PERGAMON

Phytochemistry 53 (2000) 533–538

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Purification and characterization of UDP-glucuronate: baicalein 7-*O*-glucuronosyltransferase from *Scutellaria baicalensis* Georgi. cell suspension cultures

Shigeyuki Nagashima, Masao Hirotani*, Takafumi Yoshikawa

School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Received 26 May 1999; received in revised form 22 September 1999

Abstract

UDP-glucuronate: baicalein 7-*O*-glucuronosyltransferase (UBGAT) catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to the 7-OH of baicalein. UBGAT was purified from cultured cells of *Scutellaria baicalensis* Georgi (Lamiaceae). It was purified 95-fold using various chromatography and chromatofocusing procedures to apparent homogeneity. The M_r was estimated to be 110 kDa by gel filtration chromatography with a 52 kDa subunit by SDS-PAGE. The isoelectric point was pH 4.8. UBGAT was specific to UDP-glucuronic acid as a sugar donor and flavones with substitution *ortho*- to the 7-OH group such as baicalein (6-OH), scutellarein (6-OH) and wogonin (8-OMe).

Keywords: *Scutellaria baicalensis*; Lamiaceae; Baicalin; Baicalein; Cell culture; Glucuronosyltransferase

1. Introduction

The dried roots of *Scutellaria baicalensis* Georgi (Lamiaceae) are frequently used as a Chinese herbal medicine to treat bacterial infections. The dried roots contain over 30 kinds of flavonoids (Tang & Eisenbrand, 1992). It has been reported that the major flavonoid baicalin has anti-allergic (Koda, Watanabe, Yanagihara, Nagai & Sakamoto, 1977), anti-HIV effect (Konoshima et al., 1992) and anti-tumor activities (Li, Fu, Yan, Baylor, Ruscetti & Kung, 1993). Baicalin has glucuronic acid as a sugar moiety and lacks a hydroxyl group at the 4′ position of the B-ring. Typical flavonoid glycosides have glucose as a sugar moiety and a 4′ hydroxyl group derived from 4-coumaroyl CoA as a precursor.

The UDP-glycosyltransferases (UGT) represent a super family of enzymes that catalyze transfer of the glycosyl group from a nucleotide sugar to a small hydrophobic molecule (aglycone). Mammalian UDP-glucuronosyltransferases (UDPGTs: EC 2.4.1.17) have been studied in detail because of their importance in drug and xenobiotic metabolism (Mackenzie et al., 1997). Flavonoid-specific UDP-sugar transferases from plants have been widely investigated, and they are considered to be late or terminal steps in flavonoid biosynthesis. Most are UDP-glucosyltransferases, for which some cDNAs have already been cloned and characterized (Heller & Forkmann, 1994). However, there are few reports of UBGATs from plants; in the case of flavonoids, there is only a report of partial purification from rye primary leaves (Schulz & Weissenböck, 1988), for which a cDNA has not yet been cloned.

It is known that *S. baicalensis* possesses a β-glucuronidase (EC 3.2.1.31) called baicalinase, which hydrolyzes baicalin to baicalein and glucuronic acid (Miwa, 1932, 1935, 1936). A baicalinase had already been purified and characterized from suspension cultured cells of *S. baicalensis* (Morimoto, Harioka & Shoyama, 1995) and from dried Scutellariae radix (Ikegami, Mat-

* Corresponding author. Fax: +81-3-3444-6192.
E-mail address: hirotanim@platinum.pharm.kitasato-u.ac.jp (M. Hirotani).

0031-9422/00/$ - see front matter
PII: S0031-9422(99)00593-2

Table 1
Purification of UBGAT from *S. baicalensis* cell suspension cultures

Purification step	Total activity (nkat)	Total protein (mg)	Specific activity (nkat mg^{-1})	Recovery (%)	Purification (-fold)
Crude	772.5	359.3	2.2	100	1
Ammonium sulfate	730.8	248.6	2.9	94.6	1.4
Sephadex A-50	718.8	24.4	29.4	93.0	13.7
Phenyl Sepharose CL-4B	237.2	4.0	59.3	30.7	27.6
Sephacryl S-200	133.5	0.8	171.6	17.3	79.8
Mono P	14.3	0.07	203.7	1.9	94.7

sunae, Hisamitsu, Kurihara, Yamamoto & Murakoshi, 1995). However, purification of the corresponding UDP-glucuronate: baicalein 7-*O*-glucuronosyltransferase (UBGAT) has not yet been reported. Previously, we have reported that three strains of calli from *S. baicalensis* had variations in baicalinase and UBGAT activities (Hirotani, Nagashima & Yoshikawa, 1998). We here report on the purification and characterization of UBGAT from *S. baicalensis* cell suspension culture.

