Plant Physiol. (1982) 69, 1459–1466
0032-0889/82/69/1459/08/$00.50/0

Pyrophosphorylases in *Solanum tuberosum*[1, 2]

III. PURIFICATION, PHYSICAL, AND CATALYTIC PROPERTIES OF ADPGLUCOSE PYROPHOSPHORYLASE IN POTATOES

Received for publication November 17, 1981 and in revised form January 22, 1982

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ABSTRACT

ADPglucose pyrophosphorylase from potato (*Solanum tuberosum* L.) tubers has been purified by hydrophobic chromatography on 3 aminopropyl-sepharose (Seph-C_3-NH_2). The purified preparation showed two closely associated protein-staining bands that coincided with enzyme activity stains. Only one major protein staining band was observed in sodium dodecyl sulfate polyacrylamide gel electrophoresis. The subunit molecular weight was determined to be 50,000. The molecular weight of the native enzyme was determined to be 200,000. The enzyme appeared to be a tetramer consisting of subunits of the same molecular weight. The subunit molecular weight of the enzyme is compared with previously reported subunit molecular weights of ADPglucose pyrophosphorylases from spinach leaf, maize endosperm, and various bacteria. ADPglucose synthesis from ATP and glucose 1-P is almost completely dependent on the presence of 3-P-glycerate and is inhibited by inorganic phosphate. The kinetic constants for the substrates and Mg^{2+} are reported. The enzyme V_{max} is stimulated about 1.5- to 3-fold by 3 millimolar DTT. The significance of the activation by 3-P-glycerate and inhibition by inorganic phosphate ADPglucose synthesis catalyzed by the potato tuber enzyme is discussed.

The enzyme ADPglucose pyrophosphorylase (ATP:α-glucose-1-P adenylyltransferase, E.C. 2.7.7.27), is one of the main regulatory steps in the biosynthesis of α-glucans in bacteria and plants (21, 22). This enzyme catalyzes the reversible reaction seen below:

$$\text{ATP} + \text{glucose-1-P} \longleftrightarrow \text{ADPglucose} + \text{PPi}$$

Its regulatory and catalytic properties from several sources have been recently reviewed (22). ADPglucose pyrophosphorylases from leaves of higher plants (23, 24) and nonchlorophyllous reserve tissues (4, 28) are allosterically activated and inhibited by 3-P-glycerate and Pi, respectively. The extensively studied enzyme in maize endosperm (4, 10) is less sensitive to these effectors than those studied in chlorophyllous leaf tissues (24). However, the partially purified enzyme from potato tubers (28) has demonstrated similar magnitudes and sensitivity of activation and inhibition compared to the spinach chloroplast enzyme (3, 9, 23), when the activity was measured in the pyrophosphorolysis direction. The catalytic properties of ADPglucose pyrophosphorylase in the direction of ADPglucose synthesis as well as its relative sensitivity toward metabolic effectors have not been reported in potato tubers.

This report describes the regulation of ADPglucose synthesis catalyzed by an ADPglucose pyrophosphorylase purified from potato tubers. Comparisons of the subunit and native mol wt of the purified potato enzyme to the enzymes from bacteria (22), maize endosperm (7, 10) and spinach leaves (3) are discussed.

MATERIALS AND METHODS

Reagents. [^{14}C]Glucose-1-P and [^{32}P]PPi were obtained from New England Nuclear Corporation. 3-Aminopropyl-Sepharose (Seph-C_3-NH_2) was prepared as described by Shaltiel and Er-El (25). DEAE-cellulose grade DE-52 was purchased from Reeve-Angel. All other chemical reagents used were obtained from commercial sources at the highest purity possible.[4]

Plant Material and Preparation of Acetone Powders. The potato variety Norchip (*Solanum tuberosum* L.) was used as the source of enzyme during the course of this study. Acetone powders were prepared from immature tubers (200 g average size) as previously described (27). Two hundred g 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-L explosion-proof Waring Blendor containing 1.5 L cold acetone (−20°C). The residue was washed 5 times with 150 ml of cold acetone and dried for 4 h in a vacuum desiccator. The dried samples were stored in airtight glass jars at −20°C.

Assay of ADPglucose Pyrophosphorylase. Pyrophosphorolysis of ADPglucose was followed by the formation of ATP-^{32}P in the presence of [^{32}P]PPi (26). The reaction mixture (pH 8) contained 20 μmol glycylglycine buffer, 1.25 μmol $MgCl_2$, 0.75 μmol DTT, 2.5 μmol NaF, 0.5 μmol ADPglucose, 0.38 μmol [^{32}P]PPi (1.0 to 5.0 × 10^6 cpm/μmol), 50 μg of crystalline BSA (Sigma), and enzyme in a final volume of 0.25 ml. The reaction mixture was incubated at 37°C for 10 min and the reaction terminated by the addition of 3 ml cold 5% TCA. This assay was used to quantitate the enzyme during the purification procedure. A unit of ADPglucose pyrophosphorylase activity is defined as that amount of enzyme catalyzing synthesis of 1 μmol ATP/min under the reaction conditions described. Specific activity is defined as units/mg protein.

[1] Supported in part by National Science Foundation Grant PCM78-16127.

