

Sugar Metabolism in Developing Kernels of Starch-Deficient Endosperm Mutants of Maize

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ABSTRACT

Sugar metabolism in kernels of starch-deficient endosperm mutants of maize (*Zea mays* L.) was examined to determine how single locus mutations of carbohydrate metabolism affect carbohydrate metabolism as a whole. Activities of 14 enzymes were measured in extracts from endosperms from isogenic lines of normal, *shrunken*, *shrunken-2*, *shrunken-4*, *brittle-1*, and *brittle-2* maize in an OH43 background. Nearly every enzyme activity examined was affected in some or all of the mutants. Sucrose synthase and aldolase activities were lower in all mutants compared to normal. ADP-Glc pyrophosphorylase activity in immature kernels was much higher in *brittle* endosperms than in normal, but absent in *brittle-2* and *shrunken-2* endosperms. The activity in those genotypes exhibiting activity was positively correlated with sucrose concentration in the kernels. Sucrose may be modulating the coarse control of ADP-Glc pyrophosphorylase activity by affecting the genetic transcription of message for this enzyme. Sorbitol dehydrogenase activity was negatively correlated with its substrate, fructose, supporting the hypothesis that sorbitol dehydrogenase converts fructose produced during sucrose degradation into sorbitol. Glucokinase activity was positively correlated with mature kernel dry weight. This supports the hypothesis that glucokinase activity may limit sucrose utilization. *Shrunken-4* extracts had lower activities for a number of enzymes, supporting the view that this mutant may have an impediment to protein synthesis. Elevated sucrose levels were evenly distributed throughout 20-day postpollination *shrunken-2* kernels, whereas a sucrose concentration gradient existed in normal kernels between the basal region and the upper endosperm. This gradient is apparently generated by the utilization of sugars and may facilitate the movement of sugars into developing corn kernels.

A number of mutants of maize are known that accumulate less starch and storage protein in the endosperms of their kernels than do normal kernels (2, 6, 13). The genetic lesion of several of the starch-deficient mutants are known. The *sh*¹ mutant is known to be deficient in sucrose synthase-1 (5), the endosperm specific or inducible form of sucrose synthase (4, 15). Both *sh-2* and *bt-2* mutants are deficient in ADP-Glc pyrophosphorylase activity (17). The *sh-4* mutant reportedly affects pyridoxyl phosphate metabolism (3). The nature of the *bt* mutant is not known (2).

¹ Abbreviations: *sh*, *shrunken*; *bt*, *brittle*; *bt-2*, *brittle-2*; *sh-2*, *shrunken-2*; *sh-4*, *shrunken-4*; DPP, days postpollination; *r*, correlation coefficient for linear regression; *P*, probability.

The metabolic characterization of starch-deficient endosperm mutants can provide valuable information about metabolism in normal kernels. For example, the substantial decrease in starch accumulation in *bt-2* and *sh-2* mutants indicates the importance of the enzyme ADP-Glc pyrophosphorylase in the synthesis of starch. The small amount of starch found in these mutants is attributed to a low residual activity of ADP-Glc pyrophosphorylase (8). The observation that the *sh* mutation results in only a 30% or less decrease in dry weight (5, 13) indicates that the residual sucrose synthase activity and the unaffected invertase activity (11) are capable of partially compensating for the deficiency in the sucrose synthase-1 activity.

Previous studies (2, 6, 13) have shown that the starch-deficient mutations result in profound changes in sugar, starch, and protein accumulation in developing kernels. In this study, a selected set of enzyme activities were measured in endosperm extracts from normal and starch-deficient maize kernels in order to evaluate how the genetic lesions affect the overall carbohydrate metabolism of mutant kernels. Some correlations between enzyme activities and kernel characteristics are presented that provide evidence for the roles and regulation of certain enzymes in carbohydrate metabolism. Finally, the distributions of sugars in the tissues of normal kernels and the *sh-2* mutant were evaluated in order to observe the effect of sucrose utilization on sugar concentrations in the different tissues of developing corn kernels.

