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UNPRIMED STARCH SYNTHESIS BY SOLUBLE ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE FROM POTATO TUBERS

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Abstract—Soluble ADPglucose:α-1,4-glucan α-4-glucosyltransferase from potato tubers (*Solanum tuberosum* L.) was partially purified by chromatography on DEAE-cellulose. The enzyme transferred glucose from ADPglucose to several glucan primers. In addition, the enzyme synthesized glucan in the absence of added primer in a reaction mixture containing sodium citrate and bovine serum albumin. The rate of synthesis of glucan in the absence of primer was half the rate found with amylopectin as primer and was linear with time in 0·5 M sodium citrate. The unprimed product was a methanol precipitable glucan with principally α-1,4-linkages and some branches which were cleaved by glucoamylase. The enzyme showed unprimed activity after treatment of both the enzyme and reaction mixtures with glucoamylase.

INTRODUCTION

BIOSYNTHESIS of α-1,4-glucosidic linkages of starch in higher plants is generally considered to be catalysed by ADPglucose:α-1,4-glucan α-4-glucosyltransferase (ADPglucose–starch glucosyltransferase).[1] Although, phosphorylase ctalyses the synthesis of α-1,4-glucosidic linkages *in vitro*, many workers believe that *in vivo*, it is involved primarily in starch breakdown.[2] Most enzyme preparations of both ADPglucose–starch glucosyltransferase and phosphorylase only transferred glucose from ADPglucose or glucose-1-P to a glucan primer to form α-1,4-glucosidic bonds. Thus no evidence was available as to which enzyme or enzymes are responsible for primer synthesis *in vivo*. However, recently, phosphorylases have been extracted from maize kernels and potato tubers which synthesize α-1,4-glucans in the presence of added primers[3,4] and a partially purified ADPglucose–starch glucosyltransferase from spinach leaves synthesized starch from ADPglucose without added primer.[5] The possibility that a small amount of glucan was acting as a primer for these enzymes cannot be discounted because treatment of a crystalline phosphorylase from potato tubers with glucoamylase abolished its unprimed activity.[6] Glucose-1-P has also been shown to contain traces of oligosaccharides.[7]

A soluble ADPglucose–starch glucosyltransferase from potato tubers has been described which transfers glucose from ADPglucose to various primers.[8] The present paper reports a

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soluble ADPglucose–starch glucosyltransferase from potato tubers which catalyses the formation of a polyglucan in the absence of added primer. Prior treatment of the enzyme and reaction mixtures with glucoamylase does not appreciably decrease polyglucan formation in the absence of added primer.

RESULTS

Purification of ADPglucose-starch Glucosyltransferase

Three peaks of activity (I, II and III) were obtained with primer by elution from DEAE-cellulose (Table 1). In the presence of 0·5 M Na_3 citrate and BSA, (bovine serum albumin) unprimed activity was also found in each of the three peaks. UDPglucose (0·6 mM) did not

TABLE 1. PURIFICATION OF ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE FROM POTATO TUBERS

Fraction	Vol. (ml)	Total protein (mg)	Total units* of enzyme		Specific activity (units/mg protein) × 10^4	
			Primed	Unprimed	Primed	Unprimed
Crude	138	537	9·80	0·04	186	1
45 000 *g* supernatant fluid	134	493	9·85	0·02	200	<1
0–40% satd. $(NH_4)_2SO_4$	8·5	122	7·1	1·75	582	143
DEAE-cellulose						
Fraction I (2–5)†	1·5	27	0·23	0·04	85	15
Fraction II (22–33)	1·7	2·9	1·87	0·97	6450	3350
Fraction III (44–48)	1·5	2·1	0·14	0·06	667	286

* One unit = 1 μmol [^{14}C]glucose transferred per min at 30°. Assays were carried out using the anion exchange resin method.
† Fraction number.

replace ADPglucose as a glucose donor in any of the three pooled fractions, but inucbation with [^{14}C]glucose-1-P (0·5 mM) and amylopectin resulted in some incorporation of label into methanol precipitable product with Fraction I and III. Fraction I was a brown solution containing protein which was not absorbed on the DEAE-cellulose. Only Fraction II was studied in detail and all further experiments were carried out using this cut which contained, in addition to ADPglucose–starch glucosyltransferase, branching enzyme and a small amount of amylase(s).

