Hemicellulose is a major component of primary plant cell walls. Many of the glycosyl residues found in hemicellulose are derived from the sugar precursor UDP-glucuronic acid, which can be converted into UDP-arabinose, UDP-apiose, UDP-galacturonic acid, and UDP-xylose. The enzyme controlling the biosynthesis of UDP-glucuronic acid, UDP-glucose dehydrogenase (EC 1.1.1.22), was cloned from soybean (Glycine max [L.] Merr.) by an antibody screening procedure. This enzyme is surprisingly homologous to the bovine sequence, which is the only other eukaryotic UDP-glucose dehydrogenase sequence known. The characteristic motifs of the enzyme, the catalytic center, a NAD-binding site, and two proline residues for main chain bends, are conserved within the prokaryotic and eukaryotic sequences. The soybean full-length cDNA clone encodes a protein of 480 amino acids with a predicted size of 52.9 kD. The enzyme is highly expressed in young roots, but lower expression levels were observed in expanding tissues of the epicotyl and young leaves. The expression pattern of the enzyme in different developmental stages strengthens the argument that UDP-glucose dehydrogenase is a key regulator for the availability of hemicellulose precursors.

Growing plant cells continuously synthesize new plant cell components. The hemicellulose polysaccharides are synthesized by various enzymes in the Golgi apparatus (Carpita and Gibeaut, 1993; Driouich et al., 1993). The precursors for polysaccharides of the cell wall are the corresponding nucleoside-diphosphate sugars, in particular the UDP-sugars (Feingold, 1982). Whereas cellulose components are synthesized from UDP-Glc, many of the hemicellulose sugars (e.g. UDP-Ara, UDP-Api, UDP-GalUA, and UDP-Xyl) are derived from UDP-GlcUA as shown in the scheme in Figure 1. The uronic acid can be epimerized to UDP-GalUA, the most prominent precursor of pectic compounds. Decarboxylation of UDP-GlcUA leads to UDP-Xyl, which can be further epimerized to UDP-Ara (for review, see Bolwell, 1988; Feingold and Barber, 1990; Iiyama et al., 1991; Gibeaut and Carpita, 1994). Thus, UDP-GlcUA is a precursor for sugar nucleotides, which are needed for the biosynthesis of many components of hemicellulose, including arabinans, arabinogalactans, glucuronoxylans, rhamnogalacturonans, xylans, and xyloglucans. Various biochemical studies have suggested that the production of UDP-GlcUA may be the rate-limiting step in providing precursors for the expanding cell wall. Therefore, it was hypothesized that UDP-GlcDH, the enzyme that converts UDP-Glc to UDP-GlcUA, is the key regulator in this pathway (Amino et al., 1985; Robertson et al., 1995). Nevertheless, the precise function of UDP-GlcDH in providing sugar nucleotide precursors for hemicellulose biosynthesis has not yet been elucidated.

The detailed analysis of UDP-GlcDH is difficult because the enzyme has not yet been purified from plant sources. Enzyme activity can be measured only in crude or partially purified extracts, with an important limitation being that competing reactions can degrade the substrate UDP-Glc at a high rate and the product undergoes further reactions. UDP-GlcDH is also strongly feedback-inhibited by UDP-Xyl (Davies and Dickinson, 1972), a metabolite derived from UDP-GlcUA by a decarboxylase. This biochemical regulation may distort the true enzyme activity in a crude in vitro assay.

The analysis of the role of UDP-GlcDH is further complicated by the existence of a second biosynthetic route for UDP-GlcUA called the inositol oxidation pathway (Loewus and Dickinson, 1982) (Fig. 1, right side). Within this pathway Glc-6-P is converted to GlcUA via inositol. Subsequently, GlcUA is also activated to give UDP-GlcUA. To our knowledge it is unknown at present which route for the synthesis of UDP-GlcUA is the most important one in plants. Data for the inositol oxidation pathway were obtained by feeding radioactive precursors (Roberts and Loewus, 1973; Verma and Dougall, 1979). The rate-limiting step of this pathway is not known. There is evidence for the coexistence of both pathways with changing importance of either route during plant development (Rubery, 1972; Dalesandro and Northcote, 1977a, 1977b; Witt, 1992).

