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Genetic Control of Sucrose Synthetase in Maize Endosperm

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Summary. Sucrose synthetase activity in endosperm extracts of seven *shrunken*(*sh*) mutants of spontaneous origin and three similar mutants due to the association of the controlling element *Ds* with the *Sh* locus is examined. A residual level of 3 to 5% as compared to the normal (*Sh*) endosperm is seen in all the mutants. The residual activity is similar to that of the *Sh* locus encoded endosperm sucrose synthetase by several criteria including an identical size of polypeptides and a similarity in antigenic properties. These two enzymes are, however, distinguishable by a slight difference in electrophoretic mobility in native gels and a difference in the relative abundance of enzyme molecules. The latter property is a reflection of a marked difference seen in the developmental profile of enzyme activity in the two genotypes. The earlier hypothesis (Chourey and Nelson 1976) that these two sucrose synthetases are encoded by two separate genes is strengthened by: (a) the presence of the residual enzyme in a *sh* deletion mutant and (b) an electrophoretic demonstration of two proteins, corresponding to the major and minor sucrose synthetase proteins, in the wild type (*Sh*) genotype. The two sucrose synthetase genes seem to provide a model system in plants for studying the molecular basis of temporal specificity of genes.

Introduction

Biochemical genetic analysis to elucidate gene-enzyme-phenotype relationship is a study of major significance. Such analyses have played a central role in understanding gene function and determination of rate limiting steps in biosynthetic pathways. Though advances have been made using microbial systems, very limited knowledge is available in this area in higher plants. In maize, mutants with altered quantity or quality of starch in the endosperm tissue have been of particular importance in this regard. Because their phenotype readily identifies the area of metabolic lesion, gene-enzyme relationships have been intensively examined in these mutants. To date, four starch mutants have been identified with their associated enzymatic lesions. These are: the *waxy* locus with starch granule bound glucosyl transferase (Nelson and Rines 1962; Nelson and Tsai 1964), the *shrunken-2* and *brittle-2* mutations with ADP-glucose pyrophosphorylase (Tsai and Nelson 1966; Dickinson and Preiss 1969) and the *shrunken* locus with sucrose synthetase (Chourey and Nelson 1976, 1979). In each case the mutational effect is highly specific to the endosperm tissue both at the phenotypic and the enzyme level, with the exception of the *wx* mutation which also expresses in the pollen grains. An intriguing observation remains that not a single mutant is associated with a complete loss of the respective enzyme activity. A residual level of low enzyme activity is seen in the endosperm tissue of all the mutants. The genetic as well as physiological basis of the residual activity is poorly understood.

The *shrunken* (*sh*) mutation in homozygous form conditions a shrunken or collapsed phenotype of the kernel due to a reduced level of starch content in the endosperm. At the protein level, the *sh* mutation is associated with a complete loss of the *Sh* locus encoded protein, designated as Sh protein (Schwartz 1960; Chourey and Schwartz 1971). Subsequent analysis of starch biosynthesis enzymes of normal (*Sh*) and *sh* endosperms has shown the Sh protein to be the enzyme sucrose synthetase (Chourey and Nelson 1976). Several lines of evidence show that the *Sh* locus is the structural gene for sucrose synthetase enzyme (Chourey and Nelson 1976, 1979). Enzymatic analyses also indicated that all spontaneous *sh* mutants analyzed, though shown to be 'null' alleles for the Sh protein, retained a residual level of approximately 8–10% sucrose synthetase activity as compared to the normal endosperm. Suggestive evidence indicated that the residual activity was genetically independent of the *sh* locus (Chourey and Nelson 1976). The observations discussed here strengthen this hypothesis.

The present communication focuses on a comparative analysis of sucrose synthetase activities in endosperm tissue of normal and *shrunken* genotypes. The enzyme in these two genotypes, though similar to each other by many criteria, is distinguishable by a slight difference in electrophoretic mobility in native gels. In addition, the two genotypes show a marked developmental difference leading to a significant difference in the relative levels of sucrose synthetase proteins. The genetic evidence presented here clearly indicates that the residual activity in *sh* endosperm is due to a separate gene elsewhere in the genome.

