Plant Physiol. (1985) 79, 695–698
0032-0889/85/79/0695/04/$01.00/0

Possible Control of Maize Leaf Sucrose-Phosphate Synthase Activity by Light Modulation

Received for publication April 22, 1985 and in revised form July 11, 1985

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ABSTRACT

Sucrose phosphate synthase (SPS) activity was measured in extracts of maize (*Zea mays* L.) and soybean (*Glycine max* L. [Merr.]) leaves over a single day/night cycle. There was a 2- to 3-fold postillumination increase in extractable enzyme activity in maize leaves, whereas the activity of soybean SPS was only about 30% higher in extracts prepared from light- compared to dark-adapted leaves. Alterations in extractable maize leaf SPS activity correlated with light/dark transitions suggesting that the enzyme may be light modulated. Diurnal variations of extractable maize leaf SPS activity were also observed in a greenhouse experiment. A transition from high (light) to low (dark) extractable SPS activity occurred near the light compensation point for photosynthesis (about 20 micromole photons per square meter per second). Further increases in irradiance did not increase extractable SPS activity. Substrate affinities for uridine 5′-diphosphoglucose (Michaelis constant = 3.5 and 5.1 millimolar) and fructose-6 phosphate (half maximal concentration = 1.0 and 2.5 millimolar) were lower for partially purified SPS obtained from light compared to dark acclimated maize leaves. Light-induced changes in extractable SPS activity were stable for at least one column chromatography step. The above results indicate that light-induced changes in SPS activity may be important in controlling the photosynthetic production of sucrose.

Sucrose is an important storage compound in higher plants and is the usual form of carbon transported to developing organs. Sucrose biosynthesis involves two enzymes. The first step is catalyzed by SPS[1] (EC 2.4.1.14), a cytoplasmic enzyme that uses F6P and UDPG as substrates to produce sucrose-phosphate (9, 13). Removal of the phosphate ester is performed by a specific phosphatase (7), SPP (EC 3.1.3.24). It is likely that SPS catalyzes the rate-limiting step in sucrose production (6, 11), whereas SPP may have little or no regulatory function (20). Factors controlling the activity of SPS in photosynthetic tissue are poorly understood. Recent kinetic studies (1, 5, 6) indicate that the affinity of the partially purified enzyme for UDPG is low (K_m = 1–7 mM). Estimated concentrations of UDPG in the cytoplasm are about 1 to 2 mM (19) suggesting that the metabolic production of sucrose may be limited by substrate levels in the cytosol. The observation that sucrose synthesis in higher plants is generally high in the light when the flux of intermediates from the chloroplast is maximal supports this conclusion. Inhibition of the enzyme by UDP and Pi (5, 6) and stimulation by F6P (1) or G6P (5) could provide further metabolic regulation. More recent evidence suggests that the soybean leaf enzyme is controlled by an endogenous rhythm (8, 15). Alternatively, SPS activity in leaf extracts prepared from *Hordeum* (17) or *Lolium* (12) is modulated by light. The objectives of the present study were to further characterize the mechanism by which light affects the activity of SPS in intact leaves. Results strongly support the conclusion that SPS is an important control point in the regulation of sucrose synthesis.

MATERIALS AND METHODS

Plant Materials. Maize (*Zea mays* L. cv 875) and soybean (*Glycine max* L. [Merr.] cv Williams) plants were grown from single seeds planted in 10 × 10 × 15-cm plastic pots filled with vermiculite. Plants were raised in controlled environment chambers (model M-2, Environmental Growth Chambers, Chagrin Falls, OH)[2] with irradiance (600 to 650 μmol photons m^{-2} s^{-1}), temperature (27 ± 2°C), RH (60 ± 5%), photoperiod (12-h d/12-h night cycle) and watering with complete mineral nutrient solution as previously described (17). Low light conditions were obtained by using growth cabinets with partial illumination. PAR was measured with a Li-Cor model LI-170 quantum sensor (Lambda Instruments, Lincoln, NE). Experiments were also conducted on maize plants raised in a 3 × 4-m glasshouse during the summer and fall of 1984. Irradiance and RH were ambient, whereas air temperature (25 ± 4°C) was maintained with a 15.25-kW heat pump. Experiments were conducted on the fourth leaf of maize (14- to 17-d growth) and the third trifoliolate leaf of soybean (24- to 27-d growth). Leaves (at least three per sample, except where noted) were harvested during the light or dark period, frozen in liquid N_2 and, if necessary, stored at −80°C prior to analysis (8, 17). Net carbon exchange rates by single maize leaves were determined by IR gas analysis as described elsewhere (16).