Here we report the cloning of UDP-GlcDH from soybean (Glycine max [L.]). The enzyme is highly expressed in various tissues but is almost absent in others. Most interestingly, the enzyme structure of UDP-GlcDH is highly conserved between plants and animals, although the product of the reaction is used for totally different polymers and further reactions. In animals UDP-GlcUA is used for proteoglycans, dermatan sulfate, heparan sulfate, and heparin (Hempel et al., 1994). Animals also use UDP-GlcUA for the detoxification of xenobiotics by glucuronosylation, which

Cloning of an Enzyme That Synthesizes a Key Nucleotide-Sugar Precursor of Hemicellulose Biosynthesis from Soybean: UDP-Glucose Dehydrogenase

Raimund Tenhaken* and Oliver Thulke
Universität Kaiserslautern, Fachbereich Biologie, Postfach 3049, D-67653 Kaiserslautern, Germany

* Corresponding author; e-mail tenhaken@rhrk.uni-kl.de; fax 49–631–205–2600.

Abbreviations: EST-clone, expressed sequence tags from the dbest database of GenBank; UDP-Api, UDP-apiose; UDP-GlcDH, UDP-Glc dehydrogenase (EC 1.1.1.22).
Weissenbock (1988) reported about transferases from rye. The construction of the cDNA library using UDP-GlcUA for the glucuronosylation of plant flavones.

Chomczynski and Sacchi (1987) and mRNA was further purified from total RNA by the PolyAtract system (Promega). A cDNA library was synthesized from 5 × 10^5 plaque-forming units in the primary library. Half of this ligated phage cDNA was packaged into λ phages with the Gigagold system (Stratagene), yielding more than 10^7 plaque-forming units in the primary library. Half of this library was amplified once on large Petri dishes and used for this study.

**Materials and Methods**

**Construction of the cDNA Library**

Total RNA was isolated from mid-log-phase soybean (Glycine max L.) cell suspension cultures by the method of Chomczynski and Sacchi (1987) and mRNA was further purified from total RNA by the PolyAtract system (Promega). A cDNA library was synthesized from 5 μg of mRNA using the λ-uni-Zap kit (Stratagene). About 30% of the ligated phage cDNA was packaged into λ phages with the Gigagold system (Stratagene), yielding more than 10⁷ plaque-forming units in the primary library. Half of this library was amplified once on large Petri dishes and used for this study.

**Screening of the cDNA, Sequencing, and Computer Analysis**

About 5 × 10⁵ plaque-forming units were screened with a rabbit polyclonal antiserum assumed to be directed against p47phox (obtained from A. Cross, The Scripps Clinic Research Foundation, La Jolla, CA). Screening was carried out using standard procedures (Ausubel et al., 1995). In brief, 30,000 plaque-forming units per 145-mm dish were grown for 4 h at 42°C. Plates were overlaid with a nitrocellulose filter, impregnated with 0.2 M isopropylthio-β-galactoside, and further incubated for 5 h at 37°C. The nitrocellulose filters were removed and washed extensively in TBS + Tween 20 to remove cell debris. After blocking the membrane in TBS + Tween 20 + 3% BSA, the filters were incubated with the p47phox antiserum in a 1:3000 dilution for 1 h and washed with TBS + Tween 20 four times for 5 min. Primary antibodies were visualized by incubation with a secondary antibody, conjugated with alkaline phosphatase (Bio-Rad), nitroblue tetrazolium, and X-phosphate staining typically for 15 to 30 min. Phages were purified to homogeneity using standard procedures. The cDNA inserts of the isolated λ phages were subcloned into pBluescript (Stratagene) by the in vivo excision method.

Ten individual clones were identified by the antiserum. Hybridization experiments and sequencing proved that all clones were derived from a single gene. The insert of the longest clone was sequenced by the dideoxy-chain termination method using the Sequenase system (Amersham). Both strands were sequenced from various subclones using standard primers and synthesized oligonucleotide primers. Analysis of the cDNA sequence was performed with the Blast program tool (Altschul et al., 1990) and on a local computer system using the Clone and Align programs (Scientific and Educational Software, State Line, PA).

