The Genetics, Transcriptional Profiles, and Catalytic Properties of UDP-\(\alpha\)-D-Xylose 4-Epimerases from Barley\(^1\)\(^{[OA]}\)

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Cell walls in the grasses contain relatively high levels of heteroxylans and, in particular, arabinoxylans. Enzymes and corresponding genes that are involved in the provision of sugar nucleotide substrates represent potential control points for arabinoxylan biosynthesis. Following expressed sequence tag database analyses, three genes encoding barley (\(Hordeum vulgar\)e) UDP-\(\alpha\)-xylose 4-epimerases (UXE; EC 5.1.3.5), designated \(HvUXE1\), \(HvUXE2\), and \(HvUXE3\), were cloned and their positions on genetic maps defined. To confirm the identity of the genes, a cDNA construct encoding \(HvUXE1\) was expressed in \(Pichia\) \(pastoris\). The purified, recombinant \(HvUXE1\) catalyzed the freely reversible interconversion of UDP-\(\alpha\)-D-xylopyranose and UDP-\(\beta\)-L-arabinopyranose, with \(K_m\) values of 1.8 and 1.4 mM, respectively. At equilibrium, the ratio of substrate to product was approximately 1:1. Each molecule of heterologously expressed \(HvUXE1\) enzyme contained about one molecule of non-covalently bound NAD\(^+\). Molecular modeling provided a structural rationale for the substrate specificity of the UDP-\(\alpha\)-D-xylopyranose 4-epimerase and, in particular, explained its tight specificity for UDP-\(\alpha\)-xylose compared with other sugar nucleotide epimerases.

Quantitative transcript analyses performed for each of the three genes in a range of organs showed, inter alia, that in 4-epimerase and, in particular, explained its tight specificity for UDP-\(\alpha\)-xylose compared with other sugar nucleotide epimerases.

A distinguishing feature of the grasses and commercially important cereals is that their cell walls contain higher levels of heteroxylans than walls of nongraminaceous monocotyledons and dicotyledons. Correspondingly lower amounts of xyloglucans and pectic polysaccharides are found in walls of the grasses. Additionally, \((1,3;1,4)\)-\(\beta\)-D-glucans, which are only found in isolated cases outside the Poaceae, are major constituents of walls in several organs and tissues of grasses (Gibeaut and Carpita, 1993; York and O’Neill, 2008; Fincher, 2009a, 2009b). The composition of walls of 4-d-old barley (\(Hordeum vulgar\)e) coleoptiles, which consist by weight of about 35% cellulose, 30% heteroxylan, 10% \((1,3;1,4)\)-\(\beta\)-D-glucan, 10% xyloglucan, and 10% pectin (Gibeaut et al., 2005), exemplifies the polysaccharide constituents of walls in vegetative organs of the grasses. However, the compositions of walls in grain show significant differences.

For example, walls of the starchy endosperm in mature barley grain consist of about 70% \((1,3;1,4)\)-\(\beta\)-D-glucan and 20% heteroxylan (Fincher, 1975), while wheat (\(Triticum aestivum\)) and barley aleurone walls contain about 70% (w/w) heteroxylan (Bacic and Stone, 1981). In these cases, the heteroxylans are predominantly arabinoxylans.

During the biosynthesis of arabinoxylans, monosaccharide units are believed to be provided through the activated sugar donors UDP-\(\alpha\)-D-xylose (UDP-Xyl) and UDP-\(\beta\)-L-arabinose (UDP-Ara; Reiter, 2008). UDP-Ara is also an important sugar donor for arabinogalactan proteins and pectic polysaccharides (Feingold and Avigad, 1980; Reiter and Vanzin, 2001). The UDP-Xyl and UDP-Ara substrates are drawn from a pool of sugar nucleotides that originate primarily from UDP-\(\alpha\)-D-Glc (UDP-Glc), although there are a number of pathways involved (Sharples and Fry, 2007). Oxidation of the UDP-Glc by UDP-\(\alpha\)-Glc dehydrogenase forms UDP-\(\alpha\)-glucuronate (UDP-GlcA), which is a glycosyl donor for the synthesis of glucuronoarabinoxylans. Following the epimerization of UDP-GlcA to UDP-\(\alpha\)-D-GalUA, the latter acts as a sugar donor for pectic polysaccharide synthesis (Reiter and Vanzin, 2001; Seifert, 2004). The UDP-GlcA decarboxylases, also known as UDP-\(\alpha\)-D-xylopyranose synthases (UXS), convert UDP-GlcA to UDP-Xyl (Zhang et al., 2005), which acts as the xylosyl donor not only for the biosynthesis of arabinoxylans but also for xyloglucans.

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The UXS enzyme is particularly important in sugar nucleotide interconversion pathways in the Poaceae, because the decarboxylation reaction catalyzed by UXS is essentially irreversible and the conversion of UDP-GlcA to UDP-Xyl effectively partitions glycosyl residues between the synthesis of polysaccharides composed of pentosyl residues, such as arabinoxylans, and those containing hexosyl residues, such as cellulose, (1,3;1,4)-β-glucans, and pectin. It is not known if changes in UXS activity can influence wall composition, but if so, it is likely to be a target enzyme for regulatory control of carbon flux into Glc- and pentose-containing polysaccharides.

The reversible interconversion of UDP-Xyl and UDP-Ara is catalyzed by UDP-D-xylose 4-epimerase (UXE; Feingold and Avigad, 1980). Epimerases of this type are believed to require a noncovalently bound NAD⁺ cofactor (Schutzbach and Feingold, 1970). When substrate is bound to the epimerase, a conformational change leads to the transfer of a hydride moiety from, for example, an equatorially oriented C-4 atom of one sugar nucleotide to the bound NAD⁺ and produces a keto-sugar intermediate (Bauer et al., 1992). The hydride from the NADH is transferred back to the keto-sugar intermediate but approaches from a different direction, so that the hydroxyl group on C-4 is...

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**Figure 1.** Alignment of amino acid sequences of UXEs and UGEs. Amino acid sequences of HvUXE (HvUXE1, HvUXE2, and HvUXE3) and HvUGE1 from barley and UXE from Arabidopsis (AtUXE1; locus name At1g30620) are shown. The conserved motifs GxxGxxG (NAD⁺ binding) and catalytic amino acid residues Ser/Thr and TyrxxxLys of the active site are underlined. The putative transmembrane helices for HvUXE1 and HvUXE3 are boxed. Note that the HvUXE2 sequence is not full length at its NH₂ terminus.
reformed in the axial configuration to generate the other, epimerized sugar nucleotide.

