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Light/Dark Profiles of Sucrose Phosphate Synthase, Sucrose Synthase, and Acid Invertase in Leaves of Sugar Beets

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ABSTRACT

The activity of sucrose phosphate synthase, sucrose synthase, and acid invertase was monitored in 1- to 2-month-old sugar beet (*Beta vulgaris* L.) leaves. Sugar beet leaves achieve full laminar length in 13 days. Therefore, leaves were harvested at 2-day intervals for 15 days. Sucrose phosphate synthase activity was not detectable for 6 days in the dark-grown leaves. Once activity was measurable, sucrose phosphate synthase activity never exceeded half that observed in the light-grown leaves. After 8 days in the dark, leaves which were illuminated for 30 minutes showed no significant change in sucrose phosphate synthase activity. Leaves illuminated for 24 hours after 8 days in darkness, however, recovered sucrose phosphate synthase activity to 80% of that of normally grown leaves. Sucrose synthase and acid invertase activity in the light-grown leaves both increased for the first 7 days and then decreased as the leaves matured. In contrast, the activity of sucrose synthase oscillated throughout the growth period in the dark-grown leaves. Acid invertase activity in the dark-grown leaves seemed to be the same as the activity found in the light-grown leaves.

Leaf maturation is accompanied by marked metabolic, morphological, and anatomical changes. Sink-to source transition in leaves is characterized by a change from sucrose degradation to photosynthetic carbon fixation and net sucrose synthesis and export (7). Leaf development is characterized by the beginning of photosynthetic CO_2 reduction and the appearance of enzymes involved in sucrose formation. Giaquinta (8) showed that the import-export transition began at 30% full laminar length in sugar beet (*Beta vulgaris* L.) and was characterized by an increase in photosynthesis.

The transition of sink tissue to source tissue is also characterized by significant metabolic and developmental changes which must be closely regulated. For instance, the process of sucrose hydrolysis by sink tissue must be temporally or spatially isolated from sucrose synthesis to avoid futile, enegy-dissipating cycles in the tissue. Sucrose-P synthase, sucrose synthase, and invertase are considered an important control point for sucrose synthesis and their activity appear to be regulated metabolically (4, 5).

The environmental and developmental signals which regulate leaf development are yet unknown. Light, a strong trigger of developmental and biochemical processes in plants, may act as a modulator of the enzymes involved in sucrose metabolism. The present study was designed to study the effects of light limitations on growth and metabolism in sugar beets. This paper discusses how enzymes of carbon metabolism are affected when young sugar beet leaves are grown entirely in the dark versus those grown normally in the light.

MATERIALS AND METHODS

Plant Culture

Sugar beet plants (*Beta vulgaris* L., cv Great Western multigerm hybrid) were grown for 2 months in a controlled environment in clay pots (8 cm wide × 7.5 cm high) using a sand:soil mix (1:2) for experiment 1 and a soil:vermiculite mix (2:1) for experiments 2, 3, and 4. Plants were watered daily with distilled water and fertilized every 4 d with a standard nutrient solution (22), supplemented with 0.5 mM Na_2SO_4 (6). Growth chambers were maintained at 25/20°C (day/night) temperature, 80% RH, and a 14-h photoperiod with a photosynthetic photon flux density of 400 μE $m^{-2}s^{-1}$ provided by cool-white fluorescent and incandescent bulbs.

Dark-adaption was accomplished by enclosing the newly emerging leaves with aluminum foil. Ambient CO_2 and O_2 was blown over the leaves to prevent any complications with gas exchange and temperature. The aluminum foil was changed each morning under low photon flux density light (20 μE $m^{-2}s^{-1}$) passed through an interference filter (~453 nm). This procedure allowed ample space for leaves to grow.

Sugar beet leaves emerge at 2.2-d intervals and achieve full laminar length in 13 d (7). Therefore, leaves were harvested every 2 d for 15 d to obtain a developmental profile of enzyme activity. Sugar beet leaves develop from the shoot apex as leaf pairs. Once dark-grown leaves had reached about 3 cm in length (day 8) one of the leaf pairs was left intact and illuminated for 30 min or 24 h. Day 8 was chosen so that there would be enough leaf material for enzyme assay.

