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Rice sucrose-phosphate synthase: Identification of an isoform specific for heterotrophic tissues with distinct metabolite regulation from the mature leaf enzyme

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Immunohistological analyses for rice (*Oryza sativa*) sucrose-phosphate synthase (SPS, UDP-glucose D-fructose-6-phosphate-2-glucosyltransferase, EC 2.4.1.14) show that the protein is differently localized in photosynthetic and etiolated leaves. Very little is known about SPS regulation in heterotrophic tissues; therefore, we studied the biochemical properties of the enzyme from etiolated seedlings and embryo. Two SPS forms (SPS-1 and SPS-2) were partially purified from etiolated seedlings. The effects of Glc-6-P (activator) and Pi (inhibitor) on SPS activities allowed us to differentiate the two forms. SPS-1 showed high sensitivity to Pi which also strongly decreased enzyme activation by Glc-6-P. SPS-2 was highly activated by Glc-6-P and showed low sensitivity to Pi. In vitro alkaline phosphatase treatment suggested that SPS-1 could be regulated as leaf SPS in darkness and that SPS-2 is present in a dephosphorylated state or is not regulated by protein phosphorylation. The relative MM value (116 kDa) estimated for both SPS forms in SDS-PAGE is identical to the rice leaf SPS polypeptide. Taken together, these data led us to conclude that SPS-2 is an enzyme form only present in non-photosynthetic tissues.

Introduction

Sucrose-phosphate synthase (SPS, UDP-glucose D-fructose-6-phosphate-2-glucosyltransferase, EC 2.4.1.14) has a key function in the regulation of sucrose synthesis in leaves (Stitt et al. 1987) and plays an important role in carbon partitioning during grain filling in cereals (Prioul et al. 1990), in sugar accumulation in fruits (Hubbard et al. 1990, Dali et al. 1992) and during cold stress acclimation (Tognetti et al. 1989). SPS undergoes a diversity of regulatory mechanisms including: (1) allosteric regulation by Glc-6-P (activator) and Pi (inhibitor) (Doehlert and Huber 1983, Stitt et al. 1988); (2) reversible covalent modification in response to light (Stitt et al. 1988, Huber et al. 1989, Huber and Huber 1991, 1992), accumulation of sucrose (Stitt et al. 1988) and osmotic stress (Toroser and Huber 1997); and (3) transcriptional regulation during sink-source transition (Harn et al. 1993, Klein et al. 1993).

The regulation of SPS from spinach and maize leaves has been well characterized. In darkness, SPS is inactivated by phosphorylation of a regulatory Ser residue. This effect is reversed in light by a type 2A protein phosphatase resulting in an activated SPS as a consequence of an increase in the affinity for the substrates (Fru-6-P and UDP-Glc) and for the positive modulator (Glc-6-P), and a decrease in Pi sensitivity (Huber and Huber 1996). The effect of phosphorylation on SPS activity is only evident when assayed under limiting substrate concentrations, near physiological conditions (Stitt et al. 1988, Lunn and Hatch 1997).

In contrast, there are very few studies on SPS regulation in non-photosynthetic tissues. While the wheat germ enzyme is barely affected by Pi and Glc-6-P (Salerno and Pontis 1978), potato tuber SPS is regulated by both allosteric effectors and covalent modification (Reimholz et al. 1994).

Multiple SPS forms, as an additional regulatory strategy, were reported either in photosynthetic or in non-photosynthetic tissues (Salerno et al. 1991, Reimholz et al. 1994, 1997, Salerno et al. 1998, Kumatsu et al. 1999). Changes in

Abbreviations – PGI, phosphoglucose isomerase; SPS, sucrose-phosphate synthase; SS, sucrose synthase.

SPS localization might also to be related to early events in the development of the photosynthetic apparatus (Cheng et al. 1996).

In addition to our previous work (Salerno et al. 1998), we report here a different cellular localization of the SPS protein in green and etiolated rice tissues. According to these results we were driven to study the biochemical properties of SPS isolated from non-photosynthetic tissues (etiolated seedlings and embryos). The analysis of metabolite (Glc-6-P and Pi) effects led us to conclude that there is a heterotrophic tissue specific SPS isoform.

Materials and methods

Plant material

Rice seeds (*Oryza sativa* L. cv. Blue Bell) were provided by Cámara Argentina de Arroz, Paraná, Entre Ríos, Argentina. Etiolated seedlings were grown on vermiculite in a chamber at 27°C in the dark and watered with Hoagland and Arnon solution. Seedlings with a day/night regime were grown as previously described (Salerno et al. 1998).

