Plant Physiol. (1985) 79, 530–536
0032-0889/85/79/0530/07/$01.00/0

A Comparison of Two Sucrose Synthetase Isozymes from Normal and *shrunken-1* Maize[1]

Received for publication January 25, 1985 and in revised form May 23, 1985

CRAIG S. ECHT*[2] AND PREM S. CHOUREY
USDA-ARS (P.S.C.) *and Departments of Plant Pathology* (C.S.E., P.S.C.) *and Agronomy* (P.S.C.),
University of Florida, Gainesville, Florida 32611

ABSTRACT

The maize sucrose synthetase isozyme (SS2) present in *sh1* endosperm, *sh1* seedlings, and in suspension culture cells was purified to homogeneity from each of these tissues by sequential ammonium sulfate fractionation, diethylaminoethyl-cellulose chromatography, gel filtration chromatography, and affinity elution with UTP from a carboxymethyl-cellulose column. Cyanogen bromide digests were used to demonstrate that the SS2 isozymes in these different tissues are structurally identical and are therefore the product of the same gene. The sucrose synthetase produced by the *Sh1* gene (SS1) was purified by modification of the SS2 procedure and was used in comparative analyses of the two isozymes. Ouchterlony assays demonstrated that SS1 and SS2 have partial antigenic identity. The two isozymes have similar enzyme kinetics in the sucrose cleavage reaction but differ in their relative activities with ADP and TDP. The amino acid compositions of SS1 and SS2 are similar, and proteolytic digests revealed that they share limited structural homologies.

Sucrose synthetase (UDPG: D-fructose 2-glucosyltransferase, EC 2.4.1.13) catalyzes the reversible conversion of UDP and sucrose to UDPG and fructose. The enzyme has been purified from a number of species (10, 18–22). It is unique among sugar nucleotide-glucosyltransferases in that its reaction is fully reversible and is one of the few that have a broad nucleotide specificity. Sucrose synthetase is found primarily in nonphotosynthetic tissues and in developing seeds but its roles in sucrose metabolism are not completely understood. In maize endosperm the enzyme is composed of identical subunits and its natural state is most probably a tetramer, although it may exist in higher oligomeric forms (20).

The first clear demonstration of the role of sucrose synthetase in sucrose-starch conversion reactions was provided by analysis of the shrunken (*sh1*) mutations in maize. The loss of nearly 95% of the total sucrose synthetase activity in *sh1* endosperm is associated with starch deficiency in these mutants (5). Enzyme analyses of several chemically induced *sh1* mutants, which as heterozygotes give a normal (nonshrunken) phenotype, provided a better understanding of the *in vivo* role of this enzyme. These complementing heterozygotes have higher levels of sucrose cleavage activity than their mutant parental homozygotes but their sucrose synthesis activities remain low (6). Based on this, Chourey and Nelson (6) inferred that the sucrose breakdown reaction is physiologically more critical than the sucrose synthesis reaction in the developing endosperm. Similar conclusions about the sucrose cleavage role of sucrose synthetase in other species are based on physiological and biochemical studies (11, 22).

The residual sucrose synthetase activity present in all *sh1* endosperms was demonstrated to be independent of the *Sh1* locus (4–6). This residual activity results from a low abundance sucrose synthetase isozyme, SS2,[3] that is present in endosperms (4, 16), embryos (5), and roots (P. S. Chourey, unpublished data) of *Sh1* and *sh1* plants as well as in suspension culture cells (8). SS2 from endosperm is biochemically similar to, but has a slightly faster electrophoretic mobility than, the isozyme encoded by the *Sh1* locus, SS1 (4, 16). SS1 and SS2 are differentially expressed during kernel development (4). The genes encoding the two isozymes in the endosperm appear to share a partial sequence homology based on hybridization analysis of *sh1* endosperm RNA (16). Sucrose synthetase isozymes have also been reported in cucumber (14) and pea (21).

SS1 appears to be expressed only in the endosperm since no other tissues are affected by the *sh1* mutations. Mutations of the SS2 isozyme gene that could be used to test genetically whether the SS2 present in various maize tissues is the product of a single gene or of different tissue-specific genes are not available. We therefore have purified and directly analyzed SS2 from various maize tissues and have characterized it in relation to purified SS1. The purified enzymes were compared by their immunochemical properties, kinetic parameters, amino acid compositions, and by peptide digest analyses.

MATERIALS AND METHODS

Plant Material. The stocks of *Zea mays* L. used were in a W22 genetic background and had either a *Sh1/Sh1* (normal) or *sh1 bz1-m4/sh1 bz1-m4* (shrunken) genotype. The *sh1 bz1-m4* allele is a deletion covering most or all of the *Sh1* gene (3, 12). Maize tissue culture cells were from the 'Black Mexican Sweet' suspension culture line (7) and had a *Sh1/Sh1* genotype.

