Plant Physiol. (1981) 68, 924–929
0032-0889/81/68/0924/06/$00.50/0

Pyrophosphorylases in *Solanum tuberosum*[1]

II. CATALYTIC PROPERTIES AND REGULATION OF ADP-GLUCOSE AND UDP-GLUCOSE PYROPHOSPHORYLASE ACTIVITIES IN POTATOES

Received for publication December 5, 1980 and in revised form March 25, 1981

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ABSTRACT

Pyrophosphorylytic kinetic constants ($S_{0.5}$, V_{max}) of partially purified UDP-glucose- and ADP-glucose pyrophosphorylases from potato tubers were determined in the presence of various intermediary metabolites. The $S_{0.5}$ of UDP-glucose pyrophosphorylase for UDP-glucose (0.17 millimolar) or pyrophosphate (0.30 millimolar) and the V_{max} were not influenced by high concentrations (2 millimolar) of these substances. The most efficient activator of ADP-glucose pyrophosphorylase was 3-P-glycerate ($A_{0.5} = 4.5 \times 10^{-6}$ molar). The $S_{0.5}$ for ADP-glucose and pyrophosphate was increased 3.5-fold (0.83 to 0.24 millimolar) and 1.8-fold (0.18 to 0.10 millimolar), respectively, with 0.1 millimolar 3-P-glycerate while the V_{max} was increased nearly 4-fold. The magnitude of 3-P-glycerate stimulation was dependent upon the integrity of key sulfhydryl groups (−SH) and pH. Oxidation or blockage of −SH groups resulted in a marked reduction of enzyme activity. Stimulations of 3.1-, 2.9-, 4.8-, and 9.5-fold were observed at pH 7.5, 8.0, 8.5, and 9.0, respectively, in the presence of 3-P-glycerate (2 millimolar). The most potent inhibitor of ADP-glucose pyrophosphorylase was orthophosphate ($I_{0.5} = 8.8 \times 10^{-5}$. molar). This inhibition was reversed with 3-P-glycerate (1.2×10^{-4} molar), resulting in an increased $I_{0.5}$ value of 1.5×10^{-3} molar. Likewise, orthophosphate (7.5×10^{-4} molar) caused a decrease in the activation efficiency of 3-P-glycerate ($A_{0.5}$ from 4.5×10^{-6} molar to 6.7×10^{-5} molar). The significance of 3-P-glycerate activation and orthophosphate inhibition in the regulation of α-glucan biosynthesis in *Solanum tuberosum* is discussed.

The regulatory function of ADP-glucose pyrophosphorylase in controlling α-glucan formation in bacteria and plant tissues has been well documented (16, 17). This enzyme (ATP:α-glucose-1-P adenylyltransferase) catalyzes the reversible reaction seen below.

$$\text{ATP} + \text{glucose-1-P} \leftrightharpoons \text{ADP-glucose} + \text{PPi}$$

ADP-glucose pyrophosphorylase found in the leaves of higher plants (18, 22), green algae (23), blue-green bacteria (12), and several nonchlorophyllous plant tissues (1, 3, 19, 22) are allosterically activated by 3-P-glycerate and inhibited by Pi. The enzyme from maize endosperm has been investigated in the greatest detail of the nonphotosynthetic tissues studied (3, 19). Its catalytic regulation by these allosteric effectors (*i.e.* fine metabolic control), however, is 100- to 150-fold less sensitive than that observed with ADP-glucose pyrophosphorylases from photosynthetic leaf tissues (18, 22). It was suggested that the most important phenomenon in regulating starch biosynthesis in endosperm may be control of synthesis of the starch biosynthetic enzymes (*i.e.* coarse metabolic control) (15, 17). A similar level of control has been suggested for other nonphotosynthetic tissues including pea (25) and wheat grain (26). Previous investigations with potato tubers have also indicated that there was an apparent rise in ADP-glucose pyrophosphorylase activity simultaneous with the onset of rapid starch formation during the procambial cellular division phase accompanying tuberization (24). Adenine diphosphate glucose has been shown to be superior to uridine nucleotide sugar derivatives as a glucosyl donor for particulate and soluble starch synthetases found in potato tubers (4, 5, 8). The synthesis (18) and pyrophosphorolysis (24) of ADP-glucose *via* ADP-glucose pyrophosphorylase in potato extracts is also reported to be stimulated by 3-P-glycerate. Although previous investigators have noted the presence of this enzyme in potato cells (13, 18, 22, 24), little information is available describing its catalytic properties or the nature of its regulation, *in vivo*, by physiological concentrations of intracellular effectors. This paper reports on the interaction between 3-P-glycerate and Pi in regulating the pyrophosphorolysis activity of ADP-glucose pyrophosphorylase in potato tubers. Comparisons relating the sensitivity of this fine metabolic control process to the maize endosperm (3, 19) and plant leaf (18, 22) systems are discussed.