Preparation of crude protein extracts and enzyme partial purification

Plant material was weighed and powdered under liquid nitrogen with a mortar and pestle. Homogenates were prepared by stirring the powder in a buffer (1 ml buffer [g tissue]$^{-1}$) containing 100 m*M* HEPES-NaOH pH 7.5, 20 m*M* β-mercaptoethanol, 10 m*M* $MgCl_2$, 2 m*M* EDTA, 0.5 m*M* PMSF, 2% (v/v) ethyleneglycol, 10 m*M* NaF, 1 m*M* β-glycerophosphate, 10 m*M* orthovanadate and 20% (v/v) glycerol. Extracts were filtered through sintered glass and centrifuged at 12000 *g* for 15 min. The supernatant was applied onto a DEAE-Sephacel (Pharmacia) column (1 × 20 cm) equilibrated with a buffer containing 20 m*M* HEPES-NaOH pH 6.5, 10 m*M* $MgCl_2$, 1 m*M* EDTA, 5 m*M* β-mercaptoethanol, 0.5 m*M* PMSF and 20% (v/v) glycerol (buffer A). The column was washed with 50 ml of buffer A followed by 50 ml of the same buffer containing 0.05 *M* NaCl. SPS was eluted with a 100 ml gradient of NaCl (0.05–0.5 *M*) in buffer A. Fractions of 0.5 ml were collected and assayed for enzyme activities and for protein content. Fractions containing SPS and SS activities were separately pooled and concentrated in an ultrafiltration membrane cone (Centriflo Amicon, MA, USA). The concentrated SPS fractions were further purified by chromatography onto a Sepharose 6B column (0.5 × 56 cm), previously equilibrated with a buffer containing 50 m*M* imidazol pH 7.5, 1 m*M* EDTA, 10 m*M* $MgCl_2$, 5 m*M* β-mercaptoethanol, 0.5 m*M* PMSF, and 10% (v/v) glycerol (buffer B). Elution of SPS activity was carried out with buffer B at 0.2 ml min^{-1}. Fractions containing SPS activity were assayed for phosphoglucose isomerase (PGI) activity and those fractions that were free of PGI were pooled and concentrated as indicated above. The concentrated SS fraction eluted from the DEAE-Sephacel column was chromatographed onto a Sephadex G-200 column (2.2 × 90 cm), previously equilibrated with a buffer containing 50 m*M* HEPES-NaOH pH 7.5, 10 m*M* $MgCl_2$, 1 m*M* EDTA, 5 m*M* β-mercaptoethanol, 0.5 m*M* PMSF and 150 m*M* NaCl (buffer C). The elution was carried out with buffer C at 0.15 ml min^{-1}. Fractions with SS activity were pooled and concentrated as described. Quantification of proteins was performed according to Bradford (1976) using bovine serum albumin as a standard.

Enzyme assays

SPS and SS activities were assayed as reported (Salerno et al. 1998) by quantifying sucrose-phosphate (as sucrose) and sucrose formed by the thiobarbituric acid method (Salerno et al. 1991). The standard SPS reaction mixture (V assay) contained 10 m*M* UDP-Glc, 20 m*M* $MgCl_2$, 100 m*M* HEPES-NaOH pH 7.5, and 10 m*M* Fru-6-P. A limiting substrate concentration assay (v assay) contains 1 m*M* Fru-6-P. One enzyme unit (U) is defined as the amount which catalyses the formation of 1 μmol of product per minute.

The effect of a dephosphorylation treatment on SPS activity was detected by using two different assay conditions: (1) selective assay (v_{sa}), whose incubation mixture contained 1 m*M* Fru-6-P, 3 m*M* Glc-6-P, 5 m*M* Pi, 10 m*M* UDP-Glc, 20 m*M* $MgCl_2$ and 100 m*M* HEPES-NaOH pH 7.5; (2) non-selective assay (V_{nsa}), whose incubation mixture contained 10 m*M* Fru-6-P, 30 m*M* Glc-6-P, 10 m*M* UDP-Glc, 20 m*M* $MgCl_2$ and 100 m*M* HEPES-NaOH pH 7.5.

Incubation mixtures for SS activity contained 10 m*M* UDP-Glc, 10 m*M* Fru, 20 m*M* $MgCl_2$ and 100 m*M* HEPES-NaOH pH 7.5. PGI activity was assayed following Doehlert and Huber (1983).

In vitro dephosphorylation of SPSs

Aliquots of the concentrated fractions from the partially purified enzymes (Sepharose 6B step) were incubated for 2 h at 30°C in buffer 20 m*M* Tris-glycine pH 8.8, 5 m*M* $MgCl_2$ and 0.1 m*M* $ZnCl_2$ in the presence of 5 U of alkaline phosphatase attached to beaded agarose (Sigma P-0762). As a control of SPS stability, aliquots were incubated in the presence of agarose beads (Sigma A-7431) under similar conditions.

Preparation of polyclonal rabbit antibodies and western blots

SPS from rice leaves was prepared according to Salerno et al. (1998). Polyclonal antibodies were raised by inoculating rabbits with the polyacrylamide gel band containing the 116 kDa (or the 88 kDa) polypeptide separated by SDS-PAGE and localized by staining with Coomassie blue (116- or 88-antibodies) (Salerno et al. 1998). The 116-antibodies precipitated SPS activity from both etiolated and green rice seedlings. Western blots were performed as described (Salerno et al. 1998).