UXE activity has been demonstrated in extracts of mung bean (Vigna radiata; Ginsburg et al., 1956) and wheat germ (Fan and Feingold, 1970), and a UXE gene has been isolated from Arabidopsis (Arabidopsis thaliana) by map-based cloning procedures, where mutation of one UXE gene (mur4) results in a decrease in Ara content in the cell wall (Burget et al., 2003).

Here, we have cloned three members of the barley UXE gene family and defined their locations on genetic maps. A NH2-terminal truncated HvUXE1 has been expressed heterologously in Pichia pastoris, and the enzymic and kinetic properties of the purified recombinant enzyme have been defined. Using NMR-based procedures, it has been demonstrated that the expressed HvUXE1 enzyme catalyzes the interconversion of UDP-Arap and UDP-Xylp. Transcript profiling through quantitative, real-time PCR (Q-PCR), coupled with immunoblotting techniques, indicate that transcripts of individual HvUXE and HvUXS genes increase at a time when arabinoxylans appear in the starchy endosperm of developing barley grain, in leaves, and in roots.

RESULTS

The Barley HvUXE Gene Family and Genomic Locations

Analysis of barley EST databases revealed the presence of three consensus HvUXE sequences. PCR amplification from a barley seedling cDNA preparation, using primers designed from the consensus sequences, generated three individual HvUXE cDNAs. The cDNAs were 1,508, 1,417, and 1,639 bp in length, and they were designated HvUXE1, HvUXE2, and HvUXE3, respectively (Fig. 1).

Genetic mapping showed that the HvUXE1 gene is located on the long arm of chromosome 5H in the region of the Steptoe × Morex bin 9-10 border (http://barleygenomics.wsu.edu; Fig. 2). Two possible loci

![Figure 2. Locations of HvUXS and HvUXE genes on barley chromosomes. Fragments of the HvUXS and HvUXE genes were amplified using Q-PCR primers and used as probes for RFLP mapping. The locations of the HvUXE1, HvUXE2, HvUXS2, and HvUXS3 genes were determined, but HvUXS1, HvUXS4, and HvUXE3 genes could only be mapped to chromosomes 4H, 3H, and 7H, respectively; no precise locations were determined because of the lack of useful polymorphisms. Population designations are CxS for Clipper × Sahara and GxH for Galleon × Haruna Nijo.](http://barleygenomics.wsu.edu)

Table I. Amino acid sequence identities

| Enzyme | HvUXE1 | HvUXE2 | HvUXE3 |
|--------|--------|--------|--------|
| HvUXE1 | 100    |        |        |
| HvUXE2 | 83     | 100    |        |
| HvUXE3 | 78     | 84     | 100    |
| HvUXS1 | 28     | 27     | 27     |
| HvUXS2 | 27     | 28     | 28     |
| HvUXS3 | 27     | 27     | 28     |
| HvUXS4 | 29     | 28     | 30     |
| HvUGDH | <10    | <10    | <10    |
| HvUGAE | 29     | 27     | 25     |
| AtUXE1 | 77     | 85     | 78     |
| AtUXE2 | 81     | 83     | 79     |
| AtUXE3 | 82     | 83     | 81     |
| AtUXE4 | 73     | 81     | 74     |
| HvUGE1 | 41     | 42     | 40     |
| HvUGE2 | 41     | 42     | 39     |
| HvUGE3 | 37     | 40     | 39     |
were found for the \textit{HvUXE2} gene. The more strongly hybridizing DNA fragment in Southern hybridization analyses, designated \textit{HvUXE2a} in Figure 2, is located on the long arm of chromosome 2H at about 140 centimorgan (cM). The more weakly hybridizing DNA fragment (designated \textit{HvUXE2b}) is found on the long arm of chromosome 5H, in a position close to the \textit{HvUXE1} gene. There was no sequence similarity between the \textit{HvUXE2} probe and the nearly full-length \textit{HvUXE1} cDNA, so we conclude that while the \textit{HvUXE2} gene itself is located on chromosome 2H, there is also a \textit{HvUXE2} parologue or a \textit{HvUXE} pseudogene located near the \textit{HvUXE1} gene on chromosome 5H. The \textit{HvUXE3} gene maps to chromosome 7H, but its precise position on the genetic map could not be defined because no polymorphisms were detected in the available mapping parents; its map location was defined through the wheat-barley addition lines (Islam et al., 1981).

Given the sequential functions of the HvUXS and HvUXE enzymes in the provision of pentose-based sugar nucleotides for heteroxylan biosynthesis, we also mapped the \textit{HvUXS} genes (Fig. 2). The \textit{HvUXS2} gene is located on the long arm of chromosome 1H at about 65 cM, while the \textit{HvUXS3} gene maps to about 45 cM on the long arm of chromosome 2H in the Clipper \times Sahara population. The \textit{HvUXS1} and \textit{HvUXS4} genes were mapped to chromosomes 4H and 3H, respectively, but again the lack of useful polymorphisms precluded their precise placement on the genetic map.

**Figure 3.** Phylogenetic relationships of representative UXEs, UXSs, and UGEs. The phylogenetic relationships were analyzed with the ClustalX program. The GenBank accession numbers of the enzymes are as follows: HvUXE1, AAX49504; HvUXE2, AAX49505; HvUXE3, AX49503; OsUGE1, BAF18426; OsUGE2, BAF231582; OsUGE3, BAF25641; OsUGE4, BAF24783; AtUGE1, NP_172738; AtUGE2, NP_194123; AtUGE3, NP_564811; AtUGE4, AAS76249; AtUGE5, NP_192834; PsUGE, AAB86532; HvUXS1, AAT80326; HvUXS2, AAT80327; HvUXS3, AAT80328; HvUXS4, AAT80325; AtUXS1, AAK70880; AtUXS2, AAK70881; AtUXS3, AAK70882; HvUXE1, ABC67797; HvUXE2, ABC67798; HvUXE3, ABC67799; AtUXE1, Q9SA77; AtUXE2, Q9SA77; AtUXE3, Q9SA77; and AtUXE4, Q9SA77.

**Predicted Properties of Proteins Encoded by the \textit{HvUXE} Genes**

The proteins encoded by the \textit{HvUXE} genes show amino sequence identities of 78% to 84%, but sequence identities with other barley enzymes involved in sugar nucleotide interconversions, such as HvUXS, UDP-Glc dehydrogenase, and UDP-γ-glucuronate 4-epimerase, are 30% or less (Table I). However, the HvUXE enzymes share 37% to 42% sequence identity with barley UDP-Glc epimerases (HvUGE; Zhang et al., 2006). The barley enzymes also show relatively high amino acid sequence identities (73%–85%) with the four Arabidopsis AtUXE enzymes (Fig. 1; Table I). Phylogenetic analyses show that all barley UXEs and Arabidopsis UXEs are grouped in a clade that is clearly distinct from the UXS and UGE families (Fig. 3).

