Molecular cloning and characterization of a glucosyltransferase catalyzing glucosylation of curcumin in cultured *Catharanthus roseus* cells

Yasuhisa Kaminaga[a], F. Pinar Sahin[a,b], Hajime Mizukami[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467-8603, Japan*
[b]*Faculty of Pharmacy, Hacettepe University, Ankara 06100, Turkey*

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Abstract ***Catharanthus roseus*** **cell suspension cultures are capable of converting exogenously supplied curcumin to various glucosides. The glucosylation efficiency is enhanced by addition of methyl jasmonate (MJ) to the cultures prior to curcumin administration. Two cDNAs encoding UDP-glucosyltransferases (CaUGT1 and CaUGT2) were isolated from a cDNA library of cultured *C. roseus* cells, using a PCR method directed at the conserved UDP-binding domain of plant glycosyltransferases. The sequence identity between their deduced amino acid sequences was 27%. The expression of both genes was up-regulated by addition of MJ to the cell cultures although the mRNA level of CaUGT1 was much lower than that of CaUGT2. The corresponding cDNAs were expressed in *Escherichia coli* as fusion proteins with maltose-binding protein. The recombinant CaUGT1 exhibited no glucosylation activity with either curcumin or curcumin monoglucoside as substrate, whereas the recombinant CaUGT2 catalyzed the formation of curcumin monoglucoside from curcumin and also conversion of curcumin monoglucoside to curcumin diglucoside. The use of the recombinant CaUGT2 may provide a useful new route for the production of curcumin glucosides.**

Keywords: Glucosyltransferase; Curcumin; Heterologous expression; Substrate specificity; Cell suspension culture; *Catharanthus roseus*

1. Introduction

A remarkably diverse array of glycosyltransferases is present in plant cells as exemplified by more than 110 glycosyltransferase genes in the *Arabidopsis* genome [1,2]. These glycosyltransferases are involved not only in biosynthesis of natural products but also in regulation of the cellular level of plant hormones and signal molecules, and in detoxification of xenobiotics [3]. Some glycosyltransferases have been shown to exhibit broad substrate specificity [4] which could enable them to be used in enzyme-catalyzed transfer of sugars to aglycones as an alternative approach to chemical synthesis of useful glycosides [5].

Curcumin is a yellow pigment of turmeric (dried rhizome of *Curcuma longa*, Zingiberaceae). It has been used primarily as a food colorant, but it is also a pharmacologically active principle of turmeric which has been used as a traditional medicine. Curcumin has recently attracted increased attention because of its potent anti-inflammatory and anti-leishmanial properties [6], and ability to reduce alcohol-induced liver disease [7]. Curcumin has also been reported to exhibit anti-cancer activity, based on various molecular mechanisms [8]. Furthermore, curcumin has been shown to suppress amyloid β-protein (Aβ)-induced oxidative damage and to prevent Aβ-infusion-induced spatial memory deficits in rats, suggesting a possible clinical application for treatment of Alzheimer's disease [9]. Although curcumin is an interesting compound as a novel medicine or a lead compound, its low water solubility limits further pharmacological exploration and practical application.

We previously indicated that *Catharanthus roseus* cell suspension cultures converted exogenously supplied curcumin to a series of glucosides [10]. The water solubility of curcumin glucosides is increased up to 20-million-fold compared with that of the aglycone, indicating that glucosylation is a powerful method to enhance curcumin water solubility. Furthermore, bioavailability of curcumin may be increased by glucosylation. It has been reported that, while the majority of orally administered curcumin in rats was excreted in the feces without being absorbed, some portion entered blood circulation after conjugation with glucuronic acid in the intestinal mucosa [11]. However, the glucosylation efficiency of cultured *C. roseus* cells was relatively low.

