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Substrate kinetics and substrate effects on the quaternary structure of barley UDP-glucose pyrophosphorylase

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ABSTRACT

UDP-Glc pyrophosphorylase (UGPase) is an essential enzyme responsible for production of UDP-Glc, which is used in hundreds of glycosylation reactions involving addition of Glc to a variety of compounds. In this study, barley UGPase was characterized with respect to effects of its substrates on activity and quaternary structure of the protein. Its K_m values with Glc-1-P and UTP were 0.33 and 0.25 mM, respectively. Besides using Glc-1-P as a substrate, the enzyme had also considerable activity with Gal-1-P; however, the K_m for Gal-1-P was very high (>10 mM), rendering this reaction unlikely under physiological conditions. UGPase had a relatively broad pH optimum of 6.5–8.5, regardless of the direction of reaction. The enzyme equilibrium constant was 0.4, suggesting slight preference for the Glc-1-P synthesis direction of the reaction. The quaternary structure of the enzyme, studied by Gas-phase Electrophoretic Mobility Macromolecule Analysis (GEMMA), was affected by addition of either single or both substrates in either direction of the reaction, resulting in a shift from UGPase dimers toward monomers, the active form of the enzyme. The substrate-induced changes in quaternary structure of the enzyme may have a regulatory role to assure maximal activity. Kinetics and factors affecting the oligomerization status of UGPase are discussed.

1. Introduction

UDP-Glc pyrophosphorylase (UGPase) produces UDP-Glc, a key metabolite used in a variety of glycosylation reactions. Those include synthesis of sucrose, polysaccharides (e.g. cellulose, hemicellulose), glycoproteins, glycolipids and sulfolipids as well as myriads of glycosylated secondary metabolites (Feingold and Barber, 1990; Geisler-Lee et al., 2006; Yonekura-Sakakibara, 2009). UGPase is essential for plant survival, as plants lacking or deficient in UGPase activity are male-sterile or produce less seeds (Chen et al., 2007; Meng et al., 2009b; Mu et al., 2009; Kleczkowski et al., 2010).

There are two types of UGPases, sharing little (ca. 11%) identity at the amino acid (aa) level: a mostly cytosolic UGPase-A (Kimura et al., 1992; Kleczkowski, 1994; Kleczkowski et al., 2010) and strictly plastidial UGPase-B (Okazaki et al., 2009). The barley (*Hordeum vulgare*) UGPase discussed in the present paper belongs to the UGPase-A family (Kleczkowski et al., 2010, 2011b). UGPase-A from *Arabidopsis* has already been crystallized (McCoy et al., 2007), whereas the barley protein structure has been extensively probed by site-directed mutagenesis and domain deletion analyses to elucidate structural determinants of substrate binding, catalysis and dimerization/oligomerization (Martz et al., 2002; Geisler et al., 2004; Meng et al., 2009a). Both barley and Arabidopsis UGPases-A have as much as 83% identity at the aa level and, thus, are likely to share details of their tertiary and quaternary structures (Kleczkowski et al., 2011b).

Changes in the quaternary structure of barley UGPase have been suggested to play a major role for regulation of enzyme activity (Meng et al., 2009a; Kleczkowski et al., 2010). Active monomers have been shown to associate together into inactive dimers and, frequently, higher order oligomers (Martz et al., 2002; Kleczkowski et al., 2005; Meng et al., 2009a). The dimerization/oligomerization process is reversible, with a low energy barrier between the dimeric/oligomeric and monomeric forms, and it modifies the structural environment of the active site, which is

Abbreviations: aa, amino acids; AGPase, ADP-Glc pyrophosphorylase; BSA, bovine serum albumin; Gal, galactose; GALT, Gal-1-P uridyltransferase; GDH, Glc-6-P dehydrogenase; Glc, glucose; PGM, phosphoglucomutase; PP_i, pyrophosphate; SuSy, sucrose synthase; UAGPase, UDP-N-acetylgalactosamine pyrophosphorylase; UGPase, UDP-Glc pyrophosphorylase; USPase, UDP-sugar pyrophosphorylase.

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open in the monomer and occluded at the dimer interface (Martz et al., 2002; Geisler et al., 2004; McCoy et al., 2007; Kleczkowski et al., 2011b). Oligomerization as a regulatory mechanism has also been suggested for prokaryotic UGPases from *Mycobacterium tuberculosis* and *Xanthomonas* spp. (Lai et al., 2008; Bosco et al., 2009). Since bacterial UGPases have no homology at the aa level to their eukaryotic counterparts (Kleczkowski et al., 2004, 2011b), their mechanism of oligomerization may have evolved separately from eukaryotic UGPases.

Despite substantial progress in elucidating kinetic properties and in mapping structure/function properties of plant UGPase, not much is known about its reactivity in the UDP-Glc synthesis direction of the reaction. Most previous studies involved assays of the reverse (pyrophosphorolysis) reaction (e.g. Knop and Hansen, 1970; Martz et al., 2002; Meng et al., 2009a; Coleman et al., 2007). Whereas the pyrophosphorolytic reaction does occur in vivo, especially in sink tissues (Kleczkowski, 1996; Kleczkowski et al., 2004; Chen et al., 2007; Mu et al., 2009; Kotake et al., 2010), the UDP-Glc synthesizing reaction is predominant in photosynthetic tissues, directing carbon flux toward sucrose synthesis (Kleczkowski, 1994; Kleczkowski et al., 2004; Meng et al., 2009b) and underlying the key role of UDP-Glc as a glycosylation substrate. In the present paper, we further characterize barley UGPase, especially with respect to its kinetics in the UDP-Glc synthesis direction. We also present evidence for a substrate-induced shift in the quaternary structure of UGPase, which may have a regulatory role.

