Carbohydrate Metabolism and Activity of Pyrophosphate: Fructose-6-Phosphate Phosphotransferase in Photosynthetic Soybean (Glycine max, Merr.) Suspension Cells

Received for publication March 10, 1988 and in revised form June 6, 1988

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ABSTRACT

Activity of pyrophosphate:fructose-6-phosphate phosphotransferase (PFP) was investigated in relation to carbohydrate metabolism and physiological growth stage in mixotrophic soybean (Glycine max Merr.) suspension cells. In the presence of exogenous sugars, log phase growth occurred and the cells displayed mixotrophic metabolism. During this stage, photosynthetic oxygen evolution was depressed and sugars were assimilated from the medium. Upon depletion of medium sugar, oxygen evolution and chlorophyll content increased, and cells entered stationary phase. Activities of various enzymes of glycolysis and sucrose metabolism, including PFP, sucrose synthase, fructokinase, glucokinase, UDP-glucose pyrophosphorylase, and fructose-1,6-bisphosphatase, changed as the cells went from log to stationary phases of growth. The largest change occurred in the activity of PFP, which was three-fold higher in log phase cells. PFP activity increased in cells grown on media initially containing sucrose, glucose, or fructose and began to decline when sugar in the medium was depleted. Western blots probed with antibody specific to the α-subunit of potato PFP revealed a single 56 kilodalton immunoreactive band that changed in intensity during the growth cycle in association with changes in total PFP activity. The level of fructose-2,6-bisphosphate, an activator of the soybean PFP, increased during the first 24 hours after cell transfer and returned to the stationary phase level prior to the increase in PFP activity. Throughout the growth cycle, the calculated in vivo cytosolic concentration of fructose-2,6-bisphosphate exceeded by more than two orders of magnitude the previously reported activation coefficient (Kₐ) for soybean PFP. These results indicate that metabolism of exogenously supplied sugars by these cells involves a PFP-dependent step that is not coupled directly to sucrose utilization. Activity of this pathway appears to be controlled by changes in the level of PFP, rather than changes in the total cytosolic level of fructose-2,6-bisphosphate.

Recently, the roles of PFP (EC 2.7.1.90) and PFK (EC 2.7.1.11) in pathways of glycolysis and sucrose metabolism have been intensely studied (3–5, 7, 8, 16, 28, 29). Both enzymes catalyze the phosphorylation of fru-6-P to fru-1,6-bisP, although only the reaction catalyzed by PFP is freely reversible (29).

Although PFK and PFP have been shown to be allosterically regulated by a number of metabolites (11, 20), only PFP is activated by fru-2,6-bisP (26), a compound also implicated in the regulation of sucrose biosynthesis (16, 29, 30). The total level of PFP also appears to be regulated in plant tissues. High activities of PFP are associated with sink tissues (7, 28), and changes in total PFP activity occur during developmental processes, such as leaf maturation (7) and banana ripening (3). The factors that influence the regulation of PFP activity through allosteric and coarse mechanisms are presently uncertain. Conditions favoring high glycolytic or biosynthetic activity, such as high apoplastic sucrose levels typical of sink tissues, may be important in this regard (11).

Cultures of photoautotrophic cells derived from a number of species have been increasingly employed to study primary carbon metabolism (13, 19, 27). The type of metabolism displayed by a photosynthetic cell culture depends upon several parameters that include the light regime, supplemental phytohormones, inorganic and organic composition of the medium, and the metabolic capacities of the cells under study (19). The presence of an exogenous sugar source, such as sucrose, is a predominant factor influencing metabolism in cultured photosynthetic cells (18, 19, 27). For certain photosynthetic cells, the addition of sugar to the growth medium causes the cells to change from autotrophic metabolism to a "mixotrophic" type metabolism, where carbon is assimilated from the medium as well as CO₂ (27 and references therein). The ability of these cells to shift between autotrophic and heterotrophic states makes mixotrophic cells useful for studying the regulation of glycolytic carbon metabolism.

