Biological Degradation of Taxol by Action of Cultured Cells on 7-Acetyltaxol-2″-yl Glucoside

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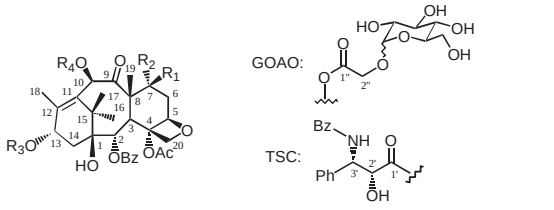
Biodegradation pathways of taxol in cultured cells of *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7942, *Marchantia polymorpha*, *Nicotiana tabacum*, and *Glycine max* were investigated using a water-soluble taxol derivative, 7-acetyltaxol-2″-yl glucoside, as the substrate. Although cyanobacteria, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, and a lower plant, *M. polymorpha*, catalyzed the epimerization at 7-position of taxol skeleton, no epimerization occurred with higher plants, *N. tabacum* and *G. max*. On the other hand, *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7942, *M. polymorpha*, and *N. tabacum* catalyzed hydrolysis at 13-position of taxol to give baccatin III and 10-deacetyl baccatin III. Both cyanobacteria cells also deacetylated 7-*epi*-baccatin III at its 10-position. *M. polymorpha* and *G. max* deacetylated at 10-position of taxol.

Taxol, a diterpenoid from the Pacific Yew *Taxus brevifiolia*, is one of the most important anticancer drugs, and is used for treatment of ovarian, breast, and various other cancers worldwide.[1] From the pharmacological point of view, the pathway of the biological degradation of taxol is interesting and important. However, little attention has been paid to the biological degradation of taxol by cultured cells, because it is hardly dissolved in aqueous solution. Use of an ester-linked taxol–sugar conjugate, which is higher water-soluble than taxol and would be hydrolyzed by hydrolytic enzymes in the living cells, is a new approach to biodegradation research of taxol. Additionally, taxol–sugar conjugates have attracted much attention clinically, because of their potential to be useful prodrugs.[2] The biodegradation pathway of taxol–sugar conjugates is also important. Herein, we report the biodegradation of taxol with cultured cells of *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7942, *Marchantia polymorpha*, *Nicotiana tabacum*, and *Glycine max* using a water-soluble taxol derivative, 7-acetyltaxol-2″-yl glucoside, as the substrate.

Biotransformation of 7-acetyltaxol-2″-yl glucoside (**1**)[3] was examined by incubating **1** with each cell cultures.[4] 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 each compounds. The structures of the products were identified based on their HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, and HMBC data (Figure 1).

Biotransformation of **1** with cultured *Synechocystis* sp. PCC 6803 cells gave taxol (**2**, 7%), 7-*epi*-taxol (**4**, 2%),[5] the cytotoxicity of which is only slightly less than that of taxol,[6] baccatin III



1: R_1 = GOAO, R_2 = H, R_3 = TSC, R_4 = Ac; **2**: R_1 = OH, R_2 = H, R_3 = TSC, R_4 = Ac;
3: R_1 = OH, R_2,R_4 = H, R_3 = TSC; **4**: R_1 = H, R_2 = OH, R_3 = TSC, R_4 = Ac;
5: R_1 = OH, R_2,R_3 = H, R_4 = Ac; **6**: R_1 = OH, R_2,R_3,R_4 = H;
7: R_1,R_3 = H, R_2 = OH, R_4 = Ac; **8**: R_1,R_3,R_4 = H, R_2 = OH.

Figure 1. Structures of substrate **1** and biotransformation products **2**–**8**.

(**5**, 33%), 10-deacetylbaccatin III (**6**, 16%), 7-*epi*-baccatin III (**7**, 27%), and 10-deacetyl-7-*epi*-baccatin III (**8**, 10%) (Table 1). No products which retained the (glucosyloxy)acetyl (GOA) group at their 7-position were detected. These suggest that the GOA group of **1** was readily hydrolyzed to give taxol (**2**), and that other hydrolysis and epimerization products **4**–**8** were produced by the action of *Synechocystis* sp. PCC 6803 cells on **2**. Feeding experiment of 7-*epi*-taxol (**4**) or baccatin III (**5**) established that 7-*epi*-baccatin III (**7**) was produced from both **4** and **5**. These findings revealed that *Synechocystis* sp. PCC 6803 hydrolyzed 10-acetoxy group of baccatin III and 7-*epi*-baccatin III, and 13-TSC (taxol side chain) group of taxol skeleton, and that epimerization of taxol and baccatin III occurred at their 7-positions. No formation of ketone intermediates was found during the incubation period. Also *Synechococcus* sp. PCC 7942 converted **1** into **2** (31%), **4** (24%), **5** (23%), **6** (4%), **7** (15%), and **8** (2%).