Extraction and Purification of Sucrose Synthetase Isozymes. The sucrose synthetase encoded by the *Sh1* locus (SS1) was isolated from whole *Sh1* kernels that were harvested 22 d after pollination, frozen in liquid N_2, and stored at −20°C in airtight containers. One hundred g of kernels were thawed and homogenized with a Virtis homogenizer using a total of 200 ml of cold (0–4°C) 50 mM Tris-HCl (pH 7.5) (at 4°C), containing 1 mM

[1] Cooperative investigation, Agricultural Research Service, United States Department of Agriculture and Institute of Food and Agricultural Sciences, University of Florida. Florida Agricultural Experiment Station Journal Series No. 6323. Supported in part by the United States Department of Agriculture, Competitive Research Grants Office, Grant No. 82-CRCR-1-1064.

[2] Present address: Department of Agronomy, University of Missouri, Columbia, MO 65211.

[3] SS2, sucrose synthetase 2; CNBr, cyanogen bromide; IEF, isoelectric focusing; SS1, sucrose synthetase 1.

DTT and 1 mM EDTA.

The homogenate was filtered, protamine sulfate was added to a concentration of 0.02%, and the filtrate was centrifuged at 37,000*g* for 30 min. This and all subsequent steps were done between 0 and 4°C. The cloudy supernatant was filtered to remove the floating material and solid $(NH_4)_2SO_4$ was added to 30% saturation (based on solubility at 25°C, *i.e.* 17.6 gm/100 ml). After 30 min the suspension was filtered through a pad of washed Celite and the cleared supernatant was brought to 45% saturation with additional $(NH_4)_2SO_4$. After 30 min the suspension was centrifuged at 10,000*g* for 20 min, the supernatant discarded, and the pellet resuspended with 5 ml of 50 mM imidazole-HCl (pH 7.0) (at 4°C) that contained 10 mM $MgCl_2$. Insoluble matter was pelleted by centrifugation and the sample supernatant was desalted by passing through a P6-DG (Bio-Rad) column equilibrated with 50 mM imidazole-HCl (pH 7.0).

The eluted protein fractions were diluted to 30 ml with the imidazole buffer and loaded onto a DEAE-Sephacel column (2.6 × 12 cm) equilibrated with the same buffer. The enzyme was eluted at a flow rate of 20 ml/cm^2·h with 500 ml of a linear 0 to 0.4 M NaCl gradient made in the imidazole buffer. Those fractions that contained the bulk of the sucrose synthetase activity, as measured by the sucrose cleavage reaction, were pooled and precipitated with 60% $(NH_4)_2SO_4$. The pellet was dissolved in 3 ml of the imidazole buffer containing 0.1 mM DTT and loaded onto a Bio-Gel A-1.5 m gel filtration column (2.6 × 100 cm) equilibrated with 40 mM NaOH-Mes (pH 6.0) containing 0.1 mM DTT and 0.1 mM EDTA. The enzyme was eluted at a flow rate of 2.8 ml/cm^2·h and collected in 5 ml fractions. The fractions having peak activity were pooled, then loaded and washed onto a CM-52 (Whatman) column (1.5 × 7 cm) equilibrated with the Mes buffer. SS1 was eluted with 50 mM NaOH-Mes (pH 6.0) containing 1 mM UDP and 0.1 mM DTT. The enzyme eluted with a sharp front near the void volume and trailed off gradually over the next 10 ml. The dilute trailing fractions were pooled and concentrated in Amicon G25 concentrator cones. These and the peak fractions were adjusted to 20% glycerol and 1 mM DTT and frozen at −80°C if they were not to be used within several days.

For purification of SS2 present in *sh1 bz1-m4* endosperm, 140 g of 22-d-old frozen kernels were used. Except for minor alterations, the procedure was similar to that used for the purification of SS1. The SS2 enzyme was precipitated in the 27.5 to 42.5% saturated $(NH_4)_2SO_4$ fraction. The gel filtration and CM-52 columns were equilibrated and washed with 30 mM NaOH-Mes (pH 6.0) (at 4°C) containing 0.1 mM DTT and 0.1 mM EDTA. Affinity elution of SS2 from the CM-52 column was accomplished by pre-elution with 3 ml of 30 mM NaOH-Mes (pH 6.7), 0.1 mM DTT, and elution with the same buffer containing 0.25 mM UTP. The elution profile of SS2 and handling of the SS2 fractions were the same as those of SS1 described above.

SS2 from seedling tissue was purified from 120 g of plumule and coleoptile tissues from etiolated 7-d-old *sh1 bz1-m4* seedlings. The harvested material was homogenized in 100 ml of cold 50 mM Tris (pH 7.8) (at 4°C) containing 1 mM DTT and 1 mM EDTA. The rest of the purification followed the protocol used for *sh1 bz1-m4* endosperm except that the sucrose synthetase was precipitated in the 37 to 50% saturated $(NH_4)_2SO_4$ fraction. The Celite filtration step was replaced by centrifugation at 37,000*g* for 20 min.

