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Production of unnatural glucosides of curcumin with drastically enhanced water solubility by cell suspension cultures of *Catharanthus roseus*

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Abstract ***Catharanthus roseus*** **cell suspension cultures converted exogenously supplied curcumin to a series of glucosides, none of which has been found in nature so far. The efficiency of glucosylation was dependent on culture stage of the cells and medium sucrose concentration. Methyl jasmonate and salicylic acid enhanced the glucoside formation only when they were added to the cultures prior to the addition of curcumin. The glucoside yield was 2.5 μmol/g fresh weight of the cells at an optimal culture condition. The water solubility of curcumin-4′,4″-*O*-β-D-digentiobioside was 0.65 mmol/ml, which was 20 million-fold higher than that of curcumin.**

Key words: Glucosylation; Curcumin; Water solubility; Cell suspension culture; *Catharanthus roseus*

1. Introduction

Curcumin (diferuloylmethane) is a yellow pigment of turmeric (dried rhizome of *Curcuma longa*, Zingiberaceae). It has been used primarily as a food colorant. It is also a pharmacologically active principle of turmeric which has been used as a folk medicine. Recently curcumin has attracted increased attention because of its potent pharmacological activities including anti-oxidative, anti-inflammatory, anti-leishmanial properties [1] and the ability to reduce alcohol-induced liver disease [2]. Curcumin has also been reported to exhibit anticancer action, based on inhibition of tumor initiation by various carcinogens and tumor promotion by phorbol esters [3], induction of apoptosis in cancer cells [4], modulation of expression of transcriptional factors such as NF-κB [5], and inhibition of angiogenesis [6]. In addition, 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 its clinical application for Alzheimer's disease [7].

Curcumin is practically insoluble in water at acidic and neutral pH. Although it is soluble in alkali, it undergoes rapid hydrolytic degradation at pH values above neutral [8]. This low water solubility limits further pharmacological exploitation and practical application of curcumin.

Higher plants accumulate a wide range of glycosides as secondary metabolites, and are capable of conjugating sugar residues not only to endogenous metabolic intermediates but also to xenobiotics. Such glycosylation has diverse functions in plants, including increasing the solubility and stability of the aglycones, decreasing the toxicity of xenobiotics and activation of biosynthetic intermediates [9]. The glycosylation reactions are catalyzed by plant UDP-glycosyltransferases, many of which have a broad substrate specificity [10,11]. Thus, glycosylation of various organic molecules by cultured plant cells has been investigated as one of the targets in biotechnological application of plant cell culture systems. It has been reported that a wide range of aromatic compounds supplied to cell suspension cultures can be efficiently converted to their glucosides, including hydroquinone [12], vanillin [13] and capsaicin [14].

In the present paper we describe the ability of *Catharanthus roseus* cell cultures to glucosylate exogenously supplied curcumin to form a series of glucosides with drastically increased water solubility. We also show that the culture stage and sucrose concentration in the medium both influence the glucosylation efficiency of the cells, and that pretreatment of the cells with methyl jasmonate (MJ) or salicylic acid (SA) greatly improves the cellular glucosylation activity towards curcumin.

2. Materials and methods

2.1. Cell cultures

C. roseus cell suspension cultures were maintained in LS medium [15] supplemented with 3% sucrose, 1 μM 2,4-dichlorophenoxyacetic acid and 1 μM kinetin. The cells were cultured at 25°C in the dark and subcultured at 2-week intervals. MJ and SA were dissolved in dimethylsulfoxide (DMSO) and water, respectively, and aseptically added to the cultures through membrane filters.

2.2. Addition of curcumin

Curcumin was purchased from Sigma and purified by silica gel column chromatography before use. Curcumin was dissolved in DMSO at a concentration of 150 mM and 0.1 ml of the solution was added to the culture through a membrane filter to make a final concentration of 0.5 mM. At this concentration, curcumin immediately precipitated from the medium but after 1 day incubation the precipitate had largely disappeared. The cells were collected at an

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Abbreviations: HMBC, ^{1}H-^{13}C heteronuclear multiple bond connectivity; MJ, methyl jasmonate; SA, salicylic acid

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appropriate time by filtration, frozen in liquid nitrogen and stored at −80°C until use.

2.3. Screening of glucosylation of curcumin

Curcumin was added to the cell suspension cultures 10 days after subculture and incubated for an additional 48 h before collection by vacuum filtration. The fresh cells (ca. 1 g) were extracted with 5 ml of methanol by sonication. Glycoside fractions were obtained by extracting the methanol extract suspended in water or the medium with ethyl acetate and then water-saturated butanol. The ethyl acetate and butanol extracts were applied to silica gel 60F (Merck) thin layer chromatography (TLC) plates and developed with chloroform–methanol (4:1).