Amylopectin, amylose, oyster glycogen and rabbit liver glycogen at 5 mg/ml final concentration were about equally effective as primers while the activity with 0·5 M maltose was 40% lower. Unless otherwise indicated, primed and unprimed reaction rates were linear with time and protein concentration in the range used to obtain the data reported.

Unprimed Reaction

Unprimed activity was not found with reaction mixtures containing low concentrations of salts. However, in the presence of sodium citrate and BSA, unprimed activity was apparent (Fig. 1) and at 0·5 M sodium citrate the rate was linear with time. Although not examined further, the stimulation is unlikely to be specific for citrate ions since many other anions stimulate the unprimed activity of spinach leaf enzyme[5] and waxy maize enzyme (unpublished results).

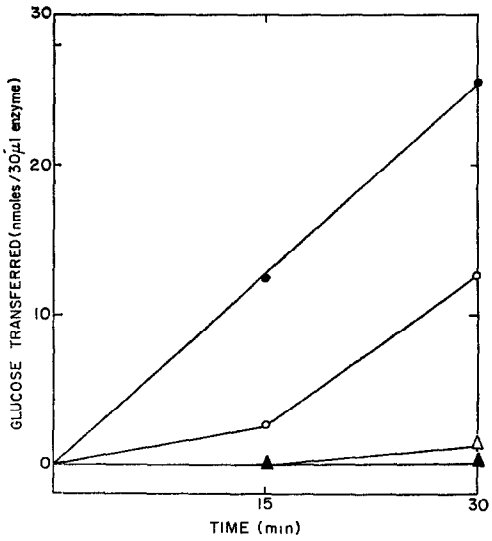

FIG. 1.

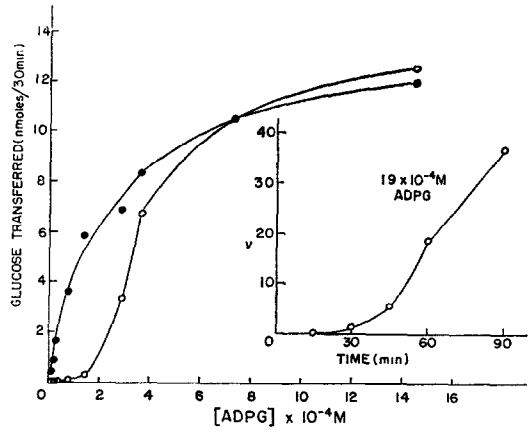

FIG. 2.

FIG. 1. EFFECT OF SODIUM CITRATE ON THE UNPRIMED ACTIVITY OF POTATO TUBER ADPGLUCOSE-STARCH GLUCOSYLTRANSFERASE.
Standard reaction mixtures for unprimed assays were used containing 0·5 M Na citrate (●), and other reaction mixtures contained 0·37 M (○), 0·25 M (△), 0·12 M and 0 Na citrate (▲). The anion exchange resin method was used.

FIG. 2. PRIMED (●) AND UNPRIMED (○) ACTIVITY OF POTATO TUBER ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE PLOTTED AGAINST ADPGLUCOSE CONCENTRATION
The insert is a time course of the unprimed reaction with 0·19 mM ADPglucose. The methanol precipitation method was used.

The K_m for ADPglucose using amylopectin as primer was 0·25 mM compared to 0·15 mM found previously for a soluble ADPglucose-starch glucosyltransferase from potato tubers.[8] At low concentrations of ADPglucose, the rate of synthesis of unprimed product was not linear with time and hence the K_m for ADPglucose could not be calculated (Fig. 2). However, the saturation curve shows that at approximately 0·4 mM the rate of glucose transfer was about half that observed at 1·45 mM indicating that the enzyme had high affinity for ADPglucose under these conditions (Fig. 2).