Enzyme Extraction and Measurement. Enzyme extractions were performed at 0 to 4°C. Leaf samples (3 to 5 g FW) were weighed while still frozen and homogenized in a mortar containing 2% (v/v) insoluble PVP, 0.5 g sand, and 7 ml buffer A (50 mM Mes-NaOH, pH 6.9, 1 mM $MgCl_2$, 0.1 mM EDTA, and 15 mM 2-mercaptoethanol). Homogenates were filtered through two layers of Miracloth and centrifuged at 27,000*g* for 20 min, and supernatants were passed over a 1.6 × 30-cm column of Sephadex G-25 preequilibrated with buffer A. Assays were performed on sample material eluting with the void volume. The soybean leaf enzyme was assayed for 10 min at 30°C as previously

[1] Abbreviations: SPS, sucrose phosphate synthase; SPP, sucrose phosphate phosphatase; UDPG, uridine 5′-diphosphoglucose; F6P, fructose-6 phosphate; G6P, glucose-6 phosphate; FW, fresh weight; F2,6BP, fructose-2,6 bisphosphate.

[2] Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture.

described (17). The maize leaf enzyme was measured as F6P-dependent UDP formation (2).

Kinetic analyses were performed on maize leaf enzyme preparations that were partially purified by a modification of the procedure described by Amir and Preiss (1). Leaves were harvested from maize plants that had been adapted to light or dark for 1-h prior to sampling as described above. Frozen leaf powder (15 to 20 g FW) was homogenized in a chilled mortar with 20 ml buffer B (50 mM Mes-NaOH, pH 6.9, 1 mM $MgCl_2$, 0.1 mM EDTA, 15 mM 2-mercaptoethanol, and 20% v/v ethyleneglycol) containing 2% insoluble PVP and 2 g washed sand. Extracts were filtered through 4 layers of cheesecloth, neutralized to pH 6.9 with 2 M NaOH (about 0.3 pH units), and centrifuged at 27,000*g* for 20 min at 4°C. Supernatants were applied directly to a 2 × 15-cm DEAE-cellulose column preequilibrated with buffer B, and SPS was eluted with a 200-ml linear salt gradient (0 to 0.5 M NaCl) in the same buffer. Active fractions were collected and stored at −80°C as described elsewhere (1). Yields for this procedure were typically 35 to 50% with a 7- to 10-fold purification.

Reagents. DEAE-cellulose (DE-23) was from Whatman and Sephadex G-25 was from Pharmacia. All other enzymes and reagents were of the highest purity available.

RESULTS AND DISCUSSION

Diurnal Variations of SPS Activity in Maize Leaves. The activity of SPS in extracts prepared from maize leaves was essentially constant throughout a normal 12-h light period (Fig. 1). Average enzyme rates during the day were about 3.3 μmol product (mg protein h)$^{-1}$ for 23 samples and this activity decreased by about one-half during darkness. In contrast to results obtained with barley (17), SPS activity in maize leaves did not increase during the second half of the dark period. Reciprocal light/dark transfers altered maize leaf SPS activity within 1 h (see below). These findings suggested that maize leaf SPS was light modulated in a manner similar to that previously reported for other monocotyledonous species (12, 17) and contradicted earlier data obtained in a field study (3). Diurnal variations in SPS activity were also observed in leaf extracts of *Sorghum bicolor* and *Digitaria decumbens* (not shown). Collectively, these findings indicate that the light-modulated properties of SPS are potentially widespread in grass species having either C_3 or C_4 photosynthetic metabolism.

