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Changes in Starch Formation and Activities of Sucrose Phosphate Synthase and Cytoplasmic Fructose-1,6-bisphosphatase in Response to Source-Sink Alterations[1]

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ABSTRACT

Short term experiments were conducted with vegetative soybean plants (*Glycine max* L. Merr. 'Ransom' or 'Arksoy') to determine whether source-sink manipulations, which rapidly changed the 'demand' for sucrose and partitioning of photosynthetically fixed carbon into starch, were associated with alterations in activities of sucrose-P synthase and/or cytoplasmic fructose-1,6-bisphosphatase in leaf extracts. When demand for sucrose from a particular source leaf was increased by defoliation of other source leaves, starch accumulation was restricted and activities of both enzymes were markedly enhanced. When demand for sucrose from source leaves was limited by excision, starch accumulation in the detached leaves was increased while activity of sucrose-P synthase declined sharply. The consistent responsiveness of sucrose-P synthase activity to changes in demand for sucrose supports the contention that regulation of sucrose-P synthase is an integral component of the system which controls sucrose biosynthesis and partitioning of carbon between starch and sucrose biosynthesis in the light.

A conceptual framework has been developed to account for regulation of partitioning of photosynthetically fixed carbon between biosynthesis of starch and sucrose in leaves (7, 12, 24, 32). In this view, an important determinant of carbon partitioning into starch may be the rate of sucrose formation and the related generation of Pi in the cytosol. Cytoplasmic Pi exchanges rapidly across the chloroplast envelope with triose phosphates, and the rate of this countertransport process relative to the rate of CO_2 fixation determines to a large extent the degree to which carbon is diverted from starch biosynthesis.

The rate of sucrose biosynthesis and Pi generation could be controlled by the activities of SPS[2] and/or cytoplasmic FBPase, which are apparently low compared to activities of other enzymes in the sucrose biosynthetic pathway (5, 11). In particular, the activity of SPS in leaf extracts has been found to be consistently correlated negatively with leaf starch content in comparisons among different species (14), and when specific genotypes were exposed to a variety of environmental and nutritional conditions (16).

Although there is some evidence to suggest that partitioning of carbon between biosynthesis of starch and sucrose is programmed genetically (15), partitioning apparently can be altered by 'source-sink' manipulation within the plant. In experiments where portions of the source (carbon exporting) leaves in the canopy were excised or shaded, it has been observed that sucrose content and export out of remaining source leaves were increased during time periods in which photosynthetic rates remained relatively stable (3, 6, 30, 31). Tissue starch has sometimes been measured and observed to be decreased (31). These results imply that partitioning of available photoassimilate into starch declined when 'demand' for sucrose was increased (7).

In other experiments where the 'sink demand' for sucrose was decreased by girdling or fruit removal, photosynthetic rates have been observed to decrease while starch accumulation either increased or was equal to that in control plants (4, 10, 18, 21, 27, 28). This suggests that partitioning of available photoassimilate into starch was increased.

Source-sink manipulations which result in rapid adjustments in demand for sucrose and partitioning of carbon into starch could be associated with alterations in the activities of SPS and/or FBPase. The present experiments were designed to investigate this hypothesis. In a series of short term experiments, demand for sucrose was either increased by defoliation of source leaves or decreased using detached source leaves. Alterations in SPS activity were consistently observed which corresponded with changes in demand for sucrose and were reciprocal to changes in starch accumulation.

MATERIALS AND METHODS[3]

The study consisted of three experiments to investigate the relationship between adjustments in demand for sucrose, accumulation of starch, and activities of SPS and FBPase in leaf extracts of vegetative soybean plants (*Glycine max* L. Merrill, 'Ransom' or 'Arksoy'). Plant culture conditions were similar in each experiment. The plants were grown in a standard soil mix in a greenhouse from April to July, 1982. Plants were watered as necessary in the morning with tap water, and in the afternoon of alternate days with approximately 600 ml of a standard nutrient solution which contained 7.5 mM NO_3^-, 0.5 mM $H_2PO_4^-$, 3.0 mM K^+, 2.5 mM Ca^{2+}, 1.0 mM Mg^{2+}, 1.0 mM SO_4^{2-}, and trace elements

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[2] Abbreviations: SPS, sucrose phosphate synthase; FBPase, fructose-1,6-bisphosphatase; F6P, fructose 6-phosphate; UDPG, uridine 5'-diphosphoglucose; CER, net CO_2 exchange rate.

