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Electrophoretic Analysis of Sucrose Synthetase Proteins in the Complementing Heterozygotes at the *Shrunken* Locus in Maize

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Summary. Electrophoretic comparisons of sucrose synthetase (SS) proteins in complementing heterozygotes and the corresponding in vitro mixtures of extracts from the homozygotes are described. The latter revealed two protein bands in the expected fashion. The SS protein pattern in the hybrid was different from that of the mixtures. The possibility of heteromeric SS molecules, formed by random polymerization of subunits of the tetramer coded by each allele in the heterozygote, was considered. Such an interaction was expected to form a multiple of five SS proteins that could be visualized after gel electrophoresis. However, only two SS bands were seen in the hybrids. The basis of this marked deviation remains to be explained.

Key words: Maize endosperm – *Shrunken* locus – Sucrose synthetase – Electrophoresis

Introduction

Interallelic complementation is the ability of one defective mutant to compensate for the defectiveness of another mutant, resulting in a partial or complete restoration of the wild type phenotype in a heterozygote. The study of this phenomenon may offer important clues to the function and regulation of the gene. A protein-protein interaction at the subunit level, which leads to the formation of the hybrid enzyme molecule, is believed to be the basis of this interaction. Association of such hybrid enzymes with complementation has been demonstrated in various microbial systems (Schlesinger and Levinthal 1963; Coddington and Fincham 1965; Kida and Crawford 1974). However, the molecular basis of the hybrid enzyme leading to the formation of an active enzyme (from the inactive enzymes in the parents) in the heterozygous genotype remains unclear. Crick and Orgel (1964) and Schwartz (1975) have proposed hypotheses to account for such molecular events. Recently Judd (1979) has proposed a rather novel molecular model to explain complementation among a cluster of alleles of certain complex loci in *Drosophila*. He has invoked post-transcriptional recombination among fragments from different transcripts during the processing steps in the heterozygote. Such a recombination is proposed to form a small amount of normal mRNA, which allows two mutant alleles to complement. No experimental evidence, however, is available in support of this model.

Interallelic complementation at the *shrunken* (*sh*) locus in maize was reported previously (Chourey 1971). Complementing heterozygotes were phenotypically indistinguishable from the wild (*Sh*) type. The occurrence of this phenomenon at the *sh* locus suggested that the protein product due to complementing alleles was either a dimer or a polymer and that a hybrid protein was formed in such heterozygotes. Electrophoretic analyses, however, revealed only the two parental proteins; no hybrid protein was seen on starch gels. Enzymatic studies of these heterozygotes became possible after Chourey and Nelson (1976) reported that the *Sh* locus coded for the enzyme sucrose synthetase. Such analyses showed a two-fold elevation in sucrose cleavage activity in the complementing heterozygotes over that in the parental homozygotes (Chourey and Nelson 1979). This increase led us to infer the existence of a heteromeric sucrose-synthetase (SS) complex in the hybrids.

Sucrose synthetase in mung bean (Delmer 1972) and rice (Nomura and Akazawa 1973) has been reported to be a tetramer. Recent studies have shown that maize SS also has four subunits (Su and Preiss 1978; Chourey unpublished), which are reported to be identical (Su and Preiss 1978; Doring, et al. 1980; Chourey unpublished). In view of these

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related findings and the lack of correspondence between the enzyme data and the electrophoretic analyses, it is critical to re-examine the complementing heterozygotes in order to understand better the basis of interallelic complementations at the *sh* locus.