SPS immunolocalization in rice leaf sections

Fully expanded leaves from 4-week-old plants (mature green leaves) and the first leaves from light (young green leaves)

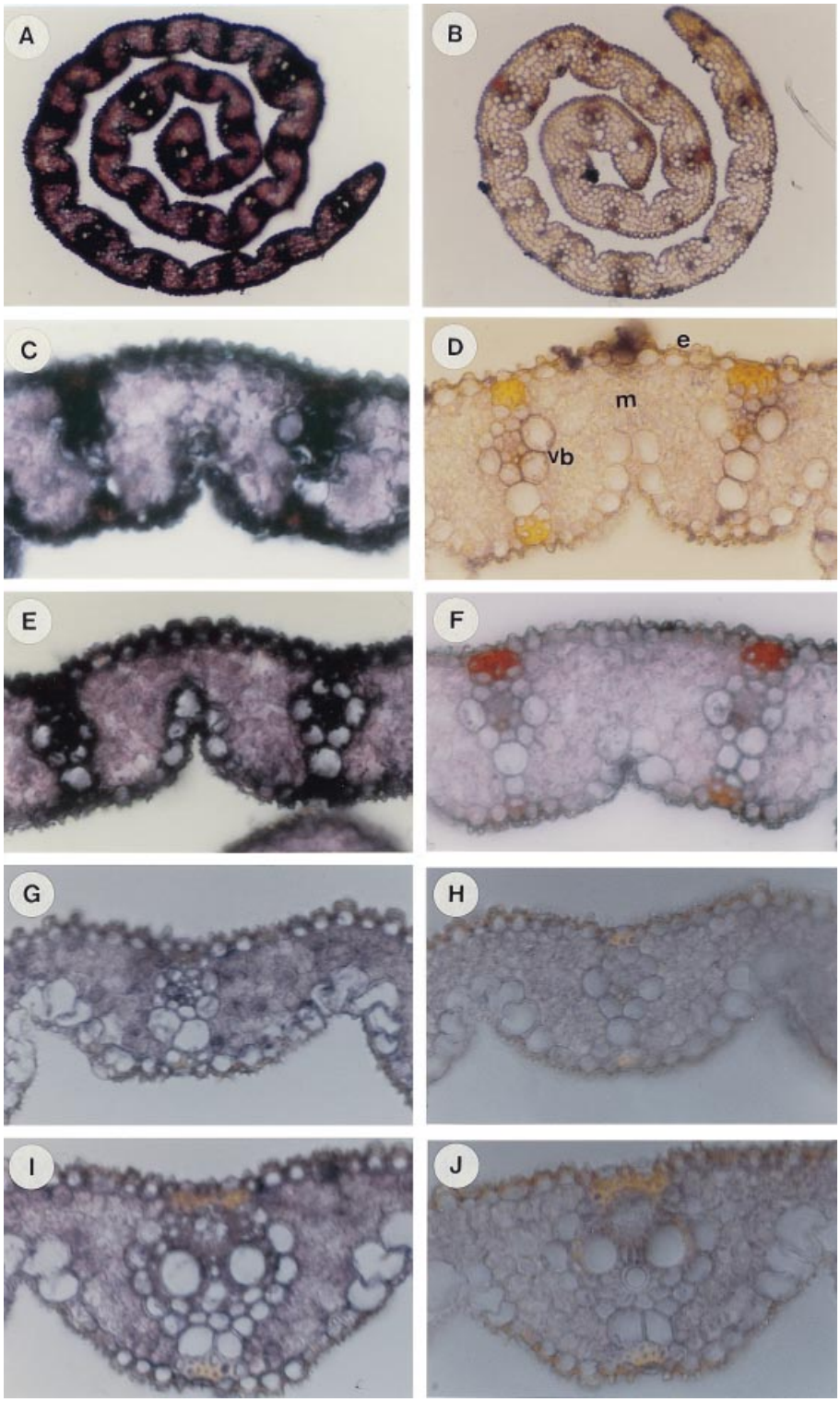

Fig. 1. Immunodetection of SPS protein in cross sections of rice leaves. A–D, etiolated leaf; E–F, young green leaf; G–H, mature green leaf minor veins; I–J, mature green leaf major vein. e, epidermis; m, mesophyll; vb, vascular bundle. Tissue sections were probed with SPS polyclonal antibodies (A, C and E, dilution 1:2000, G and F, dilution 1:4000) or with preimmune serum (B, D, F, H and J). Magnifications were 100 × for A and B and 400 × for C–J.

and dark grown 7-day-old seedlings were cut into 3 mm^3 segments in the blade midregion. Sections were fixed in an ethanol:acetone solution (3:1, v/v) for 30 min at room temperature and immediately dehydrated by a slow ethanol concentration increase. Samples were included in a paraffin matrix (Hystoplast) at 60°C and cut into 8 μm thick slices using a rotary microtome. Sections on slides were deparaffined with xylene, slowly rehydrated, and sequentially washed with water and TBS buffer. Slides were then immersed in TBS-milk-glycine solution at room temperature for 2 h. Immunolocalization was performed by incubation overnight at 4°C in a 1/500 dilution of polyclonal antibodies raised against rice SPS. A control was performed in parallel using the corresponding preimmune serum. After several TBS washes, the slices were incubated in a 1/1000 solution of secondary antibodies (alkaline-phosphatase anti-rabbit IgG conjugated, Sigma A-8025), washed with TBS and revealed with Nitro Blue Tetrazolium (Sigma N-6639) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Sigma B-6149).