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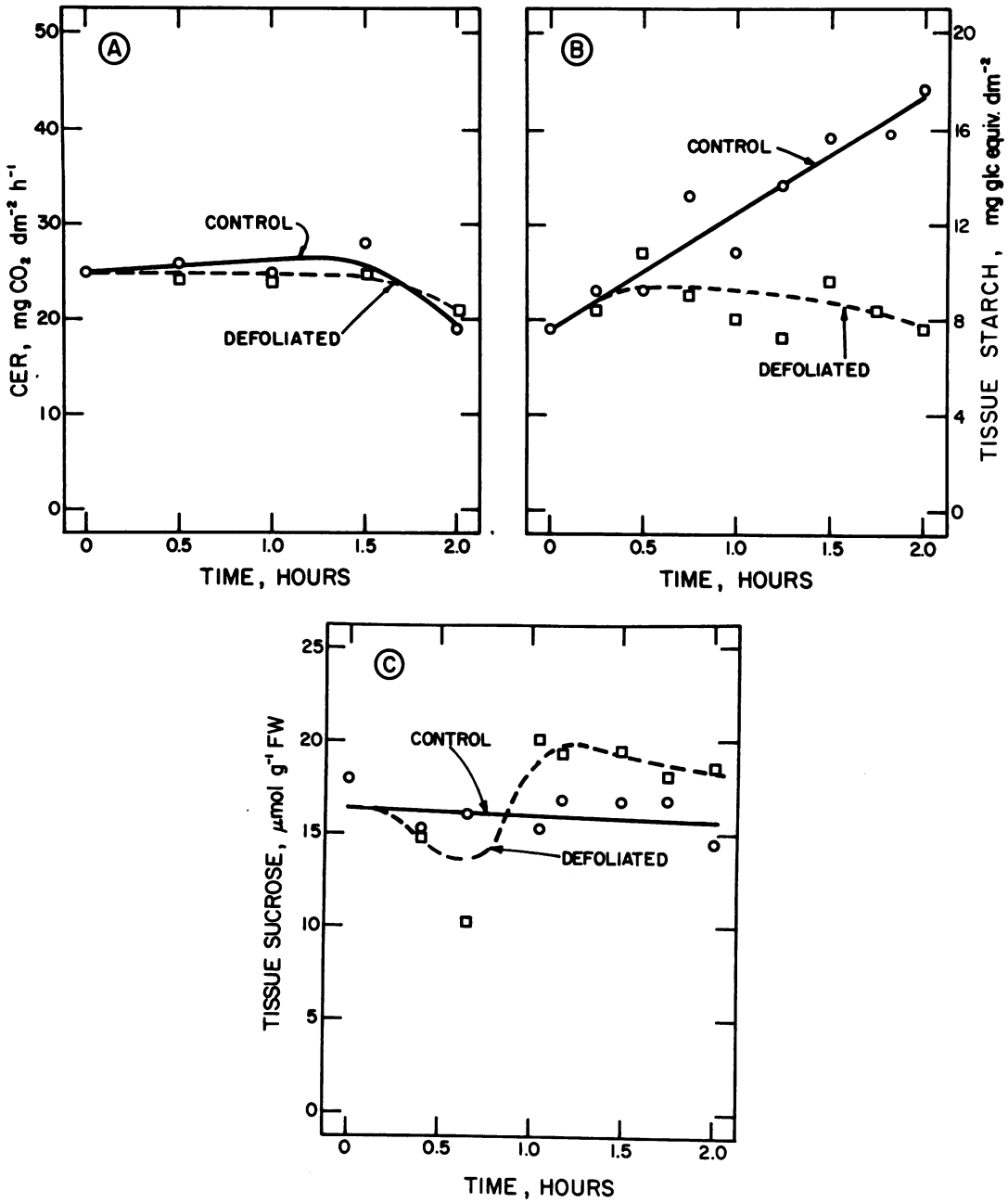

FIG. 1. Changes in (A) CER, (B) leaf starch accumulation, and (C) leaf sucrose content in the first trifoliolate of Ransom soybean plants following defoliation of all other source leaves. The first trifoliolates of other, intact plants were used as controls. glc, glucose.

at the concentration of Hoagland solution (13).

