UDP-glucose dehydrogenases of maize: a role in cell wall pentose biosynthesis

Anna KÄRKÖNEN*, Alain MURIGNEUX†, Jean-Pierre MARTINANT†, Elodie PEPEY†, Christophe TATOUT†, Bernard J. DUDLEY* and Stephen C. FRY*¹

*The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, School of Biological Sciences, The University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Edinburgh EH9 3JH, U.K., and †BIOGEMMA, Campus Universitaire des Cîteaux, 24, Avenue des Landais, 63170 Aubière, France

UDPGDH (UDP-D-glucose dehydrogenase) oxidizes UDP-Glc (UDP-D-glucose) to UDP-GlcA (UDP-D-glucuronate), the precursor of UDP-D-xylose and UDP-L-arabinose, major cell wall polysaccharide precursors. Maize (*Zea mays* L.) has at least two putative *UDPGDH* genes (*A* and *B*), according to sequence similarity to a soya bean *UDPGDH* gene. The predicted maize amino acid sequences have 95 % similarity to that of soya bean. Maize mutants with a Mu-element insertion in *UDPGDH-A* or *UDPGDH-B* were isolated (*udpgdh-A1* and *udpgdh-B1* respectively) and studied for changes in wall polysaccharide biosynthesis. The *udpgdh-A1* and *udpgdh-B1* homozygotes showed no visible phenotype but exhibited 90 and 60–70 % less UDPGDH activity respectively than wild-types in a radiochemical assay with 30 μ M UDP-glucose. Ethanol dehydrogenase (ADH) activity varied independently of UDPGDH activity, supporting the hypothesis that ADH and UDPGDH activities are due to different enzymes in maize. When extracts from wild-types and *udpgdh-*

A1 homozygotes were assayed with increasing concentrations of UDP-Glc, at least two isoforms of UDPGDH were detected, having K_m values of approx. 380 and 950 μ M for UDP-Glc. Leaf and stem non-cellulosic polysaccharides had lower Ara/Gal and Xyl/Gal ratios in *udpgdh-A1* homozygotes than in wild-types, whereas *udpgdh-B1* homozygotes exhibited more variability among individual plants, suggesting that UDPGDH-A activity has a more important role than UDPGDH-B in UDP-GlcA synthesis. The fact that mutation of a *UDPGDH* gene interferes with polysaccharide synthesis suggests a greater importance for the sugar nucleotide oxidation pathway than for the *myo*-inositol pathway in UDP-GlcA biosynthesis during post-germinative growth of maize.

Key words: arabinose, insertional mutagenesis, pentose biosynthesis, UDP-glucose dehydrogenase, UDP-glucuronate, xylose.

INTRODUCTION

UDP-D-glucuronate (UDP-GlcA) is the glucuronosyl donor for the synthesis of several structural polysaccharides, and is the precursor of other sugar nucleotides including UDP-galacturonate, UDP-Xyl (UDP-D-xylose), UDP-Ara (UDP-arabinose) and UDP-apiose, which are glycosyl donor substrates for hemicellulose and pectin biosynthesis [1–3]. Glucuronarabinoxylans (synthesized from UDP-GlcA, UDP-Ara and UDP-Xyl) constitute a substantial proportion of the maize cell wall [4], and thus the biosynthesis of UDP-GlcA is a major metabolic activity during maize cell growth.

UDP-GlcA can be formed by two routes: (i) via a sugar nucleotide oxidation pathway involving the UDPGDH (UDP-D-glucose dehydrogenase; EC 1.1.1.22)-catalysed oxidation of UDP-Glc (UDP-D-glucose) with the concomitant reduction of two molecules of NAD⁺, and (ii) from glucose 6-phosphate with the intermediate formation and oxidation of *myo*-inositol (see [5,6] for reviews). There is evidence for the existence of both pathways in plants, but the quantitative contributions of these pathways to UDP-GlcA and the pentoses of cell walls is still unknown [7].

There is evidence for transcription of maize *UDPGDH* genes in leaf, root, ear and tassel tissues [8] and in sperm cells [9], suggesting the existence of UDPGDH activity. We have confirmed that UDPGDH activity exists in developing maize (*Zea mays* L.) leaves and suspension-cultured cells [10]. In suspension-cultured cells there were at least three different isoenzymes of UDPGDH:

one (designated E_H) having an extremely high value of Michaelis constant K_m (> 50 mM) for UDP-Glc, and two others with low and medium K_m values (roughly 0.027 and 2.8 mM; designated E_L and E_M respectively). The genetic basis for these very different isoforms in maize cell cultures has not been resolved. In developing maize leaves, on the contrary, the main UDPGDH activity appears to be due to isoenzyme(s) having a K_m of 0.5–1 mM for UDP-Glc [10].

