Possible roles of nucleotide sugar interconversion enzymes in the biosynthesis of arabinoxylans in developing barley grains

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Abstract

UDP-D-Glucose, UDP-D-galactose, UDP-D-glucuronate, UDP-D-xylose and UDP-L-arabinose are important precursors for the biosynthesis of barley cell wall polysaccharides, whose compositions determine important grain quality characteristics. These precursors are synthesized in sugar nucleotide interconversion pathways. Genes encoding the key enzymes in the pathways in barley have been cloned and characterized. Expression levels of the genes and biochemical properties of some of the enzymes have been investigated. UDP-D-Xylose synthase (EC 4.1.1.35) (HvUXS) catalyzes the synthesis of UDP-D-xylose from UDP-D-glucuronate in an essentially irreversible reaction. UDP-D-Xylose is believed to commit glycosyl residues to arabinoxylans and xyloglucans. Four members of the barley *HvUXS* gene family, designated *HvUXS1* to *HvUXS4,* have been cloned and characterized. Quantitative PCR shows that the *HvUXS1* mRNA is most abundant among the four *HvUXS* genes, accounting for more than 80% of total *HvUXS* transcripts in most tissues. There is a sharp decrease in the *HvUXS* transcript levels in 13 DPA grains compared with 3 DPA grains, indicating a decrease in biosynthesis of UDP-D-xylose. UDP-D-Xylose epimerase (UXE) catalyzes the reversible interconversion of UDP-D-xylose and UDP-L-arabinose. Three members of the barley *HvUXE* gene family, named *HvUXE1* to *HvUXE3*, have been cloned and characterized. All three members contain transmembrane motifs, implying a subcellular organelle location. The mRNA levels of all three *HvUXE* genes increase substantially in 13 DPA grains compared with three DPA grains. However, it has not been

determined whether there is an increase in arabinose/xylose ratio during the grain development. Our work has also shown that transcript levels and activities of sugar nucleotide interconverting enzymes are correlated with barley cell wall polysaccharide compositions in different tissues. Studies of sugar nucleotide interconversion pathways provide opportunities to enhance the quality of barley grain and the digestibility of vegetative tissues.

Key Words: Arabinoxylans, barley grain quality, nucleotide sugars, plant cell wall, polysaccharides

Introduction

Compositions of barley cell wall polysaccharides play a critical role in determining barley grain quality. The two most important cell wall polysaccharides are (1->3, 1->4)-β-D-glucans and arabinoxylans. A high level of (1->3, 1->4)-β-D-glucan and/or arabinoxylan can cause an increase in wort viscosity, resulting in filtration difficulties during brewing. Synthesis of barley cell wall polysaccharides requires nucleotide sugars, including UDP-D-glucose, UDP-D-galactose, UDP-D-glucuronate, UDP-D-galacturonate, UDP-D-xylose and UDP-L-arabinose. These nucleotide sugars are synthesized by nucleotide sugar interconversion pathways (Figure 1).