In the present paper, we describe the isolation of two cDNAs encoding novel glucosyltransferases, designated CaUGT1 and CaUGT2 (*Ca*tharanthus *roseus* UDP-glucose glucosyltransferase), from *C. roseus* cultured cells. Functional expression of the gene products in *Escherichia coli* demonstrated that CaUGT2 converted curcumin to curcumin monoglucoside and curcumin diglucoside. Recombinant CaUGT2 enzyme thus provides us with an efficient method to produce curcumin glucosides from curcumin.

2. Materials and methods

2.1. Cell cultures

Catharanthus roseus cell suspension cultures were maintained in LS medium [12] supplemented with 3% sucrose, 1 μM 2,4-dichlorophen-

* Correspondence author. Fax: +81-52-836-3415.
E-mail address: hajimem@phar.nagoya-cu.ac.jp (H. Mizukami).

Abbreviations: CaUGT, *Catharanthus roseus* UDP-glucosyltransferase; IPTG, isopropyl 1-thio-β-D-galactoside; LS, Linsmaier and Skoog; MJ, methyl jasmonate; Pfu, plaque forming unit; PSPG, plant secondary product glycosyltransferase

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oxyacetic acid and 1 μM kinetin. The cells were cultured at 25 °C in the dark and subcultured at 2-week-intervals. Methyl jasmonate (MJ) was dissolved in dimethylsulfoxide and aseptically added to the cultures through membrane filters at a final concentration of 250 μM three days after cell inoculation. The cells were collected by vacuum filtration at the defined times, immediately frozen in liquid nitrogen, and stored at −75 °C until use.

2.2. Construction of cDNA library

Poly(A)$^+$RNA was prepared from *C. roseus* cells harvested 24 h after MJ addition, using a Quick Prep mRNA Purification Kit (Amersham). The cDNA library was constructed using a λZAP cDNA Synthesis/Gigapack Gold Packaging Kit (Stratagene).

2.3. PCR cloning of glucosyltransferase cDNAs

A 5′-sense degenerate primer (5′-TT(T/C)(T/C/G)TI(A/T)(G/C) ICA(T/C)TG(T/C)GGITGGAA) was designed based on the amino acid sequence F(L/V)(T/S)HCGWN in the conserved PSPG-box of plant glucosyltransferases [13]. A 1 μl aliquot (33 000 pfu) of the cDNA library was used as a template for PCR amplification in a 25 μl reaction mixture containing 0.2 mM dNTP, 100 nM degenerate primer, 25 nM T7 primer and 0.5 unit *Taq* Polymerase (Roche Biochemicals). PCR was carried out using the following parameters; denaturing at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 40 °C for 2 min and 72 °C for 1 min, and then a final extension at 72 °C for 10 min. PCR products of about 500 bp were recovered from an agarose gel and subcloned into pCR2.1-TOPO vector (Invitrogen). Randomly selected clone inserts were sequenced for both strands using the Thermo Sequenase Cycle Sequencing Kit (Amersham). This yielded nine clones whose sequences fell into two independent sequence classes, both of which displayed significant similarity with various plant glucosyltransferases. The 5′-fragment was obtained by PCR using a common forward primer of the T3 sequence and reverse primers specific to the 3′-fragments and sequenced. Finally, using the 5′ and 3′ sequences as specific primers the near full-length cDNA clones were amplified from the cDNA library.