MATERIALS AND METHODS

Normal OH43 maize (*Zea mays* L.) and isogenic *sh*, *sh-2*, *sh-4*, *bt*, and *bt-2* mutants in an OH43 background were grown in the field in the summer of 1986 and 1988. Enzyme activities were measured in kernels grown in 1986. The distribution of sugars in normal and *sh-2* kernels were determined on kernels grown in 1988. Ears were pollinated by hand and harvested at designated times after pollination. Whole endosperms were dissected by hand from freshly harvested kernels, frozen immediately on dry ice, and lyophilized. Lyophilized endosperm tissue was ground to a powder and stored at -80°C until used for enzyme extractions. Designated samples of freshly harvested kernels were dissected into base, pericarp, embryo, lower endosperm, middle endosperm, and upper endosperm by hand as described previously (10), frozen, and lyophilized as described above.

Enzymes were extracted from lyophilized endosperms by the procedure described earlier (10). The activities of sucrose

synthase (UDP-Glc: D-fructose 2-glucosyltransferase, EC 2.4.1.13), fructokinase (ATP: D-fructose 6-phosphotransferase, EC 2.7.1.4), glucokinase (ATP: D-glucose 6-phosphotransferase, EC 2.7.1.2), phosphofructokinase (ATP: D-Fru-6-P 1-phosphotransferase, EC 2.7.1.11), PPi:Fru-6-P P-transferase (EC 2.7.1.90), aldolase (D-Fru-1,6-bisP D-glyceraldehyde-3-P lyase, EC 4.1.2.13), phosphoglucoisomerase (D-Glu-6-P ketoisomerase, EC 5.3.1.9), phosphoglucomutase (D-Glc-1,6-bisP: D-Glc-1-P phosphotransferase, EC 2.7.5.1), UDP-Glc pyrophosphorylase (UTP: D-Glc-1-P uridyltransferase, EC 2.7.7.9), and sorbitol dehydrogenase (sorbitol: NAD 5-oxido-reductase EC 1.1.1.14) were assayed using procedures described earlier (10).

ADP-Glc pyrophosphorylase (ATP: D-Glc-1-P adenytransferase EC 2.7.7.27) activity was measured in undialyzed extracts because of the extreme lability of the activity. Activity was measured in the direction of ADP-Glc degradation by coupling Glc-1-P production with phosphoglucomutase, Glc-6-P dehydrogenase, and NAD reduction. Assays contained 50 mM Hepes-NaOH (pH 7.2), 5 mM MgCl₂, 1 mM ADP-Glc, 10 mM 3-phosphoglycerate, 1 mM NAD⁺, 5 μM Glc-1,6-bisP, 1 unit/mL phosphoglucomutase, 2.5 units/mL Glc-6-P dehydrogenase, and 25 μL of enzyme extract per 1 mL of assay volume. Assays were initiated with 2 mM PPi. Activity was derived from the increase in A₃₄₀ as NAD⁺ was reduced.

Total amylase (total amylopectin hydrolase) activity was assayed by measuring the increase of reducing power in a solution of soluble starch. Assays contained 50 mM citrate-NaOH (pH 6.0) and 2.5% (w/v) soluble potato starch. Assays were terminated after 10 min, and the reducing power was measured with dinitrosalicylic acid solution (1).

Starch-debranching enzyme (amylopectin 6-glucanhydrolase, EC 3.2.1.41) was assayed by the release of maltotriose from pullulan. Assays contained 50 mM Hepes-NaOH (pH 7.2), 5 mM MgCl₂, and 2.5% (w/v) pullulan. Samples were taken at 0, 30, 60, 90, and 120 min. The reducing power of each sample was determined with dinitrosalicylic acid solution (1) and compared with a maltotriose standard curve. Starch debranching enzyme activity was equivalent to the rate of maltotriose generation from pullulan. In addition to a zero time blank, an additional blank of the complete assay minus pullulan was allowed to incubate for the assay time and the absorbance subtracted from the complete assay in order to correct for the reducing power generated by amylases acting on soluble starch in the kernel extracts.

