Uriddine diphosphate glucose breakdown is mediated by a unique enzyme activated by fructose 2,6-bisphosphate in *Solanum tuberosum* (UDP-glucose phosphorylase/2-phosphoglyceric acid/carbohydrate metabolism/potato)

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**ABSTRACT** In the presence of inorganic phosphate, uridine 5'-diphosphate glucose (UDPG) is specifically hydrolyzed to glucose 1-phosphate and UDP by a unique enzyme, UDPG phosphorylase. The activity of the enzyme was maximally stimulated by fructose 2,6-bisphosphate, a regulatory metabolite recently discovered in both plants and animals, and by 2-phosphoglyceric acid. At 1 μM, fructose 2,6-bisphosphate stimulated UDPG phosphorylisis 10-fold, whereas 2-phosphoglyceric acid was required at higher concentrations (100 μM) to produce a similar effect. Fructose 2,6-bisphosphate appears to increase the affinity of the enzyme for inorganic phosphate, with a change in *Kₐ* from 1.6 mM to 0.3 mM. The results suggest that fructose 2,6-bisphosphate participates in the regulation of other pathways of carbohydrate metabolism in addition to playing its recognized role in glycogen and gluconeogenesis.

Uriddine 5'-diphosphate glucose (UDPG), the most abundant nucleotide sugar in living organisms, plays a key role in glyco- sidic metabolism (for recent reviews, see refs. 1 and 2). Since the discovery of this compound in 1950 by Leloir et al. (3), the involvement of UDPG in cell wall and sucrose metabolism in plants and in glycojen and mucopolysaccharide synthesis in animals (2) has been delineated in transglycosylation and interconversion reactions that use this sugar nucleotide as a central intermediate. The majority of work on UDPG has concentrated on its biosynthesis and metabolism by multiple pathways. The major pathway for sucrose utilization predominately produces this sugar nucleotide; in the presence of UDP and sucrose, sucrose synthase catalyzes a reversible transglycosylation to UDPG and fructose (4, 5). UDPG also serves as a cosubstrate with fructose 6-phosphate for sucrose formation catalyzed by sucrose synthetase (6, 7). UDPG pyrophosphorylase, the major pyrophosphorylase in plants, catalyzes the biosynthesis of UDPG in the presence of UTP and glucose 1-phosphate (Glc-1-P) (8). Several epimerases also utilize UDPG for the production of other sugar nucleotides for cell wall biosynthesis (1).

Although UDPG is such a central metabolite, little information is available on its breakdown. Scattered reports in the literature have demonstrated that nonspecific phosphodiesterases can hydrolyze UDPG to UMP and Glc-1-P (9, 10). UDPG pyrophosphorylase has been implicated in the breakdown of UDPG because this enzyme can catalyze a reversible reaction in the presence of pyrophosphate (11, 12). Because most tissues have high levels of pyrophosphatase activity, however, the physiological significance of the reverse reaction is limited. It has been suggested that a phosphorylase reaction would be a more likely means of catabolizing UDPG (13).

We have explored the latter possibility for the breakdown of UDPG in potato tissue. In this report, we describe an enzyme, UDPG phosphorylase, that specifically catalyzes the breakdown of UDPG in the presence of inorganic phosphate (P₄). This phosphorolysis is greatly stimulated by 2-phosphoglyceric acid (2-PGA) and fructose 2,6-bisphosphate (Fru-2,6-P₂), a recently discovered regulator of phosphofructokinase (14, 15), pyro-phosphate:fructose-6-phosphate phosphotransferase (16), and fructose bisphosphatase (17, 18). The following results are consistent with a role of Fru-2,6-P₂ as a regulatory metabolite in carbohydrate metabolism.

**MATERIALS AND METHODS**

**Plant Material.** Potato tubers (*Solanum tuberosum*; Frito-Lay variety 1207) were grown under standard conditions at Rhinelander, Wisconsin. After harvest, tubers were stored at 13°C until use.

**Reagents.** The following compounds contained d-[U-14C]-glucose moieties: UDPG (327 mCi/mmol; 1 Ci = 3.7 × 10¹² Bq) and Glc-1-P (294 mCi/mmol) from New England Nuclear and adenosine 5'-diphosphate glucose (ADPG) (217 mCi/mmol) from ICN Pharmaceuticals. Glucose-6-phosphate dehydrogenase and phosphoglucomutase were obtained from Sigma. For quantitation of NADPH, Picorase P and decanal were purchased from United Technologies-Packard (Downers Grove, IL). Fru-2,6-P₂ was synthesized as described (19) as well as purchased from Sigma; stability of Fru-2,6-P₂ and activation by it were dependent on obtaining a high-purity preparation. All other chemicals were reagent grade and obtained from commercial sources.