2.4. Heterologous expression in E. coli

For construction of the pMAL-CaUGT1 and pMAL-CaUGT2 expression vectors, the open reading frames of CaUGT1 and CaUGT2 were amplified by PCR with primers 5′-GCGCGAATTCATGGAAGAGATGAAGAAAGT and 5′-GCGCAAGCTTCTCATGATATAGTTTTCTTC for CaUGT1 and 5′-GCGCGGATCC-ATGGTTAATCAGCTCCATAT and 5′-GCGCGTCGACCCTAGTCTTGTTGCTTTCTT for CaUGT2. These primers correspond to the 5′-and 3′-ends of the open reading frames and include appropriate restriction sites (underlined). The 1.5 kb PCR products were subcloned into the pMAL-c2 vector (New England Biolabs) and sequenced to ensure that no mutation was incorporated. The resulting expression vectors pMAL-CaUGT1 and pMAL-CaUGT2 were used to transform *E. coli* JM109. The transformed bacteria were cultured at 37 °C in Luria–Bertani medium containing 100 μg/ml carbenicillin until they reached OD_{600} values of about 1.0, and then isopropyl 1-thio-β-D-galactoside (IPTG) was added to the culture at a final concentration of 1 mM. The induced bacteria were further cultured at 30 °C overnight and then harvested by centrifugation. Preparation and purification of the enzymes were performed at 4 °C. The crude recombinant enzyme was obtained by sonicating the bacterial pellet in 20 mM Tris–HCl buffer (pH 7.6) containing 200 mM NaCl and 5 mM EDTA followed by centrifugation at 12 000 × *g* for 15 min at 4 °C. The soluble fraction was applied to an Amylose Resin (New England Biolabs) column (bed volume, 2.0 ml). After the column was washed with column buffer (20 mM Tris–HCl, pH 7.4, 200 mM NaCl and 1 mM EDTA), the protein was eluted with 20 mM maltose in the column buffer. Protein content in the enzyme preparations was estimated using the method of Bradford [14].

2.5. Enzyme assays

For enzymatic assays of CaUGT1 and CaUGT2, 100 μl of a standard reaction mixture containing 50 mM Tris–HCl buffer (pH 7.5), 250 μM phenolic substrate, 2 mM UDP-glucose and the crude enzyme preparation (0.2 mg protein) was incubated at 30 °C for 30 min. The reaction was terminated by adding 200 μl methanol. After centrifugation at 12 000 × *g* for 10 min, the reaction products were analyzed by high performance liquid chromatography (HPLC). For determination of kinetic parameters of CaUGT2, the concentrations of the phenolic substrates were varied from 25 μM to 1 mM for curcumin and curcumin monoglucoside, and from 100 μM to 2.5 mM for other phenolics, in the presence of 2 mM UDP-glucose. For determination of K_m and V_{max} values for UDP-sugars, their concentrations were varied from 250 μM to 2 mM with the concentration of curcumin maintained at 250 μM. The incubations were carried out at 30 °C for 5 min. The kinetic values were estimated from Lineweaver–Burk plots from duplicate experiments.

HPLC analysis was performed on a reverse phase column (COSMOSIL 5C18-ARII, Nacalai Tesque) and the eluates were monitored by a photodiode array detector. The solvent conditions for separation of curcumin and curcumin glucosides were as described previously [10]. For separation of the other phenolic substrates and their glucosides, the following gradient elutions were used (flow rate 1.0 ml/min):

For esculetin, scopoletin and their glucosides: 0–8 min, 0–35% methanol; 8–9 min, 35–55% methanol; and 9–12 min, 55–100% methanol.

For *p*-nitrophenol, vanillin and their glucosides: 0–8 min, 20–55% methanol; 8–9 min, 55–100% methanol; and 9–12 min, 100% methanol.

For capsaicin, [6]-gingerol and their glucosides: 0–14 min, 40–79% methanol; 14–15 min, 79–100% methanol; and 15–20 min, 100% methanol.

The identity of the products was determined by co-chromatography on HPLC with the standard compounds and by their ultra-violet absorption spectra. The amounts of the products were determined based on the standard curves.

Curcumin, UDP-glucose and UDP-galactose were purchased from Sigma. Esculetin, esculin, scopoletin, *p*-nitrophenol and capsaicin were obtained from Wako Pure Chemicals. Vanillin and *p*-nitrophenyl-β-D-glucoside were from Nacalai Tesque. Glucovanillin was from our laboratory stocks. Curcumin monoglucoside and curcumin diglucoside were generous gifts of Profs. K. Isobe and K. Mohri of Showa Pharmaceutical University.

2.6. Analysis of gene expression by northern hybridization

Total RNA was prepared from the cultured cells using Trizol (Invitrogen). For northern hybridization, total RNA (10 μg) was electrophoresed in a formamide-containing agarose gel and blotted onto Hybond N+ (Amersham). Hybridization with digoxigenin-labeled probes and chemiluminescent detection were carried out according to a protocol supplied by Roche Biochemicals.