SS2 from tissue culture cells was purified from 100 g of cells harvested 7 d after subculture. The cells were disrupted in a Bead-Beater apparatus (Biospec Products) containing cold 50 mM Tris (pH 7.0), 1 mM DTT, and 1 mM EDTA. A total of 250 ml of buffer was used for disruption of the cells and rinsing of the glass beads. The rest of the purification followed the protocol used for the purification of SS2 from *sh1 bz1-m4* kernels except that the sucrose synthetase was precipitated in the 30 to 45% saturated $(NH_4)_2SO_4$ fraction. The Celite filtration step was replaced by centrifugation at 37,000*g* for 20 min.

The enzyme activity of SS2 from kernels and cultured cells was measured by the sucrose synthesis reaction in the $(NH_4)_2SO_4$ and DEAE fractions because high invertase activity interfered with the sucrose cleavage assay. The gel filtration and affinity elution fractions were assayed by the sucrose cleavage reaction.

Protein and Enzyme Assays. Enzyme activity was measured in the direction of sucrose cleavage using slight modifications of the assay described elsewhere (6). The sucrose cleavage reaction contained 0.1 M NaOH-Mes (pH 6.0), 0.2 M sucrose, and 4 mM UDP and was performed at 30°C for 15 min, unless indicated otherwise. The assay for activity in the direction of sucrose synthesis was performed as described elsewhere (4).

Protein was measured by a Coomassie blue binding assay (2) using purified lyophilized SS1 as the standard.

Electrophoresis and Staining. SDS-PAGE was performed according to the method of Laemmli (15). The gels were made with a 12.5 to 25% acrylamide gradient. Native polyacrylamide gels had a 5 to 7.5% acrylamide gradient, were run at 4°C, and were prepared as in Laemmli (15) but the SDS was omitted.

Native IEF gels contained 1% LE agarose (Sea-Kem) and 3% ampholines (Bio-Rad, pH 3.5–9), and were poured onto Gel-Bond films (11 × 12.5 cm) at a thickness of 0.5 mm. Focusing was performed at 10°C for 40 min with a constant 7 W. Isoelectric points of the isozymes were determined by direct pH measurement of the IEF gels using a surface pH electrode.

After separation in either polyacrylamide or IEF gels the proteins were stained with Coomassie blue R-250 or by silver staining (17).

Mol wt determinations were obtained by SDS-gradient PAGE using mol wt standards (Bio-Rad Laboratories) covering the 100,000 to 10,000 kD range.

Proteolytic Digests. Bromelain (Boehringer-Mannheim) digests were performed in 70 mM Tris-HCl (pH 7.5) at room temperature for 10 min using a bromelain concentration of 10 μg/ml (protease:isozyme, 1:40, w/w). This concentration was chosen because it gave the greatest number of clearly resolved partially digested peptide fragments. The reaction was stopped by adding one-third of the reaction volume of 8% SDS, 8% 2-mercaptoethanol, and 40% glycerol followed by heating in a boiling water bath for 3 min. The samples were assayed by SDS-gradient PAGE.

Partial digests with *Staphylococcus aureus* V8 protease (Miles Laboratories) were carried out at room temperature in 100 mM Tris-HCl (pH 7.0), containing 0.5% SDS and 1% 2-mercaptoethanol. The sucrose synthetase was first denatured by heating the reaction solution, without the V8 protease, in a boiling water bath for 2 min. The solution was cooled and the protease was added to a concentration of 33 μg/ml (protease:isozyme, 1:12, w/w). The reaction was stopped at 5 min and assayed by electrophoresis as described above.

Cyanogen bromide digests were performed at room temperature for 24 h in the dark using 10 mg CNBr/ml 68% HCOOH. Prior to digestion the isozymes were dialyzed against water overnight. The reactions were stopped by dilution with 5 volumes of water and lyophilization. The dried samples were dissolved in 0.5 ml of water and lyophilized once again. The samples were then dissolved in 8 M urea, 2% SDS, 2.5% 2-mercaptoethanol, 100 mM Tris-HCl (pH 7.0), and assayed by electrophoresis as described above.

Amino Acid Analysis. Amino acid compositions were determined by Linda Johnson at the University of Michigan Protein Sequencing Facility under the direction of George Tarr. The samples were exhaustively dialyzed against water, lyophilized, rehydrated and hydrolyzed with HCl vapors at 150°C for 1 h.

Amino acids were determined as described elsewhere (23).