In this study, we employ mixotrophic soybean suspension cells to examine sugar metabolism and regulation of PFP activity during the cell growth cycle. These cells rapidly and reversibly alternate between stationary (photoautotrophic) and log (mixotrophic) stages of growth in response to the availability of sugars to the growth medium. This capacity facilitates investigation of coarse and fine regulation of PFP activity in relation to assimilation of exogenous sugars.

MATERIALS AND METHODS

Materials. All biochemicals including coupling enzymes and Sephadex gel permeation resin were obtained from Sigma. Goat anti-fructose-2,6-bisphosphate; fru-6-P, fructose-6-phosphate; glu-6-P, glucose-6-phosphate; 3-PGA, 3-phosphoglyceric acid; PMSF, phenylmethylsulfonyl fluoride; UDPG, UDP-glucose; DHAP, dihydroxyacetone-phosphate.

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anti-rabbit IgG conjugated horseradish peroxidase, nitrocellulose membrane, horseradish peroxidase color development reagent, and Bradford protein reagent were purchased from Biorad. Compressed air (zero grade) and CO₂ (anarobic grade) were purchased from Air Products, Allentown, PA.

Cell Cultures. Suspension cultures of soybean (Glycine max Merr. var. Corsoy) were obtained from Dr. Jack Widholm, University of Illinois. Cultures were routinely maintained by subculturing on a 10-d cycle on PRB medium of Horn et al. (14) modified by the addition of 11 mM sucrose. Experiments were performed in the presence of sucrose or other sugars at the concentrations indicated in the text. Sucrose containing media were routinely sterilized by autoclaving, except for experiments involving media containing glucose or fructose, in which case all media were filter sterilized. Culture conditions were as follows: 28°C, cool white fluorescent illumination (fluence rate of 350 μE m⁻² s⁻¹), and rotation at 140 rpm. Cells were grown under humidified air enriched to 3% CO₂. Total cell volume was determined at different times by allowing cells in 100 mL of culture medium to settle for 20 min in a sterile calibrated cylinder.

Tissue Homogenization. Suspension cells were collected from culture medium on a 40 μm nylon net and washed two times with 100 mL of distilled H₂O. Cells, either frozen in liquid N₂ and stored at −80°C or freshly harvested, were homogenized with an Amino French press apparatus at 14000 p.s.i. in homogenizing buffer (50 mM Hepes, pH 8.0; 1 mM EDTA; 2 mM MgCl₂; 15 mM KCl and 2 mM DTT) containing 1 mM PMSF. Homogenates were centrifuged at 17000g for 15 min, and 1-mL aliquots were desalted by centrifugation through Sephadex G-25-50 equilibrated with homogenizing buffer.

Enzyme Assays. Assays for enzymes activities were as previously described (16) and optimized or altered as indicated. Standard assay buffer contained 50 mM Hepes-KOH pH 7.5, 1 mM EDTA, 2 mM MgCl₂, and 10 mM KCl. For PFP and UDPG pyrophosphorylase, 5 mM KF was included in the assay mixture; for glucokinase and fructokinase, hexoses were increased to 20 mM; and for PFK, Na₃-ATP replaced Mg-ATP. Fructose-1,6-bisphosphate was assayed in a 1-mL assay volume containing 0.4 mM NAD, 4 IU of glu-6-P dehydrogenase, 2 IU of phosphoglucoisomerase, and 1 mM fru-1,6-bisP. Sucrose phosphate synthase was assayed as previously described (15). Inorganic pyrophosphatase, assayed in the presence of 1 mM PPI, was determined as the release of Pi measured according to Lanzetta et al. (23).