3. Results

3.1. Isolation of UDP-glucosyltransferase cDNAs by PCR from C. roseus cDNA library

Products resulting from PCR using a degenerate primer designed based on the highly conserved amino acid sequence among plant glycosyltransferases and a 3′-non-specific antisense primer (T7 primer) complementary to a λZAP II vector sequence displayed a band of about 500 bp, which was subcloned into a pCR2.1-TOPO vector. Among 30 clones randomly sequenced, 15 were found by database search to be similar to various plant glucosyltransferases. These clones were classified into two different groups (Ca-1 and Ca-2).

Full-length clones corresponding to both groups were isolated by 5′-rapid amplification of cDNA ends by using a 5′-non-specific sense primer (T3 primer) complementary to a λZAP-II vector sequence and sequence-specific antisense primers complementary to Ca-1 or Ca-2 sequence. The resulting clones were designated CaUGT1 and CaUGT2. The nucleotide sequences of CaUGT1 and CaUGT2 have been deposited in the DDBJ/EMBL/GenBank Nucleotide Sequence Database under the accession numbers AB159212 and AB159213, respectively.

CaUGT1 (1669 nucleotides) contains an open reading frame corresponding to a protein of 480 amino acids, whereas CaUGT2 (1722 nucleotides) has an open reading frame for 487

amino acids. The sequence identity between CaUGT1 and CaUGT2 was 27% on an amino acid base. The amino acid sequence of CaUGT1 revealed 55% identity with two tobacco glucosyltransferases (Nt1a and Nt1b) [4]. CaUGT2 was 62% identical with a salicylate-induced glucosyltransferase (IS5a) [15] and a phenylpropanoid glucosyltransferase of tobacco [16], and 61% identical with an anthocyanin 3′-glucosyltransferase of *Gentiana triflora* [17].

3.2. Bacterial expression of CaUGT1 and CaUGT2 cDNAs and properties of the recombinant enzymes

To examine the catalytic function of CaUGT1 and CaUGT2, both genes were expressed in *E. coli* as fusions with maltose binding protein and the crude enzyme extract was used for enzyme activity assays using either curcumin or curcumin monoglucoside as an acceptor substrate in the presence of UDP-glucose. As shown in Fig. 1A, the crude enzyme containing recombinant CaUGT2 converted both curcumin (**3**) and curcumin monoglucoside (**2**) to curcumin monoglucoside and curcumin diglucoside (**1**), respectively. Both reactions were completely dependent on UDP-glucose. In contrast, no products were detected when the recombinant CaUGT1 was incubated with either curcumin or curcumin monoglucoside. The crude enzyme preparation from the IPTG-induced bacteria harboring the control vector pMAL-c2 produced no glucosylation products from either curcumin or curcumin monoglucoside. The time-course pattern of the glucosylation reaction with curcumin (Fig. 1B) indicated that curcumin was first converted to curcumin monoglucoside which was then further glucosylated to produce curcumin diglucoside by the single enzyme of the recombinant CaUGT2. After 120 min incubation, the complete conversion of curcumin into the diglucoside was achieved.

To analyze the biochemical properties of CaUGT2, the recombinant fusion protein was purified by affinity chromatography using Amylose Resin (Fig. 2). The pH optimum of the enzyme was 7.5–8, and the glucosylation reaction proceeded linearly over a 10-min incubation and then the rate was gradually decreased under the present assay conditions. By using the affinity-purified fusion protein, substrate specificity for various phenolic compounds (Fig. 3) was examined as shown in Table 1. Apparent K_m-values for curcumin and curcumin monoglucoside were determined to be 19 and 63 μM, respectively. Apparent K_m-values for coumarins, *p*-nitrophenol and vanillin were more than 10-fold higher than those of curcumin and curcumin monoglucosides (Table 1). The V_{max}/K_m ratios indicated that CaUGT2 exhibits the highest specificity towards curcumin (100%), followed by curcumin monoglucoside (64%), esculetin (38%), scopoletin (10%),