We investigated the role of the sugar nucleotide oxidation pathway in UDP-GlcA biosynthesis and, consequently, in cell wall pentose biosynthesis in developing organs of maize (*Z. mays*). For this we utilized maize mutants having an insertion of a transposon in either of the two putative *UDPGDH* genes. Here, we report that the disruption of either of these genes causes reduction in both UDPGDH activity and cell wall pentose content in maize.

MATERIALS AND METHODS

Maize *UDPGDH* genes and mutant plants

Two maize contigs of EST (expressed sequence tag) were identified from the GnpSeq GENOPLANTE database (<http://genoplante-info.infobiogen.fr/>) for their high amino acid sequence similarity to the soya bean *UDPGDH* gene previously isolated and characterized by [11]. Accession numbers of the contigs on GnpSeq database are 658123 and 658125, here designated as genes *UDPGDH-A* and *UDPGDH-B* respectively.

Abbreviations used: ADH, ethanol dehydrogenase; AIR, alcohol-insoluble residue; EST, expressed sequence tag; TFA, trifluoroacetic acid; UDPGDH, UDP-D-glucose dehydrogenase; UDP-GlcA, UDP-D-glucuronate; UDP-Xyl, UDP-D-xylose.

¹ To whom correspondence should be addressed (email S.Fry@ed.ac.uk).

A reverse genetics approach was performed at Biogemma on this gene family by use of a resource of 27 500 maize lines in which an endogenous transposon called 'Mutator' (Mu) had been allowed to transpose at high frequency. This mutagenesis collection was built up by use of Mu transposon stock kindly provided by B. Taylor (Commonwealth Scientific and Industrial Research Organization, Australia). The Mu transposon stock was crossed with maize hybrids developed by Limagrain (Chappes, France) in order to increase the vigour of the plants and their adaptation to European environmental conditions. The screening for Mu insertions was carried out on F1 plant material and confirmed for their germinal status (heritability) on F2 plant material. Mutant screens were accomplished through a PCR-based approach using a Mu-specific primer (OmuA: 5'-CTTCGTCAT-AATGGCAATTATCTC-3') that binds to the edges of the element, the so-called TIR (terminal-inverted repeat). OmuA was used in combination with a PCR primer (*UDPGDH_r2*: 5'-CC-AATCCTCGTGTCTTGCCC-3'), which has the ability to amplify both maize *UDPGDH-A* and *UDPGDH-B* genes. The PCR conditions were standard with 37 cycles and a $T_m = 58^\circ\text{C}$.

One insertional mutation was detected in each gene: *udpgdh-A1* and *udpgdh-B1*. Because of the process used to make the maize mutant collection, each F2 plant for each family has a heterogeneous genetic background. Therefore, in order to minimize phenotypic variation between plants belonging to the same family, we self-pollinated a single plant heterozygous for the insertion event (=mutant allele) for each family investigated. Thus, in each set of kernels, there is a genetic segregation for the mutant allele and therefore a molecular characterization based on DNA extraction and PCR analyses was made for each plant. Because of this approach, any given mutant plant should only be compared with wild-types belonging to the same family. Homozygous plants for either wild-type or a mutant allele were grown in a greenhouse in Edinburgh, U.K., and used for enzymic and cell wall analyses.

Extract preparation

Enzymes were extracted from developing leaves (basal ~3-cm pale green region of the lamina) of 2-month-old plants. Plant material was homogenized in a mortar with pestle in 2 vol. of 50 mM Tris/HCl buffer (pH 7.5) containing 2 mM EDTA and freshly added dithiothreitol (5 mM). The homogenate was centrifuged at 15 800 g at 4°C for 2 min to pellet the cell wall debris and the supernatant was re-centrifuged for 2 min. The extracts were freed of low- M_r contaminants by gel-permeation chromatography on PD10 columns (Amersham Biosciences) pre-equilibrated with the homogenization buffer and material in the void volume was used for enzymic assays.