**Preparation of Enzyme Extract.** Slices of tuber tissue (50 g) were frozen in liquid N₂, ground to a fine powder, and homogenized in a Waring Blender with 100 ml of 100 mM H₂BO₃/200 mM KH₂PO₄ containing 10 mg of 2-benzoxazolesulfonothiol, pH 6.2. The homogenate was filtered through Nitex nylon mesh 37, the residue was discarded, and the filtrate was centrifuged for 8 min at 7,000 × g. The supernatant was taken to 75% (NH₄)₂SO₄ saturation, and the pellet from centrifugation at 7,000 × g was obtained. The ammonium sulfate pellet was dissolved in a minimal volume of 50 mM Hepes, pH 6.5, containing 50 mM NaF, 1 mM NaN₄EDTA, and 2 mM dithiothreitol, then applied to a Bio-Gel P-10 column (Bio-Rad) for desalting. Approximately 25 ml was collected and centrifuged at 14,000 × g for 20 min for clarification. All of the above steps were carried out at temperatures below 6°C. All extracts were stored at −10°C.

**Abbreviations:** UDPG, uridine 5'-diphosphate glucose; Fru-2,6-P₂, fructose 2,6-bisphosphate; Glc-1-P, glucose 1-phosphate; 2-PGA, 2-phosphoglyceric acid; 3-PGA, 3-phosphoglyceric acid; ADPG, adenosine 5'-diphosphate glucose; Tricine, N-[tris(hydroxymethyl)methyl]-glycine.

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Enzyme Assays. UDPG phosphorylase was routinely assayed by measuring Glc-1-P release from radiolabeled UDPG. The reaction mixture contained the following in a 150-μl final volume: 10 mM Hepes, 10 mM KCl, at pH 7.5, 1.33 mM dithiothreitol, and 100 pmol of UDP[U-14C]G. After incubation at 20°C for 15 min, 75 μl of HCl-treated activated charcoal (Sigma) at 50 mg/ml was added to precipitate unreduced UDPG. The charcoal suspension was centrifuged, and 112 μl of the supernatant was used for radioactivity measurement. For kinetic measurements, the concentrations of P, and UDPG were varied as indicated.

Glc-1-P release was also measured by using a coupled enzyme system containing 25 mM Hepes/KOH at pH 7.5, with 2.5 mM MgCl₂, 0.1 mM NADP, 0.25 mM dithiothreitol, 0.25 μM glucose-6-phosphate, 0.5 mM EDTA, 0.1 unit of glucose-6-phosphate dehydrogenase, and 0.3 unit of phosphoglucomutase. It was necessary to perform the assay in two steps because Mg²⁺ causes decomposition of the substrate. After incubation at 20°C for 10 min, the amount of NADPH produced was measured by bioluminescence on a Packard luminometer, using Picorase P, 40 μM FMN, and decanal in 0.1 M KPF, pH 6.8, according to kit instructions. Quantitation was by comparison to a NADPH standard curve.

Glc-1-P release from radiolabeled UDPG was additionally detected by incorporation of label into starch by starch phosphorylase. The reaction mixture contained the following in a 200-μl final volume: 40 mM Hepes/KOH at pH 7.5, 0.5% amylose, 0.5 mM KCl, 0.3% dithiothreitol, and endogenous starch phosphorylase. After incubation at 20°C for 15 min, 1 ml of 75% (vol/vol) methanol/1% KCl was added to precipitate starch. After centrifugation, the pellet was washed twice with the methanol/KCl and solubilized in 1 ml of H₂O, and 0.5 ml was taken for measurement of radioactivity.

Analytical Procedures. Protein was estimated by Coomassie blue binding assay (20). Verification of reaction products was carried out by descending paper chromatography on Whatman no. 1 in ethanol/1 M ammonium acetate, pH 3.8, 7.5:3 (vol/vol). Pyrophosphate was measured as pyrophosphomolybdate as described in ref. 21.