SDS-PAGE and Western Blot Hybridization. Cell extracts were subject to SDS-PAGE using a discontinuous system with acrylamide concentrations of 4.5% in the stacking gel and 10% in the resolving gel (22). Protein was electroblotted from gels to nitrocellulose membrane using a Transphor TE 51 apparatus (Hoefler Scientific Instruments) for 3 h at an initial current of 0.6 amp as previously described (10), except that methanol was omitted from the transfer buffer to facilitate transfer of protein from the gel. Binding of protein by the nitrocellulose was shown to be unaffected by this change. Rabbit anti-potato tuber PFP β-subunit serum prepared (21) and generously provided by Dr. D. Dennis (Queen’s University, Kingston, Ontario) was used to probe nitrocellulose blots at a dilution of 1:20. Immunoreactive peptides were visualized using horseradish peroxidase conjugated goat anti-rabbit IgG and color development reagents.

Densitometry of blots was performed using a CAMAG TLC Scanner II interfaced with a CAMAG SP4290 integrator. Nitrocellulose membranes were scanned at 0.2 mm s⁻¹ in reflectance mode at a wavelength of 500 nm.

Metabolite Determination. Cells were harvested onto a 40-μm mesh under vacuum and frozen in liquid N₂ within 30 s of removal from the culture chamber. The frozen cell clumps were ground to a powder in liquid N₂ and stored at −80°C until use. Fru-2,6-bisP was extracted from 0.2 g of cells in 2 mL of 0.1 M NaOH. The partially frozen extraction slurry was allowed to thaw on ice with periodic stirring, French pressed at 14,000 p.s.i., mixed with 20 mg activated charcoal, and centrifuged at 17,000g for 15 min.

Metabolites other than sucrose were extracted from 1.2 g of cells in 3 mL of 0.45 M HClO₄, using the techniques described above. The extract was then made 50 mM in KF and centrifuged at 17,000g. Extract pH was adjusted to between 7 and 8 with 2 M K₂CO₃, mixed with 20 mg activated charcoal, and centrifuged. Activated charcoal was omitted for extracts analyzed for nucleotides. Sucrose and free hexoses were extracted from 15 mg of lyophilized cells in 3 mL of 80% ethanol at 95°C for 30 min. When it was necessary to remove exogenous sugars, cells were initially washed for 30 min with three changes of 1 mM CaCl₂ (10 mL per mL of cells) before lyophilization. Ethanol extracts were evaporated under vacuum to dryness and redissolved in 1 mL distilled H₂O.

Metabolite determinations were coupled to the reduction of NAD or oxidation of NADH as measured spectrophotometrically at 340 nm in a 1-mL assay volume. Fru-2,6-bisP was measured by stimulation of partially purified potato PFP (30). Acid lability of fru-2,6-bisP was demonstrated by treatment of cell extract at pH 2 for 10 min. Linearity of fru-2,6-bisP measurement with respect to extract volume was periodically confirmed. The in vivo concentration of fru-2,6-bisP in these cells was calculated from the empirically determined relationship, 1.54 mL packed-cell volume per g fresh wt, and estimating the cytoplasmic compartment as 10% of the total volume of the cell (12). Sucrose, hexoses, and hexose-phosphates were determined in a standard assay cocktail consisting of 100 mM Hepes-KOH, pH 7.05, 1 mM NAD, 5 mM MgCl₂, and 5 mM DTT. Sequential determinations of glu-6-P and fru-6-P were made following additions of 2 IU glu-6-P dehydrogenase and 2 IU phosphoglucoisomerase, respectively. Glucose and fructose were determined in a similar manner in the presence of 1 mM ATP and 2 IU hexose kinase, and sucrose was determined subsequently following the addition of 30 IU invertase. In some early experiments, total sugar in the culture medium was determined in assays initiated with glu-6-P dehydrogenase. The basic assay mixture for measurement of ATP and 3-PGA contained 100 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 8 mM MgCl₂, 0.2 mM NADH, 1 IU glycerol-3-P dehydrogenase, and 5 IU each of phosphoglycerate kinase, glyceraldehyde-3-P dehydrogenase, and triose phosphate isomerase. This assay mixture was supplemented with 5 mM 3-PGA or 20 mM ATP for determination of cellular ATP and 3-PGA, respectively. UDPG and UTP were determined as previously described (18a). PPI was assayed as previously described (12), and fru-1,6-bisP and triose-P were determined using this procedure with omissions of PFP or PFP and aldolase, respectively.