MATERIALS AND METHODS

Chemicals. Glycylglycine, ADP-glucose, UDP-glucose, L-cysteine, DTT, GSH, PCMB[3], AMP, ADP, $NADP^+$, BSA, phosphoglucomutase, glucose-6-P dehydrogenase, Tris, and all metabolites used (*i.e.* 3-P-glycerate, P-enolpyruvate, 2-P-glycerate, etc.) were obtained from Sigma Chemical Co. Glycerol and 2-mercaptoethanol were purchased from J. T. Baker Chemical Co, Phillipsburg, NJ. EDTA and $Na_2P_2O_7$ were obtained from Fisher Scientific Co. All other chemicals used were of analytical grade.[4]

Plant Material and Preparation of Acetone Powders. The potato variety Norchip (*Solanum tuberosum* L.) was used as the source of enzymes during the course of this study. Acetone powders were prepared from immature tubers (200-g average size), as described previously (24). Two hundred g of fresh tissue, selected at random from five healthy tubers, were thinly sliced and immediately frozen with Dry Ice. Each sample was blended at slow speed for three 1-min intervals in a 4-liter explosion-proof Waring

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[3] Abbreviation: PCMB, para-chloromercuribenzoate.

[4] Mention of company or trade names does not imply endorsement by the United States Department of Agriculture over others not named.

Blendor containing 1.5 liters cold acetone (−20 C). The acetone was removed from the resulting slurry by suction filtration through a Büchner funnel containing a Whatman No. 41 filter. The residue was washed five times with 150 ml cold acetone and dried for 4 h in a vacuum desiccator. The dried samples were stored in airtight glass jars at −20 C.

Extraction and Separation of Pyrophosphorylases from Acetone Powders. All steps were conducted at 4 C unless otherwise indicated. Aliquots of acetone powders were added to 50 mM Tris-HCl, 10 mM GSH, 1 mM EDTA buffer (pH 7.5) (10% w/v). For each g of powder, 0.1 ml 10% sodium bisulfite (pH 6) was added. The mixture was stirred slowly for 15 min. The suspension was centrifuged for 20 min at 27,000*g*, and the supernatant was saved. After $(NH_4)_2SO_4$ addition (16.4% w/v), the precipitate was discarded, and $(NH_4)_2SO_4$ (8.6% w/v) was added to the supernatant. The resulting precipitate (30–45% ammonium sulfate fraction) was suspended in a minimum volume of buffer, and $(NH_4)_2SO_4$ (30.2% w/v) was added to the supernatant. Following centrifugation, the final precipitate (45–90% ammonium sulfate fraction) was dissolved in a minimal volume of buffer, and the final supernatant was discarded. The two fractions were dialyzed for 20 h against the same buffer (5 ml of fraction per liter of buffer) with one change at 10 h. If slight cloudiness occurred during dialysis, the inactive protein was removed by centrifugation. The biuret method (7) was used to assay for protein, and crystalline BSA was used as a standard. Fractions were assayed immediately or after short-term holding (1–2 days) at −20 C (UDP-glucose pyrophosphorylase) or 4 C (ADP-glucose pyrophosphorylase).

Measurement of Pyrophosphorylase Activities. Quantitative assays were similar in principle to the procedure described by Munch-Petersen (14). The rate of α-D-glucose-1-P pyrophosphorolysis from either ADP-glucose or UDP-glucose was measured spectrophotometrically in the presence of P-glucomutase, glucose-6-P dehydrogenase, and $NADP^+$. Substrates and coupled assay components were in excess so that the rate of NADPH production was linear in respect to both time and concentration of all diluted pyrophosphorylase extracts used. All assays were run with minus PPi blanks to correct for contaminating NADPH production. Under the experimental conditions used, negligible NADPH was formed by any of the dialyzed potato pyrophosphorylase extracts prior to the addition of PPi. One unit of pyrophosphorylase activity is defined as that amount of enzyme which catalyzes the formation of 1 μmol of glucose-1-P per min. Specific activity is defined as units/mg protein.

Pyrophosphorylytic activities toward ADP-glucose or UDP-glucose were determined with a two-step assay system. This procedure prevented interference of the enzymically-coupled glucose-1-P monitoring system when pyrophosphorylytic assay conditions were varied. In step one, the standard reaction mixture for ADP-glucose pyrophosphorolysis (pH 7.5) contained 2 μmol ADP-glucose, 80 μmol glycylglycine, 5 μmol $MgCl_2$, 10 μmol NaF, 3 μmol DTT, and 0.005 to 0.05 unit of pyrophosphorylase extract (30–45% ammonium sulfate fraction.) After a 10-min incubation at 37 C, the reactions were initiated by the addition of 1.5 μmol PPi to give a total volume of 1 ml. When UDP-glucose pyrophosphorolysis was assayed, the reactions were run at pH 8.0, 1 μmol of UDP-glucose was substituted for ADP-glucose, DTT was omitted, the 45 to 90% ammonium sulfate fraction was used, and the reactions were initiated with 2.5 μmol PPi. Any variation to this standard assay system in relation to pH, substrate concentrations, reaction conditions, or additives (*i.e.* sulfhydryl-group agents and/or metabolic effectors) are indicated in "Results." Glucose-1-P production was terminated by boiling in a water bath for 1 min after an appropriate time interval (0–30 min). The mixture was centrifuged for 10 min at 800*g*, and the supernatant was saved. The precipitate was washed with 0.5 ml water and centrifuged, the two supernatants were combined, and their volume was taken to 1.5 ml with water. Samples were stored at −20 C until assayed.

