UDP-sugar pyrophosphorylase – a new old mechanism for sugar activation

Leszek A. Kleczkowski¹*, Daniel Decker¹ and Małgorzata Wilczynska²

¹Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, 90187 Umeå, Sweden; ²Department of Medical Biochemistry, Umeå University, 90187 Umeå

*Corresponding author; e-mail: leszek.kleczkowski@plantphys.umu.se

Abbreviations:  aa, amino acid; AGPase, ADP-glc pyrophosphorylase; ara, arabinose; gal, galactose; galA, galacturonic acid; GALT, gal-1-P uridyltransferase; glc, glucose; glcA, glucuronic acid; man, mannose; PPI, pyrophosphate; SuSy, sucrose synthase; UAGPase, UDP-N-acetylglucosamine pyrophosphorylase; UGE, UDP-glc epimerase; UGPase, UDP-glc pyrophosphorylase; USPase, UDP-sugar pyrophosphorylase; xyl, xylose.
INTRODUCTION

Simple sugars (e.g. glc, gal) are the building blocks of disaccharides (e.g. sucrose) and polysaccharides (e.g. cellulose, starch). To produce di- and polysaccharides, a given simple sugar needs to be “activated”. This activation involves addition of a nucleoside group to a sugar, resulting in the formation of a nucleoside sugar. Such an activated sugar can then be used by a variety of glucosyltransferases to link the sugar residue with an appropriate receptor molecule. The latter can be a carbohydrate, but also a protein, a lipid, or any other molecule which can be glycosylated (Drickamer and Taylor 2006). Among nucleoside sugars, those with uridyl group (UDP-sugars) are most prominent and they serve as precursors to primary metabolites (e.g. sucrose in plants), storage compounds (e.g. glycogen in animals and yeast), structural components (e.g. cellulose - in plants and bacteria; hemicellulose and pectin - in plants), as well as glycoproteins and glycolipids (Feingold and Barber 1990, Kleczkowski et al. 2010). UDP-sugars are the main precursors for biomass production in plants (Kotake et al. 2010).

UDP-sugar pyrophosphorylase (USPase) (EC 2.7.7.64) catalyzes a reversible transfer of the uridyl group from UTP to sugar-1-P, producing UDP-sugar and pyrophosphate (PPi). The enzyme was unequivocally identified and characterized only a few years ago (Kotake et al. 2004), but the USPase-like activities have been reported for at least 50 years now. In those early studies, USPase-like activities were observed in a wide range of organisms, from bacteria to plants to animals, with reports on e.g. “UDP-ara/UDP-xyl pyrophosphorylase(s)” (Ginsburg et al. 1956), “UDP-gal pyrophosphorylase” (Chaco et al. 1972, Lee et al. 1978, Smart and Pharr 1981, Studer-Feusi et al. 1999), “UDP-glcA pyrophosphorylase” (Hondo et al. 1983) or “UDP-ara pyrophosphorylase” (Feingold and Barber 1990). The “UDP-gal pyrophosphorylase” activities were especially significant, since they implied an alternative to the Leloir pathway believed to be the major, if not the only, mechanism of gal to glc conversion (Smart and Pharr 1981, Frey 1996). It seems likely now that at least some of those pyrophosphorylase activities correspond to the same protein, namely USPase.

The work on USPase has been hampered by its overlapping specificity with some other pyrophosphorylases. The activity with glc-1-P, which usually represents the most specific substrate for USPase, overlaps with activities of at least three other UTP-dependent pyrophosphorylases, namely UDP-glc pyrophosphorylase (UGPase) (E.C. 2.7.7.9) which exists as distinct UGPase-A and UGPase-B types, and UDP-N-acetyl-glucosamine pyrophosphorylase (UAGPase) (E.C. 2.7.7.23), known in animals as AGX. These proteins share low or very low identity at the amino acid (aa) level (below 20%) (Kleczkowski et al. 2010).
Phylogenetic analyses revealed that USPases, UGPases (either A- or B-type) and UAGPases should be all categorized into distinct groups (Geisler et al. 2004, Litterer et al. 2006a,b, Okazaki et al. 2009, Kleczkowski et al. 2010).