The open reading frames of \textit{HvUXE1} and \textit{HvUXE3} encode proteins consisting of 421 and 405 amino acid residues, respectively (Fig. 1), which correspond to molecular masses of 47 and 45 kD, respectively. The \textit{HvUXE2} cDNA was truncated at its 5’ end; the open reading frame encodes 333 amino acid residues, but the molecular mass of the mature enzyme could not be calculated.

Both \textit{HvUXE1} and \textit{HvUXE3} contain single, putative transmembrane helices near their NH2 termini, as predicted by the TMHMM topology software (www.cbs.dtu.dk/services/TMHMM-2.0), and also have GXXGXXG NAD+-binding motifs starting at amino acid residue 79 in \textit{HvUXE1} and at amino acid residue...
The product of the UXE reaction was further analyzed by \(^1\)H-NMR. Table II shows the \(^1\)H-NMR data obtained for the UDP-Xylp substrate and the reaction product and compares these with available data from known compounds (Zhang and Liu, 2001). The chemical shifts (ppm), peak integral (1H, 2H, etc.), peak splitting pattern (d, t, dd, dt, ddd, m, etc.), and coupling constants (J) from the reaction product corresponded exactly to parameters for UDP-Arapt but not to those for UDP-Arap (Table II). For example, the chemical shift of the reaction product at H-2 was 3.7 ppm, with a peak integral of 1H, which was identical to the values from UDP-Arap. These can be compared with values of 4.03 ppm in chemical shift and a peak integral of 2H for UDP-Araf (Table II). At H-4, the peak integral and splitting patterns of the product were also similar to the values for UDP-Arap but very different from the values for UDP-Araf. It was concluded, therefore, from the \(^1\)H-NMR parameters that the HvUXE1 reaction product (Table II) was UDP-Arap.

The enzyme also catalyzed the reverse reaction from UDP-Arap to UDP-Xylp. After the reactions had reached equilibrium, the ratio of UDP-Xylp to UDP-Arap was close to 1:1 for both directions of the reaction (Fig. 4). The enzyme was stable at 4°C with little or no loss of activity for at least 1 week. Optimal activity was observed at about pH 7.0 (data not shown).

The apparent \(K_m\) values of HvUXE1 were 1.8 and 1.4 mM for UDP-Xylp and UDP-Arap, respectively (Table III). The \(k_{cat}\) values of the expressed HvUXE1 were 1.5 s\(^{-1}\) for both UDP-Xylp and UDP-Arap, and catalytic efficiencies were similar for both substrates (Table III).

The purified recombinant HvUXE1 did not catalyze the interconversion of UDP-Glc and UDP-Gal, even after 16 h of incubation at a high enzyme concentration (data not shown). Using the 80% (v/v) ethanol enzyme denaturation and extraction procedures described by Zhang et al. (2006), noncovalently bound NAD\(^+\) was detected in the expressed HvUXE1 enzyme at a molar ratio of approximately 1:1. This conclusion was based on the observation that about 21 pmol of NAD\(^+\) was released from 1 \(\mu\)g of purified HvUXE1 of molecular mass approximately 46,000 D.

Neither the divalent cation Mg\(^{2+}\) nor EDTA stimulated or inhibited the activity of the recombinant HvUXE1 (Table IV). Similarly, addition of NADH or NAD\(^+\) had no significant effect on activity. The reducing agent dithiothreitol (DTT) appeared to stimulate activity, while the nucleotides UTP and UDP, but not UMP, inhibited the enzyme reaction (Table IV).

**Molecular Modeling of HvUXE1**

The human (1ek6) and *Escherichia coli* (1xel) UGE structures, which represent a mixed \(\alpha/\beta\)-protein fold based on the CATH classification (Orengo et al., 1997), served as excellent combined structural templates for
modeling HvUXE1 (Fig. 5A). The solved E. coli UGE structure included the binding positions of NAD\(^+\) and UDP-Glc, and this template therefore represented a ternary complex of UGE with bound cofactor and substrate (Thoden et al., 1996, 2000). The stereochemical quality and overall G-factors (Laskowski et al., 1993) of the final HvUXE1 model showed that none of the amino acid residues in the modeled HvUXE1s were positioned in disallowed regions. The overall G-factors for 1ek6 and 1xe1 templates were 0.19 and 0.15, respectively, while the overall G-factor for the HvUXE1 model with bound UDP-Xyl was 0.24. The Z-score value for the modeled HvUXE1 with bound UDP-Xyl was 0.24. The Z-score values evaluated by ProsaIIv3 (Sippl, 1993), which reflect combined statistical potential energy for the templates 1ek6 and 1xel, were 0.15, respectively, while the overall G-factor for the HvUXE1 model with bound UDP-Xyl was 0.24. The Z-score value for the modeled HvUXE1 with bound UDP-Xyl was 0.24. The Z-score values evaluated by ProsaIIv3 (Sippl, 1993), which reflect combined statistical potential energy for the templates 1ek6 and 1xel, were 0.15, respectively, while the overall G-factor for the HvUXE1 model with bound UDP-Xyl was 0.24. The Z-score value for the modeled HvUXE1 with bound UDP-Xyl was 0.24. The Z-score values evaluated by ProsaIIv3 (Sippl, 1993), which reflect combined statistical potential energy for the templates 1ek6 and 1xel, were 0.15, respectively, while the overall G-factor for the HvUXE1 model with bound UDP-Xyl was 0.24. The Z-score value for the modeled HvUXE1 with bound UDP-Xyl was 0.24. The Z-score values evaluated by ProsaIIv3 (Sippl, 1993), which reflect combined statistical potential energy for the templates 1ek6 and 1xel, were 0.15, respectively, while the overall G-factor for the HvUXE1 model with bound UDP-Xyl was 0.24. The Z-score value for the modeled HvUXE1 with bound UDP-Xyl was 0.24.