**Expression of the cDNA in Escherichia coli and the Generation of New Antibodies**

A full-length cDNA insert was cloned into the pQE31 expression vector (Qiagen, Chatsworth, CA) and transformed into Escherichia coli XL-1. Expression of the His-tagged fusion protein was carried out in 100-ml scale by induction of the bacteria with 1 mm isopropylthio-β-galactoside for 5 h at 30°C. The fusion protein was purified under denaturing conditions on nitrioltriacetic acid-agarose according to the Qiagen protocol. After extensive dialysis of the purified protein against 10 mM sodium phosphate buffer (pH 7.4), the enzyme was used to immunize two rabbits (white New Zealand, Thomae Pharma, Biberach, Germany). Antibodies were collected from a bleeding rabbit 11 d after the second boost injection (750 μg of protein per animal and boost). IgGs were purified from the antiserum using a ProteinA column (Hitrap, Pharmacia) and used throughout this study.

**Northern Blot Analysis**

Total RNA (10 μg per lane) was separated on a 1.1% formaldehyde agarose gel and transferred onto a nylon membrane (Hybond N+, Amersham) via downward capillary blotting. Hybridization was carried out according to the protocol of Church and Gilbert (1984) with a random primed probe (Ready To Go system, Pharmacia) of the soybean cDNA for UDP-GlcDH. The blot was rehybridized with H1, a cDNA probe from bean, believed to be constitutively expressed (Wingate et al., 1988).

**Southern Blot Analysis**

Genomic DNA from soybean and Arabidopsis was isolated as described by Taylor et al. (1993). Restricted DNA was separated on a 0.7% agarose gel, transferred to a nylon membrane (Hybond N+), and hybridized with a 32P-labeled probe.
labeled probe at 65°C for stringent conditions or at 58°C for heterologous hybridization using the buffer system of Church and Gilbert (1984).

**Immunoprecipitation**

Soybean cell-suspension cultures were homogenized in 50 mM potassium-phosphate buffer (pH 7.5) containing 2 mM EDTA, 5 mM DTT, 0.5 mM PMSF, and 0.5% (w/v) polyvinylpolypyrrolidone. The protein extract was fractionated by (NH₄)₂SO₄ precipitation. The protein corresponding to the 25 to 50% saturation fraction was desalted on a PD10 column (Pharmacia) and used for the immunoprecipitation experiment.

The IgG fraction was diluted from 1:80 to 1:1200 into the protein extract and incubated on ice for 3 h. Precipitated protein was collected by 15 min of centrifugation at 4°C. The pellet was washed once, resuspended in assay buffer, and used for the enzyme assay.

**Enzyme Assay**

UDP-GlcDH was measured spectroscopically in a 1-mL assay adapted from Roberts and Cetorelli (1973) with some modifications. In brief, the assay consists of 20 mM Tris-Cl (pH 8), 1 mM EDTA, 1 mM NAD⁺, 0.4 mM UDP-Glc, and 1 mM NaN₃. The reduction of NADH was measured as an increase at 340 nm. Controls were performed without NAD⁺ or UDP-Glc, showing a slight decrease at 340 nm, and then were subtracted.

**RESULTS**

**Isolation of the Gene**

The gene for UDP-GlcDH was isolated during our work on the oxidative burst in plant-pathogen interactions. It is thought that reactive oxygen species are formed via a plasma-membrane-bound NAD(P)H-oxidase with features similar to those of the NADPH-oxidase from neutrophil cells of the immune system (Mehdy, 1994; Tenhaken et al., 1995). Using an antibody assumed to be directed against the p47phox subunit of the mammalian NADPH-oxidase (p47phox: Lomax et al., 1989) we identified a single protein band of about 50 kD in soybean protein extracts (Fig. 2). As expected, the protein was not associated with membranes (Fig. 2A). Since the size of the protein recognized by the antibody was almost identical in human and soybean protein extracts, we decided to clone the respective gene from a cDNA expression library. A library was constructed from soybean cell culture mRNA. Poly(A)⁺ RNA was isolated from 4-d-old cell culture (mid-log-phase) and used for the synthesis of a directional cDNA expression library. Of 5 × 10⁶ plaques screened with the p47phox antiserum, 10 immune positive phages were identified and further purified. All of the positive clones cross-hybridized under stringent conditions, indicating the same cDNA for all identified clones.