Materials and Methods

The *sh* mutants used in this study, *sh-F* and *sh-S*, which were induced with ethyl methane sulfonate (EMS), have been described previously (Chourey and Schwartz 1971; Chourey and Nelson 1979). The wild type (*Sh*) and the reference *sh* allele were in the inbred W22 background. The endosperm genotype designated *sh-SSF* was obtained by crossing the *sh-S* mutant as the female parent with pollen collected from the *sh-F* parent. Conversely, the *sh-FFS* genotype represented the reciprocal cross. Developing kernels of various genotypes were harvested at 34 days after pollination, frozen on dry ice and stored at −20°. A mixture of equal parts by weight of endosperm and chilled 0.01M tris citrate buffer (pH 7.0) was homogenized. The homogenate was strained through two layers of cheesecloth and centrifuged at 30,000 g for 20 min. The supernatant fraction (endosperm extract) was dialysed overnight against the extraction buffer at 4° and was used for subsequent analyses. Protein determinations were done according to Lowry et al. (1951). Two parts of undialysed endosperm extract were mixed with one part of another extract; the mixtures were dialysed overnight and used for electrophoresis. The polyacrylamide gel electrophoresis was done according to Davis (1964), except that 1.2-mm thick slabs were used instead of tubes. Acrylamide concentrations were varied as described by Finnerty and Johnson (1979). After electrophoresis, gels were stained for proteins with Coomassie Brilliant Blue R205 (Weber and Osborn 1969).

Results

Sucrose synthetase, coded by the *Sh* locus on chromosome nine, is one of the major soluble proteins with large molecular weight in the developing endosperm of maize. Attempts to visualize this enzyme on a gel after electrophoresis, using a histochemical stain specific for the SS reaction, have not been successful to date (Chourey and Nelson 1976). However, the enzyme can be readily seen as a major protein band among the slower-migrating proteins on a gel. The major protein band/bands shown in various channels of the gel in Figures 1-3 are specified by either the *Sh* locus or its various alleles. This conclusion is based on the following analyses: (i) The major protein band seen in the extracts of the normal (*Sh*) genotype (Fig. 1H) is conspicuously absent in the mutant (*sh*) extract (Fig. 1A) at the corresponding position in the gel, as first described by Schwartz (1960) using starch gel electrophoresis. (ii) A slight alteration in the electrophoretic mobility of this protein is seen in the EMS-induced *shrunken* mutants, *sh-S* and *sh-F* (Fig. 1F, G, respectively). The *sh-S* and *sh-F* mutants code for faster- and slower-migrating proteins, respectively, as compared to the protein specified by the *Sh* locus. These mutants have been selected on the basis of the *shrunken* phenotype and their allelic relationship with the *sh* locus. Co-occurrence of electrophoretic alterations of this protein in these mutants provides strong evidence that the protein is coded by the newly induced *sh* alleles (Chourey and Schwartz 1971). Alterations in SS activities in these mutants have been reported recently (Chourey and Nelson 1979).

Figures 1 and 2 depict the SS proteins in various endosperm extracts after electrophoresis in 15% acrylamide gels. The extract from *sh-SSF* endosperms resulted in two major SS protein bands (Figs. 1D, 2C). The band pattern, however, was strikingly different from that present in the extract of the corresponding in vitro mixture (Figs. 1C, 2B). The two preparations primarily differed in the degree of separation of the SS bands from each other. The two bands of the heterozygote were closer together than those of the mixture. It was not possible to ascertain accurately the mobilities of the bands in the former in relation to the

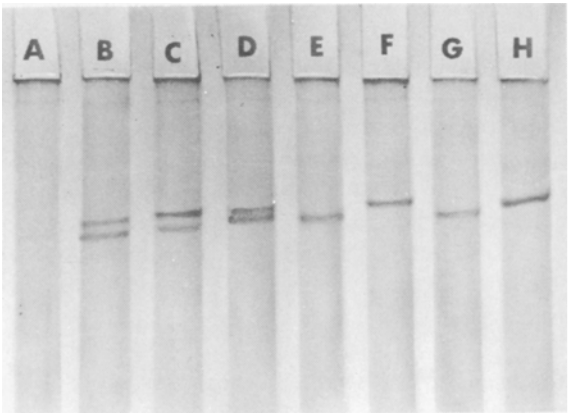

Fig. 1. 15% Polyacrylamide gel showing sucrose synthetase proteins (the arrow-marked zone) in endosperm extracts of various genotypes (20μg protein per channel). (A) *sh sh sh*, the reference W22; (B) 2:1 mixture of *sh-F*: *sh-S*; (C) 2:1 mixture of *sh-S*: *sh-F*; (D) *sh-SSF*; (E) *sh-FFS*; (F) *sh-SSS*; (G) *sh-FFF*; and (H) *Sh Sh Sh*, the wild type W22

