Biochemical Genetics, Vol. 11, No. 2, 1974

The Function of the *Waxy* Locus in Starch Synthesis in Maize Endosperm[1]

Chia-Yin Tsai[2]

Received 19 June 1973—Final 31 Aug. 1973

The soluble adenosine diphosphate glucose-starch glucosyltransferase of maize (Zea mays *L.*) *endosperm uses adenosine diphosphate glucose as a sole substrate, but the starch granule–bound nucleoside diphosphate glucose-starch glucosyltransferase utilizes both adenosine diphosphate glucose and uridine diphosphate glucose. The soluble glucosyltransferase can be bound to added amylose or to maize starch granules that contain amylose. However, binding of the soluble enzyme to the starch granules does not change its substrate specificity to that of the natural starch granule–bound glucosyltransferase. Furthermore, the soluble glucosyltransferase bound to starch granules can be removed by repeated washing without a change in specificity. The bound glucosyltransferase can be released by mechanical disruption of starch granules, and the released enzyme behaves in a manner similar to that of the bound enzyme in several respects. These observations suggest that the soluble and bound glucosyltransferases are different enzymes. The starch granule–bound glucosyltransferase activity is linearly proportional to the number of* Wx *alleles present in the endosperm. This is compatible with the hypothesis that the* Wx *allele is a structural gene coding for the bound glucosyltransferase, which is important for the normal synthesis of amylose.*

KEY WORDS: *Zea mays*; enzyme; glucosyltransferase; starch; biosynthesis.

[1] Journal Paper No. 4818 of the Purdue University Agricultural Experiment Station.

[2] Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana.

INTRODUCTION[3]

The discovery of starch granule–bound NDPglucose-starch glucosyltransferase (DeFekete *et al.*, 1960) and soluble ADPglucose-starch glucosyltransferase (Frydman and Cardini, 1964) has led to the assumption that the major portion of starch synthesis in higher plants is mediated by these two enzyme systems. Direct evidence supporting the importance of these glucosyltransferases for starch biosynthesis came from the studies of a starch-deficient maize mutant, *shrunken-2*, that was found to lack ADPglucose pyrophosphorylase activity in the endosperm (Tsai and Nelson, 1966). This mutant synthesizes only about 30% of the normal quantity of starch, presumably because ADPglucose, the substrate for glucosyltransferases, is limiting. Nevertheless, the physiological role of these two systems for the synthesis of starch fractions remains ambiguous. Amylopectin has been assumed to be converted from amylose chains by the action of Q-enzyme (Hassid, 1954). However, the argument against a role for this enzyme in the formation of amylopectin was the finding that, while the maize mutant *waxy* (*wx*) produces amylopectin exclusively (Sprague *et al.*, 1943), there is no marked difference between the normal and *wx* in Q-enzyme activity throughout the period of active starch synthesis (Fuwa, 1957). Although this observation does not rule out the precursor–product relation between amylose and amylopectin, these two components are probably synthesized independently, as the *wx* mutant has been found to produce no starch granule–bound NDPglucose-starch glucosyltransferase, suggesting an important role for this enzyme in the normal synthesis of amylose (Nelson and Rines, 1962; Nelson and Tsai, 1964). Later, it was found that the soluble ADPglucose-starch glucosyltransferase could be bound to added amylose (Akazawa and Murata, 1965), suggesting that the lack of the bound glucosyltransferase activity in the endosperm of the *wx* mutant might be a secondary consequence of that gene mutation. The association of glucosyltransferase activity with endosperm starch granules might be due to contamination by soluble glucosyltransferase that became bound to the amylose during enzyme preparation. However, preliminary studies on the bound glucosyltransferase activity prepared from both diploid (Tsai, 1965; Akatsuka and Nelson, 1969) and tetraploid (Tsai, 1965) stocks show proportional increases in enzymatic activity with increasing number of normal (*Wx*) alleles present in the endosperm tissue. This linear proportionality of activity and dosage of *Wx* alleles is compatible with either the hypothesis that the *Wx* allele is a structural gene for the bound glucosyltransferase or the hypothesis that the *Wx* allele determines the number of sites at which the enzyme can be

[3] The following abbreviations are used in this paper: ADPglucose, adenosine diphosphate glucose; UDPglucose, uridine diphosphate glucose; NDPglucose, nucleoside diphosphate glucose.

bound to the starch granule. In this paper, I present more evidence that the bound glucosyltransferase activity associated with starch granules is not due to contamination by the soluble glucosyltransferase.

