Uridine 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 phosphorolysis 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 K_m 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 glycolysis and gluconeogenesis.

Uridine 5'-diphosphate glucose (UDPG), the most abundant nucleotide sugar in living organisms, plays a key role in glycoside 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 glycogen 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 predominantly 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 phosphate 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 (P_i). This phosphorolysis is greatly stimulated by 2-phosphoglyceric acid (2-PGA) and fructose 2,6-bisphosphate (Fru-2,6-P_2), a recently discovered regulator of phosphofructokinase (14, 15), pyrophosphate:fructose-6-phosphate phosphotransferase (16), and fructose bisphosphatase (17, 18). The following results are consistent with a role of Fru-2,6-P_2 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-^{14}C]-glucose moieties: UDPG (327 mCi/mmol; 1 Ci = 3.7 × 10^{10} 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-P_2 was synthesized as described (19) as well as purchased from Sigma; stability of Fru-2,6-P_2 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 N_2, ground to a fine powder, and homogenized in a Waring Blendor with 100 ml of 100 mM H_3BO_4/200 mM KH_2PO_4 containing 10 mg of 2-benzoxazolethiol, 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% ($NH_4)_2SO_4$ 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 Na_2EDTA, 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-P_2, 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.

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 KP$_i$ at pH 7.0, 1.33 mM dithiothreitol, and 100 pmol of UDP[U-^{14}C]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 unreacted UDPG. The charcoal suspension was centrifuged, and 112 μl of the supernatant was used for radioactivity measurement. For kinetic measurements, the concentrations of P$_i$ 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 MgCl$_2$, 0.1 mM NADP, 0.25 mM dithiothreitol, 0.25 μM glucose 1,6-bisphosphate, 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 Mg^{2+} 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 KP$_i$, 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% amylopectin, 0.5 mM KCl, 0.3 mM 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 H$_2$O, 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 noncharcoal-bound material in the absence of fructose or fructose 6-phosphate. When P$_i$ 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 P$_i$ 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 phosphorolysis, little breakdown of UDPG occurred. The following compounds did not interfere with UDPG phosphorolysis at equivalent or higher concentrations: UDP-galactose, ADPG, UDP, or ADP. We have, 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 P$_i$ 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$_i$. In the absence of activator, the K_m for P$_i$ was approximately 1.6 mM (Fig. 1B). The presence of 1–5 mM dithiothreitol slightly enhanced the observed activity. The K_m 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 phosphorolysis (Table 1). The most potent activator was Fru-2,6-P_2, 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-P_2 and 2-PGA than in their absence. Other compounds such as 2,3-diphosphoglyceric acid, 2-phosphoglycollate, 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-P_2 and 2-PGA is shown in Fig. 2. It is apparent that Fru-
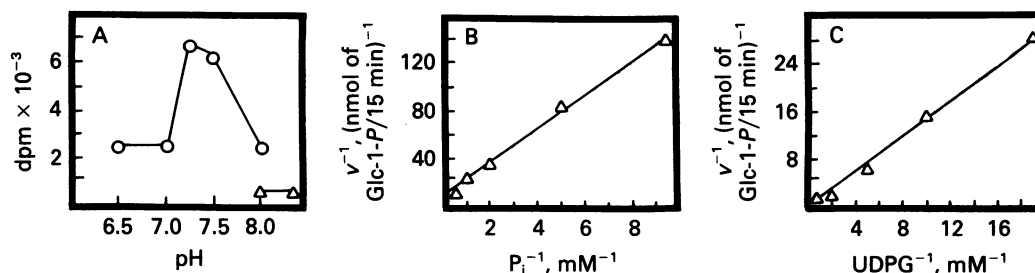

FIG. 1.   Activity profile of UDPG phosphorylase. The hydrolysis of UDPG was measured by Glc-1-P release from UDP[U-^{14}C]G in the presence of P$_i$ at 20°C for 15 min. Except for various parameters indicated, experimental conditions were as given in *Materials and Methods*. (A) pH profile. ○, Hepes/P$_i$ buffer; △, Tricine/P$_i$ buffer. (B) Lineweaver–Burk analysis of effect of P$_i$ concentration on activity. The x intercept indicates K_m = 1.6 mM for P$_i$. (C) Lineweaver–Burk plot of effect of UDPG concentration on activity. K_m = 1.1 mM for UDPG as indicated by the x intercept.