In the present review, we focus mainly on recent studies on USPase, with emphasis on its substrate specificity, structure, and role in metabolism. Recent work with purified USPases (Kotake et al. 2004, 2007, Litterer et al. 2006a,b, Dai et al. 2006, Gronwald et al. 2008, Damerow et al. 2010, Yang and Bar-Peled 2010), studies on transgenic plants with altered USPase content (Schnurr et al. 2006, Kotake et al. 2007), and resolution of the crystal structure of protozoan USPase (Dickmanns et al. 2011) have reignited the interest in USPase and provided new clues as to the possible roles of this multi-substrate-utilizing enzyme.

**EVOLUTION OF USPASE AND GENE ORGANIZATION**

In Fig. 1, we present a comprehensive phylogenetic tree for USPases, based on aa sequence identity of the derived proteins. Most literature data on USPases concern proteins from plants and protozoans (Leishmania and Trypanosoma), and there is usually about 30-35% identity between USPases from these two groups. In most cases, genomes contain a single gene (USP) for USPase. The only exception so far is genome of Physcomitrella, a moss, which has three putative USP genes. One of those genes encodes a protein which has about 60% identity to Arabidopsis USPase (31% to Leishmania USPase), whereas proteins derived from two other genes have 40 and 42% identity to the plant protein. USPase proteins from different plants share at least 60% identity at their aa sequences. Surprisingly, based on aa sequence comparisons, we have also found putative bacterial USPases (from Lentisphaera and Coralio species) with at least 38% and 29% identity to corresponding proteins from Arabidopsis and Leishmania, respectively. This fits earlier reports (Lee et al. 1978, 1980) on a bacterial pyrophosphorylase capable of pyrophosphorolysis of both UDP-glc and UDP-gal.

In the phylogenetic tree (Fig. 1), the lowest identity was for Volvox USPase (24 and 31% identity to the Leishmania and Arabidopsis proteins, respectively). It is quite possible that USPases with even lower identities exist, but at the moment, based on analyses of protein sequences derived from cDNAs and/or genomic clones, we cannot distinguish them from possible UGPases, UAGPases, or other related proteins. It is also possible that there are USPases which evolved independently in some lineages (no aa identity at all to “common” USPases), as is the case e.g. for bacterial UGPases which are not related by their aa sequence to plant or animal UGPase, but carry out the same reaction (Kleczkowski et al. 2004). For
instance, based on assays of extracts from skin fibroblasts, humans may contain separate “UDP-gal pyrophosphorylase” and UGPase proteins (Chaco et al. 1972), the former possibly analogous to USPase. However, using plant and *Leishmania* USPase aa sequences as references, we found no USPase-like protein(s) coded by human genome nor in any other mammalian/animal cDNA/genome databases.

Plant *USP* genes contain large number of introns (e.g. 17 for *Arabidopsis USP*), similar to other genes involved in UDP-gluc synthesis (Kleczkowski et al. 2010). The junctions between introns and exons are usually conserved, but the lengths of some introns differ for *USP* from different plant species. On the other hand, *Leishmania USP* gene (*LmjF17.1160*) has no introns, probably reflecting a general intron scarcity in the parasite genome (Lynn and McMaster 2008).

**GENE EXPRESSION**

Using transgenic *Arabidopsis* plants with reporter gene linked to *USP* promotor, the gene was found to be expressed throughout at various tissues and organs, suggesting a house-keeping function for USPase in nucleotide sugar metabolism (Litterer et al. 2006b, Kotake et al. 2007). The most predominant expression was in cauline leaves, vascular tissues, stem, epidermis, flowers and, especially, pollen (Litterer et al. 2006b, Schnurr et al. 2006, Kotake et al. 2007). The *USP* gene was strongly upregulated in *Arabidopsis* mutants deficient in UGPase-A activity, probably reflecting a compensatory mechanism (Meng et al. 2009b).

Analyses of microarray databases, using resources at [http://www.bar.utoronto.ca/](http://www.bar.utoronto.ca/) and [http://www.popgenie.org/](http://www.popgenie.org/) confirmed the strong expression of *USP* in the pollen of *Arabidopsis* and, generally, in flowers of different species. For instance, in aspen (a tree), *USP* was strongly expressed in both female and male flowers as well as in developing xylem and senescing leaves, but its expression was low or very low in old roots, mature leaves and in early stages of programmed cell death in wood fibers. Coexpression analyses of *USP* in relation to other genes involved in UDP-gluc synthesis/metabolism (genes coding for UGPases and sucrose synthase, SuSy) in *Arabidopsis* revealed that *USP* significantly coexpressed with genes coding for SuSy-1 and UGPase-B (Kleczkowski et al. 2010). Coexpression with a SuSy gene can, perhaps, be explained by the fact that the two enzymes, at least theoretically, may work in concert, i.e. USPase using UDP-gluc produced by SuSy during sucrose hydrolysis. The involvement of the reverse USPase reaction, using UDP-sugar and PPI as substrates, would be
expected under energy demanding conditions (e.g. anoxia) when carbon is metabolized via PPI-dependent energy transfer (Igamberdiev and Kleczkowski, 2009, 2011).