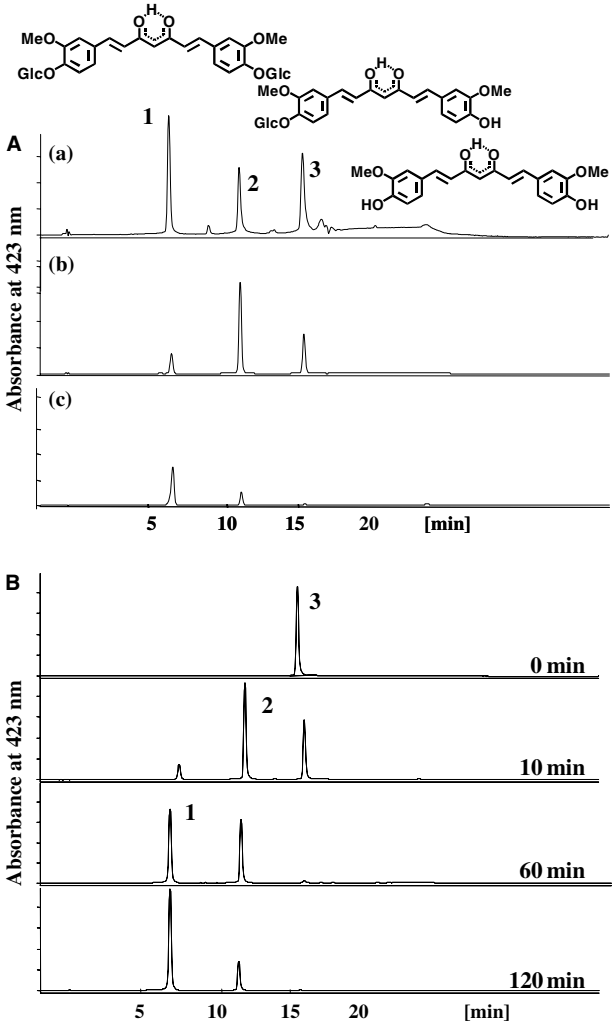

Fig. 1. Analysis of glucosyltransferase activity of recombinant CaUGT2 expressed in *E. coli*. (A) HPLC chromatogram of authentic standards of curcumin diglucoside (**1**, $R_t = 7.4$ min), curcumin monoglucoside (**2**, $R_t = 11.8$ min) and curcumin (**3**, $R_t = 15.8$ min) (a); The enzyme assay was carried out with the crude protein prepared from *E. coli* expressing recombinant CaUGT2 using curcumin (b) or curcumin monoglucoside (c) as a substrate. The eluate was monitored at $UV_{423\,nm}$. The identity of the reaction products was confirmed by comparison of the retention time and UV-spectrum (recorded on a diode array detector) with those of authentic standards. (B) Time course of changes in glucosylation of curcumin by incubation with the crude enzyme from *E. coli* producing recombinant CaUGT2. Curcumin was incubated for 0, 10, 60 and 120 min at 30 °C with 0.16 mg crude protein. The eluate was monitored at 423 nm.

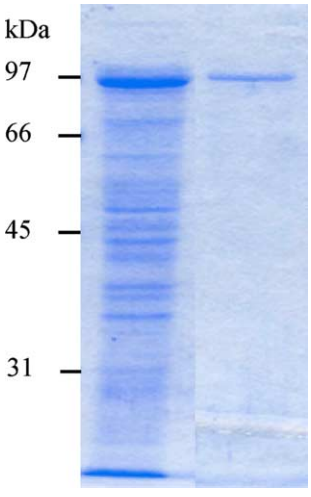

Fig. 2. SDS–PAGE analysis of the crude protein prepared from IPTG-induced *E. coli* harboring pMAL-CaUGT2 and fusion protein of recombinant CaUGT2 purified using amyrose-resin column. The crude protein (11 μg; left) and the purified protein (1 μg; right) were separated by 10% (w/v) SDS–PAGE gel and visualized with Coomassie Brilliant Blue staining.