### Table II. Identification of the HvUXE1 reaction product by \(^1\)H-NMR

| Arabinosyl/xylosyl | Reaction Substrate (UDP-Xyl) | Reaction Product (Putative UDP-Arap) | Available Data |
|--------------------|-----------------------------|------------------------------------|---------------|
| H-1                | 5.44 (1H, dd, J = 6.9, 3.4)  | 5.50 (1H, dd, J = 6.8, 3.4)        | 5.49 (dd, J = 7.5, 3.5) |
| H-2                | 3.41 (1H, dt, J = 9.7, 3.0)  | 3.70 (1H, dt, J = 10.1, 2.9)       | 3.70 (dt, J = 10.0, 3.5) |
| H-3                | 3.60 (1H, dd, J = 10.2, 3.4) | 3.82 (dd, J = 10.0, 3.5)          | 3.82 (dd, J = 10.0, 3.5) |
| H-4                | 3.52 (1H, dt, J = 9.4, 6.8)  | 3.91 (1H, m)                      | 3.91 (m)       |
| H-5a               | Approximately 3.65 (2H, m)   | 3.61 (1H, dd, J = 12.8, 1.8)      | 3.61 (dd, J = 12.5, 2.0) |
| H-5b               | approximately 3.65 (2H, m)   | 4.01 (1H, d, J = 12.9)            | 4.01 (d, J = 12.5) |
|                     |                            |                                    | 3.68 (dd, J = 12.5, 3.0) |
| Rib                |                             |                                    |               |
| H-1'               | 5.88 (1H, d, J = 4.2)        | 5.88 (1H, d, J = 4.1)             | 5.87 (d, J = 5.0) |
| H-2'               | 4.27 (2H, m)                | 4.27 (2H, m)                      | 4.26 (2H, m)   |
| H-4'               | 4.18 (1H, m)                | 4.18 (1H, m)                      | 4.17 (q, J = 3.0) |
| H-5a'              | 4.09 (1H, dd, J = 11.4, 5.2, 2.6) | 4.09 (1H, dd, J = 11.2, 5.4, 2.6) | 4.08 (ddd, J = 12.0, 5.5, 2.5) |
| H-5b'              | 4.14 (1H, m)                | 4.14 (1H, m)                      | 4.13 (ddd, J = 12.0, 4.5, 2.5) |
| Uridine            |                             |                                    | 4.12 (ddd, J = 12.0, 5.0, 2.0) |
| H-5*               | 5.87 (1H, d, J = 8.2)        | 5.87 (1H, d, J = 8.2)             | 5.86 (d, J = 8.5) |
| H-6*               | 7.85 (1H, d, J = 8.2)        | 7.85 (1H, d, J = 8.1)             | 7.85 (d, J = 8.5) |

Transcript Profiling of HvUXE and HvUXS Genes in Different Barley Organs

Using gene-specific oligonucleotide primers, Q-PCR was used to define mRNA abundance of individual HvUXE genes in extracts from several barley organs at different developmental stages (Fig. 6). Transcripts of the three genes were detected in all extracts analyzed, although the highest levels were found in the matura-
zon of roots (Fig. 6).

In view of the availability of information on the composition of cell walls in the starchy endosperm during grain development (Wilson et al., 2006), possible correlations between HvUXE transcript abundance and arabinoxylan deposition in cell walls of the developing grain were investigated. Because of the importance of UXS in diverting sugar nucleotides into the "pentose pool" (Zhang et al., 2005), the abundance of HvUXS transcripts was also monitored in these experiments. The HvUXS1 gene is the most highly transcribed of the four HvUXS genes in the organs of barley so far examined (Zhang et al., 2005).

For the developing barley endosperm series, flowers were hand pollinated and endosperm was extracted or dissected away from maternal tissues (Burton et al., 2008). Total RNA was prepared from the extracts at the syncytial stage, 2 to 5 d after pollination (DAP), at the endosperm cellularization stage (6–7 DAP), and at the endosperm differentiation stage (8–11 DAP; Brown et al., 1994; Wilson et al., 2006). Transcript levels for the HvUXE1 gene were relatively low early in endosperm...
development but started to increase at 8 DAP and peaked at 9 DAP (Fig. 7A). Thus, transcript levels for the HvUXE1 gene were about 4-fold higher at 9 DAP than in the syncytial and cellularization stages (Fig. 7A). Transcript levels of the HvUXE3 gene followed a similar developmental pattern but were considerably lower than those for the HvUXE1 gene. In contrast, HvUXE2 transcript abundance was moderate during the syncytial stage of endosperm development (2–6 DAP) but decreased to undetectable levels beyond 6 DAP. Overall, there was a substantial increase in HvUXE1 and HvUXE3 mRNA levels at the differentiation stage of endosperm development (Fig. 7A).

The HvUXS1 gene was transcribed at approximately 10-fold higher levels than the HvUXE genes in the developing endosperm from 2 to 11 DAP (Fig. 7B), and HvUXS1 transcript abundance values were also much higher than those of the other HvUXS genes. The HvUXS1 transcripts remained high and approximately constant from 2 to 8 DAP but subsequently increased to a peak at 9 DAP (Fig. 7B). The transcription of the HvUXS2 and HvUXS4 genes followed a developmental pattern that was similar to that of the HvUXS2 gene (Fig. 7B): mRNA levels for these two genes were relatively high during the syncytium stage of development but decreased during cellularization and differentiation. The levels of mRNA for the HvUXS3 gene were very low throughout endosperm development (Fig. 7B).

A comparison of HvUXE transcript levels along segments of barley leaves is shown in Figure 8, where it is clear that higher levels are found in the central regions of young barley leaves. In contrast to the developing grain, the relative abundance of the HvUXE2 mRNA was greater than that of HvUXE1 mRNA, particularly in the upper segments of the leaves (Fig. 8).

### HvUXS and HvUXE Enzyme Activities in Developing Barley Endosperm

In order to determine whether or not changes in transcript levels for HvUXE and HvUXS genes correspond to increased enzyme activity in the developing endosperm, enzyme activities of HvUXS and HvUXE were monitored in unpurified endosperm extracts. There were significant increases in both HvUXE and HvUXS activity at 8 to 9 DAP compared with activities at 6 DAP (Fig. 9, A and B), but these subsequently decreased at 11 DAP. Thus, the peaks of enzyme activity (Fig. 9, A and B) approximately matched the peaks of transcripts (Fig. 7) during grain development.