Defoliation. Twenty Ransom soybean plants were grown until the second trifoliolate had expanded fully. At this growth stage, the plants were growing exponentially; therefore, requirements for sucrose by shoot and root sinks were high. At 10 AM on a clear day, the second trifoliolate and primary leaves of 10 plants were excised, leaving the first trifoliolate as the only source (carbon exporting) leaf. At this time, two 1.1-cm diameter leaf discs were removed for starch analysis from the first trifoliolate of each defoliated plant and each of the 10 intact, control plants. The discs were immediately submerged in 80% ethanol and placed in a freezer at −10°C. The experiment lasted 2 h, with the first trifoliolate of both a defoliated and a control plant sampled every 15 or 20 min throughout. At each sampling time, two additional leaf discs were removed as before, CER was measured (see below), and then the first trifoliolate was excised, weighed, and immediately frozen at −80°C for later enzyme analysis.

Detached Leaves. In the initial experiment, Ransom soybean plants were grown until the leaf canopy was closed. At 10 AM on a clear day, six fully expanded trifoliolates were randomly selected from canopies of three plants. The trifoliolates were excised at the base of the petiole and immediately placed into 500-ml beakers filled with distilled H_2O, where the petioles were re-cut under water. A leaf was harvested just after excision and every 0.5 h over a 2.5-h experimental period; the petiole was removed, fresh weight was measured, and the leaf was immediately frozen at −80°C for later analysis of enzymes. Leaf discs were taken at excision and prior to harvest for determination of starch accumulation, and CER was measured at the time of harvest.

A second experiment was conducted with a uniform population of 20 Arksoy soybean plants which had nine fully expanded trifoliolates and were close to canopy closure. The sixth trifoliolate

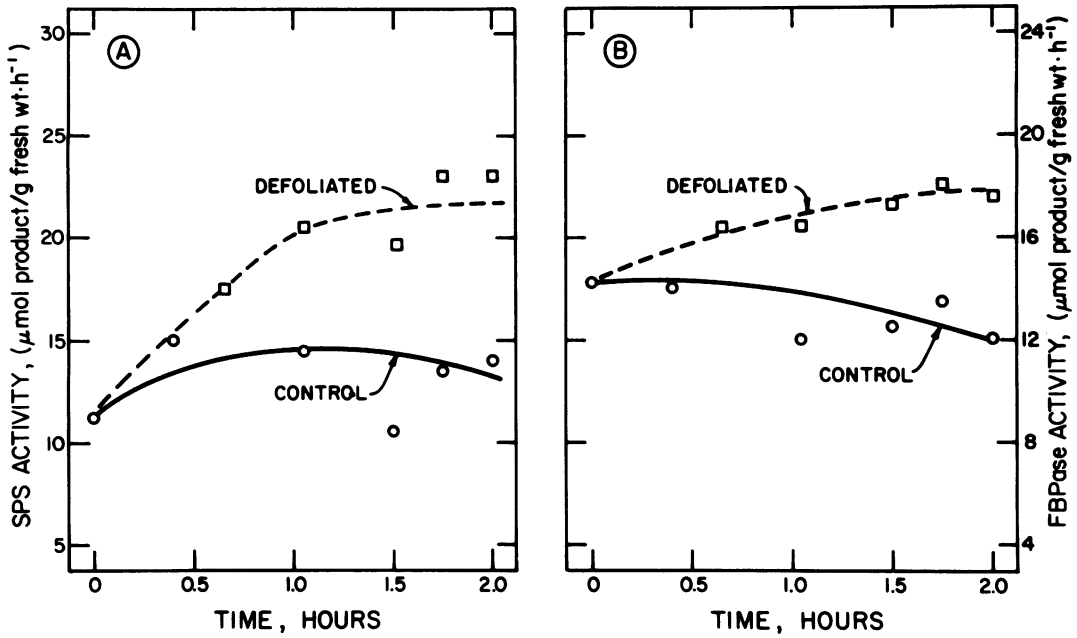

FIG. 2. Activities of (A) SPS and (B) cytoplasmic FBPase in extracts of the first trifoliolate of Ransom soybean plants which had been defoliated of all other source leaves and of the first trifoliolate of intact (control) plants.

was excised from 10 of the plants and re-cut in distilled H_2O as before. The sixth trifoliolate of the remaining 10 plants was left attached and used as a control. All leaves were fully exposed to light throughout. A detached and a control leaf were harvested as described previously at intervals (specified in results) over a 4-h experimental period.

Photosynthesis Measurements. Photosynthetic rates were measured using a Beckman model 865 differential IR CO_2 analyzer equipped with a clamp-on Plexiglas cuvette enclosing the upper and lower surfaces of a 10-cm^2 area of the appropriate leaf. Air at the same temperature and CO_2 concentration of the ambient air was passed through the cuvette for 30 to 45 s at a flow rate of 1.5 l/min. Differences between CO_2 concentration in incoming and exhaust air streams were monitored and used to calculate CER. Leaves always were fully exposed to light.