The longest cDNA insert was sequenced and it contained a putative full-length clone of 1.7 kb. The open reading frame of 1440 bases encodes a protein of 480 amino acids. The longest cDNA insert was sequenced and it contained a putative full-length clone of 1.7 kb. The open reading frame of 1440 bases encodes a protein of 480 amino acids.
encodes a protein with 480 amino acids with a predicted size of 52.9 kD. The stop codon is indicated by the asterisk at bp 1453. The 5' untranslated promoter region are probably missing. The sequence was identified from an expression library, so a few base pairs of the end of the Arabidopsis gene was obtained by sequencing two EST-clones. The bovine sequence is from Hempel et al. (1994). The NAD-cofactor binding site (amino acid positions 8–14) is boxed and shaded in gray. The catalytic site (amino acid positions 267–276) with a centered Cys residue (amino acid position 272) is underlined. Functionally relevant substitutions are shaded in gray. The catalytic site (amino acid positions 267–276) is underlined. Functionally relevant substitutions are shaded in gray.

For further analysis of the enzyme we generated antibodies against the soybean UDP-GlcDH. The enzyme was expressed as a His-tagged fusion protein in E. coli and used as an antigen after purification of the fusion protein. Two rabbits were immunized and both showed specific high-titer antibodies after two boost injections. These anti-UDP-GlcDH antibodies recognized the soybean protein initially identified with the p47phox antibody (Fig. 2B).

Immunoprecipitation and Enzyme Assay

A crude protein extract corresponding to the protein precipitated with 25 to 50% (NH₄)₂SO₄ saturation shows readily measurable activity of UDP-GlcDH (data not shown). The enzyme assay was linear over at least 1 h. Controls lacking UDP-Glc as a substrate did not show any increase of reduced NADH. A boiled enzyme control was also totally inactive.

The antibodies were used to immunoprecipitate UDP-GlcDH from the crude protein extract. A serial dilution of the IgG fraction was added to the protein extract from soybean and incubated on ice for several hours. After centrifugation enzyme activity for UDP-GlcDH was measured in the supernatant and in the resuspended pellet. The enzyme activity can be completely precipitated by the polyclonal antibody. A fraction of this activity is measurable in the pellet. The enzyme in the precipitate is partially inhibited by the antibodies, presumably by direct interaction with epitopes significant for enzymatic activity (Fig. 5).

The immunoprecipitation of UDP-GlcDH by the antibody, together with the high sequence homology to bovine UDP-GlcDH, clearly proves that the cloned gene encodes the soybean UDP-GlcDH. So far, we have been unable to demonstrate enzyme activity of the UDP-GlcDH fusion protein expressed in E. coli. The fusion protein could only be purified under denaturing conditions. Attempts to re-activate the enzyme have as yet been unsuccessful.

Figure 3. Sequence of the UDP-GlcDH from soybean. This clone was identified from an expression library, so a few base pairs of the 5' untranslated promoter region are probably missing. The sequence encodes a protein with 480 amino acids with a predicted size of 52.9 kD. The stop codon is indicated by the asterisk at bp 1453. The NAD-cofactor binding site (amino acid positions 8–14) is boxed and shaded in gray. The catalytic site (amino acid positions 267–276) with a centered Cys residue (amino acid position 272) is underlined. Functionally relevant substitutions are shaded in gray.

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The immunoprecipitation of UDP-GlcDH by the antibody, together with the high sequence homology to bovine UDP-GlcDH, clearly proves that the cloned gene encodes the soybean UDP-GlcDH. So far, we have been unable to demonstrate enzyme activity of the UDP-GlcDH fusion protein expressed in E. coli. The fusion protein could only be purified under denaturing conditions. Attempts to re-activate the enzyme have as yet been unsuccessful.