Enzymic activity assays

UDPGDH activity was assayed by spectrophotometric and radiochemical methods. The spectrophotometric assay was modified from that of Robertson et al. [12]. The final reaction mixture contained 5 mM UDP-Glc, 2 mM NAD^+ , 0.1 M Tris/HCl (pH 8.0), and desalted extract in a final volume of 1.0 ml. The enzyme was preincubated for 10 min with UDP-Glc at approx. 20°C. The reaction was initiated with NAD^+ and monitored for 10 min as the increase in A_{340} due to reduction of NAD^+ . Controls contained water instead of UDP-Glc. UDPGDH activity was calculated on the basis that 2 mol NADH were formed per mol UDP-Glc oxidized. Ethanol dehydrogenase (ADH) activity was measured in the same way as UDPGDH, except that 5 mM ethanol was used as substrate, the preincubation was omitted, and it was assumed that 1 mol NADH was formed per mol ethanol oxidized.

The radiochemical reaction mixture (10 μl) contained 30 μM UDP-D-[U- ^{14}C]Glc (3.7 kBq), 2.5 mM NAD^+ , 32 mM Tris/HCl (pH 8.0) and enzyme extract. Control samples contained the boiled enzyme. The reaction was started by addition of the enzyme and stopped after 5 min at 25°C by the addition of formic acid (to 10%). Half the reaction mixture was subjected to high-voltage paper electrophoresis at pH 6.5 (2.5 kV, 80 min) as described in [13]. The electrophoretograms were autoradiographed, and the spots of individual compounds were cut out and scintillation-counted in 1.5 ml of OptiScint 'HiSafe' (Wallac-EG&G Instruments, Evry, France). Previously, we have shown that the product of the enzymic reaction is UDP-GlcA [10].

To estimate the K_m values of different isoenzymes for UDP-Glc, we conducted the radiochemical activity assay, with increasing concentrations of UDP-Glc (0–32 mM), on extracts from wild-types and *udpgdh-A1* individuals. By subtracting the observed mutant value from that of the wild-type, we estimated the velocities of different isoenzymes and estimated their K_m values from Lineweaver–Burk or Hanes plots.

Cell wall analysis

For analysis of cell wall polysaccharides, mature leaves (first and third leaf from the soil) and the second-lowest internode were collected from greenhouse-grown plants, dried in an oven (50°C) and weighed. Dried stem tissue was ball-milled for 7 min to a fine powder. Dried leaves were crushed with a spatula. AIR (alcohol-insoluble residue) was prepared by incubation in 70% (v/v) ethanol for several days at 60°C with occasional shaking; the ethanol was changed until no more soluble sugars appeared according to an anthrone test for hexoses [13]. The AIR was washed in acetone, dried at room temperature (20°C) and weighed, and the non-cellulosic polysaccharides were hydrolysed with 2 M TFA (trifluoroacetic acid) for 1 h at 120°C (1 ml of TFA/5–20 mg of AIR). The TFA-resistant (cellulosic) residue was rinsed with water and the washings were combined with the TFA hydrolysate, which was then dried in a SpeedVac. Prior to HPLC, 200 μl of the hydrolysate was made up to 0.5 ml with 10 mM pyridinium formate, pH 5.5 (running buffer), and anions were removed by chromatography on a 0.75-ml column of Dowex 1 $\times$ 4–200 (formate form) in running buffer. The neutral fraction (2 ml) was eluted with running buffer, dried and redissolved in 200 μl of water. The samples were filtered through 0.45- μm filters and analysed by HPLC. This pre-purification removed some unidentified impurities that impaired the detection of individual sugars in HPLC.

Monosaccharides in the hydrolysates were also analysed by paper chromatography in ethyl acetate/pyridine/water (8:2:1, by vol.) and stained with aniline hydrogen-phthalate [13].

The cellulosic residue remaining after TFA hydrolysis was washed with water (until no soluble sugars appeared) and acetone, dried and assayed by a modification of the method of Updegraff [14]. For this, the residue was shaken for 1 h at approx. 20°C in 1 ml of 67% (v/v) H_2SO_4 and a portion of the solution was analysed by the anthrone test [13] with a glucose standard curve. Results were expressed as percentage of AIR after correction of free glucose (M_r 180) to glucose residues (M_r 162).

