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Pyrophosphorylases in *Solanum tuberosum*

I. CHANGES IN ADP-GLUCOSE AND UDP-GLUCOSE PYROPHOSPHORYLASE ACTIVITIES ASSOCIATED WITH STARCH BIOSYNTHESIS DURING TUBERIZATION, MATURATION, AND STORAGE OF POTATOES[1]

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ABSTRACT

Changes in ADP-glucose and UDP-glucose pyrophosphorylase activities were followed during tuber development of *Solanum tuberosum* and prolonged storage at 4 and 11 C. Potato tuberization was accompanied by a sharp increase in starch synthesis simultaneous with a marked rise in ADP-glucose pyrophosphorylase activity. When tubers reached an average diameter of 1 centimeter (0.5 gram average tuber weight) and had already established 58% starch on a dry weight basis, ADP-glucose pyrophosphorylase increased 16- to 24-fold over its activity seen in low starch containing stolon tissue. During this same period UDP-glucose pyrophosphorylase increased approximately 2- to 3-fold. Although participation of UDP-glucose in starch formation can not be neglected, it is suggested that the onset of rapid non-photosynthetic potato tuber starch biosynthesis may be closely related to the simultaneous increase in ADP-glucose pyrophosphorylase activity.

Evidence that UDP-glucose and ADP-glucose pyrophosphorylases are separate protein entities was indicated by their (*a*) activity ratio variations during tuber development and storage, (*b*) extraction stabilities, (*c*) morphological localization, (*d*) separation with ammonium sulfate, (*e*) pH optima, and (*f*) differential activation with 3-P-glycerate.

The transfer of glucose units from uridine diphosphate glucose to starch was first demonstrated in bean tissue (2, 9). This nucleoside diphosphate sugar is formed from UTP and glucose-1-P by the action of UTP:α-D-glucose-1-phosphate uridylyltransferase (UDP-glucose pyrophosphorylase: EC 2.7.7.9). Subsequent studies by Recondo and Leloir (15) indicated that adenosine diphosphate glucose reacted 10 times more efficiently than its uridine analogue. The enzymic synthesis of ADP-glucose by ATP:α-D-glucose-1-phosphate adenylyltransferase (ADP-glucose pyrophosphorylase-reaction 1) was first observed in wheat flour (1).

$$\text{ATP} + \text{glucose-1-P} \rightleftharpoons \text{ADP-glucose} + \text{PPi} \qquad (1)$$

Frydman (3) reported that ADP-glucose was superior to UDP-glucose as a glucosyl donor for particulate starch synthetase in potato tubers. Soluble forms of potato synthetase also demonstrated similar specificity properties by favoring the adenine nucleotide sugar derivatives (4–6). Enzymes affecting UDP-glucose and ADP-glucose levels in nonphotosynthetic potato tubers could influence their availability for various metabolic processes including their selective participation in starch biosynthesis. UDP-glucose (20) and ADP-glucose pyrophosphorylase (14, 19) activities have previously been noted in potatoes, but their relative physiological significance involving the sugar-starch interconversion, and their *in vivo* control mechanisms have not been clarified. This initial study of a series reports the changes in UDP-glucose and ADP-glucose pyrophosphorylase activities associated with initiation of starch biosynthesis, potato tuberization, maturation, and storage, and provides evidence that the pyrophosphorolysis of UDP-glucose and ADP-glucose is catalyzed by separate protein entities in potato extracts.

MATERIALS AND METHODS

Chemicals. Phosphoglucomutase, glucose-6-P dehydrogenase, EDTA, tris, glycylglycine, NADP, ADP-glucose, UDP-glucose, and 3-P-glycerate were obtained from Sigma Chemical Company. Glucostat was purchased from Worthington Biochemical Corporation. All other chemicals used were of analytical grade.[3]

Plant Material and Tissue Selection. Chieftan and Norchip potato varieties (*Solanum tuberosum* L.) were used during the course of this study. Cultures were grown at the Red River Valley Potato Research Farm, Grand Forks, N. D, and maintained according to commercial methods in this area. Pretuberized stolon samples (50 g each) were collected by removing the terminal 3-cm section from their apical end. As tuberization or stolon enlargement occurred, miniature tubers were removed, washed, and graded according to their diameter by passing them perpendicular to their stem-bud axis through gauged holes in a measuring plate. Several tubers of average diameter were selected at each sampling interval to give near 100 g. After tubers developed to an average diameter of 5 cm (approximately 50 g average weight), they were sampled at each successive 50-g increase in average weight until maturation was reached. Five to six tubers, representing the average weight of their respective interval, were washed, and 100 to 150 g of tissue were selected at random. Stolon or tuber samples were immediately analyzed for starch content and enzyme activity.