MATERIALS AND METHODS

Collection of Maize Samples

Ears of the normal (+), *amylose-extender* (*ae*), *wx* (standard *wx*), and *ae wx* from near-isogenic sublines of the inbred M14 were harvested at 22 days after self-pollination, frozen on dry ice, and stored at −20 C until processing. In order to study the effect of the *Wx* locus on the production of starch granule-bound NDPglucose-starch glucosyltransferase, self-pollinations and reciprocal crosses between the *wx* mutant and *Wx* were made in both diploid and tetraploid stocks. The *Wx* and *wx* tetraploid stocks (supplied by Dr. L. F. Bauman) were derived from W22 × 51A and 38–11 × Oh43E, respectively. The samples were harvested as described above.

Preparation of Enzymes

As the initial step in processing, the kernels were cut from the cob. The embryo and the pericarp were removed from the kernel. Endosperms were then homogenized in a blender (VirTis 45) with 0.01 M tris–maleate buffer (*p*H 7.0), strained through two layers of cheesecloth, and centrifuged for 20 min at 10,000 × *g*. The pellet was washed three times with 0.01 M tris–maleate buffer (*p*H 7.0), unless otherwise indicated, before being twice washed with cold acetone (−20 C). The starch granules containing bound NDP-glucose-starch glucosyltransferase were dried *in vacuo* at 4 C. The soluble ADPglucose-starch glucosyltransferase was prepared by the method described previously (Tsai *et al.*, 1970).

Preparation of the Artificially Bound ADPglucose-Starch Glucosyltransferase

About 500 mg each of the *ae*, *wx*, *ae wx*, and normal starch granules was incubated with 20 ml of 0.2 M *N*,*N*-bis(2-hydroxyethyl)-glycine (bicine) buffer (*p*H 8.5) containing 0.1% Pronase and 0.01 M $CaCl_2$ for 8 hr at 37 C to remove the endogenous bound NDPglucose-starch glucosyltransferase. After incubation, starch granules were collected by centrifugation, washed three times with 0.01 M tris–maleate buffer (*p*H 7.0), and then transferred to a 50-ml Erlenmeyer flask to which 25 ml of 90% dimethylsulfoxide was added. The mixture was stirred overnight with a magnetic stirrer and centrifuged for 20 min at 12,000 × *g*. Starch was isolated from the supernatant fraction

by adding 2 vol of 95% ethanol, centrifuging, washing the pellet once each with 95% ethanol and acetone, and air-drying the washed pellet. Duplicate 50-mg samples of each of the purified preparations, or commercial amylose or amylopectin, were weighed into centrifuge tubes containing 2 ml of the soluble ADPglucose-starch glucosyltransferase preparation. The mixture was allowed to stand for 30 min at 4 C with occasional stirring and then centrifuged. One duplicate was used as an unwashed control, and the other duplicate was washed three times with 0.01 M tris–maleate buffer (*p*H 7.0). Both duplicates were then washed twice with cold acetone (−20 C). Samples were dried *in vacuo* at 4 C. The preparations were then used to determine the substrate specificity of the artificially bound glucosyltransferase.

Release of Glucosyltransferase from the Starch Granules

Isolated normal starch granules were washed either no or three times with cold 0.01 M tris–maleate buffer, followed by two washings with cold acetone (−20 C) as described under *Preparation of Enzymes*. The unwashed and washed normal starch granule preparations (100 mg each) were then ground for 20 min with a mortar and pestle in the presence of 1.0 ml of 0.3 M bicine buffer (*p*H 8.5) and washed sand at 4 C and centrifuged for 20 min at 29,000 $\times g$. The glucosyltransferase released into the supernatant fraction was then used as an enzyme source to study its properties.