Antisera Production and Immunotechniques. Antisera were raised in New Zealand White rabbits against purified SS1 and against purified SS2 from *sh* kernels and from cultured cells. One mg of protein was emulsified in Freund's complete adjuvant and was injected intramuscularly. The secondary reaction was elicited 17 d later with 0.6 mg of purified protein in Freund's incomplete adjuvant and was also injected intramuscularly. The rabbits were bled at 7 and 19 d following the second injections. The sera were treated at 56°C for 15 min and stored at −20°C.

Double diffusion assays were performed in 0.7% agarose that contained 20 mM sodium phosphate (pH 7.0), 115 mM NaCl, 1.5% PEG, and 0.02% NaN_3. Precipitin lines were allowed to form at room temperature for 20 to 24 h and were photographed directly.

RESULTS

Two sucrose synthetase isozymes were purified from different maize tissues using the four-step isolation procedure described in "Materials and Methods." Typical yields for 100 g of tissue were 10 mg protein from *Sh1* kernels, 1 mg from *sh1* kernels, 3 mg from cultured cells (Black Mexican Sweet line), and less than 0.1 mg from etiolated *sh1* seedlings. The specific activities of the purified enzymes were 10 μmol fructose/min·mg for SS1 and 6 μmol fructose/min·mg for SS2 from *sh1* kernels and from cultured cells. The specific activity of the seedling preparation was not determined.

The preparations from *Sh1* and *sh1* kernels and from cultured cells were judged to be homogeneous by several standard criteria. A single protein band of 87,000 mol wt was seen in each sample when the purified enzymes were assayed by SDS-gradient PAGE (Fig. 1). Because of the low amounts of seedling SS2 that were available, its degree of purity was not established. Based on its cyanogen bromide digest pattern, however, it appeared to be practically free of any contaminating proteins.

IEF resolved each purified sucrose synthetase into a major species and several minor species (Fig. 2). These minor proteins most likely represent altered charge forms of the main band sucrose synthetases because they were always found close to the main band and they did not vary between different enzyme preparations. The isoelectric point of the major species of SS1 was measured to be pH 5.8 and that of SS2 from *sh1* kernels and cultured cells was pH 5.4.

Antisera were prepared using purified SS1 and SS2 from *sh1* kernels and from cultured cells. Each antiserum tested with its specific antigen by the Ouchterlony double-diffusion assay yielded a single precipitin band. No antigenic differences between the SS2 enzyme from *sh1* kernels or cultured cells were found using the three different antisera. Partial antigenic identity between SS1 and SS2 was indicated by cross-reaction with SS1 antiserum and the formation of a precipitin line spur (Fig. 3).

Unexpectedly, two precipitin lines formed when the SS2 antisera cross-reacted with SS1. Diluting the antisera or the SS1 antigen resulted in the loss of one line or the other but in neither case did the lines coverge or change their relative positions. The two precipitin lines are not due to SS1 contamination of the SS2 preparations used for producing the antisera nor do they arise from SS2 contamination of the SS1 used in the assay. This is known because isoelectric focusing (Fig. 2) and native PAGE (not shown) of the SS1 and SS2 preparations did not reveal any contaminating isozymes at their expected positions. The addition of 10 mM $MgCl_2$ to the reaction buffer to prevent possible formation of SS1 oligomers other than tetramers (20) did not alter the precipitin pattern.

The purified enzyme preparations were stable when frozen at −80°C in the presence of 20% glycerol and 0.1 to 1.0 mM DTT. Slight decreases in activity and the appearance of several discrete

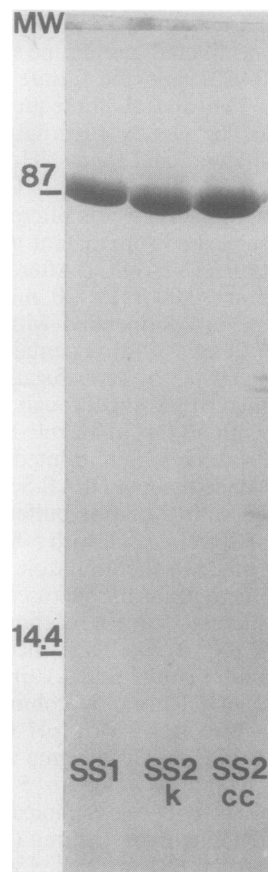

FIG. 1. Assay of purified sucrose synthetase isozymes with SDS-gradient PAGE and Coomassie blue staining. From left to right: SS1; SS2 from *sh1* kernels (k); and SS2 from cultured cells (cc). Each lane contained 12 μg of protein.