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

Table I. *Purification of ADPglucose Pyrophosphorylase*

Fraction	Volume	Protein	Activity	Specific Activity	Purification	Recovery
	ml	*mg*	*units*	*units/mg*	*-fold*	*%*
Acetone powder extract	1,000	3,140	187	0.06		100
Heat treated	1,000	1,540	205	0.13	2.2	100
$(NH_4)_2SO_4$	40	490	155	0.32	5.3	83
DEAE-cellulose	8.5	32	47	1.5	25	25
Seph C_3NH_2	3.4	1.9	5.3	2.8	47	3

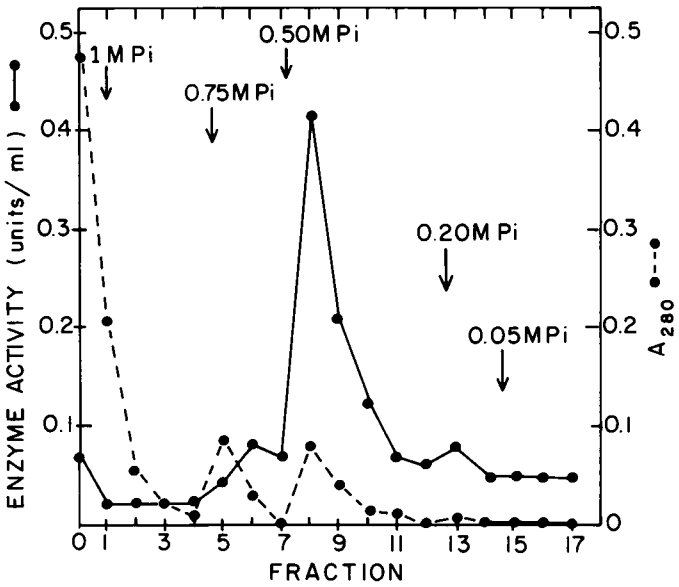

FIG. 1. Seph-C_3-NH_2 hydrophobic chromatography of potato tuber ADPglucose pyrophosphorylase. Details of the procedure are described in the text.

Assay of ADPglucose Synthesis. Synthesis of ADPglucose was measured as previously described (9). The reaction mixture (pH 8) contained 16 μmol of *N*-2 hydroxyethylpiperazine-*N*-2 ethanesulfonic acid (Hepes) buffer, 1 μmol $MgCl_2$, 0.6 μmol DTT, 0.2 μmol [^{14}C]glucose-1-P (1.0×10^6 cpm/μmol), 0.3 μmol ATP, 0.6 μmol 3-P-glycerate, 40 μg crystalline BSA, and enzyme in a final volume of 0.2 ml. The reaction mixture was incubated at 37°C for 10 min and the reaction terminated by heating in a boiling water bath for 30 s.

The rate of [^{32}P]ATP or [^{14}C]ADPglucose formation was linear with respect to both time and enzyme concentration under the experimental conditions used. Protein concentration was determined by the method of Lowry *et al.* (17).

Polyacrylamide Gel Electrophoresis. Electrophoresis in SDS was done in slab gels using the electrophoretic buffer system of Laemmli (15). The gel was 8% acrylamide and 1 mm thick. The proteins were stained by the procedure of Fairbanks *et al.* (5). The protein standards used were lactic acid dehydrogenase (mol wt, 35,000), ovalbumin (mol wt, 45,000), *Escherichia coli* B ADPglucose pyrophosphorylase (mol wt, 50,000), BSA (mol wt, 68,000), and phosphorylase A (mol wt, 92,500).

The native enzyme was also studied in the Ornstein-Davis system (8) using 7.5% (resolving) and 3% (stacking) acrylamide gels. Enzyme activity in the acryamide gel was determined by using a staining procedure previously described (19). Protein staining was done with Coomassie blue (2).

Sucrose Density Gradient Ultracentrifugation. Sucrose density ultracentrifugation was done according to the procedure of Martin and Ames (18). Lactate dehydrogenase (mol wt, 140,000) and pyruvate kinase (mol wt, 237,000) were used as marker enzymes.