Enzyme Assay

The activity of glucosyltransferases was determined by measuring the incorporation of glucose-C^{14} from either ADPglucose-C^{14} or UDPglucose-C^{14} into acceptor molecules. For the soluble ADPglucose-starch glucosyltransferase system, 40 μl of the reaction mixture contained 6 μmoles of HEPES buffer (*p*H 7.5), 0.20 μmole of ADPglucose-C^{14} or UDPglucose-C^{14} (12,000 cpm), 3 mg of amylopectin, and 20 μl of enzyme preparation. Incubation was at 37 C for 30 min. For the determination of starch granule–bound NDP-glucose-starch glucosyltransferase activity, the conditions were the same as for the soluble system except that amylopectin and 20 μl of enzyme preparation were replaced by 3 mg of starch granules and 20 μl of H_2O. For the bound glucosyltransferase system, the reaction was terminated by the addition of 0.5 ml of 0.1 N NaOH followed by precipitation with ethanol to a final concentration of 70%. The entire suspension was placed on Whatman GFA glass fiber paper (2.1-cm disc) and washed under suction with an excess of distilled water. The filter discs were dried and counted in a gas flow counter (Nuclear Chicago). For the soluble enzyme system, the reaction was terminated by the addition of 0.5 ml of 0.5 N NaOH followed by 1.5 ml of methanol.

The precipitate was then collected for counting by either of two methods.

1. Millipore method: The entire suspension was placed on Whatman GFA glass fiber filter paper and washed under suction with an excess of 70% ethanol. The filter discs were dried and counted in a gas flow counter.
2. Centrifugation method: The pellet was collected by centrifugation and washed three times with 0.5 N NaOH followed by methanol precipitation and centrifugation of the washings. After redissolving in 0.5 N NaOH, the entire amount was distributed over a ring planchet, 3.2 cm in diameter, and evaporated under an infrared lamp before counting in a gas flow counter.

The activity of glucosyltransferases was linearly proportional to time of incubation and to the amount of enzyme preparation assayed.

All data presented have been corrected by control values from reaction mixtures that contained heat-denatured enzyme or no enzyme (the control values were about 50 cpm).

Determination of Amylose Content in the *Wx* Dosage Series

The starch was isolated according to McGuire and Erlander (1966), and the amount of amylose was determined by the method of Ulmann and Augustat (1958).

RESULTS

Substrate Specificity of the Soluble and Bound Glucosyltransferase

The data in Table I indicate that starch granules prepared from the *wx* mutant contain no activity for the bound NDPglucose-starch glucosyltransferase, as was reported previously (Nelson and Rines, 1962; Nelson and Tsai, 1964). The bound glucosyltransferase prepared from both *ae* and normal endosperms is capable of using both ADPglucose and UDPglucose as substrate, and the enzyme transfers glucose from ADPglucose about twice as rapidly as from UDPglucose. The soluble ADPglucose-starch glucosyltransferase prepared from normal maize endosperm, on the other hand, utilizes only ADPglucose as a substrate, and this enzyme can be bound to added amylose but not to amylopectin. However, the binding of the soluble glucosyltransferase to amylose does not change its substrate specificity to that of the starch granule–bound NDPglucose-starch glucosyltransferase. Similar observations were made for soluble glucosyltransferase prepared from other three genotypes, *ae*, *wx*, and *ae wx*.

Table I. Substrate Specificity of the Soluble Glucosyltransferase Prepared from Normal Maize Endosperm and Starch Granule–Bound Glucosyltransferase Prepared from Various Genotypes

	Enzymatic activity (cpm)						
	Soluble glucosyltransferase			Bound glucosyltransferase			
Substrate	Soluble	Amylose bound[a]	Amylopectin bound[a]	*ae*	*wx*	*ae wx*	+
ADPglucose	262	530	50	1176	20	120	1287
UDPglucose	0	27	19	625	0	0	602

[a] Data are adapted from Table II.