2.4. Isolation of glucosylation products from C. roseus *cultured cells*

Cultured *C. roseus* cells supplemented with curcumin were sonicated in methanol as described above. After the solvent was evaporated, the residue was suspended in water and sequentially extracted with isooctane, toluene, ethyl acetate and water-saturated butanol. The ethyl acetate and butanol extracts were subjected to high performance liquid chromatography (HPLC) separation. The HPLC eluates corresponding to the possible glycosylation product peaks (G1–G5) were pooled. HPLC conditions: column, Develosil RPaqueous C30 (20×250 mm, Nomura Chemical); elution, 63% methanol for separation of the butanol extract and 70% methanol for the ethyl acetate extract; flow rate 5 ml/min. The absorbance of the eluate was monitored by a photodiode array detector.

2.5. β-Glucosidase treatment

Fractions G1–G5 were dissolved in 100 mM citrate buffer, pH 4.8, containing almond β-glucosidase (Sigma) and incubated at 37°C for 60 min. The reaction mixtures were then extracted with ethyl acetate and the extract was subjected to HPLC analysis as described in Section 2.7.

2.6. Spectrum measurements

ESI-FT mass spectroscopy (MS) spectra were recorded using an APEX III instrument (Bruker Daltonics). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured on a JNM-LA500 spectrometer (JEOL). The signals were assigned using ^{1}H-^{13}C heteronuclear multiple bond connectivity (HMBC). The ^{13}C NMR data are shown in Table 1.

Table 1
^{13}C NMR data for glucosylation products of curcumin in pyridine-d_5 (125 MHz)

Sugar residue		Chemical shift of the glucosylation products				
		CG1	CG2	CG3	CG4	CG5
Glucose	C-1a	101.8	101.7	101.8	102.1	101.6
	C-2a	74.4	74.5	74.7	74.5	74.5
	C-3a	78.2	78.3	79.0	78.3	78.8
	C-4a	70.7	70.9	71.2	70.9	71.0
	C-5a	77.5	77.6	78.3	77.6	78.2
	C-6a	69.6	69.8	62.3	69.8	62.1
Glucose	C-1b	105.1	105.2		105.3	
	C-2b	75.0	75.1		75.1	
	C-3b	78.0	78.2		78.1	
	C-4b	71.4	71.5		71.5	
	C-5b	77.9	78.0		78.1	
	C-6b	62.4	62.5		62.5	
Glucose	C-1c	101.8	101.9	101.8		
	C-2c	74.4	74.6	74.7		
	C-3c	78.2	78.8	79.0		
	C-4c	70.7	71.1	71.2		
	C-5c	77.5	78.1	78.3		
	C-6c	69.6	62.2	62.3		
Glucose	C-1d	105.1				
	C-2d	75.0				
	C-3d	78.0				
	C-4d	71.4				
	C-5d	77.9				
	C-6d	62.4				

2.7. Quantitative determination of the glucosides

The frozen cells (about 1 g) were sonicated three times in 2 ml of methanol for 30 min. The extracts were combined and evaporated to dryness. The residue was dissolved in 1.0 ml of methanol and fractionated by HPLC (Cosmosil 5C18-ARII column, 4.6×150 mm, Nacalai Tesque) with gradient elution. The following gradient was used: 0–14 min, 40–79% methanol; 14–15 min, 79–100% methanol, 15–20 min, 100% methanol. The flow rate was 1.0 ml/min. The elution was monitored at 423 nm. The amounts of the products were calculated based on a calibration curve prepared using the respective curcumin glucosides.

2.8. Measurement of water solubility

Each compound was stirred in a sufficient amount of water for 24 h at room temperature. The mixture was then centrifuged at 12 000×*g* for 15 min at room temperature. The concentration of the compound in the supernatant was estimated by HPLC as described in Section 2.7 for curcumin glucosides. The following gradient conditions were used for estimation of (A) hydroquinone and arbutin (monitored at 280 nm) and (B) luteolin and luteolin glucosides (monitored at 360 nm): (A) 0–5 min, water; 5–10 min, 0–5% methanol; 5–15 min, 5–100% methanol; (B) 0–5 min, 40–55% methanol; 5–10 min, 55–100% methanol; 10–15 min, 100% methanol.