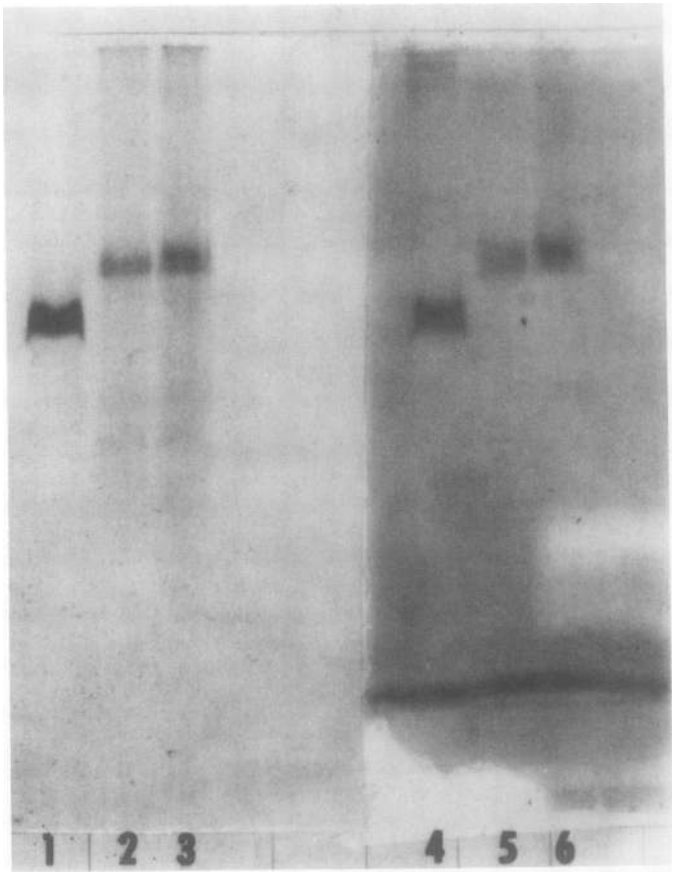

FIG. 2. Gel electrophoresis of ADPglucose pyrophosphorylases from potato tuber and *E. coli* in the Tris-glycine (pH 8.9) Ornstein system. The resolving gel polyacrylamide concentration was 7.5%. Lanes 1 through 3 are fractions stained with Coomassie blue and lanes 4 through 6 are fractions subjected to activity stains (19). The amount of proteins and fractions applied to the various lanes are; lane 1 and 4, *E. coli* ADPglucose pyrophosphorylase, 6.4 μg; lanes 2 and 5, potato Seph-C_3-NH_2 fraction, 7 μg; and lanes 3 and 6, potato Seph-C_3-NH_2 fraction, 14 μg.

RESULTS

Purification of ADPglucose Pyrophosphorylase from Potato Tubers.

Extraction, Heat Treatment, and $(NH_4)_2SO_4$ Precipitation. All steps were conducted at 4°C unless indicated otherwise. Acetone powder (100 g) was mixed with 1 L extraction buffer (pH 7.5) that contained 50 mM Hepes, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM GSH, and 20% sucrose (buffer A). The slurry was stirred slowly for 40 min and centrifuged at 16,000*g* for 20 min. The precipitate was washed once with 100 to 150 ml of buffer A and centrifuged. The two supernatants were combined. Portions of the crude extract (125 ml portions) were heated in a 70°C water bath for exactly 5 min, cooled rapidly in ice, and centrifuged. The precipitate was washed with buffer A and after centrifugation the wash and

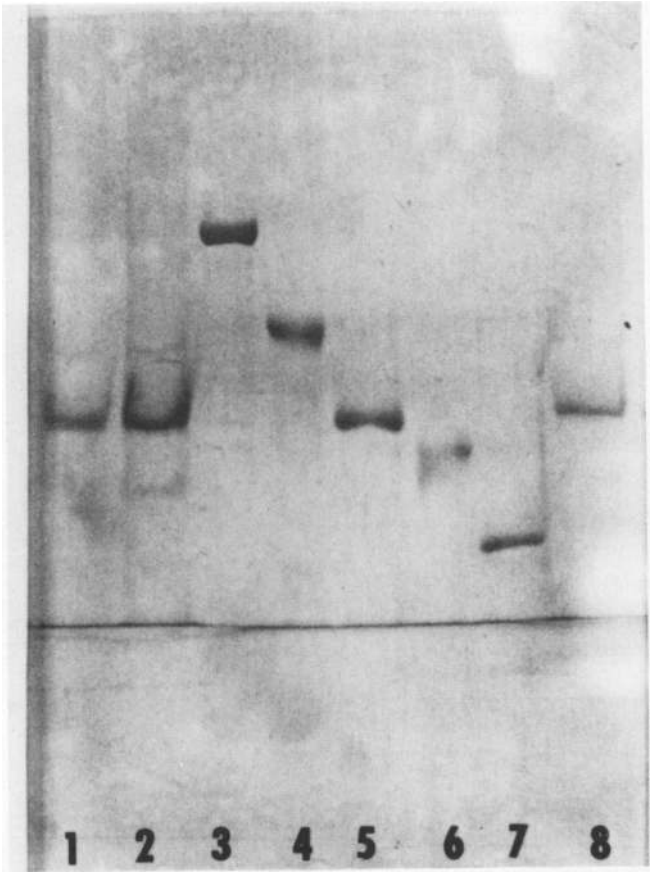

FIG. 3. Gel electrophoresis of potato tuber Seph-C_3-NH_2 fraction and various protein standards in the Tris-glycine (pH 8.8), SDS system of Laemmli (15). Lanes 1, 2, and 8 contain 4.5, 9, and 3.4 μg, respectively, of the potato tuber Seph-C_3-NH_2 fraction. Lanes 3, 4, 5, 6, and 7 contain 1 μg of the protein standards phosphorylase A, BSA, *E. coli* ADPglucose pyrophosphorylase, ovalbumin, and lactic acid dehydrogenase, respectively.

Table II. *Metabolic Activation of Potato Tuber ADPglucose Pyrophosphorylase*

Reaction mixtures in 0.2 ml (pH 8) contained 16 μmol Hepes, 1 μmol $MgCl_2$, 0.6 μmol DTT, 40 μg BSA, 0.3 μmol ATP, 0.2 μmol glucose-1-P (1×10^6 cpm/μmol), 0.6 μmol of a given metabolite and 10^{-3} units of the Seph-C_3-NH_2 fraction. All tubes were incubated for 10 min at 37°C and the ADPglucose formed was measured as described under "Materials and Methods."

