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Cloning and expression of UDP-glucose: flavonoid 7-*O*-glucosyltransferase from hairy root cultures of *Scutellaria baicalensis*

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Abstract. A cDNA encoding UDP-glucose: baicalein 7-*O*-glucosyltransferase (UBGT) was isolated from a cDNA library from hairy root cultures of *Scutellaria baicalensis* Georgi probed with a partial-length cDNA clone of a UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT) from grape (*Vitis vinifera* L.). The heterologous probe contained a glucosyltransferase consensus amino acid sequence which was also present in the *Scutellaria* cDNA clones. The complete nucleotide sequence of the 1688-bp cDNA insert was determined and the deduced amino acid sequences are presented. The nucleotide sequence analysis of UBGT revealed an open reading frame encoding a polypeptide of 476 amino acids with a calculated molecular mass of 53 094 Da. The reaction product for baicalein and UDP-glucose catalyzed by recombinant UBGT in *Escherichia coli* was identified as authentic baicalein 7-*O*-glucoside using high-performance liquid chromatography and proton nuclear magnetic resonance spectroscopy. The enzyme activities of recombinant UBGT expressed in *E. coli* were also detected towards flavonoids such as baicalein, wogonin, apigenin, scutellarein, 7,4′-dihydroxyflavone and kaempferol, and phenolic compounds. The accumulation of UBGT mRNA in hairy roots was in response to wounding or salicylic acid treatments.

Key words: Baicalin – Baicalein – Flavonoid 7-*O*-glucosyltransferase gene – Hairy root culture – *Scutellaria* flavonoids – Lamiaceae

Introduction

Glycosylation of a number of secondary plant products, including flavonoids, steroidal alkaloids, cyanohydrins and many kinds of saponin, occurs at the end of their biosynthetic pathway (Reay and Conn 1974; Sun and Hrazdina 1991; Stapleton et al. 1992). The most commonly used sugar is glucose and the reaction is catalyzed by a UDP-glucose: glucosyltransferase to produce a stable water-soluble compound that is often transported to the vacuole (Poulton 1990). The glycosides of the flavonoids are distributed in many higher plants as the glycosides of glucose, galactose and xylose. The most commonly found sugar residue of flavonoid compounds is glucose, located in the 3-*O*-position of the molecule. The glucosyl moiety is transferred from UDP-glucose to the flavonoid acceptor by the enzyme UDP-glucose: flavonoid glucosyltransferase (UFGT). Although the functions of these flavonoid glycosides in plant cells remain unclear, they are assumed to represent the accumulation forms of the flavonoid and the defense compounds against potential pathogens such as fungi and bacteria.

Two kinds of structural gene involved in glycosylation of the flavonoid have been isolated from several plants. One encodes UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UF3GT), which catalyzes glucosylation at the 3-*O*-position of flavonoids (Ralston et al. 1988; Wise et al. 1990; Ford et al. 1998). The other encodes UDP-rhamnose: anthocyanidin 3-*O*-glucoside rhamnosyltransferase, which adds a rhamnose to the 3-*O*-bond glucose of the anthocyanidin 3-*O*-glucoside molecule to produce the anthocyanidin 3-*O*-rutinosides (Brugliera et al. 1994; Kroon et al. 1994). In addition, recently the isolation and characterization of a cDNA clone of UDP-galactose: flavonoid 3-*O*-galactosyltransferase (UF3GaT) from *Vigna mungo* have been reported (Mato et al. 1998).

Thus, many glucosyltransferase genes have been isolated from various plant sources. However, the most intensively studied genes are the glycosyltransferases that catalyze the attachment of UDP-sugar to the

The cDNA sequence of UBGT has been submitted to the GenBank/EBI data bank with accession number AB031274

Abbreviations: GT = glycosyltransferase; IEGT = immediate early-induced glucosyltransferase; NMR = nuclear magnetic resonance; TOGT = tobacco glucosyltransferase; UBGT = UDP-glucose: baicalein 7-*O*-glucosyltransferase

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3-*O*-position of flavonols or anthocyanidins (Furtek et al. 1988; Martin et al. 1991; Tanaka et al. 1996). Quite recently, molecular cloning and biochemical characterization of a novel anthocyanin 5-*O*-glucosyltransferase from *Perilla frutescens* has been reported (Yamazaki et al. 1999). Although there are many reports on the purification of glucosyltransferases that catalyze the transfer of the glucosyl moiety from UDP-sugar to the 7-*O*-position of flavonoids, the isolation and characterization of the corresponding gene has not been reported until now (Schulz and Weissenböck 1988; McIntosh and Mansell 1990; McIntosh et al. 1990; Vellekoop et al. 1993; Stich et al. 1997).

