

# The Spatial Distribution of Sucrose Synthase Isozymes in Barley<sup>1</sup>

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The sucrose (Suc) synthase enzyme purified from barley (*Hordeum vulgare* L.) roots is a homotetramer that is composed of 90-kD type 1 Suc synthase (SS1) subunits.  $K_m$  values for Suc and UDP were 30 mM and 5  $\mu$ M, respectively. This enzyme can also utilize ADP at 25% of the UDP rate. Anti-SS1 polyclonal antibodies, which recognized both SS1 and type 2 Suc synthase (SS2) (88-kD) subunits, and antibodies raised against a synthetic peptide, LANGSTDNNFV, which were specific for SS2, were used to study the spatial distribution of these subunits by immunoblot analysis and immunolocalization. Both SS1 and SS2 were abundantly expressed in endosperm, where they polymerize to form the five possible homo- and heterotetramers. Only SS1 homotetramers were detected in young leaves, where they appeared exclusively in phloem cells, and in roots, where expression was associated with cap cells and the vascular bundle. In the seed both SS1 and SS2 were present in endosperm, but only SS1 was apparent in the chalazal region, the nucellar projection, and the vascular bundle. The physiological implications for the difference in expression patterns observed are discussed with respect to the maize (*Zea mays* L.) model.

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The major function of the Suc synthase enzyme (EC 2.4.1.13; Suc + UDP  $\rightleftharpoons$  UDPGlc + Fru) in sink organs, such as tubers and cereal endosperms, is the cleavage of Suc transported by the phloem, thereby catalyzing the first step of the starch biosynthesis pathway. However, several other physiological roles have recently been proposed for this enzyme in different plant tissues. Geigenberger et al. (1993) demonstrated the activity of Suc synthase and a functional glycolytic pathway in the phloem symplasm of castor bean (*Ricinus communis* L.) hypocotyls. They suggested that Suc synthase is responsible for Suc breakdown in the phloem and responds to changes in the metabolic requirement for ATP and UDPGlc for callose production. It was also noted that these processes would need to be sequestered away from the mass flow of cell sap. The localization of Suc synthase within the companion cells of maize (*Zea mays* L.) vascular bundles (Nolte and Koch, 1993; Brangeon et al., 1996) supports this proposal. Its localization in the developing trichomes of cotton (*Gossypium hirsutum* L.) ovules (Nolte et al., 1995), as well as the presence of membrane-

associated forms in cotton fibers (Amor et al., 1995) and in maize-cultured cells (Carlson and Chourey, 1996), suggest that Suc synthase is also involved in directing carbon from Suc into cell wall synthesis.

The Suc synthase reaction is competitively inhibited by its products from either direction (Wolosiuk and Pontis, 1974) and uncompetitively inhibited by Glc (Doehlert, 1987). The reversibility of the reaction is also influenced by the redox state (Pontis et al., 1981). The reaction is unique in that it is the only transglucosylation involving sugar nucleotides that is readily reversible.

Genes encoding two types of Suc synthase isozymes (SS1 and SS2) have been detected in all monocots analyzed: the SS1 and SS2 genes are more similar in sequence to their counterparts in other species than they are to each other within a given species (Shaw et al., 1994). This suggests that they have evolved from a common ancestral gene by gene duplication and divergent evolution (Sánchez de la Hoz et al., 1992). Suc synthase was first described in wheat germ (Cardini et al., 1955), but the maize enzymes have been more thoroughly studied. Comparisons of SS1 and SS2 isolated from maize (Echt and Chourey, 1985), wheat (*Triticum aestivum* L.; Larsen et al., 1985), and sugar cane (*Saccharum officinarum* L.; Buczynski et al., 1993) show a marked biochemical resemblance. Their kinetic constants and nucleotide specificities are very similar and their protein structures are closely related. However, tissue specificity and temporal expression of the two genes are quite distinct. The maize *Sus1* gene, encoding SS2, is widely expressed in the plant (McCarty et al., 1986; Nguyen-Quoc et al., 1990). The *Sh1* gene, encoding SS1, is primarily expressed in the endosperm but is inducible in leaves and roots under certain stress conditions (Springer et al., 1986). In wheat the SS2-encoding gene is predominantly expressed in the endosperm, whereas SS1 mRNA appears also in roots and leaves, where it is induced under conditions of anaerobiosis and low temperatures (Maraña et al., 1990).

Two genes corresponding to the SS1 and SS2 proteins have been characterized in barley (*Hordeum vulgare* L.; Sánchez de la Hoz et al., 1992; Martínez de Ilarduya et al., 1993). These are located on different chromosomes, 7H $\beta$  and 2HS, respectively. RNA-blot analysis, using specific probes derived from the 3' noncoding regions of the

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Abbreviations: dap, days after pollination; SS1, type 1 Suc synthase; SS2, type 2 Suc synthase.

cDNAs, shows a differential expression pattern in that *Ss2* appears only in the endosperm, whereas *Ss1* was found in all of the tissues that were analyzed.

