Plant Physiol. (1973) 51, 1-5

Starch Synthetase, Phosphorylase, ADPglucose Pyrophosphorylase, and UDPglucose Pyrophosphorylase in Developing Maize Kernels

Received for publication June 29, 1972

J. L. OZBUN,[1] J. S. HAWKER,[2] E. GREENBERG, C. LAMMEL, AND JACK PREISS

Department of Biochemistry and Biophysics, University of California, Davis, California 95616

E. Y. C. LEE

Department of Biochemistry, School of Medicine, University of Miami, Miami, Florida 33152

ABSTRACT

Soluble ADPglucose-α-glucan 4-α-glucosyltransferase (starch synthetase), ADPglucose pyrophosphorylase, UDPglucose pyrophosphorylase and phosphorylase were assayed in extracts from developing kernels of maize (*Zea mays*). Normal, waxy and amylose-extender maize at stages of development ranging from 8 days to 28 days after pollination were studied. Shrunken-4 maize at the 22-day stage was also studied. There is adequate activity of both ADPglucose pyrophosphorylase and starch synthetase at all stages of development to account for the synthesis of starch. Thus all starch could be synthesized via the ADPglucose pathway. High levels of UDPglucose pyrophosphorylase and of phosphorylase activities were also found at all stages of development. The possible role of phosphorylase in starch synthesis could not be discounted. The levels of phosphorylase, ADPglucose pyrophosphorylase, starch synthetase, and UDPglucose pyrophosphorylase activities in shrunken-4 kernels were about 20 to 40% of that found in normal maize kernels. It appears that the mutation in shrunken-4 affects the activities of more than one enzyme. The defective starch synthesis seen in this mutant could be due to the low activities of ADPglucose pyrophosphorylase and starch synthetase rather than the low activity of phosphorylase.

Biosynthesis of α-1,4-glucosidic linkages of starch in higher plants is generally considered to be catalyzed by ADPglucose-α-1,4-glucan 4-α-glucosyltransferase (starch synthetase) (13). It has recently been shown that some forms of this enzyme extracted from spinach, maize, and potato can synthesize α-1,4-glucosidic linkages in the absence of added primer (7, 11, 12). The capacity of the enzyme to catalyze unprimed synthesis of α-1,4-glucosidic linkages further supports the hypothesis that starch synthetase may also account for the initiation of starch synthesis. However, data of Tsai *et al.* (18) would suggest that ADPglucose pyrophosphorylase and soluble starch synthetase are not present at the early stages of maize kernel development (8 and 10 days postpollination) when some starch is being synthesized. These authors further suggest that the synthesis of starch in the early stages of endosperm development proceeds by a different pathway than is functional later.

In the present paper we show that ADPglucose pyrophosphorylase and soluble starch synthetase can account for starch synthesis at all stages of endosperm development from 8 to 28 days postpollination. They are present at all stages of development and at levels which can account for all starch synthesis.

MATERIALS AND METHODS

Developing kernels of normal (W64A inbred), waxy (W64A, BC-8X[2]), and amylose-extender (W64A, BC-8X[2]) maize (*Zea mays*) were harvested from the field 8, 10, 12, 14, 16, 22, and 28 days after controlled selfpollination. In addition, ears of shrunken-4 (W64A, BC-1X[2]) were harvested 22 days after pollination. The shrunken-4 allele, obtained from Dr. Roy Creech, was shrunken-4 standard (sh_4^s) and was originally obtained from Dr. Oliver E. Nelson. The ears were frozen on Dry Ice in the field and stored at −15 C until used. Kernels from three ears of each line and at each harvest date were composited for enzyme extraction and carbohydrate analysis.

The following solvent systems were used for descending paper chromatography on Whatman No. 1 filter paper: (*a*) solvent A, 95% ethanol-M ammonium acetate, pH 3.8 (5:2); (*b*) solvent B, isobutyric acid-1 M NH_4OH-0.1 M EDTA, pH 7.2, (10:6:0.16).

Carbohydrate Determinations. To 0.5 g of frozen kernels were added 5 ml of 75% (v/v) ethanol. The kernels were thawed and ground in the ethanol and then heated for 20 min in a boiling H_2O bath. After cooling, the suspension was centrifuged at 10,000*g* for 10 min. The supernatant fluid was decanted, and the starch precipitate was extracted a second time as above. The supernatant fluids were combined, evaporated to dryness, and dissolved in 1 ml of H_2O. This solution was used for analyses of reducing sugars (10), total soluble sugars (6), and sucrose (6).