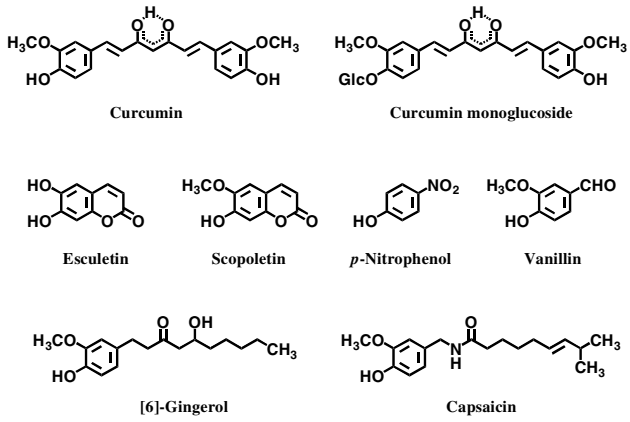

Fig. 3. Chemical structures of phenolic compounds used as glucosyl acceptors in the present investigation.

Table 1
Kinetic parameters of recombinant CaUGT2 towards various phenolic substrates

Substrates	K_m (μM)	V_{max} (pkat/mg)	V_{max}/K_m
Curcumin	19.0	273	14.9
Curcumin mono-glucoside	63.3	586	9.25
Esculetin	660	3634	5.50
Scopoletin	1120	1584	1.41
p-Nitrophenol	836	452	0.54
Vanillin	993	212	0.21
UDP-glucose	124	234	1.88
UDP-galactose	501	39.8	0.080

p-nitrophenol (4%) and vanillin (2%). In contrast, neither [6]-gingerol nor capsaicin was glucosylated although their chemical structures are somewhat similar to curcumin. The apparent K_m-values of sugar donors were 124 μM for UDP-glucose and 501 μM for UDP-galactose (Table 1).

We also examined glucosylation of the above mentioned phenolic compounds and two flavonols, kaempferol and luteolin, by crude recombinant CaUGT1 but none of these substrates was converted to the corresponding glucosides.

3.3. Methyl jasmonate-induced gene expression

It was shown previously that glucosylation of curcumin by cultured *C. roseus* cells was enhanced by addition of MJ to the cultures [10]. The effects of MJ on the mRNA level of CaUGT1 and CaUGT2 were therefore examined. As shown in Fig. 4, the expression of CaUGT2 was rapidly induced by addition of MJ to the cultures. Although the expression level of CaUGT1 was too low to be detected by northern hybridization, the CaUGT1 mRNA accumulation was also increased by MJ addition, when estimated by RT-PCR (data not shown).

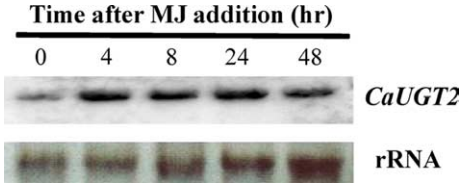

Fig. 4. Northern blot analysis of mRNA levels of CaUGT2 in *C. roseus* cell suspension cultures after the addition of MJ. The suspension cultures were supplemented with MJ at a final concentration of 250 μM three days after cell inoculation and the cells were collected for analysis 0, 4, 8, 24 and 48 h after MJ addition.

4. Discussion

Plant glycosyltransferases comprise a super-family of plant genes similar in magnitude to the *O*-methyltransferase [18] and cytochrome P450 [19] gene families. These enzyme classes are all extensively involved in modification of secondary metabolites, thus contributing to the diversity of plant chemistry. Based on the multiple alignment of a set of putative glycosyltransferases of *Cassava* and several glycosyltransferases mainly involved in flavonoid metabolism, a consensus sequence consisting of a short stretch of about 40 amino acids close to the C-terminal part of the protein has been proposed as a conserved domain for glycosyltransferases involved in plant secondary metabolism [20]. Within this PSPG-box, a peptide sequence of HCGWNS has been detected in 95% of all glycosyltransferases that catalyze inversion of the anomeric bond from the α-linkage found in UDP-sugar to the β-configuration found in the resulting glycosides [13]. By using a degenerate primer designed to target this peptide sequence, we isolated two cDNAs (CaUGT1 and CaUGT2) which encode putative glucosyltransferases from a cDNA library of cultured *C. roseus* cells.