In contrast, biotransformation of **1** by cultured cells of liverwort *M. polymorpha* or higher plants, *N. tabacum* and *G. max*, was examined. Biotransformation was performed under illumination (4000 lux, *M. polymorpha*) or in the dark (*N. tabacum*

Table 1. Biotransformation of 7-acetyltaxol-2″-yl glucoside (**1**) by cultured cells

Cell line	Products/%						
	2	**3**	**4**	**5**	**6**	**7**	**8**
Synechocystis sp. PCC 6803	7	0	2	33	16	27	10
Synechococcus sp. PCC 7942	31	0	24	23	4	15	2
M. polymorpha	17	10	8	2	8	0	0
N. tabacum	25	0	0	11	10	0	0
G. max	16	6	0	0	0	0	0

and *G. max*) in freshly prepared medium (MSK-2 medium for *M. polymorpha* and MS medium for *N. tabacum* and *G. max*). After the 7 day-incubation of **1** with cultured *M. polymorpha* cells, five products **2** (17%), **3** (10%), **4** (8%), **5** (2%), and **6** (8%) were isolated. Feeding experiment of 10-deacetyltaxol (**3**) or baccatin III (**5**) revealed that 10-deacetylbaccatin III (**6**) was predominantly formed from **5** rather than **3**. On the other hand, cultured *N. tabacum* cells converted **1** into three products **2** (25%), **5** (11%), and **6** (10%). Biotransformation of **1** with *G. max* gave only two products **2** (16%) and **3** (6%). No epimerization occurred through the transformation with both *N. tabacum* and *G. max*. In the case of the biotransformation by these three cell cultures, a large amount of unreacted substrate **1** was recovered.

The results of this experiment revealed, for the first time, diverse biodegradation pathways of taxol in cultured cells by using a water-soluble taxol derivative, 7-acetyltaxol-2″-yl glucoside, as the substrate. Two cyanobacteria, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, were able to catalyze hydrolysis at 13-position of taxol, deacetylation at 10-position of baccatin III and 7-*epi*-baccatin III, and epimerization at 7-position of taxol and baccatin III. Liverwort *M. polymorpha* could catalyze hydrolysis at 13-position of taxol, deacetylation at 10-position of taxol and baccatin III, and epimerization at 7-position of taxol. On the other hand, higher plants, *N. tabacum* and *G. max*, could not catalyze epimerization of taxol skeleton. Hydrolysis occurred at 13-position of taxol (*N. tabacum*), and 10-position of taxol (*G. max*) or baccatin III (*N. tabacum*). Further studies on the enzymes participating in the hydrolysis and epimerization of these taxoid compounds are now in progress.

References and Notes

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3 Coupling of 2′-TES ester of taxol with 2,3,4,6-tetra-*O*-benzylglucosyloxyacetic acid (1.2 equiv.) [EDCI, DMAP, CH_2Cl_2, rt] provided 7-acetyl-2′-TES-taxol-2″-yl 2,3,4,6-tetra-*O*-benzylglucoside (99%), which was deprotected with Pd black in wet acetic acid to give **1** (98%). Spectral data for **1**: HRFABMS: m/z 1096.3136 $[M + Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.10 (3H, s, H-16), 1.16 (3H, s, H-17), 1.78 (3H, s, H-19), 1.82 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.02 (1H, dd, $J = 15.2$, 9.0 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.21 (1H, dd, $J = 15.2$, 9.0 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.59 (1H, m, H-6α), 3.30–3.80 (8H, m, H-2″, 2‴, 3‴, 4‴, 5‴, 6‴), 3.90 (1H, d, $J = 7.2$ Hz, H-3), 4.19 (3H, m, H-7, 20), 4.75 (1H, d, $J = 5.2$ Hz, H-2′), 4.92–4.95 (1H, m, H-1‴), 5.01 (1H, d, $J = 9.2$ Hz, H-5), 5.60 (2H, m, H-2, 3′), 6.15 (1H, t, $J = 9.0$ Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, $J = 7.6$ Hz, *p*-H in Ph), 7.39–7.59 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.66 (1H, t, $J = 7.6$ Hz, *p*-H in OBz), 7.85 (2H, d, $J = 8.2$ Hz, *o*-H in NBz), 8.11 (2H, d, $J = 8.2$ Hz, *o*-H in OBz); ^{13}C NMR (100 MHz, CD_3OD): δ 11.3 (C-19), 14.7 (C-18), 20.7 (CH_3 in 10Ac), 22.2 (C-16), 23.1 (CH_3 in 4Ac), 26.7 (C-17), 34.2 (C-6), 36.3 (C-14), 44.5 (C-3, C-15), 57.1 (C-3′), 57.7 (C-8), 62.4 (C-6‴), 65.9 (C-2″), 71.4 (C-7, C-13), 72.1 (C-4‴), 73.6 (C-5‴), 74.1 (C-2‴), 74.8 (C-2′), 75.1 (C-3‴), 75.7 (C-2), 76.6 (C-10), 77.2 (C-20), 78.8 (C-1), 81.8 (C-4), 85.0 (C-5), 100.5, 100.6 (C-1‴), 128.6, 129.1, 129.7, 131.2, 132.5, 134.1, 134.5, 135.4, 139.8 (C-11, Ar-C in NBz, Ar-C in OBz, Ar-C in Ph), 142.1 (C-12), 167.6 (C=O in OBz), 170.0 (C=O in NBz), 170.9 (C-1″), 171.3 (C=O in 4Ac), 171.9 (C=O in 10Ac), 174.3 (C-1′), 203.1 (C-9).