Unprimed Product

The absorption spectrum of the iodine-product complex was typical of that obtained for starches and suggested that the product was an α-1,4-glucan (Fig. 3). Treatment of the product with α-amylase and β-amylase showed that the product was in fact an α-1,4-glucan with some branch points and treatment with glucoamylase suggested that these branch points were α-1,6 linkages (Table 2). However, repeated attempts to completely hydrolyse the radioactive product to maltose by treatment with either isoamylase or pullulanse followed by β-amylase were unsuccessful. Both isoamylase and pullulanase are specific for α-1,6 linkages[9,10] and glucoamylase breaks both α-1,4 and α-1,6 linkages.[11] The glucoamylase

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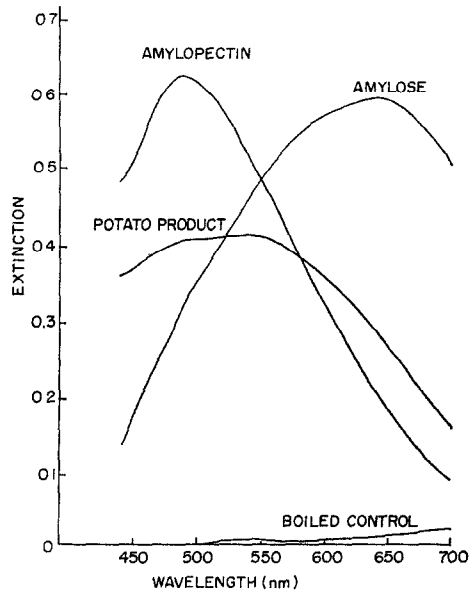

FIG. 3. ABSORPTION SPECTRA IN SATURATED $CaCl_2$ OF COMPLEXES OF IODINE WITH AMYLOSE, AMYLOPECTIN AND THE PRODUCT FORMED BY POTATO ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE IN THE ABSENCE OF PRIMER.
Heat inactivated enzyme was used in the control reaction.

used in the present work did not release glucose from nigeran (an α-1,3 glucan). The data obtained by treatment of the product with isoamylase and pullulanase cannot be explained. Pullulanase plus β-amylase treatment and isoamylase plus β-amylase treatment of spinach[5] and maize product (unpublished results) resulted in 97–99% conversion of the product to maltose and glucose. Further work on the potato product seemed unwarranted until an enzyme free of amylase could be prepared.

Glucoamylase Treatment of Potato ADPglucose-starch Glucosyltransferase

Preliminary results with sucrose density gradient centrifugation[12] showed that the potato enzyme behaved as a protein with a MW of about 90 000 suggesting that no high MW polymer such as amylopectin was bound to the enzyme.

TABLE 2. ANALYSIS OF PRODUCT FORMED BY POTATO TUBER ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE IN THE ABSENCE OF PRIMER

Product treatment	% of total ^{14}C incorporated		
	Origin	Maltose	Glucose
None	100	0	0
β-Amylase	42	58	0
α-Amylase	22	35	43
Glucoamylase	1	0	99
Isoamylase	100	0	0
Isoamylase + β-Amylase	52	48	0
Pullulanase	100	0	0
Pullulanase + β-Amylase	58	42	0

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In order to determine whether a small amount of glucan capable of acting as primer was associated with the transferase, 50 μg of protein (30 μl of enzyme preparation) was treated with 2 μg of glucoamylase (an amount of enzyme shown to release 270 μg of glucose from amylopectin under the conditions indicated in Table 3) prior to use in the primed and unprimed reaction. The results show that the glucoamylase together with contaminating α-1,4 glucan hydrolases present in the potato preparation was sufficient to convert 400 μg amylopectin from an effective primer to an almost ineffective primer (Table 3, Treatments 3 and 4, unprimed). Treatment of the enzyme with glucoamylase did not affect the rate of the unprimed reaction [Table 3, Treatments 1 and 2, unprimed (citrate + BSA)]. After incubation of reaction mixtures containing 140 nmol of ADPglucose with 50 μg of glucoamylase (enough to release 500 nmol of glucose from amylopectin under similar conditions), unprimed activity was still present (Table 3, Treatment 5). The activity of glucoamylase was 50–60-fold greater when incubated with potato enzyme at pH 7·0, than in the reaction mixture at pH 8·5 used for assaying transferase activity. The results in Table 3 also show that the primed reaction was not stimulated by citrate and BSA anywhere near as much, if at all, as the unprimed reaction. The rate of glucose transfer in the presence of amylopectin, citrate and BSA was a little greater than the rate in the presence of amylopectin alone (Table 3, Treatment 3). This may have been due to a combination of both primed and unprimed activity.