### Cell Wall Pentose Sugar Contents in Developing Endosperm, Leaves, and Roots

Because HvUXS and HvUXE activities are considered essential for the generation of pentose-containing sugar nucleotides for cell wall biosynthesis, the relationship between pentose sugar levels in crude cell wall preparations from the developing barley endosperm and levels of HvUXS and HvUXE gene transcripts was investigated. In Figure 9C, it is apparent that pentose sugar content of wall polysaccharides in developing endosperm started to increase at 8 DAP and peaked at 9 DAP. This corresponds with the peaks in transcript levels for the HvUXE1, HvUXE3, HvUXS1, and HvUXS4 genes at 9 DAP in developing endosperm (Fig. 7). In barley roots, pentose sugar contents were higher in the maturation zone than in root tips (Fig. 9D), and this is consistent with observations that transcript levels of all the HvUXE (Fig. 6) and HvUXS genes (Zhang et al., 2005) were higher in root maturation zones than in root tips. In barley leaves, pentose sugar contents were similar at the tip and base of the leaves but increased in the central regions of leaf segments (Fig. 9E), which again is consistent with transcript levels of the three HvUXE genes (Fig. 8) and with levels of HvUXS2 and HvUXS3 transcripts (Zhang et al., 2005).

### DISCUSSION

The key monosaccharide donor substrates for the synthesis of arabinoxylans in barley walls are the activated sugar nucleotides UDP-Xyl and UDP-Ara. As noted above, UXS plays a central role in irreversibly diverting hexose-containing sugar nucleotides into UDP-Xyl, which, through the action of UXE, forms a pool of UDP-Xyl and UDP-Ara for the syn-

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**Table III. Kinetic constants for the HvUXE1 reaction**

The values are means for three replicates ± sd.

| Substrate | \(K_m\) \(\mu\text{M}\) | \(k_{cat}\) \(\text{sec}^{-1} \mu\text{M}^{-1}\) | Catalytic Efficiency |
|-----------|-----------------|-----------------|-----------------|
| UDP-Xyl   | 1.8 ± 0.4       | 1.5 ± 0.2       | 0.9             |
| UDP-Ara   | 1.4 ± 0.5       | 1.5 ± 0.4       | 1.1             |

**Table IV. Effects of additives on HvUXE1 reaction rates**

The assay was conducted for 20 min at 25°C in 50 mM sodium phosphate buffer, pH 7.0. The data are means of three replicates ± s.d. The activity was 470 pmol min\(^{-1}\) mg\(^{-1}\) protein in the reaction mixture containing only sodium phosphate buffer, 0.5 mM UDP-Xyl, and recombinant HvUXE1 (0.14 μg).

| Additive | Percentage |
|----------|------------|
| None     | 100 ± 4    |
| DTT (2 mM) | 150 ± 8   |
| EDTA (2 mM) | 95 ± 2   |
| MgCl\(_2\) (1 mM) | 101 ± 2  |
| NAD\(^+\) (1 mM) | 105 ± 2  |
| NADH (1 mM) | 90 ± 3   |
| UMP (2 mM) | 93 ± 1   |
| UDP (2 mM) | 15 ± 1   |
| L-UTP (2 mM) | 54 ± 3  |
thesis of pentose-containing wall polysaccharides (Reiter and Vanzin, 2001; Seifert, 2004; Zhang et al., 2005). Furthermore, it is believed that a UDP-Ara mutase contributes to the composition of this pool through the conversion of UDP-Ara\(^+\) to UDP-Araf (Konishi et al., 2007).

Here, we have shown that the barley \textit{HvUXE} gene family contains at least three members, which are designated \textit{HvUXE1}, \textit{HvUXE2}, and \textit{HVUXE3}, and cDNAs encoding each of these enzymes have been amplified and cloned. The three genes have been mapped to chromosomes 5H, 2H, and 7H, respectively (Fig. 2). Comparisons with genome sequences that are now available for other members of the Poaceae reveal that rice (\textit{Oryza sativa}), sorghum (\textit{Sorghum bicolor}), and \textit{Brachypodium} all contain at least three \textit{UXE} genes, while Arabidopsis has four \textit{UXE} genes (Reiter and Vanzin, 2001; Seifert, 2004).

The deduced amino acid sequences of the three \textit{HvUXE} enzymes show close to 80% positional identity with the \textit{UXE} enzymes from Arabidopsis (Table I) and from other higher plants (data not shown), but the sequence identity values with other barley sugar nucleotide-interconverting enzymes are generally less than 30% (Table I). Sequence identities between the barley \textit{HvUXE} and \textit{HvUGE} enzymes, which catalyze
similar epimerase reactions, are slightly higher, at about 40% (Zhang et al., 2006). Examination of the amino acid sequences of the three HvUXEs revealed the presence of single putative transmembrane helices and NAD⁺-binding sites near the NH₂ termini of HvUXE1 and HvUXE3. However, a full-length cDNA encoding the HvUXE2 enzyme could not be obtained, and the deduced amino acid sequence of the NH₂-terminal region of the HvUXE2 protein is not complete in this region. Therefore, it is not known if the HvUXE2 isoenzyme has a transmembrane helix or a NAD⁺-binding motif (Fig. 1). The three barley HvUXEs have Thr, Tyr, and Lys residues in their catalytic sites, in a motif that is conserved in the dehydrogenase/reductase superfamily, although the Thr residue can be substituted by a Ser residue in many enzymes in the superfamily (Zhang et al., 2005, 2006). The UXE from Arabidopsis also has a Thr residue in the active site motif (Fig. 1).

To confirm that the proteins encoded by the HvUXE genes identified here actually have UXE activity and, if so, to define their kinetic properties and substrate specificities, a cDNA encoding HvUXE1 was expressed in *P. pastoris*. The cDNA was truncated, insofar as the region encoding the putative transmembrane helix near the NH₂ terminus had been removed, and it was anticipated that this would facilitate the recovery of a soluble enzyme for the enzymic analyses. The heterologously expressed enzyme did indeed have UXE activity, using either UDP-Xyl or UDP-Ara as substrate (Fig. 4), but was unable to catalyze the epimerization of UDP-Glc or UDP-Gal.

¹H-NMR showed that the arabinosyl residue of the UDP-Ara product of the reaction was in the pyranose conformation (Table II). Epimerization of the C-4 atom of the α-D-xylopyranosyl residue on UDP-Xyl by UXEs will result in the formation of a β-L-arabinopyranosyl residue on the UDP-Ara product. In cereal arabinoxylans and other wall polysaccharides, arabinosyl residues are usually found in the arabinofuranosyl (Araf) form rather than in the arabinopyranosyl (Arap) form. However, the activated donor UDP-β-L-arabinofuranose (UDP-Araf) has not been detected in plants, although the presence of UDP-β-L-arabinopyranose (UDP-Arap) has been reported (Pauly et al., 2000).