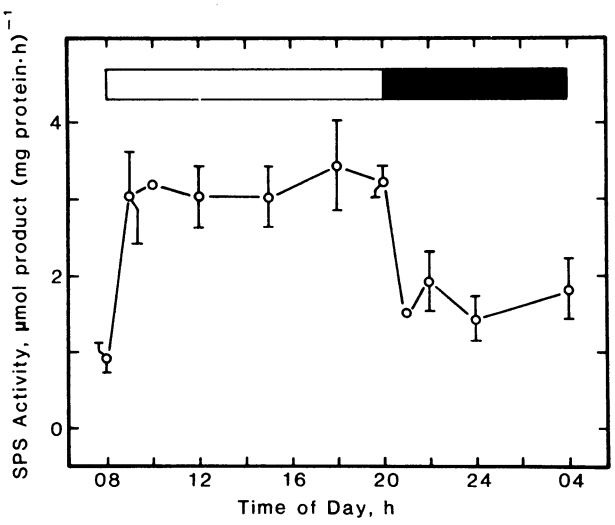

FIG. 1. Activity of maize leaf SPS during a normal 24-h day/night cycle in a controlled environment. Extracts were prepared from fourth collared leaves (4 or 5 per sample) of 14- to 17-d-old maize plants sampled at 1- to 3-h intervals throughout a 12-h light/12-h dark cycle and SPS activity (O) was measured as described in "Materials and Methods." Data are reported as means (±SE) of four determinations.

Controlled environment studies characteristically involve constant, artificial illumination with abrupt light/dark changes. More gradual day/night transitions occur in a natural environment and, in addition, shading can result in a variable light level. In the present study, diurnal variations in maize leaf SPS activity were observed when plants were raised and sampled in a temperature-controlled glasshouse, similar to those obtained in growth chamber experiments (Fig. 2). Irradiance levels until midday were below 200 and then increased to a maximum of about 800 μmol photons m^{-2} s^{-1} in the late afternoon. Maize leaf SPS activity increased about 2-fold during the first 2 h of light, remained constant throughout the light period, and decreased at the end of the day. The amount of light required to convert extractable maize leaf SPS from low (dark) to high (light) activity was less than 120 μmol photons m^{-2} s^{-1} (see 09:00 AM light readings). Subsequent increases in irradiance had little or no effect on extractable SPS activity.

The response of extractable SPS activity to changing irradiance

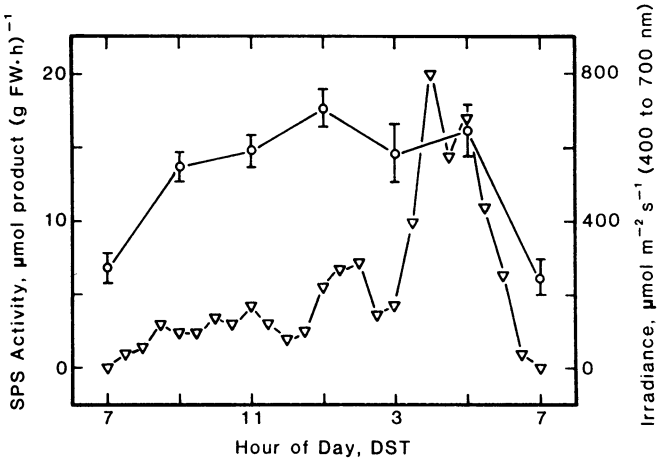

FIG. 2. Activity of maize leaf SPS during a 12-h light period in a naturally illuminated environment. Conditions were essentially as described in Figure 1 except that changes in irradiance (▽) between 400 and 700 nm are shown. Leaf extracts were prepared from 14- to 17-d-old maize plants raised in a temperature-controlled greenhouse. Enzyme activity determined for 10:00 PM samples was 4.9 μmol product formed (g FW h)$^{-1}$.