We have purified and characterized SS1 from young barley roots. Polyclonal antibodies have been raised against SS1 and against a synthetic peptide that is unique to the SS2 sequence. The antibodies against this peptide did not cross-react with SS1. Immunolocalization of Suc synthase in distinct barley tissues showed differential accumulation of the isozyme types. The spatial distribution of the two isozyme types has been found to be markedly different from that in maize. Functional implications of these findings are discussed.

## MATERIALS AND METHODS

Barley (*Hordeum vulgare* L. cv Bomi) seeds were surface-sterilized with 20% (v/v) Domestos (Lever-España, Madrid, Spain) and germinated in Petri dishes lined with moist paper. Roots, harvested for the purification procedure, were grown at room temperature for 2 d and then at 4°C for 4 d. Materials for crude extracts were grown in moistened vermiculite at 14°C, and leaves and roots were harvested after 7 d of germination. Developing endosperms were collected at approximately 15 dap. Plant material was frozen in liquid nitrogen and stored at -70°C until extraction.

### Purification of Suc Synthase from Barley Roots

The purification procedure was derived from that of Nguyen-Quoc et al. (1990). Frozen roots (23 g) were ground under liquid nitrogen with 4 volumes of 100 mM Tris-HCl (pH 7.5), 2 mM DTT, 1 mM EDTA, 1% (w/v) PVP, 1 mM PMSF, 0.7  $\mu\text{g mL}^{-1}$  pepstatin, and 0.5  $\mu\text{g mL}^{-1}$  leupeptin. The extract was subjected to centrifugation at 4°C for 15 min at 10,000g. The pellet was then re-extracted with 40 mL of the same buffer and clarified as above. The supernatants were combined and adsorbed to 50 mL of DEAE-Sephadex fast flow (Sigma) equilibrated with buffer A (50 mM Tris-HCl [pH 7.5], 1 mM DTT, 1 mM EDTA, and 5% [v/v] glycerol) containing 100 mM NaCl. The gel was washed with 300 mL of buffer A, and the protein was eluted with buffer A containing 270 mM NaCl. This fraction was concentrated by ammonium sulfate saturation at 60% (w/v) followed by centrifugation at 10,000g for 30 min and resuspension in 10 mL of buffer A. The concentrate was desalted by dialysis and loaded onto a 2.5-mL uridine 5'-diphosphoglucuronic acid-agarose affinity column (Sigma) equilibrated with buffer A. After the column was washed Suc synthase was eluted in 300 mM KCl. Active fractions were buffer-exchanged into buffer A using a 5-mL desalting column (Pharmacia). The recovered protein fraction was loaded onto a 1-mL Mono-Q column (Pharmacia) equilibrated with buffer A. Proteins were eluted over a 20-mL gradient of 55 to 75% (v/v) buffer B (buffer A containing 400 mM KCl). Active fractions were stored at -20°C in 20% (v/v) glycerol. Protein concentration was determined with a protein assay kit (Bio-Rad) using BSA as a standard.

### Enzyme Assay

Suc synthase activity was measured in the direction of Suc degradation using a modification of the assay described previously by Xu et al. (1989) to measure the production of UDPGlc. Reactions (1 mL) contained 100 mM Suc, 50 mM Hepes (pH 7.5), 0.5 mM UDP, 0.5 mM NAD<sup>+</sup>, 0.03 unit of UDPGlc dehydrogenase, and an appropriate amount of the enzyme. Concentrations of Suc or UDP were varied for the evaluation of kinetic parameters. Activity was calculated by continuous measurement of the  $A_{340}$  increase at 25°C. Samples without UDP were used as blanks.

For the evaluation of nucleoside diphosphate specificity the production of Fru was measured using a modification of the method described by Pontis et al. (1981). Reactions (50  $\mu\text{L}$ ) containing 200 mM Suc, 50 mM Hepes (pH 7.0), 1 mM UDP or ADP, 5 mM MgCl<sub>2</sub>, and an appropriate aliquot of enzyme were incubated for 30 min at 30°C. The reaction was stopped by placing the tubes in boiling water for 2 min and adding 200  $\mu\text{L}$  of 100 mM Hepes (pH 7.9). Fru in the solution was determined by the change in  $A_{340}$  after the addition of 7 units of phosphoglucose isomerase, 2 units of hexokinase, 2 units of Glc-6-P dehydrogenase, 1.2 mM ATP, and 0.5 mM NAD<sup>+</sup>.

Except for the determination of the kinetic constants, assays were done under saturating conditions linear for time and amount of enzyme.