Starch Determination. Portions of each sample (30–80 g) were sliced, dipped into 0.3% sodium bisulfite solution, pH 6, frozen,

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

and lyophilized for 48 hr at 30 C. The dehydrated tissue was blended for homogeneity and the resulting powders were stored over desiccant at −20 C until assayed. Starch was determined as previously described (23).

Preparation of Acetone Powders. Fresh tissue samples (200 g) were selected at random from immature tubers. Each sample was thinly sliced and immediately frozen with dry ice. The tissue was blended at slow speed for three 1-min intervals in a 4-liter explosion-proof Waring Blendor containing 1.5 liters of 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 of cold acetone and dried for 4 hr in a vacuum desiccator. The dried acetone powders were stored in airtight glass jars at −20 C.

Extraction of Pyrophosphorylases from Fresh Tissue. All steps were conducted at 4 C unless indicated otherwise. Each sample (20–100 g) was sliced into a Waring Blendor containing 0.05 M tris-HCl, 10 mM GSH, 1 mM EDTA buffer, pH 7.5, 50% (w/v) or 100% (w/v) for stolon or tuber tissue, respectively. For each g of tissue 0.04 ml of 10% sodium bisulfite, pH 6, was added. Two 15-sec slow-speed blending periods were followed by two 15-sec fast-speed blendings. The suspension was filtered through four layers of cheesecloth and the extract was centrifuged for 20 min at 27,000*g*. The supernatant was saved and the precipitate was washed once with buffer. After centrifugation the two supernatants were combined. The solution was taken to 47.6% (w/v) with solid $(NH_4)_2SO_4$, and the pH was maintained at 7.5 with 1N NaOH. The suspension was stirred for 20 min, allowed to stand for 15 min, and centrifuged. The supernatant was discarded and the 75% $(NH_4)_2SO_4$ precipitate was dissolved in a minimal volume of buffer (3–6 ml). This fraction was dialyzed 20 hr against 1 liter of buffer with a change at 10 hr. If slight cloudiness occurred during dialysis, the inactive precipitate was removed by centrifugation. The biuret method was used to assay for protein (8), and crystalline BSA was used as a standard. Extracts were immediately measured for pyrophosphorylase activities.

Extraction and Separation of Pyrophosphorylases from Acetone Powders. All steps were conducted at 4 C unless indicated otherwise. Aliquots of acetone powders were added to 0.05 M tris-HCl, 10 mM GSH, 1 mM EDTA buffer, pH 7.5, (10% w/v). For each g of powder, 0.1 g of 10% sodium bisulfite, pH 6, was added. The mixture was stirred slowly for 15 min, allowed to stand for 10 min at −20 C, sonicated for two 45-sec intervals, and restirred 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 minimal 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 hr against the same buffer (5 ml of fraction/liter of buffer) with one change at 10 hr. Any inactive precipitate was removed by centrifugation, and the dialyzed extracts were immediately measured for pyrophosphorylase activities.

Measurement of Pyrophosphorylase Activity. Quantitative assays were similar in principle to the procedure described by Munch-Petersen (10). The rate of α-D-glucose-1-P pyrophosphorolysis from either UDP-glucose or ADP-glucose was measured spectrophotometrically in the presence of phosphoglucomutase, glucose-6-P dehydrogenase, and NADP. Substrates and coupled assay components were in excess so that the rate of NADPH production was linear to both time and concentration of diluted pyrophosphorylase extracts used in all enzymic studies. All assays were run with minus PPi blanks to correct for any 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 μmole of glucose-1-P/min. Specific activity is defined as units/mg protein.

1. One Step Pyrophosphorylase Assay. Reaction mixtures for UDP-glucose pyrophosphorylase contained in 1 ml, pH 8: 1 μmole of UDP-glucose, 5 μmoles of $MgCl_2$, 80 μmoles of glycylglycine, 10 μmoles of NaF, 1 unit of P-glucomutase (4.4 μg), 20 μmoles of cysteine, 0.02 μmole of glucose-1, 6-diP, 0.75 unit of glucose-6-P dehydrogenase (2.1 μg), 0.6 μmole of NADP, diluted pyrohosphorylase extract (0.005–0.050 unit), and the reactions were initiated by the addition of 2.5 μmoles of PPi. When ADP-glucose pyrophosphorylase was assayed, the reaction was run at pH 7.5, 2 μmoles of ADP-glucose were substituted for UDP-glucose, and the reaction was initiated with 1.5 μmoles of PPi. In certain assays 2 μmoles of 3-P-glycerate were added to the reaction system of either enzyme. The production of NADPH at 340 nm was recorded continuously at 37 C with a Perkin-Elmer, Coleman double beam spectrophotometer model 124, until a loss of initial linear reaction rate occurred.