Table I. *Net Carbon Exchange Rate and Extractable Sucrose-Phosphate Synthase Activity of Single Maize Leaves*

Maize plants were exposed to bright light (600 μmol photons m^{-2} s^{-1}) for 2 h and were then transferred to various low-light environments. Single leaf net CO_2 exchange rates (fourth collared leaf) and SPS measurements were made after a 1-h adaptation period. Data were analyzed by 2-way analysis of variance.

Irradiance	No. of Experiments	SPS Activity	Net CER
μmol $m^{-2}s^{-1}$		μmol (mg protein h)$^{-1}$	mg CO_2 dm^{-2} h^{-1}
600	10	3.8a[a]	50.6 ± 3.4
200	4	3.2a	21.2 ± 1.7
100	4	4.0a	8.0 ± 1.4
50	4	3.4a	4.0 ± 0.2
23	12	3.1a	0.2 ± 0.5
0	13	2.0b	−3.6 ± 1.8

[a] Numbers within vertical columns followed by a different letter differ significantly ($P \leq 0.01$).

was also examined in a controlled environment study (Table 1). Maize plants were transferred 2 h after the start of the normal light cycle to varying low-irradiance environments. Net CER and extractable SPS activity of single leaves were determined after a 1-h acclimation period. Results suggested that a change from high to low extractable SPS activity occurred at or near the light compensation point for photosynthesis (about 20 μmol photons m^{-2} s^{-1}). In agreement with data obtained from glasshouse-grown maize plants, irradiances above the light compensation point increased the rate of CO_2 uptake but did not affect extractable SPS activity.

Kinetic Properties of Partially Purified Maize Leaf SPS Extracted from Leaves of Plants Adapted to Light or Dark. The diurnal variations in maize leaf SPS activity described above were probably not due to *de novo* enzyme synthesis and degradation (12, 17). Altered SPS activity could represent *in vivo* changes in the levels of active and inactive enzyme. Alternatively, the light/dark regulation of maize leaf SPS could be due to altered affinities for UDPG and F6P. Both possibilities would normally be detected in routine enzyme assays because it is difficult to saturate SPS with UDPG (1). Therefore, kinetic analyses were performed in order to distinguish between these two potential biochemical mechanisms. Hyperbolic saturation curves for UDPG were observed for SPS preparations from both light and dark adapted maize leaves. Double-reciprocal plots indicated that the K_m for UDPG was slightly higher for the enzyme from dark (5.1 mM) compared to light (3.5 mM) acclimated leaves (Fig. 3). There was a similar decrease in substrate affinity for F6P when maize leaf SPS preparations obtained in the light were compared to extracts of dark-adapted leaves (Fig. 4). In agreement with earlier reports (1, 10), the initial rate kinetics for F6P were sigmoidal. The $S_{0.5}$ values for the light and dark enzyme preparations were 1.0 and 2.5 mM, respectively. The Hill slope in the light (n = 1.4) remained essentially unchanged in the dark. Harbron *et al.* (6) have suggested that sigmoidal saturation curves for F6P are artifactual and result from the presence of contaminating enzymes in the assay that compete with SPS for this substrate. Detailed kinetic analyses of maize leaf SPS will certainly require a more highly purified preparation. Nevertheless, results shown in Figures 3 and 4 clearly indicated that affinities for F6P and UDPG were lower for dark compared to light-adapted maize leaf SPS. Note also, that like the barley enzyme (17), light-induced changes in maize leaf SPS activity were stable during extraction and for at least one column chromatography step.