Figure 4. Alignment of UDP-GlcDH from soybean, Arabidopsis, and bovine liver. The amino acid sequence from Arabidopsis was assembled from various ESTs available in the dbest database of GenBank. The 3' end of the Arabidopsis gene was obtained by sequencing two EST-clones. The bovine sequence is from Hempel et al. (1994). Identical sequences are white in black boxes, homolog exchanges are shaded in gray.
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Figure 5. Immunoprecipitation of UDP-GlcDH from soybean. Antiserum directed against the soybean UDP-GlcDH was added to a crude protein fraction (200 μL of a [NH₄]₂SO₄ precipitation [25–50% saturation]). The enzyme activity is immunoprecipitated by the antibodies (○). The remaining activity in the supernatant (□) decreases after antibody addition. Total enzyme is partially inhibited by the polyclonal antibody, presumably due to binding of antibodies to epitopes significant for enzymatic activity.

Expression of the Enzyme during Plant Development

The expression of UDP-GlcDH was analyzed at the mRNA level by northern hybridization. Total RNA was separated on an agarose gel and transferred to a nylon membrane. Northern hybridization with a ³²P-labeled probe showed a high expression in root tips and lateral roots and a moderate expression in the epicotyl and in expanding leaves (Fig. 6). In contrast, the expression in the upper part of the main root, in the hypocotyl, and in mature leaves was much lower.

Genome Structure

To test whether UDP-GlcDH is encoded by a single copy gene or by a small gene family, we hybridized restricted genomic DNA from soybean with the cDNA clone. Under stringent conditions (65°C) only two or three fragments were labeled, indicating a single-copy gene (Fig. 7A). An internal EcoRI site was present in the cDNA clone at position 481 so that at least two labeled fragments were expected. Hybridization under less stringent conditions (58°C) also detected a few other bands of lower intensity (Fig. 7B), which indicates that related dehydrogenases with different substrate specificity might be labeled by the UDP-GlcDH clone. We performed the same experiment using genomic DNA from Arabidopsis, which has a less complex genome. Only a single band of XbaI-restricted Arabidopsis DNA hybridized to the soybean cDNA clone (Fig. 7C). An internal EcoRI site was present in the Arabidopsis gene, and at least in Arabidopsis, UDP-GlcDH is a single-copy gene. These results were also confirmed by the analysis of more than 10 different Arabidopsis EST-clones, which all belong to the same gene based on sequence identity (Fig. 4).

DISCUSSION

Many of the important precursors for hemicellulose biosynthesis are formed via UDP-GlcUA, which is subsequently converted into UDP derivatives of GalUA, Xyl, and Ara for pectic polymers and hemicellulose. Regulation of the deposition of these diverse polysaccharides is thought to be controlled by various synthases in the Golgi apparatus (Carpita and Gibeaut, 1993; Driouich et al., 1993). These synthases use different UDP-sugars as substrates and the

Figure 6. Expression of UDP-GlcDH in soybean seedlings and plants. Total RNA (10 μg) from different developmental stages of the plant was separated on a denaturing agarose gel, transferred to a nylon membrane, and hybridized with a ³²P-labeled cDNA probe for UDP-GlcDH. RNA was prepared from plant organs as indicated in drawings C to E. C represents a 2-d-old seedling; D represents a 7-d-old plant; and E shows a 2-week-old plant. The expression of mRNA for UDP-GlcDH is shown in A. The blot was reprobed with H1 for loading (B).

Figure 7. Genomic Southern blot with soybean and Arabidopsis DNA, restricted with EcoRI (E) or XbaI (X). The membrane was hybridized with the ³²P-labeled soybean cDNA probe. A, Soybean DNA (high stringency, 65°C); B, soybean DNA (lower stringency, 58°C); and C, Arabidopsis DNA (lower stringency, 58°C). The sizes of the fragments are indicated in kilobase pairs (kbp).
maintenance of UDP-sugar pools is a likely prerequisite for normal cell-wall biosynthesis.

The enzyme UDP-GlcDH, which oxidizes UDP-Glc to the corresponding uronic acid and thereby controls the pool of the common precursor UDP-GlcUA, was cloned for the first time from a plant species. The sequence of the soybean gene is highly homologous to the only other known eukaryotic sequence from bovine liver (Fig. 4) (Hempel et al., 1994). Both sequences are identical to 61% and homologous, including conserved amino acid exchanges, to more than 77%. Since the enzyme was initially identified as UDP-GlcDH based on this high homology to the bovine sequence, we have confirmed the identity by biochemical means. The cDNA was expressed as a His-tagged fusion protein. Antibodies directed against the purified fusion protein recognize a single band in soybean protein extracts of the predicted size of approximately 50 to 52 kD (Fig. 2B).