3. Results

3.1. Screening for the glycosylation ability of curcumin in various plant cell cultures

Suspension cultures of 10 different plant species (*Bupleurum falcatum*, *C. roseus*, *Daucus carota*, *Duboisia* hybrid, *Gardenia jasminoides*, *Glehnia littoralis*, *Ipomoea batatas*, *Lithospermum erythrorhizon*, *Nicotiana tabacum* and *Papaver somniferum*) were screened for their ability to glycosylate curcumin. All the suspension cultures were supplied with curcumin 10 days after cell inoculation. The cells were harvested after 2 days and extracted with methanol and the medium was extracted with ethyl acetate. The cell and medium extracts were subjected to TLC analysis. Yellow spots with higher polarities (lower R_f values) than curcumin were only detected in the cellular extract of *C. roseus*.

3.2. Identification of curcumin glucosides produced in C. roseus *cell suspension cultures*

At least five potentially glycosylated products were detected when the methanol extract of the *C. roseus* cells supplied with curcumin was analyzed by HPLC as shown in Fig. 1. These products (CG1–CG5) were isolated by preparative HPLC. All the purified products yielded curcumin when digested with almond β-glucosidase. Their chemical structures were elucidated based on the MS and NMR spectra (Fig. 2).

The high resolution EI-MS spectrum of CG1 showed a pseudo-molecular ion peak at *m/z* 1039.3188 (calculated for $C_{45}H_{60}O_{26}Na$, 1039.3265), 648 mass units larger than curcumin, indicating the presence of four hexose residues. Likewise, molecular ion peaks $(M+Na)^+$ were observed at *m/z* 877.2736 (calculated for $C_{39}H_{50}O_{21}Na$, 877.2746) for CG2, 715.2198 (calculated for $C_{33}H_{40}O_{16}Na$, 715.2209) for CG3, 715.2199 (calculated for $C_{33}H_{40}O_{16}Na$, 715.2209) for CG4, and 553.1680 (calculated for $C_{27}H_{30}O_{11}Na$, 553.1689), indicating the presence of three hexose moieties in CG2, two hexose moieties in CG3 and CG4 and one hexose moiety in CG5. These hexose moieties were determined to be glucose by comparing their chemical shifts in ^{13}C NMR with those in the reference [16–19]. Furthermore, the configurations of the anomeric carbons were defined as β for all the glucose molecules from their coupling constants.

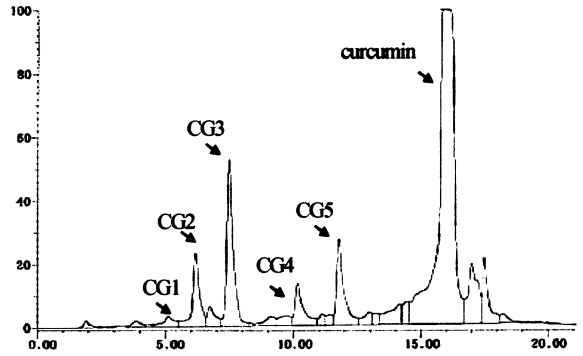


Fig. 1. HPLC profile of the methanol-soluble fraction from *C. roseus* cell suspension cultures supplemented with curcumin. The eluate was monitored at 423 nm. CG1–CG5 are possible biotransformed products of curcumin.

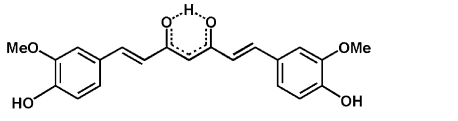
The glycosidation position in CG5 was unambiguously determined by a correlation between the glucosyl anomeric proton H-1a (δ 5.74) and curcumin C-4′ (δ 150.9) in the HMBC spectrum. In addition, ^{1}H and ^{13}C NMR data were consistent with an authentic curcumin 4′-*O*-β-D-glucoside obtained by chemical synthesis [20]. Thus CG5 was identified to be curcumin 4′-*O*-β-D-glucoside.

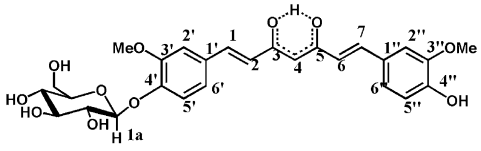
The NMR spectra of CG4 revealed that the molecule was asymmetric, suggesting that sugar residues were attached to one of two phenolic hydroxyl groups of curcumin. As observed in the HMBC spectrum, the correlation of H-1b (δ 5.09) of glucose with C-6a (δ 69.8) of glucose established the linked position between the two glucose moieties as β-D-glucopyranosyl-(1→6)-β-D-glucopyranose. The glucosidation position was determined by a correlation between the glucosyl anomeric proton H-1a (δ 5.65) and curcumin ring C-4′ (δ 149.8) using HMBC. Based on the above evidence, the structure of CG4 was established as curcumin 4′-*O*-β-D-gentiobioside.