### Preparation of Antisera

Antibodies were raised in rabbits immunized against SS1 purified from barley roots, as described in Table I, and protein eluted from the Mono-Q column (300  $\mu\text{g}$ ) was further purified by SDS-PAGE. After light staining with Coomassie brilliant blue R-250 (0.05%, w/v in water), the protein band was excised and lyophilized before immunization.

Antibodies were also raised against a synthetic peptide sequence, unique to the SS2 subunit, which was found by a comparison of the deduced amino acid sequences of the barley *Ss1* and *Ss2* cDNA clones (Martinez de Ilarduya et al., 1993). This peptide of 11 amino acids (from positions 129 to 139) plus a terminal Cys residue (LANGSTDNNFVC) was coupled to a protein carrier (keyhole limpet hemocyanin) via the Cys residue before immunization. Peptide synthesis and antibody production were done by Eurogentec (Brussels), according to their standard protocols.

### Purification of Suc Synthase-Specific Antibodies

Barley endosperm extracts, obtained by homogenization of 10 endosperms with 0.5 mL of TBS and centrifugation at 12,000g for 10 min, were separated by SDS-PAGE, transferred to PVDF membranes (Millipore), and blocked for 1 h with TBS, 5% (w/v) nonfat milk, and 0.1% (v/v) Tween 20. After the membrane was incubated with 5 mL of antisera of either SS1 or SS2, diluted 1:30 (v/v) in the TBS blocking buffer, side strips were cut and developed using the immunoblot protocol. The rest of the membrane was washed three times (10 min each) in the blocking buffer and re-

**Table 1.** Purification of Suc synthase from barley roots

Fraction	Protein	Activity	Specific Activity
	mg	$\mu\text{mol min}^{-1}$	$\mu\text{mol min}^{-1} \text{mg}^{-1}$
Crude extract	466	8.67	0.019
DEAE-Sephadex	96	8.52	0.089
UDPGlc-agarose	6	2.97	0.495
Mono-Q	0.8	2.42	3.025

aligned with the stained side strips. The region corresponding to the Suc synthase band was cut out and the antibodies were eluted in 1 mL of 100 mM Gly (pH 2.5) for 5 min. The eluted IgG fractions were neutralized with 1 M Tris (pH 7.5) and stored in aliquots at  $-20^{\circ}\text{C}$  until needed for use. Preimmune sera were treated in the same way, except that colored molecular weight markers (Bio-Rad) were used to identify the region corresponding to the Suc synthase band.

### Protein Electrophoresis and Immunodetection

Crude extracts were prepared by grinding frozen barley tissue in the TBS buffer. After the sample was centrifuged for 10 min at  $4^{\circ}\text{C}$ , protein concentration was estimated using a protein assay kit (Bio-Rad) and BSA as a standard. Separation of proteins by SDS-PAGE was done as described previously by Laemmli (1970). Native-gel electrophoresis was performed as above, without SDS, using acrylamide gradient gels (4–8%, w/v) run at  $4^{\circ}\text{C}$  for 2 h at 150 V. After electrophoretic separation, proteins were transferred to PVDF membranes (Millipore) using Towbin's buffer (Towbin et al., 1979) with an Electro Transblot apparatus (Bio-Rad). Membranes were blocked for 1 h with TBS, 5% (w/v) nonfat milk, and 0.1% (v/v) Tween 20, after which purified IgG was added at a concentration of 1:100 (v/v), and incubated for 2 h. The membranes were washed three times with TBS and 0.1% Tween 20, and the second antibody (goat anti-rabbit IgG conjugated to alkaline phosphatase, Sigma) was added at a concentration of 1:1000 (v/v) in the same buffer and incubated for 1 h. After the membranes were washed three more times, color was developed using nitroblue tetrazolium ( $0.33 \text{ mg mL}^{-1}$ ) and 5-bromo-4-chloro-3-indolyl phosphate ( $0.165 \text{ mg mL}^{-1}$ ) in a buffer containing 0.1 M Tris HCl (pH 9.5) and 0.5 mM  $\text{MgCl}_2$ .

Glycoproteins were identified with a Glycan detection kit (Boehringer Mannheim). Briefly, membrane-bound protein ( $2 \mu\text{g}$ ) was oxidized and labeled with digoxigenin, which was detected by an enzyme immunoassay using an anti-rabbit IgG-alkaline phosphatase conjugate. Transferrin and chymotrypsinogen were used as the positive and negative controls, respectively.

