Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Physiology and Biochemistry 44 (2006) 171–180
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Characterization and expression of Arabidopsis UDP-sugar pyrophosphorylase

L.A. Litterer [a], J.A. Schnurr [b], K.L. Plaisance [a], K.K. Storey [a], J.W. Gronwald [b,*], D.A. Somers [a,c]

[a] *Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, Minnesota 55108, United States*
[b] *USDA-ARS, Plant Science Research, St. Paul, Minnesota 55108, United States*
[c] *Current address: Monsanto Company, Agracetus Campus, Middleton, WI 53562, United States*

Received 28 November 2005
Available online 17 May 2006

Abstract

At5g52560, a homolog of pea (*Pisum sativum*) UDP-sugar pyrophosphorylase (*PsUSP*) was functionally annotated by expression in *Escherichia coli* and subsequent characterization of substrate specificity and kinetic properties. Arabidopsis contains a single *USP* gene (*AtUSP*) and evaluation of gene databases suggests that *USP* is unique to plants. The 69 kDa *AtUSP* gene product exhibited high activity with Glc-1-P, GlcA-1-P and Gal-1-P, but low activity with GlcNAc-1-P, Fuc-1-P, Man-1-P, inositol-1-P or Glc-6-P. AtUSP was activated by magnesium and preferred UTP as co-substrate. Apparent K_m values for GlcA-1-P, Glc-1-P and UTP were 0.13 mM, 0.42 mM and 0.14 mM, respectively. In the reverse direction (pyrophosphorolysis), the apparent K_m values for UDP-GlcA, UDP-Glc and pyrophosphate were 0.56 mM, 0.72 mM and 0.15 mM, respectively. USP enzyme activity (UDP-GlcA → GlcA-1-P) was detected in Arabidopsis tissues with highest activity found in the inflorescence. As determined by semi-quantitative RT-PCR, *AtUSP* transcript is widely expressed with high levels detected in the inflorescence. To evaluate tissue-specific expression of *AtUSP*, histochemical GUS staining of plants transformed with *AtUSPprom:GUS* constructs was performed. In 7-day-old seedlings, GUS staining was detected in cotyledons, trichomes and vascular tissues of the primary root. In the inflorescence of older plants, high levels of GUS staining were detected in cauline leaves, the epidermis of the stem and in pollen. *In silico* analysis of *AtUSP* expression in developing pollen indicates that transcript levels increase as development proceeds from the uninucleate to the tricellular stage. The results suggest that *AtUSP* plays an important role in pollen development in Arabidopsis.

Keywords: Arabidopsis thaliana; Cell wall; Pyrophosphorylase; UDP-glucuronic acid; UDP-glucose; UDP-sugar pyrophosphorylase

1. Introduction

Plant cell walls consist of cellulose, matrix polysaccharides (pectin and hemicellulose), phenolics and glycoproteins [3,8]. Matrix polysaccharides make up more than 60% of the primary cell wall of Arabidopsis leaves [36]. The amount and composition of cell wall matrix polysaccharides influence the quality of food and fiber produced by crop plants. For example, the pectin content of forage cell walls is important in determining nutritional value for ruminants [29]. The tools of molecular biology can be used to develop transgenic plants that exhibit desirable modifications in the amount and composition of cell wall matrix polysaccharides. However, progress in this area is limited by lack of understanding of key enzymes regulating pectin and hemicellulose biosynthesis.

UDP-GlcA is a major precursor for nucleotide sugars (UDP-GalA, UDP-Xyl, UDP-Ara) that are incorporated into pectin and hemicellulose [12]. Despite its importance in matrix polysaccharide biosynthesis, the regulation of UDP-GlcA synthesis is poorly understood. As seen in Fig. 1, UDP-GlcA can be synthesized by two distinct pathways in plants: (1) the nucleotide sugar oxidation (NSO) pathway where UDP-Glc dehydrogenase catalyzes the oxidation of UDP-Glc, or (2) the *myo*-inositol oxidation (MIO) pathway which involves reactions cat-

Abbreviations: Ara, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcA, glucuronic acid; GlcNAc, N-acetylglucosamine; GUS, β-glucuronidase; Man, mannose; PPi, pyrophosphate; UDP, uridine diphosphate; USP, UDP-sugar pyrophosphorylase; Xyl, xylose.

* Corresponding author. Tel.: +1 612 625 8186; fax: +1 651 649 5058.
E-mail address: gronw001@umn.edu (J.W. Gronwald).

0981-9428/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.plaphy.2006.04.004

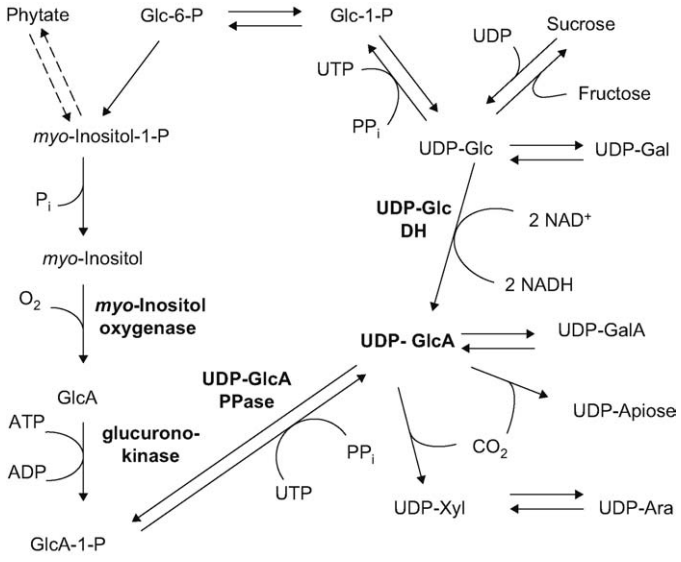

Fig. 1. Nucleotide sugar biosynthesis pathways in plants adapted from Feingold and Avigad [12] showing the *myo*-inositol oxidation and UDP-Glc dehydrogenase pathways for UDP-GlcA synthesis. Abbreviations: Glc, glucose; Gal, galactose; GlcA, glucuronic acid; GalA, galacturonic acid; Xyl, xylose; Ara, arabinose; UDP-Glc DH, UDP-glucose dehydrogenase; UDP-GlcA PPase, UDP-GlcA pyrophosphorylase.