Scutellariae Radix (Japanese name: Ougon) is the dried root of *Scutellaria baicalensis* Georgi and is a well known drug in traditional Chinese medicine, used for treatment of bronchitis, hepatitis, diarrhea, arteriosclerosis and tumors (Chiang 1977; Tang and Eisenbrand 1992). In our previous paper, we reported the isolation of sixteen flavonoids, including baicalin as the major compound, from hairy root cultures of *S. baicalensis* (Zhou et al. 1997). Baicalin, a major glycoside in Scutellariae Radix, is produced by glucuronosylation of baicalein (Hirotani et al. 1998). The presence of baicalein 7-*O*-glucoside is also known as a minor component in Scutellariae Radix (Tomimori et al. 1984).

On the other hand, two tobacco glucosyltransferase (TOGT) genes were recently isolated from elicitor-treated leaves of *Nicotiana tabacum* L. cv. Samsun NN. The TOGT proteins produced in *Escherichia coli* exhibited a broad substrate specificity for phenolic compounds such as hydroxylated coumarins and hydroxycinnamic acid (Fraissinet-Tachet et al. 1998).

In order to find the flavonoid 7-*O*-glucosyltransferase gene, we have examined a cDNA library from wounded hairy roots of *S. baicalensis*. In this paper, we report the cloning, characterization and high-level expression in *E. coli* of a cDNA encoding glucosyltransferase from *S. baicalensis* hairy roots, an enzyme which is responsible for the glucosylation of baicalein in the 7-*O*-position, and the properties of the recombinant enzyme.

Materials and methods

Hairy roots, bacterial strains and vectors. Hairy roots of *Scutellaria baicalensis* Georgi (SbpBI121 strain) were initiated from seedlings and grown in Gamborg's B5 medium (Gamborg et al. 1968) as previously described (Zhou et al. 1997). *Escherichia coli* strains XL1-Blue MRF′, SOLR, JM109 and BL21(DE3) were used for the standard molecular-biology procedures. pBluescript SKII and expression vector pET-3a were purchased from Stratagene (La Jolla, Calif., USA) and Novagene (Madison, USA), respectively.

Construction and screening of a cDNA library. Five-week-old SbpBI121 hairy roots were cut into 1-cm-long segments with a sterile knife as a wounding treatment, and then incubated for 3 h. Hairy roots were harvested by filtration through a nylon mesh. The total RNA from 20 g of hairy roots was prepared according to the guanidine thiocyanate and phenol-chloroform extraction method (Chomczynski and Sacchi 1987). Polyadenylated RNA was prepared from the total RNA using the mRNA Separator Kit (Clontech Laboratries, Palo Alto, Calif., USA). Double-stranded cDNA was prepared using 5 μg of poly(A)$^+$RNA by the Gubler and Hoffman's method (1983) according to the instructions for the Great Lengths cDNA Synthesis Kit (Clontech). After ligation with an *Eco*RI adaptor, the cDNAs were inserted into λZapII vector arms (Stratagene). Approximately 5×10^5 non-amplified plaques were screened with the ^{32}P-labeled cDNA insert of the grape (*Vitis vinifera* L.) pBS801 (UFGT). Hybridization on Hybond N$^+$ membranes (Amersham, Bucks., UK) was carried out at 65° C in 5 × SSPE (1 × SSPE = 0.18 M NaCl, 0.01 M sodium phosphate, 1 mM EDTA, pH 7.7), 1% SDS, 5 × Denhardt's solution (1 × Denhardt's solution = 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone and 0.02% bovine serum albumin) and 0.1 mg ml^{-1} salmon sperm DNA. Final washing of the membranes was conducted at 55 °C in 2 × SSPE and 0.1% SDS.

Sequencing and analysis of DNA. One positive plaque was isolated and this was subjected to in-vivo excision to produce the pBluescript SKII plasmid clone pUBGT. Sequencing was carried out by the dideoxy-chain termination method with Thermo sequenase (Amersham Pharmacia Biotech, Bucks., UK) using a DNA sequencer (model DSQ 2000L; Shimadzu, Kyoto, Japan). The results of the sequence comparison, the multiple sequence alignment, were analyzed by GENETYX version 8.0 packed programs (Software Development Co., Tokyo, Japan) and the neighbor-joining method (Saitou and Nei 1987).

Northern blot analysis. Total RNA was isolated from *S. baicalensis* hairy root cultures (3 g fresh weight) using the method outlined above. For northern hybridization analysis, equivalent amounts of RNA (10 μg) from hairy roots that had been harvested 1, 2, 4, 6, 8, 10, and 12 h after treatment by wounding or with salicylic acid (500 μM), as well as RNA isolated from non-induced hairy roots (0 h), were separated by electrophoresis in a formaldehyde denaturing 1.2% agarose gel. The separated RNA was blotted to a Hybond N$^+$ membrane (Amersham) by capillary action and then fixed by UV irradiation. ^{32}P-labeled probes corresponding to an insert fragment of the UDP-glucose: baicalein 7-*O*-glucosyltransferase (UBGT) cDNA were synthesized using a Random Primer DNA Labeling kit version 2 (Takara, Shiga, Japan). To verify the equivalent loading of RNA on the blots, membranes were hybridized using a ^{32}P-labeled fragment of the rice ribosomal RNA gene (Takaiwa et al. 1985). The blot was sequentially incubated with each probe for 16 h at 65 °C, then finally washed twice with 2 × SSPE, 0.1% SDS at 65 °C. The signals for hybridization were detected using a Fuji bioimaging BAS2000II analyzer (Fuji Film).