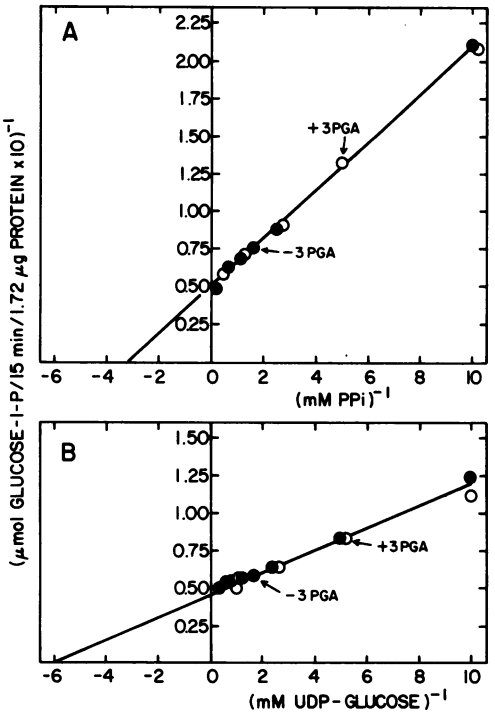

FIG. 1. Double reciprocal plots of UDP-glucose pyrophosphorolysis activity as a function of PPi (A) and UDP-glucose (B) concentrations in the absence (●——●) or presence (○——○) of 1×10^{-4} M 3-P-glycerate. The two-step assay system and other experimental details are described under "Materials and Methods."

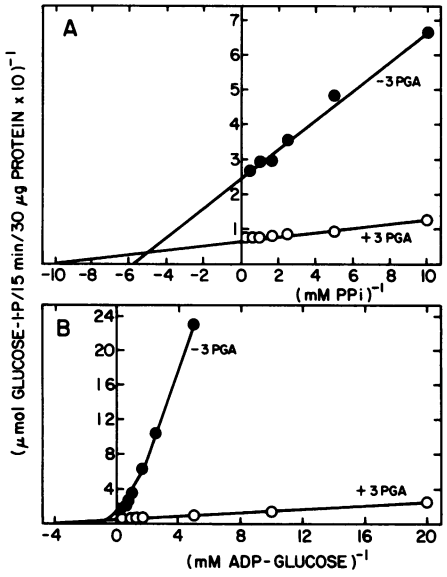

FIG. 2. Double reciprocal plots of ADP-glucose pyrophosphorolysis activity as a function of PPi (A) and ADP-glucose (B) concentrations in the absence (●——●) and presence (○——○) of 1×10^{-4} M 3-P-glycerate. The two-step assay system and other experimental details are described under "Materials and Methods."

In step two, each coupled reaction mixture in a total volume of 1.5 ml (pH 8) contained 80 μmol glycylglycine, 20 μmol cysteine, 0.6 μmol $NADP^+$, 0.02 μmol glucose-1,6-bisP, 2 units of P-glucomutase (12.1 μg), 0.75 unit of glucose-6-P dehydrogenase (3.4 μg), and an aliquot of a step-one supernatant (containing 0.15 μmol or less of glucose-1-P). The reaction was followed at 340 nm until a

Table I. *Kinetic Constants for the Pyrophosphorolysis of UDP-glucose and ADP-glucose*

Partially purified enzyme extracts were prepared from acetone powders of Norchip potatoes. Pyrophosphorolysis activity towards UDP-glucose and ADP-glucose were conducted with the 45 to 90% and 30 to 45% ammonium sulfate fractions, respectively. Concentration of 3-P-glycerate was 1×10^{-4} M. The two-step assay procedure and concentration of reaction components are described under "Materials and Methods."

Enzyme	Substrate	$S_{0.5}$		V_{max}	
		−3PGA	+3PGA	−3PGA	+3PGA
		mM		*μmol glucose-1-P/ min·mg protein*	
U-pplase[a]	UDP-glucose	0.17	0.17	8.58	8.58
	PPi	0.30	0.30	7.45	7.45
A-pplase	ADP-glucose	0.83	0.24	0.16	0.44
	PPi	0.18	0.10	0.09	0.33

[a] U-pplase, UDP-glucose pyrophosphorylase; A-pplase, ADP-glucose pyrophosphorylase.