To investigate the nature of the association of NAD⁺ with the enzyme, the purified recombinant protein obtained by heterologous expression of the truncated HvUXE1 cDNA was denatured and extracted with 80% (v/v) ethanol. The NAD⁺ was released into solution by this treatment and was identified and quantitated by HPLC, as described previously (Zhang et al., 2005, 2006). The data showed that NAD⁺ was noncovalently bound to the HvUXE enzyme and that the stoichiometry of binding was approximately 1:1 on a molar basis. This stoichiometry was also found for the noncovalent binding of NAD⁺ to barley UGE and UXS (Zhang et al., 2005, 2006).

The apparent *Kₘ* values for HvUXE1 of 1.8 and 1.4 mM for UDP-Xylp and UDP-Arap, respectively (Table III), may be compared with apparent *Kₘ* values of 1.5 and 0.5 mM that have been reported for an unpurified wheat UXE for UDP-Xyl and UDP-Ara, respectively (Fan and Feingold, 1970) and with *Kₘ* values for UDP-Xyl of 0.16 to 0.43 mM for a family of bifunctional cytosolic UGEs from pea (*Pisum sativum*) that also have UXE activity (Kotake et al., 2009). The *Kₘ* values for the
barley and wheat UXEs (Table III; Fan and Feingold, 1970) indicate that the enzymes have relatively low affinities for their UDP-Xyl or UDP-Ara substrates. The turnover rate of 1.5 s$^{-1}$ (Table III) and catalytic efficiency (1 s$^{-1}$ mM$^{-1}$) are also somewhat low compared with other sugar nucleotide-interconverting enzymes from barley, including HvUXS and HvUGE (Zhang et al., 2005, 2006). The marked contrast in substrate specificity between the HvUXE enzymes studied here and the bifunctional cytosolic UGE/UXE enzymes from pea and Arabidopsis (Kotake et al., 2009) raises a number of questions regarding the structural, cellular, and evolutionary bases for specificity in these enzymes. The HvUXE enzymes share only about 40% sequence identity with the barley UGEs (Table I), they are likely to be membrane bound, and they do not epimerize UDP-Glc. On the other hand, the bifunctional PsUGE enzymes, together with certain members of the Arabidopsis and rice UGE gene families, are cytosolic, have the ability to epimerize both UDP-Glc and UDP-Xyl or their UDP-Gal and UDP-Ara products, and can be classified in the plant UGE I subfamily (Rosti et al., 2007; Kotake et al., 2009). The availability of the three-dimensional (3D) structures for the human and E. coli UGEs, coupled with the 3D structure of a UDP-Glc dehydrogenase from Streptococcus pyrogenes and the general similarity in protein fold structure of enzymes in the dehydrogenase/reductase superfamily, enabled the structures of the plant enzymes to be modeled with high reliability. Although the reliabilities of molecular modeling programs have increased dramatically in recent years, it must be stated that they remain models only and eventually can only be confirmed by x-ray crystallography. Nevertheless, the modeling undertaken here allowed the different substrate specificities of the two groups of enzymes to be reconciled in precise structural terms.

The overall shape and distribution of the secondary structure elements in the HvUXE1 were highly conserved when compared with the human and bacterial UGE structures, and the overall geometries of the active sites were also conserved (Fig. 5). The molecular models of HvUXE1 allowed the locations of the NAD$^+$ cofactor and the UDP-Ara and UDP-Xyl substrates to be defined (Fig. 5). In particular, the models show the juxtaposition of the nicotinamide ring of NAD$^+$ with the C-4 carbon atom of the xylosyl or glucosyl residue of the respective sugar nucleotide substrate, as required for epimerases in this class of the dehydrogenase/reductase superfamily. The models provided a structural explanation for the observation that the barley HvUXE1 enzyme would accommodate UDP-Xyl in its catalytic site but not UDP-Glc. The UDP-Xyl substrate was predicted to bind in the active site of the HvUXE1 enzyme in a relaxed conformation (Fig. 5A), but the structure of a UDP-Glc molecule needed to be dramatically distorted to allow it to fit into the substrate-binding site of the HvUXE1 enzyme (Fig. 5, B and C). More specifically, the uridine moiety had to be significantly tilted in the direction of the ribosyl moiety and rotated by almost 90°, leading to a constrained conformation of UDP-Glc (Fig. 5B). These comparative analyses suggested that the HvUXE1 enzyme would not be able to accommodate the.

**Figure 7.** Normalized mRNA levels of HvUXS and HvUXE genes in developing endosperm. A, mRNA levels of HvUXE1, HvUXE2, and HvUXS3 genes. B, mRNA levels of HvUXS1, HvUXS2, HvUXS3, and HvUXS4 genes. The mRNA data are means of four separate runs. SD values are indicated by error bars.
UDP-Glc substrate in an appropriate conformation and in the correct spatial disposition with respect to the NAD$^+$ cofactor and that the HvUXE1, therefore, might not be active on UDP-Glc, as indicated by the absence of epimerase activity when the HvUXE1 enzyme was incubated with UDP-Glc. When similar modeling experiments were performed with the bifunctional UGE from pea, which can epimerize either UDP-Glc or UDP-Xyl (Kotake et al., 2009), the structural basis for its broader substrate specificity, and hence its potential bifunctional role in sugar nucleotide interconversion, could be reconciled with the 3D models. The modeling data showed that both UDP-Glc and UDP-Xyl could be accommodated in relaxed conformations in the active site pocket of the bifunctional UGE. That is, no significant distortion of the UDP-Glc was observed in the active site pocket of the bifunctional UGE model and, overall, very similar substrate-enzyme interactions were apparent when either UDP-Glc or UDP-Xyl was bound to the pea UGE enzyme (Fig. 5C). These modeling data are entirely consistent with the distinct substrate specificities of the two enzymes and provide a structural rationale for the discrimination between UDP-Xyl and UDP-Glc by the barley HvUXE1. The data are also consistent with the distinct phylogenetic relationships of the UXE, UXS, and UGE gene families (Fig. 3), but their evolutionary and functional significance have yet to be defined.

The three HvUXE genes are transcribed in all the organs from barley that we examined and at several stages of development, albeit at different rates (Fig. 6). We also examined the possibility that HvUXE transcription, together with HvUXS transcription, might be correlated in some way with the deposition of arabinoxylans in the cell wall, despite the large number of steps between gene transcription and the deposition of polysaccharide into the wall. The first experiment was to examine this potential correlation in developing barley grain. The increase in abundance of the HvUXE (Fig. 7A) and HvUXS (Fig. 7B) mRNA at 8 to 9 DAP was consistent with the appearance of arabinoxylans in walls at about this time (Wilson et al., 2006) and with the increases in both UXE and UXS activity in developing grain extracts (Fig. 9, A and B) and grain pentose content (Fig. 9C). A correlation between transcript levels and pentose content in roots (compare Figs. 6 and 9D) and leaves (compare Figs. 8 and 9E) was also observed.