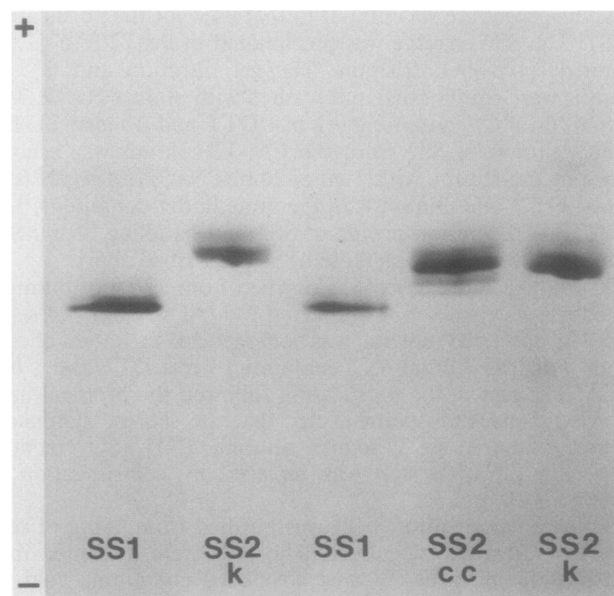

FIG. 2. IEF pattern of purified sucrose synthetase isozymes: SS1, 4 and 2 μg; SS2 from *sh1* kernels (k), 2 and 4 μg; and SS2 from cultured cells (cc), 4 μg. The proteins were stained with Coomassie blue.

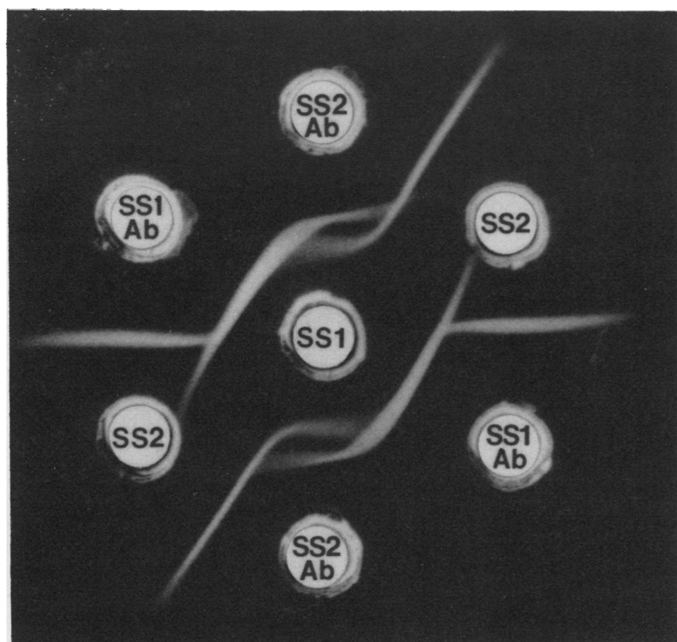

FIG. 3. Double-immunodiffusion assays of SS1 and SS2 antisera (Ab) and enzymes. Ten μl of antisera and 10 μg of purified sucrose synthetase were loaded in the wells as labeled. The SS2 antiserum and enzyme used were from cultured cell SS2 preparations but similar results were obtained when *sh1* kernel SS2 antiserum or enzyme were used.

peptide fragments were observed with repeated freezing and thawing or prolonged storage at 4°C. This occurred even when the proteins were isolated in the presence of phenylmethylsulfonyl fluoride, a serine protease inhibitor. The extent of this breakdown remained low, however, and usually did not interfere with subsequent analyses.

Kinetics and Nucleotide Specificity. The double reciprocal plots used to determine the K_m values for sucrose were linear for sucrose concentrations from 0.03 to 0.2 M but curved upward as the concentration decreased beyond 0.03 M. Using the linear portion of the plots a $K_m^{(sucrose)}$ of 52 mM for SS1 and of 63 mM for SS2 (from both *sh1* kernels and cultured cells) was determined. The K_m values for UDP could not be accurately determined because the double reciprocal plots were curvilinear along their entire lengths (from 0.02 to 2.0 mM). The velocity *versus* concentration curves of SS1 and SS2 for both sucrose and UDP appeared hyperbolic.

The nucleotide substrate specificities of SS1 and SS2 were determined for TDP, ADP, CDP, and UTP, each at a concentration of 4 mM. For SS1 the relative velocity values for each of the substrates were 100 (UDP), 91 (TDP), 6.8 (ADP), <1 (CDP), and <1 (UTP). For SS2 the relative values were, respectively, 100, 57, 1.8, 1.4, and 1.3.

Peptide Digests. Partial digests of SS1, SS2 from *sh1* kernels, and SS2 from cultured cells were performed with the nonspecific protease bromelain (Fig. 4). Partial digests of the proteins denatured by SDS were accomplished with *S. aureus* V8 protease which cleaves at the carboxyl side of glutamic and aspartic acid residues (Fig. 5). Complete digestion of the proteins, including SS2 from *sh1* seedlings, was accomplished with CNBr which cleaves at the carboxyl side of methionine (Fig. 6).