The double mutant *ae wx* also synthesizes amylopectin exclusively, as does the *wx* mutant. When the thymol–butanol method (Cowie and Greenwood, 1957) for the isolation of amylose was employed for the preparation of *ae*, *ae wx*, normal, and *wx* starch, about 90%, 80%, 30%, and 2%, respectively, were recovered as amylose (Chuang, Tsai, and Dalby, unpublished data). Although a very high percentage of *ae wx* starch is found in the amylose fraction, this is probably due to the presence of amylopectin of longer chain length, which is capable of complexing with thymol and butanol, as does the amylose, thus precipitating out from the medium. The maximum absorption for the iodine complex of the *ae wx* amylose-like material is 580 nm, which is different from that of the normal amylose–iodine complex (620 nm) (Chuang, Tsai, and Dalby, unpublished data). Furthermore, when *ae wx* starch is dissolved and subsequently fractionated on a Sepharose 4B column, none of the fractions collected can be completely degraded by β-amylase. About 40% of the total carbohydrate is recovered as β-limit dextrin, which is a characteristic of amylopectin. However, the maximum absorption for the iodine complex of the amylopectin produced by *ae wx* (580 nm) is different from that of the *wx* amylopectin (540 nm). Also, the treatment of *ae wx* starch with β-amylase released about 20% more reducing sugars than from the *wx* starch (Chuang, Tsai, and Dalby, unpublished data). These observations suggest that the amylopectin branches produced by the double mutant might have a longer chain length than those of the *wx* mutant.

The starch granules prepared from the double mutant do contain some ADPglucose-specific glucosyltransferase activity, which is a characteristic of the soluble glucosyltransferase. Thus the activity associated with *ae wx* starch granules might be due to binding of the soluble glucosyltransferase to the longer-chain amylopectin present during enzyme preparation.

Substrate Specificity of the Artificially Bound ADPglucose-Starch Glucosyltransferase

Although the binding of the soluble ADPglucose-starch glucosyltransferase to added amylose will not change its substrate specificity to utilize UDPglucose, it is important to establish whether the specificity will be maintained when the soluble glucosyltransferase is bound to starch containing amylose. The soluble glucosyltransferase was bound to starch (free of bound glucosyltransferase) by mixing the soluble enzyme preparation with the Pronase-treated starch preparations from the four genotypes.

Table II shows that, in addition to binding to amylose, the soluble glucosyltransferase is capable of binding to the Pronase-treated starch of *ae*, *ae wx*, and normal preparations. However, this bound activity can be removed completely by washing three times with 0.01 M tris–maleate buffer, according to experiments described in the succeeding section. More important, the binding of the soluble ADPglucose-starch glucosyltransferase to the Pronase-treated starch does not alter its substrate specificity to permit the utilization of UDPglucose. However, there is the possibility that the Pronase-treated starch may not permit the binding of the soluble glucosyltransferase in the right conformation so as to be able to use UDPglucose.

The binding of soluble glucosyltransferase to the amylose-free *ae wx* starch might be due to the presence of the longer-chain amylopectin available for complexing.

Table II. Substrate Specificity of ADPglucose-Starch Glucosyltransferase Artificially Bound to Amylopectin, Amylose, and Pronase-Treated Starch Preparations of Various Genotypes

Artificially bound preparation	Washings (No.)	Activity (cpm)	
		ADPglucose	UDPglucose
Amylopectin	0	50	19
	3	0	0
Amylose	0	530	27
	3	33	0
ae starch	0	1029	19
	3	30	0
wx starch	0	39	8
	3	0	0
ae wx starch	0	623	25
	3	0	0
+ starch	0	840	20
	3	0	0

Washing Effect on the Preparation of the Starch Granule–Bound NDPglucose-Starch Glucosyltransferase