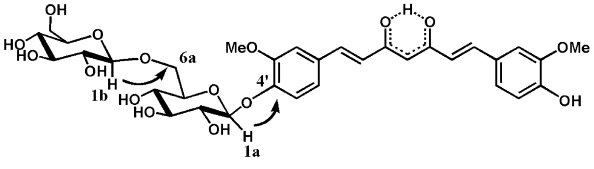
The NMR spectra data of CG3 showed that the molecule was symmetric, suggesting that single glucose residues were attached to each of the 4′- and 4″-hydroxyl groups. The ^{1}H and ^{13}C NMR spectra were consistent with those of chemically synthesized curcumin 4′,4″-*O*-β-D-diglucoside. Thus CG3 was identified as curcumin 4′,4″-*O*-β-D-diglucoside.

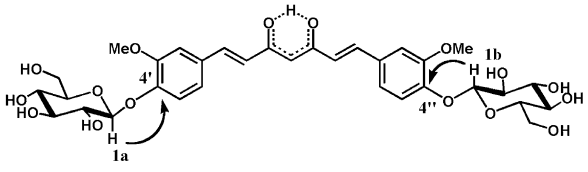
Of three anomeric protons of CG2 H-1a (δ 5.64) and H-1c (δ 5.09) exhibited correlations with curcumin C-4′ (δ 149.8) and C-4″ (δ 149.7), respectively, in the HMBC spectrum. A correlation was also observed between H-1b (δ 5.74) and C-6a (δ 69.8) of glucose using HMBC. Therefore, the structure of CG2 was determined as curcumin 4′-*O*-β-D-gentiobiosyl-4″-*O*-β-D-glucoside.

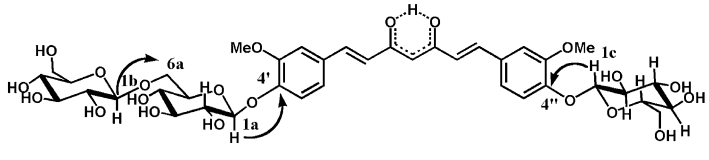
The NMR spectra of CG1 suggested that the molecule was symmetric. H-1a and H-1c (δ 5.66) of glucose exhibited correlation with either curcumin C-4′ or C-4″ (δ 149.6) in the HMBC spectrum. The correlation was also detected between either H-1b or H-1d (δ 5.08) and either C-6a or C-6c (δ 69.6).











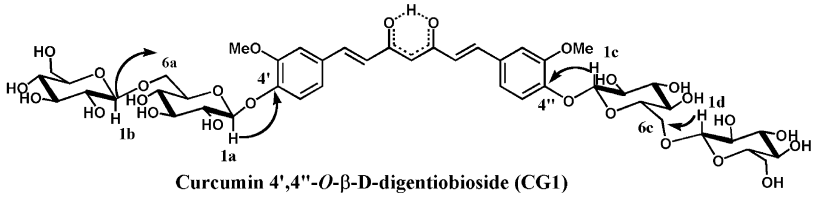


Fig. 2. Structures of curcumin and its glucosylation products isolated and identified in the present investigation. Arrows indicate the HMBC correlations.

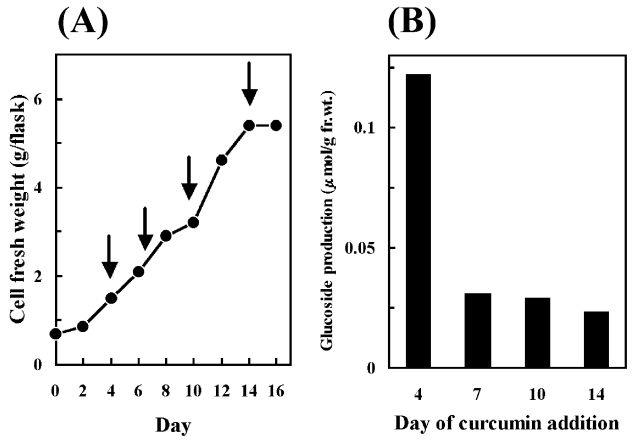


Fig. 3. Time course of changes in fresh weight (A) and glucosylation activity (B) of *C. roseus* suspension cultures. Arrows indicate curcumin application. Curcumin was supplied to the cell suspension on day 4, 7, 10 or 14, and the cells were cultured for one additional day before estimating the curcumin glucoside formation.

CG1 was thus determined as curcumin 4′,4″-*O*-β-D-digentiobioside.