Results

Immunolocalization of SPS protein

The polyclonal antibodies to be used were tested in western blot experiments which strongly revealed a specific SPS polypeptide of approximately 116 kDa, either in etiolated or in green leaf crude extracts (data not shown).

Comparative SPS immunolocalization in cross sections of etiolated and green leaves is shown in Fig. 1. Strong signals in epidermis and vascular bundles and weak signal in mesophyll cells were detected in etiolated seedling leaves (Fig. 1A–D). The presence of SPS protein in the mesophyll was markedly higher in mature green leaf than in etiolated and young green tissues (Fig. 1A–J). Epidermis and vascular associated SPS signals were barely detected in mature green leaf (Fig. 1G–J).

Isolation of two SPS forms from etiolated rice seedlings

The different cellular localization of the SPS protein in etiolated and green tissues led us to biochemically study SPS activity present in non-photosynthetic tissues. Ten-day-old etiolated seedlings were used as an enzyme source for enzyme purification. When crude extracts were chromatographed onto a DEAE-Sephacel column, two SPS activity peaks were eluted at 0.33–0.36 and 0.42–0.45 *M* NaCl (termed SPS-1 and SPS-2, respectively) (Fig. 2A). This elution profile was not modified when crude extracts were incubated with alkaline phosphatase (data not shown). The SPS-2 activity peak was not detected in green leaves sampled during the light (Salerno et al. 1998) or during the dark period (Fig. 2B). Fractions containing SPS-1 and SPS-2 activities from etiolated seedlings were separately pooled, concentrated, and submitted to gel filtration chromatography through a Sepharose 6B column. This step allowed the separation of SPS from PGI activity and resulted in a specific activity increase of 34-fold and 44-fold for SPS-1 and SPS-2, respectively (Table 1). Further purification proved to be a difficult task for both SPSs on account of very low and unstable enzyme activities.

Biochemical properties of SPS-1 and SPS-2

Partially purified SPSs were used for further studies. SPS-1 and SPS-2 showed a hyperbolic saturation kinetic for both substrates, Fru-6-P and UDP-Glc. The apparent K_m values calculated from Linweaver-Burk plots were 3.5 ± 0.8 m*M* for Fru-6-P and 3.6 ± 0.6 m*M* for UDP-Glc for SPS-1, and 4.1 ± 0.8 m*M* for Fru-6-P and 4.2 ± 0.7 m*M* for UDP-Glc for SPS-2 (data not shown).

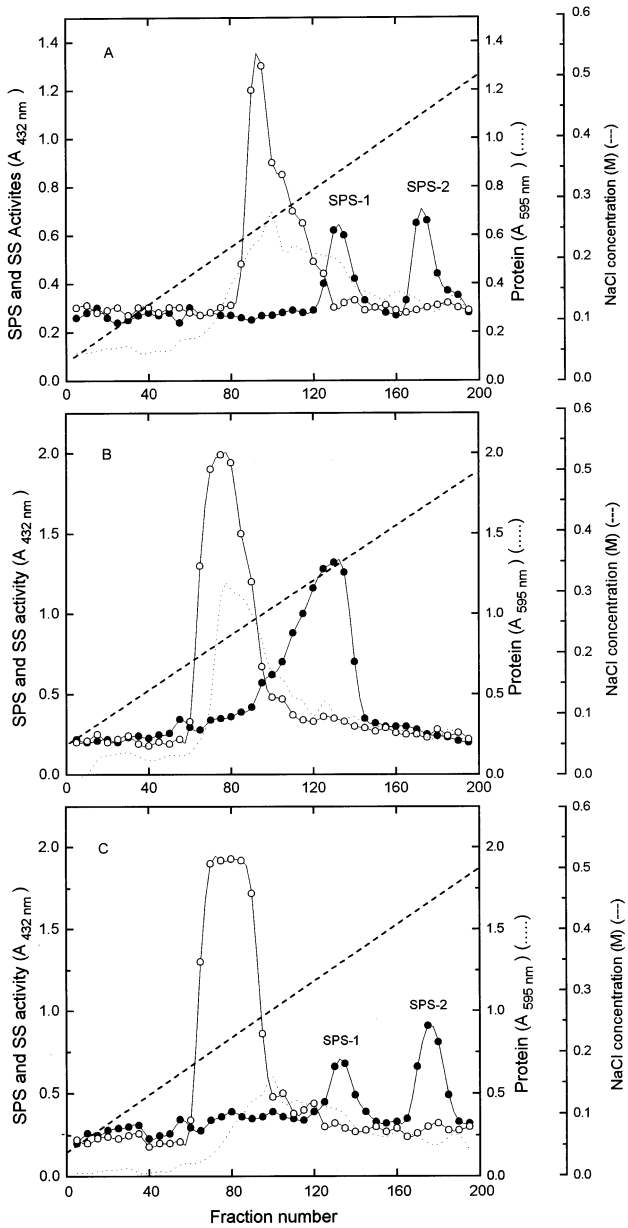

Fig. 2. DEAE-Sephacel chromatography of rice crude extracts from etiolated seedlings (A), green leaves harvested during the dark period (B) and embryo (C). SS (○) and SPS (●) activities. Protein (···). The broken line represents the NaCl gradient.