The starch precipitate was suspended in 2 ml of 0.2 N KOH, boiled for 30 min, and neutralized to pH 5.5 with M acetic acid. The starch suspension was incubated with 200 μg of purified *Rhizopus nivens* glucoamylase (Miles Laboratories, Inc.; 45 International units per mg of protein) at 37 C for 12 to 24 hr. Quantitative hydrolysis of the starch to glucose under these conditions was indicated by the complete hydrolysis of

[1] Present address: Vegetable Crops Department, Cornell University Ithaca, N. Y. 14850.

[2] Present address: Division of Horticultural Research, CSIRO, Box 350, Adelaide, Australia 5001.

30 mg of amylopectin added to 0.5 g of either the 8- or 28-day-old endosperm. Glucose was determined with glucose oxidase (5).

Enzyme Extraction. Kernels (5 g) were ground in a mortar with 10 ml of a solution containing 0.05 M tris-acetate buffer, pH 7.5, 10 mM EDTA, 2 mM DTT, and 10% sucrose for 15 min and filtered through cheesecloth. The crude enzyme extract was dialyzed overnight against the extracting buffer followed by centrifugation in an ultracentrifuge at 105,000*g* for 1 hr. The 105,000*g* precipitate was resuspended in extracting buffer. All operations were carried out at 0 to 4 C.

Enzyme Assay. ADPglucose and UDPglucose pyrophosphorylase activity were determined on the undialyzed crude extract as activity was greatly reduced during dialysis. Starch synthetase activity was determined on the crude, dialyzed 105,000*g* supernatant fluid and 105,000*g* precipitate using amylopectin or rabbit liver glycogen as primer. Unprimed α-1,4-glucan synthesis (12) was determined in the 105,000*g* supernatant fluid. Phosphorylase activity was determined in the supernatant fraction of the crude extract centrifuged for 15 min at 15,000*g*.

Pyrophosphorolysis of ADPglucose was determined by the formation of ATP-^{32}P from $^{32}PP_i$, and glucose-^{14}C-1-P was used to measure the synthesis of ADPglucose-^{14}C and UDPglucose-^{14}C (3). The pyrophosphorolysis reaction mixture contained in a volume of 0.25 ml, 20 μmoles of (N-2-hydroxyethylpiperazine-N^1-2 ethane-sulfonic acid) HEPES buffer, pH 8.0, 50 μg of crystalline bovine serum albumin, 0.2 μmole of ADPglucose, 2 μmoles of $MgCl_2$, 2.5 μmoles of NaF, 2.5 μmoles of 3-phosphoglycerate, and 0.5 μmole of $^{32}PP_i$ ($1–4 \times 10^6$ cpm/μmole). The reaction mixture was incubated 10 min at 37 C and terminated by addition of 3 ml of cold 5% trichloroacetic acid. The ATP-^{32}P formed was adsorbed onto Norit A and counted in a gas flow counter.

The reaction mixtures measuring sugar nucleotide synthesis contained 15 μmoles of HEPES buffer, pH 8.0, 50 μg of bovine serum albumin, 2 μmoles of $MgCl_2$, 0.2 μmole of ATP or UTP, 0.1 μmole of glucose-^{14}C-1-P (5.0×10^5 cpm/μmole) and enzyme in a final volume of 0.2 ml. Glycerate-3-P (2.5 μmoles) was added to the reaction mixtures measuring ADPglucose synthesis. Reaction mixtures were incubated for 10 or 20 min at 37 C and terminated by heating for 1 min in a boiling water bath and assayed as previously described (3).

Phosphorylase activity was determined by measuring the release of phosphate from glucose-1-P in the presence of maltodextrins. The reaction mixture contained in a volume of 0.1 ml, 1% maltodextrins (obtained from Corn Products, $\overline{DP} = 6$), 75 mM glucose-1P, 50 mM glycerophosphate buffer, pH 6.5, and extract. After incubation at 30 C for 5 or 10 min, the reaction is terminated by addition of 2 ml of 0.072 N H_2SO_4. Then 2 ml of a 1 N H_2SO_4 acid solution containing 1% ammonium molybdate and 4% ferrous sulphate is added. After 2 min the resultant color is read at 700 nm. Control reaction mixtures are those where the 2 ml of 0.072 N H_2SO_4 were added at zero time.