Table II. *Effect of Sulfhydryl Group Reacting Agents on the Activated and Unactivated Activity of Potato Tuber ADP-glucose Pyrophosphorylase*

The control reaction mixtures in 1 ml (pH 7.5) contained 2 μmol ADP-glucose, 80 μmol glycylglycine, 5.0 μmol $MgCl_2$, 1.5 μmol PPi, 10 μmol NaF, plus or minus 1×10^{-4} M 3-P-glycerate, and 0.0075 unit of enzyme.

Additions	μmol Glucose-1-P/min × 10^3	f[a]
Unactivated reactions		
1. Control	0.30	
2. PCMB, 1 mM	0.21	−1.4
3. Cysteine, 20 mM	0.38	+1.3
4. Cysteine, 20 mM; PCMB, 1 mM	0.23	−1.3
5. Glutathione, 10 mM	1.05	+3.5
6. Glutathione, 10 mM; PCMB, 1 mM	0.60	+2.0
7. 2-Mercaptoethanol, 10 mM	0.85	+2.8
8. 2-Mercaptoethanol, 10 mM; PCMB, 1 mM	0.42	+1.4
9. DTT, 3 mM	6.32	+21.1
10. DTT, 3 mM; PCMB, 1 mM	6.07	+20.2
Activated reactions		
11. Control	19.43	
12. PCMB, 1 mM	6.83	−2.8
13. DTT, 3 mM	19.89	1.0
14. DTT, 3 mM; PCMB, 1 mM	19.42	1.0

[a] f, Fold activity difference compared to the control respective of the unactivated or activated reactions.

maximum change in *A* occurred. Under these reaction conditions, 0.1 μmol of glucose-1-P gave a standard *A* change of 0.415.

RESULTS

Effect of 3-P-glycerate on Substrate Affinities. It was suggested previously that the pyrophosphorolysis of UDP-glucose and ADP-glucose in potato extracts was catalyzed by separate protein entities (24). Figures 1 and 2 indicate a differential effect of 3-P-glycerate on these two activities. Lineweaver-Burk plots showed that 0.1 mM 3-P-glycerate had no effect on the pyrophosphorolysis kinetic constants towards the substrates UDP-glucose or PPi and that normal Michaelis-Menten kinetics were followed (Fig. 1 and

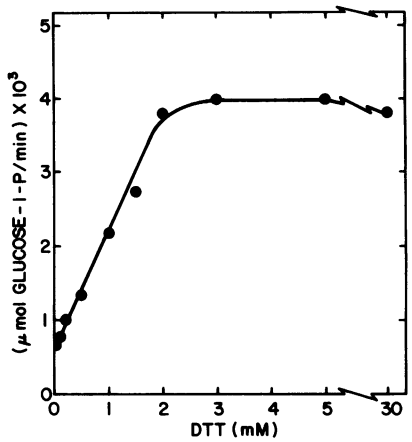

FIG. 3. ADP-glucose pyrophosphorylase activity as a function of DTT concentration. Each 1-ml reaction mixture (pH 7.5) contained 2 μmol ADP-glucose, 80 μmol glycylglycine, 5 μmol $MgCl_2$, 10 μmol NaF, 1.5 μmol PPi, and 0 to 30 μmol DTT. All tubes were incubated for 30 min at 37 C with 41 μg enzyme (0.0065 unit).

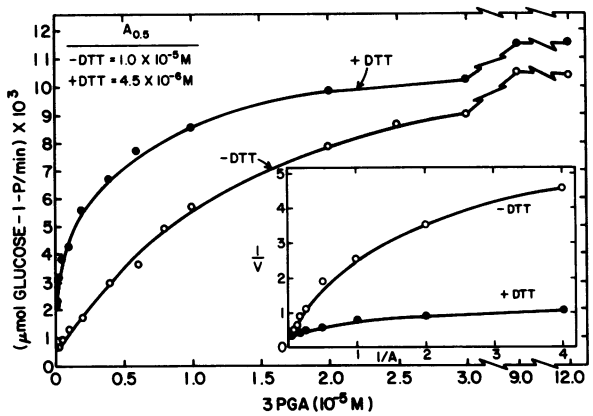

FIG. 4. Effect of DTT on 3-P-glycerate activation of potato tuber ADP-glucose pyrophosphorylase. The standard two-step assay was conducted with 3-P-glycerate ranging in concentration from 0 to 12×10^{-5} M in the absence (○——○) or presence (●——●) of 3 mM DTT. Velocity (v) is represented by μmol glucose-1-P/30 min·45 μg enzyme × 10^1. The concentration of activator (A) is represented by 10^{-6} M.

Table I). Conversely, it was noted that 3-P-glycerate had a pronounced effect on the kinetic constants of ADP-glucose pyrophosphorylase towards its pyrophosphorylytic substrates (Fig. 2 and Table I). The sigmoidal relationship observed between the unactivated enzyme and ADP-glucose was converted to hyperbolic kinetics in the presence of 3-P-glycerate (Fig. 2B).