In general terms, it can be concluded that UXE and UXS gene transcription and subsequent enzyme activity might be important in the control of carbon flux to cell wall components (Seifert, 2004) and in the composition of the sugar nucleotide pool, in particular as it relates to the diversion of hexose sugar nucleotides to pentose sugar nucleotides when the cell deposits arabinoxylans into the wall. Thus, these enzymes and their corresponding genes might control the relative concentrations of arabinoxylans in walls and could become a target for the genetic manipulation of arabinoxylan content in cereal grain and in the vegetative tissues of crop residues.

**Table V.** Q-PCR primer sequences, product sizes, and optimal acquisition temperatures

| Gene   | Forward Primer          | Reverse Primer          | bp   | T      |
|--------|-------------------------|-------------------------|------|--------|
| HvUXE1 | 5′-CCCGCAAGGATCACCCGC-3´ | 5′-GGATTTGAAACATAACCCAC-3´ | 196  | 82     |
| HvUXE2 | 5′-CCCTGGCTGCTGCTCAGAAC-3´ | 5′-GCCCATGAGTATGTCTTG-3´ | 194  | 75     |
| HvUXE3 | 5′-CTTTACTAGGCTCCCTGTGAG-3´ | 5′-ATGCTTTGATGACGACTTAGG-3´ | 155  | 75     |

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Figure 9. HvUXS and HvUXE activities and pentose sugar contents in developing endosperm, root, and leaf segments. HvUXS (A) and HvUXE (B) activities in developing endosperm were assayed and expressed as shown. Pentose sugar contents in developing endosperm (C), root (D), and leaf segments (E) were determined and expressed as shown. FW, Fresh weight. Barley roots and leaves were sectioned into segments as described by Burton et al. (2004). R1 and R4 corresponded to root tip and maturation zones, respectively, as in Figure 6. LA, LC, and LE corresponded to leaf segments A, C, and E, respectively, in Figure 8.
MATERIALS AND METHODS

Materials

UDP-Xylp and UDP-Arap were obtained from the Complex Carbohydrate Research Center at the University of Georgia. Phloroglucinol was obtained from Sigma-Aldrich. The 1-kb DNA ladder molecular mass standards and the pGEM-T Easy vector system I were from Promega, and the SuperScript II RNase H reverse transcriptase and TRIZOL reagent were from Invitrogen. Barley (Hordeum vulgare ‘Sloopy’) plants were grown in a greenhouse at a maximum daytime temperature of 23°C and a minimum overnight temperature of 15°C and hand pollinated, and the developing caryopses were harvested for RNA isolation and enzyme activity measurement as described by Burton et al. (2008).

cDNA Preparations and Q-PCR Analysis of Transcript Levels

Total RNA preparation, cDNA synthesis, and Q-PCR followed the same procedures described previously (Zhang et al., 2005). Developing endosperm, leaves, roots, and coleoptiles were harvested as described by Burton et al. (2004). HvUXE primer pairs and optimal acquisition temperatures for Q-PCR are shown in Table V. barley glyceraldehyde-3-phosphate dehydrogenase, heat shock protein 70, α-tubulin, cyclophilin, and cellulose synthase 1 were used as control genes in Q-PCR, and primer sequences were as reported previously (Zhang et al., 2005).

Cloning of HvUXE cDNAs

More than 70 cDNA sequences for putative Uxe genes were identified in barley EST databases. Analysis of the sequences with ContigExpress software (Informax) generated three contig sequences. Three gene-specific oligonucleotide primers close to the 5′ ends of the EST contigs (5′-CACTCCCGCA-ATTCTTTCCCTGTT-3′, 5′-GGATACATTGGCTCACATGTCACT-3′, and 5′-TCCTCCTCCCTCTGCTTG-3′) were used with an oligo(dT) primer to amplify cDNAs from a cDNA preparation generated from young barley seedlings. Three cDNAs were obtained and were designated HvUXE1, HvUXE2, and HvUXE3.

Analysis of HvUXS and HvUXE Activity in Developing Barley Endosperm

Barley developing endosperm preparations (0.1 g) were ground in liquid nitrogen and extracted with 1 mL of 200 mM sodium phosphate buffer, pH 6.5. All subsequent procedures were conducted at 4°C. The mixture was centrifuged at 16,000g for 15 min, the supernatant was transferred to a fresh tube, and solid ammonium sulfate was added to 45% saturation. The mixture was incubated for 15 min. The pellet was resuspended in 200 mM sodium phosphate buffer, pH 6.5, and activity was assayed immediately.

Heterologous Expression of HvUXE1 in Pichia pastoris

The truncated HvUXE1, from which the region encoding the first 50 amino acid residues had been removed, was cloned into the pPICZ alpha A vector according to the manufacturer’s instructions (Invitrogen). The HvUXE1-pPICZ alpha A construct was transformed into Pichia pastoris, and protein expression was induced with 1% (v/v) methanol. The culture medium (50 mL) containing secreted HvUXE1 was harvested by centrifugation at 16,000g for 15 min to remove yeast cells. Protein in the medium was precipitated by the addition of ammonium sulfate to 65% saturation and resuspended in 2.5 mL of 50 mM sodium phosphate buffer, pH 8.0, containing 300 mM NaCl and 10 mM imidazole. The resuspended protein was desalted on a PD 10 column (GE Healthcare Life Sciences). The recombinant HvUXE1 in the eluent was purified with Ni-NTA resin as described previously for HvUXS (Zhang et al., 2005).

Enzyme Activity Assays

The HvUXS activity was assayed as described previously (Zhang et al., 2005). The Uxe activity was determined in a reaction mixture (25 μL) containing 100 μg sodium phosphate buffer, pH 7.0, 2 mM NAD+, 2 mM DTT, 0.5 mM UDP-Xylp (or UDP-Arap), and the ammonium sulfate fraction of the tissue extract (29 μg of total protein) or recombinant HvUXE1 (0.14 μg of protein). The assay was performed at 25°C for 20 min and was stopped by incubating the mixture at 100°C. The reaction rate was linear for at least 60 min for the recombinant protein. Concentrated triethylamine acetate was added to the reaction mixture to a final concentration of 0.1 M (pH 6.8), and the mixture was heated at a HPLC Hypersil ODS column (250 × 2.1 mm, 5 μm; PTH-AA; Agilent Technologies). Sugar nucleotides were eluted using 0.1 M triethylamine acetate at a flow rate of 0.2 mL min⁻¹. Peak areas were integrated with Chemstation software (Agilent Technologies).