Enzyme Extractions and Assays

Fresh leaf material was ground with a cold mortar and pestle in an extraction buffer (8 mL/g fr cut) containing 25 mM Hepes/KOH (pH 7.5), 5 mM $MgSO_4$, 15 mM KCl, 2 mM sodium diethyldithiocarbamate, 2 mg/mL PVP (soluble), and 5 mM freshly added β-mercaptoethanol. The extract was filtered through eight layers of cheesecloth and the filtrate was

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centrifuged at 12,000 rpm for 10 min. A 1-ml aliquot of supernatant was passed through a prespun 5-ml G-50 Sephadex[1] column packed into a 5-mL syringe by centrifugation at 4750*g* for 1 min. The eluant was used for enzyme assay. All of the enzyme assays were optimized to the conditions of the experiment.

Sucrose-P synthase (EC 2.4.1.14) was assayed by measurement of sucrose produced from F6P[2] plus UDPG. The reaction mixture contained 250 μL leaf extract and 50 μL 100 mM F6P, 100 mM UDPG, 40 mM Hepes/KOH (pH 7.5), and 10 mM $MgSO_4$. The assay mixture was the same for sucrose synthase (EC 2.4.1.13) except that 100 mM fructose replaced F6P in the stock solution. Acid invertase (EC 3.2.1.26) was assayed by adding 25 μL of 20 mM sucrose, 75 mM KH_2PO_4, and 20 mM Na-citrate (pH 4.8) to 250 μL of extract. Under these assay conditions Giaquinta (8) showed that invertase in sugar beet had only one pH optimum (pH 5.0); therefore, only acid invertase was assayed in these experiments.

Mixtures were incubated at 25°C and the reactions were terminated by mixing 50-μL aliquot of enzyme preparation with 50 μL of 30% KOH. Samples were taken at 5, 10, and 15 min for sucrose-P synthase and sucrose synthase and at 10 and 20 min for acid invertase. Hexoses were destroyed by placing tubes in boiling water for 10 min. The tubes were cooled, 1 mL of anthrone reagent (76 mL H_2SO_4, 30 mL H_2O, and 150 mg anthrone) was added, the mixture was incubated at 40°C for 20 min, and the A_{620} was measured (25).

To determine the 24 h profile of enzyme activity, sucrose-P synthase was monitored for an entire 24 h light-dark cycle. Leaf samples were taken every 4 h starting at 600 h (start of the light period) through to the next 600 h (end of the dark period). Enzyme activity was determined at the same time as samples were taken.

Enzyme assays were conducted at the same time each morning for the light/dark study, approximately 4 h after the start of the light period in the growth chamber. This ensured that no differences in enzyme activity occurred due to diurnal fluctuations observed in sugar beet (Fig. 1) sucrose-P synthase (10, 11, 18). Proteins were determined by a modified Lowry method as described by Peterson (15). Chl was determined according to Arnon (1).

RESULTS AND DISCUSSION

The length of dark-grown leaves was only 40% of those which were allowed to develop normally (Fig. 2A). Protein concentration in dark-grown leaves reached only 38% of normal (Fig. 2B), whereas Chl content reached only 9% of normal (Fig. 2C). In every case, major treatment differences were not apparent until approximately 8 d after leaf emergence from the shoot apex. The mean length for leaves 8 d old was approximately 5 cm, or 29% of full laminar length. Giaquinta (8) showed that sugar beet leaves begin photosyn-

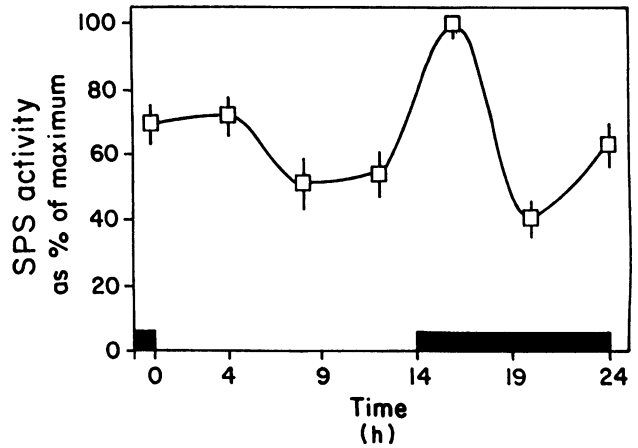

Figure 1. Sucrose-P synthase (SPS) activity in mature sugar beet leaves grown with a 14-h photoperiod. Enzyme activity was determined every 4 h starting at 0600 as the photoperiod began. Data represents mean of four experiments. For comparison, 33.1 nmol sucrose $min^{-1} \cdot mg \cdot protein^{-1}$ represented the sucrose-P synthase activity at 100%.