Generation of UBGT expression construct. *Nde*I and *Bam*HI sites were created on both sides of the coding region for the UBGT cDNA by polymerase chain reaction with two primers, 5′-ATAACTACATATGGGACAACTCCAC-3′ for upstream and 5′-CAGAACAGGATCCACACGTAATTTA-3′ for downstream. The amplified DNA was digested with *Nde*I and *Bam*HI and then ligated into the same sites of pET-3a (Novagene). *Escherichia coli* BL21(DE3) pLysS was transformed with the resulting expression vector pEUBGT.

Expression of recombinant UBGT and enzyme isolation. Transformed *E. coli* BL21 cells were precultured in LB medium (Sambrook et al. 1989) supplemented with carbenicillin (50 mg L^{-1}) and 100 μl of the culture was inoculated into 10 ml of the same medium. After incubation at 37 °C for 3 h, recombinant UBGT was induced with 1 mM of isopropyl-1-thio-β-D-galactoside (IPTG) at 30 °C for 3 h. All subsequent operations were conducted at 0–4 °C. The cell pellet was suspended in 1 ml of extraction buffer (1 mM 2-mercaptoethanol in 10 mM phosphate buffer, pH 7.0) and the cells were sonicated for 1 min, three times, at 200 W in an ice-water bath, and the resulting debris was removed by centrifugation at 5500*g* for 5 min. The resulting supernatant was used as the enzyme source.

Assay of UBGT. The reaction mixture for the glycosyltransferase (GT) contained 10 mM citrate-phosphate buffer (pH 6.5), 0.5 mM UDP-glucose, 0.16 mM substrates dissolved in methyl Cellosolve (Wako Pure Chemical Industries, Tokyo, Japan) and the crude enzyme preparation in a final volume 0.25 ml. The reaction mixture was incubated at 37 °C for 30 min and was terminated by adding 150 µl of methanol. The reaction mixture was subjected to HPLC analysis for the detection of the reaction product. For comparison, the enzyme activities for various substrates, such as baicalein, wogonin, apigenin, scutellarein, 7,4′-dihydroxyflavone, kaempferol, umbelliferone, ferulic acid and *p*-coumaric acid (Fig. 5), were measured using UDP-[U-^{14}C] glucose (Amersham; 11.5 GBq mmol^{-1}) and UDP-[U-^{14}C]-glucuronic acid (New England Nuclear, Boston, Mass., USA; 11.8 GBq mmol^{-1}). Reaction mixtures were the same as described above except for the addition of radiolabeled sugar (added 925 Bq of UDP-[U-^{14}C]glucose or glucuronic acid in each reaction mixture) as a substrate. The mixture was incubated at 37 °C for 30 min and the reaction was terminated with chilling in ice. The reaction mixture was then twice extracted with 250 µl of *n*-butanol saturated with water. The radioactivity of a 100-µl aliquot of the *n*-butanol extract was measured using a liquid scintillation counter (Beckman LS6000IC).

Identification of baicalein 7-O-glucoside as a recombinant enzyme product using proton nuclear magnetic resonance (^1H NMR). To identify the enzyme reaction product for baicalein as a substrate, a scaled-up reaction was carried out. After termination of the reaction, a 50-ml reaction mixture prepared by scale-up in the usual enzyme assay was extracted with *n*-butanol saturated with water. After evaporation of the *n*-butanol fraction, the extract was purified by HPLC using a Senshu pak ODS column (300 mm long, 10 mm i.d.) with 85% methanol as solvent at a flow rate of 2.3 ml min^{-1}. Baicalein 7-*O*-glucoside was isolated from the fraction containing the peak at Rt 7.8 min. The fraction was evaporated to complete dryness, and after dissolving in dimethylsulfoxide-d_6, the proton NMR spectrum was recorded on a Varian XL-400 spectrometer (Fig. 4).