2. Two Step Pyrophosphorylase Assay. When pyrophosphorylase reaction conditions varied (i.e., pH, cation concentration), which could interfere with the coupled glucose-1-P monitoring system, a more accurate two-part assay was used. In part one, the reaction mixtures for UDP-glucose pyrophosphorylase, pH 8, contained 1 μmole of UDP-glucose, 5 μmoles of $MgCl_2$, 80 μmoles of glycylglycine, 10 μmoles of NaF, and diluted pyrophosphorylase extract (0.005–0.050 unit). After a 10-min incubation at 37 C, the reactions were initiated by the addition of 2.5 μmoles of PPi to give a total volume of 1 ml. When ADP-glucose pyrophosphorylase was assayed, the reaction was run at pH 7.5, 2 μmoles of ADP-glucose were substituted for UDP-glucose, and the reactions were initiated with 1.5 μmoles of PPi. In certain assays 2 μmoles of 3-P-glycerate were added to the reaction system of either enzyme. Glucose-1-P production was terminated by boiling for 1 min in a water bath after appropriate time intervals (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 of water, centrifuged, the two supernatants were combined, and their volume was taken to 1.5 ml with water. In part two, aliquots of the supernatants containing 0.15 μmole or less of glucose-1-P were added to the glucose-1-P quantitating system. Each coupled assay reaction in a total volume of 1.5 ml, pH 8, contained 80 μmoles of glycylglycine, 20 μmoles of cysteine, 0.6 μmole of NADP, 2 units of P-glucomutase (8.8 μg), 0.75 unit of glucose-6-P dehydrogenase (2.1 μg), and an aliquot of a part one supernatant. The reaction was followed until a maximum change in absorbance occurred at 340 nm.

RESULTS

Changes in Starch and Pyrophosphorylase Activities during Potato Tuberization and Growth. The process of tuberization in Chieftan potatoes was accompanied by a rapid increase in starch content (Fig. 1). Compared to low starch stolon tissue, the initial tubers sampled (0.5 and 1 cm average diameter) had already established a high starch concentration of approximately 60% (w/w) on a dry weight basis. Low levels of pyrophosphorolysis activity for both UDP-glucose (11.2 units/g) and ADP-glucose (0.045 units/g) were present in stolon tissue. Both activities increased as tuberization and acceleration of starch deposition occurred. When tubers reached 1 cm in diameter

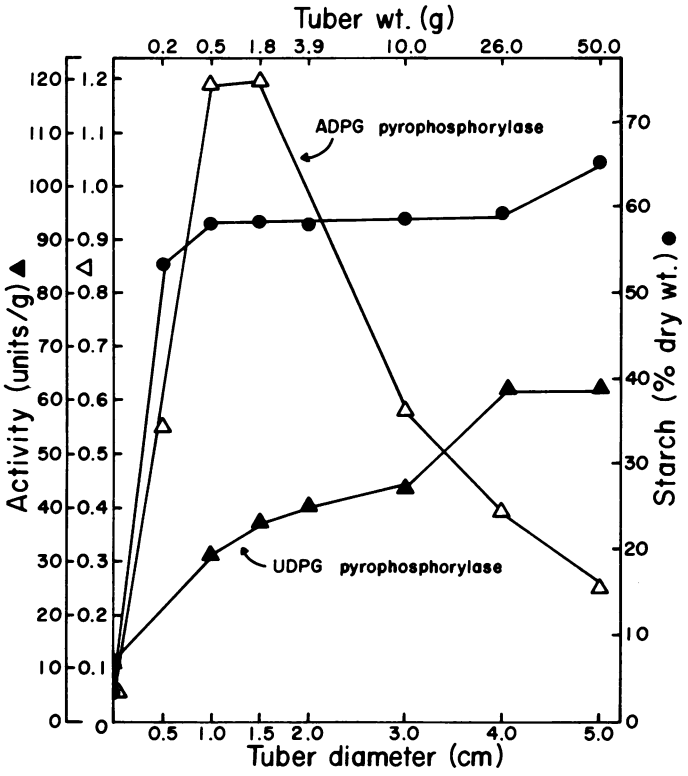

FIG. 1. Changes in starch, UDP-glucose and ADP-glucose pyrophosphorylase activities during tuberization and growth of Chieftan potatoes. Pretuberized stolon tissue is represented by a zero tuber diameter. Pyrophosphorylases were extracted from fresh tissue and were assayed according to the one-step system as described under "Materials and Methods."