Since the artificially bound soluble ADPglucose-starch glucosyltransferase may subsequently be removed by washing with buffer, a starch granule–bound NDPglucose-starch glucosyltransferase preparation largely free from contamination by the soluble glucosyltransferase ought to be obtainable. Since the contaminating soluble glucosyltransferase will not utilize UDPglucose as a substrate, it would be anticipated that the activity ratio of the starch granule–associated glucosyltransferases using ADPglucose *vs.* UDPglucose (A/U ratio) as substrate might be changed through steps of repeated washing of the starch granules with buffer during enzyme preparation. Thus a constant A/U ratio should be obtained for the natural starch granule–bound NDPglucose-starch glucosyltransferase after the removal of the soluble glucosyltransferase contamination.

Isolated starch granules from each of the four genotypes, *ae*, *wx*, *ae wx*, and normal, were washed a varying number of times with cold 0.01 M tris-maleate buffer before being twice washed with cold acetone as described in *Materials and Methods*. Table III indicates that, for both *ae* and normal starch granule–bound glucosyltransferase preparations, the A/U ratio changed from 2.8 in the unwashed preparation to about 2 in the preparation washed twice with buffer; the A/U ratio then remained constant in the subsequent washed preparations. However, other than the loss of ADPglucose activity during the first two washings, there is also a progressive loss of UDPglucose-utilizing activity. Presumably, the loss of UDPglucose activity is due to the dissociation of bound glucosyltransferase from the starch granules. If this is the case, one would expect to observe the ability to use UDPglucose by the glucosyltransferase released into the washing medium. Since the purpose of this particular experiment was to show the qualitative similarities between the bound and released glucosyltransferases, quantitative recovery was not attempted. Table III shows that the released glucosyltransferase is capable of using UDPglucose. The A/U ratio is changed from 29 in the crude homogenate to about 4 in the supernatant from the second washing with buffer. There is no bound NDPglucose-starch glucosyltransferase activity associated with the *wx* endosperm starch granules, but residual activity is detectable in the *ae wx* preparation even after six washings. However, this glucosyltransferase will not use UDPglucose as a substrate.

Glucosyltransferase Activity Released from the Normal Starch Granule Preparations

The nondialyzed preparation of the glucosyltransferase released by the

Table III. Effect of Washing on the Activity Ratio of ADPglucose/UDPglucose for the Starch Granule-Bound NDPglucose-Starch Glucosyltransferase of Various Genotypes and the Glucosyltransferases Remaining in the Supernatant Fraction of the Normal Preparation

	Bound enzyme activity										Activity in + supernatant[a]		
	ae			*wx*		*ae wx*		+					
Washings (No.)	ADPG (cpm)	UDPG (cpm)	A/U (ratio)	ADPG (cpm)	UDPG (cpm)	ADPG (cpm)	UDPG (cpm)	ADPG (cpm)	UDPG (cpm)	A/U (ratio)	ADPG (cpm)	UDPG (cpm)	A/U (ratio)
0	4644	1716	2.72	36	0	525	0	4701	1692	2.78	7028	243	29.0
1	2208	952	2.32	30	0	298	0	2850	1200	2.38	4641	417	11.1
2	1236	630	1.96	20	0	135	0	1368	638	2.14	710	162	4.4
3	1176	625	1.88	20	0	120	0	1287	612	2.10	211	51	4.0
4	1170	624	1.88	20	0	118	0	1266	600	2.11	0	0	
6	1173	619	1.89	18	0	116	0	1206	576	2.09	0	0	

[a] Data were obtained from different enzyme preparations than those used for an assay of bound activity. Reaction mixture (0.1 ml) contained 90 μl of supernatant enzyme preparation (contained 27 μmoles of bicine buffer, *p*H 8.5), 3 mg of the Pronase-treated normal starch, and 10 μl of 0.025 M ADPglucose-C^{14} or UDPglucose-C^{14} (35,000 cpm). Incubation was at 37 C for 90 min.