Starch phosphorylase (1,4-α-D-glucan: Pi α-glucosyltransferase, EC 2.4.1.1) activity was measured with a continuous spectrophotometric procedure in which the production of Glc-1-P from starch and Pi was coupled with NAD⁺ reduction through phosphoglucomutase and Glc-6-P dehydrogenase. Assays contained 50 mM Hepes-NaOH (pH 7.2), 5 mM MgCl₂, 0.5% (w/v) soluble potato starch, 1 mM NAD⁺, 50 μM Glc-1,6-bisP, 1 unit/mL phosphoglucomutase, and 4 units/mL Glc-6-P dehydrogenase. Reactions were initiated by adding 10 mM Pi. Reaction rates were derived from the rate of increase in A₃₄₀ subsequent to the addition of Pi.

All enzyme assays have been experimentally optimized for corn kernel extracts. One unit of activity is defined as the activity necessary to produce 1 μmol of product in 1 min.

Sugar analyses of whole kernels and dissected parts of normal and *sh-2* kernels were performed by the method of Kuo *et al.* (12).

Data Analysis

All measurements were made on four separate samples of endosperms, each from a different ear. Analysis of variance and correlation analysis were performed using a computer program (ABSTAT², Anderson Bell, Parker, CO). Least significant differences were calculated by the procedure described by Steel and Torrie (16). Lines drawn in all figures were the result of linear regressions.

RESULTS

Enzyme activities were measured in extracts from endosperms dissected from normal and mutant kernels harvested 20 DPP (Table I). On a fresh weight basis, sucrose synthase activity was significantly lower than normal in all mutants examined (Table I). Endosperms of *sh* had particularly low sucrose synthase activity, but *sh-4* endosperms also had less than one-half the activity of normal.

ADP-Glc pyrophosphorylase activity was absent from *bt-2* and *sh-2* endosperms, but was significantly higher than normal in *bt* endosperms (Table I). If ADP-Glc pyrophosphorylase activity was compared to sucrose levels in kernel samples, a significant linear relationship was observed among those genotypes exhibiting activity (Fig. 1).

Phosphofructokinase activity was significantly higher in *bt* and *sh* endosperms than in normal endosperms (Table I). PPi: Fru-6-P P-transferase activities were significantly lower in *bt* and *sh-4* than in normal endosperms (Table I). Aldolase activities were lower in all mutant endosperms than in normal endosperms, but were particularly low in *bt* endosperms.

Sorbitol dehydrogenase activities were significantly higher in *bt-2* endosperms than in normal endosperms (Table I). Sorbitol dehydrogenase activities among all genotypes were found to be negatively correlated with fructose concentration in kernels when both were expressed on a fresh weight basis ($r = -0.94$, $P < 0.01$) (Fig. 2).

Glucokinase activities were higher in normal endosperms than in any of the mutants. Among the mutants, glucokinase activity was highest in *sh* endosperm and lowest in *sh-2* endosperms (Table I). Glucokinase activity expressed on a fresh weight was positively correlated with mature kernel dry weight ($r = 0.91$, $P < 0.01$) (Fig. 3). Fructokinase activity was significantly lower than normal in *sh-2* and *sh-4* (Table I).

Phosphoglucoisomerase activity was significantly higher in *bt-2* endosperms than in normal, *bt*, *sh*, or *sh-4* endosperms (Table I). Phosphoglucomutase activity was significantly lower in *sh-4* endosperms than in normal endosperms (Table I).

Total amylase activity in endosperms was significantly lower in *bt*, *sh*, *sh-2*, and *sh-4* than in normal (Table I). Starch-debranching enzyme activity was significantly lower

² The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Table I. Enzyme Activities per Gram Fresh Weight in Extracts of 20 DPP Endosperms from Normal OH43 and Starch-Deficient Mutants Isogenic in an OH43 Background

Values represent the mean of four extractions, each from tissue obtained from a separate ear.