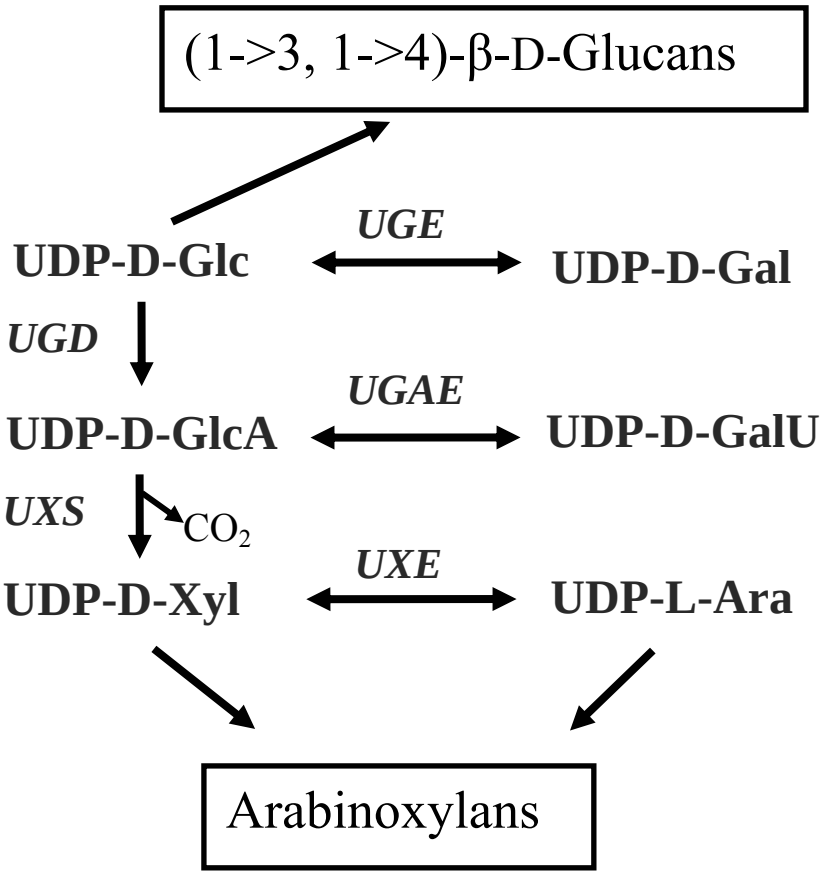

Figure 1. Nucleotide sugar interconversion pathways

UDP-D-Glucose epimerase (UGE) catalyzes the reversible interconversion of UDP-D-glucose and UDP-D-galactose. UDP-D-Glucose is used for synthesis of (1->3, 1->4)-β-D-glucan and other glucose-containing polysaccharides. UDP-D-Glucose dehydrogenase (UGD) catalyzes the formation of UDP-D-glucuronate. UDP-D-Glucuronate decarboxylase (UXS) catalyzes an irreversible reaction from UDP-D-glucuronate to UDP-D-xylose. Thus, UXS may play a critical role in control of the formation of pentose sugars. UDP-D-Galacturonate and UDP-L-arabinose are formed from UDP-D-glucuronate and UDP-D-xylose catalyzed by UDP-D-glucuronate epimerase (UGAE) and UDP-D-xylose epimerase (UXE), respectively.

Regulation of carbon flow to cell wall polysaccharides can be regulated at the transcript, enzyme activity and substrate levels. An understanding of the regulation of biochemical pathways might make it possible to alter the carbon flux through a particular pathway and to direct carbon to specific cell wall polysaccharides that may improve barley malting quality.

In this work, we have cloned members of gene families encoding nucleotide sugar interconversion enzymes. We have examined their expression patterns in developing grains and we have also discussed the effects of these enzymes on carbon flux onto arabinoxylans.

Materials and methods

UDP-D-Glucose, UDP-D-galactose and UDP-D-glucuronic acid were purchased from Sigma-Aldrich Corporation, Australia. The 1 Kb DNA ladder molecular mass standards and the pGEM-T Easy vector system I were from Promega Corporation (Madison, WI, USA) and the Superscript II RNase H Reverse Transcriptase and TRIZOL reagent were from Invitrogen Australia (Victoria, Australia).

cDNA Preparations

Total RNA from developing grains was extracted with the guanidine reagent TRIZOL. Barley tissues (50–100 mg) were homogenized in 500 μl of TRIZOL (Invitrogen), and RNA extracted according to the manufacturer's instructions. Purified RNA was treated with DNase (DNA-Free™, Ambion, Austin, TX, USA). The quality and quantity of RNA were assessed in an agarose gel (1.6% w/v) and spectrophotometrically at 280/260 nm, respectively. First strand cDNA was synthesized using 2 μg total RNA and Superscript II reverse transcriptase and used directly for Q-PCR analysis or PCR-based amplification of the *HvUXS*, *HvUGE*, *HvUXE*, *HvUGD* and *HvUGAE* cDNAs.

Quantitative PCR Analysis of Transcript Levels

Transcript levels of barley nucleotide sugar interconversion enzymes were determined by quantitative real time PCR (Q-PCR) in a RG 2000 Rotor-Gene Real Time Thermal Cycler (Corbett Research, Sydney) according to Zhang et al. (2005).