Starch was determined in HClO₄ insoluble pellets. Pellets were heated at 90°C in 5 mL of 80% ethanol containing 20 mM Tris-base to remove residual free sugars. Starch was solubilized from the pellets in 2 mL of 0.2 M KOH at 100°C for 1 h, brought to pH 5 with 1 M acetic acid, and converted to glucose with the addition of 35 IU amyloglucosidase and 50 IU α-amylase at 55°C for 1 h. The sample was then heated at 100°C for 5 min and centrifuged, and an aliquot of the supernatant was assayed for glucose as described above.

Metabolite recoveries were determined by adding known amounts of metabolites in the procedures described above. Average percent recoveries of four samples were as follows (number in the parentheses indicates micromoles of metabolite added): sucrose, 78 (0.15); glucose, 80 (0.05); fructose, 69 (0.01); glu-6-
RESULTS

Cell Growth. The soybean suspension cells entered log phase rapidly upon transfer to fresh medium containing 11 mM sucrose (Fig. 1A). Between sucrose concentrations of 11 mM and 88 mM, carbohydrate concentration was not limiting to growth rate but did determine final cell density in stationary phase. Sugar, when initially 11 mM sucrose, was depleted from the medium on the 4th d following transfer (Fig. 1A). At this time the cells abruptly entered stationary phase and cell growth ceased. During log phase, total Chl increased relatively slowly, and photosynthesis, measured as oxygen evolution, decreased (Fig. 1B). Oxygen evolution data are presented on a cell volume rather than Chl basis because the Chl level changed during the growth cycle. Concomitant with the onset of stationary phase, both the Chl content of the cells and the rate of oxygen evolution increased, indicating the onset of photoautotrophy (Fig. 1B). Stationary phase cells required elevation CO2 to remain viable and remained green and healthy for at least a month.

Growth of soybean cells on different sugars was compared since sugar type can strongly influence growth and metabolism of cultured cells (13). Cells were cultured on 58 mM sucrose or 111 mM hexoses to provide an extended log phase. Vigorous growth occurred in the presence of sucrose, glucose, and fructose (Fig. 2A), although no growth occurred and cell death ensued in the presence of galactose, arabinose, and mannose (data not shown). Exogenous sucrose was rapidly hydrolyzed to hexoses with no detectable sucrose remaining after d 2 (Fig. 2B). Glucose was taken up preferentially over fructose, which was not significantly metabolized until all glucose was removed from the medium. Identical results were obtained in cultures initiated on equimolar glucose and fructose (data not shown). Interestingly, in the presence of individual hexoses, cell growth rate and hexose uptake were faster for fructose than for glucose (Fig. 2A). Cells grown on media containing equimolar glucose and fructose grew at the same rate as cells grown on sucrose (data not shown), indicating that the difference in growth rates for these sugars was not the result of a stimulatory factor present in the commercial fructose.

Enzyme Activities. Activities of several enzymes involved in glycolysis and sucrose metabolism were measured in desalted

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**FIG. 1.** Growth cycle characteristics of cells grown in the presence of 11 mM sucrose. Cells from a stationary phase culture, 12 d post-transfer, were washed free of old medium and aliquoted into 100-mL volumes of fresh medium containing 11 mM sucrose and grown under the conditions described in the text. Three individual cultures were harvested and the data were averaged for each time point. A, Total cell volume (O); concentration in the growth medium of total free sugars expressed as sucrose equivalents as described under "Materials and Methods" (O). B, Total Chl per 100-mL cell culture (A) and photosynthesis rate (A). Photosynthesis rates are presented as oxygen evolution per unit cell volume as described in the text.

