Glycosylation and Malonylation of Quercetin, Epicatechin, and Catechin by Cultured Plant Cells

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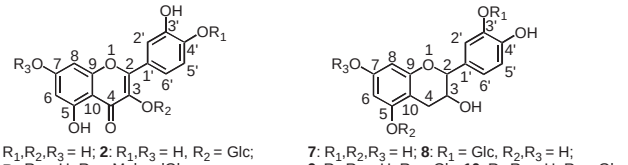
Quercetin was converted to quercetin 3-*O*-(6-*O*-malonyl)-β-D-glucoside through the regioselective glucosylation at 3-position of quercetin and the following regioselective malonylation at 6-position of sugar moiety of quercetin 3-*O*-β-D-glucoside with cultured *Nicotiana tabacum* cells. On the other hand, *N. tabacum* cells glucosylated epicatechin and catechin to the corresponding 3′-, 5-, and 7-*O*-β-D-glucosides, respectively.

Natural polyphenols such as quercetin, epicatechin, and catechin occur in plants and have diverse biological activities. Quercetin is a principal flavonoid in onion *Allium cepa*, and has anti-oxidative, anticarcinogenic, and anticancer activities.[1] Epicatechin and catechin are main flavonoids found in tea *Camellia seinensis*, and have been reported on their anti-oxidative, anti-angiogenesis, anti-inflammatory, and anticancer effects.[2] Irrespective of such physiological and pharmacological activities, the use of these polyphenols as drugs and food additives is limited because of their water-insolubility and low absorbability after oral administration. Glycosylation and acylation allows the conversion of water-insoluble and unstable organic compounds into the corresponding water-soluble and stable ones to improve their bioavailability and pharmacological properties. In addition, it has been reported that quercetin 3-*O*-β-D-glucoside was better absorbed than quercetin itself in healthy ileostomy volunteers.[3] From the physiological point of view, glycosylation and acylation of plant flavonoids are of importance and interest. However, little attention has been paid to the glycosylation and acylation of plant flavonoids such as quercetin, epicatechin, and catechin by cultured plant cells. We report, herein, the biotransformation, such as glycosylation and malonylation, of quercetin, epicatechin, and catechin by cultured plant cells of *Nicotiana tabacum*.

Cultured plant cells of *N. tabacum* (fresh weight of 50 g) were individually transplanted to 300-mL conical flasks containing 100 mL of Murashige and Skoog's medium (pH 5.7)[4] prior to this experiment, and were grown for additional 2 weeks on a rotary shaker (120 rpm) under illumination (4000 lux). A total of 1 mmol of quercetin (**1**) was administered to ten 300-mL conical flasks (0.1 mmol/flask) containing the suspension cultured *N. tabacum* 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]. Five products were isolated, and yield of the products was calculated on the basis of the peak area from HPLC using the calibration curves prepared by the HPLC analyses of authentic glycosides. The structure of the products[5] was identified using HR-FAB-MS, ^{1}H and ^{13}C NMR, H–H COSY, and C–H COSY (Figure 1). Quercetin 3-*O*-β-D-glucoside (**2**)[6] was obtained as the major product in 51% yield together with 3-*O*-(6-*O*-malonyl)-β-D-glucoside (**3**, 10%), 3-*O*-[6-*O*-(α-L-rhamnosyl)]-β-D-glucoside (β-rutinoside, **4**, 3%), 3,4′-*O*-β-D-diglucoside (**5**, 1%), and 3,7-*O*-β-D-diglucoside (**6**, 1%).

Time course experiment was carried out to investigate the ability of cultured *N. tabacum* cells to biotransform quercetin (**1**). As shown in Figure 2, formation of 3-*O*-β-D-glucoside (**2**)



1: R_1,R_2,R_3 = H; **2**: R_1,R_3 = H, R_2 = Glc; **3**: R_1,R_3 = H, R_2 = MalonylGlc; **4**: R_1,R_3 = H, R_2 = GlcRham; **5**: R_1,R_2 = Glc, R_3 = H; **6**: R_1 = H, R_2,R_3 = Glc

7: R_1,R_2,R_3 = H; **8**: R_1 = Glc, R_2,R_3 = H; **9**: R_1,R_3 = H, R_2 = Glc; **10**: R_1,R_2 = H, R_3 = Glc

Figure 1. Structures of substrates **1** and **7**, and biotransformation products **2**–**6** and **8**–**10**.