Results and Discussion

Gene families encoding nucleotide sugar interconversion enzymes

Enzymes responsible for nucleotide sugar interconversion belong to a superfamily of dehydrogenases/reductases that contain absolutely conserved GXXGXXG NAD-binding motifs and the amino acids Ser, Tyr and Lys in the catalytic site. Small gene families have been deduced from the genomic DNA sequence of *Arabidopsis*. The members of gene families for major nucleotide sugar interconversion pathways in *Arabidopsis* are listed in Table 1. Some of these genes have been cloned and their functions have been studied (Dormann and Benning, 1996; Burget et al., 2003; Seifert, 2004). In barley, small gene families of nucleotide sugar interconversion enzymes have also identified (Table 2). The gene family encoding UXS has four members (Zhang et al., 2005). One of the members (HvUXS1) lacks a transmembrane motif and is predicted to be a cytosolic enzyme. The other members (HvUXS2 to HvUXS4) all contain transmembrane motifs, as predicted by topology software, and possibly located in the Golgi apparatus. UDP-D-Glucose epimerase (HvUGE) and UDP-D-xylose epimerase (HvUXE) both contain at least three isoforms (Table 2). All HvUGE lack transmembrane motifs and are predicted to function in the cytosol, while all HvUXE have transmembrane motifs and presumably are targeted into cellular organelles.

Expression of nucleotide sugar interconversion genes in developing grains

UXS, which is also known as UDP-D-glucuronate decarboxylase, catalyzes an irreversible reaction from UDP-D-glucuronate to UDP-D-xylose, and hereby generates an important precursor of arabinoxylans. The *HvUXS1* mRNA levels were the highest among the four *HvUXS* members in three DPA grains (Table 3). This gene encodes a cytosolic enzyme, while the rest of the members encode membrane-bound enzymes. The *HvUXS2* mRNA levels were the second highest among the four *HvUXS* members in three DPA grains. There was a decrease in *HvUXS* mRNA levels in 13 DPA grains. The decrease in *HvUXS2* mRNA levels was substantial (70%). This gene encodes a membrane-bound enzyme and its corresponding mRNA is abundant in developing grains. In *Arabidopsis*, the membrane-bound UXS has been demonstrated to be located in Golgi (Pattathil et al., 2005).

HvUXE catalyzes the reversible interconversion of UDP-D-xylose and UDP-L-arabinose, both of which are precursor of arabinoxylans. *HvUXE* mRNA levels were generally lower than the levels of *HvUXS* mRNA in three DPA grains. All members have similar mRNA levels, unlike the *HvUXS* gene family where mRNA levels may differ by 100-fold among the members. There was an increase in mRNA levels of all three *HvUXE* members in 13 DPA grains, implying that there may be more arabinosyl substitution in 13 than in three DPA grains and thus a high ratios of arabinose/xylose in arabinoxylans in 13 DAP grains.

Discussion

The plant cell wall matrix of the Poaceae contains a high level of arabinoxylans and (1→3, 1→4)-β-D-glucan and small amounts of xyloglucans and pectic polysaccharides (Fincher, 1992). The relative levels of arabinoxylans and (1→3, 1→4)-β-D-glucan vary considerably between different barley tissues (Fincher, 1992). Synthesis of arabinoxylans requires the sugar nucleotides UDP-D-xylose and UDP-L-arabinose, together with several biosynthetic enzymes such as HvUXS, xylan synthase and arabinosyl transferase. The synthase and transferase have been assumed to form a complex in the process of synthesizing arabinoxylans (Seifert, 2004). However, it is also logical to assume that the enzymes responsible for generating the biosynthetic substrates may also be arranged in a multi-enzyme complex. These enzymes include HvUXS and HvUXE. Thus, regulation of the activity in one component of the complex may affect the biosynthesis of arabinoxylans. Little is known on the genetic and biochemical properties of xylan synthase and arabinosyltransferase so far. However, progress has been made in the study of HvUXS in barley (Zhang et al. 2005). This study has also shown that *HvUXS* transcript levels decreased and *HvUXE* mRNA levels increased in developing grains. This has implications on the biosynthesis of arabinoxylans in barley developing grains.