3.3. *Culture stage and glucosylation activity of* C. roseus *cells*

The glucosylation activity of the cultured *C. roseus* cells was examined during a growth cycle of cell suspension cultures (Fig. 3). Cultures at 4, 7, 10 and 14 days after cell inoculation were incubated with curcumin for 24 h before cell collection. The amount of curcumin glucosides formed per gram fresh weight of the cells was highest when curcumin was supplied to the cultures on day 4 (the exponential phase of growth) and then rapidly decreased during the later stages of the growth cycle. There was no significant difference in the ratio of various curcumin glucosides among the cells of different growth stages.

3.4. *Effects of MJ and SA on the glucosylation activity of the cultured cells*

MJ was added to the cell suspension cultures 3 days after cell inoculation at final concentrations of 100, 250 and 500 μM. The cells were incubated for 24 h and then curcumin was added to the culture. The glucosylation of curcumin was monitored for 48 h. As shown in Fig. 4A, glucosylation activity for curcumin was markedly increased by addition of MJ to the cultures prior to curcumin supply. The optimum concentration of MJ was 250 μM and the glucoside production was increased about eight-fold compared with that of the control cells. When MJ was added together with curcumin on day 4 of the culture no increase in curcumin glucosylation was observed (data not shown). Addition of SA also enhanced the glucosylation activity of the cultured *C. roseus* cells at concentrations at 250 μM and above (Fig. 4B) only when SA was added to the cultures prior to curcumin administration (data not shown).

3.5. *Effect of medium sucrose concentration on glucosylation activity of the cultured cells*

In order to examine the effect of medium sucrose concentration on the glucosylation of curcumin the cells were grown in culture media containing different amounts of sucrose. Curcumin was added to the MJ-treated cultures 4 days after cell

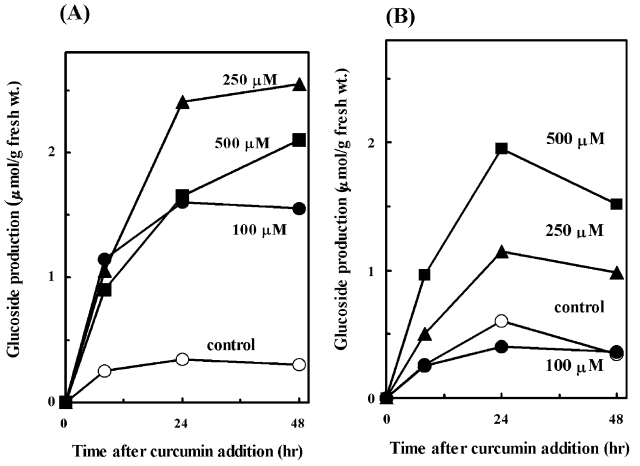


Fig. 4. Effects of MJ (A) and SA (B) on glucosylation of curcumin in *C. roseus* cell suspension cultures. Curcumin was supplied to the cell suspension on day 4 of culture. MJ or SA was added to the culture 1 day prior to curcumin supply. Each point indicates an average from triplicate cultures. The entire experiment was repeated twice with essentially the same result, and one representative data set is shown here.

inoculation and the incubation was continued for an additional 2 days (Fig. 5). The glucoside formation was enhanced at higher sucrose concentrations of 8% and 10%.

3.6. *Glucosylation of curcumin in the cultured cells of* G. jasminoides, L. erythrorhizon *and* P. somniferum

Among nine plant species whose cell culture did not exhibit glucosylation activity towards curcumin in the initial screening described in Section 3.1, *G. jasminoides*, *L. erythrorhizon* and *P. somniferum* cells were chosen to further explore their glucosylation activity. First, curcumin glucosylation was monitored at the optimal condition for *C. roseus* cells, i.e. in the presence of MJ and 8% sucrose and on day 4 of the

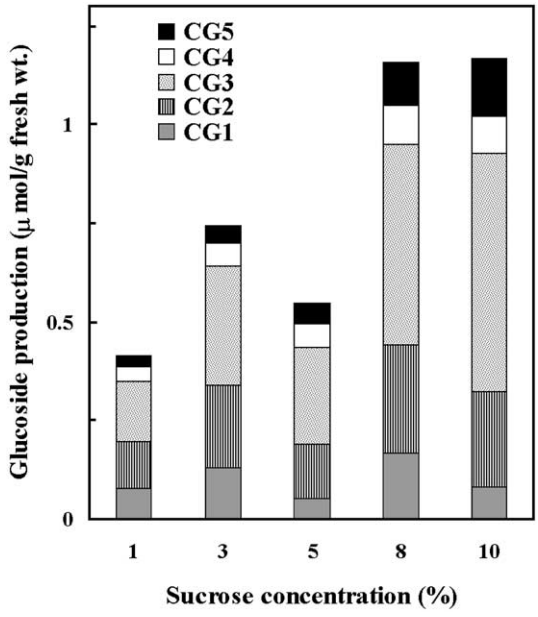


Fig. 5. Effect of medium sucrose concentration on glucosylation of curcumin in *C. roseus* cell suspension cultures. Curcumin was supplied to the cell suspension on day 4 of culture. MJ (250 μM) was added to the culture 1 day prior to curcumin supply.