Conditions for HPLC. The HPLC system used was composed of a model 510 pump (Waters, Massachusetts, USA) and an SPD-2A spectrophothometric detector equipped with a Mightysil PR-18 column (4.6 mm i. d., 150 mm long; Kanto Chemical, Tokyo, Japan) except for the isolation of baicalein 7-*O*-glucoside. For separation of substrates and reaction products, many solvent systems were used, i.e. acetonitrile: 60 mM phosphoric acid (29:71, v/v) at a flow rate of 1.1 ml min^{-1} for baicalein 7-*O*-glucoside (Rt 5.3 min), baicalein (Rt 22.1 min), apigenin glucoside (Rt 4.0 min) and apigenin (Rt 19.3 min); the same solvent at a flow rate of 0.5 ml min^{-1} for scutellarein glucoside (Rt 6.8 min) and scutellarein (Rt 14.8 min); the same solvent at a flow rate of 2.0 ml min^{-1} for wogonin glucoside (Rt 4.3 min) and wogonin (Rt 27.7 min); acetonitrile:60 mM phosphoric acid (20:80, v/v) at a flow rate of 1.5 ml min^{-1} for 7,4′-dihydroxyflavone glucoside (Rt 3.2 min) and 7,4′-dihydroxyflavone (Rt 15.5 min); CH_3CN:60 mM phosphoric acid (25:75, v/v) at a flow rate of 1.1 ml min^{-1} for kaempferol glucoside (Rt 3.0 min) and kaempferol (Rt 20.2 min). The effluent was monitored by absorption at 270–360 nm.

Results and discussion

Cloning and identification of full-length cDNA encoding UBGT from S. baicalensis. In order to isolate the cDNA encoding flavonoid 7-*O*-glucosyltransferase from *Scutellaria baicalensis*, we used low-stringency plaque hybridization with a 532-bp cDNA fragment of grape flavonol glucosyltransferase previously isolated by Sparvoli et al. (1994). One positive plaque was isolated from 5×10^5 clones of the cDNA library prepared from the poly(A)$^+$RNA of wounded hairy roots of *S. baicalensis*. After the cDNA insert was subcloned into pBluescript SKII by in-vivo excision, the resulting plasmid was designated pUBGT. Determination of the nucleotide sequence showed that the new UBGT cDNA insert of 1688 bp contained an open reading frame of 1428 bp encoding a polypeptide of 476 amino acids with a 5′-untranslated region (UTR) of 109 bp and a 3′-UTR of 151 bp. The calculated molecular mass of the UBGT protein is 53 094 Da. The initial ATG codon encoding methionine is determined by the fact that a TAG stop codon is present 9 bp upstream of the initial ATG codon.

Comparison of the deduced amino acid sequence with those of other plant flavonoid glycosyltransferases. When compared with other plant-derived UFGTs, the deduced amino acid sequence of the *S. baicalensis* enzyme shows considerable homology throughout the entire coding region, including the conserved UDP-binding domain of 44 amino acid residues located in the C-terminal region (Fig. 1) (Hundle et al. 1992). The amino acid sequence of UBGT showed 58%, 56%, 26%, 25%, 25% and 22% identity to those of IS10a, twi1, mai-3gt, gen-3gt, gra-3gt and bar-3gt, respectively. Phylogenic relationships of the amino acid sequence of the glucosyltransferase indicated that the glucosyltransferase superfamily can be divided into three groups comprised of the 3-glucosyltransferases, 5-glucosyltransferases and glucosyltransferases induced by some stress treatment. As shown in Fig. 2, the isolated UBGT protein seemed to be closer to the induced glucosyltransferases such as IS10a and twi1 than to the non-induced glucosyltransferases such as bar-3gt, mai-3gt, gen-3gt, per-3gt, gra-3gt, mai-iaa, ver-5gt and per-5gt. Therefore, this phylogenetic tree indicates that, phylogenetically, UBGT, IS10a and twil proteins belong to a new family of the glycosyltransferase superfamily.

Expression of isolated UBGT cDNA in E. coli. To clarify whether the cDNA isolated from wounded hairy roots encodes flavonoid 7-*O*-glucosyltransferase, the cDNA was expressed in *E. coli*. The coding region of the isolated UBGT cDNA insert was subcloned into the pET-3a vector, resulting in the construction of pEUBGT. The soluble fraction of *E. coli* BL2 (DE3) pLysS transformed with pEUBGT was subjected to UBGT assay using baicalein, one of the major flavonoid components in Scutellariae Radix, and UDP-glucose. The reaction mixture was separated by reversed-phase HPLC. The baicalein was converted to baicalein 7-*O*-glucoside in the presence of UDP-glucose and the enzyme solution prepared from *E. coli* transformed with pEUBGT. The baicalein 7-*O*-glucoside produced from baicalein by recombinant UBGT proteins was identified by HPLC by co-chromatography with an authentic sample (Fig. 3) and reconfirmed by the ^{1}H NMR spectrum of enzyme reaction product isolated by HPLC. The ^{1}H NMR spectrum of baicalein exhibited signals at δ_H 6.62 (1H, s, H-8), 6.92 (1H, s, H-3), 7.58 (3H, m, H-3′, H-4′ and H-5′) and 8.06 (2H, d,