Requirements for ADP-glucose Pyrophosphorolysis. Enzyme, ADP-glucose, PPi, and $MgCl_2$ were absolutely necessary for pyrophosphorolysis activity. Addition of a high concentration of BSA (200 μg) resulted in a slight stimulation of activity (*i.e.* 14–19%). Omission of the sulfhydryl-group reacting agent, DTT, resulted in a marked decline in the unactivated activity (*i.e.* 10.8–2.9 μmol glucose-1-P/min·10^2), while little effect was noted in the presence of 3-P-glycerate (26.2–25.0 μmol glucose-1-P/min·10^2).

Effect of Sulfhydryl-Group Reacting Agents on ADP-glucose Pyrophosphorylase. Relatively high levels of L-cysteine (20 mM), glutathione (10 mM), and 2-mercaptoethanol (10 mM) stimulated the unactivated enzyme 1.3-, 3.5-, and 2.8-fold, respectively (Table II, additions 3, 5, and 7). PCMB (1 mM) caused a significant reversal of these apparent activity increases (Table II, additions 4, 6, and 8). DTT was the most efficient sulfhydryl-group reacting agent, stimulating the unactivated reaction 21.1-fold. This activity

Table III. *Pyrophosphorylytic Kinetic Constants of ADP-glucose Pyrophosphorylase Plus or Minus DTT*

The partially purified enzyme represented the 30 to 45% ammonium sulfate fraction from Norchip acetone powders. The concentrations of DTT and 3PGA used were 5.0 and 0.1 mM, respectively. The remaining composition of the two-step assay system was similar to that described under "Materials and Methods."

Substrate	$S_{0.5}$				V_{max}			
	−3PGA		+3PGA		−3PGA		+3PGA	
	−DTT	+DTT	−DTT	+DTT	−DTT	+DTT	−DTT	+DTT
	mM				*μmol glucose-1-P/min·mg protein*			
ADP-glucose	3.30	0.83	0.59	0.24	0.18	0.16	0.89	0.92
PPi	5.26	0.23	0.10	0.10	0.31	0.41	0.75	0.73

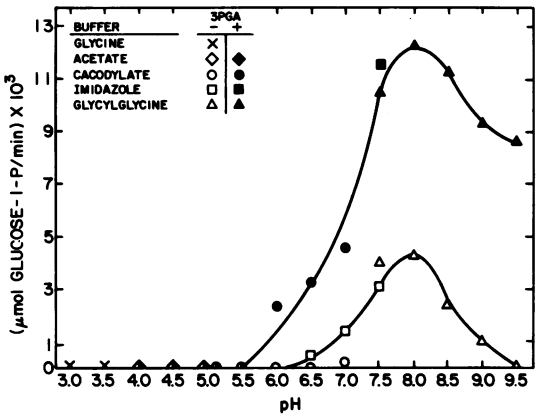

FIG. 5. ADP-glucose pyrophosphorylase activity as a function of pH in the absence or presence of 2 mM 3-P-glycerate. The pyrophosphorylase extract was prepared from Norchip acetone powders (30–45% ammonium sulfate precipitate) and assayed according to the two-step system described under "Material and Methods."

increase was not significantly reversed with PCMB. The presence of 0.1 mM 3-P-glycerate stimulated the enzyme to yield 19.43 μmol glucose-1-P/min·10^3, and this activity was decreased 64% with PCMB (Table II, additions 11 and 12). Addition of DTT to the activated enzyme did not cause an additional activity increase from the control (Table II, addition 13), but its presence did prevent the inhibition seen with PCMB (Table II, additions 12 and 14).

Because of the apparent importance of the oxidized-reduced state of the enzymes' sulfhydryl (−SH) groups on the degree of 3-P-glycerate activation, the influence of DTT was examined further. Figure 3 shows the ADP-glucose pyrophosphorylase activity as a function of DTT concentration. Under our experimental assay conditions, maximum stimulation was observed with 3 mM DTT. Figure 4 shows that DTT decreased the concentration of 3-P-glycerate required for 50% activation ($A_{0.5}$) of the potato enzyme from 1.0 to 10^{-5} M to 4.5×10^{-6} M. However, DTT did not influence the maximum velocity attained with higher concentrations of activator (*i.e.* 3×10^{-5} M) (Fig. 4, *inset*). The effects of DTT on the pyrophosphorylytic kinetic constants of ADP-glucose pyrophosphorylase are seen in Table III. It was evident that the affinity of the enzyme for its substrates was markedly influenced by its reduction state and the presence of 3-P-glycerate. The V_{max} obtained, in the presence or absence of activator, was not significantly altered with this sulfhydryl-group reacting agent (Table III). Since the presence of DTT helped to ensure saturating substrate conditions without serving as an activator, it was routinely included in all subsequent enzyme assays.