ENZYMATIC PROPERTIES AND SUBSTRATE SPECIFICITY

USPase, generally, has broad substrate specificity, efficiently using a variety of sugar-1-phosphates with UTP (forward reaction) and the corresponding UDP-sugars with PPI (reverse reaction) as substrates. The sugar-1-P substrates include glc-1-P, gal-1-P, glcA-1-P, xyl-1-P and ara-1-P (Fig. 2). On the other hand, several other enzymes that are also involved in UDP-sugar formation (Fig. 2) are usually specific for a given substrate/product. The ability to produce a variety of UDP-sugars places USPase at the very centre of mechanisms that provide UDP-sugars for glycosylation reactions.

The USPase reaction is freely reversible, with slight preference for the pyrophosphorolytic direction, with the $K_{eq}$ value of 0.2, as determined for purified pea USPase (Kotake et al. 2004). A $K_{eq}$ of 0.5 was obtained for pollen “UDP-glcA pyrophosphorylase” from *Typha latifolia* (Hondo et al. 1983), an enzyme likely corresponding to USPase. Similar $K_{eq}$ values were obtained for other pyrophosphorylases, e.g. AGPase and UGPase-A (Kleczkowski 2000, Meng et al. 2008), suggesting that $K_{eq}$ values are relatively constant across the pyrophosphorylase family of enzymes, even though the proteins share little or no homology at the aa level. The catalytic activity of USPase is initiated by binding of UTP or UDP-sugar prior to the binding of sugar-1-P or PPI, respectively, as shown both for pea and *Leishmania* USPases (Kotake et al. 2004, Damerow et al. 2010). Binding of the first substrate results in a conformational change to accommodate the second substrate. This so called Ordered Bi Bi mechanism has been demonstrated also for unrelated pyrophosphorylases (Elling 1996, Kleczkowski 2000).

In Fig. 3, we present relative UTP- and sugar-1-P-dependent activities of purified USPases from a variety of species. In those experiments, USPases were either purified from plant extracts (pea), or overexpressed in *E.coli* and purified as recombinant proteins (for *Arabidopsis*, soybean, *Leishmania* and *Trypanosoma* enzymes). In most cases, the activities with glc-1-P, gal-1-P and glcA-1-P were higher than with xyl-1-P and ara-1-P. On the other hand, the enzyme had low (below 7%) or no activity with galA-1-P, man-1-P, N-acetylglcA-1-P, fucose-1-P, inositol-1-P and gluc-6-P (Kotake et al. 2004, 2007, Litterer et al. 2006a,b, Damerow et al. 2010). For all USPases, the $K_{m}$ values for UTP were low (0.03-0.19 mM), regardless of the nature of the second substrate, whereas $K_{m}$ values for sugar-1-P were in the
range of 0.13-2.54 mM (Suppl. Table 1). With some exceptions, USPase had lower Km values
for glc-1-P, gal-1-P and glcA-1-P than for ara-1-P and xyl-1-P. This suggests that the enzyme
has higher affinity for hexose-1-P than for pentose-1-P as a substrate. In the reverse reaction
(pyrophosphorolysis direction), the reported Km values were in the range of 0.03-0.72 mM and
0.13-1.01 mM for a given UDP-sugar and PPI, respectively (Suppl. Table 1).

Interestingly, a novel plant UGPase (so called UGPase-B), a chloroplastic enzyme
involved in sulfolipids formation, has also been shown to react with gal-1-P, in addition to its
reaction with glc-1-P. However, the gal-1-P-dependent activity was 7-fold lower than that with
glc-1-P (Okazaki et al. 2009), and it is unknown whether the formation of UDP-gal via
UGPase-B occurs in vivo. Since plastid membranes have high content of galactolipids
(Kobayashi et al. 2007), which are rare in other types of cell membranes, it is tempting to
speculate that UDP-gal required for the galactolipid synthesis is indeed produced by UGPase-
B. The enzyme has about 22% identity to plant USPase, and it does not occur in animals
(Okazaki et al. 2009, Kleczkowski et al. 2010).

