Identification and Biochemical Characterization of an Arabidopsis Indole-3-acetic Acid Glucosyltransferase*

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Biochemical characterization of recombinant gene products following a phylogenetic analysis of the UDP-glucosyltransferase (UGT) multigene family of Arabidopsis has identified one enzyme (UGT84B1) with high activity toward the plant hormone indole-3-acetic acid (IAA) and three related enzymes (UGT84B2, UGT75B1, and UGT75B2) with trace activities. The identity of the IAA conjugate has been confirmed to be 1-O-indole acetyl glucose ester. A sequence annotated as a UDP-glucose:IAA glucosyltransferase (IAA-UGT) in the Arabidopsis genome and expressed sequence tag data bases given its similarity to the maize iaglu gene sequence showed no activity toward IAA. This study describes the first biochemical analysis of a recombinant IAA-UGT and provides the foundation for future genetic approaches to understand the role of 1-O-indole acetyl glucose ester in Arabidopsis.

A signature motif thought to be involved in the binding of the UDP moiety of sugar nucleotides has been identified in a wide range of enzymes that catalyze the transfer of glucose or glucuronic acid to small molecule acceptors (1). Using the motif to screen sequences in the genome and expressed sequence tag data bases of Arabidopsis, a large multigene family has been defined (2). In plants, the conjugation of glucose to small hydrophobic molecules can lead to the formation of glucose esters or glucosides. The former are high energy compounds and have long been regarded as biosynthetic intermediates (3), whereas glucosides are generally considered to represent the storage forms of the aglycones (4). Both transfer reactions are suggested to occur in the cytoplasm of cells (5), with the attachment of glucose providing access to membrane transport systems and passage into either the vacuole (6) or the extracellular space (7).

It has been known for many years that plant hormones can be glycosylated, although the role of the conjugates is not well defined (8, 9). Despite the wide occurrence of glycosylated conjugates (10–12) and the many studies of plant glucosyltransferases involved in hormone conjugation (13–15), only three genes encoding hormone glucosyltransferases have ever been identified, the iaglu gene of maize (16) and two zeatin glucosyltransferases (17, 18). The maize iaglu gene encodes a UDP-glucosyltransferase (UGT)1 that forms the 1-O-indole acetyl glucose ester (IAGluc), the glucosylated conjugate of indole-3-acetic acid (IAA).

IAA plays a central role in most, if not all, aspects of a plant's existence, providing a means of transducing external environmental changes into internal adaptive responses. The hormone is known to exist as the free acid and in conjugated form linked to a wide variety of compounds such as amino acids, peptides, and sugars (10). Many studies have used maize as a model to analyze IAA conjugates. In this species, the glucose conjugate is found in small amounts in vegetative tissue and has been shown to be an intermediate in the biosynthesis of IAA-myo-inositol (19), a putative intercellular transport form of IAA from endosperm to shoot (20). The myo-inositol conjugate has been found to be further glycosylated to form IAA-myo-inositol galactoside and IAA-myo-inositol arabinoside (21). All of these conjugates of IAA have been identified in maize seeds and are thought to represent storage forms, which upon hydrolysis could release free IAA to the seedling (22). In Arabidopsis, the principal conjugates found in vegetative tissue are the amide-linked conjugates (90%). The ester-linked conjugates are also found, but in much smaller amounts (10%). IAGluc makes up about 34% of this ester pool in 12-day-old plants (23). The IAA conjugates are suggested to be storage forms of IAA (24, 25) or catabolism products (26).

Clearly, the field of IAA conjugation is complex and, while many of the enzymes involved in these interconversions have been partially purified, the identification of their corresponding genes would open up the possibility of taking a genetic route to understanding the events that occur and their consequences in the plant. Although the iaglu gene of maize was identified 6 years ago, its product was neither purified nor characterized biochemically, and no further studies on the gene have been undertaken. Despite the availability of the iaglu for cross-hybridization and homology searching within the Arabidopsis genome, no corresponding gene product with demonstrable IAA conjugating activity has been published to date. The available-
ity of a detailed phylogenetic analysis of the Arabidopsis UGT multigene family has now made it feasible to screen likely family members for IAA-UGT activity. This study identifies an Arabidopsis gene encoding an IAA-UGT and provides detailed biochemical analysis of the gene product.