Effect of pH on 3-P-glycerate Activation. Figure 5 shows the 3-P-glycerate activation of potato tuber ADP-glucose pyrophosphorylase as a function of pH. Activations of 3.1-, 2.9-, 4.8-, and 9.5-fold were observed at pH 7.5, 8.0, 8.5, and 9.0, respectively. A relatively high activity was seen at pH 9.5 only in the presence of this effector.

Table IV. *Metabolic Activation of Potato Tuber ADP-glucose Pyrophosphorylase*

Reaction mixtures in 1 ml (pH 7.5) contained 2 μmol ADP-glucose, 80 μmol glycylglycine, 5.0 μmol $MgCl_2$, 1.5 μmol PPi, 10 μmol NaF, 3 μmol DTT, 2 μmol of a given metabolite, and 0.0075 unit of enzyme. All tubes were incubated for 15 min at 37 C, and the concentration of glucose-1-P formed (pyrophosphorylytic reaction) was measured with the enzyme coupled system described under "Materials and Methods."

Metabolite	V_{max}	Fold Activation	$A_{0.5}$
2 mM	*(μmol glucose-1-P/min·mg protein) × 10^2*		*M*
None	8.7	1.0	
3-Phospho-D-glycerate	33.4	3.8	4.5×10^{-6}
Phosphoenolpyruvate	27.9	3.2	6.2×10^{-5}
2-Phospho-D-glycerate	26.1	3.0	2.9×10^{-6}
α-Glycerol-P	22.5	2.6	3.9×10^{-4}
D-Ribose-5-P	18.6	2.1	2.8×10^{-4}
D-Fructose-6-P	18.3	2.1	
2-Deoxy-D-ribose-5-P	18.3	2.1	
D-Glycerate-2,3-bisP	13.1	1.5	
D-Fructose-1,6-bisP	12.1	1.4	
Pyruvate	10.0	1.3	
L-Lactate	10.0	1.3	
Dihydroxyacetone-P	10.0	1.3	
L-Malate	9.4	1.1	
Glycerol	9.2	1.1	
Citrate	8.8	1.0	
Acetyl-CoA	8.7	1.0	
Oxalacetate	8.0	0.9	

Table V. *Inhibition of Potato Tuber ADP-glucose Pyrophosphorylase*

Reaction mixtures in 1 ml (pH 7.5) contained 2 μmol ADP-glucose, 80 μmol glycylglycine, 5 μmol $MgCl_2$, 1.5 μmol PPi, 10 μmol NaF, 3 μmol DTT, 2 μmol of effector, and 0.0075 unit of enzyme. All tubes were incubated for 15 min at 37 C, and the concentration of α-D-glucose-1-P formed (pyrophosphorylytic reaction) was measured with the enzyme coupled system described under "Materials and Methods."

Effector	Glucose-1-P Formed	Inhibition
2 mM	*μmol/15 min × 10^2*	*%*
None	8.0	
Pi	0.6	92.5
$NADP^+$	2.1	74.0
ADP	3.6	55.0
AMP	8.3	0

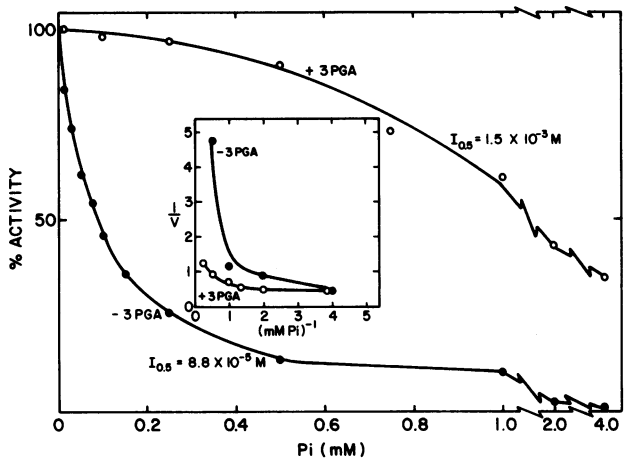

FIG. 6. Effect of Pi on the pyrophosphorolysis of ADP-glucose in the absence (●——●) or presence (○——○) of 1.2×10^{-4} M 3-P-glycerate. Velocity (v) is represented by μmol glucose-1-P/30 min·11.8 μg enzyme $\times 10^2$ and 10^1 in the absence and presence of activator, respectively.