Table 1. Summary of partial purification of SPS-1 and SPS-2 from etiolated rice seedlings.

Step	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification factor
Crude extract	58.2	66.0	1.13	100	1
DEAE-Sephacel	SPS-1 0.8	6.0	7.5	9.0	6.6
	SPS-2 1.4	8.0	5.7	12.1	5.0
Sepharose-6B	SPS-1 0.12	4.7	39.2	7.1	34.0
	SPS-2 0.02	1.0	50.0	1.6	44.2

The effects of Glc-6-P and Pi on SPS activities were also investigated. Both SPSs were activated about 1.5-fold in the presence of Glc-6-P when measured with the V assay (data not shown). At low substrate concentration (v assay) the effect was more pronounced: SPS-2 showed approximately a sevenfold increase while SPS-1 reached a fivefold activation at 6 m*M* Glc-6-P (Fig. 3A). Additionally, the affinity for the substrate Fru-6-P in the presence of Glc-6-P had an increase of more than tenfold for SPS-2 but only about twofold for SPS-1 (Fig. 3B and kinetic constants). SPS-1 was half-saturated at 1.5 m*M* Fru-6-P and SPS-2 at 0.34 m*M*.

When 10 m*M* inorganic phosphate was added to the incubation mixture, about 40–50% inhibition was obtained for both SPSs using the V assay (data not shown). With the v assay, SPS-1 activity decreased 75–90% in the presence of 5–10 m*M* Pi, while SPS-2 only had a 35–40% decrease (Fig. 3C). When the combined effect of Pi and Glc-6-P was studied, SPS-1 activation by Glc-6-P (4.5-fold) decreased to 1.5-fold in the presence of 10 m*M* Pi. However, SPS-2 activation by Glc-6-P was barely modified by the addition of Pi (Fig. 4).

Rice embryo SPS characterization

Dissected embryos from dry rice seeds were used as another non-photosynthetic SPS source. When crude extracts were chromatographed on a DEAE-Sephacel column, two SPS activity peaks were eluted at similar salt concentrations (Fig. 2C) as was the case for etiolated seedlings shown in Fig. 2A. As both enzyme activities were very unstable, further purification was not possible. SPS-1 activity was extremely unstable. Then, only some properties of embryo SPS-2 could be determined in partially purified enzyme preparations. The activation by Glc-6-P and the inhibitory action of Pi were similar to those obtained in etiolated seedling SPS-2 (data not shown).

In vitro dephosphorylation of SPSs

A pretreatment promoting dephosphorylation of SPS was carried out as an approach to determine whether protein phosphorylation may be part of the regulatory mechanism of SPS activity in etiolated seedlings and embryos. As the effect of protein phosphorylation on SPS activity is only detected under selective assay conditions, the activation state (defined as $[v_{sa} \cdot V_{nsa}^{-1}] \cdot 100$) for etiolated seedling SPS-1 and SPS-2 and for embryo SPS-2 was determined as described (Siegl and Stitt 1990). The activation state of etiolated seedling SPS-1 increased after dephosphorylation, while those of SPS-2 from both sources remained unmodified (Table 2).

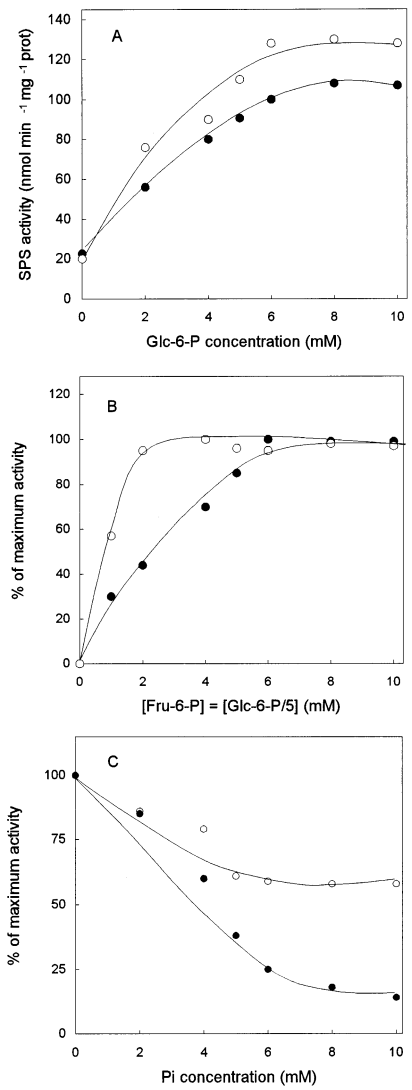

Fig. 3. Effect of Glc-6-P and Pi on rice etiolated seedling SPSs. A, effect of Glc-6-P on SPS-1 (●) and SPS-2 (○) activities measured using the v assay. B, effect of Glc-6-P on Fru-6-P saturation kinetic of SPS-1 (●) and SPS-2 (○). Activities were normalized to the highest activity obtained in each case. 100% activity was 130 μmol (mg protein)$^{-1}$ min^{-1} for SPS-1 and 112 μmol (mg protein)$^{-1}$ min^{-1} for SPS-2. Glc-6-P was added to a 10 m*M* final concentration in the incubation mixture. C, effect of Pi on SPS-1 (●) and SPS-2 (○) activities measured using the v assay. 100% activity was 32 μmol (mg protein)$^{-1}$ min^{-1} for SPS-1 and 28 μmol (mg protein)$^{-1}$ min^{-1} for SPS-2.