Table 2
Water solubility of curcumin, hydroquinone, luteolin and their glucosides

Compound	Number of glucose residues	Water solubility[a]	
		μmol/ml	fold
Curcumin[b]	0	3.0×10^{-5}	1
CG5	1	7.0×10^{-3}	2.3×10^{2}
CG4	2	7.3×10^{1}	2.4×10^{6}
CG3	2	3.7×10^{2}	1.2×10^{7}
CG2	3	4.5×10^{2}	1.5×10^{7}
CG1	4	6.5×10^{2}	2.2×10^{7}
Hydroquinone	0	5.9×10^{2}	1
Arbutin	1	3.9×10^{2}	0.66
Luteolin	0	$<2\times10^{-4}$	–
Luteolin 7G[c]	1	3.1×10^{-2}	1
Luteolin 3′,7G[d]	2	3.2×10^{-1}	10

[a]Solubility was measured at room temperature.
[b]From [31].
[c]Luteolin 7-*O*-glucoside.
[d]Luteolin 3′,7-*O*-diglucoside.

culture. None of these cells accumulated curcumin glucosides when examined 1 day after curcumin addition. Next, CG3 and CG5 were separately added to these cell suspension cultures instead of curcumin. However, production of CG1, CG2 or CG4 was not detected in any of these cultures.

3.7. Water solubility of curcumin glucosides

The water solubility of curcumin glucosides was estimated and compared with that of curcumin (Table 2). The water solubility of curcumin monoglucoside (CG5) was increased to 7.0 nmol/ml, which was 230-fold higher than that of curcumin. The solubility increased about 20 million-fold in the case of curcumin digentiobioside (CG1) (650 μmol/ml) compared with the solubility of curcumin (30 pmol/ml). In contrast, the water solubility of hydroquinone was not significantly different from that of its glucosyl conjugate, arbutin, when compared on a molar concentration basis. The solubility of luteolin 3′,7-*O*-diglucoside was about 10-fold higher than that of luteolin 7-*O*-glucoside.

4. Discussion

Curcumin was converted to a series of its glucosides when supplied to *C. roseus* cell suspension cultures. Curcumin aglycone was practically insoluble in water and a precipitate was formed in the medium when curcumin was supplied as a DMSO solution. However, it seemed to be efficiently incorporated into the cells, since after 48 h incubation only a small amount of curcumin precipitate was observed in the medium.

There have been many reports on glucosylation of xenobiotic phenols in cultured plant cells since Pilgrim first described glucosylation of some simple phenols, including hydroquinone, in callus cultures of three plant species [21]. In most cases exogenous phenolic compounds with two hydroxyl groups were shown to be converted to their monoglucosides: hydroquinone to arbutin [22], salicyl alcohol to salicin and isosalicin [23], esculetin to esculin [24] and salicylic acid to salicylic acid *O*-glucoside [25]. Furthermore, camphor was shown to be transformed to its monoglucosides at 3-, 5-, 6- or 8-position by *Eucalyptus perriniana* cultured cells after the introduction of hydroxyl groups into these positions but no diglucoside was formed [26]. In contrast, *C. roseus* cells in culture converted curcumin to various glucosides not only by introducing individual glucose residues onto its two phenolic hydroxyl groups but also by forming β-1,6-glycosidic linkages, leading to the gentiobioside derivatives. The glucosyl conjugation appeared to proceed sequentially since CG5 began to accumulate immediately after addition of curcumin while accumulation of other glucosides such as CG3 and CG1 started to increase after a lag period of about 8 h after curcumin addition (data not shown). Cellular content of curcumin glucosides decreased gradually after 24–48 h of curcumin addition. No clear difference in time course pattern of the decrease was observed among five curcumin glucosides (data not shown). It is not clear whether this decrease is due to hydrolysis by β-glucosidase or conversion to some other metabolites.

The ability to produce such diverse glucosides appears to be specific to the present system, since other species tested displayed no analogous activity. A whole set of the glucosyltransferases responsible for formation of a series of curcumin glucosides is present specifically in *C. roseus* cells because suspension cultures of other plant cells such as *G. jasminoides*, *L. erythrorhizon* and *P. somniferum* failed to glucosylate not only curcumin but also CG3 and CG5. The pattern of glycosylation may also be influenced by the nature of the substrate, curcumin, since the *C. roseus* cells converted exogenously added hydroquinone only to the monoglucoside, arbutin, and formed neither gentiobiosides nor doubly glucosylated derivatives (data not shown).