HPLC

The de-anionized samples were analysed on a Dionex HPLC with a CarboPac PA1 column. The flow rate was 1 ml $\cdot$ min $^{-1}$, NaOH was added post-column (0.2 M, 0.5 ml $\cdot$ min $^{-1}$), and sugars were quantified with a pulsed amperometric detector (with a gold electrode). In the method for monosaccharides, the programme

AAB58398	MVKICICGAGYVGPTMAVIALKCPDIEVVVDISKPRIEAWNSDTLPITYEPLDDVVVKQ
658123	MVKICICGAGYVGPTMAVIALKCPDIEVVVDISKPRIEAWNSDTLPITYEPLDDVVVKQ
658125	MVKICICGAGYVGPTMAVIALKCPDIEVVVDISKPRIEAWNSDTLPITYEPLDDVVVKQ
AAB58398	CRGKNLFFSTDEKHKVFEADIVFVSNTPTKTQGLGAGKAADLTYESAARMIAVSKSD
658123	CRGKNLFFSTDEKHKVFEADIVFVSNTPTKTQGLGAGKAADLTYESAARMIAVSKSD
658125	CRGKNLFFSTDEKHKVFEADIVFVSNTPTKTQGLGAGKAADLTYESAARMIAVSKSD
AAB58398	KIVVEKSTVPVKTAEAEIKILTHNSKGIKQILSNPEFLAEGTAIKOLFNPDRVLIGGRE
658123	KIVVEKSTVPVKTAEAEIKILTHNSKGINYQILSNPEFLAEGTAIEDLFKPDRLVIGGRE
658125	KIVVEKSTVPVKTAEAEIKILTHNSKGINYQILSNPEFLAEGTAIEDLFKPDRLVIGGRE
AAB58398	TPEGQKAIOTLKDVYAQWVPEIRILTTNLWSAELSKLAANAFQAIRISSVNAISALCEAT
658123	TPEGKAVQALQKDVYAHVWPEIRILTTNLWSAELSKLAANAFQAIRISSVNAISALCEAT
658125	TPEGKAVQALQKDVYAHVWPEIRILTTNLWSAELSKLAANAFQAIRISSVNAISALCEAT
AAB58398	GANVQGVSVSGTDRIGPKFLNASVGFGGSCFQKDIILNLVYICBNGLPEVAEWYKQVI
658123	GANVTEVAYAVGKDRIGPKFLNASVGFGGSCFQKDIILNLVYICBNGLPEVANYKQVI
658125	GANVSEVAYAVGKDRIGPKFLNASVGFGGSCFQKDIILNLVYICBNGLPEVANYKQVI
AAB58398	KINDYQKSRFVNRVVASMFNTVSNKKIAILGFAPKKDTGDTRETPAIDVCKGLLGDKAKNI
658123	KINDYQKSRFVNRVVASMFNTVAGKKIAVLGFAPKKDTGDTRETPAIDVCKGLLGDKAKNI
658125	RINDYQKSRFVNRVVASMFNTVAGKKIAVLGFAPKKDTGDTRETPAIDVCKGLLGDKAKNI
AAB58398	SIYDPQVTEQIQRDLSMNKFDWHPHILQPTSPITVKVSVVWDAYEATKGAHLGILCIT
658123	SIYDPQVTEQIQRDLSMNKFDWHPHILQPTSPITVKVSVVWDAYEATKGAHLGILCIT
658125	SIYDPQVTEQIQRDLSMNKFDWHPHILQPTSPITVKVSVVWDAYEATKGAHLGILCIT
AAB58398	EWDEFKTLDYQKIFDNMQKPAFVDFGRNIVDSEKLEIGFIVYSIGKPLDPLWKDMPAVA
658123	EWDEFKTLDYQKIFDNMQKPAFVDFGRNIVDSEKLEIGFIVYSIGKPLDPLWKDMPAVA
658125	EWDEFKTLDYQKIFDNMQKPAFVDFGRNIVDSEKLEIGFIVYSIGKPLDPLWKDMPAVA

Figure 1 Alignment of predicted UDPGDH protein from soya bean and maize

The predicted soya bean protein (id AAB58398) is compared with the proteins predicted for the two maize contigs of EST (ids 658123 and 658125 from the GENOPLANTE GnpSeq database). Proteins predicted from 658123 and 658125 are referred to here as UDPGDH-A and UDPGDH-B respectively. Underline indicates a deviation from the other two sequences; where all three sequences differ at the same site, the three residues are all underlined.

of NaOH concentrations was: 0–1.8 min, 20 mM; 1.8–30 min, 0 mM; 30–31 min, 0 → 100 mM (linear gradient); 31–35 min, 800 mM; 35–36 min, 800 → 20 mM; 36–46 min, 20 mM. Samples were run on the HPLC in batches of up to 35, with a mixture of calibration standards (0.2–1.0 mg · mL⁻¹) at the beginning of each batch. Standards of 0.5 mg · mL⁻¹ were run within batches as a check that there was no drift in retention times or sensitivity.