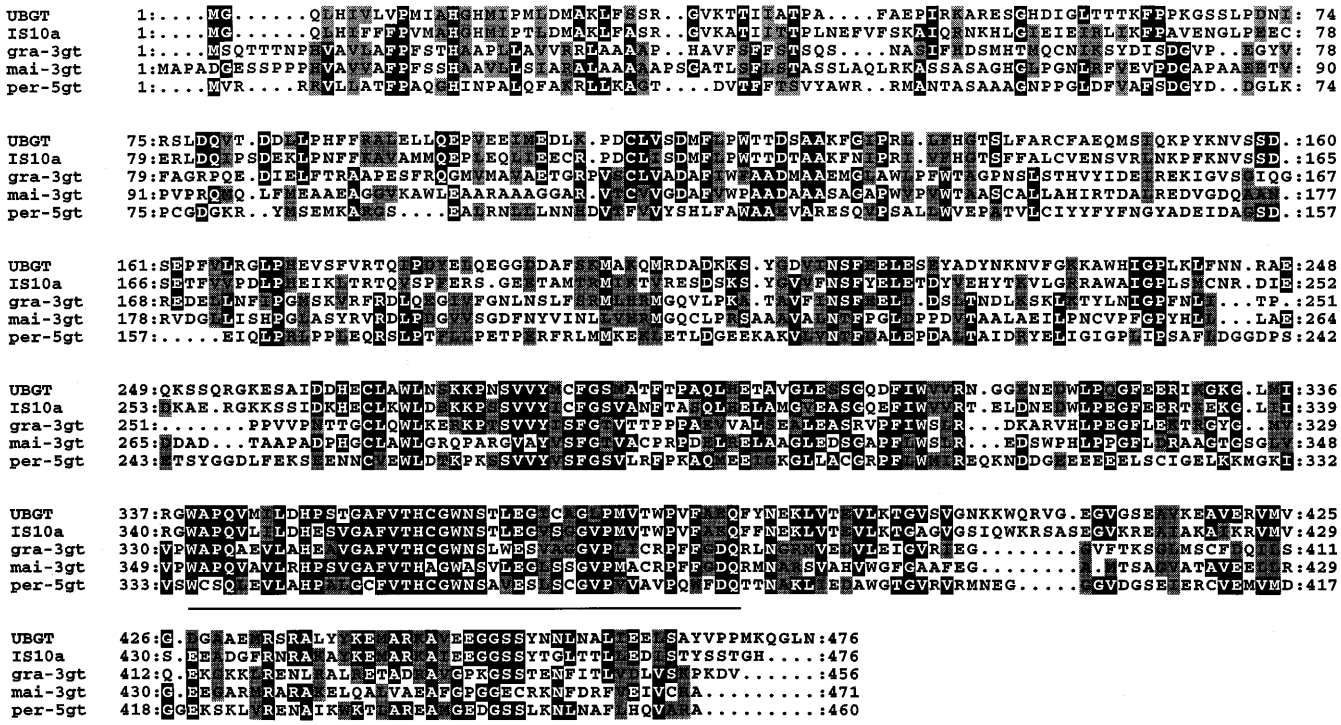

Fig. 1. Amino acid sequence comparison of five glucosyltransferases of plant origin. *Black shading* shows amino acid identities and *gray shading* shows amino acid similarity. *Underlining* indicates the signature sequence generally found in a family of glycosyltransferases. *Dots* indicate gaps introduced during alignment. The species from which the glucosyltransferases were obtained are indicated in the left column. Species abbreviations and Genbank Accession Nos. are: *UBGT*, *Scutellaria baicalensis*, AB031274 (this study); *IS10a*, *Nicotiana tabacum* (IEGT), U32643 (Horvath and Chua 1996); *gra-3gt*, *Vitis vinifera*, AF000372 (Sparvoli et al. 1994); *mai-3gt*, *Zea mays*, X13500 (Furtek et al. 1988); *per-5gt*, *Perilla frutescens*, AB013596 (Yamazaki et al. 1999)