Metabolite	Activity	Activation
3 mM	*nmol ADPglucose/ min·mg protein* $\times 10^{-2}$	*-fold*
None	0.26	
3-Phospho-D-glycerate	8.50	32.7
D-Ribose-5-P	1.51	5.8
D-Fructose-1,6-bisP	0.86	3.3
Phosphoenolpyruvate	0.67	2.6

supernatant were combined. The volume of the wash was adjusted to restore the volume of the heated supernatant to 1 L. After solid $(NH_4)_2SO_4$ addition (16.4% w/v), the precipitate was discarded and $(NH_4)_2SO_4$ (11.7% w/v) was added to the supernatant. The resulting precipitate (30–50% ammonium sulfate fraction) was collected by centrifuging at 27,000*g* for 20 min, redissolved in a minimum volume of buffer A (~35 ml) and dialyzed against the same buffer (33 volumes) for 20 h with one change at 10 h.

DEAE-Cellulose Chromatography. The dialyzed fraction was loaded onto 30 ml of a DEAE-cellulose DE-52 column equilibrated with ten bed-volumes of 10 mM K-phosphate buffer (pH 7.4) containing 20% sucrose. The column was washed with one bed-volume of equilibration buffer and eluted with a linear gradient containing 300 ml 10 mM phosphate (pH 7.4), 20% sucrose in the mixing chamber, and 300 ml of 50 mM phosphate (pH 6), 20% sucrose, and 0.3 M NaCl in the reservoir. Fractions of 10 ml were collected. Aliquots containing activity were pooled and concentrated to 9.0 ml using an Amicon thin channel ultrafiltration system with a PM 30 membrane. The concentrated fraction was dialyzed 20 h against 500 ml buffer with one change at 10 h.

Seph-C_3-NH_2 Hydrophobic Chromatography. The dialyzed DEAE-cellulose fraction was made 1 M with respect to Pi by addition of an equal volume of 2.0 M K-phosphate buffer (pH 7). The fraction was then absorbed onto 3 ml of a Sepharose-C_3-NH_2 column that had previously been washed with 1 M KCl, H_2O (three bed-volumes each) and equilibrated with 1 M phosphate (pH 7) (ten bed-volumes). The enzyme was adsorbed and the column was successively washed with phosphate buffers (pH 7) 1 M, 0.75 M, 0.50 M, 0.20 M, and 0.05 M. Ten-ml fractions were collected. Elution with each buffer was discontinued after the A_{280} decreased to about 0.05. The enzyme was eluted with 0.5 M Pi buffer (pH 7) (Fig. 1). The active fractions were pooled and concentrated to approximately 4 ml by Amicon ultrafiltration with a PM 30 membrane using 16 p.s.i. This fraction was dialyzed for 20 h against 500 ml 50 mM Hepes buffer (pH 7.5) containing 20% sucrose, 1 mM EDTA, and 2 mM GSH with one change at 10 h. Table I summarizes the purification of ADPglucose pyrophosphorylase from potato tubers. The enzyme was stable for at least 6 wk when stored at 4°C.

Physical and Catalytic Properties of ADPglucose Pyrophosphorylase from Potato Tubers.

Activity Stain, Subunit Size, and Mol Wt. Purified ADPglucose pyrophosphorylase was electrophoresed in the Tris/glycine system of Ornstein (8) with 7.5% acrylamide gel. Figure 2 shows two very closely associated protein bands staining with about equal intensity (lanes 2 and 3) that also closely coincided with enzyme activity as seen by an activity stain (lanes 5 and 6). The native gel and activity stain patterns for *E. coli* B ADPglucose pyrophosphorylase is seen in lanes 1 and 4, respectively. When the Sepharose-C_3-NH_2 fraction was denatured and subjected to electrophoresis according to the procedure of Laemmli (15), one major protein band was obtained (Fig. 3). The subunit mol wt of the potato ADPglucose pyrophosphorylase was determined to be 50,000 using the standard protein markers indicated in Figure 3. The mol wt of the enzyme was calculated to be approximately 200,000 by ultracentrifugation in a sucrose density gradient (18).

Requirements of ADPglucose Synthesis. Enzyme ATP, glucose-1-P, and $MgCl_2$ were absolutely necessary for synthesis activity. In contrast to the pyrophosphorolysis reaction (28), there was negligible activity in the synthesis direction when 3-P-glycerate was absent. Omission of BSA (200 μg/ml) or DTT (3 mM) resulted in an activity decrease of 40 and 50%, respectively. Potassium ion (50 mM) did not stimulate the synthesis of ADPglucose either in the presence or absence of 3-P-glycerate.

pH Optimum. The pH curves for ADPglucose synthesis were fairly broad. A pH optimum of 8 was noted when buffers (80 mM) Hepes, Bicine, and glycylglycine were used. A pH optimum of 8.5 was observed in the presence of Tris.