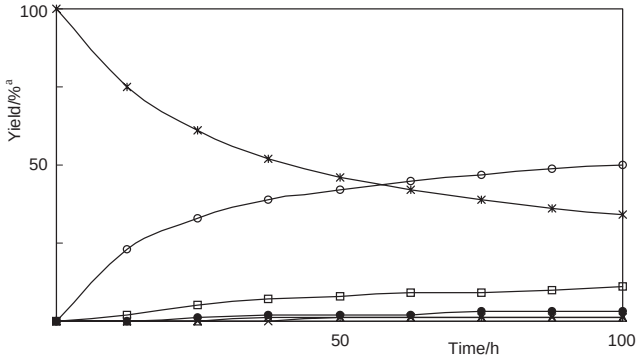


Figure 2. Biotransformation of quercetin (**1**) by cultured cells of *N. tabacum*. [a]Yield was determined by HPLC analysis using calibration curves of authentic glycosides as internal standard. Yields of **1** (∗), **2** (○), **3** (□), **4** (●), **5** (×), and **6** (△) are plotted.

occurred at early stage of incubation. Malonylglucoside **3**, rutinoside **4**, and other two disaccharides **5** and **6** were predominantly produced after 10 h. These findings suggest that quercetin 3-*O*-β-D-glucoside (**2**) was first formed and then malonylation and further glycosylation occurred to give **3**, **4**, **5**, and **6**. To our knowledge, this is the first description of the glycosylation and malonylation of exogenously added quercetin by cultured plant cells.

Next, epicatechin [(2*R*,3*R*)-**7**] and catechin [(2*R*,3*S*)-**7**] were subjected to the same biotransformation system. After the 5 day-incubation, three products were isolated from the MeOH extracts of *N. tabacum* cells treated with epicatechin [(2*R*,3*R*)-**7**]. The products were identified as 3′-*O*-β-D-glucoside [(2*R*,3*R*)-**8**, 38%],[7] 5-*O*-β-D-glucoside [(2*R*,3*R*)-**9**, 7%],[8] and 7-*O*-β-D-glucoside [(2*R*,3*R*)-**10**, 15%].[9] No further glycosylation and acylation products were obtained. Similar tendency was found in the biotransformation of catechin [(2*R*,3*S*)-**7**] with *N. tabacum* cells. Three products, 3′-*O*-β-D-glucoside [(2*R*,3*S*)-**8**, 46%],[10] 5-*O*-β-D-glucoside [(2*R*,3*S*)-**9**, 10%],[10] and 7-*O*-β-D-glucoside [(2*R*,3*S*)-**10**, 17%],[10] were isolated, and no further products were detected despite careful HPLC analyses. This is the first report on the glycosylation of epicatechin by cultured plant cells.

Thus, this study demonstrates that cultured plant cells of *N. tabacum* are able to convert quercetin into β-glucoside, malonyl β-glucoside, β-rutinoside, and two β-diglucosides. Cultured *N. tabacum* cells showed high regioselectivity in the glycosylation of quercetin, producing preferentially quercetin 3-*O*-glycosides. Also malonylation occurred regioselectively at 6-position of sugar moiety of quercetin 3-*O*-β-D-glucoside to afford quercetin 3-*O*-(6-*O*-malonyl)-β-D-glucoside. On the other hand, biotransformation of epicatechin and catechin resulted in formation of 3′-, 5-, and 7-*O*-β-D-glucosides, respectively. It is postulated that the C–C double bond at 2-position of quercetin is necessary for the regioselective glucosylation at 3-position of quercetin. Further studies on the enzymes participating in the glycosylation and malonylation of these polyphenols are now in progress.