alyzed by *myo*-inositol oxygenase, glucuronokinase and UDP-GlcA pyrophosphorylase (EC 2.7.7.44) [12,23]. UDP-Glc dehydrogenase is present in plants [17,21,33], mammals [32] and prokaryotes [25], and its properties have been well characterized. In contrast, the terminal enzyme of the *myo*-inositol oxidation pathway, UDP-GlcA pyrophosphorylase, is unique to plants and its regulation is poorly understood [23].

Our current knowledge of UDP-GlcA pyrophosphorylase is based on activity measured in partially purified fractions from seedlings of mung bean (*Phaseolus aureus* L.) [13], barley (*Hordeum vulgare* L.), broad bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) [27,28]; maize (*Zea mays* L.) seedlings and endosperm [10]; pollen of lily (*Lilium longiflorum* L.) [10] and common cattail (*Typha latifolia* L.) [18]; and liverwort (*Riella helicophylla* L.) [35]. Because these studies were conducted with semi-purified enzyme fractions, our understanding of the properties of UDP-GlcA pyrophosphorylase is incomplete. Earlier studies indicated that the enzyme has a pH optimum in the range of 8.0 to 9.0, is specific for UTP as co-substrate and requires divalent cations for activity [18,27]. However, in only two reports was cross-reactivity with other monosaccharide-1-P sugars examined. The ammonium sulfate fraction characterized from mung bean exhibited activity with both GlcA-1-P and GalA-1-P [13]. The semi-purified UDP-GlcA pyrophosphorylase from barley seedlings did not exhibit cross-reactivity with Glc-1-P [27]. In addition, there is uncertainty about the molecular wt of UDP-GlcA pyrophosporylase. UDP-GlcA pyrophosphorylase from common cattail pollen has a molecular wt of approximately 71 kDa [18]. In contrast, the enzyme from barley seedlings [27] has a molecular wt of approximately 35 kDa. Thus, both molecular wt and substrate range of UDP-GlcA pyrophosphorylase are uncertain.

Recently, Kotake et al. [22] purified and characterized a broad substrate UDP-sugar pyrophosphorylase (USP) from pea. A comparison of various monosaccharide-1-P substrates indicated that the highest activity occurred with Glc-1-P, GlcA-1-P, Gal-1-P and Ara-1-P. The recombinant PsUSP was also characterized and found to have the same properties as the purified enzyme. Kotake et al. [22] proposed that PsUSP plays an important role in the salvage pathway for the synthesis of nucleotide sugars. In this pathway, monosaccharides released during degradation of glycoconjugates and polysaccharides are recycled back into the UDP-sugar pool. Homologs of Ps*USP* were identified in rice (*Oryza sativa* L.) (AK064009) and Arabidopsis (At5g52560) [22].

The objectives of this study were to: (1) functionally annotate the putative *USP* from Arabidopsis (*AtUSP*), including characterization of kinetic parameters with substrates, and (2) investigate its expression in various tissues of Arabidopsis. Recombinant AtUSP exhibits similarities, but also significant differences compared to recombinant PsUSP [22]. Both transcripts and enzyme activity of *AtUSP* are widely expressed in Arabidopsis. Histochemical GUS staining of inflorescences of plants transformed with a *AtUSPprom:GUS* construct indicated high expression of *AtUSP* in pollen. The possibility that AtUSP serves as UDP-GlcA pyrophosphorylase, the terminal enzyme in the MIO pathway, is discussed.

2. Results

2.1. Functional annotation of AtUSP

Kotake et al. [22] identified At5g52560 as a homolog of the broad substrate UDP-sugar pyrophosphorylase (PsUSP) characterized in pea. PsUSP and the putative Arabidopsis UDP-sugar pyrophosphorylase, AtUSP (At5g52560), share 80% identity (nucleotide) and the predicted proteins share 77% identity and 88% similarity [22]. *AtUSP* has an open reading frame of 1842 bp that encodes a predicted protein of 614 amino acids. Sequence analysis using TargetP [11] predicted that AtUSP is a cytosolic protein because an identifiable target sequence was not found.

To functionally annotate At5g52560, the coding region was cloned by RT-PCR, inserted into pET28, expressed in *Escherichia coli* and purified. His-tag affinity purification of putative AtUSP yielded a single peptide that migrated at approximately 70 kDa on SDS-PAGE (Fig. 2, lane 1). The N-terminal 17 amino acids which included the poly-His motif were cleaved from the fusion peptide using thrombin. The size of the thrombin-cleaved, purified putative AtUSP was 69 kDa, consistent with removal of the His-tag (Fig. 2, lane 2). All enzyme characterization was done with thrombin-treated putative AtUSP to more accurately assess enzyme characteristics *in planta*. The purified enzyme was labile in elution buffer containing 0.5 M imidazole. Most enzyme activity (90%) was lost within 16 h of storage in elution buffer (4 °C). However, desalting into a buffer containing 20% (w/v) sucrose, 50 mM