2. Results and discussion

UBGAT was purified from cultured cells of *S. baicalensis*. The purification protocol is shown in Table 1. UBGAT was purified 95-fold with 1.9% recovery. In the polybuffer 74 used for chromatofocusing on Mono P, UBGAT showed lower activity than Sephacryl S-200 fraction. However, after the buffer exchange to buffer B, specific activities reached 200 nkat mg^{-1} protein. Fig. 1 shows the results of the SDS-PAGE of protein fractions from the chromatographic steps of UBGAT purification indicating that UBGAT was purified to apparent homogeneity.

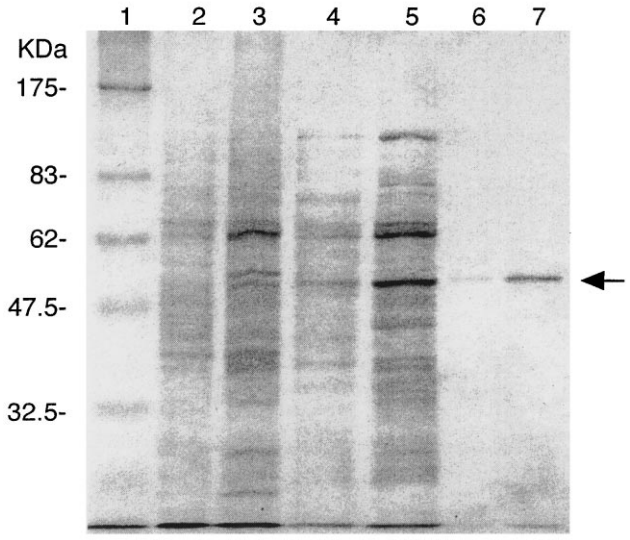


Fig. 1. SDS-PAGE of protein from the UBGAT purification steps, silver stained. Lane 1: molecular weight marker proteins; Lane 2: crude protein (6.7 μg); Lane 3: 30–50% $(NH_4)_2SO_4$ fraction (20.5 μg); Lane 4: DEAE-Sehadex A-50 fraction (1.1 μg); Lane 5: Phenyl Sepharose CL-4B fraction (0.7 μg); Lane 6: Sephacryl S-200 fraction (0.2 μg); Lane 7: Mono P fraction (0.5 μg). An arrow shows the UBGAT protein.

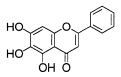
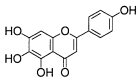
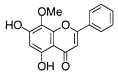
The apparent M_r of native UBGAT was estimated to be 110 kDa based on the gel filtration chromatography on a calibrated Sephacryl S-200 column and the M_r in the denatured condition was estimated to be 52 kDa from the results of SDS-PAGE, signifying that UBGAT is composed of a homo-dimer in cells. It is known that the dimeric form of a glycosyltransferase is very rare even among mammalian UDPGTs (Kleene & Berger, 1993). There is, however, a report of a dimeric UGT in the plant kingdom; Kamsteeg, van Brederode & van Nigtevecht (1978) reported that UDP-glucose: cyanidin 3-*O*-glucosyltransferase from petals of the red campion (*Silene dioica*) eluted at about 125 and 60 kDa from gel filtration chromatography, showing that the enzyme could exist as a dimer and an active monomer. In our experiments, UBGAT was eluted as a single peak from gel filtration chromatography. This result suggests that UBGAT does not exist or function as a monomer whether UBGAT can function as a monomer is a topic of interest requiring further analysis, e.g., expression of UBGAT cDNA in *Escherichia coli*.

UBGAT was eluted at pH 4.8 from chromatofocusing on Mono P, indicating a pI of 4.8. The UBGAT activity was recovered in the supernatant after ultracentrifugation at 105,000 *g* indicating that UBGAT exists in the soluble fraction of the cells.