No major regulatory mechanisms controlling USPase activity have been described.
USPase is probably regulated simply by substrate availability (Kleczkowski et al. 2010), and
having activity appropriate for the given sugar-1-P serving as substrate (Fig. 3). Studies with
purified soybean and Arabidopsis USPases using products and alternative substrates/products
as possible inhibitors, have revealed relatively small inhibition (Litterer et al. 2006a,b, Schnurr
et al. 2006, Gronwald et al. 2008).

IS USPASE PRESENT IN ANIMALS?

It seems surprising that, based on aa sequence comparisons (Fig. 1), animals have no USPase,
which otherwise exists in bacteria, plants and protozoans. One of the reasons could be the
presence of the Leloir pathway enzymes that, in animals, convert gal to UDP-glc. This
mechanism exists also in plants (Main et al. 1983, Studer-Feusi et al. 1999), but its activity is
low, or scarce, depending on plant organ/ tissue. In the Leloir pathway, the conversion from
gal to UDP-glc occurs via three enzymes: gal kinase (EC 2.7.1.6), gal-1-P uridyltransferase
(GALT) (EC 2.7.7.12) and UDP-glc epimerase (UGE) (EC 5.1.3.2) (Frey 1996) (see also Fig.
2). GALT is central to the Leloir pathway and carries out the reaction of: gal-1-P + UDP-glc
<----> glc-1-P + UDP-gal. In humans, genetic deficiency of the enzyme results in inability to
metabolize gal, and causes the disease galactosemia (Wang et al. 1998).
In mice, knockouts lacking the GALT protein allowed to identify “UDP-gal pyrophosphorylase” as an alternative route of conversion of gal-1-P to UDP-gal (Wehrli et al. 2007). This activity may belong to a yet unknown mice USPase or, more likely, to UGPase-A that has a small residual activity with some UDP-sugars, in addition to its main activity with UDP-glc. Purified human liver UGPase-A, in contrast to plant UGPase-A (Meng et al. 2008), was reported to react with several UDP-sugars, but the respective activities with UDP-gal, UDP-xyl or UDP-man were only up to 2% of those when UDP-glc served as a substrate (Knop and Hansen 1970). The ratio of activity with UDP-glc and UDP-gal was constant throughout purification of the enzyme, suggesting that human liver does not contain a UDP-gal pyrophosphorylase activity that is separate from UGPase-A (Knop and Hansen 1970). An “UDP-gal pyrophosphorylase” activity reported for liver extracts (Abraham and Howell 1969) likely corresponds to the nonspecific activity of human UGPase-A. Moreover, in yeast mutant lacking GALT and unable to grow on gal in the media, overexpression with human UGPase-A rescued the growth (Lai and Elsas 2000), suggesting that human UGPase-A may fully complement the lack of GALT in yeast. In humans, however, genetic deficiency of the GALT enzyme does result in galactosemia (Wang et al. 1998), indicating that human own UGPase-A is unable to compensate for the loss of GALT during gal metabolism.

**SUBCELLULAR LOCATION**

USPase is most likely localized in the cytosol, but other locations can not be excluded. Detailed analyses of USPase purified from *Arabidopsis* revealed presence of two isoforms slightly differing in molecular masses, possibly arising via posttranslational modification(s) or alternative splicing (Gronwald et al. 2008). Each of these processes can contribute to altered location, as found e.g. for some other enzymes involved in NDP-sugar formation: barley AGPase isoforms targeted to different compartments upon alternative splicing (Kleczkowski 1996) or phosphorylated and non-phosphorylated isoforms of maize SuSy which are cytosolic and plasmalemma-bound, respectively (Hardin et al. 2006, Kleczkowski et al. 2010)

There is also a body of evidence of UDP-glc dependent pyrophosphorylase activity associated with cellular membranes (Becker et al. 1995, Kleczkowski et al. 2004). It is unknown whether this activity belongs to USPase or reflects the involvement of other UDP-glc utilizing/producing pyrophosphorylases, i.e. UGPases or UAGPase. In plants, synthesis of both cellulose and callose occurs via the plasmalemma-bound cellulose synthase and callose synthase complexes, respectively. These enzymes are using UDP-glc as substrate and the presence of a membrane-bound pyrophosphorylase producing UDP-glc would facilitate an
efficient transfer of glic molecule to the cell wall components. Also, since synthesis of hemicelluloses and pectins as well as protein N-glycosylation reactions are occurring in endoplasmic reticulum (ER) and Golgi bodies (Gibeaut 2000), UDP-sugars used in these processes must be produced there or transported from cytosol through the ER and/or Golgi membrane by specific transporters (Bakker et al. 2009, Reyes et al. 2010). A putative membrane association of some USPase activity may be for the sake of providing UDP-sugars directly to UDP-sugar translocators.