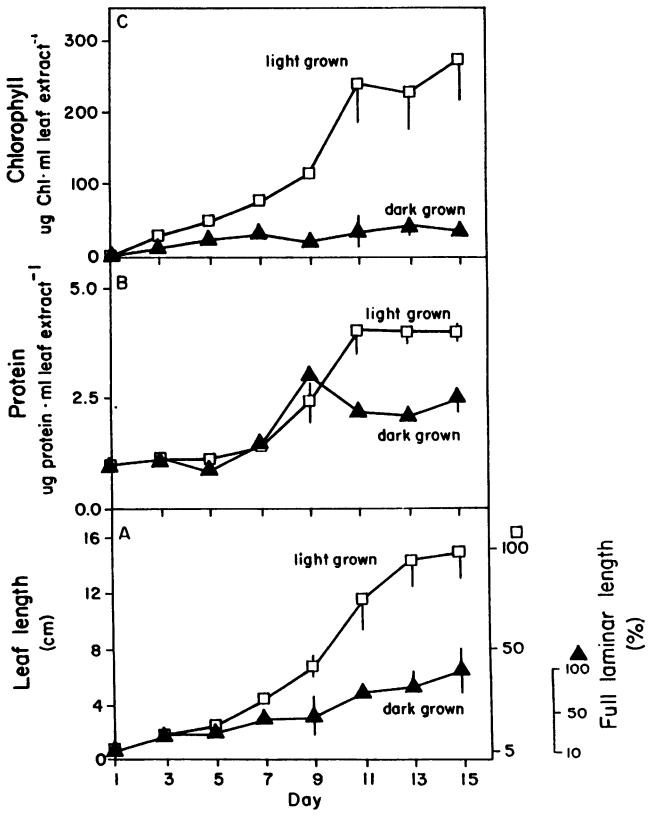

Figure 2. A, Leaf length and percent full laminar length of sugar beet leaves grown in light (□) and dark (▲) for 15 d after emergence from shoot apex. Leaves were harvested every 2 d. Leaf length represents only laminar portion of the leaf minus the petiole. Data represent mean of four experiments. B, Concentration of protein in sugar beet leaves grown in light (□) and dark (▲) for 15 d. Leaves were harvested every 2 d. Data represent mean of four experiments. C, Chl content in sugar beet leaves grown in light (□) and dark (▲) for 15 d. Leaves were harvested every 2 d. Data represent mean of four experiments.

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[2] Abbreviations: F6P, fructose 6-phosphate; F2,6-P_2, fructose 2,6-bisphosphate; UDPG, uridine 5′-diphosphoglucose; G-1-P, glucose-1-phosphate.