UBGAT from the Sephacryl S-200 fraction was stable at 4°C for more than two weeks without loss of activity. UBGAT activity remained at 50 and 25% after incubation at room temperature for 3 and 24 h, respectively. The pH optimum was 7.5 in 50 mM Tris–HCl buffer, and the optimum temperature was in the range of 30–40°C. Proteins of the Sephacryl S-200 fraction were used for further analysis, because of the unstability of the UBGAT in the buffer used for chromatofocusing.

Table 2 shows the substrate specificity and kinetic data for UBGAT. UBGAT was highly specific for UDP-glucuronic acid. V_{max} and K_m values were 257 ±

Table 2
Substrate specificity of UBGAT from *S. baicalensis* cell suspension cultures[a]

		V_{max} (nkat mg^{-1})	K_m (μM)	V_{max}/K_m (ratio)
Baicalein		257 ± 4.7	52.4 ± 2.1	4.90
Scutellarein		290 ± 62	109.0 ± 13.5	2.67
Wogonin		182 ± 9.4	106.5 ± 24.7	1.71

[a] Data are the means ± SD of three replicate assays.

4.7 nkat mg^{-1} and 72.9 ± 9.4 μM, respectively, using baicalein as the sugar acceptor. UDP-glucose and UDP-galacturonic acid did not serve as substrates. Regarding aglycones, UBGAT was specific for baicalein, scutellarein and wogonin, which are substituted at the position *ortho*- to the 7-OH (6-OH, baicalein and scutellarein; 8-OMe, wogonin). V_{max} values of UBGAT for baicalein and scutellarein were quite similar, and the K_m value for baicalein was almost half of that of scutellarein. The V_{max}/K_m ratios demonstrate that baicalein is the best sugar acceptor among the flavonoids tested. Other flavonoids lacking a substitution at the position *ortho*- to the 7-OH (chrysin, apigenin, luteolin, quercetin, formononetin and daidzein) did not serve as substrates.

Various chemical treatments suggested that the free SH group and lysine residues may play an important role in UBGAT activity (Table 3). *p*-Chloromercuribenzoic acid (PCMBA), a free SH group oxidation reagent, and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), a lysine modifier (Goffner et al., 1994), inhibited UBGAT activity completely. However, UBGAT activity was not affected by the other SH group oxidation reagent, NEM. This phenomenon was also reported by Latchinian-Sadek & Ibrahim (1991), and may be due to the different reactivity of NEM and PCMBA. DTE (dithioerythritol) had no effect on UBGAT activity, suggesting that a disulfide bond is not necessary for the protein function.

There was no divalent cation requirement noted for UBGAT activity. $CaCl_2$ and $MgCl_2$ had no effect on enzyme activity, whereas $CuSO_4$, $ZnCl_2$ and $FeCl_2$ completely inhibited UBGAT (Table 4). The inhibition of flavonoid glycosyltransferases by addition of Cu^{2+} ions is a well-known phenomenon (Latchinian-Sadek & Ibrahim, 1991; Ishikura & Mato, 1993; Vogt, Zimmermann, Grimm, Meyer & Strack, 1997; Ford, Boss & Høj, 1998). It is thought that this phenomenon is due to Cu^{2+}-mediated substrate degradation rather than by inhibition of the enzyme (Ford et al., 1998). Further analysis is needed to clarify this phenomenon.

Table 3
The effects of various chemical treatments on UBGAT activity

Reagents[a]	Specific activity (nkat mg^{-1})[b]	Ratio (%)	Type of reagent
None	99.2 ± 0.2	100	
PCMBA (100 μM)	n.d.[c]	0	Free-SH group oxidizer
NEM (100 μM)	91.9 ± 5.9	82.6	Free-SH group oxidizer
DIDS (100 μM)	n.d.	0	Lysine modifier
PG (100 μM)	99.2 ± 0.5	100	Arginine modifier
PMSF (100 μM)	90.6 ± 4.1	91.3	Serine modifier
DEPC (100 μM)	68.5 ± 2.6	69.1	Histidine modifier
DTE (10 mM)	113.7 ± 3.0	114.6	Disulfide bond reducer

[a] PCMBA: *p*-chloromercuribenzoic acid; NEM: *N*-ethylmaleimide; DIDS: 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; PG: phenylglyoxal; PMSF: phenylmethylsulfonylfluoride; DEPC: diethylpyrocarbonate; DTE: dithioerythritol.
[b] Data are the means ± SD of three replicate assays.
[c] n.d.: Enzyme activity was not detected.