The molecular phylogenetic tree (Fig. 5) based on a multiple sequence alignment of CaUGT1, CaUGT2 and 17 other plant UDP-glycosyltransferases retrieved from the database showed that CaUGT1 is placed adjacent to glucosyltransferases Nt1a and Nt1b of tobacco [4] and arbutin synthase of *Rauvolfia serpentina* [5]. All of these glucosyltransferases exhibit wide substrate specificity and catalyze glucosylation of various xenobiotic phenols such as naphthols, scopoletin and hydroquinone. CaUGT2, on the other hand, belongs to a cluster containing diverse glucosyltransferases such as flavonoid 7-glucosyltransferase of *Scutellaria baicalensis* [21], betanidin 5-glucosyltransferase of *Dorotheanthus bellidiformis* [22], flavonoid 3′-glucosyltransferase of *Gentiana triflora* [17] and *Arabidopsis* UGT73C6 [23]. The *Gentiana* and *Arabidopsis* enzymes catalyze transfer of glucose to the 3′-position of delphinidin 3, 5-*O*-diglucoside and 7-position of kaempferol 3-*O*-rhamnoside, respectively. Based on the relation of the dendrogram clusters to reported substrate specificities, we expected that CaUGT1 would be a likely candidate for catalyzing glucosylation of curcumin.

Heterologous expression experiments clearly showed, however, that only CaUGT2 was able to glucosylate curcumin, and that it did so in a sequential manner, converting curcumin to curcumin monoglucoside and then curcumin monoglucoside to curcumin diglucoside. CaUGT1, on the other hand, glucosylated neither curcumin nor curcumin monoglucoside. We have not found an efficient substrate for CaUGT1 among the various natural and unnatural phenolic compounds so far examined. The substrate specificity of CaUGT2, which was tested on a wide range of phenolic substrates, was relatively broad with the highest affinity for curcumin followed by curcumin monoglucoside and esculetin although it did not glucosylate [6]-gingerol or capsaicin, both of which show structural similarities to curcumin. Ferulic acid, which also has a similar