Enzyme	Normal	<i>bt</i>	<i>bt-2</i>	<i>sh</i>	<i>sh-2</i>	<i>sh-4</i>	LSD _{0.05}
	<i>units · g fresh wt⁻¹</i>						
Sucrose synthase	7.78	4.44	5.47	1.50	4.83	3.26	1.64
ADP-Glc pyrophosphorylase	1.71	3.31	0.00	1.83	0.00	0.99	1.25
Phosphofructokinase	0.06	0.18	0.11	0.19	0.08	0.05	0.11
PPi:Fru-6-P P-transferase	2.53	1.86	2.79	3.28	2.61	2.02	1.19
Aldolase	5.03	2.27	3.54	3.07	3.57	3.04	1.14
Fructokinase	0.15	0.14	0.16	0.23	0.09	0.10	0.09
Glucokinase	0.48	0.21	0.29	0.39	0.19	0.23	0.09
Sorbitol dehydrogenase	1.51	2.20	2.82	1.76	2.15	1.11	1.15
Phosphoglucosomerase	3.88	4.19	5.52	4.12	4.92	4.15	1.22
Phosphoglucumutase	30.50	24.80	30.40	27.80	23.60	19.40	7.80
Total amylase	3.80	2.47	2.99	2.00	2.51	1.44	0.95
Starch-debranching enzyme	0.32	0.03	0.11	0.26	0.15	0.06	0.21
Starch phosphorylase	0.43	0.49	0.65	0.36	0.49	0.24	0.17

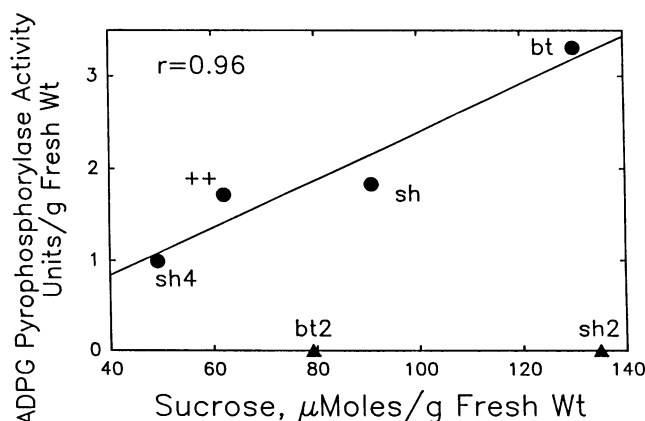


Figure 1. Correlation between ADP-Glc pyrophosphorylase activity and sucrose concentration in endosperm extracts from normal and starch-deficient endosperm mutants of maize harvested 20 DPP. Endosperms of *bt-2* and *sh-2* were deficient in activity because of their genetic lesion and were not included in the regression.

in *bt* and *sh-4* endosperms than in normal endosperms (Table I). Starch phosphorylase activity was highest in *bt-2* endosperms and lowest in *sh-4* endosperms (Table I).

An additional experiment was performed to compare the distribution of sugars in tissues of mutant and normal kernels. *Shrunken-2* kernels were chosen to examine further because of the particularly high sugar content found in that mutant. In normal kernels, the highest sugar levels were found in the base and pericarp (Table II) with particularly high glucose and fructose content in the basal region. A sucrose concentration gradient appeared to exist between the basal region and the upper endosperm of normal kernels. In contrast, all parts of the *sh-2* kernel, with the exception of the pericarp, had about the same sucrose levels. The pericarp of *sh-2* kernels had significantly higher sucrose levels than most other parts of the kernel. All parts of the *sh-2* kernels had significantly higher sucrose levels than normal kernels. *Shrunken-2* kernels also had significantly higher glucose in the middle and upper