Metabolic Activation and Inhibition. Table II shows the major activators of potato tuber ADPglucose pyrophosphorylase. The major activator of ADPglucose synthesis was 3-P-glycerate. Slight activation was noted with D-ribose-5-P, D-fructose-1,6-bisP and P-

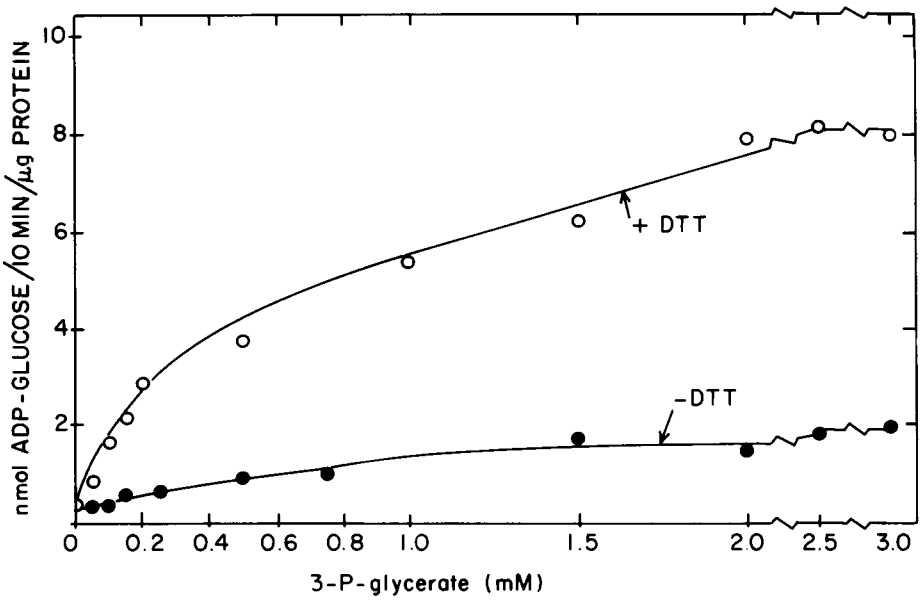

FIG. 4. Activation of potato tuber ADPglucose pyrophosphorylase with 3-P-glycerate. The synthesis reaction mixtures are described under "Materials and Methods" except that the concentration of 3-P-glycerate was varied as indicated in the presence (○——○) and absence (●——●) of 3 mM DTT.

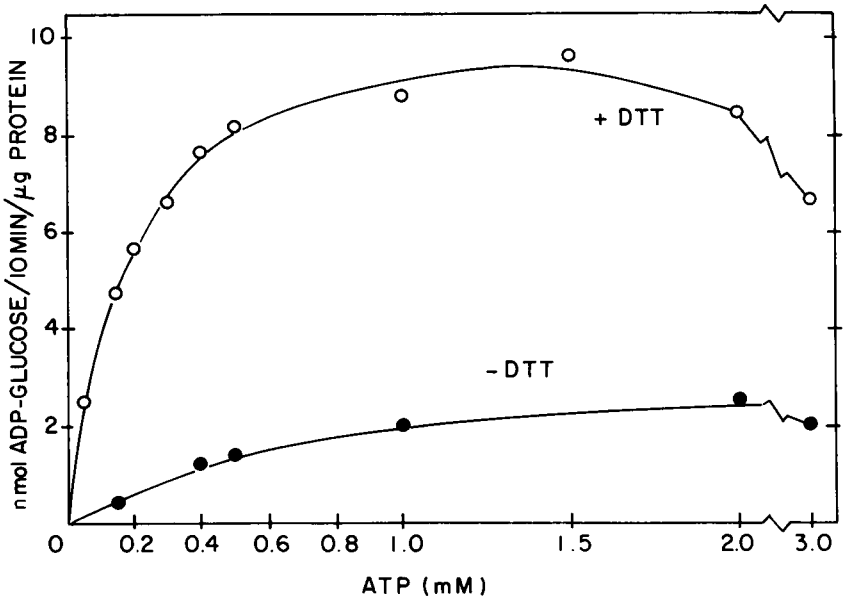

FIG. 5. Substrate saturation curve for ATP with potato tuber ADPglucose pyrophosphorylase. The synthesis reaction mixtures are described under "Materials and Methods" except that the concentration of ATP was varied as indicated in the presence (○——○) and absence (●——●) of 3 mM DTT.

enolpyruvate at 3 mM concentrations, but negligible stimulation was noted at 1 mM levels. Pyruvate, L-lactate, dihydroxyacetone-P, citrate, oxalacetate, α-glycerol-P, L-malate and α-ketoglutarate at 3 mM did not significantly activate the potato enzyme. Figure 4 shows the 3-P-glycerate activation curve for the purified enzyme in the presence or absence of DTT. The concentration of 3-P-glycerate required for 50% maximal activation ($A_{0.5}$) was found to be 0.40 mM. Omission of DTT had no effect on the $A_{0.5}$ value but V_{max} was reduced nearly 80%.

Pi was the most effective inhibitor resulting in a 93% inhibition of activity at a concentration of 1 mM. Less efficient in their inhibition were ADP, $NADP^+$, AMP, and cyclic-3',5'-AMP.

Kinetic Parameters of the Synthesis Reaction. The kinetic parameters of the purified potato tuber ADPglucose pyrophosphorylase in the synthesis direction plus or minus DTT were determined. The K_m values for ATP (0.19 mM) and glucose-1-P (0.14 mM) were very similar to the K_m values for ADPglucose (0.24 mM) and PPi (0.10 mM), respectively, in the presence of both 3-P-glycerate and DTT. Omission of DTT resulted in a higher K_m value of the adenine nucleotide substrates, while no effect was noted on the K_m values for either glucose-1-P or PPi (28). The saturation curve for ATP, plus or minus DTT, is shown in Figure 5.