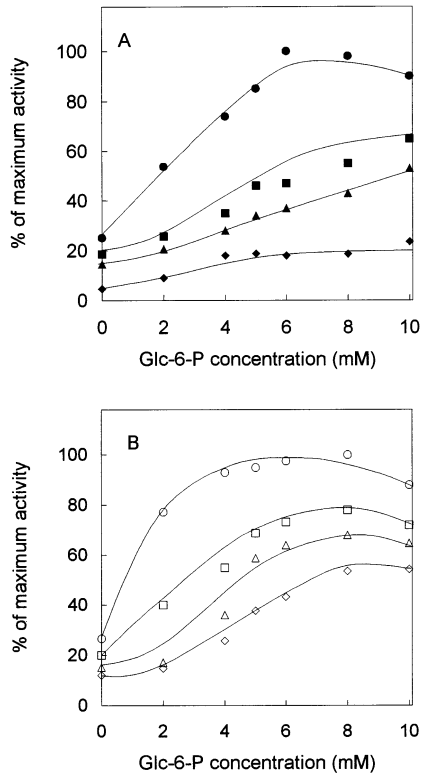

Fig. 4. Combined effect of Glc-6-P and Pi on rice etiolated seedling SPSs. (A) SPS-1, (B) SPS-2. Different Glc-6-P concentrations were tested at 0 (●, ○), 2 (■, □), 5 (▲, △) and 10 (◆, ◇) m*M* Pi. SPS-1 and SPS-2 activities were measured using the v assay. 100% activity was 139 μmol (mg protein)$^{-1}$ min^{-1} for SPS-1 and 115 μmol (mg protein)$^{-1}$ min^{-1} for SPS-2.

Molecular characterization

SDS-PAGE followed by immunological detection with polyclonal antibodies raised against the leaf rice SPS revealed a 116-kDa band in both SPS-1 and SPS-2 preparations (Fig. 5, lanes 1 and 2). In some experiments, an 88-kDa signal was also detected. Polyclonal antibodies raised against the 88-kDa polypeptide, also revealed the presence of a 116-kDa band (data not shown), indicating that the 88-kDa polypeptide was a proteolytic product of the 116-kDa polypeptide as suggested for the green leaf enzyme (Salerno et al. 1998). A 116-kDa polypeptide was also immunodetected in embryo SPS-2 preparations (Fig. 5, lane 3).

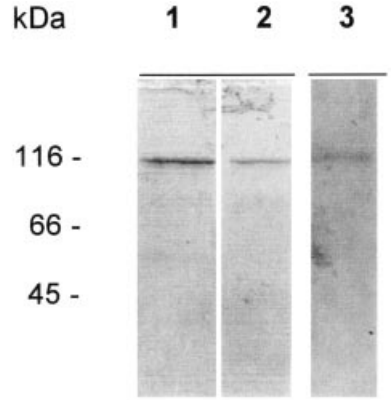

Fig. 5. Immunological detection of partially purified rice SPS from etiolated seedlings and embryos. Lane 1, etiolated SPS-1; lane 2, etiolated SPS-2; lane 3, embryo SPS-2. Partially purified enzyme fractions (DEAE-Sephacel step) were electrophoresed in SDS-PAGE. Equal amounts of protein (approximately 10 μg) were loaded in each lane. The blot was immunorevealed with polyclonal antibodies raised against rice leaf SPS.

Discussion

This paper describes the occurrence and regulation of rice SPS in non-photosynthetic tissues.

Differential SPS protein localization was shown in photosynthetic and etiolated leaves by in situ immunological analysis. As shown in Fig. 1, the SPS protein is preferentially localized in epidermis and vascular bundles of etiolated seedling leaves (Fig. 1A,C). Young leaves present an increased mesophyll-associated SPS signal in comparison with the etiolated leaves (Fig. 1E). In contrast, SPS protein is predominantly localized in mesophyll cells in completely unfolded mature green leaves (Fig. 1G,H). Similar localization was reported for SPS in *Vicia faba* leaflet (Hite et al. 1993) and in maize leaf (Furbank et al. 1985, Cheng et al. 1996). Additionally, similar results were obtained in rice *sps1* promoter studies in transgenic tobacco plants (Valdez-Alarcón 1997).