### Immunolocalization

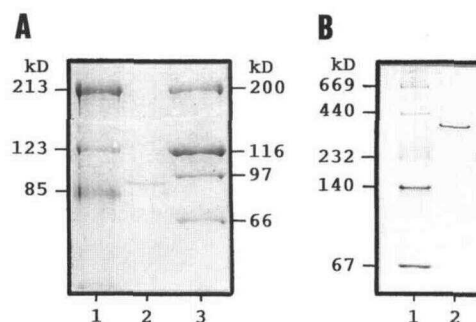
Barley (given previously) tissue was harvested from roots (3 d) and leaves (9 d) or from approximately 12 dap endosperms and fixed immediately in 4% (w/v) paraformaldehyde, included in paraffin, and cut into  $8\text{-}\mu\text{m}$  sections using a microtome (Jung-Autocut model, Leica). Paraffin was removed with xylol, and the sections were

hydrated in an ethanol-to-water dilution series and then blocked with 5% (w/v) BSA in TBS 0.1% (v/v) and Tween 20 for 1 h at  $37^{\circ}\text{C}$ . IgG fractions for Suc synthase, or specific for SS2, were diluted with an equal volume of the blocking buffer and placed over the sections for 1 h at  $37^{\circ}\text{C}$ . The slides were then washed three times for 10 min each with TBS and 0.1% Tween 20. Sections were incubated for 1 h at  $37^{\circ}\text{C}$  with the second antibody (goat anti-rabbit IgG conjugated to alkaline phosphatase, Sigma), diluted 1:25 (v/v) in the blocking buffer, and then washed as above. After the sample was washed a final time in TBS, color was developed with nitroblue tetrazolium ( $0.33 \text{ mg mL}^{-1}$ ) and 5-bromo-4-chloro-3-indolyl phosphate ( $0.165 \text{ mg mL}^{-1}$ ) in a buffer containing 0.1 M Tris HCl (pH 9.5), and 0.5 mM  $\text{MgCl}_2$ . After dehydration in a water-to-ethanol dilution series, slides were mounted in Entellan mounting media (Merck), viewed, and photographed under an Axiophot light microscope (Zeiss).

## RESULTS

### Purification of the Root Suc Synthase Isozyme

Suc synthase was purified from barley roots, where *Ss1* mRNA had been previously shown to be abundantly present, without traces of *Ss2* mRNA (Martinez de Ilarduya et al., 1993). The enzyme was extracted after 2 d of germination at room temperature, followed by 4 d at  $4^{\circ}\text{C}$ , and purified by using affinity- and ion-exchange chromatographies (Table I). This purification procedure resulted in an approximately 160-fold purification, with a yield of approximately 30%. The final fraction contained one main protein band of 90 kD after SDS-PAGE (Fig. 1A) with an approximate pI of 5.1, as determined by IEF (data not shown). The native protein ran as one single band with an estimated molecular mass of approximately 360 kD in native gradient PAGE (Fig. 1B). Although this method of estimating molecular mass is subject to aberrations due to the charge on the native protein, the sample was subjected to electrophoresis for 5 h over a steep (4–20%, w/v) acrylamide gradient. Because the gel-sieving effect is greater for larger macromolecules (Goldenberg and Creighton, 1984),



**Figure 1.** Molecular mass estimation of Suc synthase purified from barley roots. A, SDS-PAGE (10%, w/v); B, native PAGE (4–20%, w/v, acrylamide gradient). Molecular mass markers, as indicated, were loaded in lanes 1 and 3; purified root Suc synthase ( $4 \mu\text{g}$ ) was loaded in lanes 2. Gels were stained with Coomassie brilliant blue.

the results are sufficient to substantiate that the native form of the root Suc synthase in barley is a homotetramer.

Four putative *N*-glycosylation sites are present in the deduced amino acid sequence from the *Ss1* cDNA (Sánchez de la Hoz et al., 1992). However, analysis for carbohydrate conjugation gave a negative result (data not shown).

### Kinetics and Nucleoside Diphosphate Specificity

Kinetic parameters of the root Suc synthase in the direction of Suc degradation were determined using *s/v* versus *s* plots (data not shown). The  $K_m$  values were 30 mM for Suc and 5  $\mu$ M for UDP, indicating a much greater affinity for UDP than for Suc. Michaelis-Menten plots were hyperbolic and double-reciprocal plots were linear for both of the substrates (data not shown). Nucleoside diphosphate specificity was tested by comparing the rate of Fru production in the presence of Suc (200 mM) and UDP (1 mM) or ADP (1 mM). When ADP was used as a substrate, the reaction rate was only 25% of that with UDP.

### Distribution of SS1 and SS2 in Different Tissues

The distribution in barley tissues of the two types of Suc synthase was investigated by immunoblot electrophoretic

analyses using two different polyclonal antisera obtained from rabbits. These antibodies were raised against the purified SS1 and against a synthetic peptide containing 11 residues (LANGSTDNNFV) unique to the predicted amino acid sequence of *Ss2* (Martinez de Ilarduya et al., 1993). As shown in Figure 2A (I), the first antibody recognized both the 90-kD SS1 subunit and the 88-kD SS2 subunit, also present in endosperm, whereas the second antibody recognized only SS2 (Fig. 2A, II). These results were in agreement with the previously reported 79% identity of the deduced amino acid sequences of the two subunits and with the presence of the two types of mRNA in endosperm (Martinez de Ilarduya et al., 1993).