Starch synthetase activity was determined as described previously (11) at 30 C for 10 min. Protein determinations were by the method of Lowry *et al.* (9).

Table I. *Carbohydrate Level, Dry Weight, and Kernels per 5 g Fresh Weight for Maize at Different Stages of Development*

The values for starch are indicated as μmoles of anhydroglu-glucose units per kernel. Glucose was used as a standard for the reducing sugar and total soluble sugar determinations.

Line	Time after Pollination	Dry Weight	Kernels per 5 g Fresh Wt	Content of Carbohydrate			
				Starch	Reducing sugar	Total soluble sugar	Sucrose
	days	*per cent*		*μmoles/kernel*			
Normal	8	10.5	98	1.58	9.7	11.0	0.65
	10	11.4	91	1.97	11.2	15.2	2.0
	12	12.8	67	4.00	15.2	25.2	5.0
	14	17.6	50	17.8	17.6	40.3	11.4
	16	22.8	37	45.9	14.9	37.3	11.2
	22	33.0	25	219	18.0	46.0	14
	28	50.3	20	365	20.0	50.0	15
Waxy maize	8	9.8	93	1.21	16.2	17.1	0.45
	10	12.2	81	2.32	12.0	17.4	2.72
	12	14.3	63	5.73	15.4	29.5	7.0
	14	17.0	52	17.3	13.6	36.5	11.5
	16	25.6	34	70.0	18.2	54.4	18.1
	22	34.8	22	238	19.5	52.3	16.4
	28	44.2	18	425	9.4	55.0	22.8
Amylose-extender	8	10.7	89	1.33	12.4	12.9	0.25
	10	12.3	81	1.97	13.1	16.1	1.5
	12	15.1	62	5.12	15.5	31.4	7.9
	14	17.8	47	8.63	19.6	44.1	12.3
	16	20.3	34	26.0	20.9	58.6	18.8
	22	28.7	23	146	17.8	62.2	22.2
	28	35.0	17	227	11.7	70.6	29.5
Shrunken-4	22	24.3	28	87.2	30.4	111	40.2

RESULTS

Carbohydrate analyses for normal, waxy, amylose-extender and sh$_4$ maize at each harvest date are given in Table I. Starch levels were quite low in all three lines for the 8th and 10th day with a definite lag in starch synthesis continuing through the 12th or 14th day. Starch levels in the early stages of development are somewhat higher than reported by Tsai *et al.* (18). However, the pattern of synthesis is identical with maximum accumulation occurring after the 16th day.

Reducing sugars tend to reach a maximum between the 16th and 22nd day, but the changes during development are quite small compared with the other carbohydrate fractions measured. If the reducing sugar data are expressed on a fresh or dry weight basis, the level decreases with time.

The content of sucrose and total soluble sugars continues to increase during kernel development. The values for starch, sucrose, and reducing sugar depict a similar pattern to that reported by Tsai *et al.* (18).

Protein in the crude extract and 105,000*g* supernatant fluid are given in Table II. The protein content per kernel increased during development. At all stages of development about 50% of the protein was present in the 105,000*g* precipitate fraction. The protein content in the 22-day-old sh$_4$ kernel is only 25% of that found in the normal maize kernel.

The activities of the ADPglucose and UDPglucose pyrophosphorylases are shown in Table III. In contrast to the results of Tsai *et al.* (18) there were low but significant levels of ADPglucose pyrophosphorylase present in the 8- and 10-day kernels. The activity of ADPglucose synthesis in the presence or absence of glycerate-3-P at all stages of development was more than sufficient to synthesize ADPglucose necessary for the synthesis of all the starch present in the endosperm by soluble ADPglucose-starch glucosyltransferase. During early stages of development there was greater activity for ADPglucose pyrophosphorolysis than synthesis. At 12, 14, 16, 22, and 28 days the rates of synthesis and pyrophosphorolysis were approximately equal. The low activity of synthesis at 8 and 10 days most probably is due to interfering reactions, since greater quantities of the crude extract is required to detect ADPglu-

Table II. *Total Extractable Protein (Crude) and Soluble Protein (105,000g Supernatant) for Developing Maize Kernels*