Materials and Methods

Developing kernels of various genotypes at 22 days after pollination (DAP) or at various development stages were harvested, frozen on dry ice and stored at −20° C. The embryo and pericarp

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were removed prior to homogenization unless specified otherwise. A mixture of equal parts by weight of endosperm and chilled 0.01 M tris-maleate buffer (pH 7.0) was homogenized, strained through two layers of cheesecloth and centrifuged at 30,000 g for 20 min. The supernatant was dialyzed overnight against the extraction buffer. The preparation thus obtained, referred to as endosperm extract or crude extract, was used as the enzyme source for the routine assays.

Enzyme Assay. Enzyme activity was assayed in the direction of sucrose synthesis as well as sucrose cleavage. The reaction mixture for sucrose synthesis contained 60 μmoles of glycine-NaOH buffer (pH 9.0), 2 μmoles $MgSO_4$, 10 μmoles fructose, 2 μmoles uridine diphosphate glucose (UDPG), and 1,5 or 10 μl of crude enzyme preparation from various genotypes in a total volume of 0.4 ml. The control tubes lacked UDPG. The reaction was carried out at 30° C for 15 min followed by the addition of 0.1 ml of 10 N NaOH. The mixture was boiled in a water bath for 12 min to destroy the unreacted fructose. The sucrose formed was then measured by the method of Roe (1934). The reaction mixture for sucrose cleavage consisted of 64 μmoles MES buffer (pH 6.0), 125 μmoles sucrose, 0.5 μmoles uridine diphosphate (UDP), and 1,5 or 10 μl of the crude enzyme preparation from various genotypes in a total volume of 0.4 ml. Entries lacking UDP constituted the controls. The tubes were incubated in a 30° C water bath for 15 min and the reaction was terminated by adding Nelson's reagent (1944) for the assay of reducing sugars.

Partial Purification. Crude extract obtained from 40–50 gms of endosperm tissue was used for ammonium sulfate fractionation. Ammonium sulfate (12.57 g/100 ml) was added to the crude extract, stirred for 20 min and centrifuged as above. Ammonium sulfate was added the second time (6.27 g/100 ml), stirred and centrifuged. The pellet was dissolved in the extraction buffer, dialyzed overnight and then loaded on a Bio-gel A 1.5 m column (diameter: 2.5 cm; length: 90 cm) in a total volume of 10 ml. Elution was performed with extraction buffer at a flow rate of 11 ml/hr and 5 ml fractions were collected. A total of four tubes surrounding the peak specific activity tube were pooled, concentrated by ammonium sulfate precipitation, dialyzed overnight and used for Km determinations.