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**FIG. 2.** Growth and carbohydrate assimilation by cells grown in the presence of different sugars. Cells from a stationary phase culture, 12 d post-transfer, were washed free of old medium and aliquoted into 200-mL cultures. For each time point, cells from individual cultures were harvested and divided into three samples prior to processing as described under "Materials and Methods". A, Total cell volume per 100 mL of media for cultures initially containing 58 mM sucrose (O), 111 mM glucose (O) or fructose (O). B, Concentration in the growth medium of glucose (O, O), fructose (A, A) and sucrose (O). Closed symbols are for culture media initially containing only the indicated sugar; open symbols are for sugars produced in media initially containing only sucrose. Error bars indicate standard deviations, where visible.
extracts of cells harvested in log and stationary phases (Table I). The activities of a number of these enzymes increased during log phase. Fructokinase (ATP- and UTP-dependent) activity was higher than glucokinase activity at both growth stages and increased during log phase. High activity of UDPG pyrophosphorylase was present in both phases of growth and increased slightly during log phase. Neutral invertase and sucrose synthase, both enzymes implicated in initial catabolic steps of sucrose metabolism, had similar activities in the cells, although the activity of sucrose synthase decreased during log phase. ATP-dependent glucokinase and fructose-1,6-bisphosphatase, an enzyme implicated in sucrose biosynthesis, also decreased during log phase. Addition of 10 mM EDTA to the assay mixture had no effect on this activity, indicating that the predominant fructose-1,6-bisphosphatase activity was cytosolic in origin, and no change in activity was observed in the presence of 20 μM fru-2,6-bisP.

The largest difference was observed in the activity of PFP; activity of this enzyme was almost three-fold higher in log phase cells. In contrast to PFP, the activity of PFK did not show a difference between log and stationary phase cells. The activity of PFP was entirely dependent upon the presence of fru-2,6-bisP.

Activities of PFP and other enzymes were examined on different days following cell transfer to better characterize enzyme activities throughout the cell growth cycle (Fig. 3). The increase in PFP activity occurred on the first 3 d following cell transfer during log phase growth (Fig. 3A). When the external sugar was exhausted and the cells entered stationary phase, PFP activity decreased while remaining higher than that of PFK, which remained essentially unchanged at 26 ± 3 nmol min⁻¹ mg⁻¹ of protein. Sucrose synthase activity (Fig. 3B) decreased 40 to 50% as cells entered log phase and then rose again starting on d 3. In contrast, neutral invertase activity essentially remained unchanged throughout the growth cycle. UDPG pyrophosphorylase activity (Fig. 3C) increased during log phase and peaked with a 58% increase in activity when cells entered stationary phase. Activity then declined slightly during stationary phase.

The effect of exogenous sugar type on PFP activity during log phase was examined for cells cultured on glucose, fructose, or sucrose. Total PFP activity increased during log phase in cells grown on either glucose or fructose alone, as well as sucrose (Fig. 4). Maximum PFP activity was similar for cells on each sugar, and also for cells grown in the presence of sucrose at initial concentrations of 11 mM (Fig. 2A), 58 mM (Fig. 4), and 88 mM (data not shown). In all cases, PFP activity began to decline when the external sugar was depleted and cells entered stationary phase.