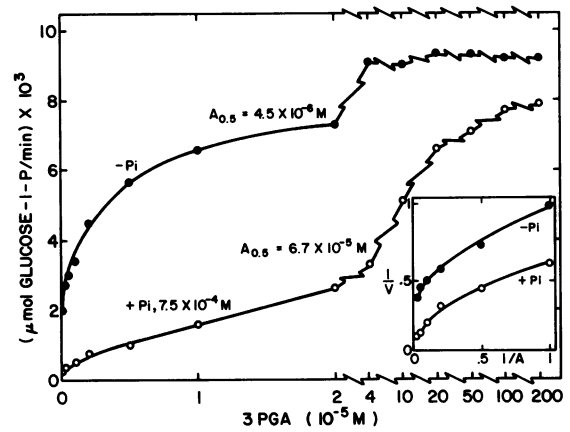

FIG. 7. Effect of 3-P-glycerate on the pyrophosphorolysis of ADP-glucose in the absence (●——●) or presence (○——○) of 7.5×10^{-4} M Pi. Velocity (v) is represented by μmol glucose-1-P/30 min·23 μg enzyme $\times$ 10^2 and 10^1 in the presence and absence of inhibitor, respectively. The concentration of activator is represented by 10^{-6} M (see inset).

Metabolite Activation of Potato Tuber ADP-glucose Pyrophosphorylase. In addition to 3-P-glycerate, P-enolpyruvate and 2-P-glycerate activated ADP-glucose pyrophosphorolysis 3.2- and 3.0-fold, respectively, at physiological concentrations. Their respective $A_{0.5}$ values ranged from 2.9×10^{-6} to 6.2×10^{-5} M (Table IV). One hundred-fold less efficient in their activation (*i.e.* $A_{0.5} = 10^{-4}$ M or higher) were α-glycerol-P, D-ribose-5-P, D-fructose-6-P, and 2-deoxy-D-ribose-5-P. These effectors stimulated the potato enzyme between 2.1- and 2.6-fold. The remaining metabolites, including D-glycerate-2,3-bisP, D-fructose-1,6-bisP, and pyruvate, had no effect on the potato tuber enzyme.

Inhibition of Potato Tuber ADP-glucose Pyrophosphorylase. Inorganic phosphate was the most effective inhibitor resulting in a 92.5% inhibition of activity at a concentration of 2 mM (Table V). Other inhibitors included $NADP^+$ (*i.e.* 74.0% inhibition) and ADP (*i.e.* 55.0% inhibition). No effect was seen with AMP.

Interaction between Pi and 3-P-glycerate. The effect of Pi on the rate of ADP-glucose pyrophosphorolysis is shown in Figure 6. Fifty percent inhibition ($I_{0.5}$) occurred at 8.8×10^{-5} M Pi, in the presence of 1.2×10^{-4} M 3-P-glycerate, and increased $I_{0.5}$ of 1.5 $\times 10^{-3}$ was obtained. Figure 7 indicates the effect of Pi on the potato enzyme activity *versus* 3-P-glycerate concentration. Fifty percent activation occurred with 4.5×10^{-6} M 3-P-glycerate; in the presence of 7.5×10^{-4} M Pi, an increased $A_{0.5}$ of 6.7×10^{-5} M was obtained.

DISCUSSION

The most effective activator of ADP-glucose pyrophosphorylase from nonphotosynthetic potato tubers was 3-P-glycerate (Table IV). The concentration of this activator, required to give 50% of the maximum velocity ($A_{0.5}$), was 4.5×10^{-6} M. This $A_{0.5}$ was similar in magnitude to those noted for the spinach (6) and barley leaf (22) enzyme (range, 10^{-5} to 10^{-6} M), while it was considerably lower than $A_{0.5}$ values observed with the sorghum leaf (22) and maize endosperm (3) enzyme (range, 10^{-3} and 10^{-4} M) when ADP-glucose synthesis was followed. P-enolpyruvate and 2-P-glycerate activated the potato enzyme approximately 3-fold, and gave $A_{0.5}$ values of 6.2×10^{-5} and 2.9×10^{-6} M, respectively. It is possible that activation with these two metabolites could have been mediated through their conversion to 3-P-glycerate by contaminating enzymes (*i.e.* enolase and P-glyceromutase) in the partially purified enzyme preparations used. The $S_{0.5}$ for ADP-glucose and PPi were decreased 3.5-fold (*i.e.* 0.83 to 0.24 mM) and 1.8-fold (0.18 to 0.10 mM), respectively, by 0.1 mM 3-P-glycerate while the V_{max} was increased 3- to 4-fold (Table I). The $S_{0.5}$ values of the spinach leaf enzyme were similarly decreased from 0.93 to 0.15 mM (*i.e.* ADP-glucose) and from 0.50 to 0.04 mM (*i.e.* PPi) in the presence of 3-P-glycerate (21). These substrate saturation curves were hyperbolic and obeyed Michaelis-Menten kinetics in the presence or absence of 3-P-glycerate. The potato enzyme, however, demonstrated a sigmoidal relationship to the substrate ADP-glucose in the absence of 3-P-glycerate (Fig. 2B). This saturation curve became hyperbolic when the modifier 3-P-glycerate was present. Similar shifts from sigmoidal to linear Michaelis-Menten kinetics were noted with the maize endosperm enzyme relative to substrates ADP-glucose and ATP (3).

