the compound 48/80-induced histamine release from rat peritoneal mast cells, that is, % inhibitions of **1** and **8** were 85 and 77%. The inhibitory activities of genistein 7-*O*-β-glycosides **5**–**7** were 90, 85, and 69%, respectively. On the other hand, genistein 4′-*O*-β-glycosides **2**–**4** showed no inhibitory effects on histamine release. In the case of quercetin β-glycosides **9**–**14**, **9** and **12**–**14** exhibited potent inhibitory effects on histamine release.

Thus, 4′- and 7-*O*-β-maltooligosaccharides of genistein, and 3- and 7-*O*-β-maltooligosaccharides of quercetin[9] were synthesized by sequential glycosylation with cultured plant cells and CGTase. Genistein 7-*O*-β-maltooligosaccharides, quercetin 3-*O*-β-glucoside, and quercetin 7-*O*-β-maltooligosaccharides showed potent inhibitory effects on histamine release from rat peritoneal mast cells, indicating that the β-glycosides at C-7 of genistein and quercetin, and β-glucoside at C-3 of quercetin did not attenuate the anti-inflammatory effects of aglycones, i.e., genistein and quercetin. The present sequential glycosylation system using plant cell cultures and CGTase would be useful for preparation of water-soluble glycosides of isoflavonoid and flavonoid. Use of isoflavonoid glycosides and flavonoid glycosides would be a new approach to research of the structure–activity relationship of their aglycones, i.e., isoflavonoid and flavonoid. Further studies on the physiological properties of β-maltooligosaccharides of genistein and quercetin are now in progress.

References and Notes

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4 Typical procedure of glycosylation with cultured plant cells is as follows. A total of 1 mmol of genistein (**1**) was administered to ten 300-mL conical flasks (0.1 mmol/flask) containing 70 g of suspension cultured *I. batatas* cells, and the cultures were incubated at 25 °C for 5 days on a rotary shaker (120 rpm). After the incubation period, the cells and medium were separated by filtration with suction. The cells were extracted (×3) by homogenization with MeOH and the extract was concentrated. The residue was partitioned between H_2O and EtOAc. The H_2O layer was applied to a Diaion HP-20 column, and the column was washed with H_2O followed by elution with MeOH. The MeOH eluate was subjected to HPLC [column: YMC-Pack R&D ODS column (150 × 30 mm); solvent: MeOH-H_2O (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 mL/min] to give products.

5 Typical procedure of CGTase-catalyzed glycosylation is as follows. To a solution containing 10 mL of 25 mM sodium phosphate buffer (pH 7.0), 0.2 mmol of genistein 4′-*O*-β-D-glucoside (**2**), and 5 g of soluble starch was added 300 units of CGTase (Amano Pharmaceutical Co., Ltd). The mixture was incubated for 24 h at 40 °C. After centrifugation of the mixture at 3000 *g* for 10 min, the supernatant was subjected to Sephadex G-25 column chromatography. The glycoside fractions were lyophilized, and subjected to purification by preparative HPLC.

6 Each compound was stirred in water for 24 h at 25 °C. The mixture was centrifuged at 100000 *g* for 30 min at 25 °C. The concentration of test compounds was estimated on the basis of their peak areas using calibration curves prepared by HPLC analyses of authentic samples.

7 Peritoneal mast cells were collected from the abdominal cavity of rats (Male Wistar rats, Nippon SLC) and purified to a level higher than 95% according to a method previously described.[8] The purified mast cells were suspended in a physiological buffered solution (PBS) containing 145 mM NaCl, 2.7 mM KCl, 1.0 mM $CaCl_2$, 5.6 mM glucose, and 20 mM HEPES (pH 7.4) to give approximately 10^4 mast cells/mL. Cell viability was always greater than 90% as judged by the trypan blue exclusion test. Mast cells were preincubated with the test compound (1 μM) for 15 min at 37 °C, and subsequently exposed to compound 48/80 at 0.35 μg/mL. Histamine release was determined by a fluorometric assay according to the previously reported method, and was expressed as a percentage of total histamine.[8]

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9 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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