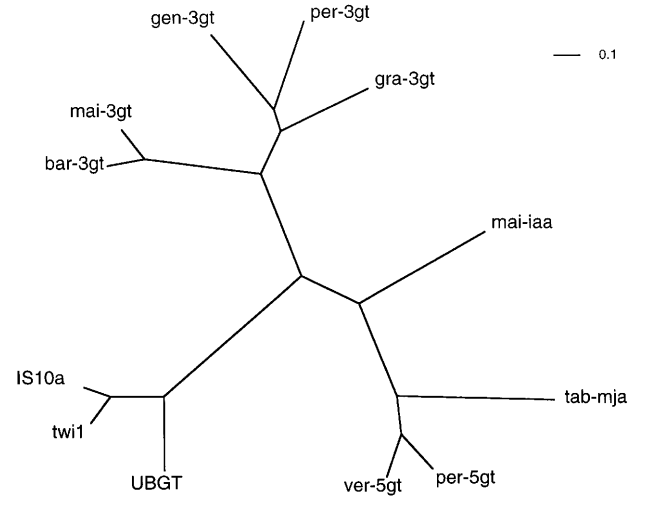

Fig. 2. Molecular phylogenic tree of plant-derived glycosyltransferase sequences based on amino acid similarity. The tree was constructed by the neighbor-joining method. Lengths of lines indicate the relative distances between nodes. UBGT, IS10a, mai-3gt, gra-3gt and per-5gt are as defined in the Fig. 2 legend. *bar-3gt* and *per-3gt*, UDP-glucose: flavonol 3-*O*-glucosyltransferase from *Hordeum vulgare* (X15694) and *P. frutescens* (AB002818); *gen-3gt*, UDP-glucose: flavonioid 3-*O*-glucosyltransferase from *Gentiana triflora* (D85186); *ver-5gt*, UDP-glucose: anthocyanidine 5-*O*-glucosyltransferase from *Vervena hybrida* (AB013598); *mai-iaa*, indole-3-acetic acid glucosyltransferase from *Zea mays* (L34847); *tab-mja*, a protein induced by methyl jasmonate from tobacco (AB000623); *twi1*, wound-induced from tomato (X8513)

J = 8.0 Hz, H-2′ and H-6′) in the aromatic region. As shown in Fig. 4, in the enzyme reaction product and standard sample of baicalein 7-*O*-glucoside the signal at δ_H 6.62 (1H, s, H-8) seen in baicalein shifted downfield to δ_H 7.04 (1H, s, H-8). This assignment was based on the ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC) spectral data (data not shown). These results revealed that the enzyme transfers the glucosyl moiety from UDP-glucose to the 7-*O*-position of baicalein (Markham and Geiger 1994).

Characterization of recombinant UBGT protein. The substrate specificity of recombinant UBGT protein was examined using two kinds of UDP-sugar, and different flavonoids and phenolic compounds as acceptor substrates (Fig. 5). Extracts of *E. coli* containing the recombinant protein were incubated with radiolabeled UDP-[U-^{14} C]glucose as the donor substrate. As shown in Table 1 the UBGT enzyme was active to a greater or lesser extent with all substrates tested except for ferulic acid and *p*-coumaric acid. On the other hand, only UDP-glucose, not UDP-glucuronic acid, was able to support the glycosylation of baicalein. From these results, the recombinant UBGT protein is clearly identified as flavonoid 7-*O*-glucosyltransferase. These results agree with data obtained for non-homogeneous preparations of other flavonoid 3-*O*-glucosyltransferases showing exclusive acceptor-substrate specificity for anthocyanidins and flavonols (Heller and Forkmann 1994).

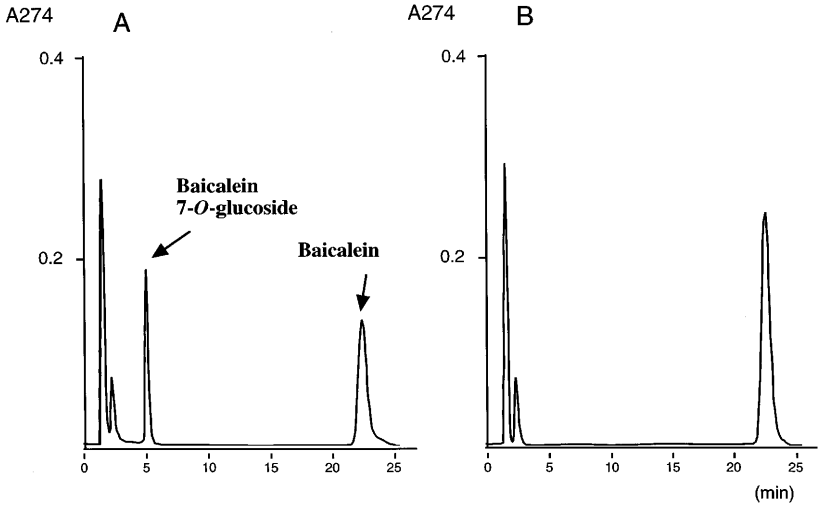

Fig. 3A,B. Analysis by HPLC of the enzymatic reaction catalyzed by UBGT expressed in *E. coli*. See *Materials and methods* for HPLC conditions. **A** The elution profile of the reaction product with the protein extracts from the transformed *E. coli* expressing UBGT. The reaction mixture was reacted with baicalein and UDP-glucose at 37 °C for 30 min. B Reaction mixture at time point 0 min