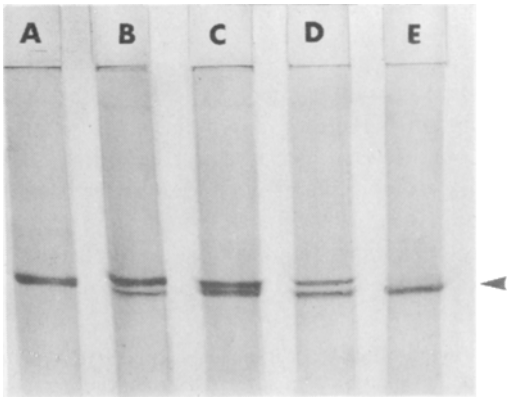

Fig. 2. 15% Polyacrylamide gel showing sucrose synthetase proteins (the arrow-marked zone) in endosperm extracts of various genotypes (40μg protein per channel). (A) *Sh Sh Sh* (W22); (B) 2:1 mixture of *sh-S*: *sh-F*; (C) *sh-SSF*, (D) 2:1 mixture of *sh-F*: *sh-S*; and (E) *sh-FFS*

bands in the latter. The reciprocal hybrid *sh-FFS* revealed only one major band (Figs. 1E, 2E). Electrophoretic mobility of this protein band seemed to coincide with the faster band in the corresponding mixture (Figs. 1B, 2D). The slower protein band was either missing or was in trace amounts in the extracts of this hybrid. However, a twofold increase in the amount of protein for electrophoresis (i.e. 40 μg/channel compared with 20 μg) led to the appearance of a very faint band (Fig. 2E) at a position corresponding to the slow band from the mixture (Fig. 2D). Though this heterozygote and the corresponding mixture revealed a similar pattern of separation of the SS bands, the slower band in the former was considerably fainter than the slower band in the latter.

The same preparations were also electrophoresed in a 7% acrylamide gel. The SS protein bands in endosperm extracts of heterozygotes, mixtures, and the wild type genotype are shown in Figure 3. This gel system, as expected, provided a greater resolution among the bands, consequently enabling an important observation: one of the two bands seen in the *sh-SSF* extract (Fig. 3C) was slightly retarded in its mobility and occupied an intermediate position relative to the two bands seen in the corresponding mixture (Fig. 3B). The second band (darker stained) corresponded to the slower band of the mixture. The reciprocal hybrid, *sh-FFS* (Fig. 3E), revealed a single major SS band, as seen in the previous gel. A very faintly stained minor band, however, was also seen in this extract. Both the major and the minor band seemingly corresponded to the faster and slower bands seen in the mixture. Four such separate crosses were examined, and the same pattern was seen in all the *sh-FFS* heterozygotes.

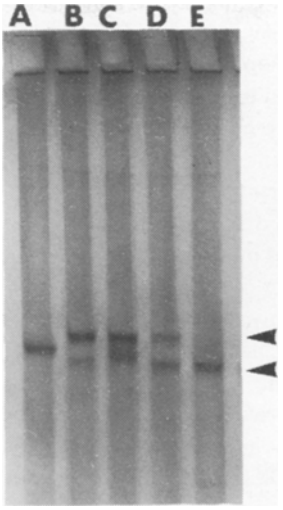

Fig. 3. 7% Polyacrylamide gel showing sucrose synthetase proteins (the arrow-marked zone) in endosperm extracts of various genotypes. (A) *Sh Sh Sh*; (B) 2:1 mixture of *sh-S*: *sh-F*; (C) *sh-SSF*; (D) 2:1 mixture of *sh-F*: *sh-S*; and (E) *sh-FFS*

Discussion

The results presented here do not conclusively show that heteromeric polymers of SS are present in the complementing heterozygotes. However, the lack of similarity between the SS protein profile in heterozygotes and their corresponding mixtures suggests the presence of unique polymers in the hybrids. A similar conclusion was reached in previous analyses of SS activities in these preparations (Chourey and Nelson 1979). In a tetrameric enzyme system such as SS, a multiple of five SS polymers can be expected in the heterozygote on the basis of random polymerization of different kinds of subunits. Electrophoretic visualization of the five isozymes from such allelic interactions has been shown for catalase in maize (Beckman et al. 1964). On the other hand, in vitro mixtures (without dissociation and reassociation of subunits) of two electrophoretically separable proteins have yielded only two homomeric parental bands, as expected.