Inorganic phosphate was the most efficient inhibitor of potato tuber ADP-glucose pyrophosphorylase (Table V). Both $NADP^+$ and ADP demonstrated significant inhibition at relatively high concentration (2 mM). The concentration of Pi required to cause 50% inhibition ($I_{0.5}$) increased from 8.8×10^{-5} M to 1.5×10^{-3} M in the presence of 1.2×10^{-4} M 3-P-glycerate (Fig. 6). The $I_{0.5}$ value for the spinach leaf enzyme, in the direction of ADP-glucose synthesis, was similarly increased from 2.2×10^{-5} M to 1.3×10^{-3} M with 1 mM 3-P-glycerate (18).

Although the presence of key sulfhydryl (−SH) groups at the catalytic and/or allosteric sites of the potato tuber ADP-glucose pyrophosphorylase remains to be confirmed, their role in maintaining the enzyme in a more catalytically active state cannot be excluded. Previous studies indicated an instability of the potato tuber enzyme in reaction to freezing and storage at 4 C (24). Apparently, once this enzyme becomes solubilized, it rapidly loses its specially active conformation. UDP-*N*-acetylglucosamine pyrophosphorylase from yeast was shown to be labile at 4 C, and addition of 0.1 mM DDT resulted in a full restoration and stabilization of activity (11). Addition of DTT (0.1 to 0.8 mM) to partially purified potato tuber ADP-glucose pyrophosphorylase extracts did not restore its original activity or demonstrate significant stabilization of the enzyme, but it did increase the apparent affinity of the tuber enzyme for its substrates (Table III) and the efficiency of the allosteric activator 3-P-glycerate (Fig. 4). Preliminary Hill equation plots (2) suggested that DTT addition may decrease interaction between 3-P-glycerate binding sites (J. R. Sowokinos, unpublished observations). The blockage or oxidation of −SH groups resulted in a marked reduction in the apparent enzyme activity (Table II). Addition of DTT did help to prevent the activity loss seen in the presence of PCMB. At this point, an indirect effect of DTT via some component in the partially purified potato enzyme preparations used can not be eliminated. Studies with more highly purified fractions are necessary before

the absolute requirement of, or the mode of action of, DTT in enhancing the activity of potato tuber ADP-glucose pyrophosphorylase can be confirmed.

These observations might be related to the physiological control of starch synthesis in *Solanum tuberosum*. Elevated levels of glycolytic intermediates (*i.e.* 3-P-glycerate, P-enolpyruvate, etc.) during rapid tuber growth would increase the synthesis of ADP-glucose and subsequent starch deposition. Accumulation of Pi would decrease the efficiency of the allosteric activator, reducing the rate of starch formation. Partial regulation of α-glucan formation by altering the *in vivo* concentration of ADP-glucose pyrophosphorylase (*i.e.* coarse metabolic control) has been suggested for the potato as well as for other nonphotosynthetic tissues including pea (25), wheat grain (26), and maize endosperm (15). However, unlike the endosperm enzyme, which apparently has evolved to a form that is comparatively insensitive to 3-P-glycerate activation and Pi inhibition (3), the potato enzyme is quite sensitive to allosteric regulation (*i.e.* fine metabolic control) by these effectors. In this regard, ADP-glucose pyrophosphorylase from nonchlorophyllous potato tuber tissue is more closely related to the enzyme found in the leaves of higher plants (12, 22), green algae (23), and blue-green bacteria (12). Because of the sensitivity of the potato enzyme to 3-P glycerate, it would appear that the intracellular level of this metabolite would continuously be several-fold in excess of its $A_{0.5}$ value of 4.5×10^{-6} M. As tuber growth ceases, the level of 3-P-glycerate may still exceed this concentration by nearly 10-fold (*i.e.* 4×10^{-5} M) (9). The concentration of Pi in the potato cell has been estimated to be as high as 12 to 20 mM (20). This level is several-fold higher than its $I_{0.5}$ value of 1.5 mM seen in the presence of 1.2×10^{-4} M 3-P-glycerate (Fig. 6). However, while most of the ester-P is found in the cytoplasm, a large portion of the potato Pi is suggested to be vacuolar (10). The actual *in vivo* concentration of Pi at its effector sites on potato tuber ADP-glucose pyrophosphorylase is now known. When intracellular 3-P-glycerate concentrations ranged from 0.5×10^{-3} to 4×10^{-5} M, however, Pi levels as small as 0.75 mM would inhibit the enzyme 24 to 64%, respectively (Fig. 7). This negative effector (Pi) has been indicated to elicit the major regulatory control on ADP-glucose pyrophosphorylase in spinach leaves (18). It is apparent that small concentration changes of intracellular Pi, regulated by membrane permeability and metabolic processes, could be of great physiological importance in regulating starch synthesis during tuberization and maturation of potato tuber.

Acknowledgments—The technical assistance of E. A. Paulson and I. Lay is gratefully appreciated.

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