Table IV. Properties of the Bound, Soluble, and Released Glucosyltransferases from Normal Maize Endosperm[a]

Enzyme source	Activity (ADPglucose/UDPglucose) cpm	A/U	K_m (mM)	Heat inactivation[b] (%)
Bound glucosyltransferase	2124/1016	2.1	2.85	70
Soluble glucosyltransferase	7028/ 243	29	0.15	100
Released glucosyltransferase from washed granules	763/ 154	4.9	2.50	76
Released glucosyltransferase from unwashed granules	1939/ 193	10		

[a] For the soluble enzyme system, reaction mixture (0.12 ml) contained 10 μmoles of bicine buffer (*p*H 8.5), 90 μl of enzyme preparation, 3 mg of Pronase-treated starch, and 10 μl of ADPglucose-C^{14} or UDPglucose-C^{14} (0.025 M, 35,000 cpm). Incubation time was 90 min. For the released enzyme system, conditions were similar to those of the soluble system except that 50 μl of enzyme preparation was used. For the bound enzyme system, the conditions are described under *Materials and Methods*. Incubation time was 10 min.

[b] Percent loss in activity resulting from preincubation of enzyme at 55 C for 5 min. Activity was assayed at 37 C in each case using ADPglucose as a substrate.

mechanical disruption of buffer-washed starch granules was active to the extent of 840 cpm compared with 763 cpm using Pronase-treated starch as primer. However, this "unprimed activity" is probably due to the presence of dialyzable primers in the medium. Dialysis results in substantially decreased glucosyltransferase activity, and dialyzed preparations require added primer for activity. Because of this and the possibility that the released enzyme does not exist in a particulate form, the nondialyzed preparation was used as a source for investigating the properties of this enzyme. When the glucosyltranferase is released from the unwashed normal endosperm starch granules, the enzyme is most active with ADPglucose as a substrate, with the A/U ratio being about 10 (Table IV). Presumably, the high A/U ratio observed is largely due to the release of the contaminating soluble ADPglucose-starch glucosyltransferase. On the other hand, the A/U ratio for glucosyltransferase released from the washed starch granules is about 5. Thus a larger portion of this activity must be derived from the starch granule–bound glucosyltransferase. The attachment of glucose-C^{14} from either ADPglucose-C^{14} or UDPglucose-C^{14} to primer by the released glucosyltransferase is β-amylase sensitive. When the product was treated with β-amylase and chromatographed with butanol–pyridine–water (6:4:3) (Jeanes *et al.*, 1951), the radioactivity was found to migrate to a position characteristic of maltose. This observation is indicative of the formation of α-1,4 glucosidic linkage by the glucosyltransferase.

In addition to the substrate specificity, the released glucosyltransferase

also behaves in a manner similar to the bound form with respect to both K_m and heat stability (Table IV). The K_m value, using ADPglucose as a substrate and starch granules as primer, for the bound or released glucosyltransferase is about 17 times higher than for the soluble enzyme. Soluble glucosyltransferase is more thermolabile than the bound and released enzymes. Further kinetic studies using both purified soluble and released glucosyltransferases might be helpful in further establishing their differences.

Dosage Effect of *Wx* Alleles on the Production of Starch Granule–Bound NDPglucose-Starch Glucosyltransferase and Amylose Content

Endosperm starch granules were prepared from kernels resulting from self-pollinations and reciprocal crosses between *wx* and *Wx* in both diploid and tetraploid stocks. These samples contain zero, one, two, three, four, or six doses of *Wx* alleles. From each of these samples, 1 mg of starch granules was weighed out to determine the starch granule–bound NDPglucose-starch glucosyltransferase activity using ADPglucose as a substrate. Table V shows that the bound glucosyltransferase activity in endosperm starch granules is linearly proportional to the number of normal (*Wx*) alleles present in the endosperm tissue. Similar results were obtained when UDPglucose was used. On the other hand, the amount of amylose formed in the dosage series is not linearly proportional to the dose of *Wx* alleles.