The digests revealed that the SS2 preparations from different tissues are practically identical and that SS1 and SS2 share a number of proteolytic fragments of similar size. The differences observed between the *sh1* kernel and cultured cell SS2 samples were limited to certain minor fragments that SS2 from cultured cells had in common with some of the major SS1 fragments

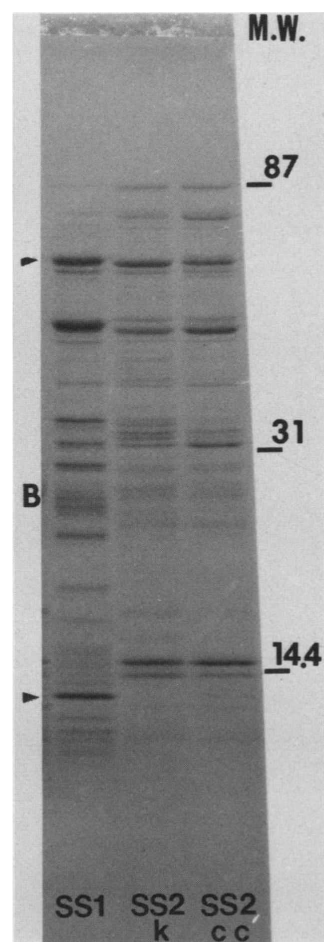

FIG. 4. SDS-gradient PAGE pattern of proteolytic fragments of sucrose synthetase isozymes partially digested with bromelain. From left to right: SS1; SS2 from *sh1* kernels (k); and SS2 from cultured cells (cc). The position of migration of bromelain is marked (B) and appears as a cluster of several proteins. Arrows indicate position of SS1 peptides that appear to be present in the SS2 preparation from cultured cells. Twelve μg of isozyme and 0.3 μg of bromelain were used in each digestion. Mol wt are expressed in kD. The peptides were stained with Coomassie blue.

(arrows in Figs. 4–6; see "Discussion"). Other minor differences that were seen between the two SS2 preparations were not reproducible among replicate digests.

Amino Acid Analyses. The amino acid compositions of SS1 and SS2 (from *sh1* kernels) are given in Table I. The two isozymes have similar overall compositions. Tryptophan content was not determined. The estimated number of total amino acid residues (excluding tryptophan) is 769 for SS1 and 778 for SS2 but no mol wt differences between the two isozymes were detected by SDS PAGE or gel filtration chromatography.

DISCUSSION

The procedure developed for the purification of maize sucrose synthetase isozymes 1 and 2 was a more convenient and less expensive alternative to that described by Su and Preiss (20) for maize sucrose synthetase. The major difference between the two procedures is in the last step, where we used affinity elution of the enzymes from a CM-cellulose column instead of affinity binding and elution from a UDP-hexanolamine-agarose column. The purity of the preparations appears to be comparable for the two procedures but our yield of SS1 was one-third of that reported by Su and Preiss.

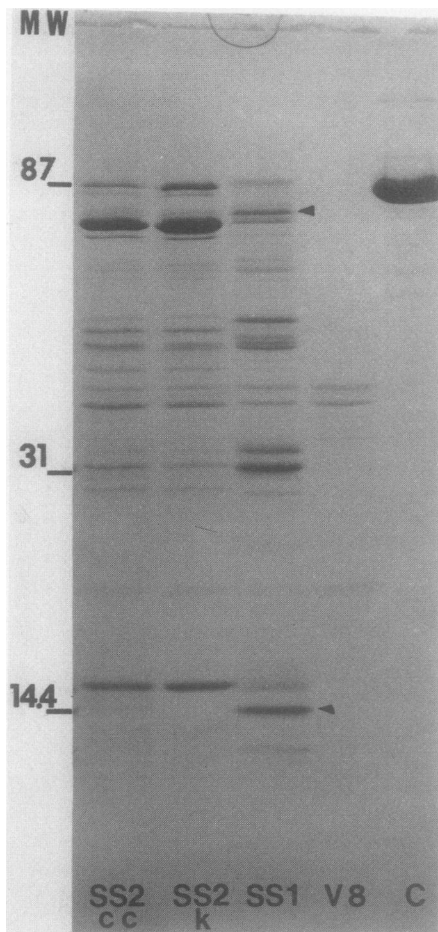

FIG. 5. SDS-gradient PAGE pattern of proteolytic fragments of denatured sucrose synthetase isozymes partially digested with V8 protease. From left to right: SS2 from cultured cells (cc); SS2 from *sh1* kernels (k); SS1; V8 protease; and undigested sucrose synthetase. Arrows indicate position of SS1 peptides that appear to be present in the SS2 preparation from cultured cells. Twelve μg of isozymes and 1 μg of V8 were used in the digestions and controls. Mol wt are expressed in kD. The peptides were stained with Coomassie blue.