The Km determinations were made according to Lineweaver-Burk reciprocal plot analysis. Protein determinations were done according to Lowry et al. (1951). The specific details for electrophoretic analysis of proteins on non-denaturing discontinuous acrylamide slab gels (0.8 mm thickness) are as follows (adapted from Hoeffer Scientific Instrument Catalog, 1980): The separating gel was prepared at 7.0% T and 5.0% C in 239 mM tris, 0.072 N HCl buffer and the stacking gel consisted of 3.125% T, and 20% C in 39.5 mM tris, 0.064 N H_3PO_4 buffer. Crude extracts were mixed with 50% glycerol and electrophoresed at a constant voltage of 200 volts for 6 h, unless specified otherwise. The buffer in the upper and lower chambers consisted of 37.6 mM tris, 40 mM glycine and 63 mM tris, 50 mM HCl, respectively. Electrophoresis was done at 4° C. Standard discontinuous SDS gels (Laemmli 1970) were prepared as follows: 5% acrylamide in 0.125 M tris HCl (pH 6.8) with 0.1% SDS as stacking gel over 10% acrylamide in 0.375 M tris-HCl (pH 8.8) with 0.1% SDS as separating gel. Crude extracts were diluted 1:1 in 2× treatment buffer which consisted of 0.125 M tris – HCl (pH 6.8), 4% SDS, 20% glycerol and 10% 2-mercaptoethanol. The sample was then put in a boiling water bath for 90 s and chilled in ice until ready to use. Electrophoresis was carried out at 20 ma for approximately 3 h using 0.25 tris 0.192 M glycine, pH 8.6–8.7 and 0.1% SDS, as the electrode buffer. Gels were stained at room temperature for 1 h in a solution containing 0.1% coomassie blue (Biorad) in 50% methanol and 10% acetic acid. Destaining was done in 50% methanol, 10% acetic acid solution until an optimum contrast between protein bands and background of the gel was obtained. Preparatory acrylamide gels (native) were 1.2 mm thick and were electrophoresed overnight. After electrophoresis, the position of the desired protein band was visualized by staining a part of the gel. The remainder of the gel was saved at 4° C. Soon after visualization of the disired protein band, the piece corresponding to it in the unstained gel was sliced out, cut into small pieces of desired length, minced and subsequently added to the reaction mixture for the assay of sucrose synthetase activity or tested for homogeneity in native as well as in SDS gels.

Immuno-Inactivation Analysis. The preparation of the antiserum has been described previously (Chourey and Nelson 1976). A constant volume of normal or *sh* endosperm extract, equilibrated for the level of soluble protein, was treated with the antiserum raised against the partially purified sucrose synthetase from the normal endosperm. The 200 μl reaction mixture consisted of crude extract, a variable amount of the antiserum, 30 μl of 3% polyethylene glycol (6,000 M.W.) in 35 mM tris – HCl buffer (pH 8.2) and the remainder of the volume consisted of the grinding buffer. The control reaction employed variable amounts of nonspecific serum. The mixtures were incubated for 4 h at 4° C and then centrifuged at 12,000 g for 30 min at 4° C. Supernatant aliquots were assayed for sucrose synthetase activity.

Results

The sucrose synthetase activity in 22 day old endosperms of various genotypes is shown in Table 1. A previous study (Chourey and Nelson 1976) included five *sh* mutants of spontaneous and independent origin; all the mutants were associated with a loss of approximately 90% of the total sucrose synthetase activity. A subsequent study (Chourey and Nelson 1979) showed that the optimal levels of sucrose synthesis and sucrose cleavage reactions, due to this enzyme, were obtained at pH 9.0 and pH 6.0, respectively. The analyses reported in the first communication were done at pH 8.0 which significantly inhibited the

Table 1. Sucrose synthetase activity in 22 day old endosperms of normal spontaneous *shrunken* and *Ds* suppressed *Sh* alleles (Stocks # 1–8 and # 9–13 were grown in Wisconsin and Florida, respectively.)

#	Stock	Synthesis (pH 9.0)		Cleavage (pH 6.0)	
		Sp. Act.[a]	%	Sp. Act.[a]	%
1	Normal-W22	1,080	100	1,568	100
2	*shrunken*-W22	48.5	4.88	78.5	5.05
3	*shrunken*-7196	29.0	2.92	36.9	2.37
4	*shrunken*-7205	50.4	4.67	79.5	5.07
5	*shrunken*-7342	29.9	2.77	42.6	2.72
6	*shrunken*-7611	41.8	3.87	53.6	3.42
7	*shrunken*-7650	56.0	5.18	76.7	4.89
8	*shrunken*-7731	60.4	5.59	83.1	5.30
9	Normal-W22	1,748	100	2,206	100
10	*shrunken*-W22	81.7	4.67	126.5	5.70
11	*shrunken*-m6233	43.8	2.51	55.0	2.50
12	*shrunken*-m5933	64.6	3.70	84.0	3.80
13	*shrunken-bz-m4*	69.3	3.96	95.6	4.30