Table V. Starch Granule–Bound NDPglucose-Starch Glucosyltransferase Activity and the Percentage of Amylose of Starch Granules in Both Diploid and Tetraploid Series with Regard to the Number of *Wx* Alleles

Wx alleles (No.)	Activity (nmoles glucose-C^{14} incorp./ mg starch granules)	Amylose (% starch)
Diploid		
0	0.2	0
1	4.2	13.6
2	7.6	19.5
3	10.8	23.1
Tetraploid		
0	0.3	0.5
2	6.8	18.5
4	13.3	22.0
6	19.6	23.5

DISCUSSION

Kinetic studies of the soluble ADPglucose-starch glucosyltransferase and starch granule–bound NDPglucose-starch glucosyltransferase isolated from normal maize endosperm have indicated that these enzymes behave differently in several respects (Akatsuka and Nelson, personal communication). The soluble glucosyltransferase uses ADPglucose exclusively, while the bound glucosyltransferase utilizes both ADPglucose and UDPglucose. These observations suggest that the soluble and bound glucosyltransferases are probably different enzymes. However, the fact that the soluble glucosyltransferase can be bound to added amylose (Akazawa and Murata, 1965) suggests that the difference in specificity between the soluble and bound forms could be due to changes in the conformation of the soluble enzyme arising from its bound state. In this state, the soluble enzyme would need to become active toward UDPglucose, and its affinity toward ADPglucose would need to decrease, in order to correspond to the properties of the naturally bound glucosyltransferase. Also, Frydman and Cardini (1967) have shown that the properties of the starch granule–bound glucosyltransferase were considerably changed when the structure of the granules was modified by mechanical disruption. After this treatment, UDPglucose was no longer a substrate, while the activity with ADPglucose was enhanced. Since the new specificity is similar to that of the soluble ADPglucose-starch glucosyltransferase, these workers suggest that both glucosyltransferases could be different forms of the same enzyme.

The experimental data presented above indicate that the soluble and bound glucosyltransferases are different enzymes. Contrary to the observation made by Frydman and Cardini (1967), the bound glucosyltransferase released from the starch granule will utilize both ADPglucose and UDPglucose, although the A/U ratio is changed. Also, the released glucosyltransferase behaves in a similar manner to the starch granule–bound form with respect to the K_m value and heat stability. The binding of the soluble enzyme to amylose or starch granules does not change its specificity to that of the natural granule-bound glucosyltransferase. This is in agreement with the findings of Tanaka and Akazawa (1968), who showed that binding spinach chloroplast soluble glucosyltransferase to amylose did not change its substrate specificity. The activity of the soluble form, but not of the bound glucosyltransferase, can be removed completely by washing. The pattern of glucose incorporation into the amylose and amylopectin fractions of the starch granule also suggests that the bound and soluble glucosyltransferases are different enzymes. The bound glucosyltransferase incorporates glucose from either substrate more rapidly into the amylose fraction than into amylopectin. The soluble enzyme, on the other hand, incorporates glucose from

only ADPglucose into the amylopectin fraction (Tsai, 1973). Furthermore, the starch granule–bound glucosyltransferase activity is proportional to the number of *Wx* alleles present in the endosperm. However, the amount of amylose produced in the dosage series is not linearly proportional to the dose of *Wx* alleles. Based on the available evidence, it is unlikely that the bound glucosyltransferase activity arises from the binding of the soluble glucosyltransferase to the amylose. The dosage effect observed suggests that the *Wx* locus is probably a structural gene coding for the starch granule–bound NDPglucose-starch glucosyltransferase.