EXPERIMENTAL PROCEDURES

Plant Materials—Wild-type Arabidopsis, ecotype Columbia, were grown in Levington’s seed and modular compost in a controlled environment of 16/8 h light-dark cycle (22 °C, 220 microeinsteins·s⁻¹·m⁻² light, 18 °C, dark). After 4 weeks, tissue from leaf, stem (all vertical nonleaf or nonflower tissue), root, and inflorescence (flowers at all stages of development including siliques) were harvested for RNA extraction. 6-Week-old tissue was used to analyze the steady-state levels of mRNA in siliques. Young siliques were the first nine from the tip, and all of the subsequent siliques were classified as old.

Recombinant UGT Purification—Escherichia coli strain XL-1 Blue carrying the recombinant GST-UGT protein expression plasmid (27) was grown at 20 °C in 75 ml of 2× YT medium containing 50 µg/ml

![Graphical representation of experimental procedures](https://example.com/graphic.png)
ampicillin until the $A_{600}$ reached 1.0, after which the culture was incubated with 1 mM isopropyl-1-thio-D-galactopyranoside for 24 h at 20 °C. The cells were harvested by centrifugation at 5,000 × g for 5 min and were resuspended in 2 ml of Spheroblast buffer (0.5 mM EDTA, 750 mM sucrose, 200 mM Tris-HCl, pH 8.0) (28). Lysozyme (1 mg) and 14 ml of half-strength Spheroblast buffer were added immediately. After incubation at 4 °C for 30 min, the cells were harvested again by centrifugation and osmotically shocked in 5 ml of phosphate-buffered saline containing 0.2 mM phenylmethylsulfonyl fluoride. Cell debris was removed by centrifugation at 10,000 × g for 15 min. The protein in the supernatant fraction was collected by adding 100 ml of 50% glutathione-coupled Sepharose gel (Amersham Pharmacia Biotech) and recovered in elution buffer (20 mM reduced form glutathione, 100 mM Tris-HCl, pH 8.0, 120 mM NaCl), according to the manufacturer’s instructions. The protein assays were carried out with Bio-Rad Protein Assay Dye using bovine serum albumin as reference. The purified recombinant proteins were also analyzed by SDS-PAGE following the methods described by Sambrook et al. (29).

**Glucosyltransferase Activity Assay**—The general glucosyltransferase activity assay mix (200 μl) contained 2 μg of purified recombinant proteins, 14 mM 2-mercaptoethanol, 2.5 mM UDPG, 1 mM IAA, 50 mM Tris-HCl, pH 7.0. The reaction was carried out at 30 °C for 1 h and stopped by the addition of 20 μl of trichloroacetic acid (240 mg/ml). The reaction mix was analyzed using the HPLC method.

**HPLC Analysis**—Reverse phase HPLC was performed with a Waters HPLC system (Waters Separator 2690 and Waters Tunable Absorbance Detector 486; Waters Ltd., Herts, UK) and a Columbus 5-μm C18 column (250 × 4.60 mm; Phenomenex). A linear gradient with increasing methanol (solvent B) against distilled H2O (solvent A) at a flow rate of 1 ml/min over 30 min was used to separate the glucose conjugates from their aglycone. Both solvents contained 0.01% H3PO4 (pH 3.0). The following elution conditions were used: IAA, 10–48% B, λ detection 230 nm; IBA, IPA, NAA, 5-OH-IAA, and 2-ox-IAA, 10–70% B, λ detection 230 nm; 2,4-dichlorophenoxyacetic acid and pipicloram, 10–100% B, λ detection 287 nm. The cinnamic acids were analyzed under the conditions described previously (27).

**Identification of the 1-O-Glucose Ester Using Gas Chromatography-Mass Spectrometry**—The reaction was carried out in 200 μl of 100 mM HEPES/NaOH, pH 7.0, containing 14 mM 2-mercaptoethanol, 5 mM MgCl2, 10 mM KCl, 5 mM UDPG, 1 μM IAA. After 1 h of incubation (or 6 h for isomer analysis) with 5 μg of the enzyme at 37 °C, the reaction was stopped with 1 ml of acetonitrile. Subsequently, samples were centrifuged, and the supernatant was dried in vacuo. The product of the reaction was then separated from the substrates using two Isolute SPE columns, SAX (anion exchanger) and Env+ (hydrophobic), arranged in tandem. Eluate from the Env+ column was dried, dissolved in 500 μl of acetonitrile, and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide/trichloroacetic acid/trimethylchlorosilane (Pierce), and 1 μl was injected into the gas chromatography-mass spectrometry system. Half of the sample for isomer analysis was treated with hydroxylamine prior to the silylation with N,O-bis(trimethylsilyl)trifluoroacetamide/trichloroacetic acid. The column used for analysis was a 15-m × 0.25-mm Chrompack CP-SIL 22CB, and injector temperature was set to 280 °C. After the injection (splitless), the column temperature was held at 80 °C for 2 min and then increased at the rate of 20 °C/min to 200 °C, and 4 °C/min to 280 °C. Column effluent was introduced into the ion source of a JEOL JMS-700 mass spectrometer. Ion source and a gas chromatography interface were held at 270 °C. Ions were generated with 70 eV at an ionization current of 300 μA. A mass spectrometer operated in full scan mode with an acceleration voltage of −10 kV, scan range between m/z 40 and 800, and ion multiplier set to −1.2 kV.
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### RESULTS