RESULTS

UDPGDH genes

Two contigs of EST (GnpSeq ids 658123 and 658125) were identified for their high amino acid sequence similarity to the first isolated gene of the *UDPGDH* family in soya bean [11]. Each contig contains the whole coding sequence, and ids 658123 and 658125 probably correspond to two different homologous genes named *UDPGDH-A* and *UDPGDH-B* respectively. Although identity in nucleic acid sequence between the two contigs and the soya bean sequence is only approx. 78 %, alignment of the predicted protein sequences shows at least 95 % similarity between the maize and soya bean proteins, 94.5 % similarity between maize and poplar proteins [15], 93 % similarity between maize and *Arabidopsis* proteins [16] (accession numbers in [3]), and 84 % similarity between maize and tobacco [17]. This result illustrates a very high intra- and inter-species amino acid conservation rate (Figure 1).

Two insertion mutants, *udpgdh-A1* and *udpgdh-B1*, were discovered through the screening of a collection of 27 500 maize mutants. The mutations were shown by their flanking sequences to occur near the middle of the single exon of each respective gene (Figure 2); therefore a decrease of UDPGDH activity (due to an abnormal enzyme) is expected for each mutant family.

Enzymic activities

There were no obvious phenotypic differences in growth, morphology or survival between wild-types and mutants grown in greenhouse conditions in Edinburgh. Developing leaves of homozygous *udpgdh-A1* plants contained approx. 90 and 60 % less UDPGDH activity than the corresponding wild-type, as detected by radiochemical and spectrophotometric assays respectively (Figures 3A and 4A). ADH activities varied independently of UDPGDH activities (Figure 4). Absolute activities were somewhat variable between individuals; however, the homozygous mutants consistently had approx. 2–3-fold lower UDPGDH/ADH ratios than wild-types (Table 1).

Homozygotes for *udpgdh-B1* showed approx. 60–70 and 40–50 % reductions in UDPGDH activity in the radiochemical and spectrophotometric assays respectively (Figures 3B and 4B). These mutants also showed a reduction in UDPGDH/ADH ratio (Table 1).

When increasing concentrations of non-radioactive UDP-Glc were added to the radiochemical UDPGDH assay, a clear difference was observed between the mutant and the wild-type (data for *udpgdh-A1* mutants; Figure 5). The activity of the mutant never exceeded approx. 20 % of that of the wild-type even when the activities approached $V_{\max}$. This result supports our earlier finding that majority of the UDPGDH activity in developing maize leaves is due to enzyme(s) other than those (E_H) having an extremely high K_m for UDP-Glc [10]. From the total enzymic activities measured in the wild-type and *udpgdh-A1* mutant, the activities of individual isoenzymes can now be estimated. We assume that the wild-type has at least two functional isoenzymes, with genes *A* and *B* encoding isoenzymes UDPGDH-A and UDPGDH-B respectively; thus the homozygous mutant defective in *UDPGDH-A* is devoid of isoenzyme A. By subtracting the velocity observed in the mutant (which possesses active isoenzyme B) from that of the wild-type we can estimate the velocity of isoenzyme A (Figure 5), assuming the mutant has the same UDPGDH-B activity as the wild-type. The K_m values of approx. 380 and 950 μ M for UDP-Glc and $V_{\max}$ values of 900 and 240 pkat · (g fresh weight)⁻¹ for isoenzymes A and B respectively, can now be estimated by use of Lineweaver–Burk or Hanes plots (Figure 6). These K_m values are comparable with the range (0.5–1.0 mM) that we previously estimated for enzymes extracted from wild-type developing maize leaves [10]. Thus both isoenzymes A and B more closely resemble isoform E_M than E_L or E_H (see the Introduction section).

Cell wall analysis

Cell wall matrix polysaccharides were acid-hydrolysed and the monosaccharides analysed by paper chromatography and HPLC. The Xyl/Gal and Ara/Gal ratio was chosen to check the pentose/hexose ratio because Xyl and Ara are synthesized from the product of UDPGDH while the route to Gal, on the contrary, diverges before the enzyme of interest. Gal was taken as the representative hexose because some of the Glc in AIR would have arisen from starch rather than the cell wall matrix. It was shown that Ara/Gal and Xyl/Gal ratios were clearly reduced (by ~35–45 %) in the cell wall polysaccharides of leaves defective in *UDPGDH-A* (Figures 7A and 7B), indicating an important role for UDPGDH-A in pentosan biosynthesis. In stems, on the contrary, the reduction in Xyl/Gal ratio was only 10 %, suggesting that the mutants are capable of producing adequate UDP-GlcA, and hence UDP-Xyl, via a UDPGDH other than isoenzyme A in xylan-rich stems. The other possibility is that the *myo*-inositol oxidation pathway can produce sufficient UDP-GlcA in stems of maize, at least when UDPGDH activity is deficient.