[a] Specific Activity: nmoles of sucrose or reducing sugar synthesized per mg of protein per minute

Table 2. Developmental profile of sucrose synthetase activity in normal and *shrunken* endosperms. (The values in parenthesis represent % of W22 +/+)

Days after pollination	Specific activity (nmoles sucrose synthesized/mg protein/min)		
	W22 +/+	W22 sh/sh	
8	38.6[a]	16.8[a]	(43.50)
12	339.6	40.4	(11.89)
16	1,034.0	42.0	(4.10)
22	1,046.0	52.0	(4.97)
28	1,301.0	52.0	(4.00)
32	1,512.0	54.7	(3.61)
40	1,537.0	48.8	(3.17)
48	619.0	19.0	(3.70)

[a] Whole kernels were used for this analysis

sucrose cleavage reaction. The assays shown in Table 1 were conducted at pH 9.0 and pH 6.0 and also included newly defined substrate concentrations based upon Km determinations for various substrates reported here in Table 3. The genetic stocks in Table 1, with the exception of alleles in W22 inbred line and *sh-7205,* represent a new addition to the previously analyzed *sh* alleles for sucrose synthetase activity. All the mutants show a range of 2.5–5% activity as compared to the normal endosperm. This level of residual activity is manifested regardless of whether the analysis is done for the sucrose cleavage or for the sucrose synthesis reaction. Also included in Table 1 are three *Ds* suppressed *Sh* alleles (originally isolated by Dr. B. McClintock). These stocks were grown in Florida. The 22 DAP developmental stage of the kernel in Florida (Table 1, entries #9–13) is significantly advanced as compared to those (entries # 1–8) grown in Wisconsin. A higher level of specific activity in kernels grown in Florida is a reflection of this difference. However, a similar relative level of residual enzyme activity is also seen in these stocks.

The developmental profile of enzyme activity in the mutant and the wild type endosperm is shown in Table 2. Samples at the 4 DAP stage were not analyzable as maternal nucellus tissue constituted the predominant mass of material in the kernel. At the 8 DAP stage, enzyme assays were conducted on crude extracts obtained from whole kernels which included endosperm, embryo and pericarp. Subsequent analyses, i.e. at the 12–48 DAP stage were restricted to the endosperm tissue. The enzyme activity in the mutant at the 8 DAP stage constitutes nearly 43% of the total activity in the normal genotype. At the 12 DAP stage, the level of activity drops to 12% while during the following time periods up to 48 DAP, the level of activity remains at less than 5% of the normal endosperm. The wild type genotype shows a sharp increase in enzyme activity at the 16 DAP stage and continues at a gradual increase until the 40 DAP stage. An approximate 40 fold increase in enzyme activity is seen in the wild type genotype during development between 8–40 DAP. The mutant endosperm, during the same period shows only a four fold increase in enzyme activity.

Electrophoretic analysis of endosperm extracts under non-denaturing conditions, shows a major protein band which is completely missing in the mutants (Fig. 1). This protein designated as Sh protein (Schwartz 1960) is the enzyme sucrose synthetase (Chourey and Nelson 1976). The *sh* mutants, however, show a faintly stained protein band in that region of the gel. After purification to homogeneity by preparatory acrylamide slab gel electrophoresis and subsequent enzymatic analysis, we were able to identify this protein band as sucrose synthetase. Similar analyses were previously conducted using preparatory starch gels. It was also possible to show that sucrose synthetase proteins from normal and *sh* genotypes were electrophoretically separable (Chourey and Nelson 1976). However, it was not possible to demonstrate that both forms of sucrose synthetase were present in the normal genotype (Chourey and Nelson 1976; Chourey 1981). For the first time, the present analysis (Fig. 1B), allows detection of two proteins in the normal endosperm extracts. It shows, in addition to the Sh protein, a second protein co-migrating with the weakly stained protein identified as sucrose synthetase in the *sh* endosperm. Such a demonstration is possible only under certain restrictive conditions such as overloading of crude extract (approx. 50 µg protein per channel) and rather long durations of electrophoresis (18–24 h at 4° C).