**Table I. Enzyme Activities in Cells from Log Phase and Stationary Phase Cultures**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Culture Growth Stage</th>
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<tbody>
<tr>
<td></td>
<td>Log</td>
<td>Stationary</td>
</tr>
<tr>
<td></td>
<td>nmol product min⁻¹ mg protein⁻¹</td>
<td></td>
</tr>
<tr>
<td>PFP</td>
<td>173 ± 51*</td>
<td>59 ± 19**</td>
</tr>
<tr>
<td>PFK</td>
<td>32 ± 2</td>
<td>29 ± 0.6</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
<td>93 ± 12</td>
<td>136 ± 13*</td>
</tr>
<tr>
<td>Sucrose synthase</td>
<td>22 ± 0.1</td>
<td>30 ± 9*</td>
</tr>
<tr>
<td>Neutral invertase</td>
<td>32 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Glucokinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP-dependent</td>
<td>4 ± 0.9</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td>UTP-dependent</td>
<td>0.9 ± 0.2</td>
<td>2.5 ± 0.8*</td>
</tr>
<tr>
<td>Fructokinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP-dependent</td>
<td>13 ± 1</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td>UTP-dependent</td>
<td>29 ± 2</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>UDPG pyrophosphorylase</td>
<td>1965 ± 158</td>
<td>1384 ± 265*</td>
</tr>
<tr>
<td>Pyrophosphatase</td>
<td>600 ± 61</td>
<td>622 ± 86</td>
</tr>
<tr>
<td>Sucrose-phosphate synthetase</td>
<td>49 ± 9</td>
<td>61 ± 6</td>
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*Standard deviation (n = 3). **Difference between means for log and stationary phase cells significant at 0.05 (*) or 0.01 (**) levels using Student t-test.
Materials and Methods. A single major immuno-reactive band, with a calculated M_r of 56 kD, was detected in the cell extracts. This was smaller than the M_r of the potato PFP β-subunit (Fig. 5A, lane 6), which was calculated as 61 kD in close agreement with previous results (21). The amount of PFP, as determined from relative intensities of the 56 kD band, increased in extracts from days 0 to 3, and decreased again on days 5 to 8. The change in PFP level correlated with the PFP activity during the growth cycle (Fig. 5B; the correlation coefficient, r, between band intensity and PFP activity was 0.939). No reaction was observed for blots probed with preimmune serum (data not shown).

Metabolite Levels. Changes in PFP activity and the level of the activator metabolite fru-2,6-bisP have been reported to be correlated in some whole plant systems (4, 5, 8). Therefore, we determined the levels of this metabolite and others during the growth cycle of the soybean cells (Figs. 6 and 7). Significant levels of all metabolites measured existed in the cells prior to transfer (day 0) and reflected the stationary phase pool sizes supported photoautotrophically. Transfer of stationary phase cells to fresh medium resulted in rapid transient increases in the levels of most metabolites. In this experiment cells were grown on glucose, so that hexose-P would be the expected initial metabolite of incorporation. The levels of both glu-6-P and fru-6-P peaked during the first 24 h after transfer (Fig. 6A) followed by a gradual decline throughout log phase. UDPG is the principal entry point for carbon into structural carbohydrates, and throughout log phase the UDPG level was two- to three-fold elevated (Fig. 6B). Increased carbon flux into this pathway and higher PFP activity during log phase may both contribute to the decline in hexose-P pools during log phase. Levels of both 3-PGA (Fig. 6B) and ATP (Fig. 6C) transiently peaked during the first 12 h after transfer. ATP levels again increased during log phase. 3-PGA levels decreased during log phase and subsequently increased as cells entered stationary phase, and the change probably reflected changes in photosynthetic rate (Fig. 1B).

The level of fru-2,6-bisP increased during the first 12-h period following transfer, and then returned to the stationary phase level prior to the increase in PFP activity (Fig. 7A). Following this transient increase, fru-2,6-bisP remained relatively constant throughout log phase, and declined slightly when cells entered stationary phase. Fru-2,6-bisP has been reported to be restricted to the cytosolic compartment (29), and its concentration in these cells was calculated to range from a maximum of 5.3 μM at time 12 h, to 2.7 μM in late stationary phase (d 14). These values are very close to estimates of fru-2,6-bisP made by similar means for cultured cells of Catharanthus roseus (3).