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Figure 3 UDPGDH activities in developing maize leaves: radiochemical assay

(A) UDPGDH activities in two wild-types (wt) and two homozygous *udpgdh-A1* mutant individuals (mut). (B) UDPGDH activities in two wild-types (wt) and two homozygous *udpgdh-B1* mutant individuals (mut). Activities in 'pkat' are reported for the particular assay conditions used (30 μ M UDP- 14 C)Glc, no account being taken of the K_m value(s) for this substrate. FW, fresh weight.

Table 1 UDPGDH/ADH ratios in leaves of wild-type and mutant maize individuals having an insertion in gene *UDPGDH-A* or *UDPGDH-B*

UDPGDH and ADH activities were assayed by the spectrophotometric method.

Gene	Individual plant	UDPGDH/ADH ratio
<i>UDPGDH-A</i>	Wild-type 11	0.98
	Wild-type 14	0.70
	Wild-type 15	0.44
	Mutant 1	0.24
	Mutant 7	0.19
	Mutant 8	0.23
	Wild-type 10	0.38
	Wild-type 26	0.67
<i>UDPGDH-B</i>	Mutant 11	0.18
	Mutant 24	0.29

It has been suggested that different pathways for UDP-GlcA synthesis predominate in different organs of the *Arabidopsis* seedling [16]. In seedlings up to 5 days old the *UDPGDH* gene was mainly expressed in roots; however, older plants showed more uniform gene expression with preference for the vascular system. UDPGDH protein concentration and enzymic activity correlated well with the mRNA levels. Radioactivity from [3 H]inositol supplied in the culture medium, on the other hand, was more efficiently incorporated into cell walls of the hypocotyl and

Figure 4 UDPGDH and ADH activities in developing maize leaves: spectrophotometric assay

The assay mixture contained 5 mM UDP-Glc or ethanol as substrate. (A) Enzymic activities in three wild-types (wt) and three homozygous *udpgdh-A1* mutant individuals (mut). (B) Enzymic activities in two wild-types (wt) and two homozygous *udpgdh-B1* mutant individuals (mut). FW, fresh weight.

Figure 5 Effect of UDP-Glc concentration on UDPGDH activity in extracts from wild-type and homozygous *udpgdh-A1* mutants

UDPGDH activity in extracts from developing leaves of the wild-type and homozygous *udpgdh-A1* mutants was assayed by the radiochemical method with increasing concentrations of UDP-Glc. By subtraction of the observed mutant activity (isoenzyme B only; dotted line) from that of the wild-type (isoenzymes A and B; solid line), the activity due to isoenzyme A is estimated (broken line). FW, fresh weight.

cotyledons than into those of the roots, suggesting the potential for an active *myo*-inositol oxidation pathway in these aerial organs (if sufficient inositol is present in them). *Arabidopsis* has three additional *UDPGDH* genes that are expressed in a developmentally regulated way and often in a transient pattern that suggested that they are only expressed in cells with a need for precursors for cell wall biosynthesis [20].

Figure 6 Deduced Hanes plots for isoenzymes UDPGDH-A (▼) and UDPGDH-B (○)

In the present study, two different isoenzymes of UDPGDH were observed with K_m values for UDP-Glc of approx. 380 and 950 μM , coded for by genes *UDPGDH-A* and *UDPGDH-B* respectively. Both these K_m values for UDP-Glc are high when compared with some published elsewhere. For example, $K_m \sim 18.7 \mu\text{M}$ for UDP-Glc was estimated for UDPGDH purified from another gramineous plant, *Saccharum* spp. [21]. In the present study, K_m values were estimated by use of crude cell extracts. It is possible that maize also contains an additional UDPGDH isoenzyme with a very low K_m for UDP-Glc. In suspension-cultured cells of maize, there was an indication of the existence of a UDPGDH isoenzyme ('E_L') with a K_m value of $\sim 28 \mu\text{M}$ for UDP-Glc [10]. It will be of interest to identify the gene that encodes E_L. Purified isoenzymes are needed to resolve the definitive kinetic values.