Several other properties of the enzyme in normal and *sh* endosperms are listed in Table 3. Gel chromatography on a Biogel A 1.5 column showed a superimposable elution peak of enzyme activity in the two genotypes indicating a native enzyme of similar size. This is further substantiated by SDS gel electrophoresis of monomeric polypeptides of the respective genotypes (Fig. 2). The purified sucrose synthetase marker polypeptide (a single band) allows identification of the sucrose synthetase monomer in the wild type as well as in *sh* crude extracts. A

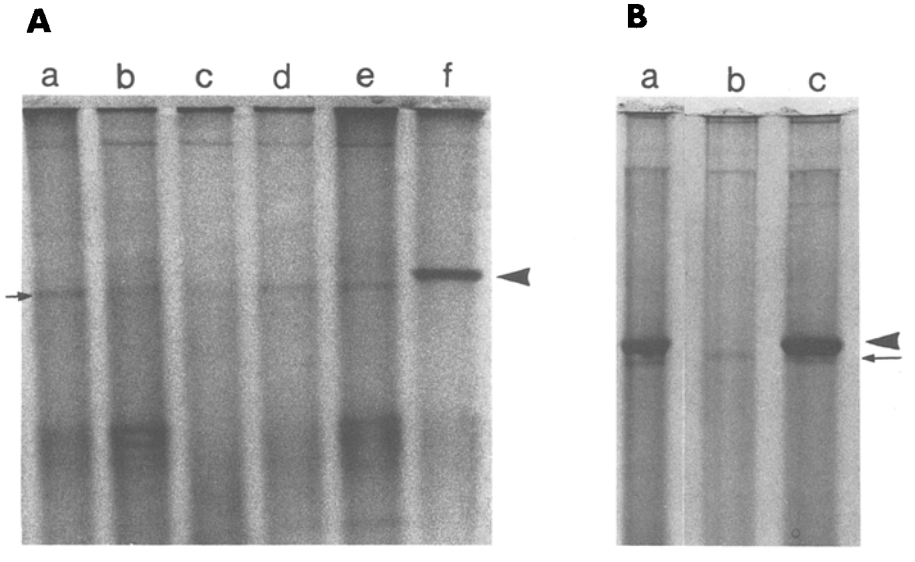

Fig. 1A, B. Representation of native polyacrylamide gel showing sucrose synthetase protein in endosperm extracts of various genotypes. (A dark and a light arrow mark the major and minor sucrose synthetase protein respectively).
A (a) *sh*-m 5,933, (b) *sh*-m 6,233, (c) *sh*-7,196, (d) *sh*-7,731, (e) *sh*-W22, (f) Sh-W22.
B (a) mixture of *Sh*-W22 and *sh*-W22 extracts, (b) *sh*-W22 and (c) *Sh*-W22

Table 3. Properties of sucrose synthetase in normal and *shrunken* endosperm extracts

Criteria	+/+ Endosperm	sh/sh Endosperm
1. Ammonium sulfate cut	30–50%	30–50%
2. Biogel A 1.5 m column, Peak sp. act. tube #	34–35	33–34
3. Electrophoretical	Sh protein band	forward band
4. Immunochemical[a]	similar	similar
5. pH optima for:		
Sucrose synthesis	9.0	9.0
Sucrose cleavage	6.0	6.0
6. Km's for:		
UDP-Glucose $\times 10^{-3}$ M	0.435	0.215
Fructose $\times 10^{-3}$ M	2.93	2.68
UDP $\times 10^{-3}$ M	0.85	0.62
Sucrose $\times 10^{-3}$ M	192.0	127.0