Analysis of the immunoblots after SDS-PAGE showed that SS1 and SS2 were strongly expressed in developing endosperm, whereas in root and leaf extracts, only the SS1 subunit was detected (Fig. 2B, I and II). Both enzyme subunits were also detected in crude extracts of barley anthers and in immature embryos (data not shown).

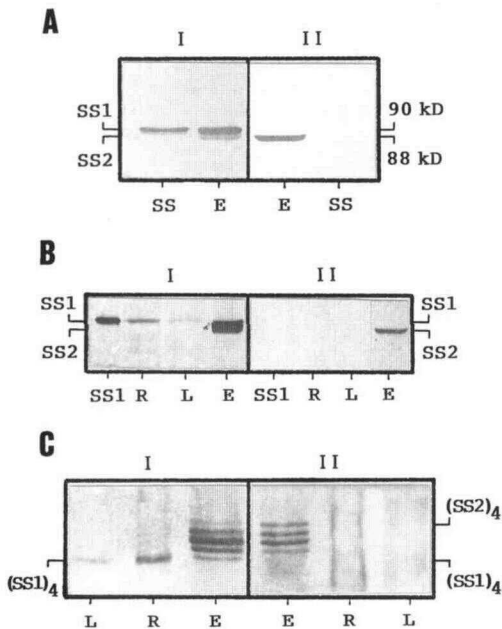
Analysis of the immunoblots after native gradient (4–8%, w/v) PAGE indicated that in endosperm where the two types of subunits are expressed, all five possible homo- and heterotetrameric forms of the enzyme are present (Fig. 2C, I). With the SS2-specific antiserum (Fig. 2C, II) only four bands appeared, because these antibodies do not recognize the homotetramer of SS1. In the leaf and root extracts only the SS1 homotetramer was detected.

### Immunolocalization of SS1 and SS2

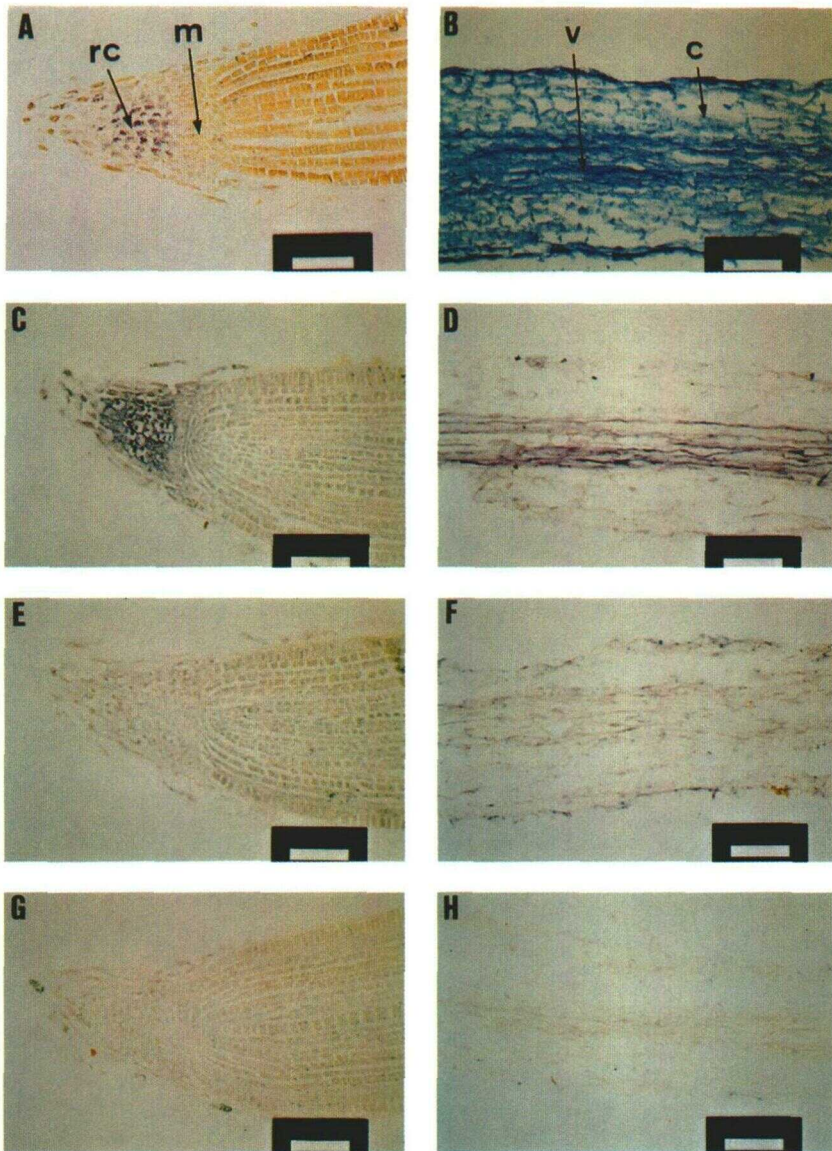
To localize the expression of SS1 and SS2 in different cell types within a given tissue, immunohistochemical analyses were done (Figs. 3 and 4). In 3-d-old roots the SS1 label was detected in cap cells and along the vascular strand starting from about 600  $\mu$ m from the root tip, just after the meristematic region (Fig. 3, C and D). We could not identify whether the vascular label was associated with the xylem or the phloem, because the cellular structure was not well fixed in that region. In cap cells the label was associated with the presence of amyloplasts, which stained blue with an I-KI stain (Fig. 3A). Neither the SS2 IgG nor the preimmune treatment gave any signal (Fig. 3, E–H).