Interaction Between 3-P-Glycerate and Pi. The activation curve with 3-P-glycerate is hyperbolic in shape yielding a Hill plot slope value $\bar{n}$, (13) of 1. However, the activation curve became increasingly sigmoidal in shape as the concentration of Pi increased from 0.1 to 1.0 mM (Fig. 6). Corresponding Hill plot slopes increased to 1.6 and 2.8 in the presence of 0.2 and 1.0 mM pi, respectively (Fig. 6B). The effect of Pi on the rate of ADPglucose synthesis is shown in Figure 7. Fifty % inhibition ($I_{0.5}$) occurred with Pi concentrations of 0.12, 0.18, and 0.33 mM in the presence of 0.25, 0.50, and 3.0 mM 3-P-glycerate, respectively. Hill plot slopes decreased from

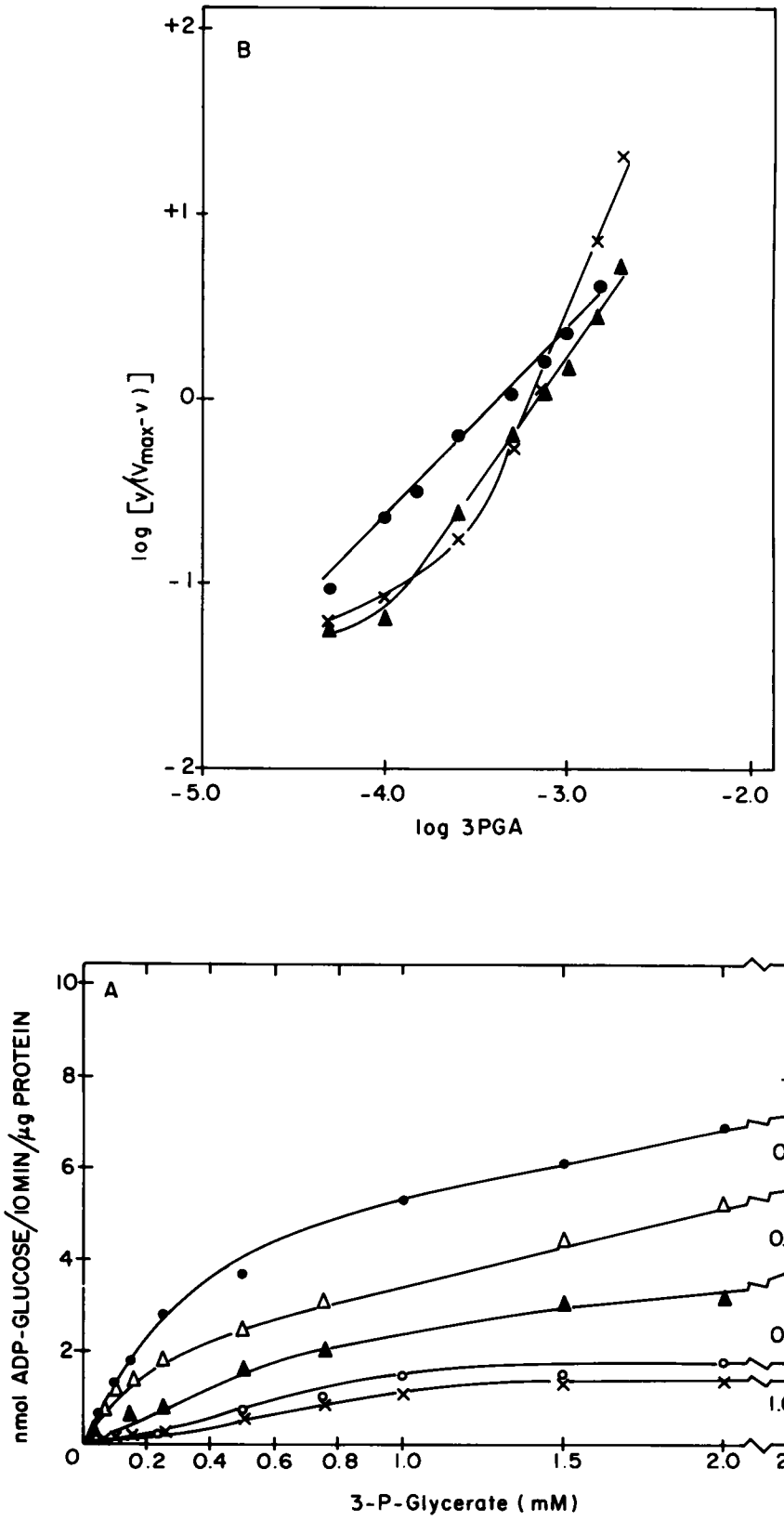

FIG. 6. Effect of Pi on the activation of ADPglucose synthesis by 3-P-glycerate. The synthesis reaction mixtures are described under "Materials and Methods" except that the concentrations of 3-P-glycerate and Pi were varied as indicated. B, a Hill equation plot of the curves obtained with 0, 0.2 and 1.0 mM Pi.