(0.5 g) and established near 58% (w/w) starch. ADP-glucose pyrophosphorylase increased 24-fold over levels seen in pretuberized stolons. UDP-glucose pyrophosphorylase increased approximately 3-fold during this same period. Similar changes were also observed in tuberizing Norchip potatoes. As Chieftan tubers continued to develop from an average diameter of 1 cm to 5 cm (50 g) (Fig. 1), a high starch concentration was maintained, UDP-glucose pyrophosphorylase increased to a high near 60 units/g, while apparent ADP-glucose pyrophosphorylase decreased to about 0.25 unit/g. Throughout the remainder of the growth and maturation period the pyrophosphorolysis activities and starch content were maintained relatively constant.

Changes in Pyrophosphorylase Activities during Potato Storage. The high level of UDP-glucose pyrophosphorylase activity seen in mature Chieftan and Norchip potatoes was constant after harvest and no significant variation occurred during storage for 7 months at either 4 or 11 C. Mature levels of ADP-glucose pyrophosphorylase were maintained constant in potatoes stored at 4 C, while a gradual drop occurred at 11 C. The decrease seen at the higher temperature was slight, so that 25 to 35% of its mature activity still remained after prolonged storage (7 months).

Stability and Localization of Pyrophosphorylase Activities. No loss of total UDP-glucose or ADP-glucose pyrophosphorylase activity was observed during dialysis of the 75% ammonium sulfate fraction from fresh potatoes. A slight precipitate formed, which was removed by centrifugation, resulting in a loss of protein. Dialysis improved the assay system by eliminating a slight absorbance increase seen at 340 nm with minus PPi or enzyme blanks, possibly by removing endogenous substrates (*i.e.*, glucose-1-P, glucose-6-P) of the glucose-6-P quantitation system.

The two pyrophosphorylytic activities in the dialyzed extracts demonstrated differential stabilities when subjected to storage at 4 and −20 C (Fig. 2). UDP-glucose pyrophosphorylase lost only 14 to 19% of its original activity when stored for 16 days, and the majority of this loss occurred during the first 24 hr. It was equally stable at either temperature. Conversely, ADP-glucose pyrophosphorylase lost activity rapidly at 4 C and demonstrated a sharp lability upon freezing. At −20 C, only 24% of its original activity remained after 1 day, while 42% was lost after 4 days at 4 C. In routine analysis, however, extracts were assayed immediately after dialysis and not subjected to these storage conditions.

The specific activity of UDP-glucose pyrophosphorylase was extremely constant in most tuber areas studied, but was slightly lower in the outer cortical region (Table I). Its total activity, based on 100 g of tissue, was high in each area and increased slightly as one progressed from the outer cortical (5535 units) to the inner pith region (7330 units). ADP-glucose pyrophosphorylase was present in each area, and its total and specific activities were lowest in the pith area. Due to the respective changes in the two activities, the ratio of UDP-glucose to ADP-glucose

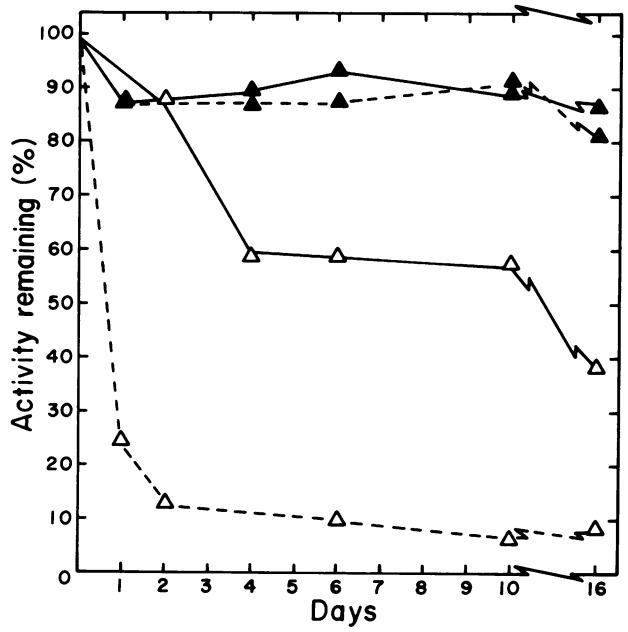

FIG. 2. Stability of UDP-glucose (▲) and ADP-glucose (△) pyrophosphorylase in extracts prepared from fresh Norchip tubers and stored at 4 C (——) and −20 C (– – –). Extracts were prepared and assayed according to the methods indicated in Fig. 1.