2. Results and discussion

2.1. Substrate kinetics with Glc-1-P, Gal-1-P and UTP of barley UGPase

We used double-reciprocal plots to study kinetic characteristics of the UGPase reaction. The assays were carried out at varying concentrations of Glc-1-P or UTP, with the other substrate kept at constant level. Over the range of substrate concentrations applied, the activity plots in Fig. 1 yielded straight lines for both Glc-1-P and UTP. The K_m values determined from the plots were 0.33 and 0.25 mM for Glc-1-P and UTP, respectively (Fig. 1). This should be compared to in vivo concentrations of Glc-1-P and UTP, which are likely in the mM range, especially in sink tissues (Roessner-Tunali et al., 2003). Thus, the enzyme is likely to operate close to near-saturating concentrations of its substrates under physiological conditions.

In preliminary assays, we have found that the enzyme also reacted with Gal-1-P, and the activity, at 0.5 mM Gal-1-P, was about 8-fold lower than that with 0.5 mM Glc-1-P. Similar to Glc-1-P, kinetics with Gal-1-P (as well as with UTP) were linear when either Gal-1-P or UTP were varied (Fig. 2). In the former case, however, the line in Fig. 2A crossed the X-axis very close to Y-axis, and it was impossible to determine the exact K_m value for Gal-1-P. However, we are confident that K_m with Gal-1-P is higher than 10 mM, since activities with 20 mM Gal-1-P were nearly double than those with 10 mM Gal-1-P (data not shown). Even assuming the K_m for Gal-1-P of 10 mM, this would be at least 30-fold higher than that for Glc-1-P (Fig. 1). Gal-1-P is, generally, toxic in vivo (Gross and Schnarrenberger, 1995; Lang and Botstein, 2011) and its physiological levels are likely lower than those of Glc-1-P. In such a case, given that Glc-1-P and Gal-1-P bind to the same site at the active center of UGPase (Kleczkowski et al., 2011b), Glc-1-P would simply outcompete Gal-1-P in accessing the active site.

Plant UGPases that belong to the so called UGPase-A family are believed to be fairly specific for Glc-1-P (Meng et al., 2008; Kleczkowski et al., 2011b). This is in contrast to the so called UDP-sugar pyrophosphorylase (USPase) which shows comparable activity with a variety of sugar-1-phosphates (Kotake et al., 2004; Litterer et al., 2006; Damerow et al., 2010; Dickmanns et al., 2011; Kleczkowski et al., 2011a). For UGPase-A, however,

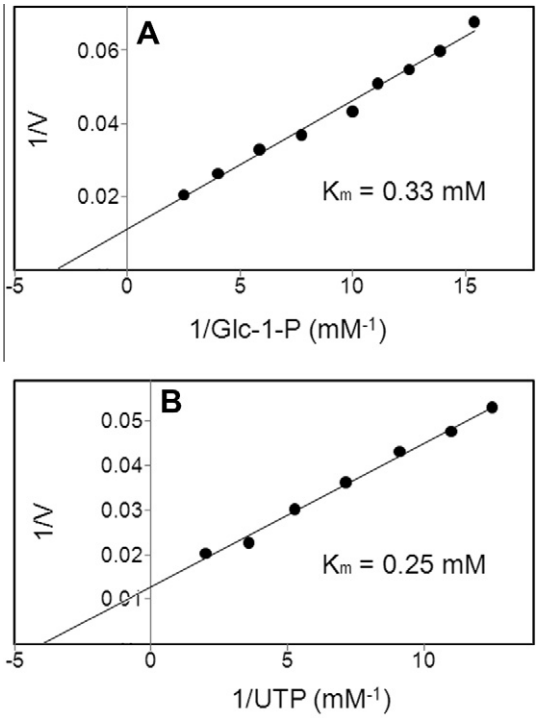

Fig. 1. Kinetics of barley UGPase with Glc-1-P (A) and UTP (B). The kinetics were studied using double reciprocal plots which allowed for estimation of K_m values. Concentrations of Glc-1-P and UTP were varied (0.065–0.4 mM and 0.08–0.5 mM, respectively) and the other substrate was kept at 0.5 mM.

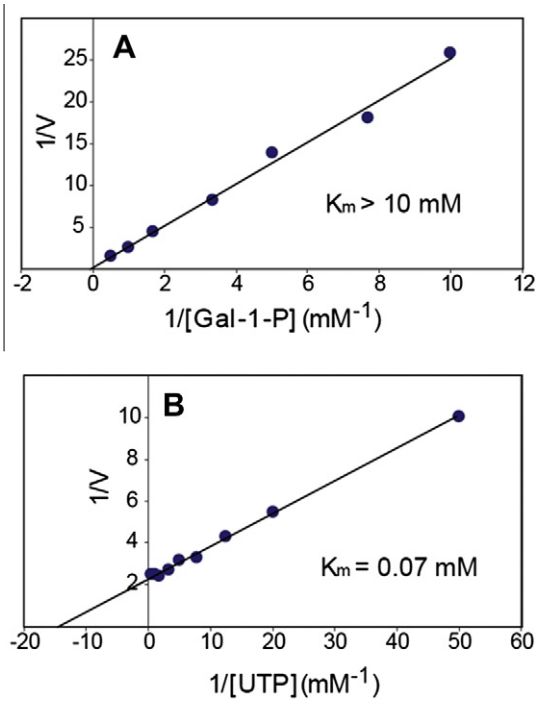

Fig. 2. Kinetics of barley UGPase with Gal-1-P (A) and UTP (B). The kinetics were studied using double reciprocal plots which allowed for estimation of K_m values. Concentrations of Gal-1-P and UTP were varied (0.1–2 mM and 0.02–5.0 mM, respectively) and the other substrate was kept at 1 mM.