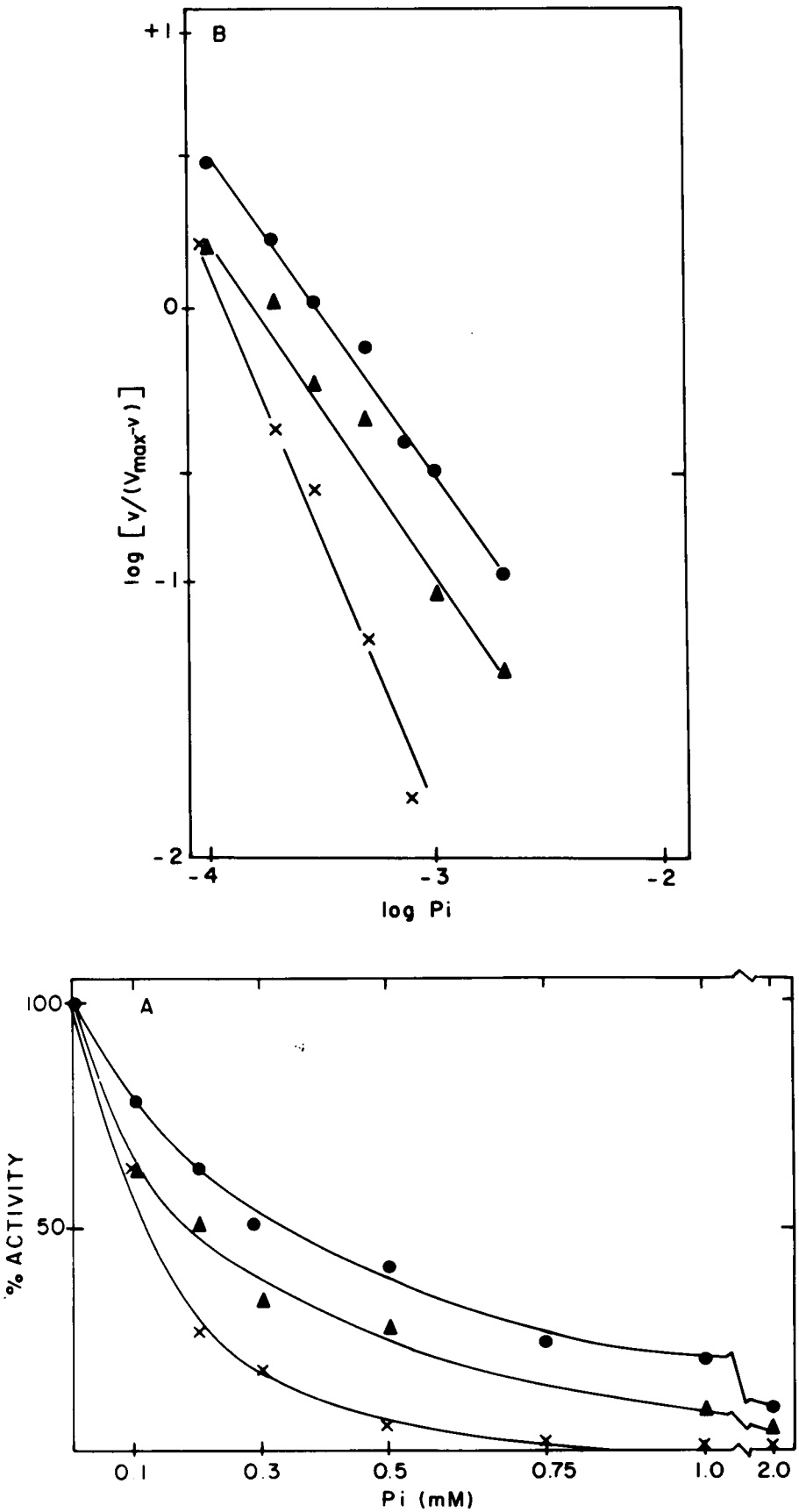

FIG. 7. Inhibition by Pi of potato tuber ADPglucose pyrophosphorylase in the presence of 3 mM (●——●), 0.5 mM (▲——▲), and 0.25 mM (×——×) 3-P-glycerate. The synthesis reaction mixtures are described under "Materials and Methods" except that the concentrations of Pi and 3-P-glycerate were varied as indicated. B, a Hill equation plot of data.

an $\bar{n}$ value of 2.0 (0.25 mM 3-P-glycerate) to an $\bar{n}$ of 1.1 (3.0 mM 3-P-glycerate) (Fig. 7B).

DISCUSSION

Purification of potato tuber ADPglucose pyrophosphorylase is summarized in Table I. Hydrophobic chromatography yielded a fraction with a specific activity of 2.8 μmol/min$^{-1}\cdot$mg^{-1} protein. Although this activity is much lower than that reported for spinach leaf (3) or bacterial ADPglucose pyrophosphorylase (21), it was equivalent to that shown for the enzyme purified to homogeneity from developing maize seeds (7). A subunit mol wt of 50,000 (Fig. 4) for the potato enzyme was in the order of that demonstrated by *E. coli* (11) and the spinach leaf enzyme (3). These subunits are different from the molecular size of 96,000 reported for the maize ADPglucose pyrophosphorylase (7). The potato enzyme, which has a native mol wt of 200,000 as determined by sucrose density centrifugation, appears to be a tetramer of four similar mol wt subunits.