TABLE 3. EFFECT OF GLUCOAMYLASE ON UMPRIMED ACTIVITY OF ADPGLUCOSE–STARCH GLUCOSYLTRANSFERASE FROM POTATO TUBERS

Type of assay Prior treatment of enzyme	Glucose transferred (nmol/7 μg protein/30 min)					
	Primed		Unprimed		Unprimed (Citrate + BSA)	
	Resin	Methanol	Resin	Methanol	Resin	Methanol
1 Potato enzyme* − glucoamylase	44	40	0	0	27	27
2 Potato enzyme* + glucoamylase	44	38	0	0	23	24
3 Potato enzyme† + amylopectin	46	41	34	26	45	49
4 Potato enzyme* + amylopectin + glucoamylase	38	31	0·5	0·2	20	16
5 Potato enzyme + BSA* + glucoamylase	—	—	0‡	0‡	19‡	19‡

* Enzyme (50 ug) was incubated at pH 7·0 (7·5 mM Hepes buffer) and 30° for 80 min with 2 μg glucomylase, 500 μg amylopectin or 500 μg BSA as indicated, prior to assay with [^{14}C]ADPglucose.

† Amylopectin (500 μg) added at end of 80 min incubation.

‡ Reaction mixtures minus enzyme and BSA were incubated for 1 hr at 30° with 50 μg glucoamylase and boiled for 2 min prior to assay.

DISCUSSION

The primed reaction of the soluble ADPglucose–starch glucosyltransferase partially purified from potato tubers is similar to that found previously.[8] However, the unprimed

reaction has not been observed before and this is probably due to the conditions required for the reaction to proceed. Although high concentrations of sodium citrate are non-physiological, other anions below sulphate ions in the chaotropic series[13] stimulate the activity of spinach enzyme.[5] It is possible that the conditions used *in vitro* simulate the environment *in vivo*. The high salt concentration and bovine plasma albumin may cause the transferase to be in the conformation active for unprimed synthesis. *In vivo* other factors may serve in this role.

The product of the unprimed reaction has been shown to contain α-1,4 linkages and some branch points which may be α-1,6 linkages even though not broken by either isoamylase or pullulanase. The potato enzyme preparation converted amylose to a compound which had an iodine absorption spectrum characteristic of a branched glucan and it seems likely that branching enzyme resulted in a branched product being formed in the unprimed reaction. The presence of unprimed activity even after glucoamylase treatment of the ADPglucose–starch glucosyltransferase strongly suggests the absence of an α-1,4 or α-1,6 glucan primer attached to the enzyme. Treatment of a crystalline potato phosphorylase with glucoamylase abolished unprimed activity.[6] However, the presence of a small glucoamylase resistant primer cannot be discounted.

Potato tubers contain ADPglucose pyrophosphorylase[14] which synthesizes ADP-glucose, the substrate for both primed and unprimed reactions of ADPglucose–starch glucosyltransferase. Activity of phosphorylase in potato tubers is about 1 μmol Pi released from glucsoe-1-P/min/g fresh wt at 37°.[15] The activity found for ADPglucose-starch glucosyltransferase in the present work was 0·1 μmol glucose transferred from ADPglucose/min/g fresh wt at 30°. It seems possible that the latter enzyme might initiate and maintain starch synthesis in potato tubers.

EXPERIMENTAL

Materials. ADP[^{14}C]glucose was prepared from α-glucose-1-P.[16] Glucoamylase from *Rhizopus* was purchased from Agaku Kogyo Co., Japan. *Cytophaga* isoamylase and pullulanase were kindly provided by Dr. W. J. WHELAN and E. Y. C. Lee of the Department of Biochemistry, University of Miami, Florida.

Purification of ADPglucose–starch glucosyltransferase. Freshly harvested immature (5 cm dia.) tubers of *Solanum tuberosum* L. variety, White Rose, were peeled and macerated (100 g) in a Waring blender with 100 ml of a solution containing 50 mM Tris-acetate buffer, pH 7·5, 0·1% bovine serum albumin (BSA), 10 mM EDTA, 2 mM $Na_2S_2O_5$ and 10% sucrose for 1 min. All operations were carried out at 0–4°. The supernatant fraction resulting from centrifugation at 45 000 *g* for 20 min was made to 40% saturation with solid $(NH_4)_2SO_4$ and centrifuged at 30 000 *g* for 15 min. The precipitate was dissolved in about 8 ml of 50 mM Hepes buffer, pH 7·0, containing 5 mM dithiothreitol (DTT), 10 mM EDTA, and 10% sucrose and dialyzed overnight against the same solution.