Sucrose and starch also increased during the first 12 h after transfer (Fig. 7B). Internal sucrose followed a pattern similar to that of fru-2,6-bisP during the first 24 h and then began to increase again during log phase. Internal sucrose pools of similar sizes formed in cells grown on sucrose, glucose, or fructose (Table II). Free hexose pool sizes were much lower than that of sucrose and were not influenced by the type of exogenous sugar present (Table II). After an initial increase, starch levels decreased slowly

Fig. 5. Immunological detection of soybean PFP. Samples were subject to SDS-PAGE and blot transferred to nitrocellulose as described under "Materials and Methods". A, Antigenic peptides were probed with anti-potato PFP β-subunit antibody as the primary antibody and goat anti-rabbit IgG horseradish conjugate as the secondary antibody. Lanes 1 through 5 contain 50 μg protein from extracts of cells harvested on different days after transfer from the same experiment shown in Figures 1 and 3: lane 1, d 0; lane 2, d 1; lane 3, d 3; lane 4, d 5; lane 5, d 8. Lane 6 contains 5 μg of partially purified potato tuber PFP. The position of molecular mass markers transferred to nitrocellulose and stained with amido black are shown to the left of the figure, and were, from largest to smallest, phosphorylase b, BSA, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor. B, Reflective intensity of the 56 kD band on Western blots. Blots were scanned as described under "Materials and Methods", and reflective intensity (open bars) and total PFP activity (shaded bars) were normalized to 100% for d 3. Data for PFP activity were derived from Figure 2.
CARBOHYDRATE METABOLISM BY SOYBEAN CELLS

Table II. Internal Sugar Levels in Cells Grown in the Presence of Different Sugars

<table>
<thead>
<tr>
<th>Medium Carbohydrate</th>
<th>Internal Level of Carbohydrate</th>
<th>μmol g dry wt⁻¹</th>
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<tbody>
<tr>
<td>Sucrose</td>
<td>173 ± 1*</td>
<td>7.6 ± 1.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>218 ± 3</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>Fructose</td>
<td>262 ± 10</td>
<td>4.7 ± 0.6</td>
</tr>
</tbody>
</table>

* Standard deviation (n = 3).

Throughout log phase (Fig. 7B). Starch most likely was not mobilized during log phase, and its decrease per unit fresh weight probably reflected increased carbon flux into structural carbohydrates during cell division. UTP, fru-1,6-bisP, and triose-P were also measured but were found not to occur at detectable levels during the growth cycle.

DISCUSSION

The soybean mixotrophic cells used for this study were originally derived from a photoautotrophic suspension culture (14), but after several years of mixotrophic growth the cells have lost the ability to divide photoautotrophically. Measurable growth only occurs during log phase and is dependent on an external supply of carbohydrate. Metabolism of external sugars has the effect of repressing photosynthetic activity, and this repression is a common feature of cultured cells (18). When the carbohydrate is depleted from the culture medium, the soybean cells immediately shift into stationary phase. The onset of photoautotomy is indicated by increased photosynthetic capacity and by requirements for elevated atmospheric CO₂ and illumination in excess of 200 μE m⁻² s⁻¹ for continued cell viability. The inability of these cells to grow photoautotrophically indicates that either the photosynthetic rate is not sufficient to support cell division or the cells have changed developmentally. Therefore, these soybean cells provide a unique system to study carbon metabolism under heterotrophic and autotrophic conditions.

External inversion of sucrose is common to many species in culture (13), including the soybean cells used in this study. Thus, the soybean cells metabolize exogenous hexoses rather than sucrose during most of the log phase. However, sucrose metabolism in these cells is clearly complex in view of the large internal pool of sucrose produced in log phase cells. A portion of the UDPG and fru-6-P present in log phase cells may be diverted through sucrose-phosphate synthase into sucrose. In contrast with sucrose, internal levels of hexoses remain very low even when the cells are grown exclusively on glucose or fructose, suggesting that sugar uptake and phosphorylation may be coupled (13). In this context, the higher activity of fructokinase than glucokinase in these cells is interesting, and the contribution of these enzymes to the more rapid growth on fructose than on glucose warrants further investigation.