RESULTS

Specificity of Reaction. While monitoring for sucrose synthase and sucrose-6-phosphate synthetase activities, we noted that a portion of radiolabeled UDPG was converted to non-charcoal-bound material in the absence of fructose or fructose 6-phosphate. When P₁ was added to the reaction mixture, a greater amount of activity was observed. There was no detectable pyrophosphate in the reaction mixture, and the activity in the extract was dependent on the amount of P₁ present. Because ADPG is hydrolyzed by a specific ADPG phosphorylase in potato tuber (22), we tested whether this activity could also account for UDPG degradation. Under conditions optimal for ADPG phosphorylase, little breakdown of UDPG occurred. The following compounds did not interfere with UDPG phosphorylation at equivalent or higher concentrations: UDP-galactose, ADPG, UDP, or ADP. We, therefore, named this enzyme UDPG phosphorylase.

Activity was greatest at pH 7.25–7.50 with Hepes buffer; when the assay was conducted with N-[tris(hydroxymethyl)]-methyl]glycine (Tricine) buffer, a loss of activity was observed (Fig. 1A).

Verification of Reaction Products. Release of Glc-1-P from UDPG in the presence of P₁ was verified by three independent methods. When the reaction products remaining after charcoal adsorption were separated by paper chromatography, all of the radioactivity in the mixture migrated the same distance as a Glc-1-P standard. When unlabeled UDPG at higher concentrations was used in the assay, the presence of UDP in the reaction mixture was detectable on the chromatogram by UV light and comparison with the migration of a UDP standard.

When the charcoal-adsorbed supernatant was incubated in a starch-synthesizing system in the presence of phosphorylase activity, the radioactivity was incorporated into the starch pellet. The radioactive material incorporated was similar to the amount present when labeled Glc-1-P was used under identical conditions.

The third method employed an enzyme-coupled system monitoring NADPH production in the presence of glucose-6-phosphate dehydrogenase and phosphoglucomutase. When the charcoal-treated product mixture was added, NADPH was formed in direct proportion to the amount of UDPG hydrolyzed.

Kinetics of Reaction. The breakdown of UDPG had an absolute requirement for P. In the absence of activator, the Kₚ for P₁ was approximately 1.6 mM (Fig. 1B). The presence of 1–5 mM dithiothreitol slightly enhanced the observed activity. The Kₚ for UDPG was also in the range of 1.1 mM (Fig. 1C).

Effect of Metabolites on Activity. Several compounds were tested for their effectiveness in stimulating UDPG phosphorylase (Table 1). The most potent activator was Fru-2,6-P₂, which stimulated the activity more than 10-fold at 1 μM. Fructose 1,6-bisphosphate was without effect at equivalent concentrations. Both 2-PGA and 3-PGA also stimulated activity, but 2-PGA was active at much lower concentrations than 3-PGA; only at 2-PGA concentrations above 10 μM was significant activation observed. The reaction products, Glc-1-P and UDP, accumulated in larger amounts in the presence of the activators Fru-2,6-P₂ and 2-PGA than in their absence. Other compounds such as 2,3-diphosphoglyceric acid, 2-phosphoglycolate, and sucrose, each at 0.1 mM, had no effect on UDPG hydrolysis.

A concentration profile of the activation of UDPG by Fru-2,6-P₂ and 2-PGA is shown in Fig. 2. It is apparent that Fru-

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Fig. 1. Activity profile of UDPG phosphorylase. The hydrolysis of UDPG was measured by Glc-1-P release from UDP[U-14C]G in the presence of P₁ at 20°C for 15 min. Except for various parameters indicated, experimental conditions were as given in Materials and Methods. (A) pH profile. (B) Hepes/P₁ buffer; (C) Tricine/P₁ buffer. (B) Lineweaver–Burk analysis of effect of P₁ concentration on activity. The X intercept indicates Kₚ = 1.6 mM for P₁. (C) Lineweaver–Burk plot of effect of UDPG concentration on activity. Kₚ = 1.1 mM for UDPG as indicated by the X intercept.
Table 1. Metabolite activation of UDPG phosphorylase

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Activity, % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100*</td>
</tr>
<tr>
<td>Dihydroxyacetone phosphate, 1 mM</td>
<td>127</td>
</tr>
<tr>
<td>2-PGA, 10 μM</td>
<td>350</td>
</tr>
<tr>
<td>3-PGA, 1 mM</td>
<td>455</td>
</tr>
<tr>
<td>Pyruvate, 1 mM</td>
<td>110</td>
</tr>
<tr>
<td>6-Phosphogluconate, 1 mM</td>
<td>83</td>
</tr>
<tr>
<td>Fru-2,6-P₂, 1 μM</td>
<td>1,200</td>
</tr>
<tr>
<td>Fru-1,6-P₂, 3 μM</td>
<td>99</td>
</tr>
</tbody>
</table>

* Activity in the absence of activation was 106 pmol/min per mg of protein.