Table 4
Effects of divalent cations and EDTA ON UBGAT activity

Reagent	Concentration (mM)	Specific activity (nkat mg^{-1})[a]	Ratio (%)
None	-	140.1 ± 11.9	100
CuSO4	1	n.d.[b]	0
$ZnCl_2$	1	n.d.	0
$FeCl_2$	1	n.d.	0
$CaCl_2$	1	129.2 ± 6.2	92.2
$MgCl_2$	1	109.9 ± 7.7	78.4
EDTA	5	116.9 ± 1.0	83.4

[a] Data are the means ± SD of three replicate assays.
[b] n.d.: Enzyme activity was not detected.

3. Experimental

3.1. Plant material

The callus tissue was derived from the petiole of a sterile young plant of *S. baicalensis* Georgi in April 1991. It was subcultured at 3-week intervals on MS medium (Murashige & Skoog, 1962) containing 3 mg l^{-1} naphthaleneacetic acid, 0.1 mg l^{-1} kinetin and 3% sucrose. For the suspension culture, approximately 4 g of 2-week-old callus tissues were transferred to 125 ml of liquid medium in a 500 ml flask and cultured at 25°C in the dark on a reciprocal shaker at 80 spm.

3.2. Column materials and substrates

Sephadex G-25, DEAE Sephadex A-50, Phenyl Sepharose CL-4B, Sephacryl S-200HR and Mono P HR 5/20 were obtained from Pharmacia (Uppsala, Sweden). Baicalin and baicalein were from Kanto (Tokyo, Japan). UDP-glucuronic acid, UDP-glucose and UDP-galacturonic acid were obtained from Nacalai Tesque (Kyoto, Japan). Wogonin and wogonin 7-*O*-glucuronoside were from our laboratory collection. Scutellarin and scutellarein were kindly provided by Professor Tomimori (Hokuriku University, Ishikawa, Japan). All the amino acid modifying reagents were obtained from Sigma (MO, USA).

3.3. Buffers

Buffer A: 100 mM phosphate buffer, 10 mM 2-ME, pH 7.0. Buffer B:10 mM phosphate buffer, 1 mM 2-ME, pH 7.0.

3.4. Reversed phase-HPLC

The HPLC system consisted of a model 510 pump (Waters, MA, USA) and an SPD-2A spectrophotometric detector equipped with a Mightysil RP-18 column (150 mm length, 4.6 mm i.d.; Kanto). The elution was performed with CH_3CN–60 mM phosphoric acid (29:71) at a flow rate of 2.2 ml min^{-1}; the eluent was monitored by absorbance at 274 nm. The peak intensity was determined with a C-R3A Chromatopac (Shimadzu, Kyoto, Japan). The amounts of enzymatic reaction products were estimated from standard curves obtained with authentic samples.

3.5. Assay of UBGAT activity

The standard assays consisted of 50 mM Tris–HCl buffer (pH 7.5), 0.16 mM substrate, 0.5 mM UDP-sugar and 5 μl of enzyme solution in a final volume of 125 μl. The sample mixtures were incubated for 10 min at 37°C, and the enzyme reaction was terminated by the addition of 75 μl of MeOH. Identification and quantitation of the enzyme reaction products were carried out by reversed phase-HPLC as described above.

3.6. Purification of UBGAT

All the purification procedures were carried out at 4°C. Protein concentration was determined by the Bradford method (Bradford, 1976) with BSA as a standard.

Ten-day-old suspension cultured cells of *S. baicalensis* were filtered and washed with distilled water. Two ml of buffer A per g fr. wt. was added. Homogenization was performed using a glass homogenizer. The homogenate was filtered through four-layers of cheese cloth and centrifuged at 12,000 *g* for 10 min to remove the cell debris. Proteins were precipitated by 30–50% saturated ammonium sulfate, centrifuged at 12,000 *g* for 10 min, resuspended in a minimum volume of buffer B, centrifuged at 12,000 *g* for 10 min and desalted on a Sephadex G-25 column (30 cm length, 4.7 cm i.d.) with buffer B.

Desalted protein solution was applied to a DEAE Sephadex A-50 (8.0 cm length, 3.2 cm i.d.) anion-exchange column, pre-equilibrated in buffer B. Protein fractions were eluted with a linear salt gradient of NaCl in buffer B (from 0 to 0.4 M) within 1000 ml. Ten ml samples of the fractions were collected and assayed for enzyme activity and protein concentration.