Table I. *Localization of UDP-Glucose and ADP-Glucose Pyrophosphorylase in Cellular Areas of Solanum tuberosum*

Pyrophosphorylases were extracted from fresh Norchip potatoes (avg weight 107 g) and were assayed according to the one step system described under "Materials and Methods." Results are based on 100 g fresh weight of each tuber area.

Cellular Area	Total Soluble Protein	Total Units		Specific Activity		U-/A-ppase
		U-ppase[1]	A-ppase	U-ppase	A-ppase	
	mg	*μmole glucose-1-P/min*		*μmole glucose-1-P/min·mg protein*		*ratio*
Cortical	407	5535	50.5	13.6	0.124	110
Vascular ring	442	6807	57.0	15.4	0.129	119
Premedullary	487	7451	48.7	15.3	0.100	153
Pith	461	7330	41.5	15.9	0.090	177

[1] U-ppase: UDP-glucose pyrophosphorylase; A-ppase: ADP-glucose pyrophosphorylase.

pyrophosphorylase increased gradually from 110 in the cortex to 177 in the pith.

Separation of ADP-Glucose and UDP-Glucose Pyrophosphorolysis Activities. The pyrophosphorolysis activites towards UDP-glucose and ADP-glucose could be separated by ammonium sulfate fractionation of extracts from potato acetone powders (Table II). The 30 to 45% ammonium sulfate precipitate contained 78.5% of the ADP-glucose pyrophosphorolysis activity evident in the initial acetone powder extract, while only 2.8% of UDP-glucose pyrophosphorolysis activity was present. The former activity was purified 3.2-fold in this fraction. The 45 to 90% ammonium sulfate precipitate contained 97.1% of the original UDP-glucose pyrophosphorolysis activity, which represented a purification of 2.5-fold. The remaining studies of the two pyrophosphorylases were conducted with their appropriate ammonium sulfate fraction.

Effect of Mg^{2+} on Pyrophosphorolysis. Optimum pyrophosphorolysis of UDP-glucose and ADP-glucose was observed in the presence of 5 to 8 mM Mg^{2+}. At these Mg^{2+} concentrations the highest UDP-glucose pyrophosphorylase actitivity occurred with a UDP-glucose to PPi ratio of 0.4 (UDP-glucose-2 mM/5 mM-PPi) (Fig. 3). The highest ADP-glucose pyrophosphorylase activity was evident with an ADP-glucose to PPi ratio of 1.3 (ADP-glucose-2 mM/1.5 mM-PPi) (Fig. 4).

Effect of pH and 3-P-Glycerate. UDP-glucose pyrophosphorylase activity as a function of pH plus or minus 3-P-glycerate is shown in Figure 5. A very broad pH curve was observed with very little change in optimum activity occurring between pH 7 and pH 9. A high concentration of 3-P-glycerate had no effect on its activity at any pH.

ADP-glucose pyrophosphorylase activity as a function of pH plus or minus 3-P-glycerate is shown in Figure 6. Both curves demonstrated an optimum activity near pH 7.5. 3-P-Glycerate activated the nonphotosynthetic potato enzyme over 40-fold at pH 7.5. No ADP-glucose pyrophosphorylase activity was demonstrated at the most alkaline pH values used unless this effector was present.

DISCUSSION

Both UDP-glucose and ADP-glucose pyrophosphorylases were present throughout tuberization, maturation, and storage of potato tubers. In stolon tissue and developing potatoes the activity of UDP-glucose pyrophosphorylase was always many-fold higher than the activity of ADP-glucose pyrophosphorylase. This has also been observed in other starch-forming tissues such as pea seed (22), rice and bean leaf (13), wheat grain (21), and maize endosperm (24). As tuberization and a simultaneous rapid increase in starch content occurred (Fig. 1), there was a sharp rise in ADP-glucose pyrophosphorylase activity while UDP-glucose pyrophosphorylase activity increased gradually over its level seen in low starch stolon tissue. Consequently, the ratio of UDP-glucose to ADP-glucose pyrophosphorylase activities in Chieftan potatoes decreased from 222 to 27 during initiation of maximal starch formation. A similar ratio change was