SS1 and SS2 differed in their CM-cellulose binding and elution parameters. SS2 bound to the column with a slightly lower ionic strength buffer (30 *versus* 40 mM NaOH-Mes) than did SS1, and it eluted, in the presence of an affinity ligand, at a higher pH (6.7 *versus* 6.0) than did SS1. UMP and UDP eluted SS2 but not nearly as effectively as UTP. This is somewhat surprising because UTP was found to be a poor substrate for SS2 in the sucrose cleavage reaction. The higher negative charge density of UTP may have contributed to its greater effectiveness. UMP and UTP were not tested for their abilities to elute SS1.

Even though SS2 is present both in the endosperm and scutellum of *Sh1* kernels (5) no SS2 was found in the SS1 preparations from *Sh1* kernels. This was probably due to their different CM-cellulose binding and elution parameters, their different points of elution from the DEAE column (0.15 *versus* 0.18 M NaCl), and the comparatively low amount of SS2 in *Sh1* endosperm. The absence of SS2 in purified SS1 preparations has been reported by others (20).

Double immunodiffusion assays revealed partial antigenic identity between SS1 and SS2 and complete identity between SS2 isolated from *sh1* kernels and from cultured cells. Contamination of one isozyme preparation with the other was not the cause of the unusual precipitin reaction between SS2 antisera and purified SS1 enzyme (Fig. 3). The immunoprecipitate pattern is one that might be expected if SS2 were able to acquire a SS1-like conformation in the rabbit but we have no direct evidence for such conformational switching. The degrees of oligomerization of SS1, SS2, or both, might also account for the observed results. Conditions that would be expected to favor only the tetrameric form of SS1, *i.e.* high ionic strength (ionic strength 0.27) and 10 mM Mg^{2+} (20), were present in the Ouchterlony plates, however. The oligomeric state(s) of SS2 in the rabbit or the factors that affect SS2 oligomerization are not known.

The complex kinetics of the sucrose cleavage reaction of sucrose synthetase from *Sh1* kernels (SS1) that was previously described by Su and Preiss (20) were also found for SS2. The similar $K_m^{(sucrose)}$ values of SS1 and SS2 (52 and 63 mM) are in approximate agreement with the reported value for purified SS1 of 30 to 40 mM (20). Differences between SS1 and SS2 sucrose cleavage activities were found using TDP and ADP as substrates.

The relative activity of SS2 with TDP (57% of UDP activity) is similar to the reported values of other plant sucrose synthetases, although the value for SS1 (90% of UDP activity) is higher than any previously reported value (11, 18, 22). The efficient TDP utilization of SS1 raises the possibility that this enzyme functions in sucrose metabolic pathways other than starch biosynthesis because TDP is not known to be involved in starch synthesis in maize endosperm. Mutations at the *Sh1* locus, however, are not known to affect any function other than starch synthesis in the endosperm.

Comparative proteolytic digests were used to study in more detail the structural similarities between SS1 and SS2 and among the SS2 enzymes prepared from different tissues. Different proteins that share certain tertiary and quaternary structures have similar exposed sites that are more sensitive to attack by proteases resulting in the generation of partially digested fragments of similar size (1, 9). Similarities in the proteins' primary structures, on the other hand, are revealed by the generation of peptides of similar size upon cleavage to completion by a proteolytic agent such as CNBr.

The CNBr digest patterns of SS2 from *sh1* endosperm, etiolated seedlings, and cultured cells and the partial protease digest patterns of the enzyme from *sh1* kernels and cultured cells are virtually identical. The only consistent differences found between the *sh1* kernel and cultured cell preparations are some minor fragments in the cultured cell SS2 digest that appeared to be SS1 fragments (Figs. 4–6). Analyses of the cultured cell SS2 by IEF (Fig. 2) and by native PAGE (not shown) revealed no protein at the SS1 position, but our isolation procedure may not have excluded SS1-SS2 heterotetramers from co-purifying with the cultured cell SS2. Such heterotetramers would carry various charges intermediate to those of SS1 and SS2 and may be represented by some of the minor cathodal charge variants seen with IEF (Fig. 2) and native PAGE of the cultured cell SS2 samples. Heterotetramers would not be found in the seedling or kernel SS2 preparations because the *sh1* deletion mutation carried by these tissues precludes SS1 synthesis. Endosperm is the only tissue known to be morphologically affected by mutations at the *Sh1* locus, but there is little evidence to date to rule out the possibility of low level expression of SS1 in other tissues.