The pooled fractions containing enzyme activity from the anion-exchange chromatography were precipitated by 0–50% saturated ammonium sulfate, centrifuged at 12,000 *g* for 15 min, and resuspended in 1 ml of buffer B containing 1 M of ammonium sulfate. Samples were applied to a Phenyl Sepharose CL-4B column (4.5 cm length, 1.4 cm i.d.) pre-equilibrated with 1 M ammonium sulfate in buffer B. Protein fractions were eluted with a linear salt gradient of ammonium sulfate (from 1 to 0 M) within 200 ml. Two ml fractions were collected and assayed as above.

The pooled fractions containing enzyme activity from hydrophobic chromatography were concentrated by ultrafiltration (Amicon, MA, USA) to 1 ml. Concentrated samples were applied to a Sephacryl S-200 column pre-equilibrated with buffer B. Protein fractions were eluted with buffer B and 1.5 ml samples of the fractions were collected.

The pooled fractions containing enzyme activity from gel filtration chromatography were concentrated by ultrafiltration to 3.0 ml, followed by the buffer exchange on a PD-10 column (Pharmacia) pre-equilibrated with start buffer. Proteins were applied to a Mono P HR 5/20 column (20 cm length, 5 mm i.d.) pre-equilibrated with 25 mM 2-methylpiperazine, pH 5.7 (the pH was adjusted with saturated iminodiacetic acid (IDA)). The pH gradient was created by elution

with 10% polybuffer 74, pH 4.0 (adjusted with saturated IDA) at a flow rate of 1.5 ml min^{-1} and 1.5 ml fractions were collected.

3.7. Determination of the molecular mass

To determine the M_r of the purified proteins, SDS-PAGE and gel filtration chromatography were performed. SDS-PAGE was done according to Laemmli (1970) with standard protein markers (New England Biolabs, MA, USA). Proteins were stained with Silver Stain Kanto II (Kanto, Tokyo, Japan). Gel filtration chromatography was done as above, and the M_r was determined with the standard protein markers (Pharmacia), chymotrypsinogen A (25 kDa), ovalbumin (45 kDa), BSA (67 kDa), aldolase (158 kDa) and ferritin (440 kDa).

3.8. Kinetic properties

Apparent K_m and V_{max} values of the enzyme were determined using various concentrations of the donor molecule (UDP-sugar) at fixed concentrations of the acceptors and vice versa. The kinetic values were estimated from a Lineweaver–Burk plot.

3.9. Effects of various chemicals on UBGAT activity

Various chemicals were added to the standard assay mixture. The final concentrations in the assay mixture of PCMBA, *N*-ethylmaleimide, DIDS, phenylglyoxal, phenylmethylsulfonylfluoride and diethylpyrocarbonate were 100 μM and that of DTE was 10 mM.

Copper sulfate, zinc chloride, iron (II) chloride, calcium chloride and magnesium chloride were added to the standard assay mixture at a concentration of 1 mM.

Acknowledgements

We thank Professor Tomimori (Hokuriku University) for providing us with the flavonoid substrates. This research was financially supported by a Sasakawa Scientific Research Grant from the Japan Science Society.

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*, *72*, 248–254.

Ford, C. M., Boss, P. K., & Høi, P. B. (1998). Cloning and characterization of *Vitis vinifera* UDP-glucose : flavonoid 3-*O*-glucoyltransferase, a homologue of the enzyme encoded by the maize *Bronze-1* locus that may primarily serve to glucosylate anthocyanidins in vivo. *J. Biol. Chem*, *273*, 9224–9233.

Goffner, D., Campbell, M. M., Campargue, C., Clastre, M., Borderies, G., Boudet, A., & Boudet, A. M. (1994). Purification and characterization of cinnamoyl-coenzyme A: NADP oxidoreductase in *Eucalyptus gunnii*. *Plant Physiol*, *106*, 625–632.

Heller, W., & Forkmann, G. (1994). Biosynthesis of flavonoids. In J. B. Harborne, *The flavonoids. Advances in research since 1986* (pp. 499–535). London: Chapman & Hall,.

Hirotani, M., Nagashima, S., & Yoshikawa, T. (1998). Baicalin and baicalein productions of cultured *Scutellaria baicalensis* cells. *Natural Medicines*, *52*, 440–443.