Table 2 Cellulose content of wild-type and *UDPGDH* insertion mutants of maize

Data are means $\pm$ S.E.M. ($n=5$ for wild-type-A and *udpgdh-A1*, $n=4$ for wild-type-B and $n=3$ for *udpgdh-B1*). Means do not differ statistically significantly from each other as analysed by Student's *t* test ($P > 0.05$). n.d., not determined.

Genotype	Cellulose content (% of total AIR)		
	First leaf	Third leaf	Stem
Wild-type-A	16.0 $\pm$ 1.0	19.2 $\pm$ 1.3	34.7 $\pm$ 4.4
<i>udpgdh-A1</i> homozygote	19.4 $\pm$ 1.3	16.0 $\pm$ 0.8	37.4 $\pm$ 1.6
Wild-type-B	20.9 $\pm$ 1.8	n.d.	42.5 $\pm$ 1.3
<i>udpgdh-B1</i> homozygote	17.6 $\pm$ 0.5	n.d.	38.2 $\pm$ 2.1

The decrease in cell wall pentose content in *udpgdh-A1* homozygotes suggests that isoenzyme A is essential for adequate UDP-GlcA and hence UDP-Xyl and UDP-Ara synthesis in maize leaves. The remaining UDPGDH activity (isoenzyme B) was evidently not enough to produce sufficient UDP-GlcA for cell wall pentose synthesis in these mutant plants. It seems that the *myo*-inositol oxidation pathway is not active enough to compensate for the reduced UDPGDH activity in UDP-GlcA production in developing leaves of maize. In the pathogenic fungus *Cryptococcus neoformans* a disruption of the *UDPGDH* gene led to a total lack of both UDP-GlcA and UDP-Xyl [22]. Interestingly, the Xyl/Gal ratio was reduced by only 10% in the stems of maize mutants defective in *UDPGDH-A*, and this decrease was not statistically significant (Figure 7B), suggesting that other isoenzymes of UDPGDH or the alternative *myo*-inositol pathway do (or can when necessary) produce enough UDP-GlcA in these tissues.

Figure 7 Sugar residue composition of cell wall polysaccharides from wild-type and *udpgdh* mutants

The graphs show pentose/hexose ratios of non-cellulosic cell wall polysaccharides in the first and the third mature leaves (counting from the soil) and in the second-lowest stem internode of wild-type maize plants and homozygotes of *udpgdh-A1* (graphs A and B) or *udpgdh-B1* (graphs C and D). Black = wild-type; grey = mutant. Error bars indicate the standard errors of the ratios measured on ($n=5$ for wt-A and *udpgdh-A1*, $n=4$ for wt-B and $n=3$ for *udpgdh-B1*) individuals of each genotype. Means of the mutant ratios that differ statistically significantly from those of the corresponding wild-types are indicated (Student's *t* test, $*P < 0.05$).

Developing stems were not assayed for UDPGDH enzymic activities in the present study.

Cellulose content was not significantly affected in either of the mutants so any surplus UDP-Glc, accumulated because of the block in UDPGDH activity, must be directed elsewhere than into cellulose synthesis in these plants.

Conclusion

Mutants obtained by insertion of a transposon into *UDPGDH* genes offer a valuable means to study the importance of this enzyme activity in UDP-GlcA and hence cell wall pentose biosynthesis. According to our results it seems that knocking out UDPGDH-A reduces the cell wall pentose content, suggesting an important role for this enzyme in the biosynthesis of UDP-GlcA and other cell wall polysaccharide precursors in maize. However, loss of UDPGDH-B led to no change in cell wall composition. If there are multiple isoenzymes or alternative routes to the product of interest, as in the case of UDPGDH-B, then the knocking out of this may lead to no change in cell wall composition. Cell wall composition mutants like those discovered in this study give an excellent way of elucidating the role of sugar nucleotide interconverting enzymes in cell wall biosynthesis.