Line	Time after Pollination	Crude Extract	105,000g Supernatant
	days	*mg protein/kernel*	
Normal	8	0.45	0.20
	10	0.33	0.20
	12	0.67	0.39
	14	1.13	0.58
	16	2.39	1.31
	22	4.40	2.40
	28	5.20	3.00
Waxy maize	8	0.39	0.22
	10	0.44	0.27
	12	0.60	0.42
	14	1.09	0.68
	16	2.88	1.42
	22	4.91	2.42
	28	6.28	3.04
Amylose-extender	8	0.38	0.23
	10	0.53	0.29
	12	0.72	0.53
	14	1.21	0.72
	16	2.49	1.35
	22	4.00	2.31
	28	7.00	3.65
Shrunken-4	22	1.17	0.69

cose synthesis. In the 8- to 10-day period the ADPglucose pyrophosphorylase activity is about 20 times in excess over the rate of starch synthesis. During the latter periods of endosperm development the ADPglucose pyrophosphorylase activity was four to eight times greater than the rate of starch synthesis.

The rate of UDPglucose synthesis from UTP and glucose-1-P was about 1000-fold greater than the rate of ADPglucose synthesis in the early stage of development and about 200- to 250-fold in the latter stages. Chromatography of the reaction mixtures used for measuring sugar nucleotide synthesis in solvents A and B indicated formation of a radioactive product in reaction mixtures containing ATP that co-chromatographed with authentic ADPglucose and formation of a product in reaction mixtures containing UTP that co-chromatographed with UDPglucose. Synthesis of both sugar nucleotides depended on the presence of the respective nucleoside triphosphates and ADPglucose synthesis was stimulated about 2- to 3-fold by the addition of 10 mM glycerate-3-P to the reaction mixture. UDPglucose synthesis was not stimulated by the addition of glycerate-3-P to the reaction mixtures. These differences were consistent for all three lines and for the different stages of development. Activity of ADPglucose pyrophosphorylase and UDPglucose pyrophosphorylase per kernel in shrunken-4 at 22 days after pollination was about one-third the level of that found in the normal maize.

Activity of starch synthetase under the primed conditions was the same following dialysis as observed in the crude extract, and the total activity in the 105,000*g* supernatant fluid plus 105,000*g* precipitate was equal to that found in the dialyzed crude extract. Soluble starch synthetase was found at all stages of development studied using either amylopectin or rabbit liver glycogen as a primer and in the absence of added primer (Table IV). In all cases soluble enzyme activity increased with maturity of the kernels. The rate of activity with rabbit liver glycogen was greater than that with amylopectin at all stages of development and for all three lines. However, the ratio at 8 days is about 2.3:1, whereas at 22 and 28 days it was less than 1.4:1. The ratio of primed to unprimed activity increases with maturity. Thus during early stages of development when starch synthesis is initiated there is relatively more unprimed activity than is the case at later stages of development.

Treatment of the soluble enzyme preparation with glucoamylase (0.2 ml extract with 5 μl of glucoamylase [1 mg/ml] for 30 min) as described by Hawker *et al.* (7) did not affect the rate of unprimed synthesis.

Under the conditions of the transferase assay used, less than 10% of the starch synthetase activity was in the 105,000*g* precipitate fraction (Table IV). In all cases the insoluble enzyme activity increased with kernel maturity. The reaction rate was nearly the same with amylopectin or rabbit liver glycogen as a primer.

The level of starch synthetase activity (glycogen as primer) during all stages of endosperm development is about two to eight times in excess of the rate of starch synthesis observed. The level of soluble starch synthetase activity in the normal maize endosperm with amylopectin as a primer is about the same as the level reported by Tsai *et al.* (18) at 14, 16, 22, and 28 days after pollination. However in contrast to Tsai *et al.* (18) very little granule-bound enzyme (105,000*g* precipitate) was found. This is most probably due to the conditions of the assay used by us. The particulate starch synthetase has a high K*m* for ADPglucose (2–3.3 mM) (1, 4), and the concentration of ADPglucose used in the starch synthetase reaction mixtures in our studies is only 0.7 mM. The K*m* for ADPglucose for the soluble starch synthetases is about 0.1 mM (12). The activity of starch synthetase present in the 22-day-old sh_4 endosperm is only about ⅓ the level found in the corresponding normal endosperm (Table IV).