[a] As judged by immuno-inactivation of the enzyme activity by the antiserum

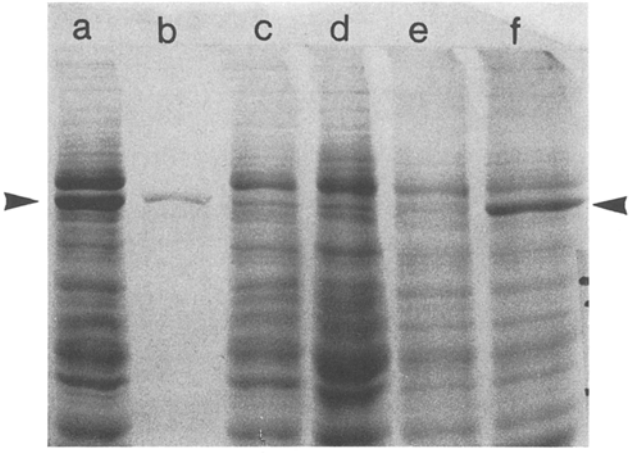

Fig. 2. SDS gel electrophoretic analysis of endosperm extracts in various genotypes (the arrows mark sucrose synthetase protein): (a) and (f) *Sh*-W22, (b) purified sucrose synthetase polypeptide (c) *sh-bz-m4*, (d) *sh*-m 5933 and (e) *sh*-W22. (Fast migrating lower M.W. polypeptides are not included)

polypeptide co-migrating with the purified sucrose synthetase of approximate MW of 89000 (molecular weight marker proteins not shown in Fig. 2), is seen in both genotypes. A considerable difference in relative staining intensity of this polypeptide is seen in the two genotypes. It is more intense in the wild type than in either *sh* or *sh bz-m4*; the relative staining intensity of other polypeptides is not significantly altered.

Immuno-precipitation (Fig. 3) reactions show that sucrose synthetase activity from both genotypes is completely inactivated upon reaction with antiserum raised against the partially purified enzyme from the wild type genotype. However, as expected, a quantitative difference is clearly noticeable. In the case of the normal genotype, 80 µl of antiserum is required to completely inactivate enzyme activity in 10 µl of crude endosperm extract. Thus, a ratio of 1:8 between endosperm homogenate and the antiserum is obtained. Similar analysis with the mutant endosperm extract yielded a ratio of 1:1 between the extract and the antiserum (50 µm of crude extract required 50 µl of the antiserum for complete inactivation of the enzyme activity).

No marked differences between the two genotypes are detectable for Km estimates of sucrose, UDP, UDP glucose and fructose (Table 3). The Km for sucrose is exceptionally high in both genotypes, as compared to the other three substrates.

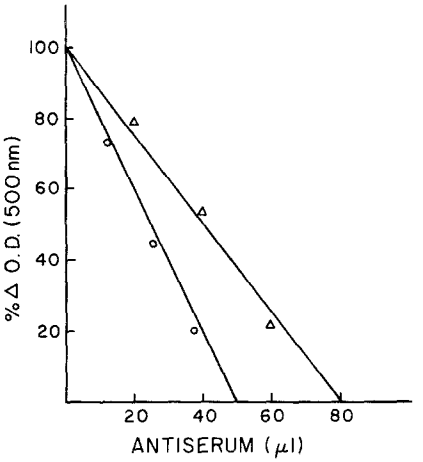

Fig. 3. Immuno-inactivation of sucrose synthetase activity in *Sh*-W22 (△–△) and *sh*-W22 (○–○) endosperm extracts by the antiserum raised against the enzyme from the normal genotype

Discussion

These studies clearly show that all *sh* alleles analyzed here, either of spontaneous origin or those due to association of the controlling element *Ds* with *Sh* locus, are associated with a substantial loss of sucrose synthetase activity. At the protein level, these alleles are CRM^- for the Sh protein. Schwartz (1960) reported CRM^- behavior for several *sh* alleles, including four alleles due to *Ds* suppression of the *Sh* locus. However, a residual level of approximately 5% of normal endosperm enzyme activity is seen in all the mutants.