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REFERENCES

- Feingold, D. S. and Avigad, G. (1980) Sugar nucleotide transformations in plants. In *The Biochemistry of Plants, A Comprehensive Treatise*, vol. 3 (Preiss, J., ed.), pp. 101–170, Academic Press, New York
- Mohnen, D. (2002) Biosynthesis of pectins. In *Pectins and their Manipulation* (Seymour, G. B. and Knox, J. P., eds.), pp. 52–98, Blackwell Publishing/CRC Press, Oxford, U.K.
- Seifert, G. J. (2004) Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. *Curr. Opin. Plant Biol.* **7**, 277–284
- Carpita, N. C. (1996) Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 445–476
- Loewus, F., Chen, M. S. and Loewus, M. W. (1973) The *myo*-inositol oxidation pathway to cell wall polysaccharides. In *Biogenesis of Plant Cell Wall Polysaccharides* (Loewus, F., ed.), pp. 1–27, Academic Press, New York
- Loewus, F. and Murthy, P. P. N. (2000) *myo*-Inositol metabolism in plants. *Plant Sci.* **150**, 1–19
- Kärkönen, A. (2005) Biosynthesis of UDP-GlcA: via UDPGDH or *myo*-inositol oxidation pathway? *Plant Biosystems* **139**, 46–49
- Casati, P. and Walbot, V. (2004) Rapid transcriptome responses of maize (*Zea mays*) to UV-B in irradiated and shielded tissues. *Genome Biol.* **5**, R16 [Supplementary material therein]
- Engel, M. L., Chaboud, A., Dumas, C. and McCormick, S. (2003) Sperm cells of *Zea mays* have a complex complement of mRNAs. *Plant J.* **34**, 697–707 [Supplementary material therein]
- Kärkönen, A. and Fry, S. C. (2005) Novel characteristics of UDP-glucose dehydrogenase activities in maize: non-involvement of alcohol dehydrogenases in cell wall polysaccharide biosynthesis. *Planta*, in the press
- Tenhaken, R. and Thulke, O. (1996) Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. *Plant Physiol.* **112**, 1127–1134
- Robertson, D., Smith, C. and Bolwell, G. P. (1996) Inducible UDP-glucose dehydrogenase from French bean (*Phaseolus vulgaris* L.) locates to vascular tissue and has alcohol dehydrogenase activity. *Biochem. J.* **313**, 311–317
- Fry, S. C. (2000) *The Growing Plant Cell Wall: Chemical and Metabolic Analysis*. 2nd edn, Blackburn Press, Caldwell, NJ
- Updegraff, D. M. (1969) Semi-micro determination of cellulose in biological materials. *Anal. Biochem.* **32**, 420–424
- Johansson, H., Sterky, F., Amini, B., Lundeberg, J. and Kleczkowski, L. A. (2002) Molecular cloning and characterization of a cDNA encoding poplar UDP-glucose dehydrogenase, a key gene of hemicellulose/pectin formation. *Biochim. Biophys. Acta* **1576**, 53–58
- Seitz, B., Klos, C., Wurm, M. and Tenhaken, R. (2000) Matrix polysaccharide precursors in *Arabidopsis* cell walls are synthesized by alternate pathways with organ-specific expression patterns. *Plant J.* **21**, 537–546
- Bindschedler, L. V., Wheatley, E., Gay, E., Cole, J., Cottage, A. and Bolwell, G. P. (2005) Characterisation and expression of the pathway from UDP-glucose to UDP-xylose in differentiating tobacco tissue. *Plant Mol. Biol.* **57**, 285–301
- Roberts, R. M. and Cetorelli, J. J. (1973) UDP-D-glucuronic acid pyrophosphorylase and the formation of UDP-D-glucuronic acid in plants. In *Biogenesis of Plant Cell Wall Polysaccharides* (Loewus, F., ed.), pp. 49–68, Academic Press, New York
- Roberts, R. M. and Loewus, F. (1973) The conversion of D-glucose-6-¹⁴C to cell wall polysaccharide material in *Zea mays* in presence of high endogenous levels of *myo*-inositol. *Plant Physiol.* **52**, 646–650
- Tenhaken, R., Kanter, U. and Klinghammer, M. (2004) Biosynthesis of UDP-glucuronic acid, an important precursor for plant cell wall matrix polysaccharides. In *X Cell Wall Meeting*, 29 August–3 September 2004, Sorrento, Italy, Programme and Abstract book, p. 93
- Turner, W. and Botha, F. C. (2002) Purification and kinetic properties of UDP-glucose dehydrogenase from sugarcane. *Arch. Biochem. Biophys.* **407**, 209–216
- Griffith, C. L., Klutts, J. S., Zhang, L., Levery, S. B. and Doering, T. L. (2004) UDP-glucose dehydrogenase plays multiple roles in the biology of the pathogenic fungus *Cryptococcus neoformans*. *J. Biol. Chem.* **279**, 51669–51676

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