Table III. *Synthesis and Pyrophosphorolysis of ADPglucose and Synthesis of UDPglucose by Crude Extracts from Developing Maize Kernels*

Line	Time after Pollination	Enzyme Activities		
		ADPG pyrophosphorolysis	ADPG synthesis	UDPG synthesis
	days	*nmoles/10 min·kernel*		*μmoles/10 min·kernel*
Normal	8	34	10.5	14.5
	10	27	15.9	26.0
	12	62	63	37.4
	14	278	260	96.6
	16	1028	855	149.2
	22	1300	1470	464
	28	1544	1845	476
Waxy maize	8	43.6	27.2	15
	10	35.8	21.5	29
	12	90.8	72.6	46.3
	14	287	252	68.2
	16	1300	1210	130
	22	1595	1805	390
	28	1766	1978	454
Amylose-extender	8	28	7.3	13.5
	10	39	17.7	21
	12	92.4	96	59
	14	357	321	72
	16	1626	1318	144
	22	3020	3300	463
	28	3550	3230	688
Shrunken-4	22	394	454	165

Table IV. *Activity of ADPglucose-α-1,4-glucan α-4-Glucosyltransferase with Amylopectin and Rabbit Liver Glycogen as Primers and in the Absence of Added Primer for Developing Maize Kernels*

Line	Time after Pollination	ADPglucose-Starch Glucosyltransferase				
		105,000*g* Supernatant Primed		105,000*g* Precipitate Primed		105,000*g* Supernatant
		Amylopectin	Glycogen	Amylopectin	Glycogen	Unprimed
	days	*nmoles glucose transferred 15 min·kernel*				
Normal	8	5.8	14	0.24	0.23	6.1
	10	10.9	19.1	0.23	0.19	8.4
	12	39.2	58.2	1.33	1.39	21.1
	14	138	185	6.37	7.18	52
	16	277	340	20.0	15.2	80
	22	460	554	20.5	11.7	114
	28	350	495	12.9	6.87	97
Waxy maize	8	10	21.9	0.26	0.26	6.7
	10	25.3	34.7	0.37	0.42	8.5
	12	72.4	92.4	1.18	1.42	23.1
	14	124	158	2.41	3.04	48.5
	16	295	373	7.85	8.80	102
	22	409	530	9.66	11.4	157
	28	365	515	2.28	5.92	132
Amylose-extender	8	4.9	12	0.16	0.10	6.4
	10	21.4	32	0.33	0.40	10.1
	12	62	79	1.72	1.86	22.8
	14	139	177	6.17	6.74	48
	16	272	322	18.2	20.6	95
	22	598	599	36.7	34.8	93
	28	610	688	35.6	36.4	113
Shrunken-4	22	91	159	5.06	6.57	76

Table V. *Phosphorylase Activity in Developing Maize Kernels*

Line	Time after Pollination	Phosphorylase Activity
	days	*μmoles/10 min·kernel*
Normal	8	0.68
	10	0.93
	12	1.27
	14	1.94
	16	2.85
	22	4.61
	28	5.56
Waxy maize	8	1.03
	10	1.18
	12	1.52
	14	2.10
	16	3.50
	22	5.80
	28	6.4
Amylose-extender	8	0.74
	10	1.10
	12	1.45
	14	2.19
	16	3.37
	22	5.86
	28	7.68
Shrunken-4	22	2.17

The phosphorylase activity is in considerable excess over that observed for the transferase being about 40-fold greater in the early stages and about 10-fold greater in the later stage of development (Table V). The activity in the endosperm at 28 days after pollination is 10-fold greater than what is observed at 8 days after pollination. The level of phosphorylase in the 22-day-old sh, endosperm is about 35 to 45% of that found in the normal maize.

DISCUSSION

The results presented show that ADPglucose pyrophosphorylase and soluble starch synthetase were present in corn kernels 8 days after pollination and while starch synthesis was in the lag phase. The ADPglucose pyrophosphorylase present in the 8-day-old kernel appears to be mainly the one associated with the endosperm as greater than 80% of the ADPglucose pyrophosphorylase activity is labile to heat treatment for 5 min. at 60 C. The embryo enzyme was reported to be stable to this treatment (14). Unpublished experiments indicate that all the ADPglucose pyrophosphorylase activity present in the 8- and 10-day-old kernels reside in the endosperm tissue.