A comparative analysis shows a similarity between the enzyme in the mutant and the enzyme present in the wild type. Both are of similar size at the polymer as well as at the monomer level. The Km estimates for the four substrates are similar for the two enzymes. The high Km for sucrose is a noteworthy observation as it is indicative of a poor affinity between the enzyme and the substrate, sucrose. Genetic evidence indicates that sucrose breakdown reaction of this enzyme is a critical in vivo function in the endosperm cells (Chourey and Nelson 1979). Such a situation would require endosperm cells to have excessive amounts of sucrose synthetase molecules for an efficient mobilization of sucrose into subsequent reactions. Indeed, this enzyme is present in large amounts in wild type developing endosperm and is easily detectable as a major protein after gel electrophoresis (Schwartz 1960; Chourey and Schwartz 1971; Chourey and Nelson 1976; Su and Preiss 1978). Sucrose synthetase in the mutant endosperm is also present in significant amounts and is thus detectable as a protein band after gel electrophoresis (Chourey and Nelson 1976 and Fig. 1B, this report). Nomura and Akazawa (1973) similarly reported a high Km (290 mM) for sucrose synthetase from developing rice grains. Whether or not it is a major protein species in developing rice grains is not known.

A major difference between the two genotypes is that the relative abundance of sucrose synthetase molecules is significantly altered. This is evident from native as well as SDS gels and is further substantiated by immuno-inactivation experiments. A considerably higher amount of antiserum is required to titrate-out enzyme activity in normal than in the mutant. This clearly indicates a relatively higher level of cross reacting material (CRM) in the former than in the latter.

The genetic basis of the residual enzyme activity has been proposed to be independent of the *sh* locus (Chourey and Nelson 1976). The following observations strengthen this hypothesis:

1) One of the *Ds* suppressed alleles analyzed here and designated as *sh bz-m4* has presumably lost (deleted) the *sh* locus. This stock was originally isolated by McClintock (1956) as a *sh bz* double mutant (4 map units apart) owing to the *Ds* distal to the *Sh* locus. Because of the reduced recombination in the *sh bz* region and a lack of *Ac* response at the *sh* locus, while *bz* reponse persisted, Dooner (1981) concluded that the *sh* locus was deleted or was associated with a rearrangement in this stock. Burr and Burr (1981) were able to single out the *sh bz-m4* from a total of six *sh* alleles, for in this case the restriction endonuclease digested genomic DNA was found to lack a homologous fragment when hybridized with a rather small cDNA probe of the *Sh* locus. Similar molecular observations have been obtained independently by separate investigators, Federoff and Starlinger (pers. comm.). It is concluded from these observations that the *sh* locus is deleted (completely or incompletely, not known) in this stock. The enzyme and protein analysis, reported here, however, fail to distinguish the *sh bz-m4* allele from the *sh-R* (a reference allele) in W22 inbred line. This strongly indicates that another locus is responsible for the residual sucrose synthetase activity.

2) According to the two gene hypothesis, the wild type endosperm should possess both sucrose synthetases. Such a demonstration, however, remained inconclusive (Chourey and Nelson 1976; Chourey 1981) until the present observations discussed here. Electrophoretic analyses of wild type endosperm reveal a second protein in addition to the Sh protein which co-migrates with a protein identified as sucrose synthetase in the *sh* endosperm.