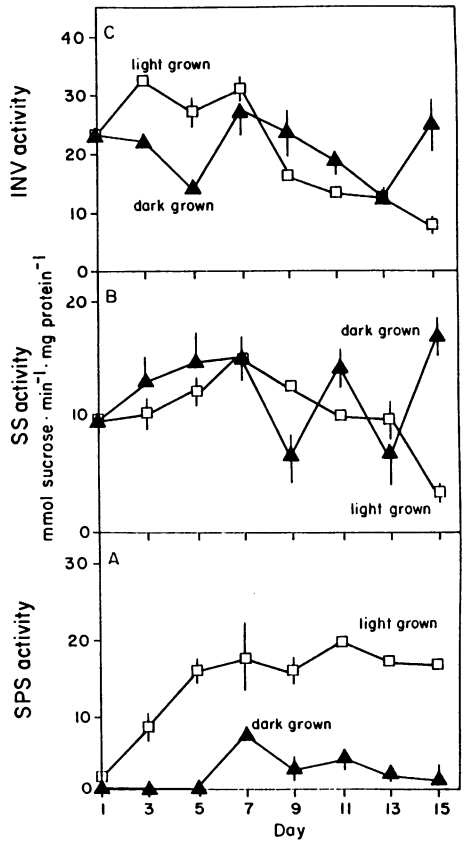

Figure 3. A, Extractable activity of sucrose-P synthase in sugar beet leaves grown in light (□) and dark (▲) for 15 days. Leaves were harvested every 2 d at 1000 h. Data represent mean of four experiments. B, Extractable activity of sucrose synthase in sugar beet leaves grown in light (□) and dark (▲) for 15 d. Leaves were harvested every 2 d at 1000 h. Data represent mean of four experiments. C, Extractable activity of acid invertase in sugar beet leaves grown in the light (□) and dark (▲) for 15 d. Leaves were harvested every 2 d at 1000 h. Data represent mean of four experiments.

thetic activity and sucrose synthesis and were importing about 38% less carbon from source leaves at approximately 30% full laminar length. The data presented here, with those of Giaquinta (8), suggest the conversion from sugar beet sink to source may begin at approximately 30% full laminar length.

Sucrose-P Synthase Activity

Initial experiments were conducted to examine the diurnal pattern in sucrose-P synthase activity. Figure 1 shows the changes in extractable activity of sucrose-P synthase in mature sugar beet leaves over a 24-h period. An early activity peak which lasted about 4 h was followed by a 50% decrease in activity by 1400 h. Highest extractable activity occurred about 2 h into the dark period (2200 h), whereas the lowest activity occurred about 4 h later (0200 h). Changes in sucrose-P synthase activity did not result from changes in extractable protein concentration as protein did not change in the 24-h period (2.3 μg protein mL^{-1} leaf extract ± 0.004). These data are very similar to those obtained for soybean by Rufty *et al.* (18).

The peak during the dark period may accommodate starch breakdown and resynthesis of the liberated carbon into sucrose. However, Rufty *et al.* (18) showed that in soybean, under their growth conditions, starch breakdown and formation and export of sucrose remained constant throughout the dark period. However, Mullen and Koller (14) have shown that in soybean starch breakdown and mobilization increased 200-fold in the cultivar Amsoy '71 and approximately 250-fold in the cultivar Wells II by 6 h into the dark period. Their data for starch mobilization and assimilate export correlate well with data concerning the maximal peak in sucrose-P synthase activity observed in soybean and sugar beet during the dark period.

Young, newly expanding sugar beet leaves (0.6 cm) show no detectable sucrose-P synthase activity whether leaves are grown in the light or dark (Fig. 3A). However, light-grown leaves had sucrose-P synthase activity 2 d after leaves began to elongate. No detectable activity was observed in dark-grown leaves for more than 6 d. After 7 d, sucrose-P synthase activity in dark-grown leaves had reached only 54% of that for the light-grown leaves (Fig. 3A). It is interesting that even though leaves were grown in the dark, sucrose-P synthase was measurable after 7 days. This indicates that the sucrose-P synthase protein or the modulation of enzyme activity was responding to some signal other than light or photosynthesis. Ultimately sucrose-P synthase activity may be programmed and controlled by some developmental factor as young leaves begin to develop.

When one of the 8-d-old dark-grown leaf pairs was illuminated for 30 min, there was no significant change in enzyme activity. Sucrose-P synthase activity remained approximately 50% of that found in the grown sugar beet leaves (18.3 nmol sucrose·min^{-1}·mg $protein^{-1}$). However, when dark-grown leaves were illuminated for 24 h (through an entire light/dark cycle), sucrose-P synthase activity increased to about 82% of that found in normally grown leaves. These results are very different from those observed in a monocotyledonous plant by Sicher and Kremer (21), who found that decreased sucrose-P synthase activity in dark adapated primary barley leaves was fully reversible within 10 min.