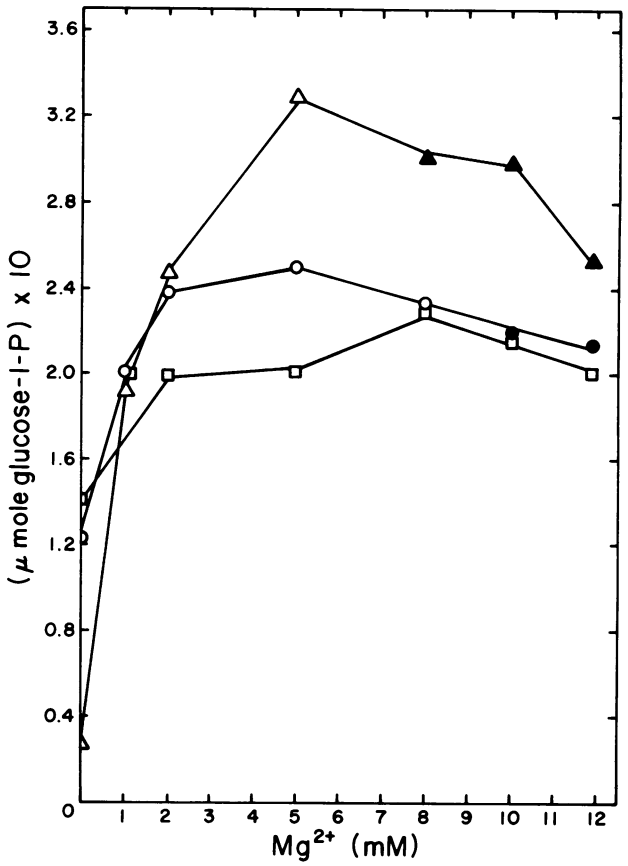

FIG. 3. Effect of Mg^{2+} concentration on UDP-glucose pyrophosphorylase activity in the presence of various UDP-glucose to PPi ratios. The pyrophosphorylase extract was prepared from Norchip acetone powders (45–90% ammonium sulfate precipitate, see Table II) and assayed according to the two-step system described under "Materials and Methods." Each assay mixture, pH 8, contained 2 μmoles of UDP-glucose, 80 μmoles of glycylglycine, 10 μmoles of NaF, MgCl (0–12 μmoles), and 0.02 unit of diluted enzyme (1.7 μg). Reactions were initiated by addition of 5 μmoles (△), 2 μmoles (○), or 1 μmole (□) of PPi to give a total volume of 1 ml. The closed symbols represent precipitation of a Mg-pyrophosphate chelate during the incubation period.

Table II. *Separation of UDP-Glucose and ADP-Glucose Pyrophosphorylase from Norchip Acetone Powders: Ammonium Sulfate Precipitation*

Pyrophosphorylases were extracted from Norchip acetone powders (prepared from tubers of 200 g avg weight), and were subjected to ammonium sulfate precipitation, and activities were assayed according to the one step system described under "Materials and Methods." Results are based on sampling 15 g of powder.

Fraction	Total Soluble Protein	Total Units		Specific Activity		Yield		Purification		Specific Activity U-/A-ppase
		U-ppase[1]	A-ppase	U-ppase	A-ppase	U-ppase	A-ppase	U-ppase	A-ppase	
	mg	*μmole glucose-1-P/min*		*μmole glucose-1-P/min · mg protein*		*%*		*-fold*		*ratio*
Acetone powder extract	336	5678	57.1	16.9	0.17	100	100			99.4
Ammonium sulfate										
ppt 0–30%	22	33	0.7	1.5	0.03	0.6	1.2	0.1	0.2	50
ppt 30–45%	83	158	44.8	1.9	0.54	2.8	78.5	0.1	3.2	3.5
ppt 45–90%	131	5515	3.9	42.1	0.03	97.1	6.8	2.5	0.2	1403.3

[1] U-ppase: UDP-glucose pyrophosphorylase; A-ppase: ADP-glucose pyrophosphorylase.