References and Notes

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5 Spectral data for selected products; product **3**: Mp: 195–201 °C; HR-FAB-MS: m/z 573.0855 $[M + Na]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07 (2H, s, malonyl CH_2), 3.21–4.22 (6H, m, H-2″, 3″, 4″, 5″, 6″), 5.37 (1H, d, $J = 7.2$ Hz, H-1″), 6.22 (1H, d, $J = 2.0$ Hz, H-6), 6.41 (1H, d, $J = 2.0$ Hz, H-8), 6.85 (1H, d, $J = 8.8$ Hz, H-5′), 7.50 (1H, dd, $J = 8.8$, 1.8 Hz, H-6′), 7.55 (1H, d, $J = 2.0$ Hz, H-2′); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.0 (malonyl CH_2), 63.3 (C-6″), 69.2 (C-4″), 73.7 (C-2″), 73.9 (C-5″), 76.1 (C-3″), 93.1 (C-8), 98.5 (C-6), 101.2 (C-1″), 103.9 (C-10), 115.1 (C-2′), 116.3 (C-5′), 120.9 (C-6′), 121.3 (C-1′), 133.5 (C-3), 144.9 (C-3′), 148.7 (C-4′), 156.1 (C-9), 156.5 (C-2), 161.2 (C-5), 164.1 (C-7), 166.7 (malonyl CO), 167.9 (malonyl CO), 177.5 (C-4). Product **4**: Mp: 189–196 °C; HR-FAB-MS: m/z 633.1435 $[M + Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.12 (3H, d, $J = 6.0$ Hz, H-6‴), 3.34–3.80 (10H, m, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴, 6″), 4.52 (1H, d, $J = 1.6$ Hz, H-1‴), 5.11 (1H, d, $J = 7.6$ Hz, H-1″), 6.21 (1H, d, $J = 2.0$ Hz, H-6), 6.39 (1H, d, $J = 2.0$ Hz, H-8), 6.87 (1H, d, $J = 8.8$ Hz, H-5′), 7.63 (1H, dd, $J = 8.8$, 2.0 Hz, H-6′), 7.67 (1H, d, $J = 2.0$ Hz, H-2′); ^{13}C NMR (100 MHz, CD_3OD): δ 17.9 (C-6‴), 68.5 (C-6″), 69.6 (C-5‴), 71.2 (C-4″), 72.0 (C-2‴), 72.2 (C-3‴), 73.9 (C-4‴), 75.7 (C-2″), 77.1 (C-3″), 78.1 (C-5″), 94.8 (C-8), 99.8 (C-6), 102.3 (C-1‴), 104.6 (C-10), 105.5 (C-1″), 115.9 (C-2′), 117.6 (C-5′), 123.0 (C-6′), 123.4 (C-1′), 135.5 (C-3), 145.7 (C-4′), 149.6 (C-3′), 158.3 (C-2), 159.1 (C-9), 162.8 (C-5), 165.8 (C-7), 179.2 (C-4). Product **5**: Mp: 185–189 °C; HR-FAB-MS: m/z 649.1382 $[M + Na]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.09–3.75 (12H, m, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴, 6″, 6‴), 4.86 (1H, d, $J = 7.6$ Hz, H-1‴), 5.50 (1H, d, $J = 7.6$ Hz, H-1″), 6.25 (1H, d, $J = 2.0$ Hz, H-6), 6.38 (1H, d, $J = 2.0$ Hz, H-8), 7.20 (1H, d, $J = 8.8$ Hz, H-5′), 7.57 (1H, dd, $J = 8.8$, 2.0 Hz, H-6′), 7.67 (1H, d, $J = 2.0$ Hz, H-2′); ^{13}C NMR (100 MHz, DMSO-d_6): δ 61.0 (C-6‴), 61.1 (C-6″), 70.0 (C-4‴), 70.3 (C-4″), 73.7 (C-2‴), 74.5 (C-2″), 76.3 (C-3‴), 76.7 (C-3″), 77.6 (C-5‴), 77.9 (C-5″), 94.1 (C-8), 99.2 (C-6), 101.3 (C-1″), 101.7 (C-1‴), 105.0 (C-10), 115.9 (C-2′), 117.0 (C-5′), 121.5 (C-6′), 125.0 (C-1′), 134.3 (C-3), 146.5 (C-4′), 147.7 (C-3′), 156.0 (C-2), 156.8 (C-9), 161.6 (C-5), 164.7 (C-7), 174.8 (C-4). Product **6**: Mp: 181–186 °C; HR-FAB-MS: m/z 649.1390 $[M + Na]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.01–3.70 (12H, m, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴, 6″, 6‴), 4.88 (1H, d, $J = 7.6$ Hz, H-1‴), 5.51 (1H, d, $J = 7.6$ Hz, H-1″), 6.41 (1H, d, $J = 2.0$ Hz, H-6), 6.80 (1H, d, $J = 2.0$ Hz, H-8), 7.22 (1H, d, $J = 8.8$ Hz, H-5′), 7.63 (1H, dd, $J = 8.8$, 2.0 Hz, H-6′), 7.67 (1H, d, $J = 2.0$ Hz, H-2′); ^{13}C NMR (100 MHz, DMSO-d_6): δ 60.9 (C-6‴), 61.5 (C-6″), 69.9 (C-4‴), 70.2 (C-4″), 73.1 (C-2‴), 74.2 (C-2″), 76.5 (C-3″, C-3‴), 77.7 (C-5″, C-5‴), 94.6 (C-8), 99.5 (C-6), 99.7 (C-1‴), 100.5 (C-1″), 105.6 (C-10), 115.5 (C-5′), 116.6 (C-2′), 120.5 (C-6′), 124.4 (C-1′), 134.2 (C-3), 146.2 (C-4′), 147.8 (C-3′), 156.1 (C-9), 156.3 (C-2), 160.8 (C-5), 163.0 (C-7), 177.6 (C-4).

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