In transverse sections of vascular bundles of 9-d-old leaves, the toluidine blue stain distinguished the lignified xylem cells (stained green in Fig. 4A). The SS1 label was confined within the phloem and had a strong affinity with the smallest cells (Fig. 4C). Although it is difficult to distinguish between the phloem cell types with a light microscope, it is clear that the label was not present in all of the phloem cells and appeared to adhere to the companion cells, as reported for maize leaves (Nolte and Koch, 1993). This discrete signal was seen in all vascular bundles of the leaf sections and was not evident in any other cell type (data not shown). SS2 was not detected in any cell type and the preimmune treatment gave no signal (Fig. 4, E and G).

In developing seeds there were strong signals for both SS1 and SS2, which were distributed throughout the endosperm (Fig. 4, D and F). SS1, but not SS2, was also apparent in the assimilate-unloading tissues: the nucellar



**Figure 2.** Distribution of SS1 and SS2 in barley tissues by immunoblot analysis after SDS-PAGE (7%, w/v; A and B) or after native gradient PAGE (4–8%, w/v; C). Panels I were incubated with IgG fractions purified from antisera raised against root SS1. Panels II were incubated with IgG fractions purified from antisera raised against the synthetic peptide unique to the SS2 subunit. A, Specificity of IgG. SS, Suc synthase purified from roots (4  $\mu$ g); E, endosperm extract (10  $\mu$ g). B, Detection of SS1 and SS2 subunits in barley tissues. SS1, Suc synthase purified from roots (4  $\mu$ g); R, root extract; L, leaf extract; and E, endosperm extract. Equal amounts of protein (15  $\mu$ g) were loaded for these crude extracts. C, Detection of Suc synthase tetramers in barley tissues. L, Leaf extract; R, root extract; and E, endosperm extract. Fifteen micrograms of protein was loaded in each sample.



**Figure 3.** Immunolocalization of different Suc synthase types (SS1 and SS2) in barley roots. Paradermal sections (8  $\mu\text{m}$ ) of 3-d-old root tips (A, C, E, and G) and in the region approximately 700  $\mu\text{m}$  above the root tip (B, D, F, and H) were treated with I-KI stain (A), toluidine blue stain (B), the IgG purified from root SS1 antisera (C and D), the SS2-monospecific IgG (E and F), and the preimmune IgG (G and H). rc, Root cap; m, meristematic region; c, cortex; and v, vascular bundle. Bars = 100  $\mu\text{m}$ .

projection, the vascular area, and, at a high concentration, the chalazal region.