Levels of starch synthetase were essentially the same in developing kernels of normal, waxy, and amylose-extender maize as was the level of starch synthesis. Less than 10% of the total enzyme activity was precipitated by centrifugation with the starch granules. Greater activity most probably could have been obtained from the starch granule precipitate had the concentration of ADPglucose been higher in the reaction mixture. The K*m* of the soluble starch synthetases for ADPglucose appears to be lower than those found for the starch-bound enzyme (1, 4). It has also been shown that mechanical disruption of waxy maize starch grains increases the activity of the bound starch synthetase about 4- to 5-fold (2, 4).

In a previous study it was shown that there were two forms of soluble ADPglucose-α-4-glucan 4-α-glucosyltransferase in waxy maize (12). One form of the enzyme was active in the absence of added primer. The activity of this form with rabbit liver glycogen as primer was 380% greater than the activity with amylopectin as primer. With the other form, there was no unprimed activity and amylopectin was a slightly better primer than rabbit liver glycogen. In the present study the different forms of the enzyme were not separated. However, the relatively higher ratio of unprimed to primed activity in the earlier stages of development and the relatively greater activity with rabbit liver glycogen as compared with amylopectin in the early stages would suggest that the contribution from the two enzyme forms changes during development. The data would support the concept that during early stages of development when starch synthesis is in the lag phase, the form of the enzyme responsible for unprimed activity predominates.

Furthermore, there is an excess of ADPglucose pyrophosphorylase and starch synthetase activities to account for all the starch accumulation present in the endosperm throughout the 8- to 28-day stage. However, since very little is known about the turnover of the starch molecules in the endosperm during this period, it is difficult to assess from these data the quantitative contribution of the ADPglucose enzymes towards starch formation. Both ADPglucose pyrophosphorylase and ADPglucose-α-1,4-glucan 4-α-glucosyl transferase activities increase about 40- to 100-fold, whereas starch levels increase about 200- to 300-fold in the various maize kernels. A similar result in developing pea seeds was observed by Turner (19). It should be pointed out that the activities of both UDPglucose pyrophosphorylase and phosphorylase, enzymes implicated in starch synthesis (8, 16–18), are about 20- to 500-fold in excess of the ADPglucose pyrophosphorylase and transferase levels in the developing endosperm. The activities found for these en-

zymes in the kernel at the 8-day-old stage could account for all the starch synthesis occurring at any stage of development of the endosperm. However, the activity of the UDPglucose-α-1,4-glucan 4-α-glucosyltransferase activity in maize is only associated with the starch bound fraction and has only 10 to 16% of the activity observed for the starch synthetase activity and could not account for the major portion of starch accumulation. The K*m* of the bound enzyme for UDPglucose is also very high. It is likely that the main function for UDPglucose pyrophosphorylase is to supply precursors for cell wall formation or sucrose formation.

There is also a 6- to 10-fold increase in the phosphorylase level during the period where there is 200- to 300-fold increase in the level of starch. It has been postulated that phosphorylase is involved in the initiation of starch synthesis (8, 16–18). The major support for this is based on the observation that sh_4 maize endosperm, which contains only 30 to 40% of the level of starch found in normal maize endosperm, contains only about 30% of the phosphorylase level found in the normal endosperm (16). Although these results have been confirmed in our present experiments, it was also found that sh_4 endosperm on a kernel basis has only 20 to 25% of the total protein found in the normal kernel. It also has only 20% of the ADPglucose pyrophosphorylase activity, 20% of the starch synthetase activity, and 33% of the UDPglucose pyrophosphorylase activity found in the normal endosperm. Thus the sh_4 mutation is not specific for the phosphorylase genome and appears to cause a general defect on protein synthesis in the sh_4 kernel. These findings weaken considerably the argument that phosphorylase is involved in the biosynthesis of starch.

In addition, the rates of unprimed synthesis of α-1,4-glucan catalyzed by phosphorylase in maize endosperm (17) are quite low and could not account for the rates of starch synthesis observed in these studies. High concentrations or unphysiological concentrations of glucose-1-P (15–20 mM) are also required for the unprimed reaction. At present there appears to be no data pro or con on the involvement of phosphorylase in starch synthesis.