2,6-P₂ is a potent activator of UDPG phosphorylase, because this compound is active at 1/1,000th the concentration of 2-PGA. UDPG phosphorylase activity showed a strong requirement for Fru-2,6-P₂ (Kᵣ for Fru-2,6-P₂ = 3.9 nM) (Fig. 2 Inset). This concentration of Fru-2,6-P₂ compares favorably with the binding constant of 12 nM observed with cytoplasmic pyrophosphate: fructose-6-phosphate phosphotransferase from green leaves (18) and the constant of 10 nM for muscle phosphofructokinase (14).

Fru-2,6-P₂ activation of UDPG phosphorylase was observed over a broad range of pH values and substrate concentrations; overall total activity increased at higher pH (Fig. 3). The data indicate that Fru-2,6-P₂ increases the affinity for Pᵢ (Fig. 4), with a slight change in affinity for UDPG (Fig. 5). The addition of Fru-2,6-P₂ at 1 μM decreased the Kᵣ for Pᵢ from 1.6 mM to 0.34 mM, and the Kᵣ for UDPG changed from 1.1 mM to 0.5 mM.

**DISCUSSION**

We have demonstrated a unique enzyme activity in potato tuber tissue, UDPG phosphorylase, that specifically hydrolyzes UDPG in the presence of Pᵢ. Metabolic activation was observed both with Fru-2,6-P₂ and with 2-PGA, although Fru-2,6-P₂ appears to be the more potent regulatory metabolite. As little as 1 μM causes a 10-fold increase in UDPG phosphorylase activity, a concentration that compares favorably with the concentrations required for Fru-2,6-P₂ activation (1 μM) of pyrophosphate: fructose-6-phosphate phosphotransferase (16, 18). We have examined potato tissue for the latter enzyme as well and have observed its activation in the presence of Fru-2,6-P₂. Also, the soluble fructose 1,6-bisphosphatase from potato is inhibited by Fru-2,6-P₂, as demonstrated previously in mammalian systems (17, 23) and in green leaves (18). Thus, the results of the present investigation suggest that metabolites (and pathways) other than those related to glycolysis are regulated by the activator Fru-2,6-P₂.
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FIG. 4. Effect of $P_i$ concentration on the activity of UDPG phosphorylase in the presence of 1 $\mu$M Fru-2,6-P$_2$ (a) or in its absence (d). Except for varied $P_i$ concentrations, assays were performed at pH 7.0 as given in the text.

The reaction product, Glc-1-P, formed from UDPG phosphorolysis, would be channeled into glycolysis or into starch formation, depending on the metabolic state. Because Fru-2,6-P$_2$ appears to stimulate both glycolysis and the breakdown of UDPG, this metabolite would increase the flow of Glc-1-P in the direction of glycolysis. However, as glycolysis is inhibited through feedback loops, 2-PGA (and 3-PGA) would accumulate. When UDPG phosphorylase is activated by 2-PGA, Glc-1-P would continue to be produced, but the reaction product would now be channeled to starch biosynthesis. Previous studies have demonstrated that both 3-PGA and 2-PGA activate ADPG pyrophosphorylase, the rate-limiting enzyme in starch biosynthesis (24, 25). The relative levels of Fru-2,6-P$_2$ and 2-PGA would regulate the production and utilization of Glc-1-P. In the absence of both Fru-2,6-P$_2$ and 2-PGA or in the presence of possible inhibitors of UDPG phosphorylase, UDPG would be available for use in sucrose formation, cell wall biosynthesis, and other reactions using this sugar nucleotide.

The fact that the activity of UDPG phosphorylase had previously been undetected is most probably due to its strong requirement for activators. However, its discovery reinforces the central role in carbohydrate metabolism that is occupied by UDPG and the important role played by regulatory metabolites such as Fru-2,6-P$_2$ and 2-PGA in directing carbon flow. The present investigation opens up the possibility that Fru-2,6-P$_2$ may play a more generalized role in carbohydrate metabolism.


FIG. 5. Effect of UDPG concentrations on activity of UDPG phosphorylase in the presence of 1 $\mu$M Fru-2,6-P$_2$ (a) or in its absence (d). Assays were performed at pH 7.0 as given in the text, but with various UDPG concentrations.