### DISCUSSION

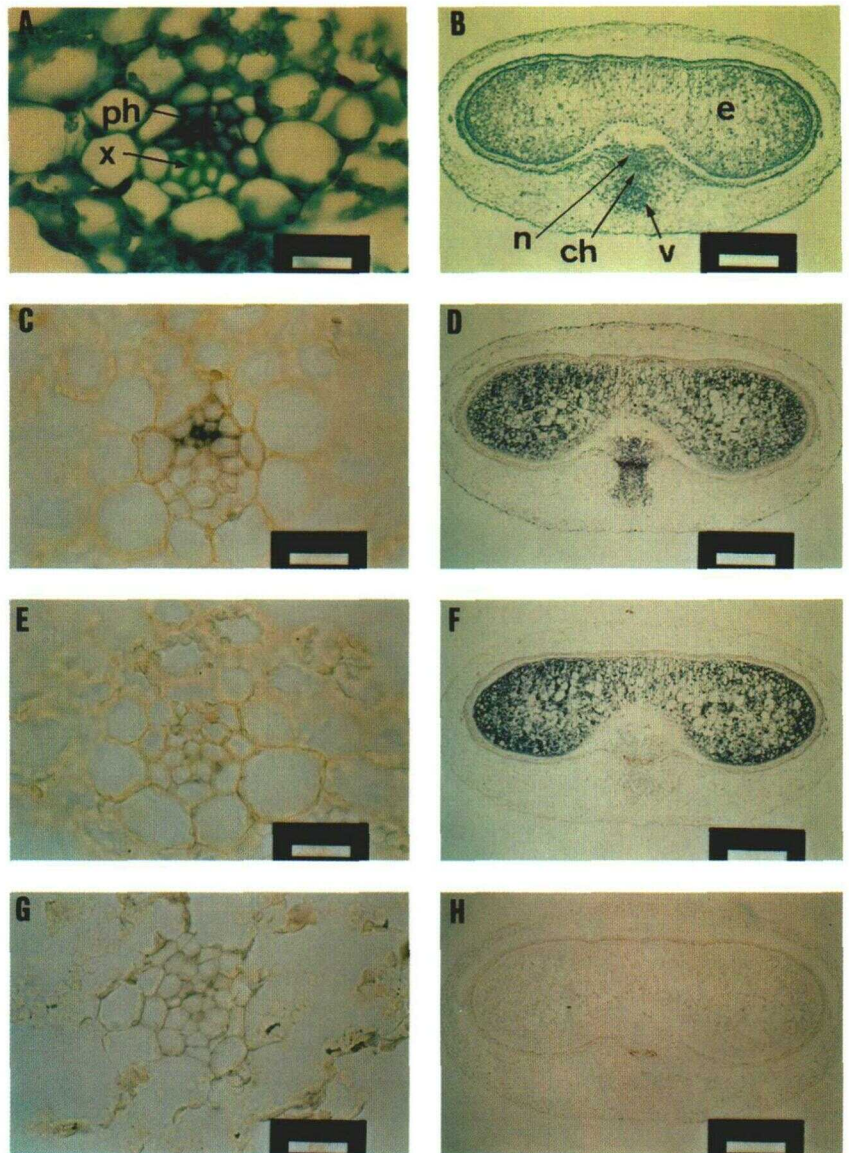
Suc synthase from barley roots has been shown here to be a homotetramer composed of 90-kD SS1 subunits. It had a lower affinity for Suc than for UDP, as shown by the  $K_m$  values of 30 mM and 5  $\mu\text{M}$ , respectively. These  $K_m$  values were lower than reported previously for Suc synthase purified from other cereals such as wheat, maize, and sugarcane, which were in the range of 40 to 60 mM for Suc and of 22  $\mu\text{M}$  to 2 mM for UDP (Larsen et al., 1985; Nguyen-Quoc et al., 1990; Buczynski et al., 1993). However, they were very similar to those reported for the Suc synthase isolated from soybean nodules (Morell and Copeland, 1985).

The Suc cleavage reaction was not UDP-specific, since there was appreciable activity in the presence of ADP (25%

of that with UDP). Reaction with ADP has also been reported for Suc synthases purified from maize, faba beans, and soybean nodules (Morell and Copeland, 1985; Nguyen-Quoc et al., 1990; Ross and Davies, 1992). The formation of ADPGlc by this enzyme has important implications in the light of recent experiments, supporting the idea of an alternative pathway for starch biosynthesis in nonphotosynthetic organs, where ADPGlc, synthesized in the cytosol by either Suc synthase or by a cytosolic form of ADPGlc pyrophosphorylase, would be transported across the amyloplast membrane via an adenylate translocator and incorporated directly into starch (Pozueta-Romero et al., 1991; Thorbjørnsen et al., 1996). However, more evidence would be necessary to ascertain that this is a major pathway of starch synthesis (Okita, 1992; Smith and Martin, 1993).

The Suc synthase expression pattern in barley markedly differs from that in maize, which is the most thoroughly characterized cereal to date. In contrast to barley, both SS1 and SS2 have been detected in maize roots, where they are

**Figure 4.** Immunolocalization of different Suc synthase types (SS1 and SS2) in barley leaves and developing seeds. Transverse sections (8  $\mu\text{m}$ ) of 9-d-old leaf vascular bundles (A, C, E, and G; bar = 20  $\mu\text{m}$ ) and developing seeds harvested 12 dap (B, D, F, and H; bar = 400  $\mu\text{m}$ ) were treated with toluidine blue stain (A and B), the IgG fraction purified from root SS1 antisera (C and D), the SS2-monospecific IgG (E and F), and the preimmune IgG (G and H). x, Xylem; ph, phloem; e, endosperm; n, nucellar projection; ch, chalazal region; and v, vascular bundle.



thought to exist in the same cellular compartments, based on the formation of heterotetramers (Chourey et al., 1986). In barley roots only SS1 was present in the vascular region and in the root cap cells, where starch accumulation was also detected. The Suc synthase *sus4* gene from potato is also expressed in root caps (Fu and Park, 1995). The presence of Suc synthase in barley root cap cells may indicate a role in the biogenesis of amyloplasts, in which accumulation and sedimentation have been associated with gravity sensing (Volkman and Sievers, 1979). The expression of Suc synthase in the root tips of maize has been shown to be dependent on sugar supplies (Koch et al., 1992; Koch, 1996). In 7-d-old maize roots, Suc synthase was most abundant in and near the stele; however, at higher Glc levels (2.0%) the Suc synthase protein was uniformly distributed throughout the root profile.