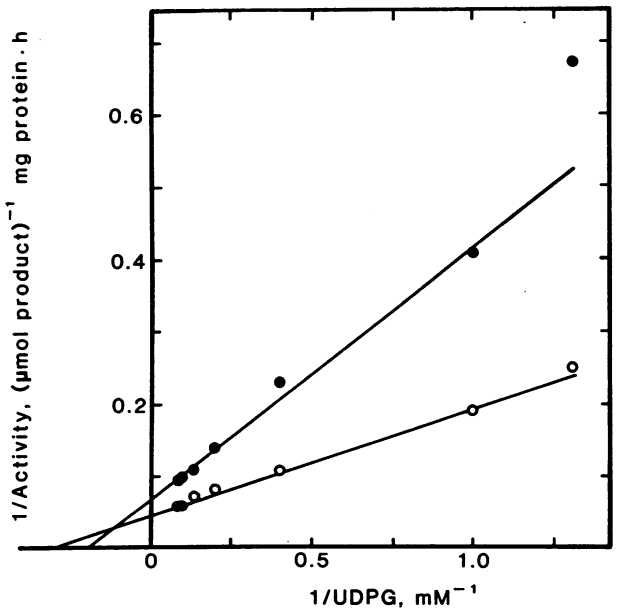

FIG. 3. Double-reciprocal plot showing the effects of UDPG concentration on maize leaf SPS activity. Leaf extracts were prepared from maize plants adapted either to light (O) or dark (●). Assays were performed as described in "Materials and Methods" with 10 mM F6P. Results are representative of three separate experiments. Curves were fitted by linear regression.

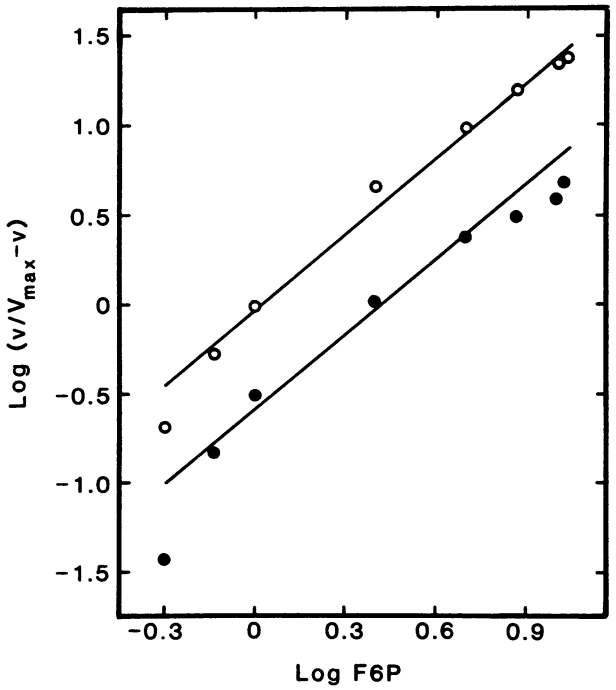

FIG. 4. Hill plot showing the effects of F6P concentration on maize leaf SPS activity. Conditions and symbols were as in Figure 3 except that F6P was the varied substrate and UDPG was 10 mM. Curves were fitted by linear regression.

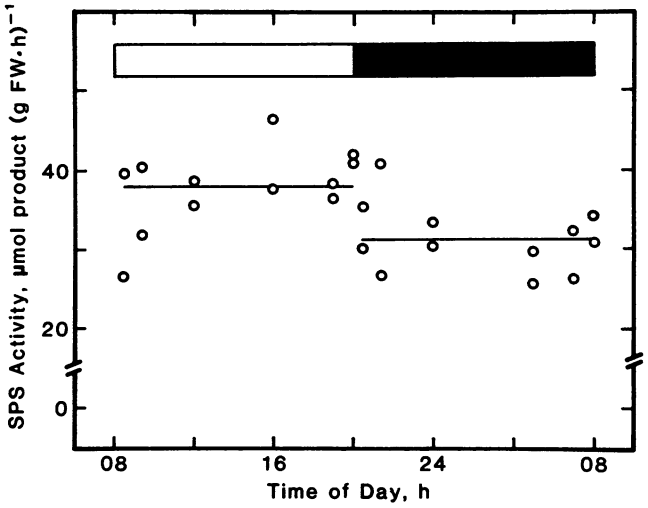

FIG. 5. Diurnal variation in soybean leaf SPS activity. Enzyme extracts were prepared from the third trifoliate leaf (3 per sample) of 24- to 27-d-old soybean plants raised in controlled environment chambers. Horizontal lines indicate mean (n = 12 each) SPS activity (O) for enzyme extracts prepared from light and dark adapted plants. Data for light and dark samples were significantly different ($P \leq 0.01$, 2-way analysis of variance test).