It has been reported that expression of some glucosyltransferase genes can be enhanced by stress treatment such as wounding, infection, elicitor treatment and treatments with MJ or SA [27–30]. Such reports led us to examine the effects of MJ or SA on in vivo glucosylation of curcumin by *C. roseus* cells. The glucosylation efficiency of the cells was markedly up-regulated by pretreatment of the cells with either MJ or SA. Although MJ exhibited a positive effect on curcumin glucosylation at 100 μM, the effect of SA was negligible at this concentration. Simultaneous addition of MJ and curcumin produced no change in the glucosylation activity, and pretreatment of the cells with MJ was necessary to up-regulate the cellular glucosylation activity, indicating that MJ-inducible glucosyltransferases are probably involved in glucosylation of curcumin.

Tønnesen et al. [31] investigated the effect of ionic and non-ionic cyclodextrins on the solubilization of curcumin in aqueous medium. The highest curcumin concentration was approximately 0.8 μmol/ml when 11% randomly methylated β-cyclodextrin solution was used. This concentration was about 26 000-fold higher than the maximum solubility of curcumin in aqueous buffer (30 pmol/ml). Glucosyl conjugation of curcumin drastically enhanced the water solubility when at least two glucose residues were conjugated to the molecule because CG4, curcumin 4′-*O*-gentiobioside, and CG3, curcumin 4′,4″-*O*-diglucoside, were 10 000-fold and 50 000-fold, respectively, more soluble than CG5, curcumin 4′-*O*-monoglucoside. This is in sharp contrast to the result that luteolin 3′,7-*O*-diglucoside was only 10-fold more soluble than luteolin 7-*O*-glucoside. The solubility reached 0.65 mmol/l when four glucosyl residues were conjugated to curcumin, and this concentration was more than 20 million-fold higher than that of curcumin. Thus, glucosyl conjugation was far more effective than cyclodextrin complexation at enhancing the water solubility of curcumin. It has been shown that, in rats, the majority of orally

administered curcumin was excreted in the feces without being absorbed but some portion entered the blood circulation after conjugation with glucuronic acid in the intestinal mucosa, and further conjugation with sulfate in the liver [32]. Therefore, the soluble curcumin glucosides should not only facilitate investigation of the pharmacological activities of curcumin but also may be useful as prodrugs of curcumin.

Curcumin glucosides have also been chemically synthesized. Hergenhahn [33] obtained curcumin-4′,4″-*O*-β-D-diglucoside (CG3) and curcumin-4′-*O*-β-D-monoglucoside (CG5) by condensing curcumin with α-D-acetyl-bromoglucose followed by deacetylation but the yields were only 3% for CG3 and 8% for CG5. Recently, more efficient novel synthetic routes to CG3 and CG5 from vanillin-*O*-tetraacetylglucoside have been presented, with total yields of 35% and 21%, respectively [17]. Presently, the maximum productivity of curcumin glucosides is about 2.5 μmol/g fresh weight of *C. roseus* cells, when the cells are cultured in LS medium containing 8% sucrose and supplemented 4 days after cell inoculation with 250 μM MJ 24–48 h prior to curcumin addition. This productivity corresponds to a yield of 32% because average biomass yield per culture was 1.5 g and 15 μmol curcumin was added to a 30 ml culture. However, it may be possible to further improve the productivity by manipulating the culture conditions and/or the protocols for applying curcumin. Furthermore, cloning of genes encoding glucosyltransferases involved in curcumin glucosylation may lead to biotechnological production of curcumin glucosides using recombinant enzymes or recombinant bacteria, which would avoid the tedious protection and deprotection processes necessary in chemical synthesis.

In conclusion, exogenously added curcumin to *C. roseus* suspension cultures was efficiently transformed to various naturally unknown glucosides with greatly enhanced water solubility. The glucosylation efficiency was increased by elevating medium sucrose concentration up to 8%. The productivity was also enhanced by adding MJ or SA to the cell suspension 24–48 h prior to curcumin supply, suggesting the involvement of stress-inducible glucosyltransferases. Purification and cDNA cloning of the glucosyltransferases responsible for curcumin glucosylation are now under investigation. Absorption, metabolism and pharmacological activities of the curcumin glucosides in laboratory animals should also be examined to evaluate the potential for use of the glucosides as prodrugs.