Identification of UGT Activity toward IAA—Earlier work suggested that only one subgroup of putative glucosyltransferase sequences in the Arabidopsis genome encodes enzymes forming glucosyl esters (2, 27). This subgroup was analyzed for activity in synthesizing the glucose ester of IAA. The sequences were cloned and used to produce soluble recombinant fusion proteins with GST in Escherichia coli (Fig. 1A). Following purification, some of these fusion proteins proved to be particularly unstable, releasing GST (26 kDa) as a separate polypeptide. This is a common observation with this fusion system (34). Each of these 10 recombinant proteins was screened for putative UGT activity toward IAA including the protein corresponding to the previously annotated IAA-UGT in Arabidopsis data bases (35) (Fig. 1B). Only four out of all of the sequences consistently produced a more hydrophilic compound eluting more rapidly in the methanol gradient. The data shown correspond to an assay time of 1 h at 30 °C to visualize the trace activities seen for UGT75B1, UGT75B2, and UGT84B2. The major activity was always observed with UGT84B1, which reached equilibrium rapidly and did not go beyond 32% conversion under any of the conditions assayed. A pH profile of enzyme activity indicated a broad optimum from pH 6 to 7.5 (data not shown). Under conditions described above, in which the assay was stopped by the addition of trichloroacetic acid, only a single peak of product was observed, and this comigrated with the glucose esters of IAA (30) could be observed when the reaction was not stopped by trichloroacetic acid addition (Fig. 1C). These multiple peaks were found to be hydrolyzable by 1 M NaOH (Fig. 1C), and all, as shown in Fig. 1D, contained radio-

### Table I

| Substrate                  | UGT84B1 | UGT84B2 | UGT75B1 | UGT75B2 |
|----------------------------|---------|---------|---------|---------|
| ICA                        | 5.9     | ND      | ND      | ND      |
| IAA                        | 159.0   | 7.2     | 2.7     | 2.8     |
| IPA                        | 97.0    | 3.7     | 10.8    | 5.5     |
| IBA                        | 112.0   | 6.2     | 23.3    | 2.8     |
| 5-OH IAA                   | 57.4    | 2.3     | 1.3     | 3.6     |
| tryptophan                 | 22.2    | ND      | ND      | ND      |
| 2-Ox-IAA                   | 2.2     | ND      | ND      | ND      |
| Cinnamic acid              | 71.6    | 2.3     | 10.7    | 1.4     |
| Caffeic acid               | 2.8     | ND      | ND      | ND      |
| Ferulic acid               | 28.8    | ND      | ND      | ND      |
| Sinapic acid               | 5.6     | ND      | ND      | ND      |
| NAA                        | 33.0    | 2.7     | 0.8     |         |
| 2,4-Dichlorophenoxyacetic acid | 2.1 | ND      | ND      | ND      |
| Pichloram                  | 0.7     | ND      | ND      | ND      |
| IAA + UDP-galactose        | 0.2     | ND      | ND      | ND      |
| IAA + UDP-xylose           | 0.2     | ND      | ND      | ND      |

Coupled Enzyme Assay—The IAA-UGT activity was determined as the release of UDP, which can be measured using a coupled assay containing UGT, pyruvate kinase, and lactate dehydrogenase (30). The reaction mechanisms are shown as the following.

\[
\text{IAA + UDPG} \leftrightarrow \text{IAA-Glc + UDP} \\
\text{PEP + UDP} \leftrightarrow \text{UTP + pyruvate} \\
\text{Pyruvate + NADH + H}^+ \leftrightarrow \text{lactate + NAD}^+ \\
\]

### REACTIONS 1–3

The reaction mix, in a total volume of 1.0 ml, contained 50 mM HEPES-NaOH, pH 7.6, 2.5 mM MgSO4, 10 mM KCl, 1 mM potential substrate, 5 mM UDPG, and 0.5–25 μg/ml enzyme. The reactions were incubated at 30 °C for 20–60 min. UDP-galactose and UDP-xylose were tested at 2.5 mM in the same conditions. ND, not determined.