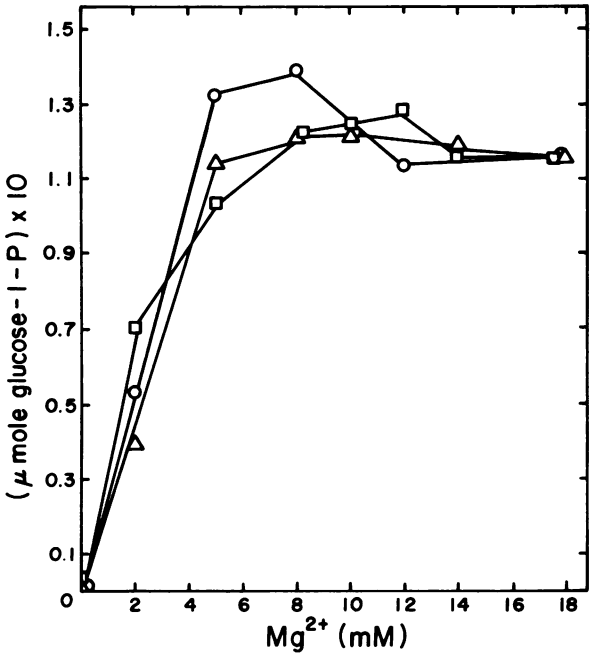

FIG. 4. Effect of Mg^{2+} concentration on ADP-glucose pyrophosphorylase activity in the presence of various ADP-glucose to PPi ratios. The pyrophosphorylase extract was prepared from Norchip acetone powders (30–45% ammonium precipitate, see Table II) and assayed according to the two-step system described under "Materials and Methods." Each assay mixture, pH 7.5, contained 2 μmoles of ADP-glucose, 80 μmoles of glycylglycine, 10 μmoles of NaF, $MgCl_2$ (0–18 μmoles), 2 μmoles of 3-P-glycerate, and 0.01 units of diluted enzyme (20 μg). Reactions were initiated by the addition of 2 μmoles (△), 1.5 μmoles (○), or 1 μmole (□) of PPi to give a total volume of 1 ml. See the text for other experimental details.

observed in tuberizing Norchip potatoes. It is indicated that the onset of α-glucan synthesis in potato tubers may be closely related to the rapid increase in ADP-glucose pyrophosphorylase activity.

The increase in ADP-glucose pyrophosphorylase activity/g of tissue coincided principally with the procambial cellular division phase of the tuberization process (17, 18). After a fairly constant starch content was attained, the ADP-glucose pyrophosphorylase activity/g of tissue decreased (Fig. 1). This apparent drop occurred during the period when tuber growth is primarily by cellular enlargement (18). The decrease in its activity may partially reflect a "dilution effect" where its unit activity/g of tissue drops but its elevated activity per cell may remain fairly constant. Also, if the enzyme is associated with the starch granule as suggested by Murata *et al.* in leaf tissue (11) and rice grains (12), this drop in apparent activity may reflect an increase in the affinity of the enzyme for the established starch particle. If this occurs, then the ADP-glucose pyrophosphorylase activity indicated in this study may be considered minimal and could represent that portion of enzyme solubilized by the extraction procedure utilized. The instability of the extracted enzyme to freezing and storage at 4 C compared to the stability of UDP-glucose pyrophosphorylase (soluble enzyme) (Fig. 2), may lend support to a particulate origin. Once ADP-glucose pyrophosphorylase becomes solubilized it rapidly loses its specially active conformation. It was noted that increasing the number of short-interval sonication periods (4–8 C) did not result in higher yields of either pyrophosphorylase activity in extracts from acetone powders. Studies with starch particle samples prepared and subjected to various treatments will be necessary to clarify any affiliation of ADP-glucose pyrophosphorylase with potato starch granules.

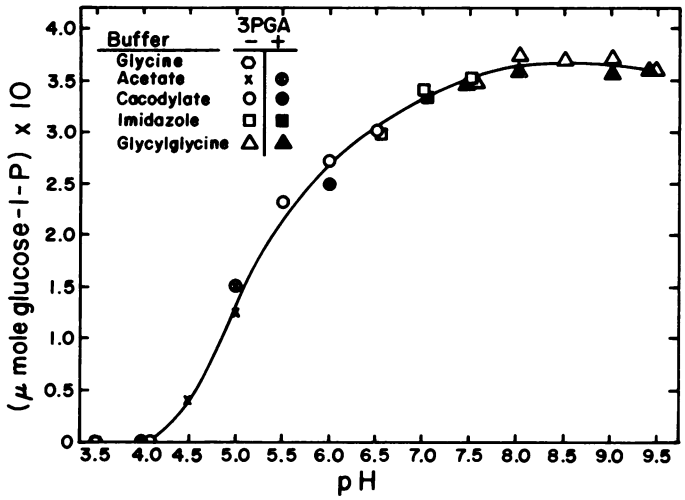

FIG. 5. UDP-glucose pyrophosphorylase activity as a function of pH in the absence or presence of 2 mM 3-P-glycerate (3PGA). The pyrophosphorylase extract was prepared from Norchip acetone powders (45–90% ammonium sulfate precipitate, see Table II) and assayed according to the two-step system described under "Materials and Methods."