The UDP-GlcDH sequence contains a cofactor binding site for NAD (Fig. 3, amino acid positions 8-14), which is identical to the bovine sequence (Hempel et al., 1994) and is also conserved in other NAD-linked dehydrogenases. The catalytic center of the bovine enzyme was initially identified by chemical modifications of amino acids and subsequent peptide sequencing (Franzen et al., 1981). It contains a Cys residue at position 275 (amino acid position 272 in soybean; Fig. 3). The surrounding amino acids are strictly conserved in prokaryotic and eukaryotic sequences of sugar-nucleotide dehydrogenases. Two Pro residues (amino acid positions 92 and 159 in bovine liver, amino acid positions 89 and 156 in soybean; Fig. 3) that are thought to present turns in the structure of the protein main chain are also maintained in both sequences. Since various motifs of the enzymes are absolutely conserved, one can expect a very similar structure of the plant and the animal enzyme for UDP-GlcDH.

Using the dbest database of GenBank (Newman et al., 1994) we were able to assemble the nearly complete Arabidopsis sequence of UDP-GlcDH. The 3' end of the gene was obtained with identical results by sequencing two different EST-clones. All of the available homologous sequences belong to the same gene, indicating a single-copy gene for UDP-GlcDH in this weed. The Southern blot data with soybean DNA (Fig. 7A) as well as with Arabidopsis DNA (Fig. 7C) also point to a single-copy gene.

UDP-GlcUA can be synthesized in plants via two different pathways (see Fig. 1). The simple route is the direct conversion of the ubiquitous UDP-Glc to the uronic acid by the enzyme described in this paper. Alternatively, UDP-GlcUA can also be formed within the inositol oxidation pathway (Loewus et al., 1973). This route involves several enzymatic steps. There is clear evidence for the existence of both pathways in plants but the relative contribution of both routes to the UDP-GlcUA pool is largely unknown. In cambium cells from sycamore, UDP-GlcDH rather than the inositol pathway seems to contribute the major portion to the UDP-GlcUA pool (Dalessandro and Northcote, 1977b). In germinating Lilium longiflorum pollen tubes, Maiti and Loewus (1978) were able to demonstrate the flow of Glc into hemicellulose via inositol. Nevertheless, the same preparation contains significant amounts of UDP-GlcDH, since this tissue was used to partially purify the enzyme. A 12-fold enrichment of specific enzyme activity for UDP-GlcDH was obtained by Davies and Dickinson (1972).

In contrast, Roberts and Cetorelli (1973) were unable to measure significant activity of UDP-GlcDH in various monocotyledons and dicotyledons and concluded that the inositol oxidation pathway must be the major route for hemicellulose sugars. The incorporation of $^{14}$C into the hemicellulose fraction. One can speculate that the inositol oxidation pathway is predominantly active in seedlings to metabolize the liberated inositol from phytic acid, a common storage compound in seeds. Taken together, most data are conflicting in some points and the application of radioactive precursors changes pool sizes of metabolites and should be interpreted with caution.

Cloning of UDP-GlcDH provides a new tool with which to dissect the expression pattern of the two pathways. From the present study it becomes clear that at least during particular steps of the seedling's developmental program, UDP-GlcDH is highly expressed. This implies that the enzyme plays an important role for providing hemicellulose precursors in roots and expanding leaves. The low expression level in other parts of soybean plants (upper root, hypocotyl, and leaves) can be explained by the lack of demand for UDP-GlcUA-derived sugars in differentiated mature cells. Alternatively, the inositol oxidation pathway takes over in all other tissues. Although we cannot rule out the latter possibility, this explanation is somehow unlikely. Enzyme activity of UDP-GlcDH is strictly correlated with growing and expanding tissues that have a demand for hemicellulose precursors. At the moment we do not know if UDP-GlcDH is expressed in all types of cells; a careful examination of this question is underway. In addition, a probe for the inositol oxidation pathway recently became available when the gene for inositol-1-phosphate phosphatase was cloned from tomato (Gillaspy et al., 1995). The enzyme exists in three different isoforms, and at least one of them is involved in the generation of inositol phosphates as second messengers. Another isoform is believed to be a part of the inositol oxidation pathway leading to UDP-GlcUA. By using gene-specific probes for individual isoforms of inositol-1-phosphate phosphatase, it should be possible to analyze the expression for genes in both pathways that fill the UDP-GlcUA pool. This will definitely clarify if both routes are inversely expressed or if they contribute jointly to the pool of hemicellulose precursors.