A wide range of heterogeneous arabinoxylans has been found in barley. They differ in degree of arabinose content, length of xylan backbone (400 - 500 residues) and in the degree of various substitutions with other residues such as ferulic acid and *p*-coumaric acid (Fincher 1992). Biosynthesis of arabinoxylans presumably occurs in Golgi apparatus, where it is catalyzed by xylan synthase and glycosyl transferases for the extension of xylan backbone and addition of arabinosyl substituents, respectively (Seifert, 2004). Control of carbon flux into either arabinoxylans or other polysaccharides may be regulated by coarse and fine regulatory mechanisms. It has been demonstrated that *HvUXS* mRNA levels differ in different tissues

(Zhang et al. 2005). The tissues with higher *HvUXS* mRNA activities contain a higher level of arabinoxylans (Fincher 1992, Zhang at al. 2005). It has also been demonstrated that the mRNA levels correlate with protein levels and enzyme activity (Zhang et al. 2005). A decrease in *HvUXS* mRNA in 13 DPA grain may indicate a reduction of arabinoxylan content and an increase in *HvUXE* mRNA could mean an increase in arabinose substitution. The fine regulatory mechanism includes the activation or inhibition of an enzyme by a ligand. UXS activity is feed-back inhibited by its product UDP-D-xylose (Zhang et al. 2005). Another regulatory mechanism is by compartmentation. The barley *HvUXS* gene family encodes a cytosolic and three membrane-bound enzymes. The expression levels of the isoforms vary in different tissues and at different developing stages. The biochemical properties are also different in the UXS isoforms from *Arabidopsis* (Pattathil et al., 2005). Thus, the study of genes encoding nucleotide sugar interconversion enzymes helps us understand the process of arabinoxylan biosynthesis in developing grains.

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Table 1 Gene families of nucleotide sugar interconversion pathways in Arabidopsis (Seifert, 2004)

Enzyme family	**Isoform**	**Locus**	**Number of amino acid**	**EST**
UDP-glucose dehydrogenase	UGD1	At5 g39320	480	23
	UGD2	At3 g29369	480	24
	UGD3	At5 g15490	480	16
	UGD4	At1 g26570	481	3
UDP-xylose synthase	UXS1	At3 g53520	433	10
	UXS2	At3 g62830	445	18
	UXS3	At5 g59290	342	9
	UXS4	At2 g47650	443	17
	UXS5	At3 g46440	341	5
UDP-xylose epimerase	UXE1	At1 g30620	419	3
	UXE2	At4 g20460	379	Nf
	UXE3	At2 g34850	385	1
	UXE4	At5 g44480	436	nf
UDP-glucuronate epimerase	GAE1	At4 g30440	429	29
	GAE2	At1 g02000	434	5
	GAE3	At4 g00110	430	6
	GAE4	At2 g45310	437	5
	GAE5	At4 g12250	436	6
	GAE6	At3 g23820	460	51
UDP-glucose epimerase	UGE1	At1 g12780	351	17
	UGE2	At4 g23920	350	4
	UGE3	At1 g63180	351	4
	UGE4	At1 g64440	348	7
	UGE5	At4 g10960	351	1

Table 2 Gene families of nucleotide sugar interconversion pathways in barely

Enzyme family	Isoform	Accession numbers	Number of amino acid	EST
UDP-glucose dehydrogenase	HvUGD	Un-published		
UDP-xylose synthase	HvUXS1	AY677177	348	255
	HvUXS2	AY677178	>400	73
	HvUXS3	AY677179	436	25
	HvUXS4	AY677176	408	19
UDP-xylose epimerase	HvUXE1	Un-published	424	25
	HvUXE2		421	10
	HvUXE3		>400	7
UDP-glucuronate epimerase	HvGAE	Un-published		
UDP-glucose epimerase	HvUGE1	AY943955	352	74
	HvUGE2	AY943956	>353	7
	HvUGE3	AY943954	370	10

Table 3 mRNA levels of nucleotide sugar interconversion genes in developing grains
The mRNA levels (copies /μg total RNA) were determined by quantitative real time PCR. The data are means of 4 replicates.

Genes	3 DPA	13 DPA
	Copies/μg total RNA	
UXS1	10,259,000	8,049,000
UXS2	2,406,000	722,000
UXS3	213,000	303,000
UXS4	744,000	408,000
UXE1	83,000	154,000
UXE2	20,000	24,000
UXE3	75,000	132,000