Phospholipid Preparation and Binding Assay—Phospholipid vesicles were prepared using the sucrose solution method (31). A 100-μl bed volume of phospholipids (Sigma) was homogenized in distilled H2O, followed by centrifugation for 5 min at 12,000 × g, the pellet was resuspended in 240 mM sucrose and was incubated at room temperature for 2 h. The liposomes were harvested by centrifugation and then resuspended in 100 μl in binding buffer (50 mM HEPES, pH 7.6, with or without 1 mM CaCl2, 1 mM MgCl2). An aliquot of 10 μl was used for inhibition assays, and 80 μl was used for binding assays.

UGT84B1 or a recombinant tomato annexin Rp35 (32) was incubated for 15 min at room temperature with liposomes in the presence of binding buffer. Nonbound protein in the supernatant was separated from the liposomes by centrifugation at 12,000 × g for 10 min. The supernatant (100 μl) was added to 20 μl of SDS loading buffer. The pellet was washed in 5 volumes of binding buffer, and 100 μl of 50 mM HEPES containing 5 mM EDTA was added to elute protein bound to the liposomes. After centrifugation, the supernatant containing EDTA-eluted protein was added to 20 μl of SDS loading buffer. All of the samples were subsequently boiled for 5 min, and 25 μl of each sample was analyzed by 10% SDS-PAGE.

Reverse Transcription-PCR—Total RNA was extracted from plant tissue by the hot phenol method (33) and quantified by spectrophotometry. The integrity was checked by formaldehyde-agarose gel electrophoresis. Reverse transcription was carried out using a superscript premplification system (Life Technologies, Inc.) for first strand cDNA synthesis according to the manufacturer's instructions using an oligo(dT) primer. UGT84B1-specific PCR was then performed using primers 5'-CCGCTATGATGCGCGATGTACGGGCTC-3' (for 84B1) and 5'-CGGGTCCGCCGCGATGTACGGGCTC-3' (for 84B1). The PCR (100 μl) was set up by mixing 2 μl of first strand cDNA, 1× PCR buffer (Promega), 1.5 mM MgCl2, 0.5 μl each primer, 0.25 mM each deoxynucleoside triphosphate, and 5 units of Taq DNA polymerase (Promega). The PCR was carried out in an MJ Research PTC-200 thermal cycler with 5 min at 94 °C, followed by 30 cycles of 1 min of denaturation at 94 °C, 2 min of annealing at 62 °C, and 4 min of extension at 74 °C. The reaction was completed by one cycle of 5 min at 74 °C. The PCR products were then analyzed by 1% (w/v) agarose gel. After separation, the PCR products were transferred onto nylon membrane and probed with a radiolabeled UGT84B1 DNA fragment (29).

### TABLE I

| Substrate                  | Specific activity (mkat/kg) |
|----------------------------|-----------------------------|
| ICA                        | 5.9                         |
| IAA                        | 159.0                       |
| IPA                        | 97.0                        |
| IBA                        | 112.0                       |
| 5-OH IAA                   | 57.4                        |
| tryptophan                 | 22.2                        |
| 2-Ox-IAA                   | 2.2                         |
| Cinnamic acid              | 71.6                        |
| Caffeic acid               | 2.8                         |
| Ferulic acid               | 28.8                        |
| Sinapic acid               | 5.6                         |
| NAA                        | 33.0                        |
| 2,4-Dichlorophenoxyacetic acid | 2.1 |                  |
| Pichloram                  | 0.7                         |
| IAA + UDP-galactose        | 0.2                         |
| IAA + UDP-xylose           | 0.2                         |

All assays were carried out in 50 mM HEPES, pH 7.6, 2.5 mM MgSO4, 10 mM KCl, 1 mM potential substrate, 5 mM UDPG, and 0.5–25 μg/ml enzyme. The reactions were incubated at 30 °C for 20–60 min. UDP-galactose and UDP-xylose were tested at 2.5 mM in the same conditions. ND, not determined.