Characterization of Reaction Product by NMR

A preparative HPLC procedure was used for separation of UDP-Xylp and putative UDP-Arap in the HvUXE1 reaction mixture. The reaction mixture was separated on a Preval Carbodhydrate ES column in ion-exchange mode (4.6 × 150 mm, 5 μm; Alltech) with 300 mM ammonium formate, pH 7. The fractions were collected, thoroughly dried, and subjected to proton-deuterium exchange by dissolving in D_2O (DLM-6; 99.96%; Cambridge Isotope Laboratories). After the samples were freeze-dried, the exchange procedure was repeated. The samples were redissolved in D_2O, and NMR spectra were recorded at room temperature using a Bruker-Biospin Avance 800 MHz NMR spectrometer, with a spectral width of 11.16 kHz, an acquisition time of 2.94 s, and a relaxation delay of 1 s, for 256 scans. The reference was D_2O.

Analysis of Pentose Sugars in the Cell Wall

Barley developing endosperm and root fragments were ground in liquid N_2 and extracted with 80% (v/v) ethanol at 75°C. The crude cell wall material was washed seven times with 80% ethanol, each once with 100% acetone and 100% methanol, and air-dried. Starch was removed by incubating the cell wall material with α-amylase at 40°C for 1 h. This process was repeated once following the addition of fresh enzyme. Degradation products released by the α-amylase treatment were removed by washing three times with 80% ethanol. Cell wall material was hydrolyzed by 0.5 M sulfuric acid at 100°C for 30 min, and pentose sugars were determined essentially as described by Bell (1985). The colorimetric reagent was prepared by mixing 11 mL of acetic acid, 0.2 mL of HCl, 0.1 mL of 1.5% (w/v) Glic, and 0.5 mL of 20% (w/v) ethanol) phloroglucinol. One milliliter of the reagent was added to 0.2-ML samples containing zero to 100 μg of pentose sugars, the mixture was heated at 100°C for 25 min and cooled for 5 min, and absorption was measured at 552 and 510 nm. The difference between A_552 and A_510 was taken for the calculation of pentose sugar concentration (Bell, 1985). Xyl was used as standard in the assay.

Genetic Mapping of HvUXS and HvUXE Genes

DNA fragments were amplified from cDNA preparations with the Q-PCR primers of HvUXS (Zhang et al., 2005) and HvUXE (Table V) genes and were used as probes for RFLP analysis of wheat-barley addition lines (Islam et al., 1981) and of the barley doubled haploid mapping populations. The doubled haploid mapping populations used comprised 146 lines of the Clipper × Sahara population (Karakousis et al., 2003a) and 107 lines of the Galeon × Haruna Nijo population (Karakousis et al., 2003b). Loci were positioned using the “Find links” function of Map Manager QTXb20 (Manly et al., 2001). DNA extractions and Southern hybridization analyses were performed using standard methods (Sambrook et al., 1989; Rogowsky et al., 1991). Relative locations on the Septeope × Morex bin maps (http://barleygenomics.wsu.edu) were determined using common markers.

Generation of a 3D Model of Barley HvUXE1

A 3D molecular model of HvUXE1 was constructed essentially as described previously (Zhang et al., 2006) using the Modeller 9v1 program (Sali and Blundell, 1993; Sanchez and Sali, 1998). The first step in the computational approach was the identification of a known 3D structure (template) related to
the target HvUXE1 sequence. Searches with the Structure Prediction Meta-Server (Ginalski et al., 2003), SeqAlert (Bioinformatics and Biological Computing, Weizmann Institute of Science), Protein Data Bank (http://www.rcsb.org/pdb/), and 3D-PSSM Server (Imperial College of Science, Technology, and Medicine) identified UGE from human and Escherichia coli with the respective Protein Data Bank entries 1ek6 and 1xel (Thoden et al., 1996, 2000) as the best templates. The 1xel structure represents a ternary complex with bound NAD + and UDP-Glc (Thoden et al., 1996). In the 1ek6 structure, a NAD + analog (1,4-dihydroroticamidine adenine dinucleotide) and UDP-Glc are bound, and this complex can also be classified as a ternary complex. In the second step of the modeling process, template structures were aligned with the target sequence (Devereux et al., 1984; Notredame et al., 2000), and the three sequences were truncated at their NH2 and COOH termini to eliminate parts of the sequences that did not have structural counterparts. The sequence alignment of the 1ek6, 1xel, and HvUXE1 entries contained eight one- to six-amino acid insertions and deletions. The sequence identity and similarity scores were calculated by the Water program of BioManager version 2.0 at the Australian National Genomics Information Service (www.angis.org.au), with the implemented gap penalty function and dynamic programming algorithm of Smith and Waterman (1981). The identity and similarity scores between 1ek6 and HvUXE1 were 41% and 55%, and those between 1xel and HvUXE1 were 40% and 55%. In the final step, the structurally aligned HvUXE1 and UGE sequences containing NAD +, UDP-Glc, or UDP-Xyl (generated from the coordinates of UDP-Glc bound to 1xel) were used as input parameters to construct 3D molecular models on a Linux Red Hat workstation running a Fedora Linux Core 4 operating system. The final 3D molecular models of HvUXE1 (with bound UDP-Glc and UDP-Xyl) were selected from 120 models. The models with the lowest value of the Modeller 9v1 objective function were chosen for further refinement using conjugate gradient energy minimization within a suite of CNS programs (Brunger et al., 1998), in particular a “model_minimize.inp” script with 200 gradient steps, and the NAD+, UDP+, and UDP-Xyl topology and parameter specifications provided by the Hic-Up Database (Kleywegt and Jones, 1998).

The stereochemical quality and overall C-factors of the final HvUXE1 models were calculated with PROCHECK (Laskowski et al., 1993). Z-score values were calculated by ProsAll (Sipp, 1993), and the program O (Jones et al., 1991) was used to determine the root mean square deviation values in the Cα positions between the 3D structures and their templates. The molecular graphics were generated with PyMol (http://www.pymol.org).

Sequence data for the cDNAs have been deposited in the GenBank/EMBL databases under accession numbers DQ336893, DQ336894, and DQ336895 for HvUXE1, HvUXE2, and HvUXE3, respectively.

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