**PROTEIN STRUCTURE**

The only USPase to be crystallized is that from *Leishmania* (Dickmanns et al. 2011). This protein has an overall kidney-shaped structure and it is composed of three distinct domains, including large central domain and N- and C-terminal domains (Fig. 4). The central domain contains a classic Rossmann fold (mixed β-sheet surrounded by α-helices) that is characteristic of proteins that use nucleotides as substrates. The β-sheet of Rossmann fold is a structural basis for active site of the enzyme. This general structural blueprint is shared by other UDP-glc-producing pyrophosphorylases (UGPase-A, UGPase-B and UAGPase) (Peneff et al. 2001, Geisler et al. 2004, Roeben et al. 2006, McCoy et al. 2007, Steiner et al. 2007, Maruyama et al. 2007, Okazaki et al. 2009, Kleczkowski et al. 2010, Yang et al. 2010). Differences concern mostly details of the C-terminal domain which, in USPase, is composed of two parts: a distorted β-sheet resembling that in C-terminal domain of human AGX (UAGPase), and a left-handed β-helix that is characteristic of C-terminal domain of eukaryotic UGPase-A. The distorted β-sheet of USPase contains a loop similar to the so called “I-loop” of AGX. For the latter protein, the loop was shown to facilitate formation of an inactive dimer from active monomers (Peneff et al. 2001). Oligomerization as a regulatory mechanism has also been described for plant UGPase-A, but there the molecular determinants of oligomerization differed from those of AGX (Martz et al. 2002, McCoy et al. 2007, Meng et al. 2009a). Whether such a regulatory mechanism exists for USPase is unknown at present.

The active site of USPase resembles that of other pyrophosphorylases, and it is in the form of elongated cavity that is flanked from one side by nucleotide-binding loop (NB-loop) and from the other side by sugar-binding loop (SB-loop) (Fig. 4). Upon substrates’ binding, the NB- and SB-loops move toward the substrates (Dickmanns et al. 2011). This tightens the active center and brings the substrates into a strained conformation which allows catalysis to occur. The active site cavity of USPase is larger than that of UGPase-A or UAGPase, apparently allowing for accommodation of a variety of substrates. The USPase binding sites for C-5 and
C-6 of the sugar substrate are flexible and are less shielded from the environment than the corresponding region of UGPase-A (Dickmanns et al. 2011). This is consistent with USPase using several alternative substrates, in contrast to e.g. plant UGPase-A which is specific for glc-1-P and UDP-glc, depending on direction of the reaction (Meng et al. 2008).

ON THE ROLE OF USPASE

UDP-sugars produced by USPase can be used in a plethora of biochemical reactions carried out by hundreds of specific glycosyltransferases (Geisler-Lee et al. 2006, Yonekura-Sakakibara 2009) (Fig. 5). For instance, UDP-glc can be used in the formation of polysaccharides such as cellulose and callose, but also simple disaccharides, such as sucrose and trehalose, the latter metabolically related to trehalose-6-P which is an essential signalling molecule (Schluepmann et al. 2003). On the other hand, UDP-glc is also produced by both UGPase and SuSy, and those two enzymes are believed to provide the bulk of UDP-glc in plants, based on their specific activities (Kleczkowski et al. 2010). However, the USPase reaction can be the major mechanism for synthesis of several other UDP-sugars, i.e. UDP-gal, UDP-glcA, UDP-xy1 and UDP-ara. All of those activated sugars are used in the formation of pectin and hemicellulose, two of the most abundant biomolecules in nature, and are required for glycosylation of proteins and lipids (Karr 1972, Hayashi and Maclachlan 1984, Drickamer and Taylor 2006). In plants, UDP-gal is also essential for synthesis of raffinose and stachyose, which are derivatives of sucrose containing one and two gal molecules, respectively. Those carbohydrates are the main carbon transporting compounds in the phloem of Cucurbitaceae family of plants (e.g. melon, cucumber) (Dai et al. 2006).