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TABLE II
Effect of potential activators and inhibitors on the activity of UGT84B1

| Potential inhibitor or activator | Relative activity |
|---------------------------------|-------------------|
| 3 mM MgCl₂                     | 125.2 ± 3.5       |
| 3 mM CaCl₂                      | 122.2 ± 3.7       |
| 3 mM MnCl₂                      | 151.4 ± 6.8       |
| 5 mM EDTA                       | 98.4 ± 6.3        |
| 5 mM EGTA                       | 96.0 ± 3.7        |
| 3 mM MgCl₂ + 5 mM EDTA + 5 mM EGTA | 96.2 ± 4.2     |
| 3 mM CaCl₂ + 5 mM EDTA + 5 mM EGTA | 94.1 ± 1.8      |
| 3 mM MnCl₂ + 5 mM EDTA + 5 mM EGTA | 92.9 ± 2.4      |
| 4 mM pyrophosphate              | 96.0 ± 5.3        |
| 1 mM UDP                        | 37.3 ± 2.3        |
| 1 mM zeatin                     | 97.3 ± 2.0        |
| 1 mM dihydrosietin              | 95.9 ± 1.0        |
| 1 mM cinnamic acid              | 95.2 ± 1.2        |

The effect of metal ions on UGT activity is shown in Table II. Each assay contained substrate at a range from 0 to 1 mM. A saturating concentration of UDPG at 5 mM was used to measure the enzyme activity towards IBA, IPA, and cinnamic acid. The results shown represent the mean of three independent experiments ± S.D.

Relative Activity of UGTs with Different Substrates—Enzyme activity was first compared using two assays: one based on HPLC and a second based on a coupled assay in which NADH oxidation was followed. Both methods gave comparable results (data not shown). UGT84B1 has the highest activity toward IAA, with significant activity also shown toward IBA, IPA, and cinnamic acid. The other proteins only showed low activity toward these additional substrates. The activity of UGT84B1 toward indole acetyl aspartate, indole acetyl glutamate, indole acetyl alanine, and indole acetaldehyde was also tested and found to be zero (data not shown). In addition to UDPG, the ability of UDP-galactose and UDP-xylose to act as sugar donors was investigated and found to be negative.

Effect of Potential Inhibitors and Activators of Enzyme Activity—The effect of metal ions on UGT activity is shown in Table II. Some increase in relative activity was observed with Mg²⁺, Ca²⁺, and Mn²⁺, which was abolished by the addition of...
Expression of UGT84B1 in Arabidopsis Plants—The sequence UGT84B1 was identified in the genome data base as a putative UGT on the basis of a UDP-glucose binding motif previously reported (1). However, no expressed sequence tag had been found to correspond to UGT84B1; therefore, there was no direct evidence to show the gene is expressed in planta. Fig. 7 shows the results of analyzing UGT84B1 mRNA levels. Clearly, the data, based on RT-PCR, show that UGT84B1 transcripts are highly abundant in siliques and inflorescence with relatively low levels of expression observed in root tissue.

DISCUSSION

The near completion of the Arabidopsis genome sequencing project provides an opportunity to identify novel plant genes on the basis of homology searching. In this study, we have used a characteristic signature motif, previously shown to define a family of UGTs encompassing UDP-glucuronosyltransferases and UDP-glucosyltransferases from prokaryotic and eukaryotic organisms (1). A detailed phylogenetic analysis of the Arabidopsis multigene family encoding the enzymes has shown that 12 groups (A–L) can be distinguished on the basis of their sequence and pattern of intron gain (2). In particular, a subfamily in group L has been identified in biochemical analyses of recombinant proteins to form glucose esters (27). These studies provided the basis for screening Arabidopsis UGTs for their ability to form the glucose ester conjugate of IAA. When the recombinant proteins were analyzed for their ability to glucosylate IAA, only one, UGT84B1, showed high activity. Interestingly, the closest relative of UGT84B1, with greater than 80% identity, had only trace activity toward IAA. Six of these recombinant proteins, including UGT84B1, are active toward cinnamates (27).