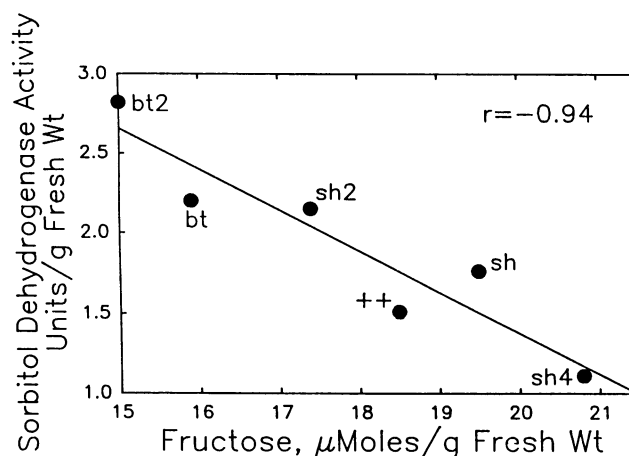


Figure 2. Correlation between sorbitol dehydrogenase activity and fructose concentration in endosperm extracts from normal and starch deficient endosperm mutants of maize harvested 20 DPP.

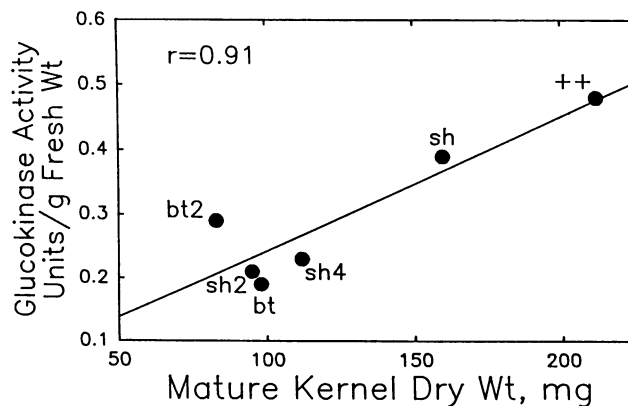


Figure 3. Correlation between glucokinase activity in endosperm harvested 20 DPP and mature kernel dry weights levels in normal and starch-deficient mutants of maize.

Table II. Distribution of Sugars in Normal OH43 and *sh-2* Maize Kernels Harvested 20 DPP

Kernels were obtained from plants grown in the field in 1988. Each value represents the mean from four extractions, each from tissue obtained from a separate ear.

	Sucrose		Glucose		Fructose		Sorbitol	
	Normal	<i>sh-2</i>	Normal	<i>sh-2</i>	Normal	<i>sh-2</i>	Normal	<i>sh-2</i>
	<i>mg sugar · g fresh wt⁻¹</i>							
Base	47.2	59.2	16.7	21.5	14.0	18.9	1.2	2.2
Pericarp	49.1	68.3	8.5	9.6	3.5	3.7	0.3	0.2
Embryo	34.4	54.7	0.3	0.4	0.6	0.8	0.4	0.7
Lower endosperm	31.7	57.5	2.6	4.8	2.7	4.1	1.0	2.3
Middle endosperm	10.5	54.9	0.3	2.9	0.7	2.4	0.4	1.9
Upper endosperm	3.2	59.9	0.2	3.4	0.4	1.6	0.2	2.1
LSD _{0.05}	5.5	8.5	1.9	3.8	1.9	3.1	0.4	0.6

endosperm and higher fructose in the upper endosperm than did normal kernels and had significantly higher sorbitol levels in the base and endosperm than did normal kernels.

DISCUSSION

It is clear that many diverse enzyme activities are affected by the endosperm mutants examined in this study. Perhaps the most interesting of these is the apparent relationship between sucrose levels and ADP-Glc pyrophosphorylase activity (Fig. 1). ADP-Glc pyrophosphorylase is thought to be a key enzyme controlling starch biosynthesis in developing maize endosperm (7). The correlation between sucrose and the ADP-Glc pyrophosphorylase activity among the genotypes exhibiting activity (Fig. 1) supports the hypothesis of the sucrose-modulated expression of this enzyme. Such a mechanism might utilize sucrose accumulation as a signal to increase starch synthesizing capacity in developing endosperm cells, thus utilizing sugars at a faster rate. The increase in ADP-Glc pyrophosphorylase activity could be achieved by increasing the genetic transcription and subsequent translation of the message for the enzyme. Other mechanisms affecting the coarse control of this enzyme activity are also possible. Two of the mutants tested (*bt-2* and *sh-2*) are known to be deficient in ADP-Glc pyrophosphorylase activity (17) and thus may be genetically unable to respond to the increased sucrose as a physiological signal. Starch levels in *bt* may be low in spite of the increase in ADP-Glc pyrophosphorylase activity because of the unknown genetic lesion of *bt*. Additional experimentation will be necessary to test whether the differences in activity are because of the transcriptional differences.