Diurnal Variations in Soybean Leaf SPS Activity. In a prelim-

inary study (17), *in vivo* changes of SPS activity in spinach, soybean, or pea leaves were not detected during the 1st h of the dark period. This result suggested that SPS activity in leaves of dicotyledonous species was not affected by light. However, results shown in Figure 5 indicate that extractable SPS activity in soybean leaves varies diurnally when measured in a controlled environment over a full 24-h period. The mean enzyme activity of samples harvested in the light was only 20 to 30% greater than that of leaf extracts prepared from dark-adapted plants (significantly different at $P \leq 0.01$). The activity of soybean SPS was consistently higher in extracts prepared from light compared to dark-adapted leaves over several experiments (not shown). However, as a result of sample variability, significant differences at the 1% confidence level were only detected when a large number of extracts were analyzed. The above findings indicate that there is a small light/dark difference in extractable soybean leaf SPS activity that can be difficult to detect. In contrast to results of the present study, Rufty *et al.* (15) have proposed that the activity of SPS in soybean leaves is controlled by an endogenous rhythm. These enzyme oscillations did not coincide with light/dark transitions, and furthermore, minimum SPS activities were about 70% less than the peak rate (8, 15). Any potential discrepancies between these earlier findings and results shown in Figure 5 may be a consequence of using different sample preparation procedures.

Concluding Remarks. During rapid photosynthesis, triose phosphates are exported from the chloroplast and are used to manufacture sucrose in the cytoplasm. It is generally agreed that sucrose biosynthesis is subject to stringent metabolic regulation in order to allow a coordination of photosynthetic metabolism between the cytosolic and chloroplastic compartments (14, 18). Recent evidence indicates that cytosolic FBPase is inhibited by a powerful effector metabolite, F2,6BP (4). This compound is synthesized by a kinase that uses F6P as a substrate and as an allosteric activator (4, 14). Preliminary results suggest that changes in cellular concentrations of F2,6BP could control photosynthetic sucrose production (18). However, it is also apparent that the terminal steps in sucrose biosynthesis should be regulated in order to coordinate photosynthetic sucrose production with other major metabolic pathways in the cell that use F6P and UDPG (*i.e.* glycolysis and cell wall synthesis, respectively).

Results of the present study confirm and extend earlier reports (12, 17) that sucrose synthesis in intact leaves is controlled, at least in part, by light-induced changes in the activity of SPS. Enzyme activity extracted from maize and soybean leaves was high in the light and low in the dark. Note that SPS activity did not go to zero in the dark. This residual activity may be important for allowing a low rate of sucrose synthesis from starch reserves formed during the day (17). Light/dark changes in extractable SPS activity have been detected in several monocotyledonous species. In addition, results shown in Figure 5 indicate that there may also be a small light-mediated change in extractable soybean leaf SPS activity. These findings raise the possibility that the control of SPS by light is essentially a universal property of the higher plant enzyme. A transition from low to high extractable SPS activity occurs at or near the light compensation point for photosynthesis. Increased SPS activity in the light could be due to the initiation of photosynthetic metabolism (*i.e.* 17). However, since the irradiance response is sensitive to low light, it remains possible that a photoreceptor could be involved in light perception. Light modulation of maize leaf SPS involves a stable modification of the enzyme that affects substrate affinities for both UDPG and F6P. Therefore, it is likely that the high and low activity extracts of SPS prepared from light and dark acclimated leaves, respectively, represent two kinetically different forms of the enzyme. The biochemical mechanism responsible for modifying SPS activity in intact leaves is unknown at present. It also remains to be seen if SPS can be interconverted from high to low activity *in vitro*.

Acknowledgments—The authors thank Dr. C. J. Pollock for kindly providing information in advance of publication, and also acknowledge the technical assistance of D. R. Lee and W. G. Harris.

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