The localization of the SPS protein in the mesophyll of green leaves is in agreement with the predominant role of these cells in the photoassimilatory biosynthesis of sucrose. The vascular bundle associated SPS protein observed in etiolated and young green leaves could be related to the non-photosynthetic biosynthesis of sucrose (probably

Table 2. In vitro dephosphorylation of rice etiolated seedling and embryo SPSs. SPS activity was measured using a selective assay (v_{sa}) or a non-selective assay (V_{nsa}). Activation state is defined as ($v_{sa} \cdot V_{nsa}^{-1}$) · 100 (see Materials and methods). [Et] From etiolated seedlings; [Em] from embryo.

Enzyme	Pretreatment	SPS activity (μmol [mg protein]$^{-1}$ min^{-1})		Activation state (%)
		v_{sa}	V_{nsa}	
SPS-1^{Et}	Control	8.79 ± 0.62	18.98 ± 0.51	46
SPS-1^{Et}	Alk-PP	18.05 ± 0.81	20.83 ± 0.88	86
SPS-2^{Et}	Control	12.11 ± 0.55	20.37 ± 0.33	59
SPS-2^{Et}	Alk-PP	11.11 ± 0.64	19.34 ± 0.51	57
SPS-2^{Em}	Control	13.88 ± 0.44	26.30 ± 0.98	52
SPS-2^{Em}	Alk-PP	13.20 ± 0.64	22.30 ± 0.89	59

through starch turnover) as suggested by Cheng et al. (1996).

The different localization pattern detected for SPS protein in etiolated seedlings and green leaf led us to investigate the presence of a heterotrophic-specific SPS form by analysing the biochemical properties of the enzyme from etiolated seedlings. Two SPS forms were separated when crude extracts were chromatographed onto a DEAE-Sephacel column. Consequently, a comparative study of biochemical and regulatory properties for rice etiolated SPSs was carried out. The apparent K_m values estimated for UDP-Glc and Fru-6-P either for SPS-1 or SPS-2 were similar to constants reported for other sources (Stitt et al. 1987, Reimholz et al. 1994, Salerno et al. 1998). The analysis of Glc-6-P (activator) and Pi (inhibitor) effects on SPS activities allowed us to show the differences between the two forms. Significantly, the presence of Glc-6-P increased the affinity for Fru-6-P more than tenfold for SPS-2, but only about twofold for SPS-1 (Fig. 3B and kinetic constants). SPS-1 displayed a high sensitivity to Pi, which besides produced a strong decrease on Glc-6-P enzyme activation (Fig. 4). Thus, SPS-1 responds to metabolite regulation similar to SPSs from green leaves harvested in darkness (Huber and Huber 1991, 1992, 1996, Salerno et al. 1998). In contrast, SPS-2 was highly activated by Glc-6-P and showed a low sensitivity to Pi (Figs. 3 and 4). Additionally, SPS-1 activation state increased after in vitro dephosphorylation as reported for leaf SPS in darkness (Huber and Huber 1996). This was not the case for SPS-2 whose activation state was not modified by dephosphorylation (Table 2). These results may indicate that either rice etiolated SPS-2 is present in a dephosphorylated state or is not regulated by protein phosphorylation as SPS from green leaves. The possibility that SPS-2 originates from SPS-1 dephosphorylation is ruled out since both activity peaks were observed in a DEAE-Sephacel chromatography profile after alkaline phosphatase treatment of the loaded extracts. Besides, the simultaneous occurrence of SPS-1 and SPS-2 activities in etiolated seedlings and embryos is not likely to be a consequence of partial proteolysis. Both SPS-1 and SPS-2 polypeptides were revealed with leaf SPS polyclonal antibodies in western blot analysis (Fig. 5). The relative MM value estimated for both forms was 116 kDa (Fig. 5) and is identical to that of rice leaf SPS (Salerno et al. 1998). This agrees with the predicted MM for the 1048-aa polypeptide deduced from the rice SPS gene (Valdez-Alarcón et al. 1996). Taken together, the data presented led us to conclude that SPS-2 is an enzyme form characteristic of non-photosynthetic tissues.

Rice SPS regulation in heterotrophic tissues could involve the expression of different enzyme forms that differ in their regulatory properties. More than one gene was proposed to explain the existence of 4 SPS polypeptides in potato (Reimholz et al. 1997). The existence of two SPS genes, which are differentially regulated in response to light, was described in sugarcane (Sugiharto et al. 1997). However, only one SPS gene was reported in rice (Sakamoto et al. 1995, Valdez-Alarcón et al. 1996). Consequently, a non-homologous gene, an alternative splicing, or another undescribed post-translational modification may explain the results obtained herein.