Starch Analysis. Leaf discs were extracted with hot 80% ethanol until the tissue was pigment free. Particulates including starch were pelleted by centrifugation and then suspended in 1.0 ml of 0.2 N KOH and placed in boiling water for 30 min. After cooling, the pH of the mixture was adjusted to about pH 5.5 with 200 μl of 1.0 N acetic acid. To each sample, 1.0 ml of dialyzed amyloglucosidase (from *Aspergillus oryzae*, Sigma) solution (35 units/ml in 50.0 mM Na acetate buffer, pH 4.5) was added, and the tubes were incubated at 55°C for 30 min. After digestion, the tubes were placed in boiling water for 1 min and centrifuged, and the glucose in the supernatant was analyzed enzymically using hexokinase and glucose-6-P dehydrogenase (17).

Enzyme Extraction and Assays. The frozen leaf tissue was ground with a Polytron high speed homogenizer in a grind medium (8.0 ml of medium/g fresh weight) containing 50.0 mM Hepes-NaOH (pH 7.5), 5.0 mM $MgCl_2$, 1.0 mM EDTA, 2.5 mM DTT, 2% polyethylene glycol 20 (w/v), and 1% BSA (w/v). The brei was then filtered through eight layers of cheesecloth, and cells were disrupted by passage through a French pressure cell (330 kg/cm^2). Debris was pelleted by centrifugation at 38,000*g* for 10 min and enzyme assays were conducted on the supernatant fluid.

Sucrose-P synthase was assayed by measurement of fructose-6-P-dependent formation of sucrose (+sucrose-P) from UDP-glucose (2). The assay mixture (70 μl) contained 7.5 mM UDP-glucose, 7.5 mM fructose-6-P, 5 mM $MgCl_2$, 50.0 mM Hepes-NaOH (pH 7.5), and an aliquot of leaf extract. The assay mixture for sucrose synthase was the same, except that fructose replaced fructose-6-P. Mixtures were incubated at 25°C for 10 min, and reactions were terminated by addition of 70 μl 1.0 N NaOH. Unreacted fructose-6-P (or fructose) was destroyed by placing the tubes in boiling water for 10 min. After cooling, 0.25 ml of 0.1% (v/v) resorcinol in 95% ethanol and 0.75 ml of 30% HCl were added, and the tubes were incubated at 80°C for 8 min (25). The tubes were allowed to cool and were centrifuged at 1,500*g* for 5 min, and the A_{520} was measured.

Cytoplasmic FBPase was assayed by a continuous spectrophotometric assay. The 1.0-ml reaction mixture contained 50 mM Hepes-NaOH (pH 7.0), 5 mM $MgCl_2$, 100 μM FBP, 0.2 mM NADP, 2 units each of phosphoglucoisomerase and glucose-6-P dehydrogenase, 1 unit of phosphogluconate dehydrogenase, and an aliquot of leaf extract (under these conditions, only the cytoplasmic FBPase is active; Ref. 11). Estimates of Mg^{2+}-independent FBPase activity were obtained by conducting the assay in the presence of 10 mM EDTA (D. M. Pharr and S. C. Huber, submitted).

The tissue extract also was used for hexose and sucrose determinations. Following centrifugation, an aliquot of the extract was removed, diluted with redistilled H_2O, boiled for 1 min, and analyzed enzymically (17).

RESULTS

Defoliation of Source Leaves. Defoliation of all source leaves except the first trifoliolate had little discernible effect on the photosynthetic rate of this remaining source leaf (Fig. 1A), but markedly affected accumulation of leaf starch (Fig. 1B). While the first leaf of control plants accumulated starch continually, accumulation in the same, source leaf of defoliated plants was severely restricted.

Defoliation and the related decrease in accumulation of starch apparently were associated with increased formation and export of sucrose. As noted previously, in similar defoliation studies which employed ^{14}C labeling, increased formation and export of sucrose have been observed consistently (3, 6, 30, 31). In the present experiment, after an initial decline, the tissue sucrose content was increased in the second h after defoliation and stabilized at a level which exceeded the sucrose content in leaves of