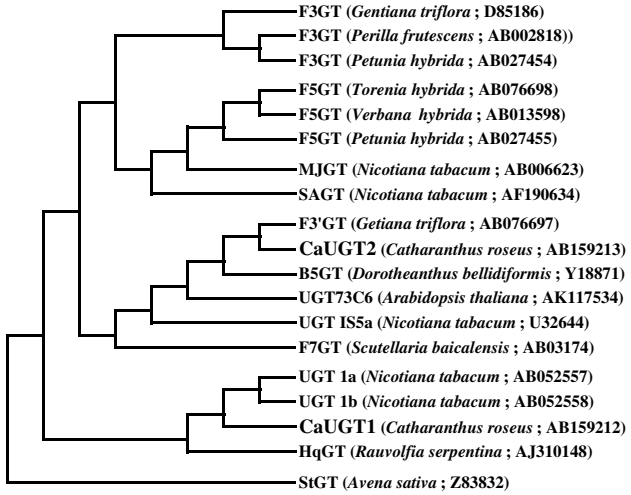

Fig. 5. Phylogenetic tree showing the relationship between two UDP-glucosyltransferases from *C. roseus* and some other plant secondary product glucosyltransferases retrieved from the database. The amino acid sequences were aligned using Clustal X and the tree was constructed by the neighbor-joining method in PHYLIP software package (J. Felsenstein, Phylogeny Inference Package, version 3.573, University of Washington, Seattle, WA, USA). Applying the parsimony-based method gave essentially the same topology. F3′H, flavonoid 3′-*O*-glucosyltransferase; F3H, flavonoid 3-*O*-glucosyltransferase; F5H, flavonoid 5-*O*-glucosyltransferase; MJGT, jasmonate-induced glucosyltransferase; SAGT, salicylic acid glucosyltransferase; F7GT, flavonoid 7-*O*-glucosyltransferase; B5GT, betanidin 5-*O*-glucosyltransferase; UGT1a, tobacco glucosyltransferase Nt1a; UGT1b, tobacco glucosyltransferase Nt1b; HqGT, hydroquinone *O*-glucosyltransferase; StGT, sterol *O*-glucosyltransferase. The species names of the original plants and database accession numbers are indicated in parentheses.

structure to curcumin, was glucosylated to some extent but further kinetic analysis was not performed because authentic ferulic acid glucoside was not available. The kinetic constants of recombinant CaUGT2 towards various phenolic substrates were within the same range displayed by other phenolic glucosyltransferases so far examined. Both UDP-glucose and UDP-galactose could support the glycosylation of curcumin by CaUGT2 although apparent K_m-value for UDP-galactose was 4-fold higher than that for UDP-glucose and the V_{max}/K_m ratio was only 4% of that for UDP-glucose, a pattern analogous to that reported for the tobacco glucosyltransferase Nt1a, which preferred UDP-glucose but also utilized UDP-xylose [3].

It is interesting to note that although esculetin was efficiently glucosylated by CaUGT2, the sole product was esculetin 6-*O*-β-monoglucoside (esculin); i.e., formation of esculetin 6,7-*O*-β-diglucoside was not detected. This indicates that conjugation of a glucose molecule to one phenolic hydroxyl group of curcumin does not create steric hindrance for the second glucosylation, whereas a glucosyl residue attached to 6-hydroxyl position of esculetin may interfere with further glucosyl conjugation to the neighboring 7-hydroxyl group. The result also suggests that CaUGT2 might have a strict position specificity for the hydroxyl group it can act upon.

Northern hybridization analysis indicated that expression of CaUGT2 was rapidly up-regulated by addition of MJ to the cells. This is consistent with our previous result [10] that glucosylation of curcumin in the cultured cells of *C. roseus* was markedly enhanced by adding MJ to the cell suspension cultures prior to curcumin addition, and may suggest, together with its relatively broad substrate specificity for both acceptor and donor molecules, that CaUGT2 is a defense-related UDP-glucosyltransferase whose function is detoxification of xenobiotic phenols or biosynthesis of defense molecules.

There have been some reports describing chemical synthesis of curcumin glucosides. Direct glucosylation of curcumin by acetobromoglucose followed by deacetylation gave curcumin monoglucoside and curcumin diglucoside at yields of 8% and 3%, respectively [24]. Recently, more efficient routes to curcumin monoglucoside and curcumin diglucoside starting from vanillin were reported with total yields of 35% and 21%, respectively [25]. In contrast to these, incubation of 1 μmole curcumin and 20 μmole UDP-glucose in the presence of 4 mg crude bacterial protein containing recombinant CaUGT2 in a total volume of 1 ml for 3 h at 30 °C yielded 735 nmole curcumin diglucoside, corresponding to 74% yield, without tedious purification of synthetic intermediates or any need for protection and deprotection of reactive groups. In a preliminary experiment, we also examined in vivo glucosylation by using intact recombinant *E. coli* cells expressing CaUGT2, in order to circumvent the use of relatively expensive UDP-glucose in the in vitro synthesis. However, the glucosylation yield was 4% at maximum and the sole product was curcumin monoglucoside.

In conclusion, whatever the physiological function of CaUGT2 may be, the use of recombinant CaUGT2 may provide an efficient tool for the production of pharmacologically useful curcumin glucosides. Furthermore, integration and expression of CaUGT2 gene may result in an efficient biotransformation of this and other natural products in planta.

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