Partial bromelain digestion of native SS1 and SS2 and V8 protease digests of the denatured isozymes yielded a number of fragments of similar size that were common between the two proteins (Figs. 4 and 5). Complete digestion using CNBr also yielded several peptides of similar size common to SS1 and SS2 (Fig. 6). More extensive digestions using either of the two proteases, however, resulted in fewer and fewer apparent similarities between SS1 and SS2 (not shown). We conclude that SS1 and SS2 have certain topological features in common and share a

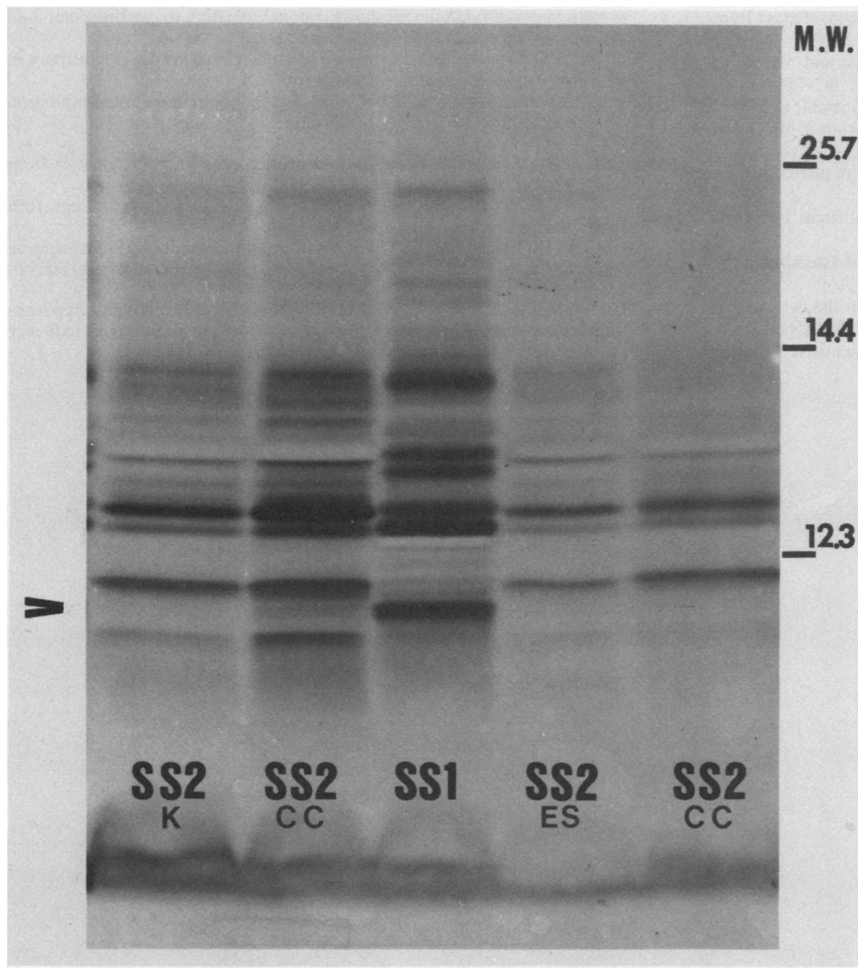

FIG. 6. SDS-gradient PAGE pattern CNBr digest peptides from sucrose synthetase isozymes. From left to right: SS2 from *sh1* kernels (k); SS2 from cultured cells (cc); SS1; SS2 from etiolated-seedlings (es); and SS2 from cultured cells. Arrows indicate position of SS1 peptides that appear to be present in the SS2 preparation from cultured cells. Mol wt are expressed in kD. The peptides were stained by the silver staining method (16).

Table I. *Amino Acid Compositions of SS1 and SS2*

Amino Acid	No. of Residues Based on Mol Wt 87,000	
	SS1	SS2
Ala	53.1	54.4
Cys	7.9	7.3
Asx	75.6	70.8
Glx	83.2	87.4
Phe	40.7	37.3
Gly	54.2	61.1
His	22.3	27.2
Ile	43.7	38.8
Lys	50.0	43.4
Leu	90.5	87.7
Met	15.4	15.8
Pro	35.1	39.5
Arg	47.3	47.0
Ser	46.6	49.7
Thr	34.0	39.7
Val	43.9	48.4
Tyr	33.5	29.4

limited homology between their amino acid sequences. The similar amino acid compositions (Table I) of the two isozymes and the apparent limited sequence homology of the two genes (16) support this view.

In the absence of polyploidy, the presence of genetically distinct isozymes in plants indicates that the genes encoding the isozymes arose by gene duplication(s) (13). Proteins of common ancestry would be expected to share some structural homologies. Such homologies between sucrose synthetase 1 and 2 of maize were revealed by the immunological, proteolytic digest and amino acid composition data that we have presented. From the CNBr digest data it appears that the SS2 enzymes present in endosperms, seedlings, and cultured cells are the products of the same gene. We have found no evidence for additional genetically independent sucrose synthetase isozymes in maize.

Acknowledgments—Technical assistance from Ms. D. Z. Sharpe and Dr. P. E. Still is gratefully acknowledged.

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