As far as we know, UDP-GlcDH has not been fully purified from any plant species, with the possible exception of an enzyme preparation from French bean contain-
ing UDP-GlcDH activity, which was recently largely purified (Robertson et al., 1996). Those authors describe the co-purification of UDP-GlcDH and alcohol dehydrogenase. Several chromatographic procedures were unable to separate the two enzyme activities. An antibody raised against a partially purified enzyme fraction, however, recognized a 40-kD band in bean protein extracts. The predicted size of UDP-GlcDH in soybean is 52 kD and is identical with the size of the bovine enzyme. The sequences from soybean and Arabidopsis are conserved to more than 95% (Fig. 4).

In addition, our antibody raised against UDP-GlcDH cross-reacts with a 52-kD band in bean plants as expected (data not shown). Therefore, it is highly unlikely that the characterized protein(s) from bean is UDP-GlcDH, especially since soybean and bean plants are closely related. The reported $K_m$ value for UDP-Glc for the bean enzyme is 5.5 mM (Robertson et al., 1996). In contrast, the soybean enzyme has a $K_m$ of about 0.2 mM, which agrees with a report from Davies and Dickinson (1972) in which they estimated the $K_m$ for the Lilium enzyme to be 0.3 mM. The pool size of UDP-Glc in soybean cell cultures is about 0.1 mM (Hayashi and Matzuda, 1981).

Robertson et al. (1996) emphasized the similarity of their partially purified enzyme fraction to alcohol dehydrogenase. Since this enzyme is cloned from various species, we compared the molecular mass and the amino acid sequence of alcohol dehydrogenase from Arabidopsis (GenBank accession no. X77943) with those of UDP-GlcDH. The sequence shows no significant homology to UDP-GlcDH. Hempel et al. (1994) showed that there is no obvious homology between UDP-GlcDH and alcohol dehydrogenase except for the NAD-binding site. It is interesting that the molecular mass of the Arabidopsis alcohol dehydrogenase is about 40 kD, and taken together, it seems likely that Robertson et al. (1996) purified an alcohol dehydrogenase rather than UDP-GlcDH from bean plants.

The detailed expression pattern of UDP-GlcDH in plants remains to be analyzed within our transgenic plants carrying a promoter-GUS fusion. The function of UDP-GlcDH needs further clarification that we hope to provide from the current analysis of various transgenic plants, in which the UDP-GlcDH is expressed in sense or anti-sense orientation.

Modified cell walls with a changed composition in hemicellulose are very helpful in elucidating the complex structural network of plant cell walls. A genetic approach for cell-wall composition mutants with Arabidopsis was recently described by Reiter et al. (1993). Our transgenic plants with manipulated levels of UDP-GlcDH might have similar phenotypes to particular mutants. Since we currently do not understand why cell-wall matrix polysaccharides from grasses are so different from those from dicotyledonous plants (Carpita and Gibeaut, 1993), transgenic plants with lower or altered hemicellulose content are promising tools to further address this problem. In addition, plants with such genetically modified cell walls are of potential interest in the area of biotechnology.

ACKNOWLEDGMENTS

We thank Heinrich Kauss for comments on the paper and for continuous support of the project. We thank Christine Rübel for technical help and Ralf Kaps for taking care of the animals. We also thank Andrew Cross for the generous gift of p47phox antibodies and Chris Lamb for the H1 cDNA probe.

Received May 22, 1996; accepted August 16, 1996

Copyright Clearance Center: 0032-0889/96/112/1127/08.

The GenBank accession number for the soybean UDP-GlcDH sequence reported in this article is U53418.

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