The starch-deficient maize mutant, shrunken-2, which has only about 10 to 12% of the ADPglucose pyrophosphorylase activity, synthesizes only 25 to 30% as much starch as normal maize (3, 15). These data would indicate that the major portion if not all of the starch synthesized in the normal endosperm is via the ADPglucose pathway. Since it has been shown that the ADPglucose-α-glucan 4-α-glucosyl transferase is capable of catalyzing synthesis of a polyglucan in the absence of primer (7, 11, 12), it is possible that initiation of starch synthesis also occurs via the ADPglucose pathway.

Acknowledgments—The authors thank Dr. R. Creech for generously supplying the maize seed used in this study and the Vegetable Crops Department at the University of California, Davis, for the space, equipment, and supplies for growing the plants. E. Y. C. Lee wishes to acknowledge the technical assistance of Miss G. G. Gutten in the phosphorylase studies. This work was supported in part by U.S. Public Health Services, National Institutes of Health Grant AIO5520, and Southeast Asia Treaty Organization Fellowship VII (J.S.H.).

LITERATURE CITED

1. Akatsuka, T. and O. E. Nelson. 1966. Starch granule bound adenosine diphosphate glucose starch glucosyltransferases of maize seeds. J. Biol. Chem. 241: 2280–2286.
2. Chandorkar, K. R. and N. P. Badenhuizen. 1966. How meaningful are determinations of glucosyltransferase activities in starch-enzyme complexes? Staerke 18: 91–95.
3. Dickinson, D. B. and J. Preiss. 1969. Presence of ADPglucose pyrophosphorylase in shrunken-2 and brittle-2 mutants of maize endosperm. Plant Physiol. 44: 1058–1062.
4. Frydman, R. B. and C. E. Cardini. 1967. Studies on the biosynthesis of starch II. Some properties of the adenosine diphosphate glucose: starch glucosyltransferase bound to the starch granule. J. Biol. Chem. 242: 312–317.
5. Glucostat Technical Bulletin, Worthington Biochemical Corporation, Freehold, N. J.
6. Handel, E. van. 1968. Direct microdetermination of sucrose. Anal Biochem. 22: 280–283.
7. Hawker, J. S., J. L. Ozbun, and J. Preiss. 1972. Starch synthesis in the absence of primer by soluble ADPglucose-starch glucosyltransferase from potato tubers. Phytochemistry. 11: 1287–1294.
8. Illingworth, B., D. H. Brown, and C. F. Cori. 1961. The *de novo* synthesis of polysaccharide by phosphorylase. Proc. Nat. Acad. Sci. U.S.A. 47: 469–478.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
10. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375–380.
11. Ozbun, J. L., J. S. Hawker, and J. Preiss. 1971. Multiple forms of α-1,4-glucan synthetase from spinach leaves. Biochem. Biophys. Res. Commun. 43: 631–636.
12. Ozbun, J. L., J. S. Hawker, and J. Preiss. 1971. ADPglucose-starch glucosyltransferases from developing kernels of waxy maize. Plant Physiol. 48: 765–769.
13. Preiss, J. 1969. The regulation of the biosynthesis of α-1,4-glucans in bacteria and plants. *In:* E. R. Stadtman and B. L. Horecker, eds., Current Topics in Cellular Regulation. Academic Press Inc. p. 125–160.
14. Preiss, J., C. Lammel, and A. Sabraw. 1971. A unique adenosine diphosphoglucose pyrophosphorylase associated with maize embryo tissue. Plant. Physiol. 47: 104–108.
15. Tsai, C. Y. and O. E. Nelson. 1966. Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity. Science 151: 341–343.
16. Tsai, C. Y. and O. E. Nelson. 1969. Mutations at the shrunken-4 locus in maize that produce three altered phosphorylases. Genetics 61: 813–821.
17. Tsai, C. Y. and O. E. Nelson. 1969. Two additional phosphorylases in developing maize seeds. Plant Physiol. 44: 159–167.
18. Tsai, C. Y., F. Salamini, and O. E. Nelson. 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol. 46: 299–306.
19. Turner, J. F. 1969. Physiology of pea fruits VI. Changes in uridine diphosphate glucose pyrophosphorylase and adenosine diphosphate glucose pyrophosphorylase in the developing seed. Aust. J. Biol. Sciences 22: 1145–1151.