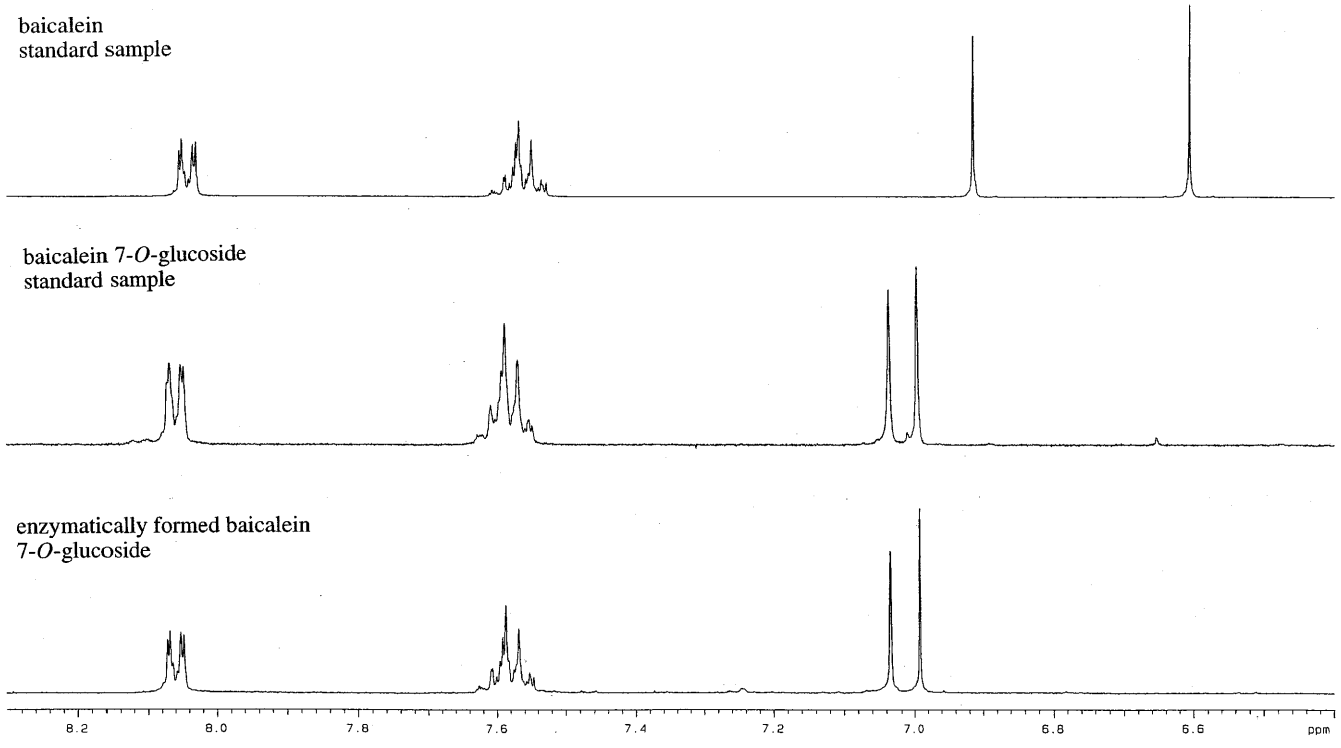

Fig. 4. The ^{1}H NMR spectrum of enzymatically formed baicalein 7-*O*-glucoside from baicalein catalyzed by UBGT expressed in *E. coli*. Only the aromatic region of the spectrum is shown. Spectra were recorded in dimethylsulfoxide d_6

Changes in UBGT mRNA levels in response to wounding and salicylic acid treatments. Changes in gene expression were determind at various times after the induction treatments. Figure 6 shows an RNA-blot analysis, using a probe for UBGT, of total RNA isolated from hairy roots. There was a rapid increase in the steady-state mRNA levels after wounding or treatment with salicylic acid. In the case of wounding, accumulation of mRNA was first apparent at 1 h; RNA levels rose rapidly until 2 h and then gradually decreased (Fig. 6A). Similarly, in the case of salicylic acid treatment, accumulation of mRNA was also first apparent at 1 h; RNA levels rose rapidly until 4 h and then decreased with time (Fig. 6B). The kinetics for these induced responses of *S. baicalensis* hairy roots to wounding and salicylic acid are comparable to those of the elicitor-induced tobacco IEGT (Horvath and Chua 1996) glucosyltransferase gene. Similary, a tomato twi1 gene with homology to UBGT, and also induced during hypersensitive response or by exogenous salicylic acid has been recently shown to be induced by wounding (O'Donnell et al. 1998).

In conclusion, the data in this study demonstrate the isolation from *S. baicalensis* of a glucosyltransferase