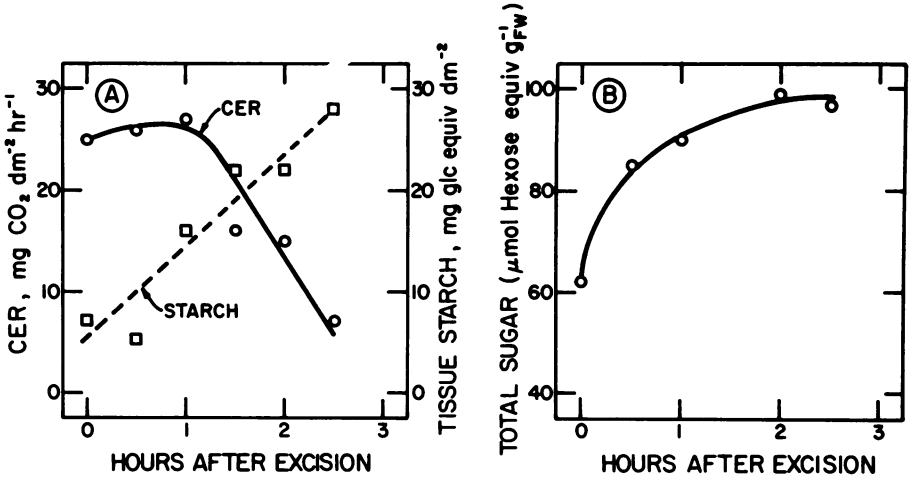

FIG. 3. Changes in (A) CER and leaf starch content, and (B) leaf sugar content in mature trifoliolates from Ransom soybean plants following excision. glc, glucose.

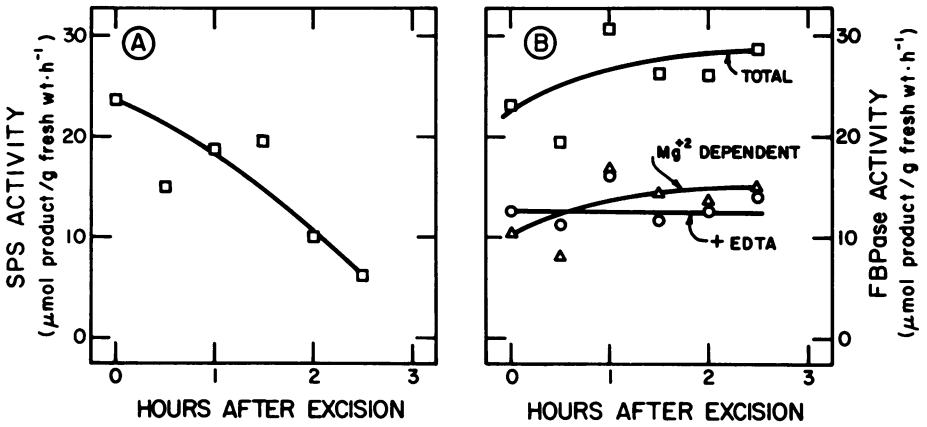

FIG. 4. Activities of (A) SPS and (B) cytoplasmic FBPase in leaf extracts from mature trifoliolates from Ransom soybean plants following excision.

control plants by 12 to 20% (Fig. 1C). The leaf sucrose content generally has been found to be closely related with the steady state rate of sucrose formation and export out of source leaves (9); therefore, the response pattern observed is consistent with increased sucrose biosynthesis and equilibration of the transient sucrose pools in the leaf with the enhanced rate of sucrose export.

The measured decrease in net synthesis of starch and presumed increase in sucrose formation and export in defoliated plants was associated with markedly increased activities of both SPS (Fig. 2A) and cytoplasmic FBPase (Fig. 2B) in leaf extracts. Increased enzyme activities relative to control plants were apparent within the first h after defoliation and were maximized at the end of 2 h. The stimulation of SPS activity ranged from 50 to 77% relative to activity in leaves of control plants during the second h of the experiment, whereas total activity of FBPase was enhanced 33 to 45%. Crude soybean leaf extracts contain both Mg^{2+}-dependent and independent FBPase activities (Pharr and Huber, submitted). The Mg^{2+}-dependent enzyme is thought to be involved in sucrose biosynthesis in the cytoplasm. The FBPase activities in Figure 2B represent the summation of Mg^{2+}-dependent and independent activities. Both were increased similarly by defoliation (data not shown).

Decreased partitioning of carbon into starch and increased activities of SPS and FBPase have been observed in a number of other, similar defoliation experiments with vegetative soybean plants (data not shown). In four experiments, the maximum enhancement of SPS activities during the 2-h time interval after defoliation was 74 ± 8% relative to activities of controls. Maximum enhancement of FBPase activities has been more variable, ranging from 5 to 45% relative to controls, with a mean enhancement of 24 ± 20%. In each experiment, increases in SPS activities were substantially larger than increases in FBPase activities.