We have shown that only SS1 is present in the phloem of barley leaves and might be localized in the companion cells. In maize and citrus leaves Suc synthase has also

been described in the phloem (Nolte and Koch, 1993) and, recently, ultrastructural detection has confirmed its presence in companion cells (Brangeon et al., 1996). Although it is known that SS2 is the most abundant isoform in maize leaves (Nguyen-Quoc et al., 1990), the Suc synthase type was not specified in the immunolocalization studies cited above. The presence of SS1 in barley phloem cells would most likely have functions similar to those suggested for SS2 in the same cells of maize. It should be noted that SS1 mRNA is induced by anaerobiosis in both wheat and maize leaves (Springer et al., 1986; Marañón et al., 1990).

In barley seeds polymerization of the SS1 and SS2 subunits results in heterotetramers, whereas in maize there is spatial segregation of the subunits (Chen and Chourey, 1989) and only homotetramers are formed (Chourey et al., 1986). Furthermore, only SS1 is present in the nucellar projection, the chalazal region, and the vascular bundle at the base of the barley endosperm, whereas in maize SS2 is

specifically present in the basal endosperm transfer cells (Chen and Chourey, 1989).

The conservation of the two types of subunits among cereals would suggest that they have evolved into specialized roles. However, the lack of significant differences in their catalytic properties (Echt and Chourey, 1985; Larsen et al., 1985; Nguyen-Quoc et al., 1990; Buczynski et al., 1993), and their dissimilar expression patterns between cereal species such as maize and barley, suggest that the functions of the isozymes are to a large extent exchangeable *in vivo*.

Several recent study groups reported the expression of Suc synthase in the phloem cells of plants (Yang and Russell, 1990; Martin et al., 1993; Nolte and Koch, 1993; Shi et al., 1994; Brangeon et al., 1996). Although the role of Suc synthase in the phloem is still unclear, there is increasing evidence that it may be required to provide energy for Suc loading and substrates for callose biosynthesis in these cells (Geigenberger et al., 1993; Nolte and Koch, 1993).

Sugars are transported from the phloem to the developing endosperm via the chalazal tissue and the nucellar projection. Suc is reported to be the major form in which carbon is transported to the endosperm (Jenner, 1973; Sakri and Shannon, 1975; Felker et al., 1984). The strong Suc synthase signal present in the endosperm tissues strongly supports these results and suggests that this is the major site of Suc degradation. The expression of SS1 in tissues adjacent to the endosperm is therefore unlikely to have the function of bulk Suc cleavage prior to transport into the endosperm. Large amounts of callose are deposited in the crease region next to the endosperm cavity, which may be a means of stabilizing the rigid morphology that is necessary for assimilate transport (Duffus and Cochrane, 1992). Suc synthase may provide UDPGlc for the callose synthase reaction.

Assimilate transport from the phloem to the endosperm occurs primarily via a symplastic pathway (Wang and Fisher, 1994). Whether the process is driven by diffusion along a Suc gradient or by active membrane transport is unclear. Suc synthase activity in this region could be involved either in the maintenance of a Suc gradient and/or in providing substrates for respiration reactions. Studies performed in the wheat grain suggest that the cells in the nucellar projection have a sufficient membrane surface area to support *in vivo* rates of Suc transport into the endosperm cavity. Furthermore, the dense cytoplasm and abundant mitochondria in these cells indicate high levels of metabolic activity, which could provide energy for active transport across the plasma membrane (Wang et al., 1994). Nolte and Koch (1993) also localized Suc synthase in the phloem-unloading site of citrus fruit and speculated that products may be used to meet elevated respiratory requirements in this region. The localized expression in the vascular area and nucellar projection presented here suggest that SS1, but not SS2, has an integral role in the transport of nutrients into the barley endosperm.

Membrane-associated forms of SS1 and SS2 have been recently reported in plasma membrane fractions from maize (Carlson and Chourey, 1996). Delmer and Amor

(1995) have described a model of a complex composed of membrane-associated forms of Suc and callose synthases, which could directly channel carbon from Suc to callose. The immunological studies presented here are unlikely to distinguish between membrane-associated and cytosolic Suc synthase forms. However, if SS2 was membrane-associated, it is possible that the peptide epitope could be obscured. Nevertheless, the immunological data reported here and the RNA electrophoretic analyses previously described by Martinez de Ilarduya et al. (1993) are in agreement.

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