Sorbitol dehydrogenase is thought to function to convert fructose produced during sucrose degradation into sorbitol as part of the sorbitol pathway (10). A negative correlation between fructose and sorbitol dehydrogenase activity (Fig. 2) appears to support this hypothesis.

Glucokinase activity at 20 DPP was positively correlated with mature kernel dry weight (Fig. 3). It was observed earlier that glucokinase activity (as well as fructokinase activity) were particularly low relative to other enzymes of sugar metabolism, and it was hypothesized that these activities could limit

sucrose utilization (10). The relationship between glucokinase activity and kernel dry weight is consistent with this hypothesis, but clearly other factors appear to be limiting dry weight accumulation in mutant kernels.

The *sh-4* mutant was reportedly deficient in pyridoxyl phosphate metabolism (3), thereby affecting starch phosphorylase activity. In the present study, in addition to *sh-4* kernels containing lower starch phosphorylase activity (Table I), *sh-4* endosperm also contained significantly lower sucrose synthase, aldolase, glucokinase, phosphoglucosmutase, total amylase, and starch-debranching enzyme (Table I). The results are consistent with the presence of some type of impediment to protein synthesis, possibly caused by deficient pyridoxyl phosphate metabolism (3).

It has been shown previously that starch-deficient mutants contain higher sugar levels than normal kernels (2, 6, 13), but the distribution of sugars in these kernels has apparently not been studied. Perhaps one of the most interesting differences in the distribution of sugars in normal and *sh-2* mutant kernels (Table II) is the presence of a sucrose concentration gradient between the basal region and the upper endosperm in normal kernels and the absence of this gradient in *sh-2* kernels. This sucrose concentration gradient in normal corn kernels was observed previously by Shannon (14). Since the major difference between normal and *sh-2* kernels is that normal kernels are utilizing sugars to accumulate dry weight whereas *sh-2* kernels are not, the sucrose concentration gradient in normal kernels appears to be generated by the utilization of sucrose by the developing kernel. Furthermore, the maintenance of this concentration gradient by sugar utilization could facilitate the passive movement of sucrose into the kernel, since sucrose entering the kernel in the basal region would diffuse towards regions of lesser sucrose concentration. Thus, the utilization of sucrose by developing endosperm may provide some of the driving force necessary to draw more sucrose into developing corn kernels. The failure of kernels to utilize sucrose appears to result in its accumulation whereby sucrose in all parts of the kernel (except the pericarp) reached an equilibrium with the basal region as observed in *sh-2* kernels (Table II). The presence of sucrose in *sh-2* pericarp at higher levels than in the rest of the kernel indicates that a sucrose concentrating mechanism may exist in the pericarp, possibly associated with the vacuole. Increased hexoses in the basal region of normal and *sh-2* kernels reflects the high invertase activity in those regions (9). Increased sorbitol in mutant endosperm reflects the increased hexoses in that tissue (Table II). Relative to hexoses, sorbitol concentrations are low in the basal region. This may be due to the lack of ketose reductase (sorbitol dehydrogenase) in that region of the kernel (10).

In conclusion, several enzyme activities in normal and mutant maize kernels appeared to vary in relation to the altered properties of sugar metabolism in the mutants. Both sucrose synthase and aldolase activities were lower in all mutants examined in comparison to normal endosperms. ADP-Glc pyrophosphorylase activity appeared to be dependent on sucrose levels in the immature kernel. Glucokinase activity was positively correlated with mature kernel dry weight. This information provides insight into the control of

grain fill in normal kernels and provides the basis for future studies on the metabolic control of gene expression.

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