Detached Leaves. Two additional experiments were conducted in which source leaves were detached from the plant in order to severely limit the demand for sucrose, and thus increase partitioning of photosynthate into starch. It has been reported previously that detached leaves accumulate considerable amounts of starch (20). In an initial experiment, the photosynthetic rate of detached source leaves remained stable for about 1 h after excision and then declined sharply (Fig. 3A). Starch accumulation, however, was maintained at about the same rate throughout the 2.5-h experimental period (Fig. 3A), which suggested that a larger proportion of the fixed carbon was partitioned into starch after the first h. As shown in Figure 3B, soluble sugar (sucrose + hexose) content in the leaves increased sharply in the first h after excision and then tended to level off. This response likely reflected the abrupt restriction of sucrose export out of the leaf.

The accumulation of sugars and apparent increase in partitioning of fixed carbon into starch was associated with a decline in SPS activity (Fig. 4A). After 2.5 h, SPS activity had decreased to only 26% of the activity at the time of excision. In contrast, total activity of FBPase was not altered as both the Mg^{2+}-dependent and Mg^{2+}-independent activities remained relatively stable (Fig. 4B).

To investigate further the effects of leaf excision, changes in detached leaves were monitored over a 4-h period and compared to processes in attached leaves from the same canopy position. Photosynthetic rates of detached leaves were similar to the rates