Increases in the *in vivo* concentrations of key biosynthetic enzymes (*i.e.* coarse metabolic control) is one level of regulation affecting starch synthesis in developing reserve tissues. Increases in both ADPglucose pyrophosphorylase (27, 29, 30) and starch synthase (ADPglucose specific [1, 20]) have been correlated with increased rates of starch accumulation in barley, peas, wheat, maize endosperm, and potatoes during development. Among these nonchlorophyllous tissues, ADPglucose pyrophosphorylase from maize (4, 10) and potatoes (28) have been studied in detail in regard to their catalytic regulation by 3-P-glycerate and Pi (*i.e.* fine metabolic control). The potato enzyme is almost completely dependent on the metabolite 3-P-glycerate for ADPglucose synthesis activity. An activation of over 30-fold is observed with this effector (Table II) and 50% of the maximal stimulation ($A_{0.5}$) is noted at a concentration of 0.4 mM (Fig. 4). The maximum velocity observed was enhanced 4-fold when the enzyme was assayed in the presence of 3 mM DTT. The sulfhydryl-group reacting agent also increased the apparent affinity towards the substrate ATP (K_m from 0.54 to 0.19 mM). Similarly, DTT increases the apparent affinity for ADPglucose (K_m from 0.59 to 0.24 mM) when the reaction was measured in the pyrophosphorolysis direction (28). The K_m values for glucose-1-P or PPi (28) were not affected by DTT. All substrate saturation curves were hyperbolic except for ADPglucose in the absence of 3-P-glycerate (28). The mechanism of the DTT activity enhancement or the presence of key sulfhydryl (—SH) groups at the catalytic and/or allosteric sites of potato tuber ADPglucose pyrophosphorylase remains to be demonstrated.

ADPglucose pyrophosphorylase from nonchlorophyllous maize endosperm is activated only 3- to 4-fold by 3-P-glycerate with an $A_{0.5}$ value of 2.2 mM (4). This is compared with 3-P-glycerate $A_{0.5}$ values ranging from 0.007 to 0.37 mM with leaf enzymes (24) and 0.40 mM with the potato enzyme (Fig. 4). Similarly, the maize endosperm enzyme is less sensitive to inhibition with Pi. Fifty % inhibition ($I_{0.5}$) was noted with 10 mM Pi in the presence of 10 mM 3-P-glycerate. With the potato enzyme $I_{0.5}$ values for Pi of 0.12 and 0.33 mM were obtained in the presence of 0.25 and 3.0 mM 3-P-glycerate, respectively (Fig. 7). With regard to sensitivity to the effectors, 3-P-glycerate and Pi, the potato enzyme is somewhat similar to the leaf enzymes. The hyperbolic activator saturation curve found for 3-P-glycerate in Figure 6 became increasingly sigmoidal as Pi concentrations increased from 0.1 to 1.0 mM. Similar shifts to sigmoidicity were reported with the spinach leaf enzyme (9, 23). A Hill-equation plot (Fig. 6B) showed $\bar{n}$ values which increased from 1.0 to 1.5 and 2.4 in the presence of 0.2 and 1.0 mM Pi, respectively. This negative effector (Pi) apparently increased interaction between activator binding sites. Hill plots of Pi inhibition yielded $\bar{n}$ values that did not change significantly (*i.e.* 1.15 and 1.23) when 3-P-glycerate ranged from 0.5 to 3.0 mM (Fig. 7B). When activator concentration was low (*i.e.* 0.25 mM) and $\bar{n}$ value of 2.0 was obtained.

The significance of activation by 3-P-glycerate and inhibition by Pi of ADPglucose synthesis and its relationship to starch synthesis in amyloplasts of nonchlorophyllous tissues is still not explained. It has been suggested that both chloroplasts and amyloplasts are similar in their metabolite transfer across the membrane (16). The products of photosynthesis (3-P-glycerate and dihydroxyacetone-P) move out of spinach chloroplasts in exchange with Pi via the phosphate translocator (12). Liu *et al.* (16), after constituent analysis of maize amyloplasts, suggested that hexoses are converted to triose-P via glycolysis prior to entering the amyloplast via the Pi translocator. A similar mechanism involving the transport of 3-P-glycerate and/or dihydroxyacetone-P across wheat grain amyloplasts in exchange for Pi has been postulated (14). The physiological importance of the potato tuber's ADPglucose pyrophosphorylase sensitivity towards the allosteric effectors 3-P-glycerate and Pi may take on added significance in view of the above findings. The potato enzyme has been suggested to be closely associated with/or located inside the amyloplast of potato cells which is the site of starch synthesis (27). Metabolites formed from sucrose via cytoplasmic sucrose synthetase and glycolytic reactions could enter the potato amyloplast via a phosphate translocator. This would increase activator levels of ADPglucose pyrophosphorylase inside the amyloplast as well as the activator efficiency due to the removal of Pi. Increasing the 3-P-glycerate:Pi ratio could increase synthesis of ADPglucose which is the primary glucosyl donor for starch synthases found in potato tubers (6). At present, however, the actual *in vivo* concentrations of 3-P-glycerate and Pi at their effector sites on potato tuber ADPglucose pyrophosphorylase are not known. Before actual regulatory mechanisms controlling starch synthesis in potato cells can be clarified, identification and quantitation of the major constituents (*i.e.* enzymes, substrates, effectors) inside and outside the amyloplasts will have to be completed.

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