A shift in carbon metabolism during the transition from photoautotrophic stationary phase to mixotrophic log phase growth was indicated by changes in the activities of several enzymes. The largest change was in the activity of PFP, and Western blot analysis indicated that coarse regulation of total PFP peptide occurs during the growth cycle. The magnitude of the change in PFP activity and its correlation with the period of sugar import would argue that PFP activity is linked to the metabolism of exogenous sugars during the log phase of cell growth. However, measurements of flux through PFP and PFK would be necessary to determine the actual contributions of each enzyme to overall metabolism.
of these pathways. Coarse regulation of PFP activity in response to availability of exogenous sugar appears not to be a phenomenon restricted to photosynthetic sugar cultures. PFP activity was also reported to fluctuate during the growth cycle of C. roseus (3) and to decline in heterotrophic Acer pseudoplatanus suspension cells when starved of sucrose (17). In whole plants, highest PFP activity is often found in rapidly growing tissues, such as young leaves, root tips, and shoot apices (7, 28). Thus, in cultured cells, as in whole plants, PFP activity may be induced under conditions of high biosynthetic activity (11).

The increase in PFP activity during log phase was not dependent upon the specific presence of sucrose, but also occurred in the presence of either glucose or fructose alone. Thus, coarse regulation of PFP activity is not linked directly to the presence or uptake of exogenous sucrose. Hexitol assimilated from the medium would be expected to be phosphorylated by hexose kinase and then fed through phosphoglucoisomerase to PFK or PFP operating in the glycolytic direction. Alternatively, PFP may be coupled to a gluconeogenic direction to the sucrose synthase-dependent pathway of sucrose breakdown (16). Although we feel that this pathway is less likely, PFP could function in this manner if hexose breakdown by these cells involves an initial conversion into sucrose. Knowledge of turnover rates for the internal sucrose pool would be helpful in resolving this question.

Fru-2,6-bisP concentration and changes in total enzyme activity could both potentially regulate the rate of glycolysis in the PFP-linked pathway. In certain whole plant systems, coordinated changes in total PFP activity and fru-2,6-bisP levels occurred during plant development (7, 28) and banana ripening (5) and upon perturbations in source/sink relationships (8) and photosynthesis (4). The increase in fru-2,6-bisP immediately following transfer of the soybean cells to medium is similar to that reported for cultured C. roseus cells (2). This transitory peak in fru-2,6-bisP may be related to the rapid fluctuations in the levels of fru-6-P and ATP, which are the substrates for the enzyme fru-6-P, 2-kinase (EC 2.7.1.105), that occur after the cells are transferred to the medium containing sugar. Allosteric interaction of this enzyme and fructose-2,6-bisphosphatase (EC 3.1.3.46) (24) with regulatory metabolites also fluctuating after cell transfer may also modulate fru-2,6-bisP. In soybean cells, this peak in fru-2,6-bisP was not associated with significant total PFP activity nor with the period of mixotrophic growth on exogenous sugars. Furthermore, the estimated stationary phase in vivo concentration of fru-2,6-bisP exceeds by more than two orders of magnitude the previously reported activation coefficient for soybean PFP (0.017 μM) (25), suggesting that PFP remains in a fully activated state. These results indicate that during the soybean cell cycle the activity of the PFP-dependent pathway is not modulated by changes in total cytosolic fru-2,6-bisP level, but rather by coarse regulation of total PFP activity alone.

Acknowledgments—We thank S. C. Huber for generously providing the potato PFP utilized in this study and for helpful suggestions and advice, and D. T. Dennis for providing the PFP antibody.

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