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References

[1] Gomes, D.C.F., Alegrio, L.V., Leon, L.L. and de Lima, M.E.F. (2002) Arzneim.-Forsch. 52, 695–698.
[2] Nanji, A.A., Jokelainen, K., Tipoe, G.L., Rahemtulla, A., Thomas, P. and Dannenberg, A.J. (2003) Am. J. Physiol. Gastrointest. Liver Physiol. 284, G321–G327.
[3] Kawamori, T., Lubet, R., Steele, V.E., Kelloff, G.I., Kaskey, R.B., Rao, C.V. and Raddy, B.S. (1999) Cancer Res. 99, 597–601.
[4] Choudhuri, T., Pal, S., Agwarwal, M.L., Das, T. and Sa, G. (2002) FEBS Lett. 512, 334–340.
[5] Bharti, A.C., Donato, N., Singh, S. and Aggarwal, B.B. (2003) Blood 101, 1053–1062.
[6] Gururaj, A.E., Belakavadi, M., Venkatesh, D.A., Marme, D. and Salimath, B.P. (2002) Biochem. Biophys. Res. Commun. 297, 934–942.
[7] Frautschy, S.A., Hu, W., Kim, P., Miller, S.A., Chu, T., Harris-White, M.E. and Cole, G.M. (2001) Neurobiol. Aging 22, 993–1005.
[8] Tønnesen, H.H. and Karlsen, J. (1985) Z. Lebensm.-Unters.-Forsch. 180, 132–134.
[9] Jones, P. and Vogt, T. (2001) Planta 213, 164–174.
[10] Taguchi, G., Yazawa, T., Hayashida, N. and Okazaki, M. (2001) Eur. J. Biochem. 268, 4086–4094.
[11] Hefner, T., Arend, J., Warzecha, H., Siems, K. and Stöckigt, J. (2002) Bioorg. Med. Chem. 10, 1731–1741.
[12] Inomata, S., Yokoyama, M., Seto, S. and Yanagi, M. (1991) Appl. Microbiol. Biotechnol. 36, 315–319.
[13] Kometani, T., Tanimoto, H., Nishimura, T. and Okada, S. (1993) Biosci. Biotechnol. Biochem. 57, 1290–1293.
[14] Kometani, T., Tanimoto, H., Nishimura, T., Kanbara, I. and Okada, S. (1993) Biosci. Biotechnol. Biochem. 57, 2192–2193.
[15] Linsmaier, E.M. and Skoog, F. (1965) Physiol. Plant. 18, 100–127.
[16] Hiller, K., Otto, A. and Grundeman, E. (1980) Pharmazie 35, 113–114.
[17] Aritomi, M., Komori, T. and Kawasaki, T. (1986) Phytochemistry 25, 231–234.
[18] Yasukawa, K. and Takido, M. (1986) Phytochemistry 26, 1224–1226.
[19] Merfort, I. and Wendisch, D. (1987) Planta Med. 53, 434–437.
[20] Watanabe, Y., Mouri, K. and Isobe, K. (2003) 123rd Annual Meeting of The Pharmaceutical Society of Japan, Nagasaki, Abstract 28-P1-II-129.
[21] Pilgrim, H. (1970) Pharmazie 25, 568.
[22] Tabata, M., Ikeda, F., Hiraoka, N. and Konoshima, M. (1976) Phytochemistry 15, 1225–1229.
[23] Mizukami, H., Terao, T., Miura, H. and Ohashi, H. (1983) Phytochemistry 22, 679–680.
[24] Tabata, M., Umetani, Y., Shima, K. and Tanaka, S. (1984) Plant Cell Tissue Organ Culture 3, 3–9.
[25] Umetani, Y., Kodakari, E., Yamamura, T., Tanaka, S. and Tabata, M. (1990) Plant Cell Rep. 9, 325–327.
[26] Orihara, Y., Noguchi, T. and Furuya, T. (1994) Phytochemistry 35, 941–945.
[27] Imanishi, S., Hashizume, K., Kojima, H., Ichihara, A. and Nakamura, K. (1998) Plant Cell Physiol. 39, 202–211.
[28] Farissinet-Tachet, L., Baltz, R., Chong, J., Kauffmann, S., Fritig, B. and Saindrenan, P. (1998) FEBS Lett. 437, 319–323.
[29] O'Donnell, P.J., Truesda, M.R., Calvert, C.M., Dorans, A., Roberts, M.R. and Bowles, D.J. (1998) Plant J. 14, 137–142.
[30] Hirotani, M., Kuroda, R., Suzuki, H. and Yoshikawa, T. (2000) Planta 210, 1006–1013.
[31] Tønnesen, H.H., Másson, M. and Loftsson, T. (2002) Int. J. Pharmacol. 244, 127–135.
[32] Asai, A. and Miyazawa, T. (2000) Life Sci. 67, 2785–2793.
[33] Hergenhahn, M. (2002) German patent application, DE 2337.