Ikegami, F., Matsunae, K., Hisamitsu, M., Kurihara, T., Yamamoto, T., & Murakoshi, I. (1995). Purification and properties of a plant β-D-glucuronidase from Scutellaria root. *Biol. Pharm. Bull*, *18*, 1531–1534.

Ishikura, N., & Mato, M. (1993). Partial purification and some properties of flavonol 3-*O*-glycosyltransferases from seedlings of *Vigna mungo*, with special reference to the formation of kaempferol 3-*O*-galactoside and 3-*O*-glucoside. *Plant Cell Physiol*, *34*, 329–335.

Kamsteeg, J., van Brederode, J., & van Nigtevecht, G. (1978). Identification and properties of UDP-glucose: cyanidin-3-*O*-glucosyltransferase isolated from petals of the red campion (*Silene dioica*). *Biochem. Genet*, *16*, 1045–1058.

Kleene, R., & Berger, E. G. (1993). The molecular and cell biology of glycosyltransferases. *Biochim. Biophys. Acta*, *1154*, 283–325.

Koda, A., Watanabe, S., Yanagihara, Y., Nagai, H., & Sakamoto, K. (1977). A comparative study of the anti-allergic effects of disodium baicalein 6-phosphate (BPS) and disodium cromoglycate (DSCG). *Japan J. Pharmacol*, *27*, 31–38.

Konoshima, T., Kokumai, M., Kozuka, M., Iinuma, M., Mizuno, M., Tanaka, T., Tokuda, H., Nishino, H., & Iwashima, A. (1992). Studies on inhibitors of skin tumor promotion. Part XI: Inhibitory effects of flavonodis from *Scutellaria baicalensis* on Epstein–Barr virus activation and their anti-tumor-promoting activities. *Chem. Pharm. Bull*, *40*, 531–533.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Latchinian-Sadek, L., & Ibrahim, R. K. (1991). Partial purification and some properties of a ring B-*O*-glucosyltransferase from onion bulbs. *Phytochemistry*, *30*, 1767–1771.

Li, B. Q., Fu, T., Yan, Y. D., Baylor, N. W., Ruscetti, F. W., & Kung, H. F. (1993). Inhibition of HIV infection by baicalin — a flavonoid compound purified from Chinese herbal medicine. *Cell. Mol. Biol. Res*, *39*, 119–124.

Mackenzie, P. I., Owens, I. S., Burchell, B., Bock, K. W., Bairoch, A., Bélanger, A., Fournel-Gigleux, S., Green, M., Hum, D. W., Iyanagi, T., Lancet, D., Louisot, P., Magdalou, J., Chowdhury, J. R., Ritter, J. K., Schachter, H., Tephly, T. R., Tipton, K. F., & Nebert, D. W. (1997). The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics*, *7*, 255–269.

Miwa, T. (1932). Baicalinase, a flavone glucuronide-splitting enzyme: Part I. *Acta Phytochim. (Japan)*, *6*, 155–171.

Miwa, T. (1935). Baicalinase, a flavone glucuronide-splitting enzyme: Part II. *Acta Phytochim. (Japan)*, *8*, 231–243.

Miwa, T. (1936). Baicalinase, a flavone glucuronide-splitting enzyme: Part III. *Acta Phytochim. (Japan)*, *9*, 89–102.

Morimoto, S., Harioka, T., & Shoyama, Y. (1995). Purification and characterization of flavone-specific β-glucuronidase from callus cultures of *Scutellaria baicalensis* Georgi. *Planta*, *195*, 535–540.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant*, *15*, 473–497.

Schulz, M., & Weissenböck, G. (1988). Three specific UDP-glucuronate: flavone-glucuronosyltransferases from primary leaves of *Secale cereale*. *Phytochemistry*, *27*, 1261–1267.

Tang, W., & Eisenbrand, G. (1992). *Scutellaria baicalensis* Georgi. In *Chinese drugs of plant origin* (pp. 919–929). Berlin: Springer–Verlag.

Vogt, T., Zimmermann, E., Grimm, R., Meyer, M., & Strack, D. (1997). Are the characteristics of betanidin glucosyltransferases from cell-suspension cultures of *Dorotheanthus bellidiformis* indicative of their phylogenetic relationship with flavonoid glucosyltransferases? *Planta*, *203*, 349–361.