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Table 1. Metabolite activation of UDPG phosphorylase

Metabolite	Activity, % of control
None	100*
Dihydroxyacetone phosphate, 1 mM	127
2-PGA, 10 μM	350
3-PGA, 1 mM	455
Pyruvate, 1 mM	110
6-Phosphogluconate, 1 mM	83
Fru-2,6-P_2, 1 μM	1,200
Fru-1,6-P_2, 3 μM	99

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

2,6-P_2 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-P_2 (K_a for Fru-2,6-P_2 = 3.9 nM) (Fig. 2 *Inset*). This concentration of Fru-2,6-P_2 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-P_2 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-P_2 increases the affinity for P_i (Fig. 4), with a slight change in affinity for UDPG (Fig. 5). The addition of Fru-2,6-P_2 at 1 μM decreased the K_m for P_i from 1.6 mM to 0.34 mM, and the K_m 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 P_i. Metabolic activation was observed both with Fru-2,6-P_2 and with 2-PGA, although Fru-2,6-P_2 appears to be the more potent regulatory metabolite. As little as 1 μM causes a 10-fold increase in UDPG phosphorylase activity, a
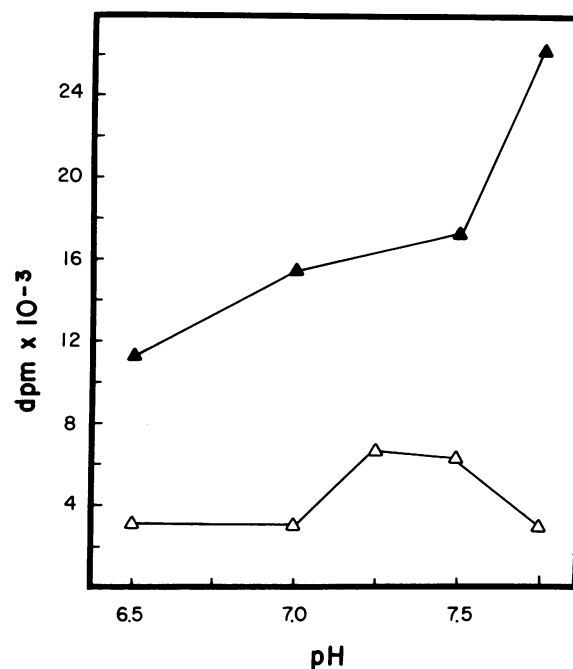

FIG. 3. pH activity profile of UDPG phosphorylase in the presence of 1 μM Fru-2,6-P_2 ($\blacktriangle$) or in its absence ($\triangle$). All assays were performed in 10 mM Hepes/P_i buffer.