The physiological significance of two rather similar sucrose synthetases in the same tissue, encoded by two separate genes, is not clear. The *Sh* locus encoded activity clearly has a critical role in the mobilization of sucrose into starch biosynthesis in the endosperm (Chourey and Nelson 1976). A loss of this enzyme, as in the case of the *sh* mutant, leads to a rate limiting step in starch biosynthesis in endosperm tissue. The in vivo role of the minor enzyme, however, is not known. The starch deficiency of the *sh* mutant is far less severe than several other starch deficient mutants in maize. The *sh* endosperm has nearly 60% as much starch as the normal endosperm (Chourey and Nelson 1976). We presume on this basis that all or a significant part of the residual enzyme is also involved in sucrose → starch conversion reactions. A possibility of two separate pathways for unloading sucrose into starch biosynthetic reactions as first suggested by Tsai et al. (1970) is not ruled out. In this regard studies concerning the developmental profile of enzyme activity in the two genotypes are of some significance, particularly at the 8 DAP stage. The mutant kernels have nearly 43% of the total activity seen in the normal genotype. Though embryos were also included in the preparation for this stage of analysis, it is assumed that embryos of the two genotypes are not significantly different from each other. This assumption is based on the results of an earlier analysis of embryos at the 22 DAP stage (Chourey and Nelson 1976) where no difference in sucrose synthetase activity was detected in embryos of the two genotypes. Thus, the smallest difference between the two genotypes is seen at the earliest stage of the development. A sharp increase in the difference occurs during the subsequent development of the kernel as a result of a tremendous increase in the *Sh* locus encoded enzyme activity. This increase is also detectable at the protein level. The relative staining intensity of the Sh protein band increases during the early developmental phases of the kernel (Chourey, unpublished). Burr and Burr (1981) and Wostemeyer et al. (1981) have reported sucrose synthetase mRNA to be one of the predominant mRNA species in the developing maize kernel. It is quite probable that the increase in enzyme activity during endosperm development in the normal genotype is of transcriptional origin. The gene encoding the residual enzyme, however, fails to elicit such a developmental regulatory response seen with the *Sh* locus.

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References

Burr B, Burr FA (1981) Detection of maize controlling element at the *Shrunken* locus. Genetics 98:143–156

Chourey PS (1981) Interaction among sucrose synthetase genes in maize endosperm. Genetics 97:21

Chourey PS, Schwartz D (1971) Ethyl methanesulfonate induced mutations at the *Sh 1* protein in maize. Mutat Res 12:151–157

Chourey PS, Nelson OE (1976) The enzymatic deficiency conditioned by the *shrunken-1* mutations in maize. Biochem Genet 14:1041–1055

Chourey PS, Nelson OE (1979) Interallelic complementation at the *sh* locus in maize at the enzyme level. Genetics 91:317–325

Dickinson DB, Preiss J (1969) ADP – glucose pyrophosphorylase from maize endosperm. Arch Biochem Biophys 130:119–128

Dooner HK (1981) Regulation of the enzyme UFGT by the controlling element *Ds* in *bz-m4*, an unstable mutant in maize. Cold Spring Harbor Symp Quant Biol 45:457–462

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T_4. Nature 227:680–685

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagent. J Biol Chem 193:265–275

McClintock B (1956) Mutation in maize. Carnegie Institute of Washington Yearbook 55:323–332

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153:375–380

Nelson OE, Rines HW (1962) The enzymatic deficiency in waxy mutant of maize. Biochem Biophys Res Commun 9:297–300

Nelson OE, Tsai CY (1964) Glucose transfer from adenosine diphosphate glucose to starch in preparations of waxy seeds. Science 145:1194–1195

Nomura T, Akazawa T (1973) Enzymatic mechanism of starch synthesis in ripening rice grains. VII. Purification and enzymatic properties of sucrose synthetase. Arch Biochem Biophys 156:644–652

Roe JH (1934) A colorimetric method for the determination of fructose in blood and urine. J Biol Chem 107:15–22

Schwartz D (1960) Electrophoretic and immunochemical studies with endosperm proteins of maize mutants. Genetics 45:1419–1427

Su J, Preiss J (1978) Purification and properties of sucrose synthetase from maize kernels. Plant Physiol 61:389–393

Tsai CY, Salamini F, Nelson OE (1970) Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol 46:299–306

Wostemeyer J, Behrens U, Merckelbachi A, Muller M, Starlinger P (1981) Translation of *Zea mays* endosperm sucrose synthetase mRNA *in vitro*. Eur J Biochem 114:39–44

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