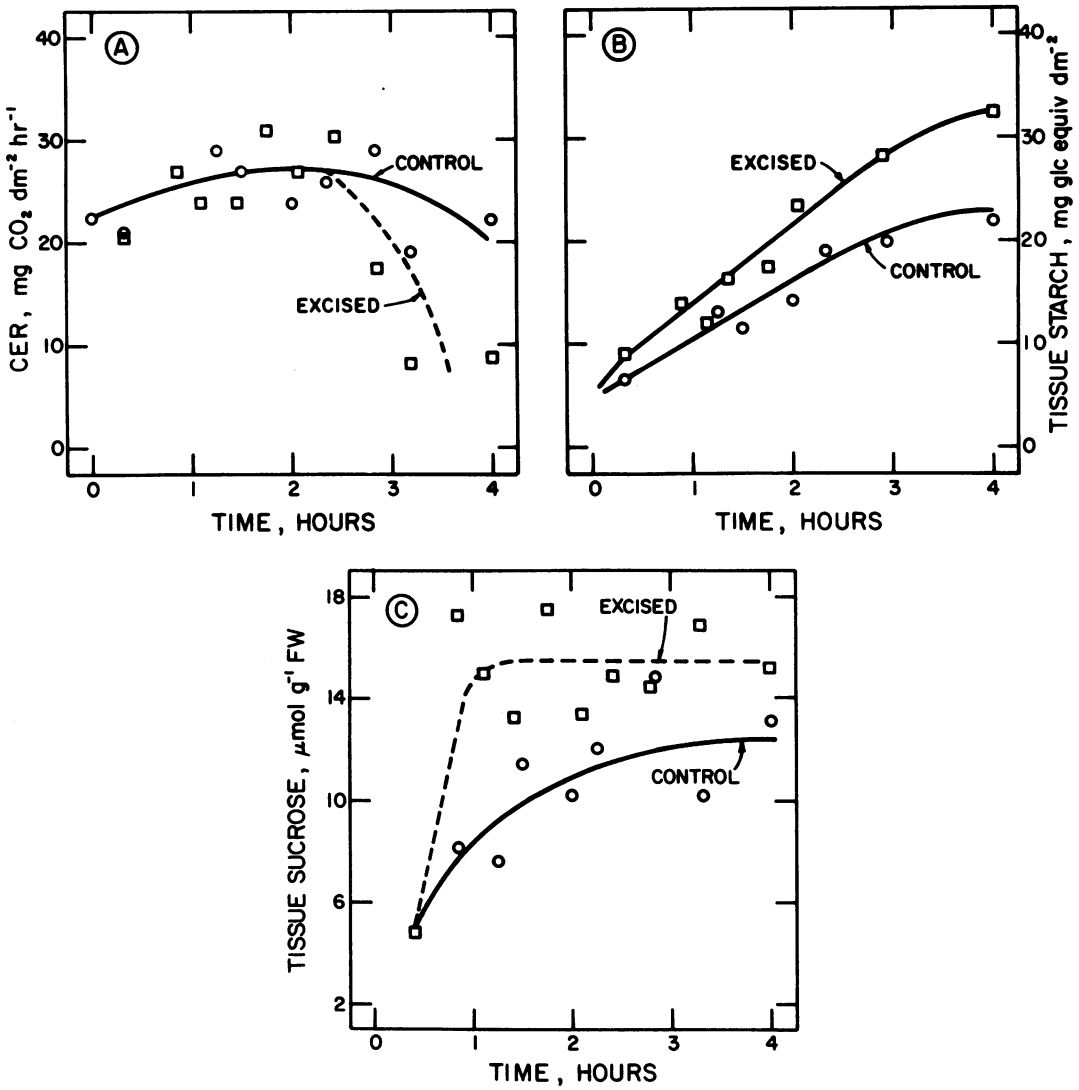

FIG. 5. Changes in (A) CER, (B) leaf starch accumulation, and (C) leaf sucrose content of excised leaves and of intact leaves from the same canopy position of Arksoy soybean plants. glc, glucose.

of intact, control leaves until the third h after excision when the CER of detached leaves declined sharply (Fig. 5A). Even though carbon fixation of detached leaves always was similar to or less than that of controls, starch accumulation was increased (Fig. 5B), again suggesting that partitioning of carbon into starch was enhanced. Excision also was associated with an initial, rapid increase in sucrose content which stabilized after the first h (Fig. 5C).

Extractable SPS activities were markedly reduced in detached leaves (Fig. 6A). Decreased SPS activities were apparent within the first h after excision and the activities declined steadily throughout the experimental period (Fig. 6A). At the end of 4 h, SPS activity in detached leaves was only 16% of the activity in control leaves. Total activity of FBPase in detached leaves, however, was similar to activity in control leaves throughout (Fig. 6B). The activity of sucrose synthase also was determined, but only a small effect of leaf excision was apparent (Fig. 6C) in comparison to the changes observed in SPS activity (Fig. 6A).

DISCUSSION

Activities of enzymes involved in sucrose biosynthesis were clearly affected following source-sink manipulations to alter demand for sucrose and partitioning of carbon into starch. In the defoliation experiments, increased demand for sucrose was associated with a restriction of starch accumulation and enhancement of activities of SPS and FBPase. In experiments with detached source leaves, the demand for sucrose was decreased, and partitioning of carbon into starch increased while activity of SPS declined. These results are consistent with regulation of the activities of SPS, and to a lesser extent FBPase, being intimately involved in the control of sucrose formation and the related partitioning of photosynthetically fixed carbon between the sucrose and starch biosynthetic pathways.

A number of characteristics have been attributed to SPS which can be related to its suitability as a central control point in sucrose formation. Involvement in sucrose biosynthesis apparently is the only metabolic function of SPS, and the equilibrium constant of the SPS reaction heavily favors formation of sucrose phosphate (19). It has been reported that SPS from plant leaves may exhibit sigmoidal substrate saturation kinetics (1, 22), characteristic of a regulatory enzyme. Activity of SPS is very low or absent in newly emerging sink leaves, but is increased appreciably as leaves approach full expansion when significant amounts of sucrose accumulate and leaves become exporters of sucrose (8, 23, 29). Even though activity of SPS is maximized in fully expanded leaves actively exporting sucrose, the activity in leaf extracts is still low relative to that of most other enzymes involved in sucrose formation (5, 11), and consequently, could be rate limiting. Furthermore, as noted previously, activity of SPS was negatively correlated with partitioning of carbon into starch in species comparisons (14) and when partitioning was altered by environmental and nutritional