The role of USPase has been most thoroughly studied in Arabidopsis, using both loss-of-function knockouts as well as “antisense” and overexpression strategies. However, the exact physiological role of plant USPase is still obscure, since no homozygous mutants could be produced. This was because the loss-of-function mutation in USP could not be transmitted through the male gametophyte due to pollen sterility (Schnurr et al. 2006, Kotake et al. 2007). The usp pollen lacked pectocellulosic inner layer in the cell wall (Schnurr et al. 2006), and was shrunken and collapsed in shape. It is unknown which particular UDP-sugar deficiency leads to the usp pollen phenotype. In this respect, it is interesting to note that plants deficient in UGPase-A activity were also male-sterile (Chen et al. 2007, Mu et al. 2009, Park et al. 2010) or producing less seeds (Meng et al. 2009b). Thus, both UGPase-A and USPase are essential in reproductive processes. Since UGPase-A carries out only UDP-glc synthesis (Meng et al.
and assuming that UGPase-A is active in the *usp* pollen, it appears that UDP-sugars in general, not just UDP-glc, are essential for proper functioning of the pollen.

“Antisense” inhibition of *USP* expression in *Arabidopsis* led to a 75% decrease of USPase activity, whereas overexpression of *USP* resulted in an up to 2.5-fold increase of USPase activity in transgenic plants (Kotake et al. 2007). However, neither the “antisense” nor overexpression strategies led to any change in phenotype in transgenic plants, suggesting that USPase is not rate-limiting in plant growth/development. In plants, USPase was proposed to be involved in myo-inositol oxidation pathway, with UDP-glcA as an intermediate (Gronwald et al. 2008) and in recycling of monosaccharides released from cell walls during rapid cell growth and cell division (Kotake et al. 2004, 2007, 2010). On the other hand, there are also other ways of making UDP-sugars, catalyzed by distinct enzymes (Johansson et al. 2002, Suzuki et al. 2003, Kotake et al. 2010) (Fig. 2), and they may compensate for USPase deficiency.

In *Leishmania*, USPase is a major mechanism to produce UDP-gal which is essential for synthesis of several glycoconjugates. This was indirectly, but elegantly, demonstrated (Lamerz et al. 2010) by targeted deleting of UGPase-A gene, depriving the parasite of UDP-glc which otherwise can be used to produce UDP-gal via UGE (Fig. 2). The *ugp* mutant was not affected in the content of abundant gal-containing glycoinositolphospholipids suggesting that synthesis of UDP-gal in *Leishmania* is independent of UDP-glc supply. USPase was recently found in *Trypanosoma cruzi* (Yang and Bar-Peled 2010), but not in *T. brucei*, possibly because of diverse nature of glycan structures and glycoconjugate composition in the trypanosomatid parasites (Damerow et al. 2010, Yang and Bar-Peled 2010).

**PERSPECTIVES**

Despite USPase involvement in the production of variety of UDP-sugars, not much is known on how essential this process is. There are other enzymes contributing to synthesis of UDP-sugars (Fig. 2) and they may substitute for USPase reaction. Also, the exact role of plant USPase is difficult to study because deletion of the *USP* gene results in male sterility, and it has not been possible to produce homozygous mutants (Litterer et al. 2006b, Kotake et al. 2007). This perhaps can be overcome by using an inducible expression system (Zuo and Chua 2000), where *USP*-gene in an inducible construct (in the background of heterozygous *usp/USP* mutant) is induced during the reproductive stage. This should facilitate the formation of viable *usp* pollens and, subsequently, the production of homozygous mutants...
which would be crucial to study USPase role in vivo. Also, a recent study on male-sterile UGPase-A knockout plants has suggested that male sterility can be circumvented by UDP-glc supplementation to growth media (Park et al. 2010). Assuming that uptake of other nucleotide-sugars occurs in vivo, this might prove to be an effective approach to produce homozygous USPase mutants, and might also reveal which of UDP-sugar(s) produced by the enzyme may overcome the reported male sterility of the usp/USP plants.