ADPglucose–starch glucosyltransferase was further purified on a 30 ml column of DEAE-cellulose as described previously[5] except that 1 l. of buffer with increasing concentrations of KCl (linear gradient 0–0·4 M KCl) was used and collected in 9·8 ml fractions. The enzymes were stored in 50 mM Hepes buffer, pH 7·0, containing 5 mM DTT, 10 mM EDTA and 10% sucrose at 0°.

Assay of ADPglucose–starch glucosyltransferase. Transfer of [^{14}C]glucose to a primer, or formation of a [^{14}C]-labelled glucan in the absence of primer was determined as previously described[5] at 30°. Primed reaction mixtures contained 140 nmol of ADP[^{14}C]glucose (400–600 cpm/nmol), 20 μmol of Bicine buffer, pH 8·5, 5 μmol of K OAc, 2 μmol of GSH, 1 μmol of EDTA, 1 mg of amylopectin (amylose free) and enzyme in a final vol. of 0·2 ml. Unprimed reaction mixtures were as above except KOAc and amylopectin were replaced by 100 μmol of Na_3 citrate and 100 μg of BSA. In the assay for unprimed activity, the reaction was stopped by heating at 100° for 1 min and carrier amylopectin (0·1 ml, 1 mg) was added prior to methanol precipitation.

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In some experiments the reaction was stopped by heating for 1 min at 100° and either 2 or 4 ml of a slurry containing 400 mg of anion exchange resin (Dowex 1-X8,200–400 mesh) were added to absorb the remaining ADP[^{14}C]glucose. In the assay for unprimed activity 4 ml of resin were needed to absorb the ADP[^{14}C] glucose in the presence of 0·5 M Na_3 citrate. After filtration through Whatman No. 1 filter paper, the radioactivity in 0·5 ml of the filtrate was determined in a liquid scintillation counter.

Identification of product. Radioactive product containing carrier amylopectin was incubated at 37° and pH 5·5 (100 mM Na acetate buffer) with either sufficient β-amylase, α-amylase, glucoamylase, isoamylase, pullulanase, isoamylase plus β-amylase or pullulanase plus β-amylase to cause the reactions to reach completion within 4 hr. The extent of hydrolysis and identity of hydrolysis products was determined by chromatography on Whatman No. 1 paper in both BuOH–pyridine–H_2O (6:4:3, by vol.) and 95% EtOH–M NH_4OAc, pH 3·8 (5:2, by vol.). Radioactive spots were detected by a strip counter and identified by co-chromatography with standards. These spots were cut out and counted in a liquid scintillation counter with toluene scintillation fluid.

The I_2 absorption spectrum of the unprimed product formed by incubating enzyme with unlabelled ADPglucose in the reaction mixture with 0·5 M Na_3 citrate and BSA but no GSH, was determined on a solution containing 50 μl of heat inactivated reaction mixture (about 25 μg of product) in 0·95 ml of I_2 reagent with saturated $CaCl_2$.[17]

Treatment of enzyme with glucoamylase. Samples (30 μl) of the partially purified ADPglucose–starch glucosyltransferase were incubated with glucoamylase in a final volume of 0·2 ml as indicated in Table 3. Samples (30 μl) were assayed for primed and unprimed activity and also assayed using the primed assay conditions but with amylopectin (the primer) omitted. The amount of [^{14}C]glucose in polysaccharide or in polysaccharide plus oligosaccharides was determined by using both methanol precipitation and anion exchange resin methods respectively. The activity of the glucoamylase under the above conditions was determined by adding 2 mg of amylopectin to the reaction mixtures and measuring the glucose released (Glucostat, Worthington).

Other enzymes. Qualitative tests for α-1,4-glucan hydrolysases and branching (Q) enzyme in the potato preparation were carried out by incubating enzyme with amylose and observing the decrease in the extinction at both 630 and 520 nm or decrease in the ratio of extinction at 630–520 nm of the amylose-I_2 complex.[17]

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Key Word Index—Solanum tuberosum; Solanaceae; potato; starch synthesis; ADPglucose–starch glucosyltransferase.