4 Typical biotransformation procedures were as follows. Prior to this experiment, 5 g (fresh weight) of mature cultured cells of cyanobacterium, *Synechocystis* sp. PCC 6803, was individually transplanted to 300-mL conical flasks containing 100 mL of freshly prepared BG-11 medium. The cultures were grown for 2 weeks on a rotary shaker (120 rpm) under illumination (4000 lux). A total of 0.03 mmol of a water-soluble taxol derivative, **1**, was added to three 300-mL conical flasks (0.01 mmol/flask) containing the cultured cyanobacterium cells. The flasks were incubated at 25 °C for further 7 days on a rotary shaker (120 rpm) under illumination (4000 lux). After incubation, the cells and medium were separated by centrifugation at 10000 g for 5 min. The cells were extracted (×3) by homogenization in MeOH, and the extract was concentrated. The residue was partitioned between H_2O and CH_2Cl_2. The medium was extracted (×3) with CH_2Cl_2. The CH_2Cl_2 fractions were combined, concentrated, and purified by HPLC [column: YMC-Pack R&D ODS column (150 × 30 mm); solvent: MeOH–H_2O (13:7, v/v); detection: UV (227 nm); flow rate: 1.0 mL/min] to give products.

5 Spectral data for product **4**: HRFABMS: m/z 876.3207 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$): δ 1.16 (3H, s, H-16), 1.21 (3H, s, H-17), 1.68 (3H, s, H-19), 1.81 (3H, s, H-18), 2.17 (3H, s, CH_3 in 10Ac), 2.25 (1H, dd, $J = 15.4$, 9.0 Hz, H-14a), 2.29 (1H, m, H-6a), 2.35 (1H, m, H-6b), 2.41 (1H, dd, $J = 15.4$, 9.0 Hz, H-14b), 2.51 (3H, s, CH_3 in 4Ac), 3.70 (1H, d, $J = 12.0$ Hz, H-7), 3.90 (1H, d, $J = 7.2$ Hz, H-3), 4.39 (2H, m, H-20), 4.81 (1H, d, $J = 3.0$ Hz, H-2′), 4.91 (1H, d, $J = 9.2$ Hz, H-5), 5.76 (1H, d, $J = 7.2$ Hz, H-2), 5.82 (1H, d, $J = 9.2$ Hz, H-3′), 6.25 (1H, t, $J = 9.2$ Hz, H-13), 6.80 (1H, s, H-10), 7.35–7.58 (10H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph, *p*-H in Ph), 7.62 (1H, t, $J = 7.6$ Hz, *p*-H in OBz), 7.75 (2H, d, $J = 8.2$ Hz, *o*-H in NBz), 8.17 (2H, d, $J = 8.2$ Hz, *o*-H in OBz); ^{13}C NMR ($CDCl_3$): δ 14.7 (C-18), 16.0 (C-19), 20.8 (CH_3 in 10Ac), 21.4 (C-16), 22.5 (CH_3 in 4Ac), 26.0 (C-17), 35.5 (C-14), 36.2 (C-6), 40.3 (C-3), 42.7 (C-15), 55.0 (C-3′), 57.7 (C-8), 72.5 (C-13), 73.2 (C-2′), 75.5 (C-2), 75.7 (C-7), 77.5 (C-20), 78.0 (C-10), 79.0 (C-1), 82.0 (C-4), 83.0 (C-5), 126.9, 128.5, 129.0, 129.2, 133.5, 133.7, 138.1, 139.6 (C-11, C-12, Ar-C in NBz, Ar-C in OBz, Ar-C in Ph), 167.0 (C=O in NBz), 167.3 (C=O in OBz), 170.0 (C=O in 10Ac), 172.3 (C=O in 4Ac), 173.0 (C-1′), 207.5 (C-9).

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