Previously, the only gene that has been identified to encode a UGT of IAA is the maize inlglu (16). In that study, the gene was found by screening an expression library with antibodies raised against a maize UGT. Following heterologous expression of the gene in E. coli, the bacterial extract was shown to have UGT activity toward IAA. The maize sequence has been used for many years to identify putative IAA-UGT in the Arabidopsis genome and expressed sequence tag data bases. For example, based on their close sequence homology, UGT77D1 has been annotated as an IAA-UGT (34). However, analysis of recombinant protein from this sequence for activity toward IAA

| Soluble | Bound |
|---|---|
| Rp35 UGT 84B1 | Rp35 UGT 84B1 |
| - | - | - | - | + | + | + | + |
| Ca²⁺ | | | | | | | |

Fig. 6. Interaction of UGT84B1 with phospholipids. Phospholipid vesicles prepared from a mixture of phosphatidylserine and phosphatidylcholine were incubated with tomato annexin GST fusion (Rp35) or UGT84B1 in the presence (+) or absence (−) of 1 mM Ca²⁺, 1 mM Mg²⁺ in 50 mM HEPES, pH 7.6. Protein bound in a Ca²⁺-dependent manner was separated from nonbound protein by centrifugation and subsequently eluted with 5 mM EDTA. Samples were analyzed on a 10% SDS-PAGE gel, and proteins were visualized by Coomassie staining.

Fig. 7. RT-PCR analysis of different plant tissues. The expression of UGT84B1 in different plant tissues was analyzed by RT-PCR on 1% (w/v) agarose gel (upper) and by Southern hybridization using radiolabeled UGT84B1 DNA fragments following exposure of 10 s (middle), or longer exposure of 1 h (lower). The controls are samples prepared without reverse transcriptase.
has proved negative. The fact that UGT84B2 with greater than 80% identity to UGT84B1 and existing within the same plant species has only negligible activity toward IAA demonstrates that sequence comparison alone cannot be used to predict substrate specificity.

This study shows that in the in vitro assay, the recombinant enzyme from UGT84B1 conjugates IBA, IPA, and cinnamic acid in addition to IAA. The $K_m$ values for IAA, IBA, IPA, and cinnamic acid are nearly identical, although competition experiments indicate that IAA is the preferred substrate for UGT84B1 when compared with cinnamic acid and IBA (Table III, Fig. 5). The interaction of UGT84B1 and IAA is complex and can be affected by a number of factors. The specificity studies indicate that the chain length of the carboxyl group is important, since UGT84B1 has very low activity toward 2-oxIAA, an IAA shows the lowest activity among the cinnamates. In addition, a 64% decrease in the activity on the indole and benzyl rings. A 64% decrease in the activity has been observed with 5-OH IAA compared with that with IAA. A similar effect has been found with the cinnamates. Caffeic acid, which has two hydroxyl groups on the benzyl ring, shows the lowest activity among the cinnamates. In addition, the enzyme has extremely low activity toward 2-oxIAA, an IAA derivative containing a carbonyl group at position 2 on the indole ring (Table I, Fig. 3).

There are no previous data available on a purified IAA-UGT, since the maize enzyme was analyzed only as a partial purified extract (38, 39) and the recombinant product of the maize iaglu gene was neither purified nor characterized (16). The properties of the recombinant enzyme analyzed in this study are different from those described for the partially purified IAA-UGT from maize, with respect to the lack of inhibition by cytokinins and lack of effect of phospholipids, whereas the dependence on metal ions and reducing conditions are identical. Cinnamic acid, IPA, and IBA were not tested as potential substrates of the maize enzyme. Similar levels of trace activities for enzymes of the two species were observed toward ferulic acid and p-coumaric acid.

This study is a biochemical characterization of a recombinant protein using an in vitro assay. As yet, the relationships of this activity to events that occur within the plant are unknown. For example, both IBA and IAA glucose conjugates have been identified in plants, including Arabidopsis (23, 40, 41). IAA is the preferred substrate for UGT84B1 in vitro, but in planta the enzyme may glucosylate both IAA and IBA dependent on cell specificity of expression, relative availability of substrate(s), and relative compartmentalization of enzyme and substrate(s).

Preliminary analysis of the tissue-specific expression of UGT84B1 in Arabidopsis plants showed the highest level of expression in the siliques, when steady-state levels of UGT84B1 mRNA were detectable even in Northern analysis of total RNA. Results from RT-PCR, however, demonstrate transcripts of the gene are also present in the root. Experiments are currently under way using a UGT84B1 promoter-reporter gene system to define more closely which cells express the UGT. Parallel phenotypic characterization of transgenic Arabidopsis plants either overexpressing UGT84B1 or with the gene knocked out by a nonautonomous dSpm transposable element insertion will provide essential information on the function of the IAA-UGT.

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