Sicher and Kremer (20) suggested that for monocotyledonous species, sucrose-P synthase is light-modulated and that control may involve a stable modification of the enzyme affecting substrate affinities for both UDPG and F6P. Any differences in activity between light and dark grown leaves, therefore, could represent two kinetically different forms of the enzyme (20, 23). Present data with sugar beets suggest that light is not as important in short term instantaneous modulation of sucrose-P synthase activity in light versus dark grown sugar beet leaves. For leaves illuminated for 24 h, the increase in sucrose-P synthase activity probably reflects an increased demand for sucrose synthesis as a result of newly fixed carbon and increased substrates of sucrose synthesis (23).

Sucrose Synthase and Invertase

Differences were observed between the activities of sucrose synthase in light- and dark-grown leaves. However, the differences seem to be in the pattern of activity and not in the appearance of activity (Fig. 3B). Unlike results of Giaquinta (8) and Arthur *et al.* (2), sucrose synthase activity increased

initially in light-grown leaves and then decreased as the leaves began to mature. Dark-grown leaves showed some oscillations in sucrose synthase activity (Fig. 3B). Also, the maximum activity of sucrose synthase as well as that of acid invertase was greater in the dark-grown leaves than in the light-grown leaves. The inconsistent activity of sucrose synthase in the dark-grown leaves may reflect a perpetuated sink activity of dark-grown leaves compared to light-grown leaves. However, the dark-grown leaves may have been programmed to ultimately die prematurely and these high concentrations of enzyme activity may have been designed to remobilize carbon skeletons to other parts of the plant.

There was no difference in the activity of acid invertase in the light-grown leaves versus dark-grown leaves except for day 15 (Fig. 3C). The data presented here are consistent agree with those of Pollock and Lloyd (16), Morris and Arthur (13), and Arthur *et al.* (2) with respect to the activity of acid invertase in sugar beet leaves. Initially, acid invertase increases followed after 7 d by a decrease as leaves began to mature. Also, the activity of acid invertase was greater than that of sucrose synthase, throughout the entire growth period.

Sucrose synthase, located in the cytosol of leaf tissue, is involved in hydrolysis of sucrose for polysaccharide synthesis leading to cell wall precursers (9). Claussen *et al.* (4) demonstrated that sucrose synthase activity was closely regulated by sucrose import and was a good marker for the ability of sink organs to attract assimilates. With a decrease in sucrose import there followed a decrease in sucrose synthase activity. Preiss (17) suggested that sucrose synthase activity correlated well with complex carbohydrate synthesis. This is not to say that products of sucrose synthase activity do not enter glycolysis. It has been shown that G-1-P produced by sucrose synthase plus UDPG pyrophosphorylase activity feeds directly into glycolysis (3, 24).

Acid invertase is located in the apoplasmic free space and vacuole and is involved in providing hexoses for respiration, sucrose transplant gradients and vacuolar osmotic-turgor related cell expansion (9, 12). Therefore, both enzymes are found to a higher degree in young actively growing tissue which is utilizing sucrose more rapidly than storing it (3).

The data presented here indicate that activities of sucrose synthase and acid invertase in dark-grown plants are similar to the activities of these enzymes in light-grown leaves. However, there seems to be some inconsistency in activity of sucrose synthase in the dark-grown leaves, and this may indicate that these leaves are still acting as sinks or are prematurely dying. As growth does not keep pace with metabolism decreased activity may serve as a control of sucrose synthase, preventing an accumulation of hexoses. This may account for the inconsistent activity of sucrose synthase and possibly acid invertase. The apparent 2-d cycle suggests this is not a circadian effect.

This study also shows the activity of sucrose-P synthase was reduced by a light limitation and most likely by the absence of any photosynthesis. Dark adaption caused a delay in the appearance of extractable sucrose-P synthase activity and a reduced activity once it was initiated. Based on the illumination data, it appears that there is an effect of light on enzyme activity but not necessarily the short term regulation as seen in barley by Sicher and Kremer (20). It is more likely that the change in sucrose-P synthase was a result of changes in metabolism, mainly photosynthesis, and an increase in substrates of sucrose synthesis (23). This needs further investigation. It is also not known if changes in any of the extractable activities of sucrose-P synthase were a result of protein changes or changes in the way the protein is modulated. These are interesting questions and should be examined carefully.

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