Currently, no USPase inhibitors are known. Since USPase is not present in animals, but occurs in Leishmania or Trypanosoma which are parasites killing tens of thousands of people every year (Yang and Bar-Peled 2010, Dickmanns et al. 2011), such an inhibitor could be used as pharmacological means of neutralizing the parasite. Search for suitable inhibitors may involve high throughput screening of chemical libraries (Kaiser et al. 2008) or the so called structure-based inhibitor design strategy, based on architecture of active site of a protein (von Itzstein 2008). The latter approach may be most promising given that the protozoan USPase structure has already been solved (Dickmanns et al. 2011). Besides pharmacological applications, specific inhibitors of USPase could be essential to distinguish, for instance, between UDP-glc-producing activity of USPase from those of UGPases and UAGPases in crude extracts or partially purified preparations. The extent of sensitivity to a given inhibitor may represent a distinctive feature of USPase from a given species (Kleczkowski 1994).

The crystallization of Leishmania USPase (Dickmanns et al. 2011) has become a milestone in our understanding of function/structure properties of this protein. However, given that the protozoan USPase has at most 35% identity to corresponding proteins from other species, those USPases also need to be crystallized in order to have detailed understanding of their structures. This especially concerns details of the active site which, for different USPases, can accommodate distinct substrates with differing Km values and differing specific activities (Fig. 3, Suppl. Table 1). On the other hand, the structure of Leishmania protein can already provide a blueprint for studies on other USPases, where the role of critical aa groups can be experimentally tested, e.g. by site-directed mutagenesis approaches. Similar approaches with respect to barley UGPase-A revealed regions crucial for (de)oligomerization and those affecting substrate binding (Meng et al. 2009a).

ACKNOWLEDGEMENTS

This work was supported (to L.A.K.) by The Swedish Research Council.
LITERATURE CITED


Chaco CM, McCrone L, Nadler HL (1972) Uridine diphosphoglucose pyrophosphorylase and uridine diphosphogalactose pyrophosphorylase in human skin fibroblasts derived from normal and galactosemic individuals. Biochim Biophys Acta 268: 113-120


Drickamer K, Taylor ME (2006) Introduction to Glycobiology (2nd ed.). Oxford University Press, USA


Karr AL (1972) Isolation of an enzyme system which will catalyze the glycosylation of extensin. Plant Physiol 50: 275-282


Reyes F, León G, Donoso M, Brandizzi F, Weber AP, Orellana A (2010) The nucleotide sugar transporters AtUTr1 and AtUTr3 are required for the incorporation of UDP-glucose into the endoplasmic reticulum and are essential for pollen development and needed for embryo sac progress in Arabidopsis thaliana. Plant J 61: 423-435


Legends to Figures

**Fig. 1.** Evolutionary tree of USPase proteins. Publicly available ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used to analyze aa sequences acquired at NCBI, and the resulting tree subsequently visualized using Treeview v.1.6.6 and Adobe Photoshop CS3 Extended. The bar represents 0.1 substitutions per aa.

**Fig. 2.** The central role of USPase in UDP-sugar production. Green boxes represent products of USPase, whereas gray boxes refer to other enzymes producing UDP-sugars. GALT, gal-1-P uridylyltransferase; MUR4, UDP-xyl 4-epimerase; SuSy, sucrose synthase; UAGPase (AGX), UDP-N-acetylglucosamine pyrophosphorylase; UDPG-DH, UDP-gluc dehydrogenase; UGDase, UDP-galacturonate decarboxylase; UGE, UDP-gluc epimerase; UGPase, UDP-gluc pyrophosphorylase.

**Fig. 3.** Relative UTP-dependent activities with different sugar-1-phosphates of purified USPases from pea (Kotake et al. 2004), Arabidopsis A (Litterer et al. 2006b), Arabidopsis B (Kotake et al. 2007), soybean (Litterer et al. 2006a), Leishmania major (Damerow et al. 2010) and Trypanosoma cruzi (Yang and Bar-Peled 2010). Only activities with substrates showing over 7% activity in comparison to that with the most active substrate are shown.

**Fig. 4.** Overall structure of USPase from Leishmania major. We show the structure as a ribbon model, with the central, N- and C-terminal domains marked. Substrate (UDP-gluc) is shown in cyan, NB- and SB-loops that interact with nucleotide and sugar part of the substrate (respectively) are shown in green and red. The loop (“I”) located at C-terminal domain corresponds to the I-loop in human AGX and is shown in pink. The model is based on X-ray structure with pdb code 3OH4 (Dickmanns et al. 2011), and prepared using VMD v1.8.7 software.

**Fig. 5.** The role of products of enzymatic reaction of USPase. Green boxes represent products of USPase reaction.