Kramer *et al.* (1958) showed that the double mutant, *ae wx*, contained about 15% of amylose, determined on the basis of iodine–amylose complex formation. Since *wx* was shown to be completely epistatic to other starch-forming mutants, such as *dull*, *sugary-1*, and *sugary-2*, but not to *ae*, for the production of amylose, these workers suggested that *ae* might condition a different pathway of amylose synthesis. However, the amount of amylose-like material detected in *ae wx* may be due to the presence of amylopectin containing more helical structure available for complexing with iodine. Chuang, Tsai, and Dalby (unpublished data) have shown the *ae wx* produces amylopectin exclusively, as does *wx*, but the maximum absorption for the iodine spectrum of the *ae wx* amylopectin is 580 nm, as compared with 540 nm for the *wx* amylopectin. The facts that the lack of the starch granule–bound NDPglucose-starch glucosyltransferase is the primary consequence of the *wx* gene mutation and that the *wx* gene is epistatic to all other starch-forming mutant genes for the synthesis of amylose suggest that the starch granule–bound glucosyltransferase is responsible for the normal synthesis of amylose in maize endosperm.

REFERENCES

Akatsuka, T., and Nelson, O. E. (1969). Studies on starch synthesis in maize mutants. *J. Jap. Soc. Starch Sci.* **17**:99.

Akazawa, T., and Murata, T. (1965). Absorption of ADPG-starch transglucosylase by amylose. *Biochem. Biophys. Res. Commun.* **19**:21.

Cowie, J. M. G., and Greenwood, C. T. (1957). Physicochemical studies on starches. Part VI. Aqueous leaching and the fractionation of potato starch. *J. Chem. Soc. Lond.*, p. 2862.

DeFekete, M. A. R., Leloir, L. F., and Cardini, C. D. (1960). Mechanism of starch biosynthesis. *Nature* **187**:918.

Frydman, R. B., and Cardini, C. E. (1964). Biosynthesis of phytoglycogen from adenosine diphosphate D-glucose in sweet corn. *Biochem. Biophys. Res. Commun.* **14**:353.

Frydman, R. B., and Cardini, C. E. (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.

Fuwa, H. (1957). Phosphorylase and Q enzyme in developing maize kernels. *Arch. Biochem. Biophys.* **70**:152.

Hassid. W. Z. (1954). In Greenberg, D. M. (ed.), *Chemical Pathways of Metabolism*, Vol. 1, Academic Press, New York, p. 235.

Jeanes, A., Wise, C. S., and Dimlev, R. J. (1951). Improved techniques in paper chromatography of carbohydrates. *Anal. Chem.* **23**:415.

Kramer, H. H., Pfahler, P. L., and Whistler, R. L. (1958). Gene interactions in maize affecting endosperm properties. *Agron. J.* **50**:207.

McGuire, J. P., and Erlander, S. R. (1966). Quantitative isolation and dispersion of starch from corn kernels without degradation. *Die Stärke* **19**:337.

Nelson, O. E., and Rines, H. W. (1962). The enzymatic deficiency in the *waxy* mutant of maize. *Biochem. Biophys. Res. Commun.* **9**:297.

Nelson, O. E., and Tsai, C. Y. (1964). Glucose transfer from adenosine diphosphate glucose to starch in preparations of waxy seeds. *Science* **145**:1194.

Sprague, G. F., Brimhall, B., and Hixon, R. M. (1943). Some effects of the waxy gene in corn on properties of the endosperm starch. *Agron. J.* **55**:817.

Tanaka, Y., and Akazawa, T. (1968). Substrate specificity of the granule-bound and chloroplastic starch synthetase. *Plant Cell Physiol.* **9**:405.

Tsai, C. Y. (1965). Correlation of enzymatic activity with *Wx* dosage. *Maize Genet. Coop News Letter* **39**:153.

Tsai, C. Y. (1973). The activity of maize α-1,4 glucan glucosyltransferases *in vitro*. *Bot. Bull.* **14**:75.

Tsai, C. Y., and Nelson, O. E. (1966). Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity. *Science* **151**:341.

Tsai, C. Y., Salamini, F., and Nelson, O. E. (1970). Enzymes of carbohydrate metabolism in the developing endosperm of maize. *Plant Physiol.* **46**:299.

Ulmann, M., and Augustat, S. (1958). Die quantitative Bestimmung des Gehaltes an Amylose in Stärke nach der "Blauwert" Methode unter Verwendung des Universalcolorimeters von B. Lange. *Z. Anal. Chem.* **162**:337.