In diploid tissues, the proportions of the possible combinations of the five tetramers are expected to be 1:4:6:4:1, on the assumption that each allelic gene produces equal amounts of its respective polypeptide. However, in the case of triploid endosperm, where the female contribution is twice that of the male parent, the gene product equivalence is altered. Thus in a *sh-SSF* endosperm, the relative frequencies are expected to be 16:32:24:8:1 for *SSSS*, *SSSF*, *SSFF*, *SFFF*, and *FFFF* tetramers, respectively (assuming *sh-S* and *sh-F* alleles code for S and F polypeptides, respectively). In the case of the reciprocal hybrid *sh-FFS*, the relative frequencies of the same tetramers were expected to be 1:8:24:32:16, respectively. On this basis, some of the least-frequently occurring polymers in the heterozygotes would be below the limit of resolution in the gel. Consequently, a set of the five bands would not have been seen. However, the reciprocal hybrids were expected to reveal qualitatively the same profile, even though the band intensity of one heterozygote was expected to be the inverse of that of the other. The polymer SSFF, expected to have the same relative frequency (24/81) in the two heterozygotes, would appear as a common band (in mobility as well as staining intensity) in both profiles. However, these two expectations were not met; the reciprocal hybrids were different qualitatively as well as quantitatively.

The *sh-FFS* endosperm extracts revealed only one major SS protein band; the second band was stained very faintly. The faint band corresponded to the polymer(s) predominantly consisting of subunits from the *sh-S* allele (the parent in single dose). It is proposed, on the basis of this observation, that the hybrid expresses the *sh-S* allele more weakly than the *sh-F* allele. Alternatively, the SS polymers in the extracts of reciprocal hybrids may have had in vitro or in vivo differential stabilities. This was, however, unlikely due to the nearby isogenic genetic backgrounds of

the homozygous parental stocks. Though the differential expression was clearly manifested in the *sh-FFS* heterozygotes, the present demonstrations did not show it clearly in the *sh-SSF* hybrid. The band intensities of the *sh-FFF* and *sh-SSS* endosperm extracts were not markedly different, suggesting equal levels of gene product in the homozygotes. These results were similar to those of the alcohol dehydrogenase (ADH) system in maize, in which homozygous alleles had equal expressions but in the heterozygote, one allele predominated over the other (Schwartz 1971). These observations have led Schwartz (1971) to propose the competition hypothesis of gene regulation. If, indeed, an allelic competition among the *sh* alleles is similar to that for the ADH alleles, the relative frequencies of various tetramers would be significantly altered. Because quantitative information is not available on the relative levels of the allelic products in the *sh* heterozygotes, it is not possible to estimate the relative frequencies of various tetramers. Consequently, it is not possible in this preliminary report to propose any explanation of the unexpected SS band pattern seen here in the heterozygotes.

In conclusion, the complementing heterozygotes showed two SS protein bands with gel electrophoresis, as was reported previously (Chourey 1971). However, no comparisons were made then between the in vitro mixtures and the heterozygotes. Such a comparison here enabled the detection of SS protein bands (patterns) that were unique to the hybrids. In this study the reciprocal hybrids were also shown to be different from each other, something not quite as apparent in the first study. The basis of this discrepancy was not clear, although the two investigations differed on several points. The important difference was that the present studies were conducted on 34-day-old endosperms, whereas the previous study used rather young endosperms, 16-20 days old. Schwartz (1971) observed that interallelic competition at the ADH locus was tissue-specific. Assuming a similar competition-like interaction among the *sh* alleles, a temporal specificity may explain the observed differences between young and old *sh-FFS* endosperms. This hypothesis is being tested.

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