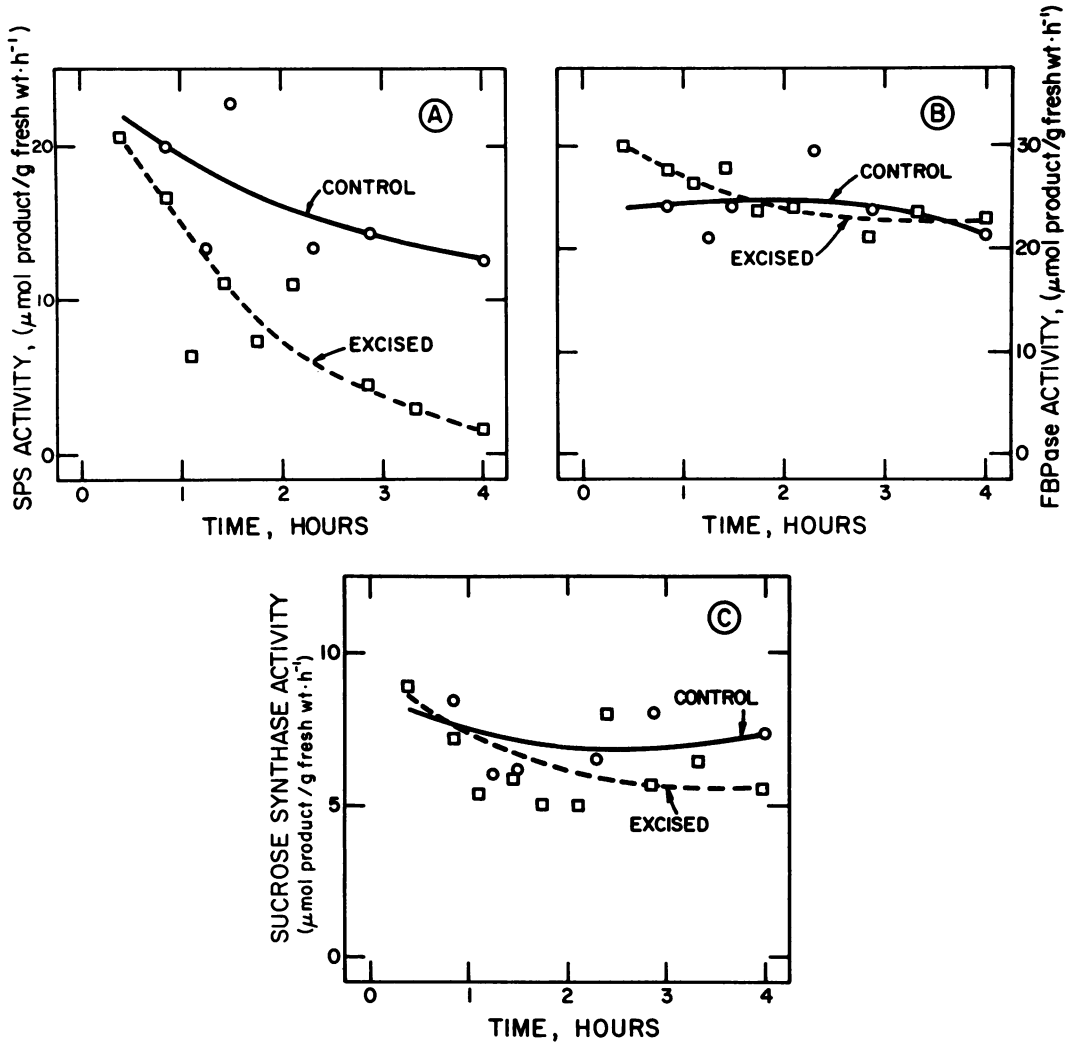

FIG. 6. Activities of (A) SPS, (B) cytoplasmic FBPase, and (C) sucrose synthase in extracts of excised Arksoy soybean leaves and of intact leaves from the same canopy position.

treatments (16). These negative correlations, which imply that SPS activity and sucrose formation were correlated positively, have persistently recurred in a large number of our unpublished nutritional experiments with vegetative soybean plants. When the body of evidence concerning SPS is considered collectively, along with the observed responsiveness of SPS activity to changes in demand for sucrose in the present study, there is strong support for the contention that SPS is an important regulatory component of carbohydrate metabolism in the light.

Alterations in SPS activity would presumably influence the activities of other enzymes in the sucrose biosynthetic pathway. For example, decreased activity of SPS would be expected to result in accumulation of F6P which, in turn, could inhibit the activity of cytoplasmic FBPase *in situ* (11). Such metabolic regulation of FBPase activity would be undetected in the enzyme analyses performed in the present study. However, FBPase activity evidently is subject to other types of regulation, as the activity was enhanced in leaf extracts when the demand for sucrose was increased (Fig. 2B). Because FBPase activity appears close to rate limiting, such enhancement could be essential to allow rapid increases in sucrose formation and flux to sink regions.

It is not known whether the altered enzyme activities in the present study were the result of modification of pre-existing enzyme molecules or alterations of net synthesis of the enzyme. The signal(s) which elicits the changes in enzyme activities also is unknown. Sucrose appears to be a potential inhibitor of SPS (14, 26). In these experiments, however, the tissue sucrose content was increased following source-sink alterations both when SPS activity was observed to increase (Figs. 1C and 2A) and decrease (Figs. 5C and 6A). Although this apparent inconsistency can be explained by differential compartmentation of sucrose, it is conceivable that factors other than sucrose, *i.e.* abscisic acid (28), could be responsive to changes in sucrose demand and be involved in regulation of SPS.

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