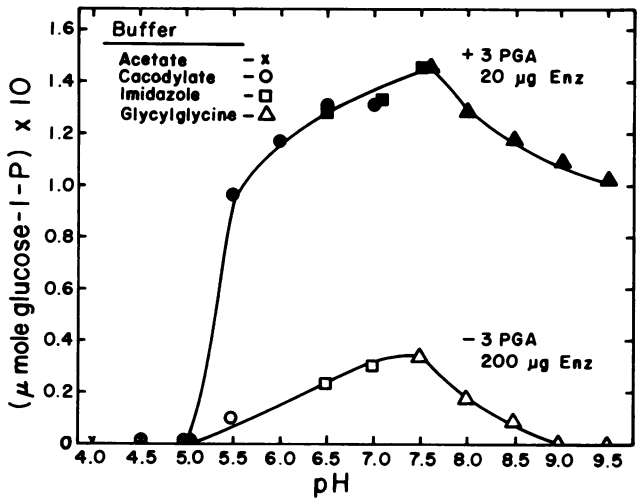

FIG. 6. ADP-glucose pyrophosphorylase activity as a function of pH in the absence or presence of 2 mM 3-P-glycerate (3PGA). The pyrophosphorylase extract was prepared from Norchip acetone powders (30–45% ammonium sulfate precipitate, see Table II) and assayed according to the two-step system described under "Materials and Methods."

There appeared to be a good correlation between the cellular area localization of ADP-glucose pyrophosphorylase (Table I) relative to starch gradients in potato tubers (18). Both ADP-glucose pyrophosphorylase activity and starch content are highest in outer cortical and premedullary regions, and decrease slightly as one approaches the inner "water core" pith area.

It is doubtful that free sugar accumulation in cold-stored potatoes is significantly influenced by either UDP-glucose or ADP-glucose pyrophosphorylase, since their activities showed little fluctuation during prolonged storage at 4 C.

The pyrophosphorylytic activity ratio change towards UDP-glucose and ADP-glucose seen during tuberization of potatoes suggests that separate protein entities were involved in the potato extracts. Additional evidence was provided by their differential stabilities, and by their ability to be separated by ammonium sulfate precipitation. When extracts from fresh potato extracts were stored at −20 C for only 2 days (Fig. 2), the

UDP-glucose to ADP-glucose pyrophosphorylase activity ratio increased 7-fold. Separation of their two activities was accomplished by precipitation with ammonium sulfate (Table II). Their activity ratio demonstrated a striking change from 99.4 in the initial acetone powder fraction to 3.5 and 1403 in the 30 to 45% and 45 to 90% ammonium sulfate fractions, respectively. Maximum pyrophosphorolysis of UDP-glucose or ADP-glucose occurred in the presence of 5 to 8 mM Mg^{2+}. Although some UDP-glucose pyrophosphorylase activity was observed in the absence of this cation (Fig. 3), ADP-glucose pyrophosphorylase demonstrated an absolute requirement for Mg^{2+} regardless of saturating levels of ADP-glucose and PPi used (Fig. 4). Optimal pyrophosphorolysis of UDP-glucose in pea seeds also occurred with 5 mM Mg^{2+} (20). The substitution of other cations for Mg^{2+} remains to be studied. These two partially purified fractions also exhibited differential pH requirements and differential activation with 3-P-glycerate. The broad pH curve seen with potato UDP-glucose pyrophosphorylase (Fig. 5) was very similar to that observed with the pea seed enzyme (20). The metabolite 3-P-glycerate did not effect its activity at any pH. Conversely, ADP-glucose pyrophosphorylase demonstrated little activity from pH 5.5 to 8.5 compared to when 3-P-glycerate was present (Fig. 6). The activation with this effector appeared to be pH-dependent. Activations of 40-, 70-, and 131-fold were observed at pH 7.5, 8, and 8.5, respectively, under the experimental conditions used. Above pH 8.5, no activity was seen in the absence of 3-P-glycerate. The physiological significance of activation of nonphotosynthetic potato tuber ADP-glucose pyrophosphorylase by 3-P-glycerate, which is a positive effector of this enzyme in plant leaves (7, 14, 19), is presently under investigation.

This study supports the importance of adenine diphosphate glucose (ADPG) in potato starch synthesis. Previous investigations by Frydman and Cardini (3–6) have shown that adenine nucleotide sugar derivatives serve as superior glucosyl donors for potato particulate and soluble starch synthetases. In studying nucleotide patterns isolated from potato starch granules, Rees and Duncan (16) found a predominance of adenine nucleotides, while ADP-glucose was the only sugar nucleotide present. Accompanying ADP-glucose were always other metabolites that could lead to its continued production. They supported the view that enzyme systems forming and utilizing ADP-glucose are of great physiological significance in potato starch synthesis. The sharp rise in potato ADP-glucose pyrophosphorylase accompanying tuberization observed in this study correlates well with the metabolic trends and experimental findings listed above. Although participation of UDP-glucose in starch formation can not be neglected, it is suggested that the onset of rapid tuber starch biosynthesis may be closely related to the simultaneous increase in potato ADP-glucose pyrophosphorylase activity.

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