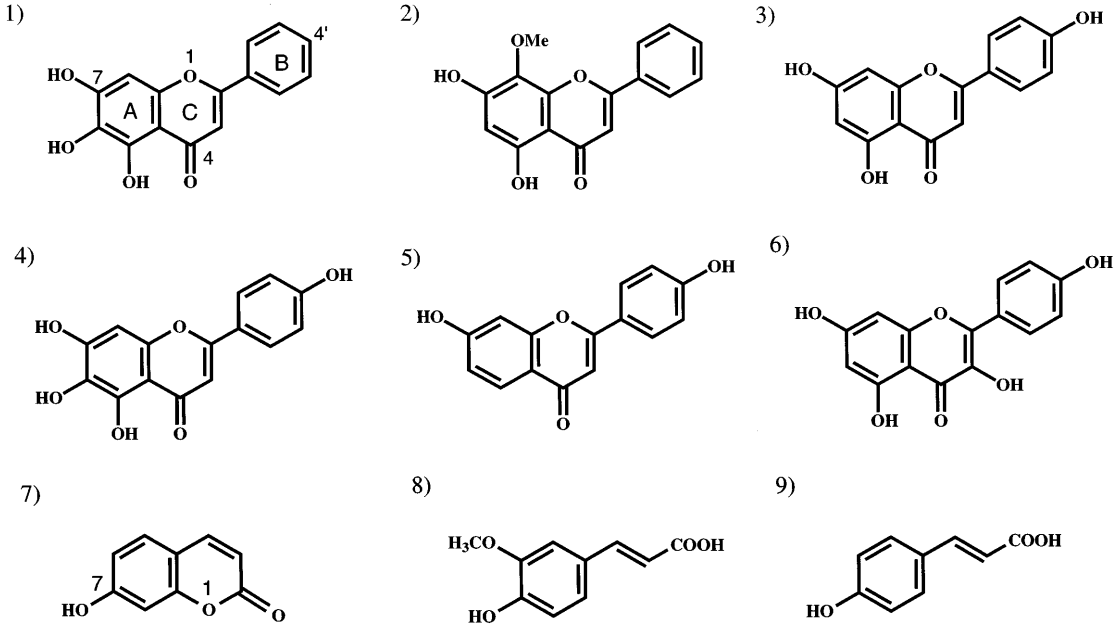

Fig. 5. Chemical structures of flavonoids and phenolic compounds used as the substrates for recombinant UBGT. 1) baicalein; 2) wogonin; 3) apigenin; 4) scutellarein; 5) 7,4′-dihydroxyflavone; 6) kaempferol; 7) umbelliferone; 8) ferulic acid; 9) *p*-coumaric acid

Table 1. Substrate specificity of recombinant UBGT measured by glucosylation of the indicated substrates. Radioactivity was not detected from butanol extract of the reaction product with the protein extracts from the control *E. coli* transformed with an empty vector. Data are means ± SD of three replicate assays. –, not determined

Substrate	Radioactivity in butanol extract (dpm)	Relative activity (%)	K_m (μM)	V_{max}/K_m
1) Baicalein	1804 ± 63	100	137	136
2) Wogonin	799 ± 47	44	98	127
3) Apigenin	1666 ± 134	92	–	–
4) Scutellarein	1217 ± 91	67	–	–
5) 7,4′-Dihydroxyflavone	317 ± 29	18	–	–
6) Kaempferol	2460 ± 69	136	–	–
7) Umbelliferone	127 ± 12	7	–	–
8) Ferulic acid	−50 ± 27	0	–	–
9) *p*-Coumaric acid	22 ± 8	1	–	–
Baicalein + UDP-glucuronic acid	−8 ± 4	0	–	–

cDNA which, compared with other glucosyltransferase cDNAs, is remarkably specific because the enzyme is able to catalyze the transfer of a glucosyl moiety from UDP-glucose to the 7-hydroxy group of a flavone. Expression of the UBGT gene is induced by stresses such as wounding and treatment with salicylic acid. The expression of the UBGT gene in response to stress is very rapid and transient, similar to that of the tobacco IEGT gene (Horvath and Chua 1996). Recently, two tobacco genes (*TOGT* s) with homology to glucosyltransferase genes known to be induced by salicylic acid were isolated from the tobacco cultivar Samsun. Recombinant TOGT proteins have been found to have a high glucosyltransferase activity for hydroxylated coumarins and (hydroxy)cinnamic acids (Fraissinet-Tachet et al. 1998). However, UBGT proteins showed a weak glucosyltransferase activity for umberiferone but not for ferulic acid and *p*-coumaric acid. Therefore, UBGT seems to play different role from TOGT. Elucidation of

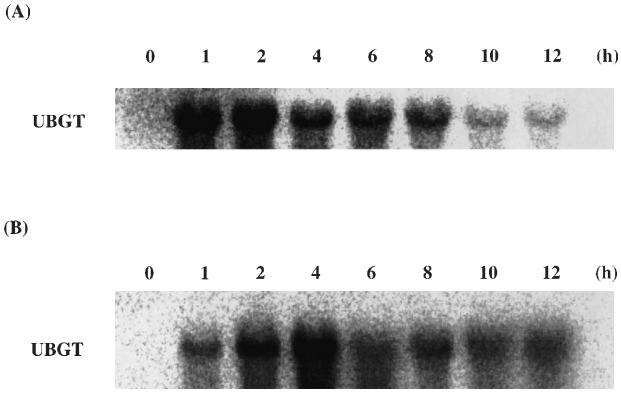

Fig. 6A,B. Accumulation of UBGT mRNA after wounding or treatment with salicylic acid. *Scutellaria baicalensis* hairy roots were incubated after wounding (**A**) or treatment with salicylic acid (**B**) for the following times: 0, 1, 2, 4, 6, 8, 10, 12 h. A 10-μg portion of total RNA was loaded per lane. These filters were hybridized with a rice ribosomal probe to confirm equal loading

UBGT enzymatic activity with respect to the other phenolic compounds is in progress and will be important for understanding the role of this protein in hairy roots of *S. baicalensis*.

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