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References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Cheng W-H, Im KH, Chourey PS (1996) Sucrose phosphate synthase expression at the cell and tissue level is coordinated with sink-to-source transitions in maize leaf. Plant Physiol 111: 1021–1029

Dali N, Michaud D, Yelle S (1992) Evidence for the involvement of sucrose phosphate synthase in the pathway of sugar accumulation in sucrose-accumulating tomato fruits. Plant Physiol 99: 434–438

Doehlert DC, Huber SC (1983) Spinach leaf sucrose phosphate synthase. Activation by glucose-6-phosphate and interaction with inorganic phosphate. FEBS Lett 153: 293–297

Furbank RT, Stitt M, Foyer CH (1985) Intercellular compartmentation of sucrose synthesis in leaves of *Zea mays* L. Planta 164: 172–178

Harn C, Khayat E, Daie J (1993) Expression dynamics of genes encoding key carbon metabolism enzymes during sink to source transition of developing leaves. Plant Cell Physiol 34: 1045–1053

Hite DRC, Outlaw WH Jr, Tarczynski MC (1993) Elevated levels of both sucrose-phosphate synthase and sucrose synthase in *Vicia* guard cells indicate cell-specific carbohydrate interconversions. Plant Physiol 101: 1217–1221

Hubbard NL, Pharr DM, Huber SC (1990) Role of sucrose phosphate synthase in sucrose biosynthesis in ripening bananas and its relationship to the respiratory climateric. Plant Physiol 94: 201–208

Huber SC, Huber JL (1991) Regulation of maize leaf sucrose-phosphate synthase by protein phosphorylation. Plant Cell Physiol 32: 319–326

Huber JLA, Huber SC (1992) Site specific serine phosphorylation of spinach leaf sucrose phosphate synthase. Biochem J 283: 877–882

Huber SC, Huber JL (1996) Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol 47: 49–73

Huber JLA, Huber SC, Nielsen TH (1989) Protein phosphorylation as a mechanism for regulation of spinach leaf sucrose-phosphate synthase activity. Arch Biochem Biophys 270: 681–690

Klein RR, Crafts-Brandner SJ, Salvucci ME (1993) Cloning and developmental expression of the sucrose-phosphate synthase gene from spinach. Planta 190: 498–510

Kumatsu A, Takanokura Y, Moriguchi T, Omura M, Akihama T (1999) Differential expression of three sucrose-phosphate synthase isoforms during sucrose accumulation in citrus fruits (*Citrus unshiu* Marc.). Plant Sci 140: 169–178

Lunn JE, Hatch MD (1997) The role of sucrose-phosphate synthase in the control of photosynthate partitioning in *Zea mays* leaves. Aust J Plant Physiol 24: 1–8

Prioul JL, Ryerr A, Schwebel-Dugue N (1990) Relationships between carbohydrate metabolism in ear and adjacent leaf during grain filling in maize genotypes. Plant Physiol Biochem 28: 485–493

Reimholz R, Geigenberger P, Stitt M (1994) Sucrose-phosphate synthase is regulated via metabolites and protein phosphorylation in potato tubers, in a manner analogous to the enzyme in leaves. Planta 192: 480–488

Reimholz R, Geiger M, Haake V, Deiting U, Krause KP, Sonnewald U, Stitt M (1997) Potato plants contain multiple forms of sucrose-phosphate synthase which differ in their tissue distributions, their levels during development and their responses to low temperature. Plant Cell Environ 20: 291–305

Sakamoto M, Satozawa T, Kishimoto N, Higo K, Shimada H, Fujimura T (1995) Structure and RFLP mapping of a rice

sucrose-phosphate synthase (SPS) gene that is specifically expressed in the source organ. Plant Sci 112: 207–217
Salerno GL, Pontis HG (1978) Sucrose-phosphate synthetase. Separation from sucrose synthetase and a study of its properties. Planta 142: 41–48
Salerno GL, Crespi MD, Zabaleta EJ, Pontis HG (1991) Sucrose-phosphate synthase from wheat. Characterization of peptides by immunoblotting analysis. Physiol Plant 81: 541–547
Salerno GL, Pagnussat GC, Pontis HG (1998) Studies on sucrose-phosphate synthase from rice leaves. Cell Mol Biol 44: 407–416
Siegl G, Stitt M (1990) Partial purification of two forms of spinach leaf sucrose-phosphate synthase which differ in their kinetic properties. Plant Sci 66: 205–210
Stitt M, Huber SC, Kerr PS (1987) Control of photosynthetic sucrose formation. In: Hatch MD, Boardman NK (eds) The Biochemistry of Plants. A Comprehensive Treatise, Vol. 10. Academic Press, San Diego, CA, pp 327–409
Stitt M, Wilke I, Feil R, Heldt HW (1988) Coarse control of sucrose-phosphate synthase in leaves: Alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. Planta 174: 217–230
Sugiharto B, Sakakibara, Sumadi H, Sugiyama T (1997) Differential expression of two genes for sucrose-phosphate synthase in sugarcane: Molecular cloning of the cDNAs and comparative analysis of gene expression. Plant Cell Physiol 38: 961–965
Tognetti JA, Calderón PL, Pontis HG (1989) Fructan metabolism: Reversal of cold acclimation. J Plant Physiol 134: 212–216
Toroser D, Huber SC (1997) Protein phosphorylation as a mechanism for osmotic-stress activation of sucrose-phosphate synthase in spinach leaves. Plant Physiol 114: 947–955
Valdez-Alarcón JJ (1997) Molecular characterization and expression analysis of the of rice (*Oryza sativa* L.) sucrose-phosphate synthase gene
Valdez-Alarcón JJ, Ferrando M, Salerno GL, Jimenez-Moraila B, Herrera-Estrella L (1996) Characterization of a rice sucrose synthase encoding gene. Gene 170: 217–222

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