concentration that compares favorably with the concentrations required for Fru-2,6-P_2 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-P_2. Also, the soluble fructose 1,6-bisphosphatase from potato is inhibited by Fru-2,6-P_2, 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-P_2.
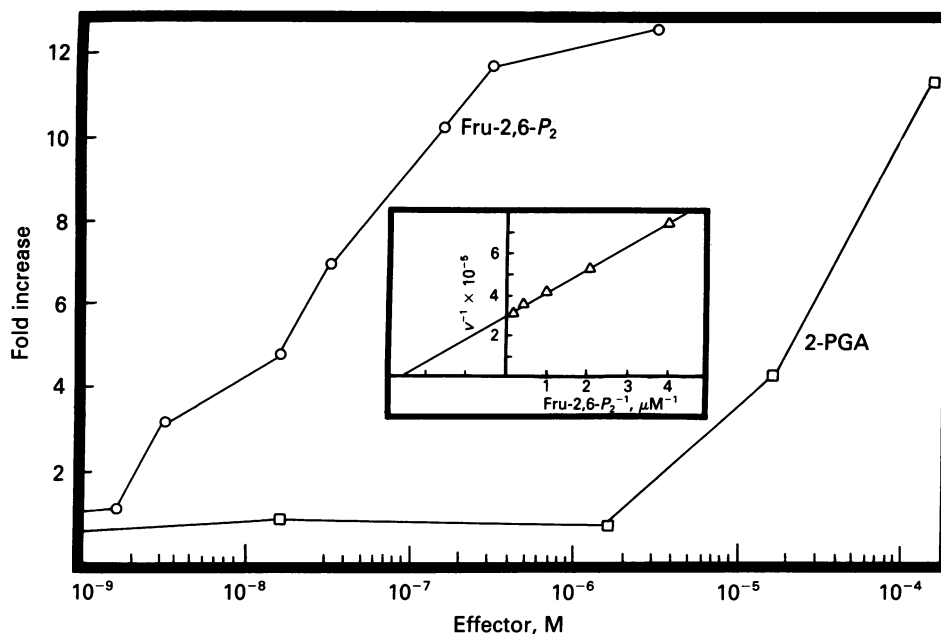

FIG. 2. Effect of concentration of Fru-2,6-P_2, (○) and 2-PGA (□) on the activity of UDPG phosphorylase. Reactions were carried out at pH 7.0 under standard conditions. Activity in the absence of activators was 132 pmol/min per mg of protein. (*Inset*) Double-reciprocal plot of the data for Fru-2,6-P_2. The K_a calculated for Fru-2,6-P_2 is 3.9 nM.
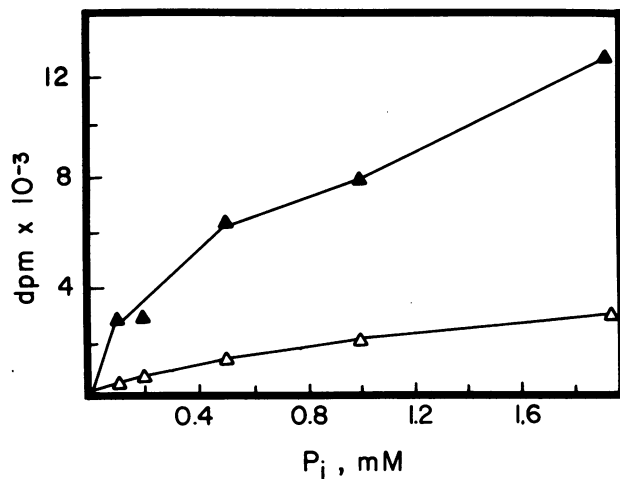
FIG. 4. Effect of P_i concentration on the activity of UDPG phosphorylase in the presence of 1 μM Fru-2,6-P_2 (▲) or in its absence (△). Except for varied P_i concentrations, assays were performed at pH 7.0 as given in the text.

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 stud-
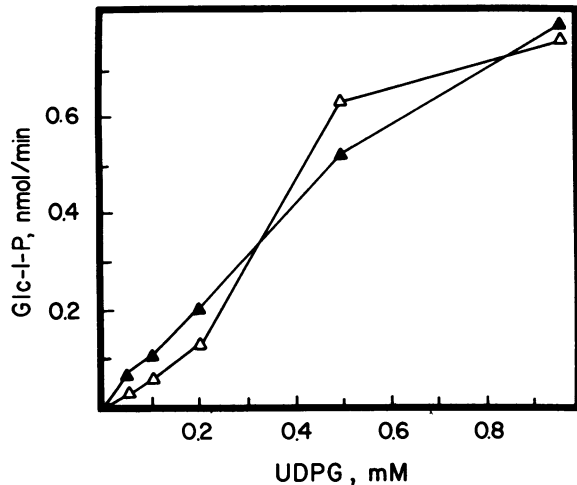

FIG. 5. Effect of UDPG concentrations on activity of UDPG phosphorylase in the presence of 1 μM Fru-2,6-P_2 (▲) or in its absence (△). Assays were performed at pH 7.0 as given in the text, but with various UDPG concentrations.

ies 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.

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